

STUDIES OF
MARSSONINA AND DREPANopeziza
SPECIES PATHOGENIC TO POPLARS

VOLUME IIB
FIGURES 87-169

ADRIAN SPIERS
1981

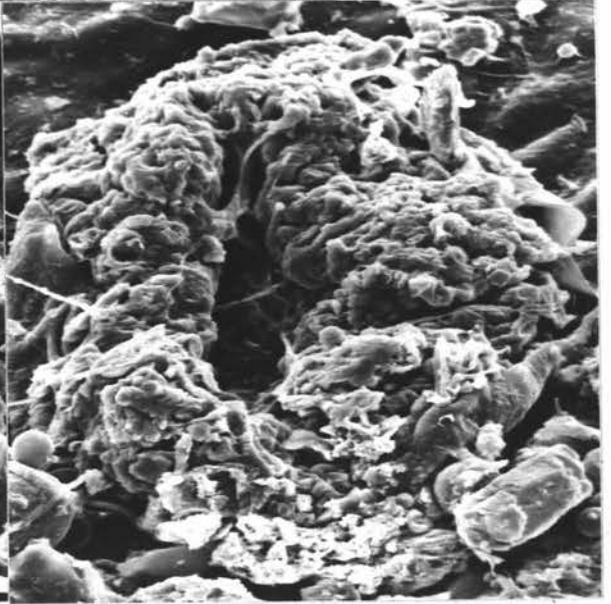
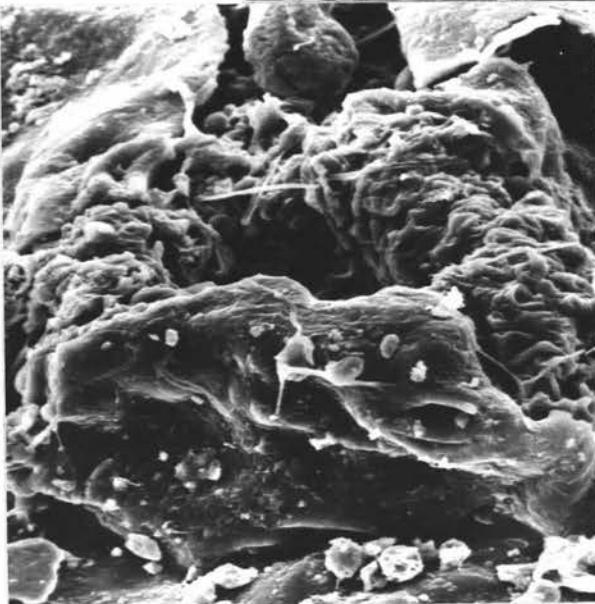
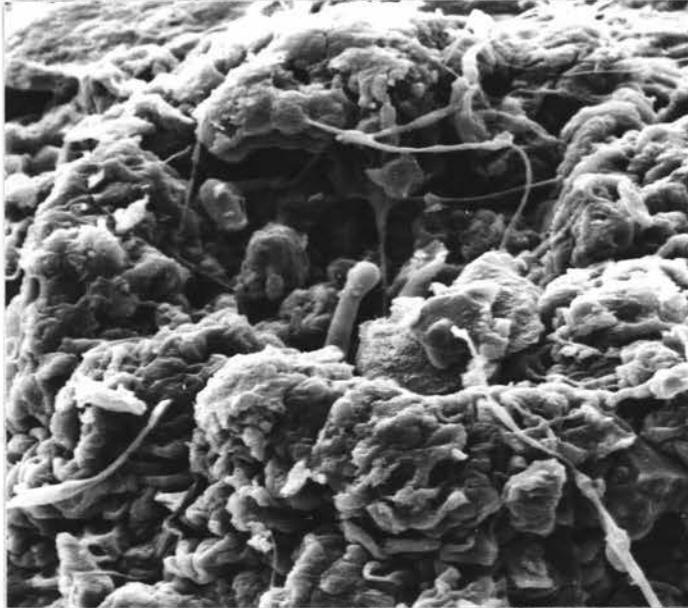
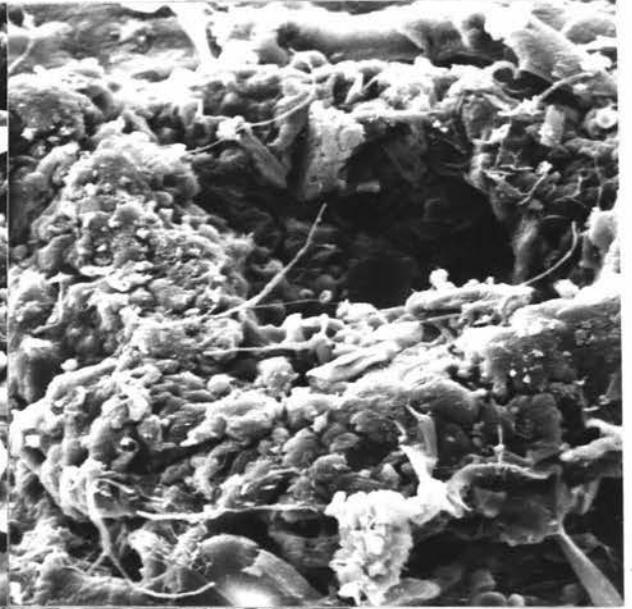
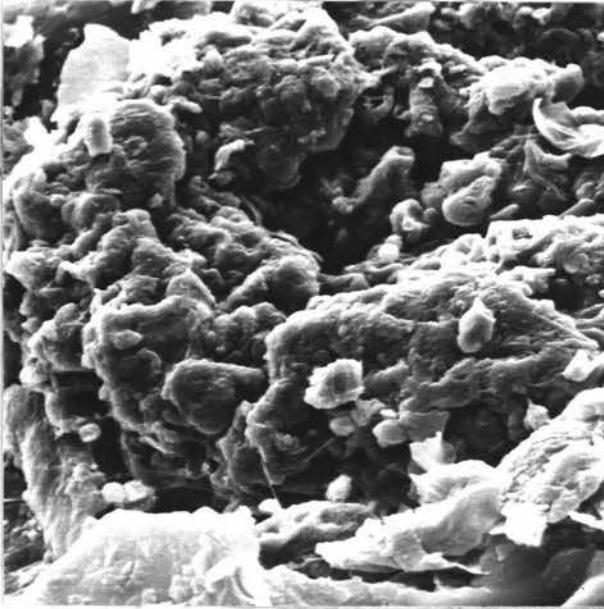


FIG. 87: External morphology of apothecia of *Drepanopeziza tremulae*.

TOP LEFT & RIGHT: *P. tremula*, Brandenburg Germany, collected by Fahrendorff, 11.10.1940. Syntype, X800.

CENTRE: *P. tremula*, Osthavelland Germany, collected by Kirschstein, 24.4.1937. Syntype. Note the protruding paraphyses. X900.

BOTTOM LEFT & RIGHT: *P. tremula*, Osterreich Germany, ex herbarium Von Hohnell no. 1241. X600.

Note that apothecia of *D. tremulae*, *D. populorum* (Figs. 77, 78) and *D. punctiformis* (Fig. 96) are morphologically inseparable.

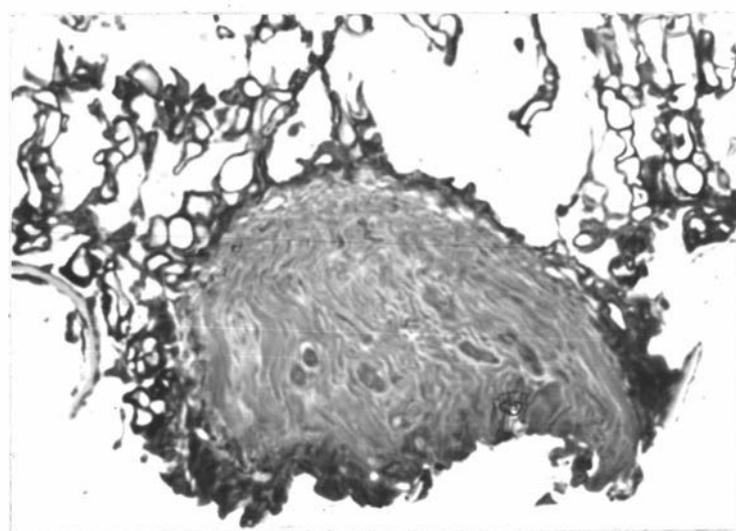
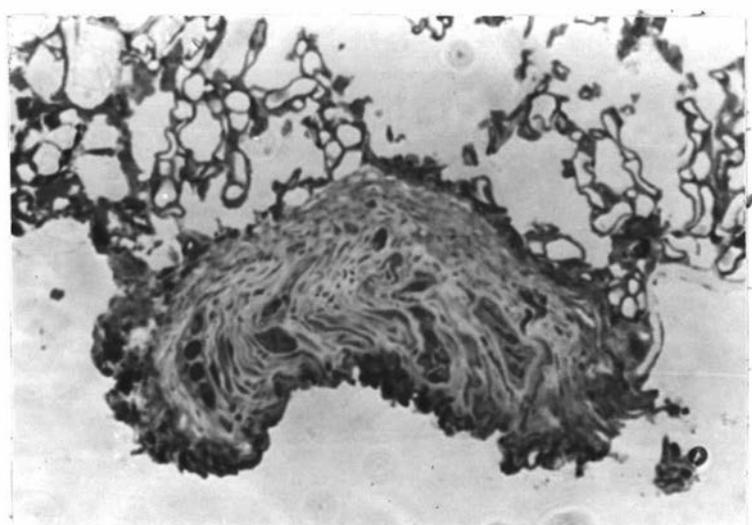
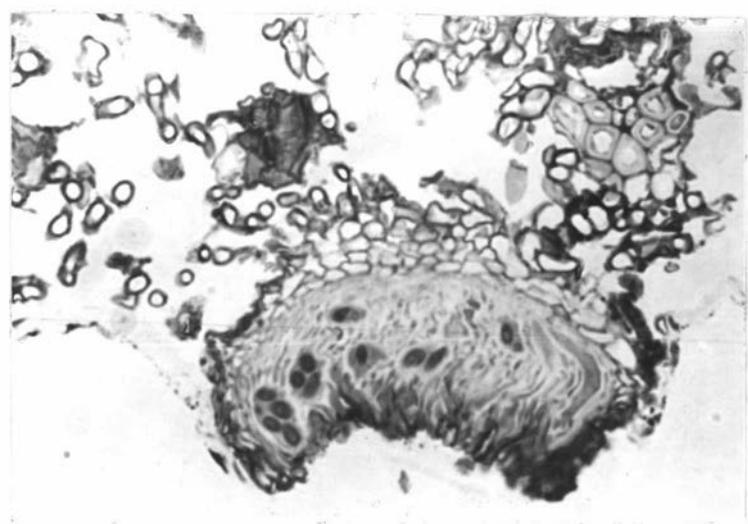


FIG. 88: Vertical sections through apothecia of
Drepanopeziza tremulae.

TOP: *P. tremula*, Brandenburg Germany, Fahrendorff,
11.10.1940, syntype. Note barrel like shape.
X530.

CENTRE: *P. tremula*, Osthavelland Germany, Kirschstein,
24.4.1937, syntype. Note the conical shape in
section. X530.

BOTTOM: *P. tremula*, Osterreich Germany, ex herbarium
Von Hohnel no. 1241. Note the well developed
medullary excipulum. X430.

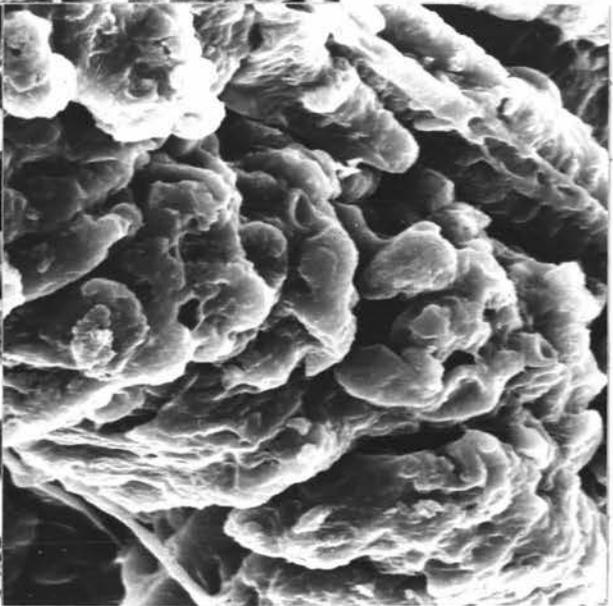
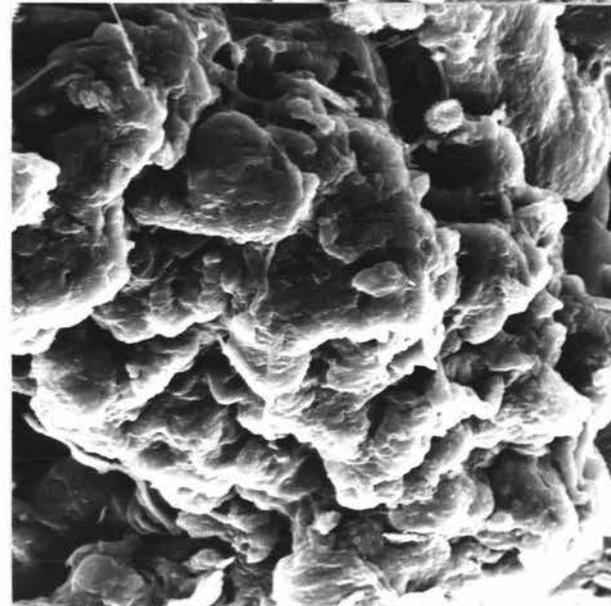
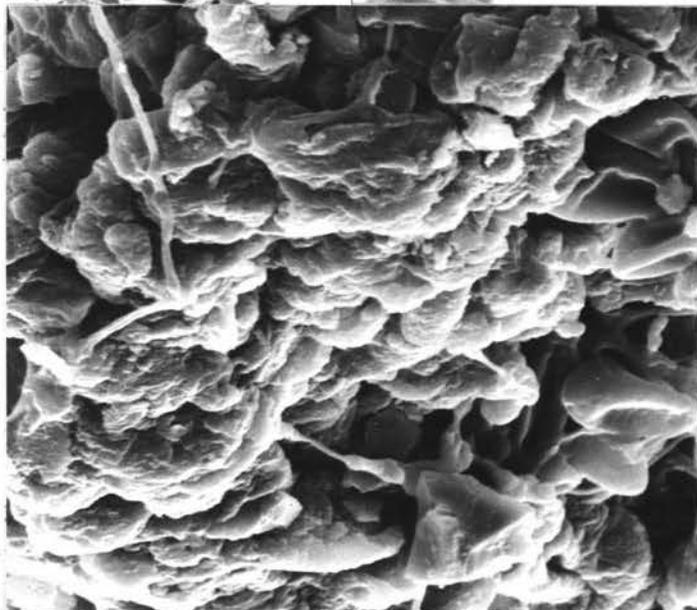
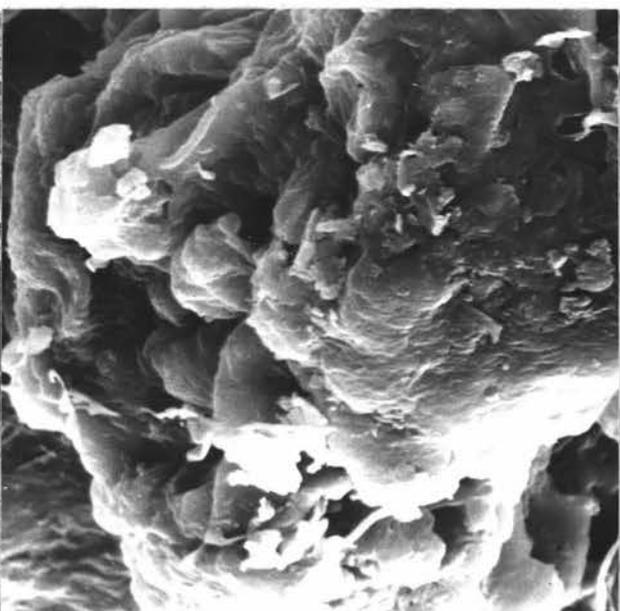
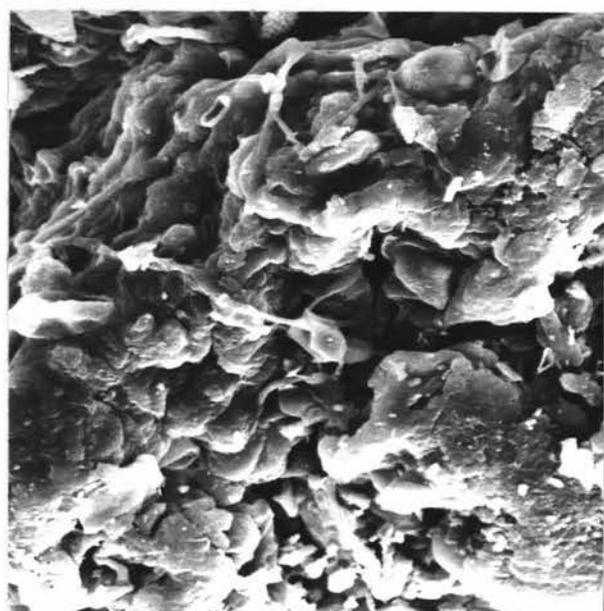


FIG. 89: External morphology of apothecia of *Drepanopeziza tremulae*.

TOP LEFT & RIGHT: *P. tremula*, Brandenburg Germany, Fahrendorff, 11.10.1940. Side wall. X1,800.

CENTRE: *P. tremula*, Osthavelland Germany, Kirschstein, 24.4.1937. Top of apothecium, X1,900.

BOTTOM LEFT & RIGHT: *P. tremula*, Osterreich Germany, ex herb. Von Hohnel no. 1241. X2,400.



FIG. 90: Ectal excipulum *Drepanopeziza tremulae*, *P. tremula*,
Brandenburg, Germany, Fahrenedorff, 11.10.1940.
Note the outer 2-3 layers of large irregular to
globular cells embedded in a dark matrix
bounded on the inside by 2-5 layers of closely packed
elongate cells and paraphyses. X2,800.

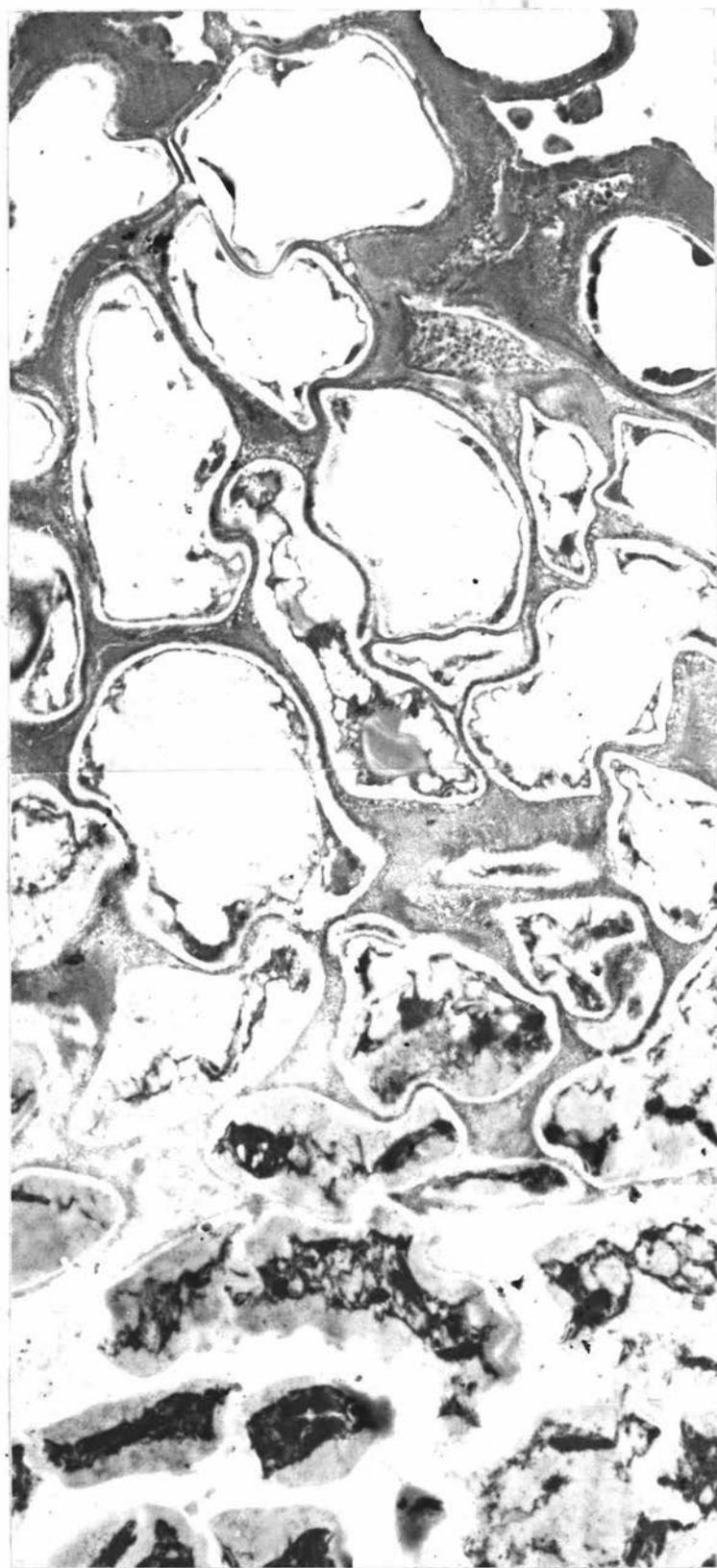


FIG. 91: Medullary excipulum and hypothecium of
Drepanopeziza tremulae.

P. tremula, Osterreich Germany, Von Hohnel no. 2141.

Vertical section through apothecium showing large (5-7 μ diam.) angular globose to sub-globose cells of the medullary excipulum and the small globose to subglobose cells of the hypothecium regularly arrayed around the bases of asci. Note the sharp delimitation between the two tissue layers.

X6,400.

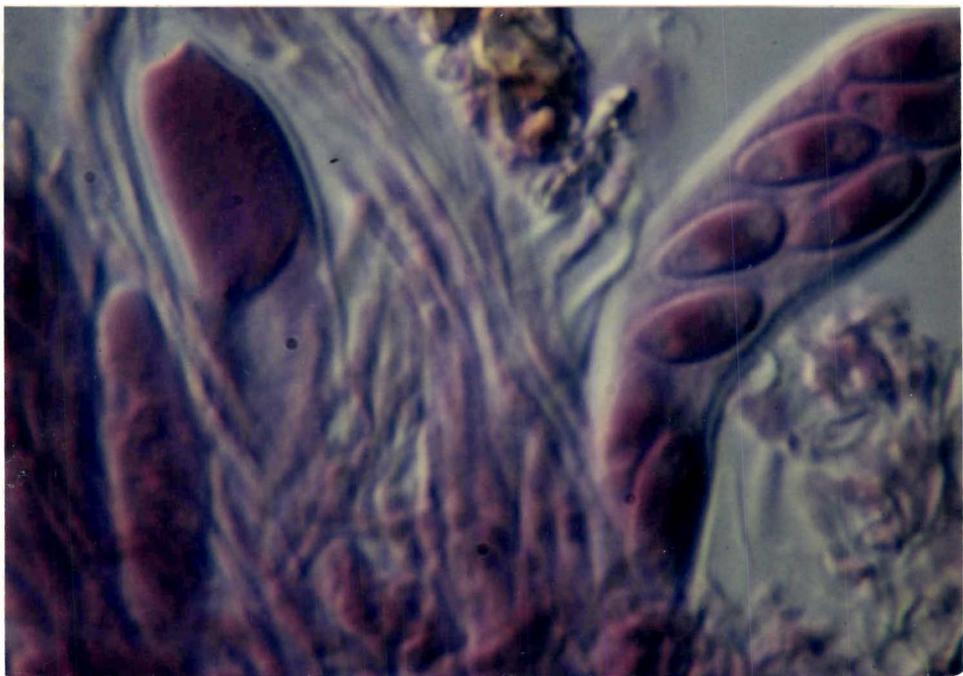


FIG. 92: Asci and ascospores of *Drepanopeziza tremulae* X1,600.

Cylindrical unitunicate asci with thickened apices.
Ascospores unicellular, ellipsoidal, smooth walled,
hyaline (in this instance stained 0.5% acid
fuchsin) with two conspicuous polar bodies.

TOP: *P. tremula*, Brandenburg Germany, collected by
Fahrendorff, 11.10.1940, Syntype.

BOTTOM: *P. tremula*, Osterreich Germany, ex herb. Von Hohnel
no. 1241.

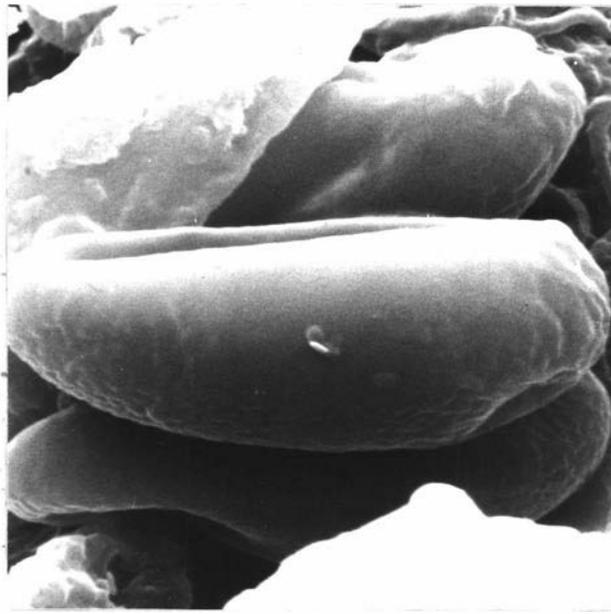
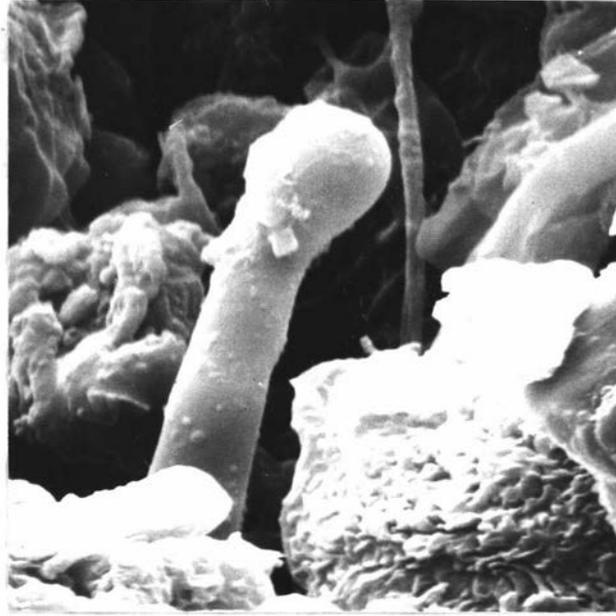


FIG. 93: Paraphyses of *Drepanopeziza tremulae*. *P. tremula*,
Osthavelland, Germany, Kirschstein, 24.4.1937.
Filiform paraphyses protruding from the apothecium
showing clavate terminal cell. X4,000.

FIG. 94: Ascospores of *Drepanopeziza tremulae*, *P. tremula*,
Brandenburg, Germany, Fahrendorff, 11.10.1940.
Note the smooth outer wall. X8,000.

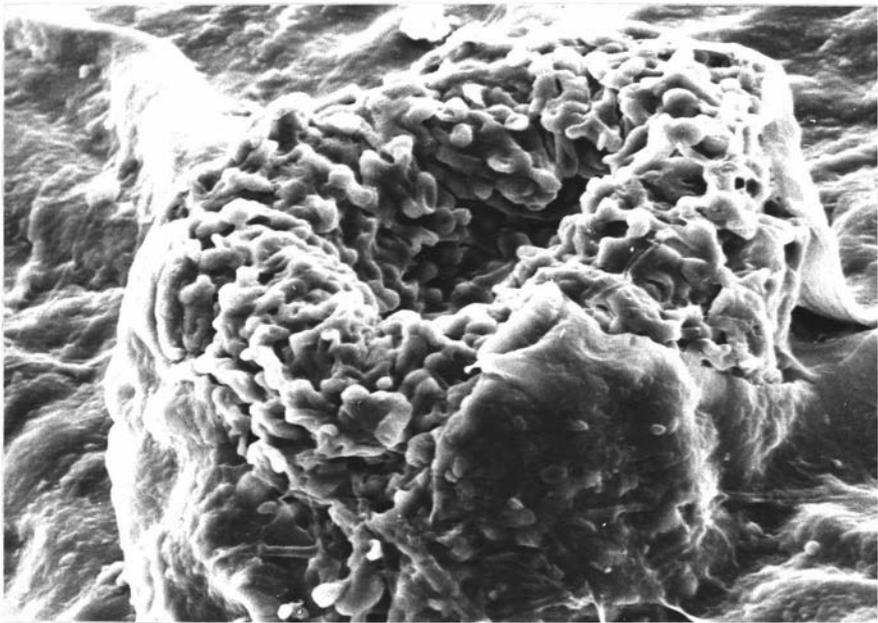
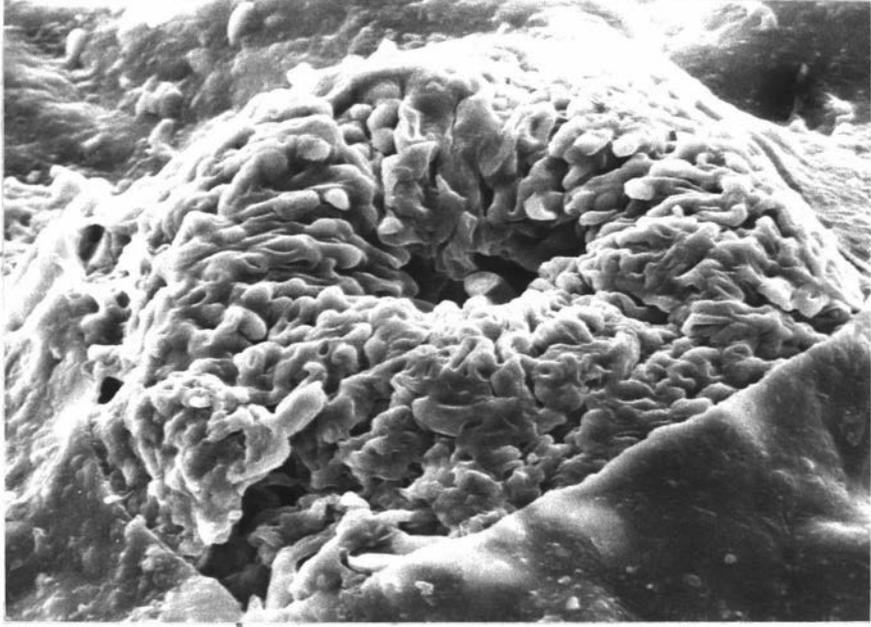


FIG. 95: External morphology of apothecia of
Drepanopeziza punctiformis.

TOP & BOTTOM: *P. deltoides*, Colorado USA, collected by
Hinds, 30.10.1979. Note the circular outline
and the uplifted epidermis. X1,500.

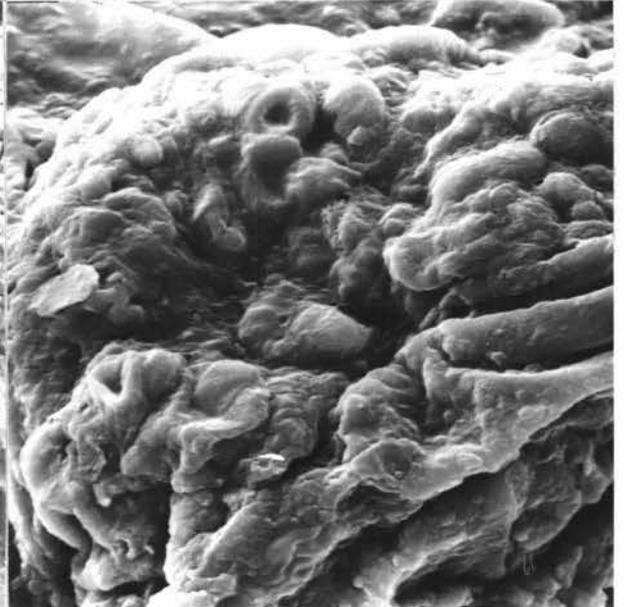
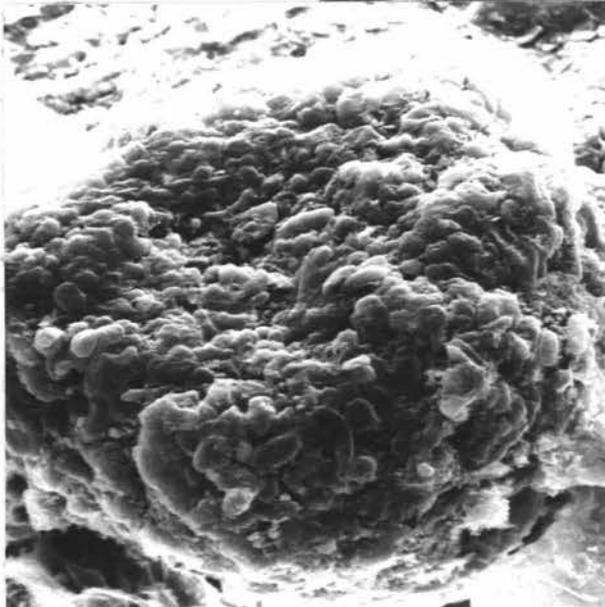
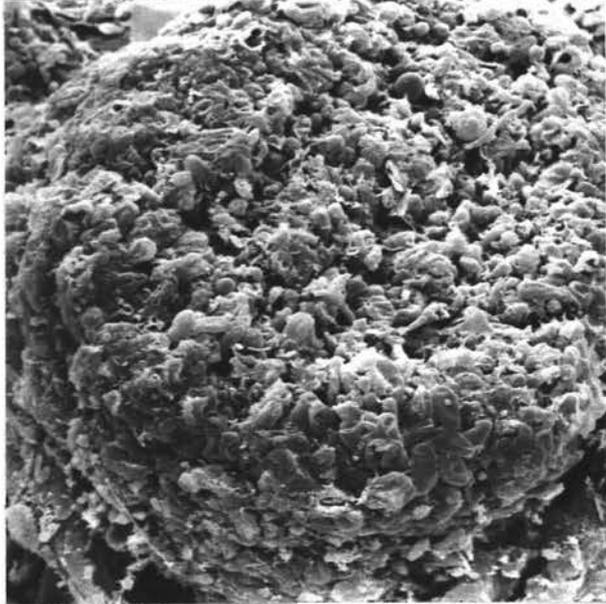
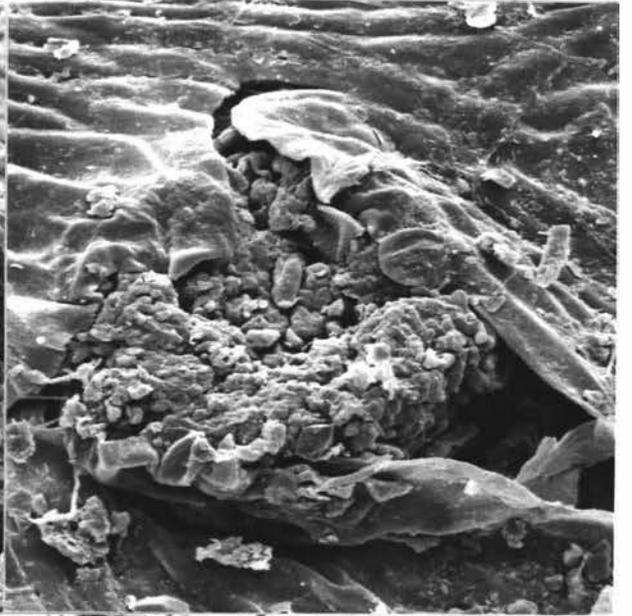
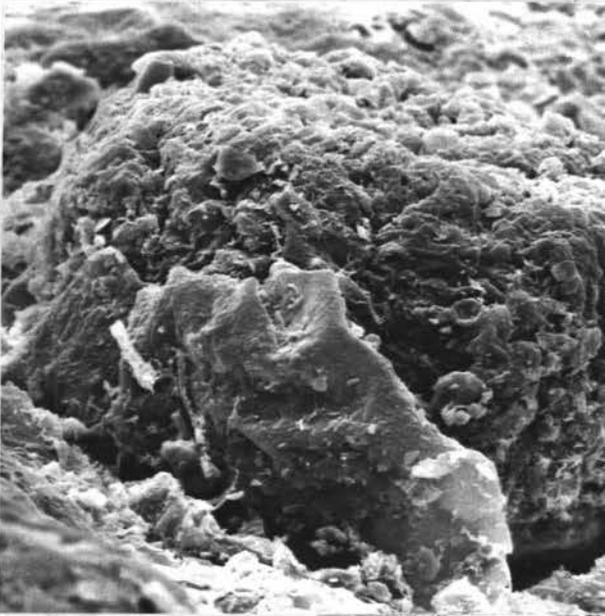


FIG. 96: External morphology of apothecia of *Drepanopeziza punctiformis*.

TOP LEFT, CENTRE, *P. x euramericana*, Nancy France, collected
 BOTTOM LEFT & by Pinon.
 RIGHT:

TOP LEFT, CENTRE, BOTTOM LEFT: X800.
 BOTTOM RIGHT: X1,600.

TOP RIGHT: *P. canadensis* cv. Marilandica, Flevoland
 Netherlands, collected by Gremmen, 21.5.1965.
 Type specimen. Apothecium emerging through
 the adaxial leaf surface. X400.

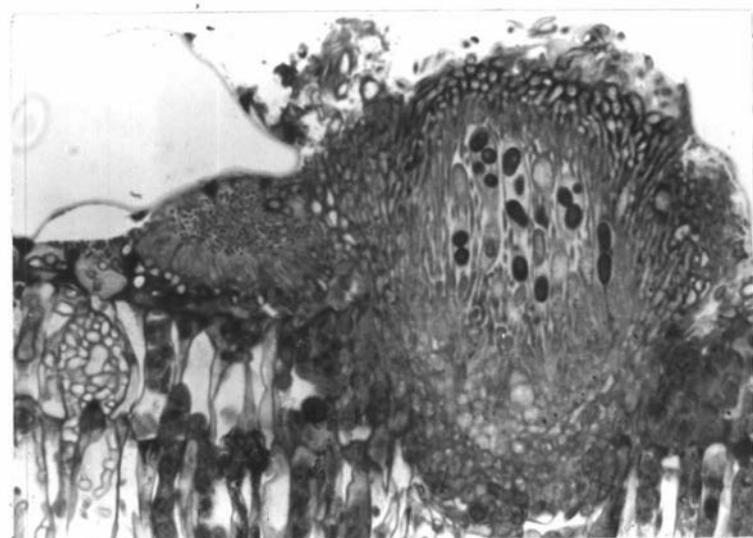
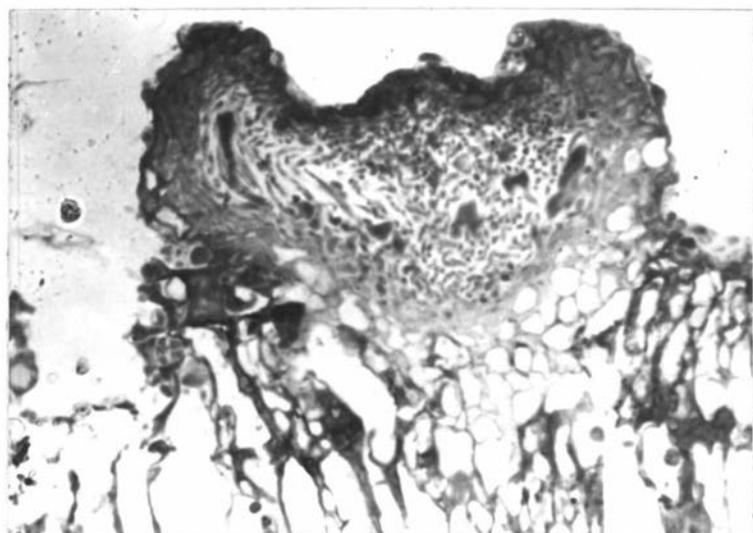


FIG. 97: Vertical sections through apothecia of
Drepanopeziza punctiformis.

TOP: Type specimen *P. canadensis* cv. Marilandica,
Flevoland Netherlands, Gremmen, 21.5.1965.
Barrel shaped apothecium. Note the well
developed ectal excipulum. X550.

CENTRE: *P. x euramericana*, Nancy France, Pinon. Conical
apothecium. Note the poorly developed ectal
excipulum. X550.

BOTTOM: *P. deltoides*, Colorado USA, Hinds, 30.10.1979.
Barrel shaped apothecium with a well developed
ectal and medullary excipulum. X400.

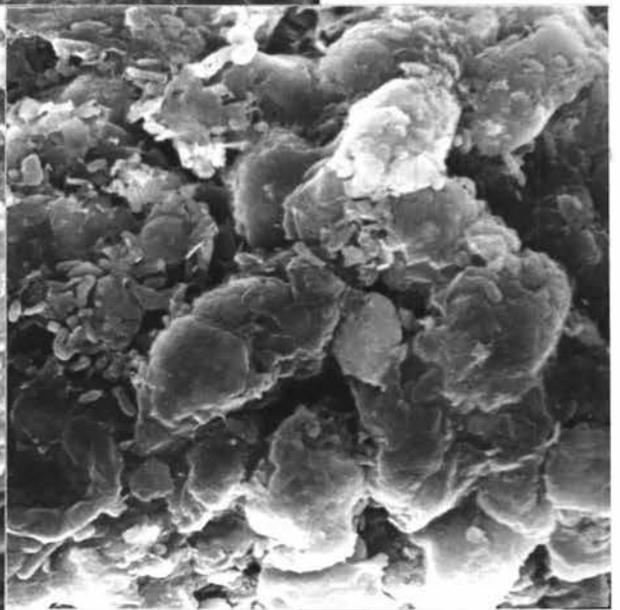
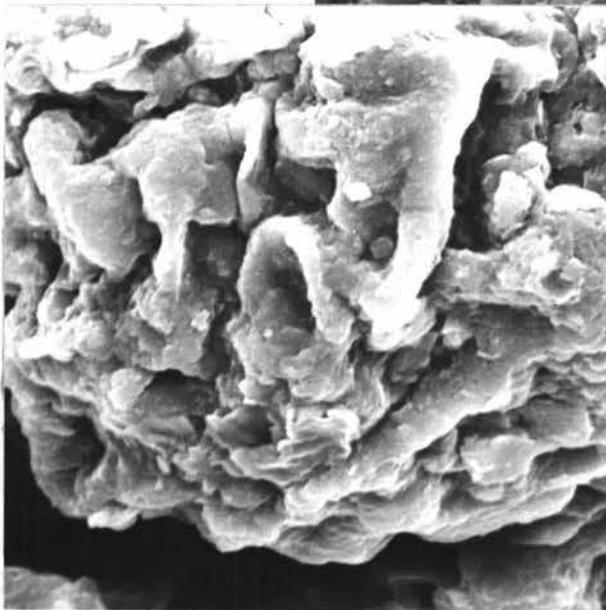
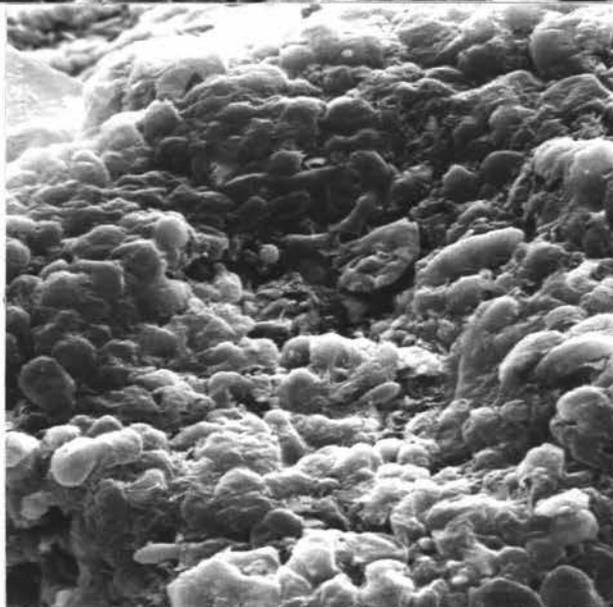
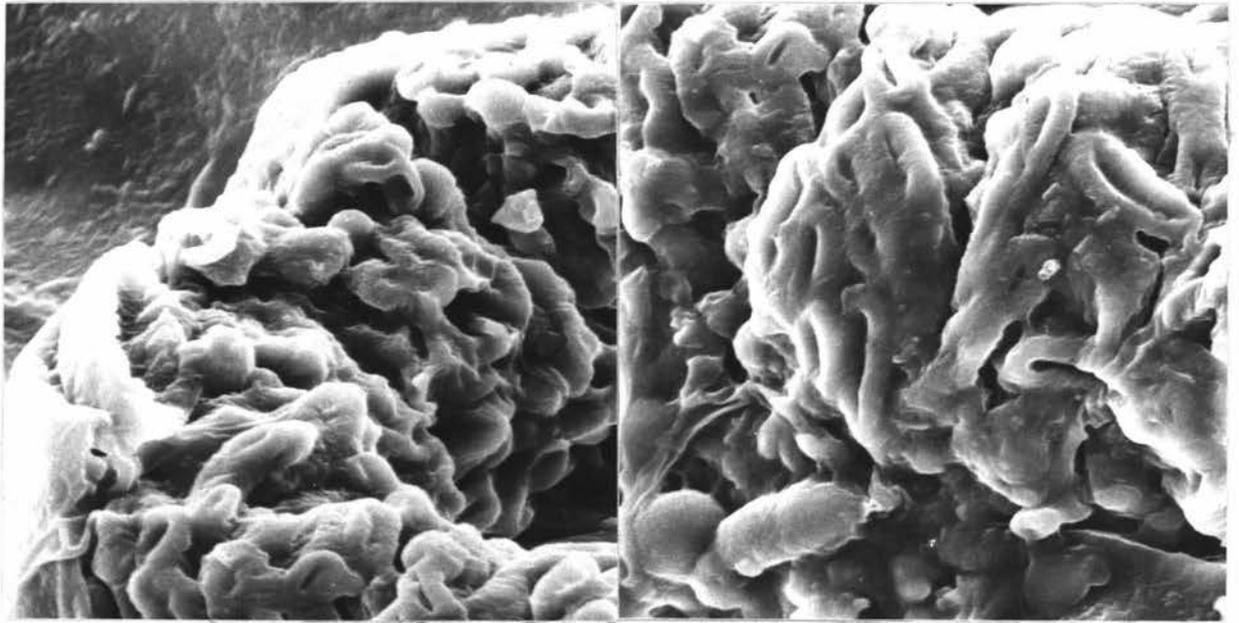


FIG. 98: External morphology of Apothecia of
Drepanopeziza punctiformis.

TOP: *P. deltoides*, Colorado USA, Hinds,
30.10.1979.

LEFT: Side wall of apothecium viewed from
above showing lumpy texture. X800.

RIGHT: Side wall of apothecium. X1,600.

CENTRE &
BOTTOM: *P. x euramericana*, Nancy France, Pinon.

CENTRE: Apothecium viewed from above, X1,400.

BOTTOM LEFT & RIGHT: Side wall of apothecium.

LEFT: X2,400.

RIGHT: X3,200.

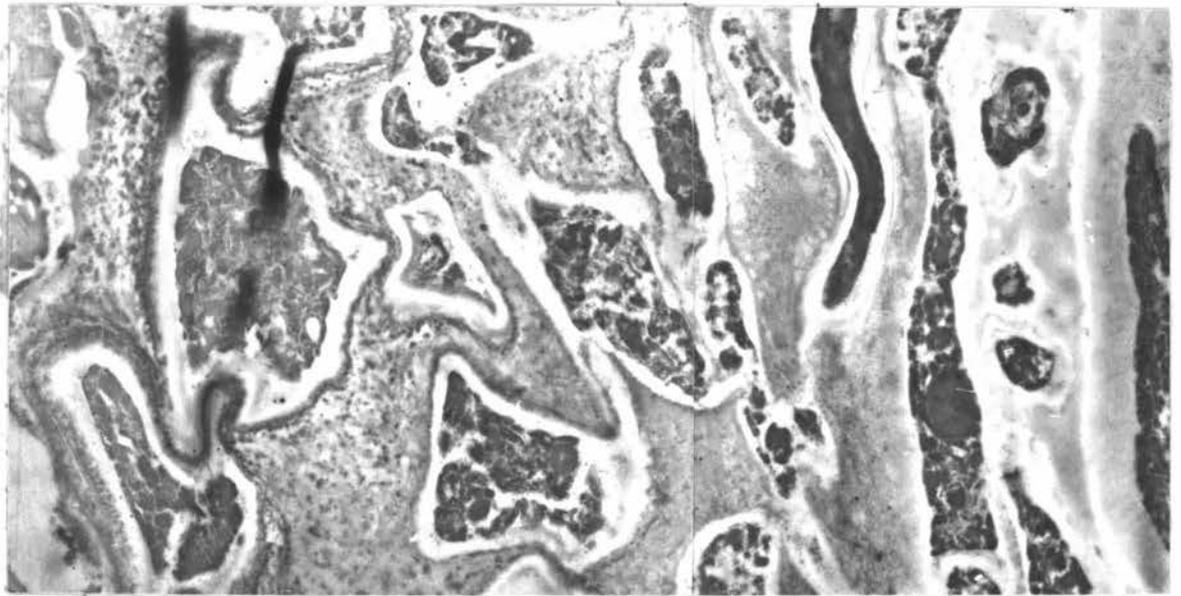
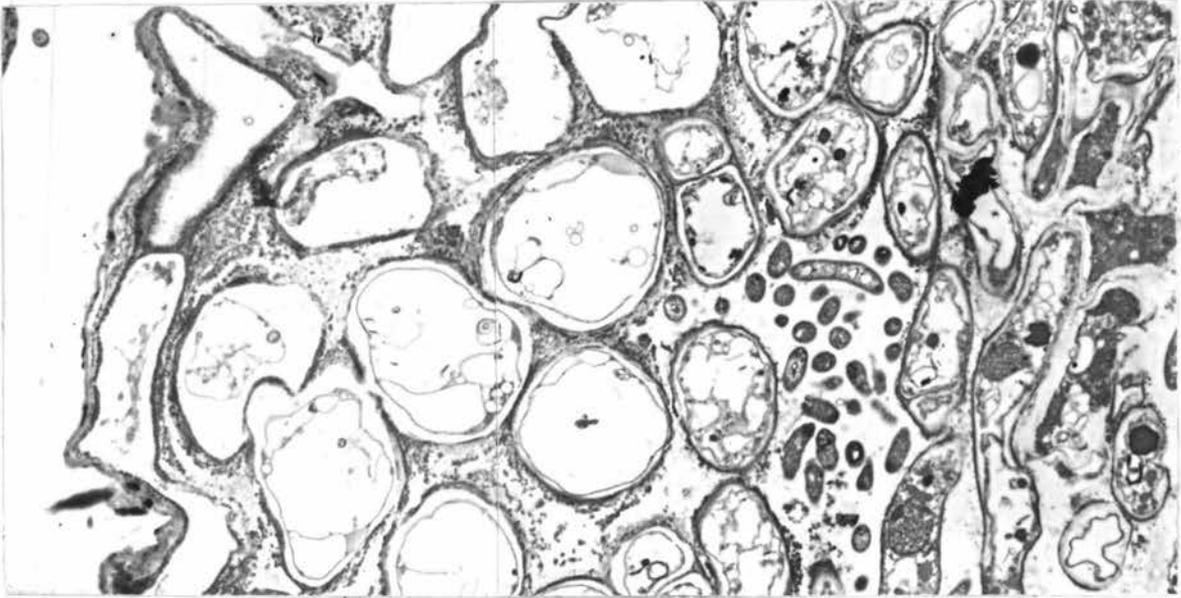
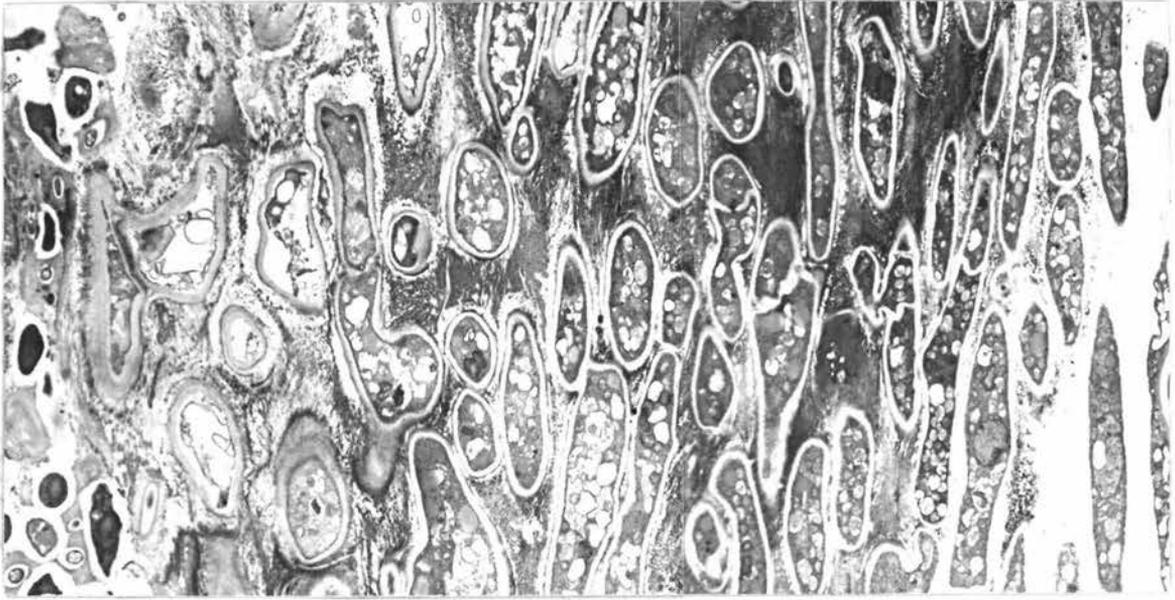


FIG. 99: Ectal excipulum of *Drepanopeziza punctiformis*.

- TOP: *P. deltoides*, Colorado USA, Hinds, 30.10.1979, From left, 2-3 rows of globular to irregular cells bounded inside by several layers of elongated paraphyses-like cells. To the extreme right paraphyses. X3,800.
- CENTRE: *P. x euramericana*, Nancy France, Pinon. From left, 3-4 rows of globular to irregular cells bounded on the inside by 3-4 rows of smaller, closely packed globular cells. To the extreme right paraphyses. X4,500.
- BOTTOM: *P. canadensis* cv. Marilandica, Flevoland Netherlands, Gremmen, type specimen, From left 2-3 rows of globular to angular cells embedded in a dark matrix bounded inside by 2-3 rows of smaller elongate cells and paraphyses. X5,000.

Note Specimens of Hinds and Pinon were fixed in glutaraldehyde when fresh whereas the specimen of Gremmen was rehydrated herbarium material.

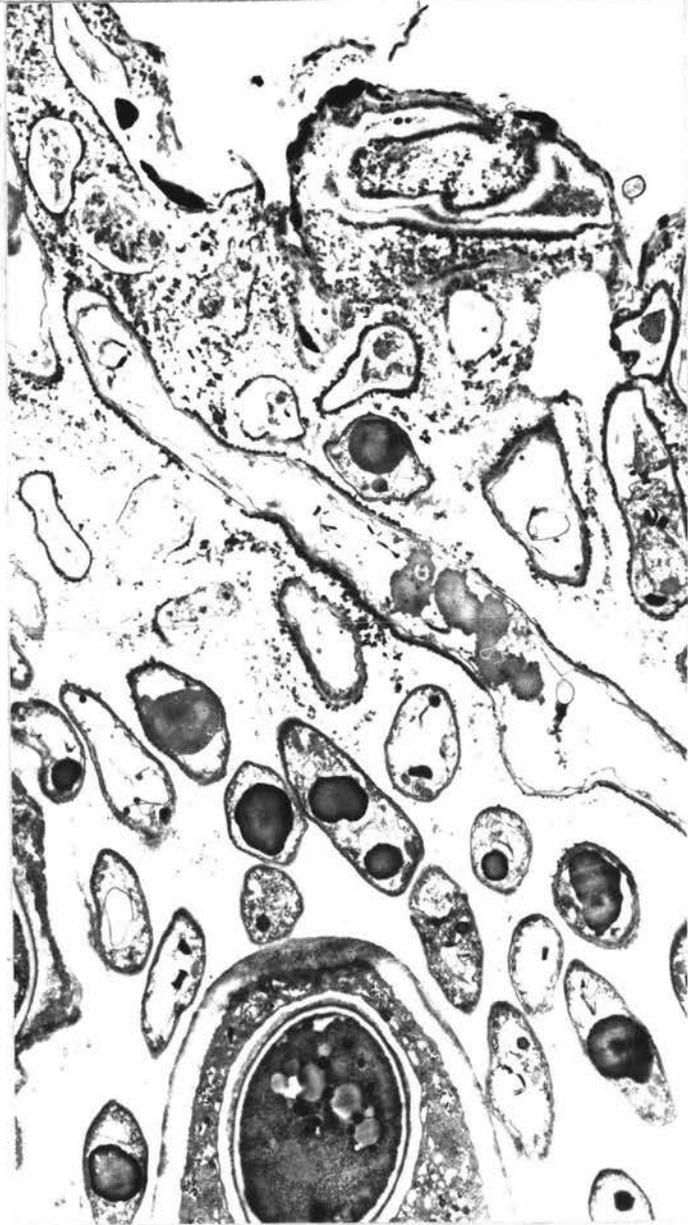


FIG. 100: Epithecium of *Drepanopeziza punctiformis*.

TOP: *P. deltoides*, Colorado USA, Hinds, 30.10.1979.
Epithecium comprised of globular to irregular
cells loosely embedded in a dark matrix above
asci. X6,400.

BOTTOM: *P. x euramericana*, Nancy France, Pinon.
Globular to irregular cells of the epithecium
and paraphyses above asci. X6,400.



FIG. 101: Asci and ascospores of *D. punctiformis*. X1,600.

Cylindrical unitunicate asci with thickened apices. Ascospores biserially or uniserially arrayed, unicellular, ellipsoidal, smooth walled, hyaline (in this instance stained 0.5% acid fuchsin) with two conspicuous polar bodies.

P. x euramericana, Nancy France, collected by Pinon.

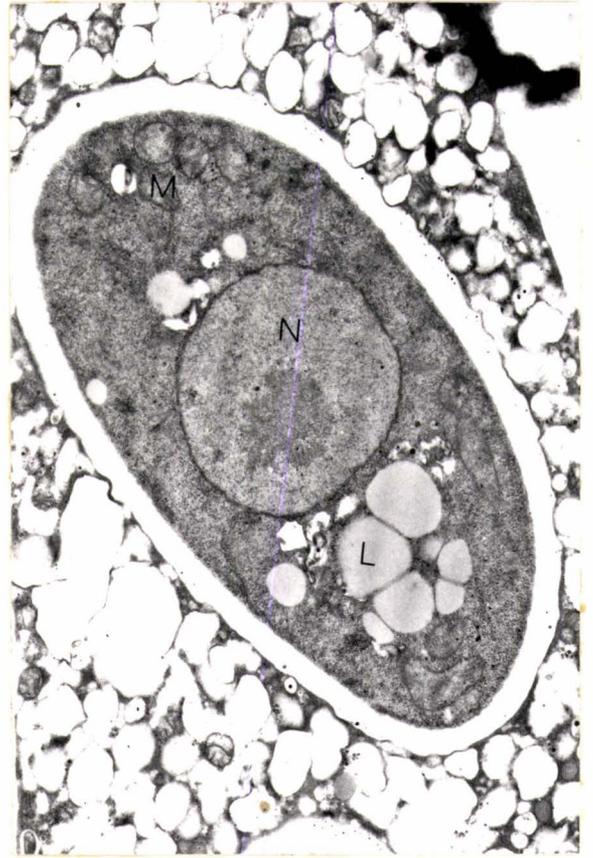


FIG. 102: Ascospores of *Drepanopeziza punctiformis*,

TOP LEFT: *P. x euramericana*, Nancy France, Pinon.

TOP RIGHT: *P. deltoides*, Colorado USA, Hinds.

RIGHT: Note the single central nucleus (N), mitochondria (M) and polar lipid bodies (L). X13,500.

BOTTOM: *P. x euramericana*, France. Unicellular, ellipsoidal, smooth-walled ascospores each with two conspicuous polar bodies. X1,200.

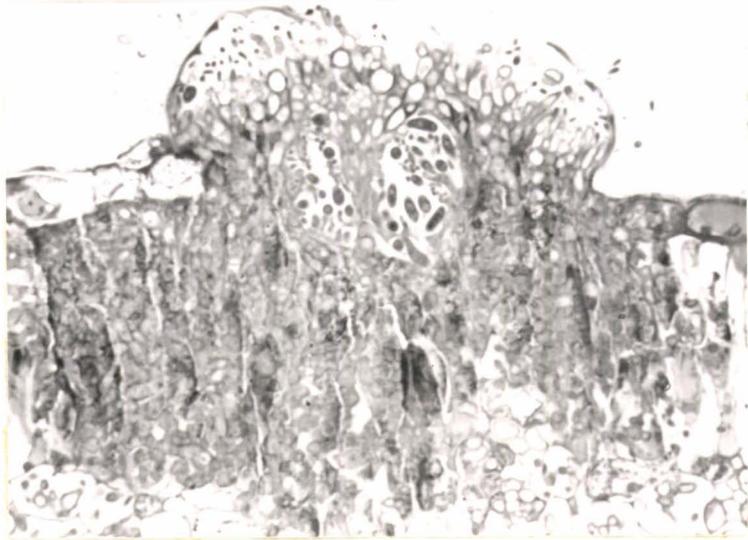


FIG. 105: Vertical sections through stromatal bodies of
M. brunnea.

TOP: Stromatal body formed on leaf discs inoculated
with conidia from leaves of *P. deitoides*,
Colorado USA. Note the conical shape and super-
ficial resemblance to an apothecium. X350.

CENTRE &
BOTTOM: Stromatal bodies formed on leaf discs inoculated
with a blend of 16 international isolates of
M. brunnea. Note the formation of conidia and
microconidia both within and on top of stromata.
X350.

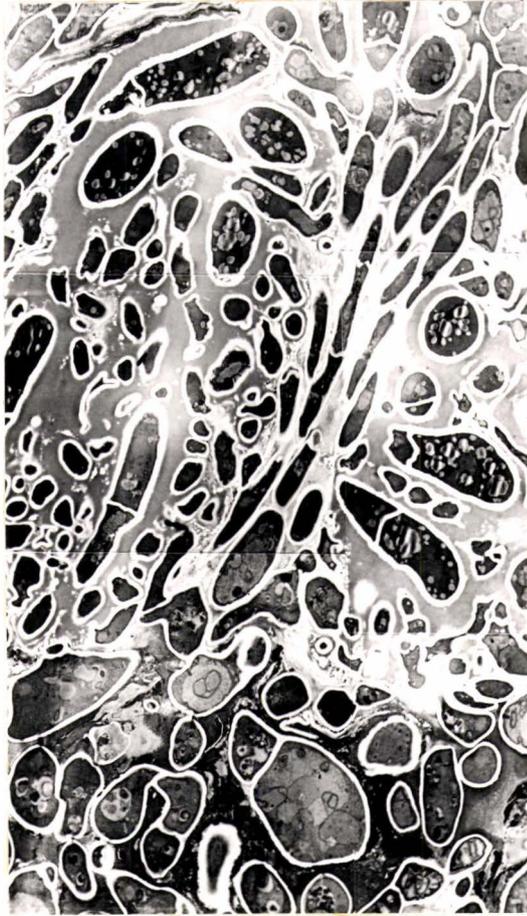


FIG. 106: Vertical sections through stromatal bodies of
M. brunnea.

TOP: Side wall of stromatal body composed of closely packed oval to elongate thick-walled cells.
Note the large number of lipid globules. X2,000.

BOTTOM: Central stromatal cavity showing accumulated conidia and microconidia. Note the irregular shape and septation of conidia. X2,000.

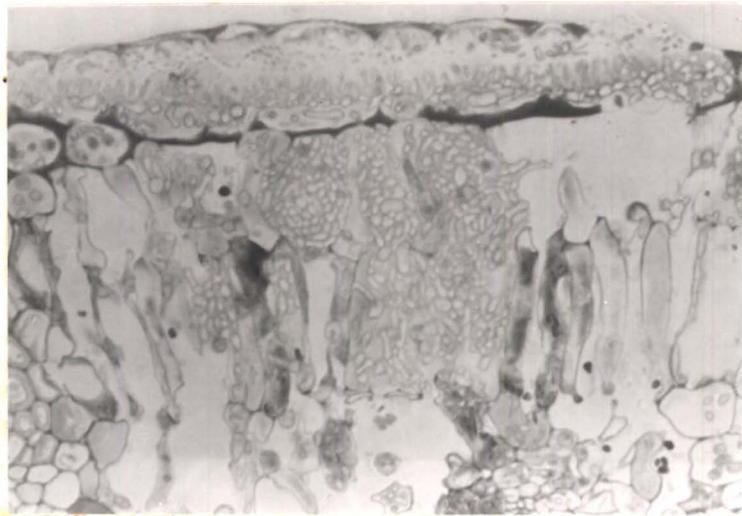
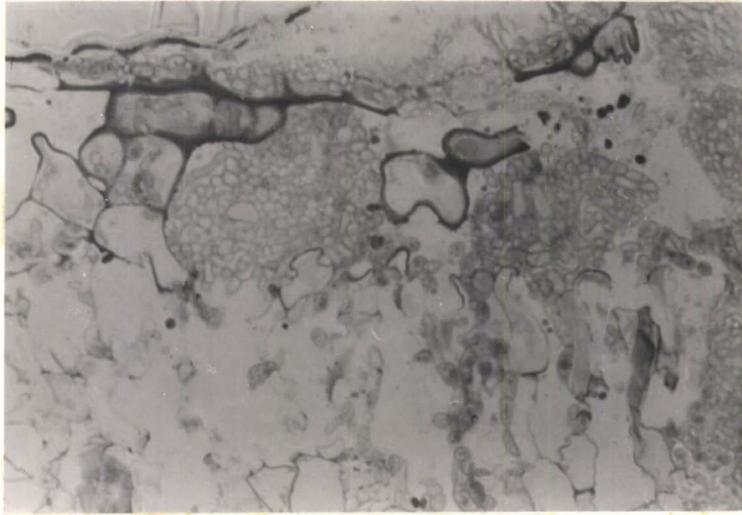
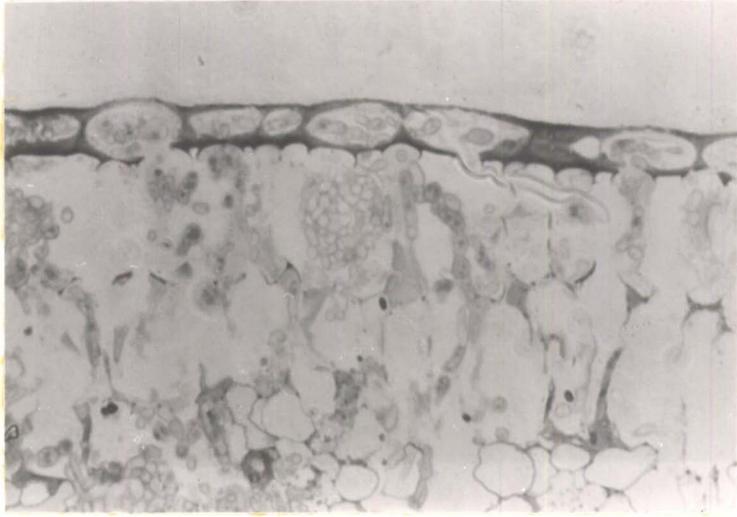


FIG. 107: Stages in the formation of apothecia by
Drepanopeziza tremulae.

TOP: Formation of an ascogonium surrounded by somatic
hyphae just under the epidermis. X350.

CENTRE &
BOTTOM: Expansion of the apothecial initial to form a
dark-brown sub-epidermal pseudoparenchymatous mass.

CENTRE: X450 .

BOTTOM: X350.

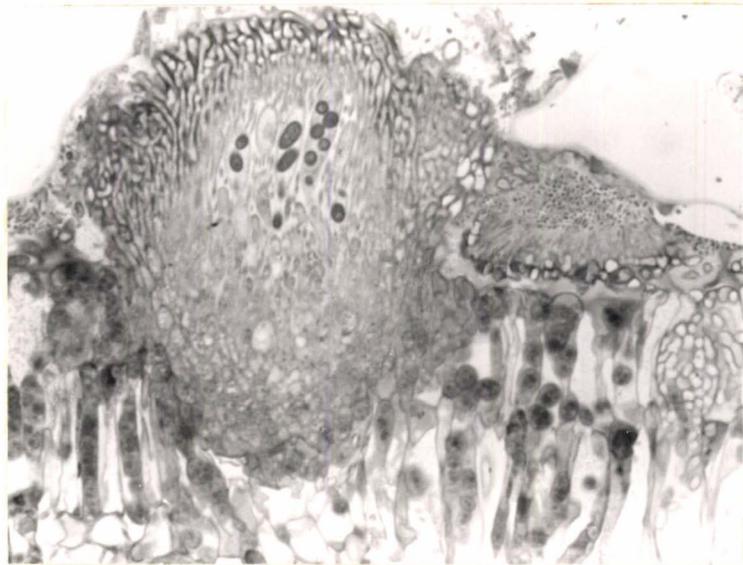
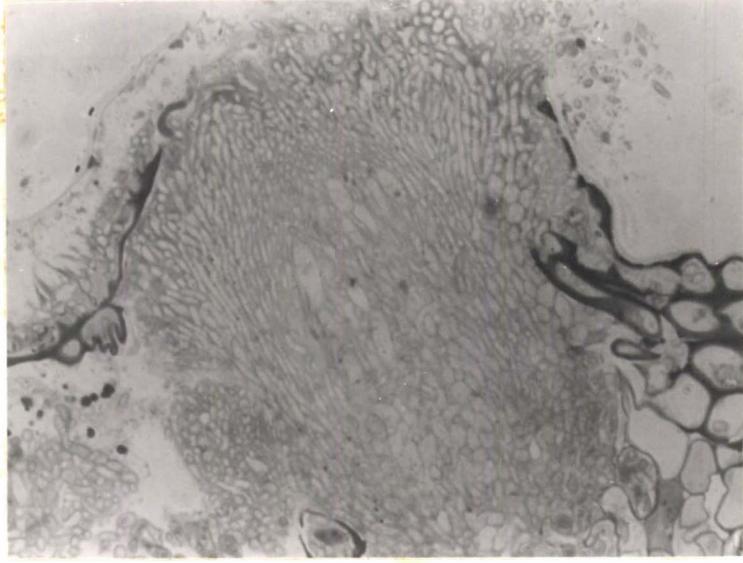


FIG. 108: Stages in the formation of apothecia by
D. tremulae.

TOP: Rupture of the epidermis by the expanding
apothecial initial. X800.

BOTTOM: Mature apothecium containing asci with ascospores.
Note to the right an acervulus forming micro-
conidia and below the epidermis an ascogonium.
X400.

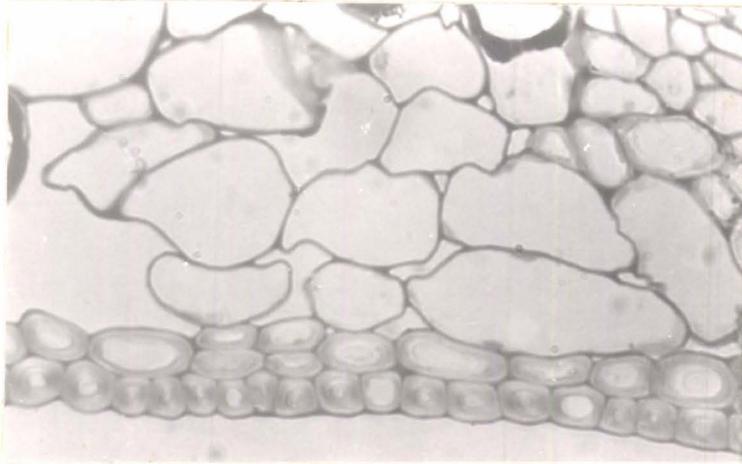


FIG. 109: Structure of the seed capsule wall.

TOP: Inner layers of the seed capsule wall showing the considerably thickened walls of the innermost 2-3 layers of cells adjoining the capsule lumen. X700.

BOTTOM: Considerably thickened walls of the innermost 2-3 layers of cells. X8,100.

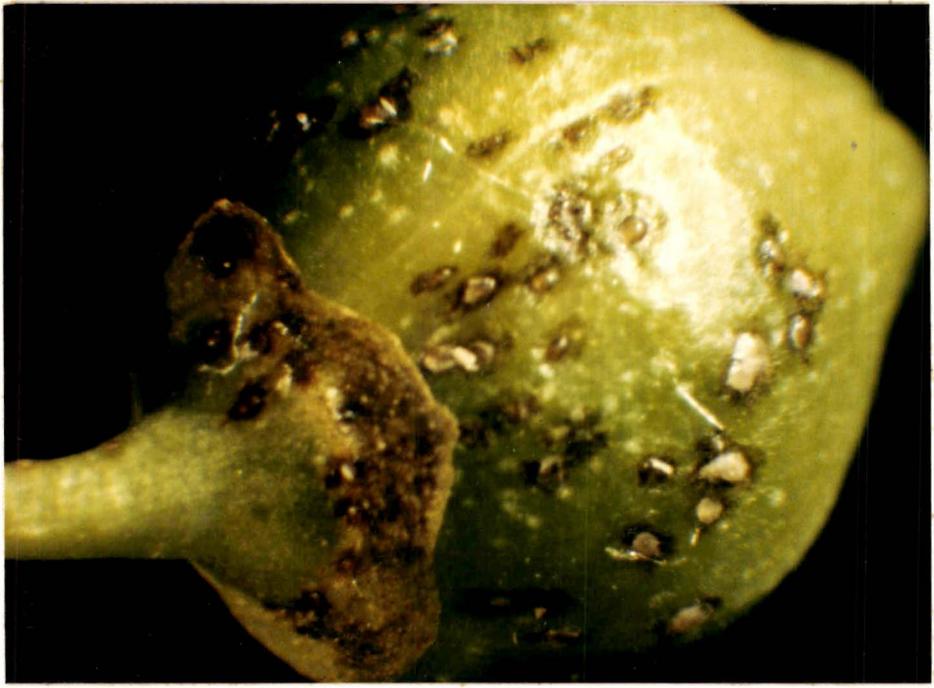


FIG. 110: Laboratory infected seed capsules of
P. deltoides cv. NE 245.

TOP: Inoculated seed capsule showing lesions and
acervuli on the outer wall.

BOTTOM: Vertical section through the infected capsule
wall showing the formation of conidia in
acervuli and hyphae throughout the capsule wall
except the innermost 2-3 layers of thick-
walled cells adjoining the capsule lumen. X1,150.

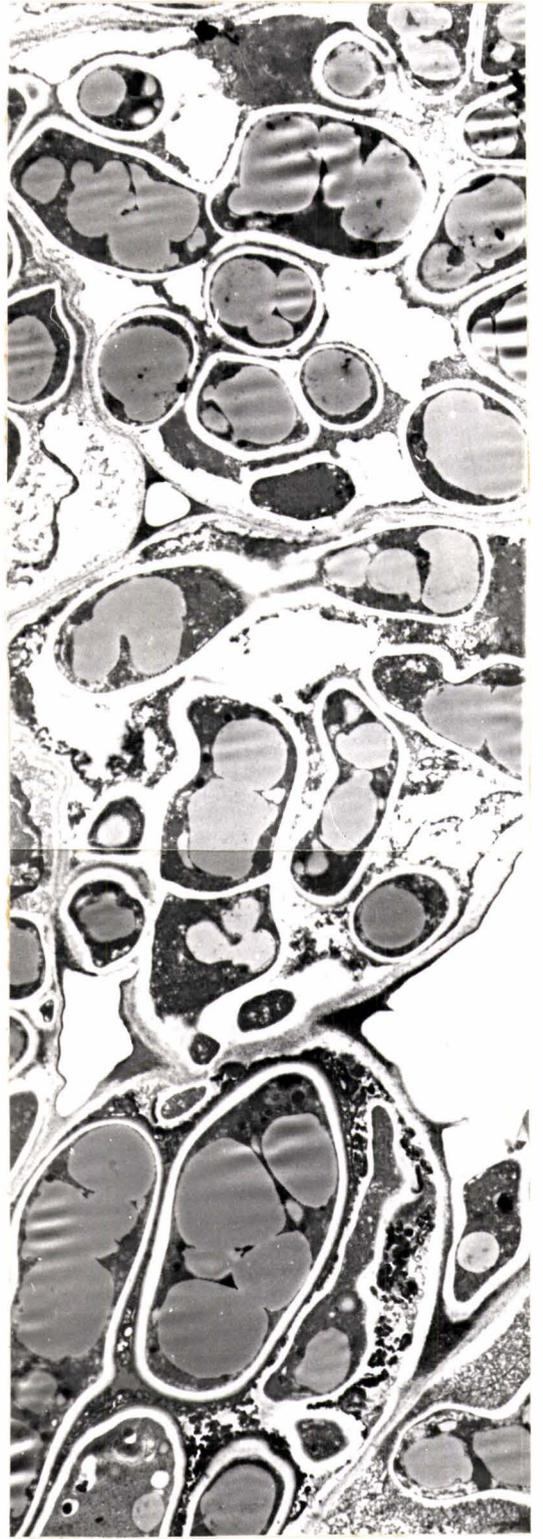


FIG. 111: Vertical sections through infected seed capsule walls. X6,400.

LEFT: Outermost four layers of the capsule wall, heavily infected with hyphae of *M. brunnea*.

RIGHT: Inner layers of the capsule wall heavily infected with hyphae of *M. brunnea*. Note the complete disruption of cellular contents and the large amounts of lipid within hyphae.



FIG. 112; Levels of infection established on laboratory inoculated seed capsules.

TOP: Lightly infected seed capsules of *P. deltoides* cv. NE245 spray inoculated 10 days previously with a conidial suspension of 1000 conidia/ml.

BOTTOM: Heavily infected seed capsules of *P. x euramericana* cv. I214 inoculated 10 days previously with a conidial suspension of 10,000 conidia/ml.

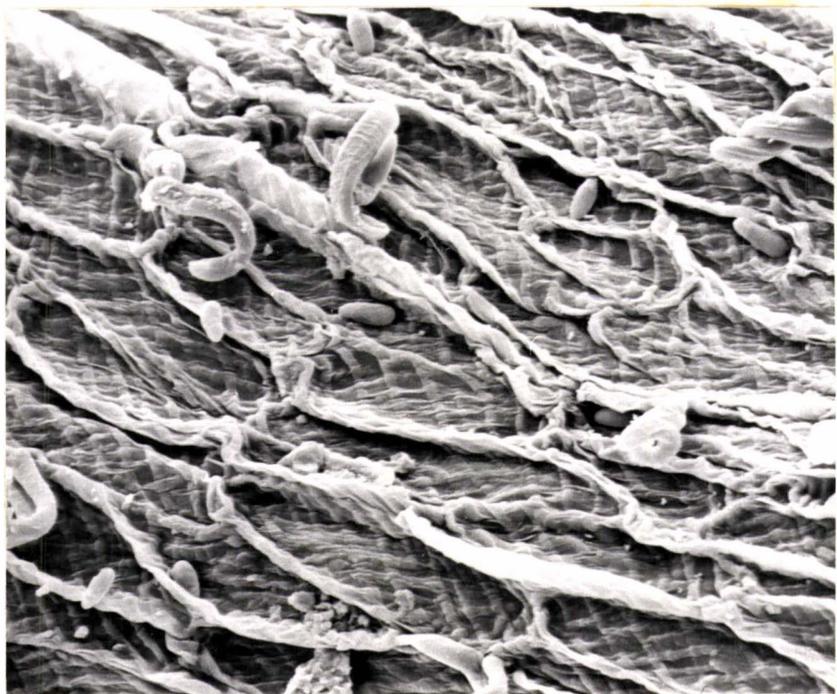


FIG. 113: Association between seed-borne inoculum and the testa.

TOP &
BOTTOM: Seed of *P. deltoides* cv. NE 245 extracted from heavily infected capsules showing the convoluted testa and the loosely associated conidia of *M. brunnea*.

TOP: X1,200

BOTTOM: X4,000.

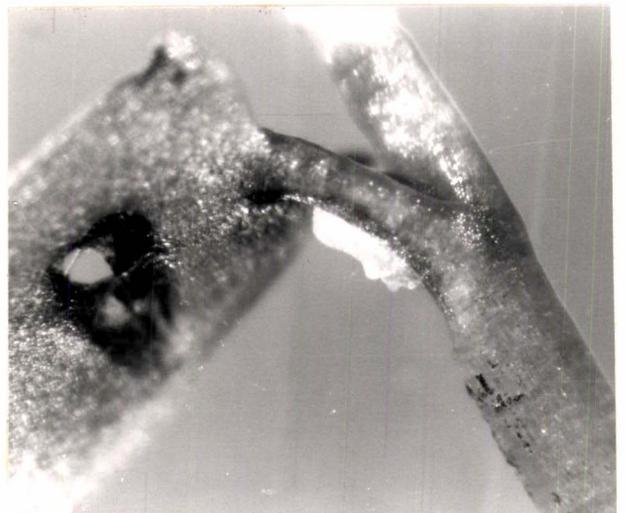
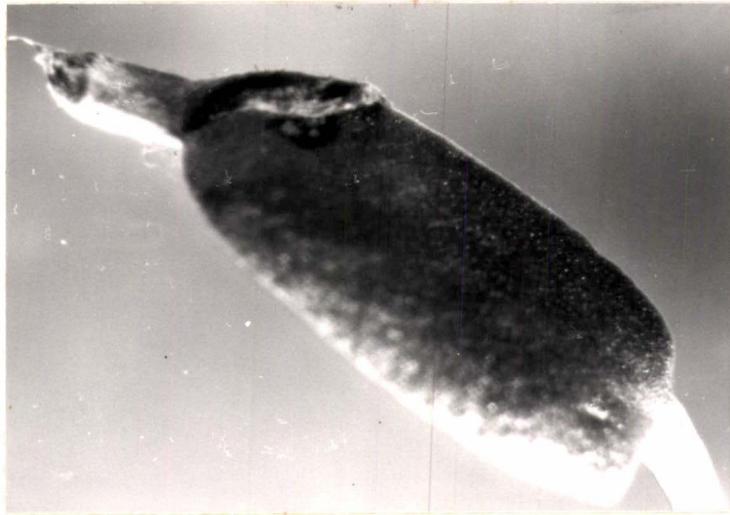
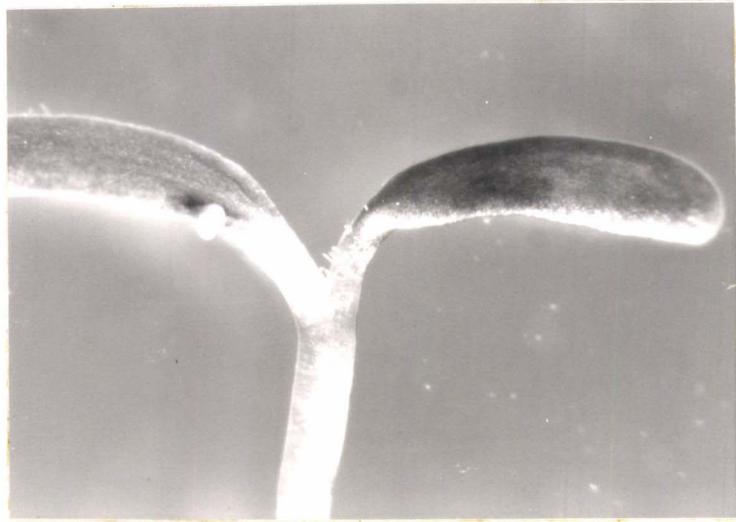


FIG. 114: Symptoms exhibited by seedlings infected with
M. brunnea.

TOP: Lesion on the cotyledon covered with a white mass
of conidia.

CENTRE &
BOTTOM
LEFT: Lesions formed on the cotyledon at the testa/
cotyledon interface.

BOTTOM
RIGHT: Lesion and conidial mass formed on the hypocotyl.

Note the inconspicuous disease symptoms and the
abundant sporulation.



FIG. 116: Photomicrograph of the resuspended pellet (Bolley technique) from Dutch seedlines.

TOP: Seedline 6156.

BOTTOM: Seedline 6150.

Conidia of *M. brunnea* are indicated (/). Note the large number of yeast cells contaminating seed. X1,200.



FIG. 117: Seed capsules of *P. deltoides* (78074) imported from Illinois, USA. Note the lesions in the capsule wall induced by *M. brunnea*.



FIG. 118: Symptoms exhibited by infected seedlings from Dutch seedlines sown on blotters.

TOP: Lesions formed on the abaxial surface of the cotyledon adjacent to the testa. Note the mass of conidia.

CENTRE: Lesion formed on the hypocotyl. Note the mass of conidia.

BOTTOM: Lesion formed on the abaxial surface of the cotyledon.

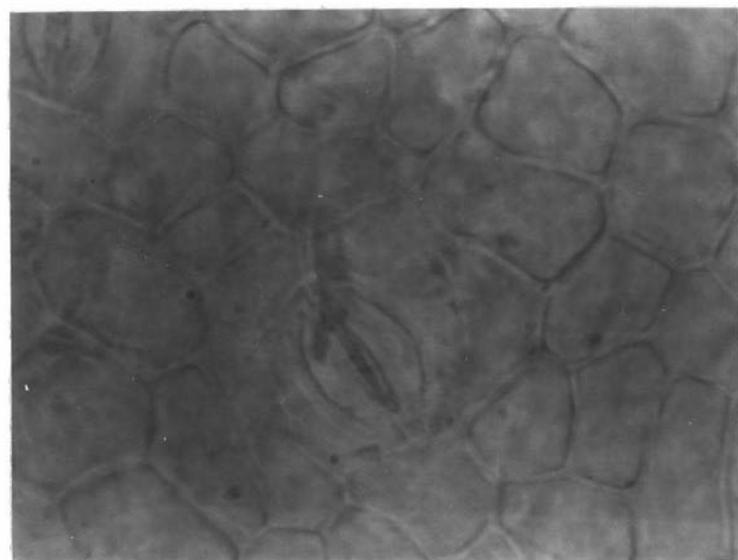
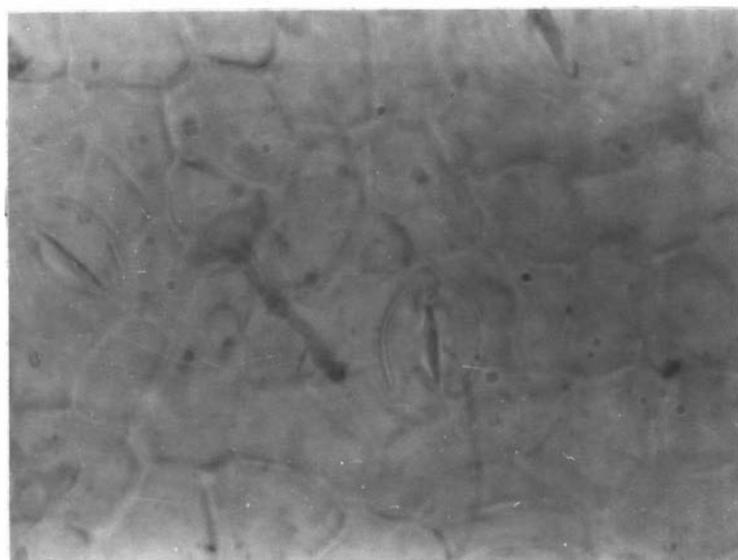


FIG. 119: Direct penetration of *P. nigra* cv. Italica
'Aurea' by *M. brunnea*. Note the short germtube.
X500.

FIG. 120: Stomatal penetration of *P. nigra* cv. Italica
'Aurea' by *M. brunnea*. Note the short germtube.
X500.

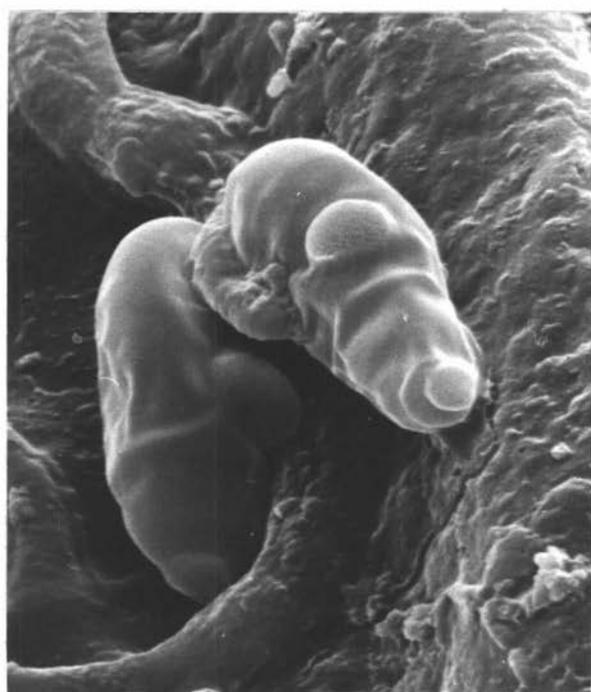


FIG. 121: Initiation of conidial germination. Note the cracking of the conidium wall.

LEFT: X4,800.

RIGHT: X20,000.

FIG. 122: Direct penetration without appressorium formation. Note the short germ tube.

LEFT: X4,000.

RIGHT: X18,000.

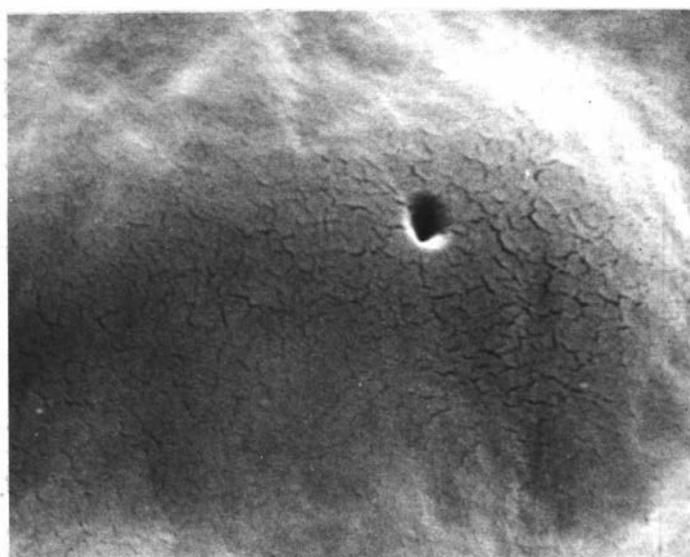
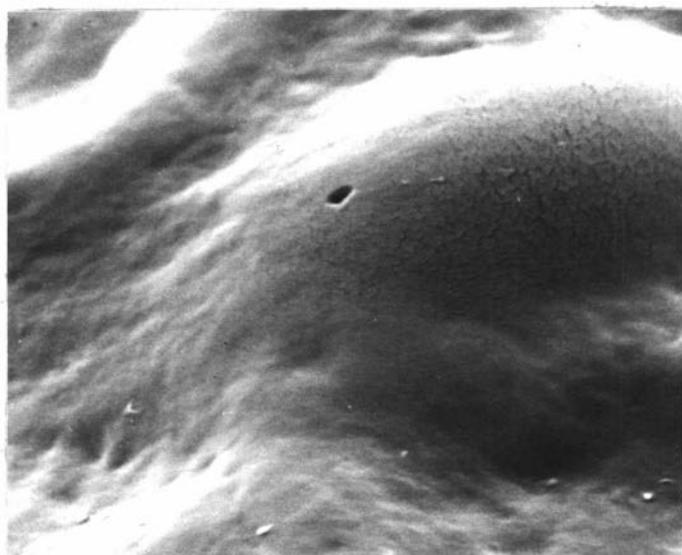


FIG: 123: Penetration holes revealed following removal of conidia and germtubes with gelatine. Note the smooth inturned edges of the perforations and the bifurcated hole (Bottom).

TOP: X4,000.

CENTRE: X5,000.

BOTTOM: X15,000.

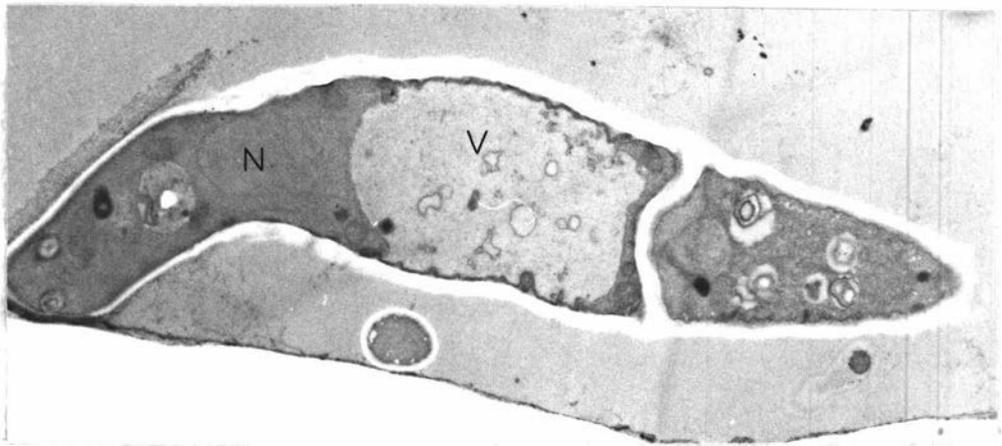


FIG. 124: Conidial germination. Isolation of the two cells of the conidium by septal pore plugging and wall overgrowth. The left cell of the conidium is vacuolate whereas the right cell is unchanged. Note the nucleus (N) and mitochondria (M). X25,000.

FIG. 125: Vertical section through germinated conidium. Note the formation of a large vacuole (V) on migration of cytoplasm into the germtube. The apex of the germtube is in contact with the cuticle. Note the location of the nucleus and the independence of the two cells of the conidium. (X6,400.)

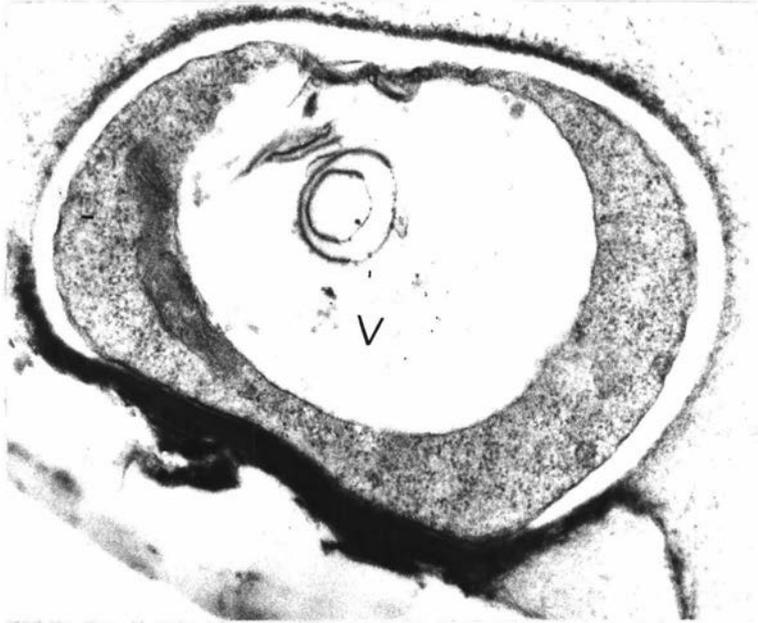
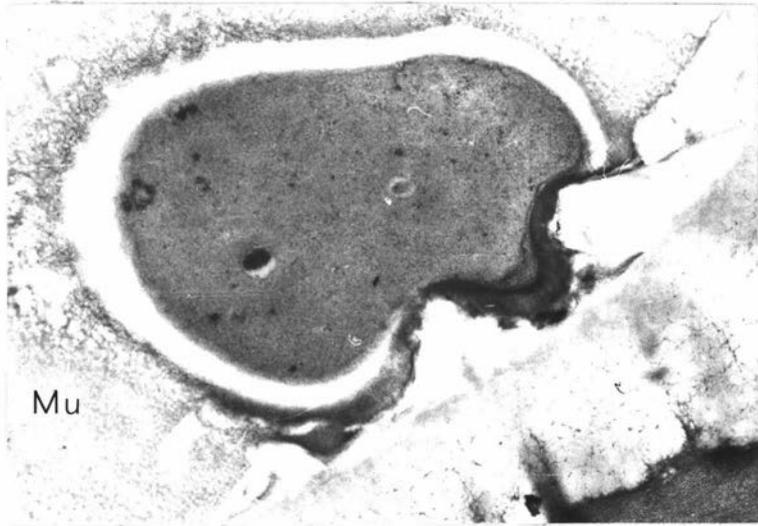


FIG. 126: Host penetration: germ pore formation.

TOP: The germ tube is in contact with the cuticle and has conformed closely to surface irregularities. The lower germtube wall has become darkly stained and the plasmalemma has moved away from the germ tube wall. Note the germ tube is surrounded by mucilage (Mu). X22,500.

CENTRE: The germtube wall in contact with the cuticle has become darkly stained. Note the central vacuole (V) and the mucilage. X25,000.

BOTTOM: The germtube wall in contact with the cuticle has become thinner. Note the lomasome between the plasmalemma and the thin germtube wall. X46,000. For lower magnification see Fig. 125 (X6,400).

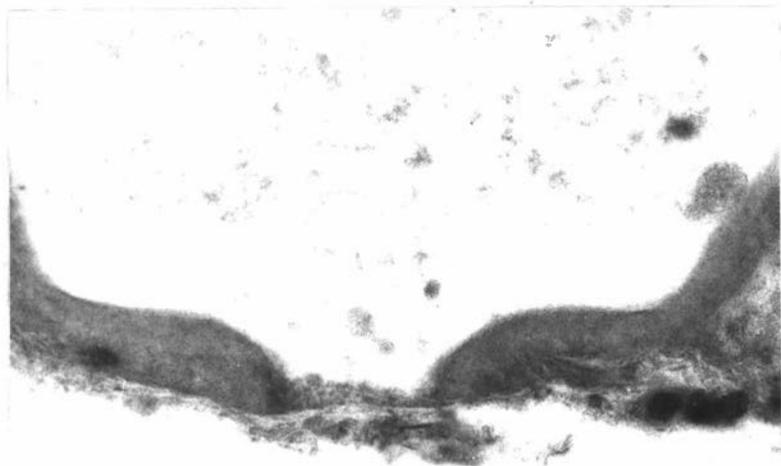
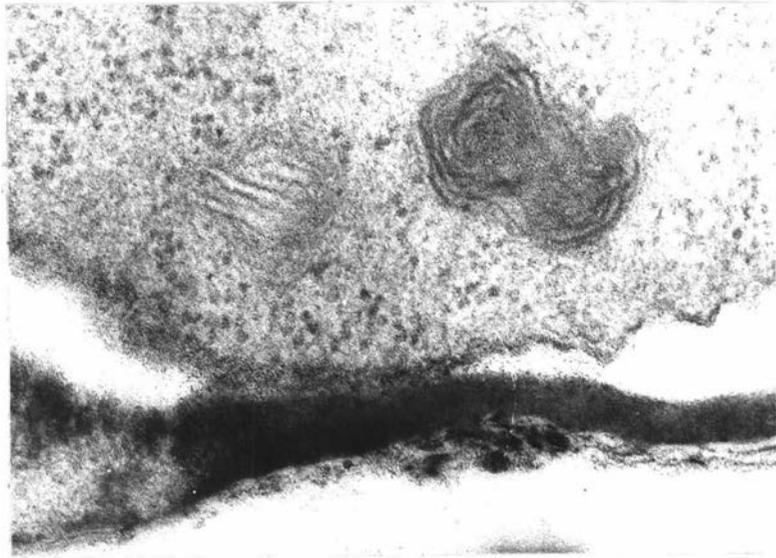
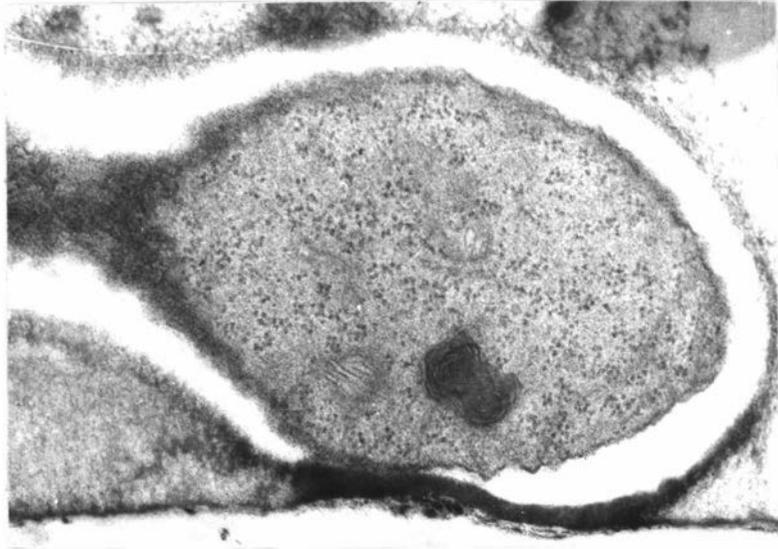


FIG. 127: Host penetration: germ pore formation.

TOP &
CENTRE: The germtube wall in contact with the cuticle is becoming thinner leading to the formation of a pore. The plasmalemma is almost in contact with the cuticle.

TOP: X34,000

CENTRE: X82,500.

BOTTOM: The germtube wall has been eroded away completely leading to the formation of a pore. X46,000.

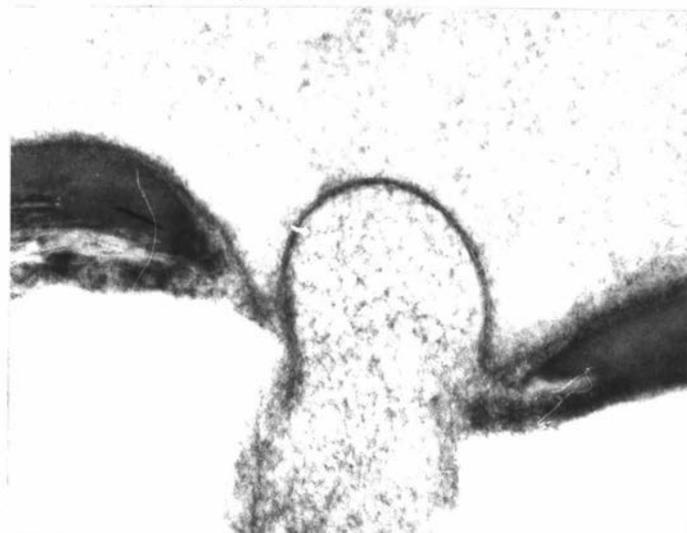
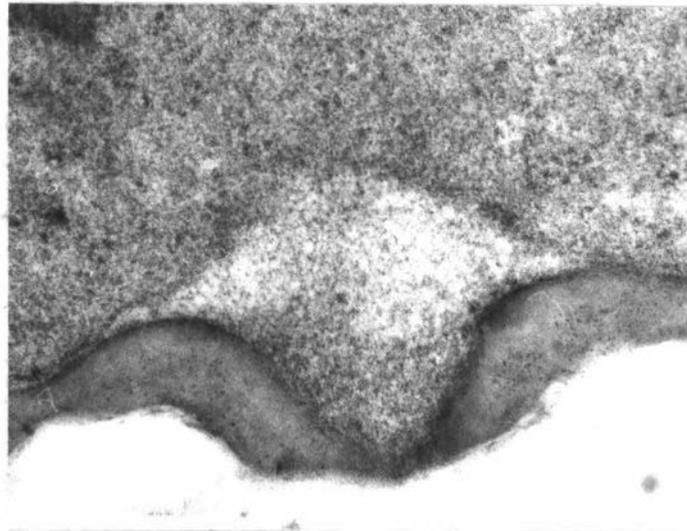
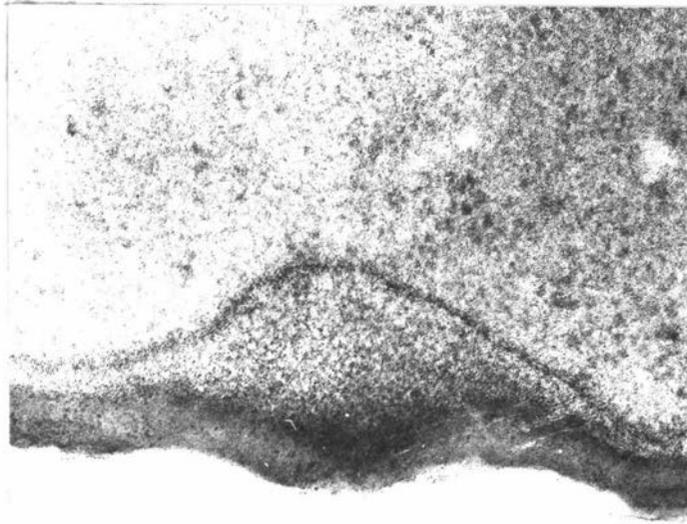


FIG. 128: Host penetration: germ pore formation.

TOP &
CENTRE: Prior to dissolution of the lower germtube wall
the plasmalemma appears to move upwards and away
from the site of pore formation.

TOP: X82,500,

CENTRE: X61,000,

BOTTOM: The gerpore has formed and naked cytoplasm has
moved through the pore. Note that the plasma-
lemma has looped upwards away from the pore.

X61,000,

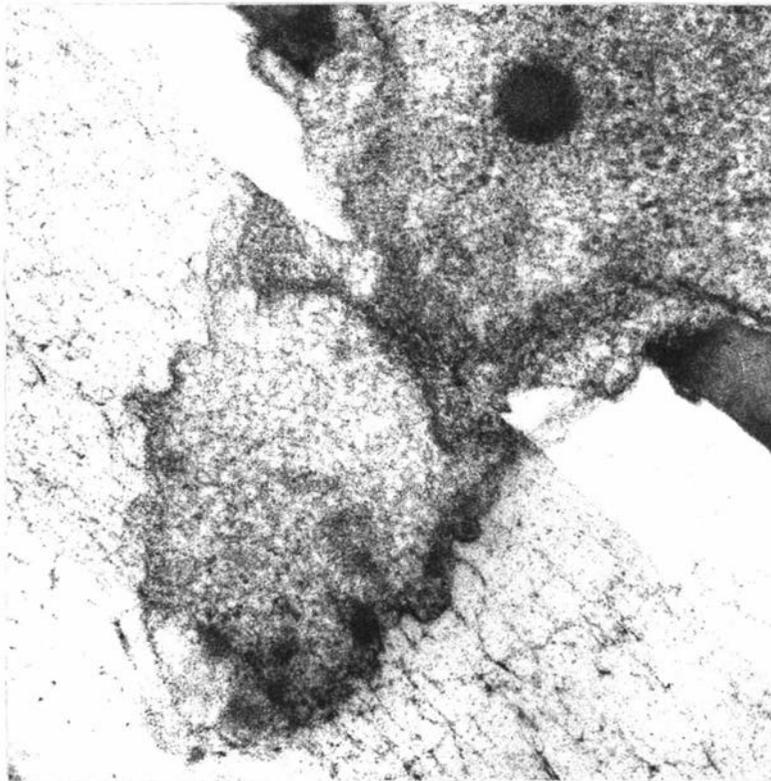


FIG. 129: Host penetration: germtube entry.

TOP & BOTTOM: Penetration of the cuticle by a naked penetration peg. Note that the germtube has become detached from the cuticle.

TOP: X25,000.

BOTTOM: X82,500

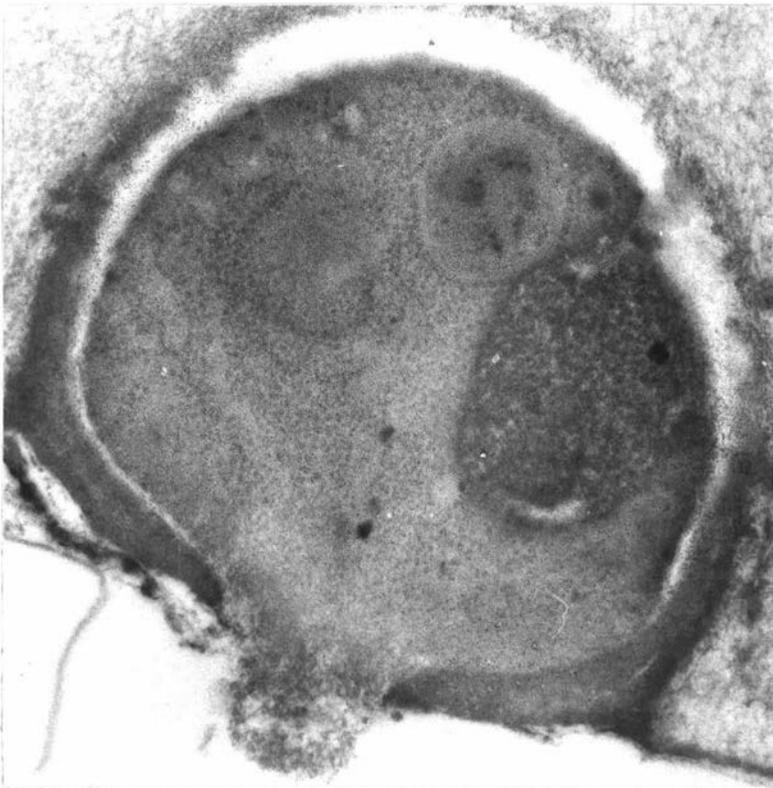


FIG. 130: Host penetration: germtube entry.

- TOP & BOTTOM: Penetration of the cuticle by a naked penetration peg. Note the mucilage surrounding the germtube.

TOP: X18,000.

BOTTOM: X46,000.

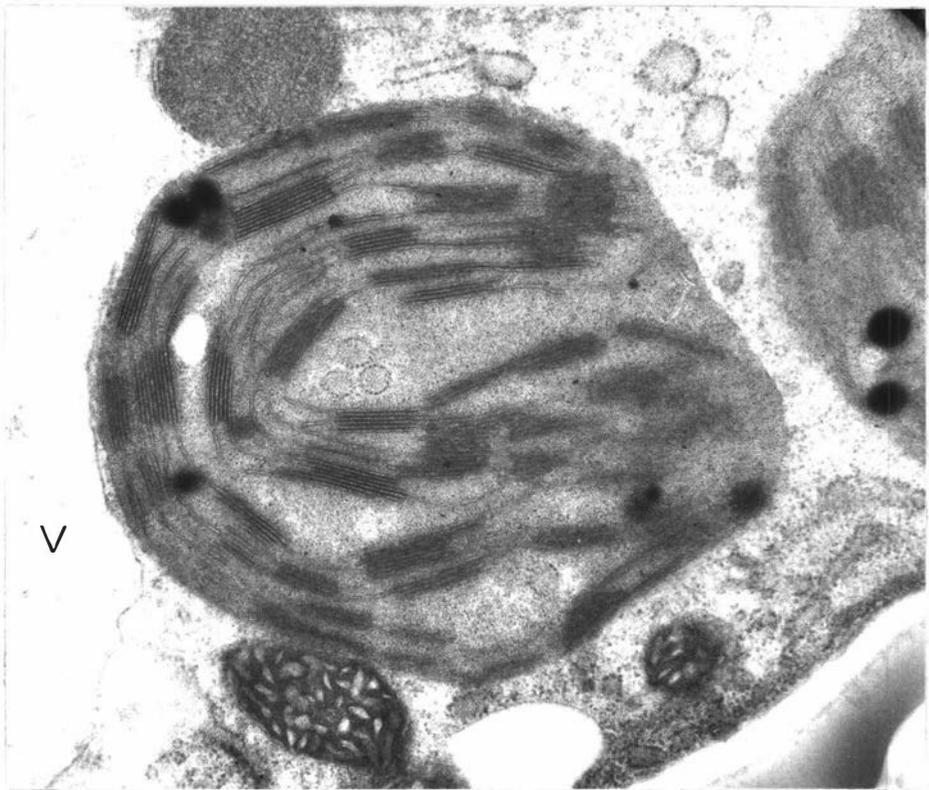
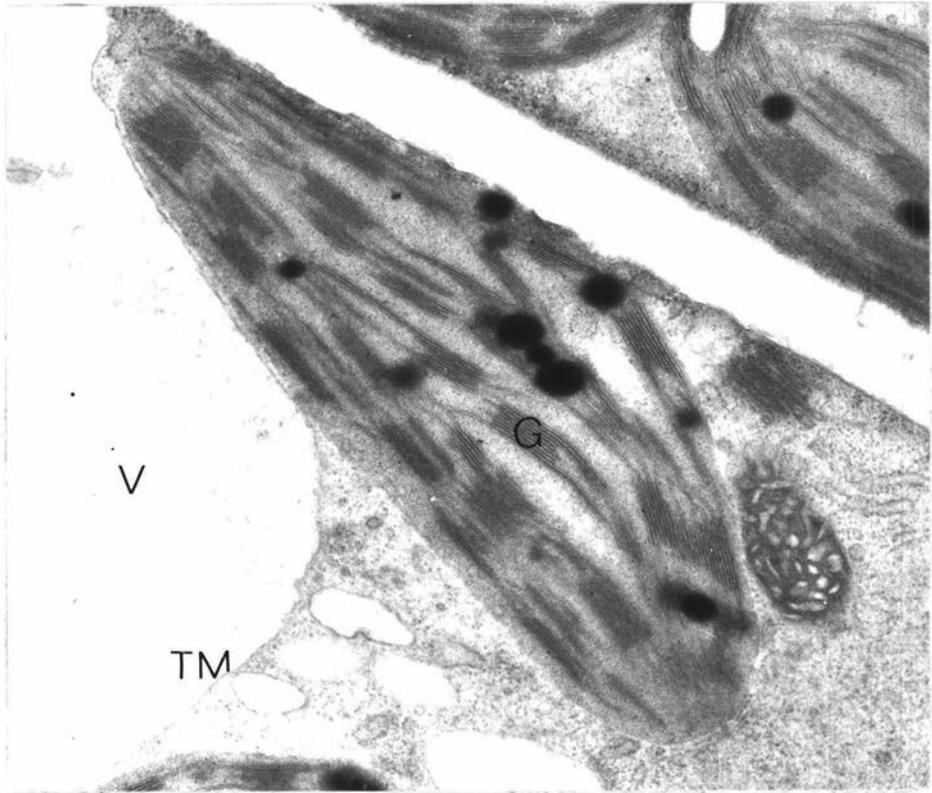


FIG. 131: Ultrastructure of healthy leaf tissue.

TOP &
BOTTOM: Typical chloroplasts with grana (G), intergranal lamellae and osmiophilic plastoglobuli
Note the vacuoles (V), tonoplast membrane (TM), and ribosomes, both free and attached to the endoplasmic reticulum. *P. alba* x *P. nigra* cv. *Sempervirens* cv. Mareg 2. X22,500.



FIG. 132: Hyphae of *M. brunnea* within leaf tissue of *P. nigra* cv. Vert de Garonne 3 days following inoculation. The contents of epidermal cells have been completely disrupted and hyphae have penetrated palisade tissue. Note in the left hand palisade cell the amorphous electron opaque deposit formed following hyphal penetration. X6,400.

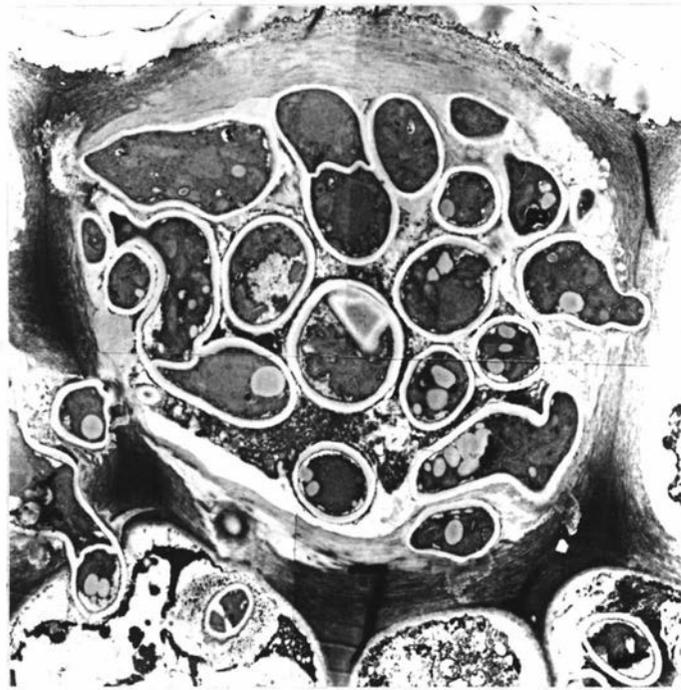


FIG. 133: Hyphae of *M. brunnea* within guard and subsidiary cells of a stoma. (*P. x euramericana* cv. Robusta). Note the stoma is closed. X3,100.

FIG. 134: Hyphae of *M. brunnea* within an epidermal cell (*P. x euramericana* cv. I154) 6 days following inoculation. Note the direct penetration of cell walls by hyphae. X5,100.



FIG. 135: Hyphae of *M. brunnea* within epidermal and palisade tissue of *P. x euramericana* cv. Robusta 6 days following inoculation. Hyphae are both intercellular and intracellular. Contents of the left and central palisade cells have been disrupted with only the chloroplasts remaining discernable. The palisade cell to the right has been penetrated however cellular organelles have not been disrupted. X3,000.

FIG. 136: Hyphae of *M. brunnea* within palisade tissue of *P. nigra* cv. Vert de Garonne 12 days following inoculation. Cellular contents are moribund and hyphae are ramifying indiscriminately. X2,600.



FIG. 137: Hyphae of *M. brunnea* with epidermal cells of *P. x euramericana* cv. Robusta (top) and *P. nigra* cv. Vert de Garonne (bottom) 12 days following inoculation. The lateral walls of the epidermal cells have been eroded by enzymatic action enabling them to bulge upwards with the pressure exerted by conidia formed by underlying conidiophores.

TOP: X3,100

BOTTOM: X2,800.

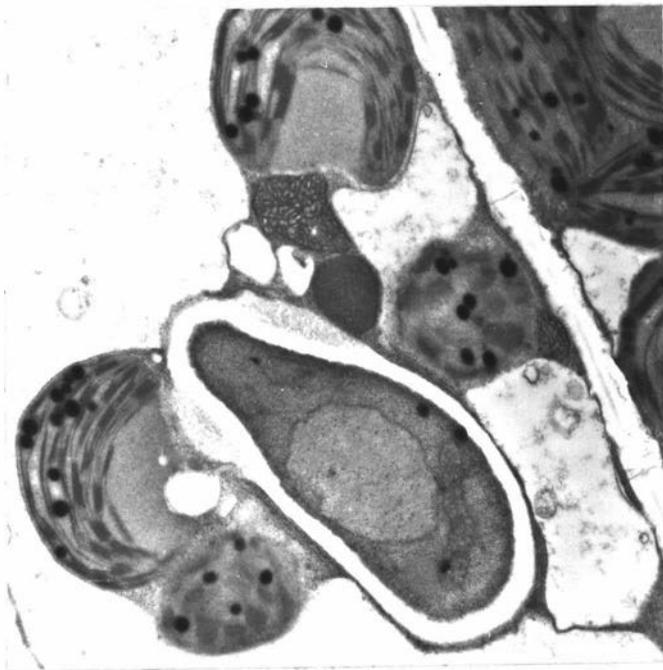


FIG. 138: Vertical sections through acervuli of *M. brunnea* formed on host tissue.

TOP: *P. nigra* cv. Italica 'Aurea'. X800.

CENTRE: *P. nigra* cv. Vert de Garonne. X1,000.

BOTTOM: *P. x euramericana* cv. Robusta, 700X.

On all three hosts note that acervuli are formed intraepidermally.

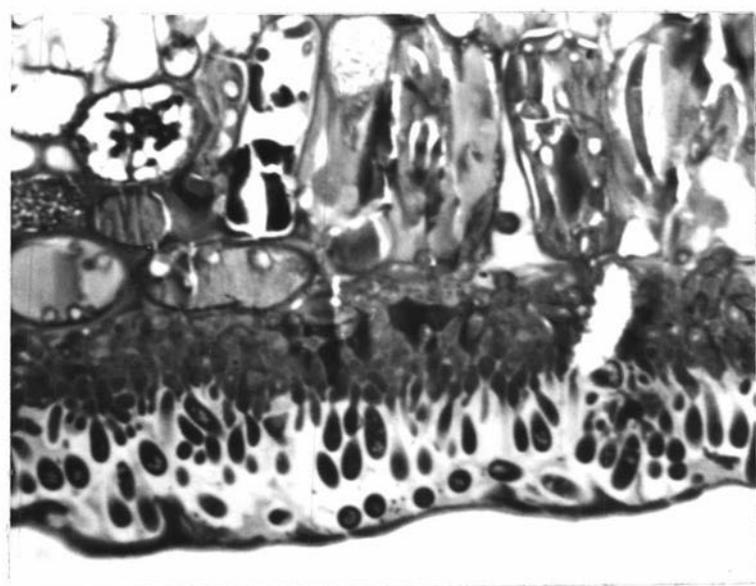
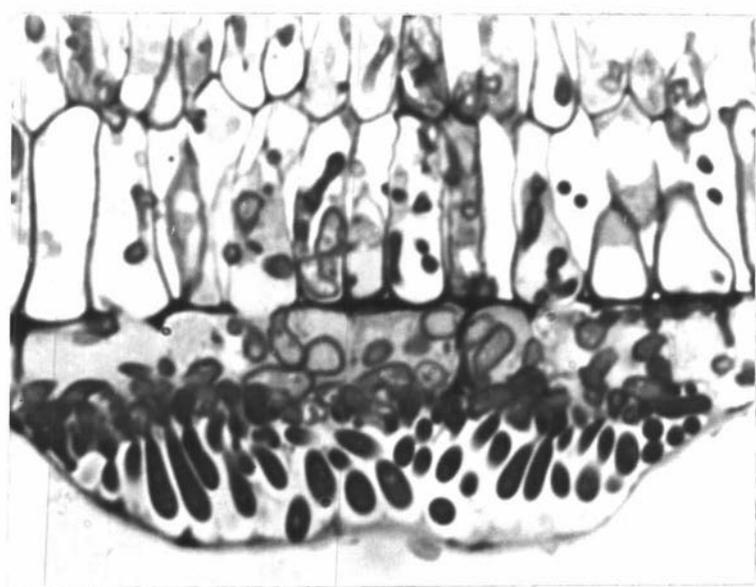
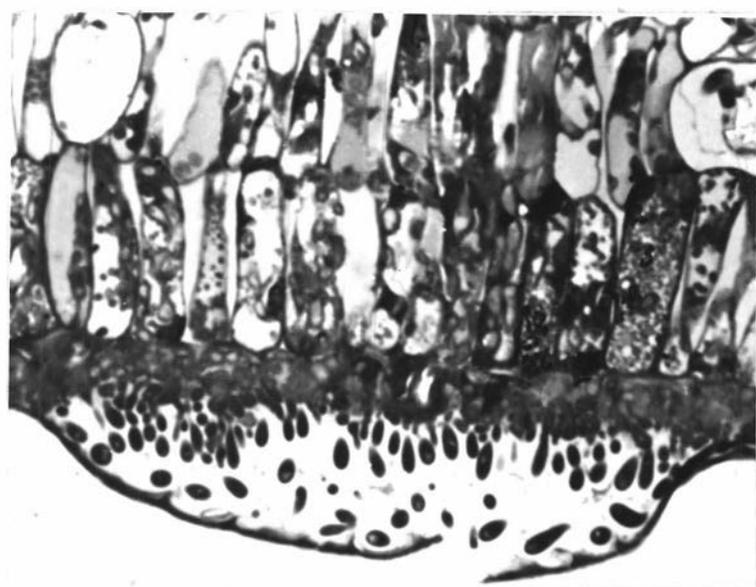


FIG. 139: Hyphae of *M. brunnea* within palisade cells of
P. alba 'Morocco' x *P. nigra* Sempervirens cv. Mareg 2.

TOP & BOTTOM: The hyphae is surrounded by cytoplasm, the tonoplast membrane and the plasmalemma have not been ruptured and accordingly cellular contents are still intact. That is, the fungus is acting like a biotrophic parasite. X8,500.

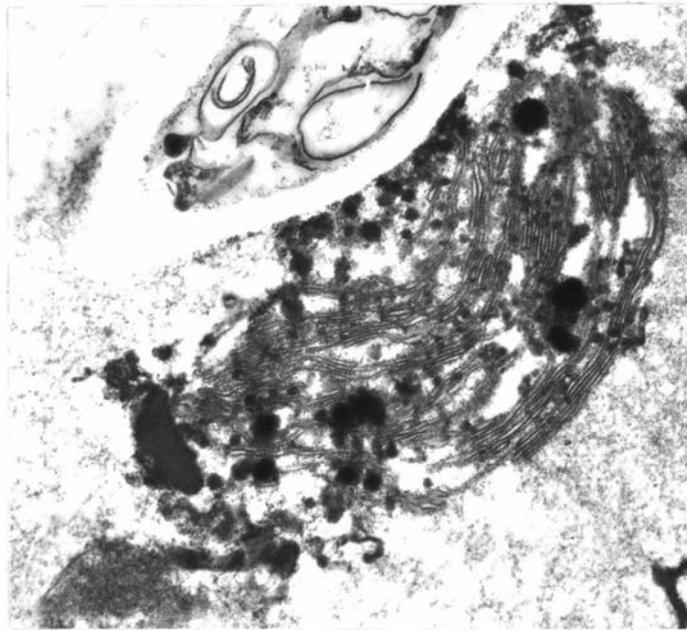
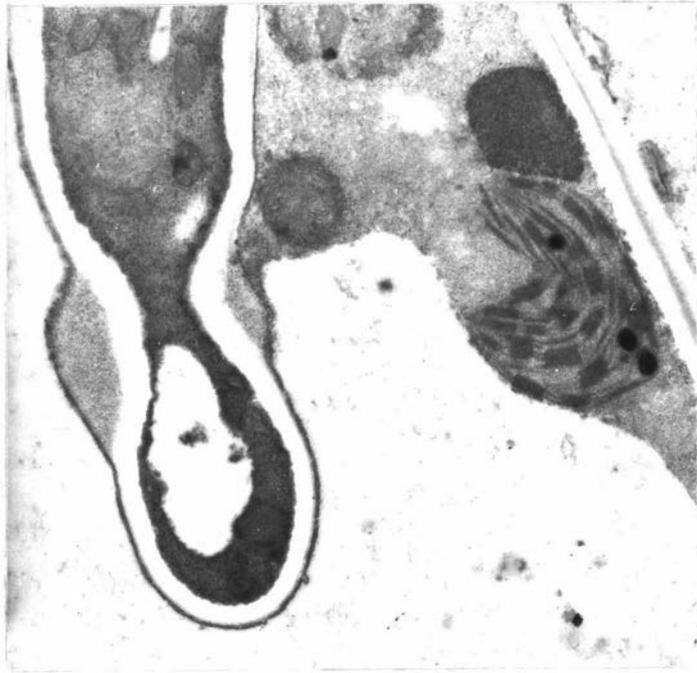


FIG. 140: Hyphae of *M. brunnea* within palisade cells of *P. alba* 'Morocco' x *P. nigra* Sempervirens cv. Mareg 2.

TOP: Further growth of the hyphae is about to breach the tonoplast causing vacuolar and cytoplasmic contents to be intermixed completely disrupting the cell. X12,500.

BOTTOM: Disrupted cell showing chloroplast with discernable intergranal lamellae and osmiophilic plastoglobuli within the granulated cytoplasm. X18,000.

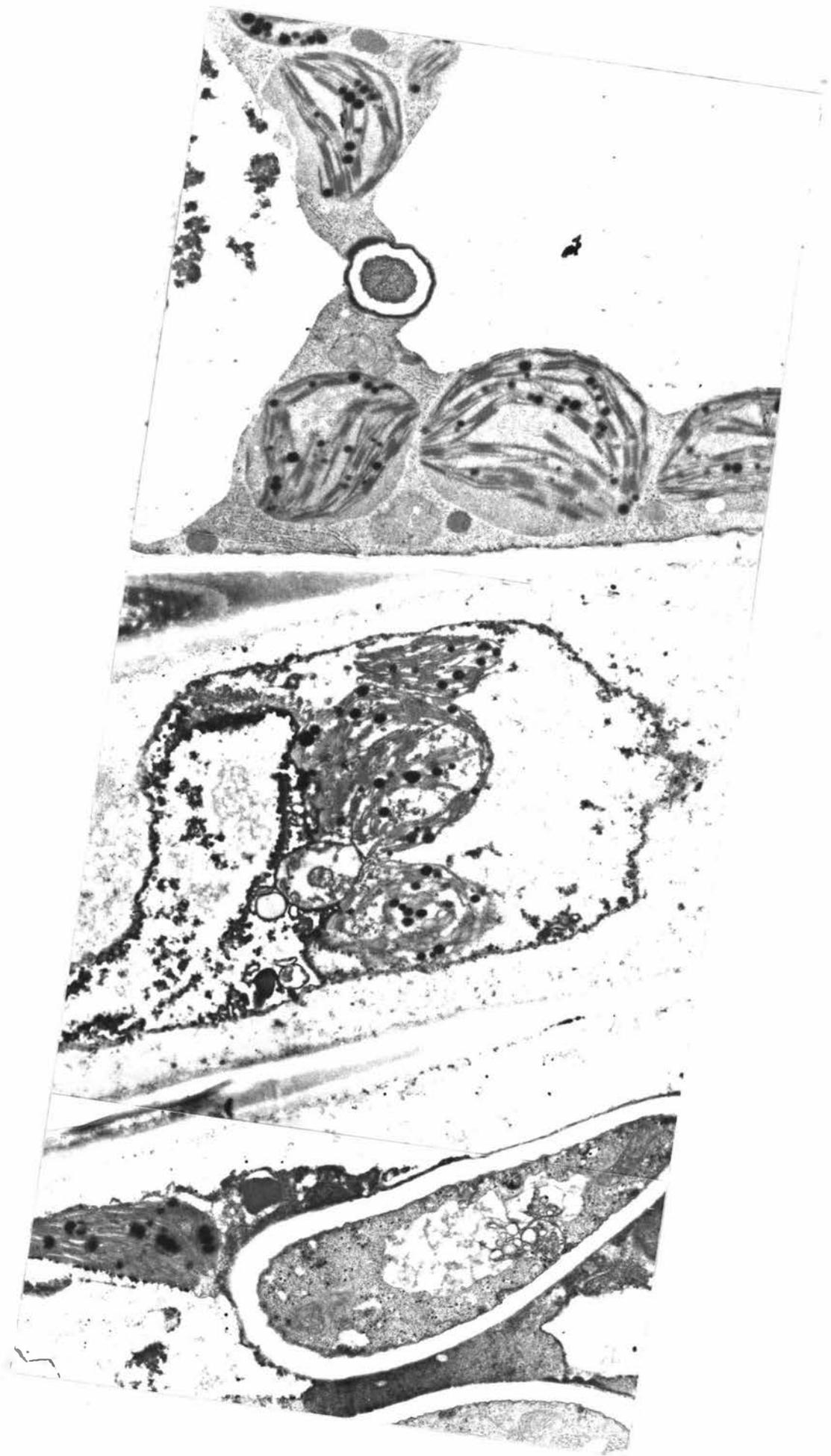


FIG. 141: Hyphae of *M. brunnea* within palisade tissue of *P. nigra* cv. Italica 'Aurea' showing juxtaposition of severely disrupted and healthy cells. In the central palisade cell only the intergranal lamellae and osmiophilic plastoglobuli are discernable. Note in the palisade cell to the right the presence of a hypha within the cytoplasm without apparent cellular degradation. X10,500.

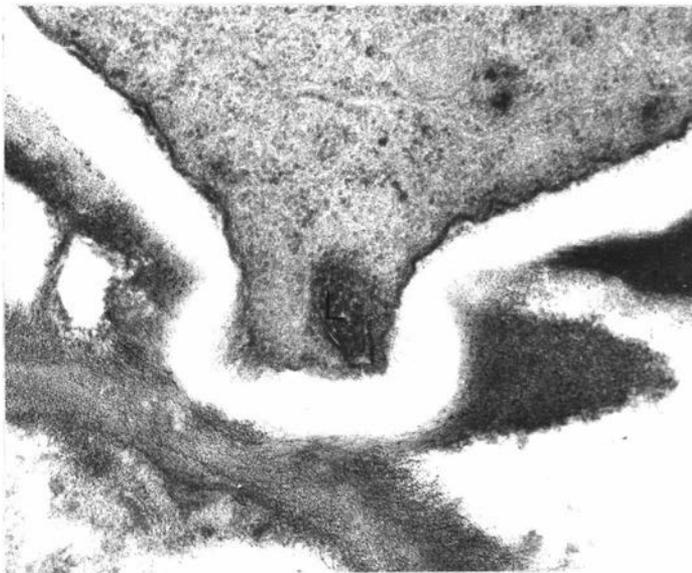
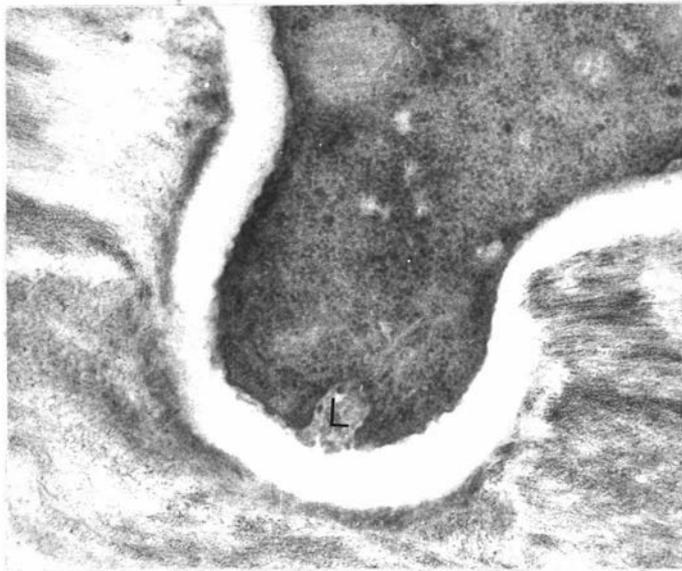


FIG. 142: Hyphae of *M. brunnea* penetrating cell walls.
X35,000.

TOP: *P. x euramericana* cv. Robusta. The hypha has penetrated halfway through the cell wall (the apex is in contact with the middle lamella). Note the clean passage of the hypha through the wall and slight inward depression of cell wall microfibrils. Note also the lomasomes (L) within the hypha.

CENTRE: *P. x euramericana* cv. Robusta. Direct penetration of the cell wall showing digested ends of cell wall microfibrils. Note the lomasome (L) in the hyphal apex.

BOTTOM: *P. x euramericana* cv. I154. Penetration of the cell wall, the altered staining properties of the wall suggesting leakage of enzymes from the hypha. Note the lomasome (L).

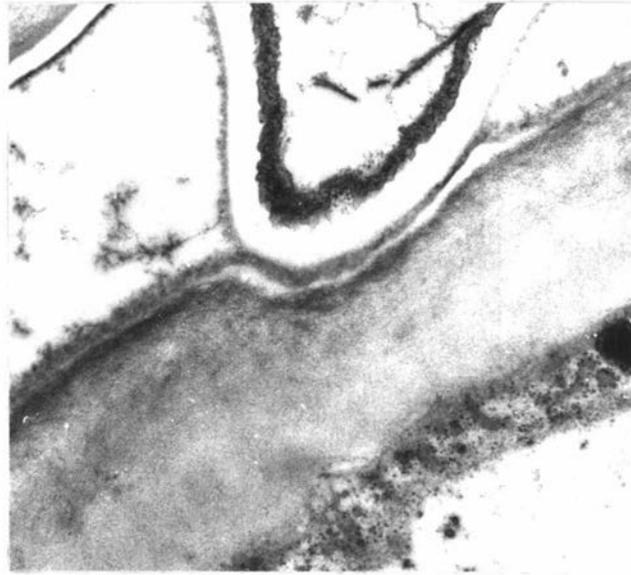
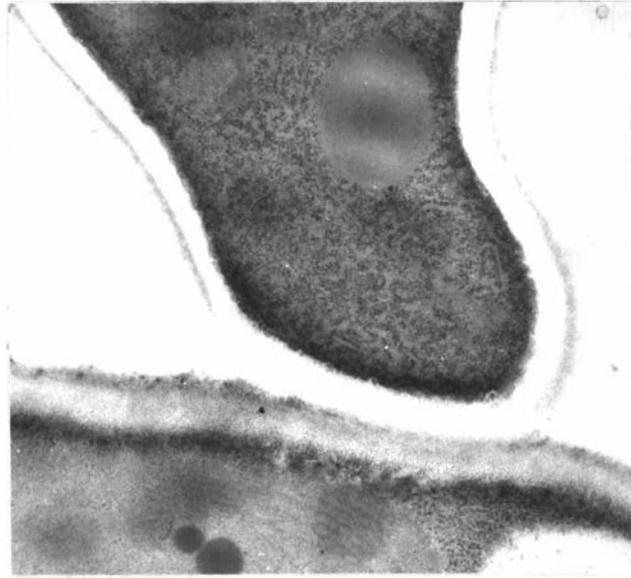


FIG. 143: Penetration of cell walls by hyphae from within intercellular air spaces.

TOP: *P. nigra* cv. Vert de Garonne. Initial contact between hypha and cell wall. Note the slight inward depression of the cell wall at the point of contact suggesting involvement of physical pressure. X19,000.

CENTRE: *P. x euramericana* cv. I154. The hypha is penetrating the cell wall directly, probably by enzymatic action since there is little distortion of the cell wall. X19,000.

BOTTOM: *P. x euramericana* cv. I154. The hypha has penetrated the cell wall directly and entered the cell. X16,500.

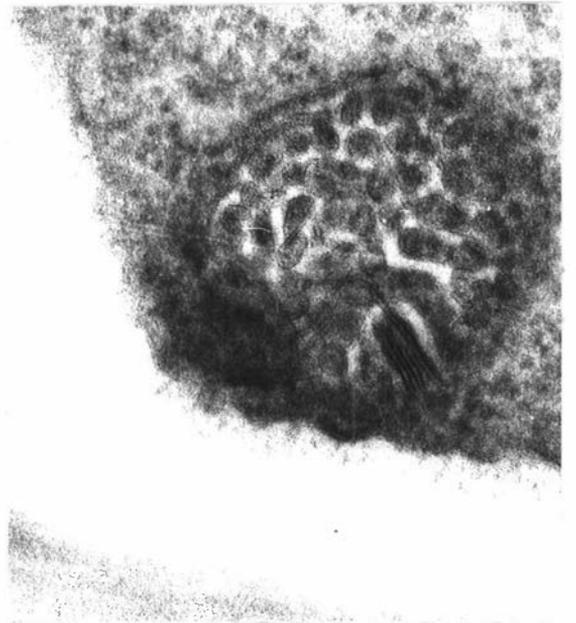
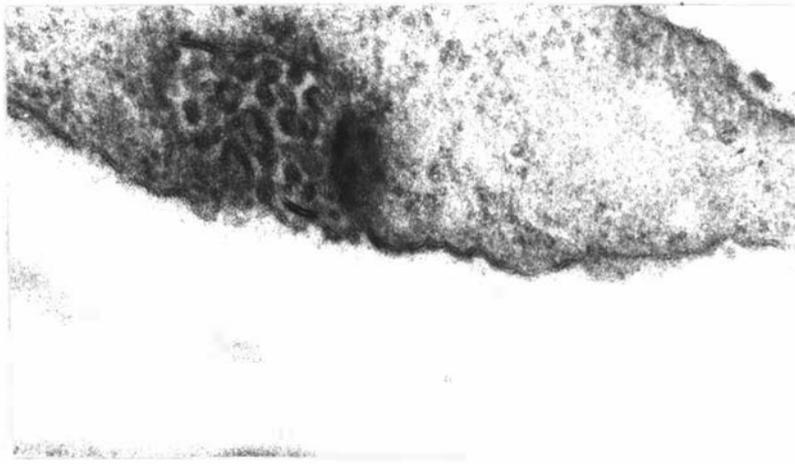
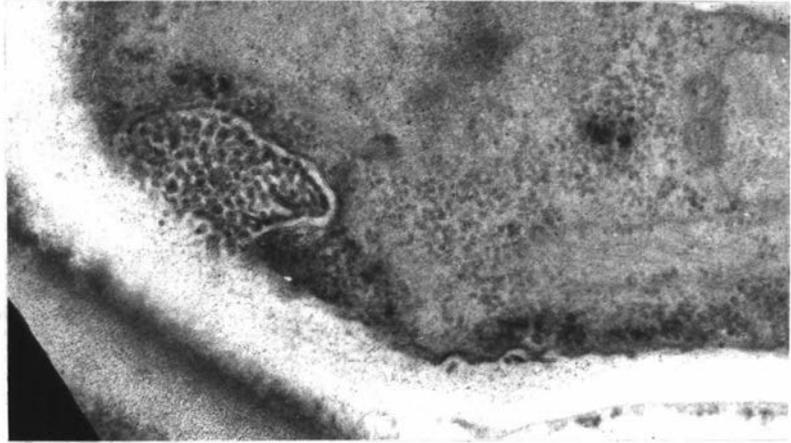


FIG. 144: Lomasomes within apices of hyphae penetrating cell walls.

TOP: *P. x euramericana* cv. I154. The lomasome and hyphal wall are continuous, the lomasome being bounded on the inside by the plasmalemma. X46,000.

CENTRE: *P. nigra* cv. Vert de Garonne. As for above. Note the tightly wound coils of plasmalemma. X82,500.

BOTTOM LEFT: *P. alba* x *P. nigra* Sempervirens cv. Mareg 2, Apex of penetration hypha showing lomasome and mitochondrion (M). X82,500.

BOTTOM RIGHT: *P. x euramericana* cv. I154. Lomasome showing tightly wound coils of plasmalemma. X110,000.

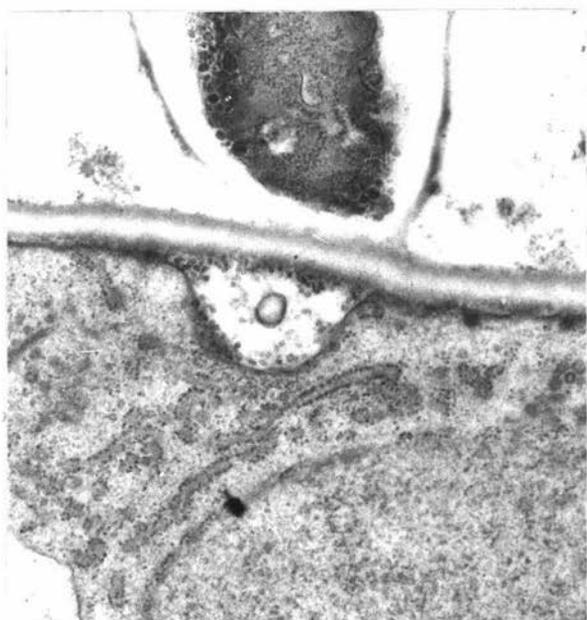
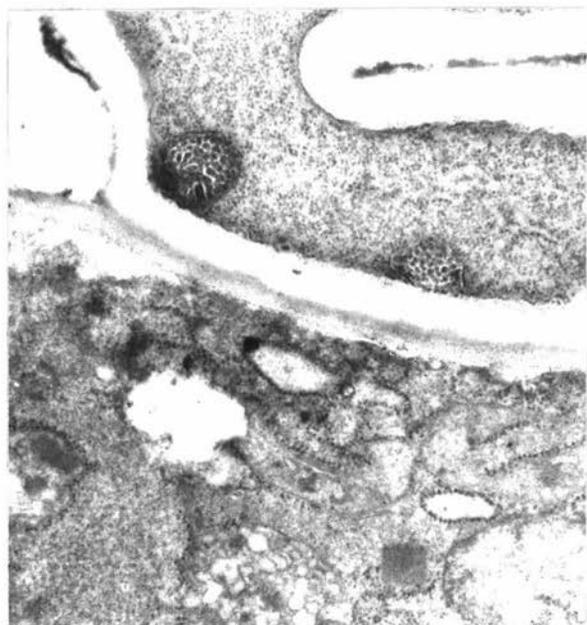


FIG. 145: Reaction of healthy cells to hyphal penetration.
P. x euramericana cv. I154. X24,000.

TOP LEFT: Initial contact. The penetration hypha is closely appressed to the cell wall and the cell has reacted by increasing the amount of endoplasmic reticulum and number of ribosomes in the cytoplasm adjacent to the point of contact. The plasmalemma has moved away from the cell wall. Note the lomasomes in the hypha.

TOP RIGHT: The plasmalemma has moved further away from the cell wall which has become depressed.

BOTTOM RIGHT: The cell wall has become more depressed and the plasmalemma has moved further away from the cell wall. The chloroplast appears to be moribund.

BOTTOM LEFT: The plasmalemma has invaginated to enclose an amorphous electron opaque deposit. Note the extensive endoplasmic reticulum and large number of ribosomes in the surrounding cytoplasm.

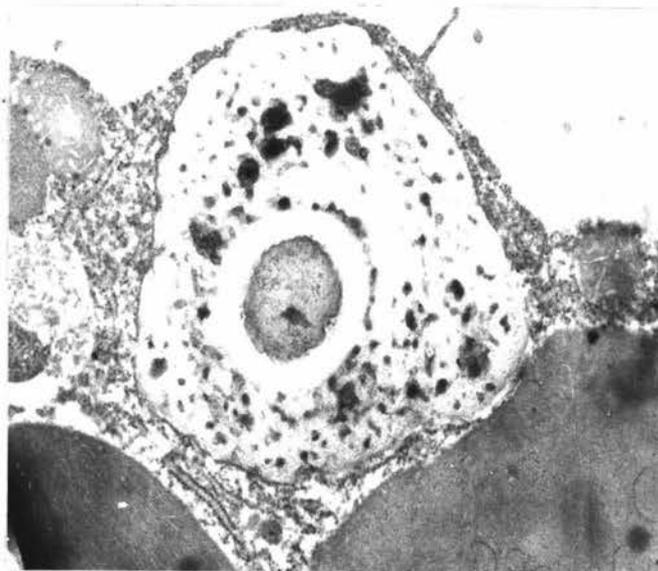
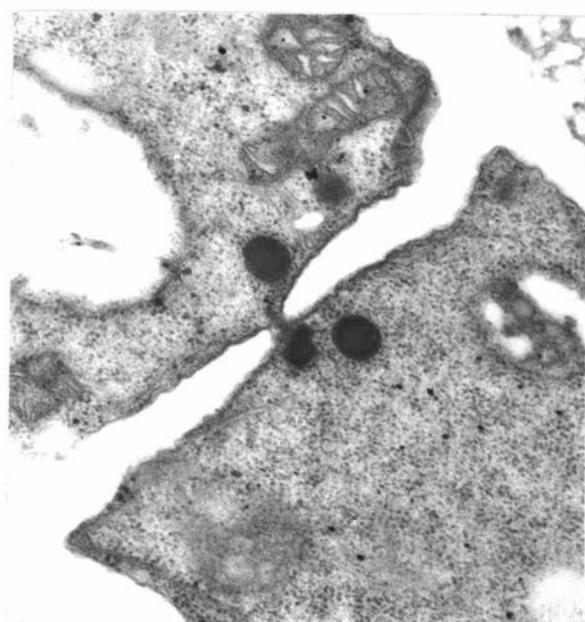


FIG. 146: Reaction of healthy cells to hyphal penetration.
M. brunnea, *P. x euramericana* cv. I154.

TOP: Amorphous electron opaque deposit formed in palisade cell following hyphal penetration. The contents of both cells have become moribund. Note the direct penetration of the cell wall by the invading hypha and the altered staining properties of the cell wall surrounding the point of entry. X10,000.

CENTRE: Amorphous electron opaque deposit formed in a mesophyll cell with an embedded penetration hypha. Note that the structure is enclosed by an invaginated plasmalemma. X11,200.

BOTTOM: Amorphous electron opaque deposit formed in lateral wall of palisade cell with embedded hypha. Note that the deposit is bound by the plasmalemma and also contains embedded fragments of plasmalemma. X18,200.



FIG, 147: Hyphae of *M. brunnea* within host tissue. X34,000.

P. nigra cv. Italica 'Aurea'. Hyphae within epidermal cell showing simple perforate septum flanked by Woronin bodies.

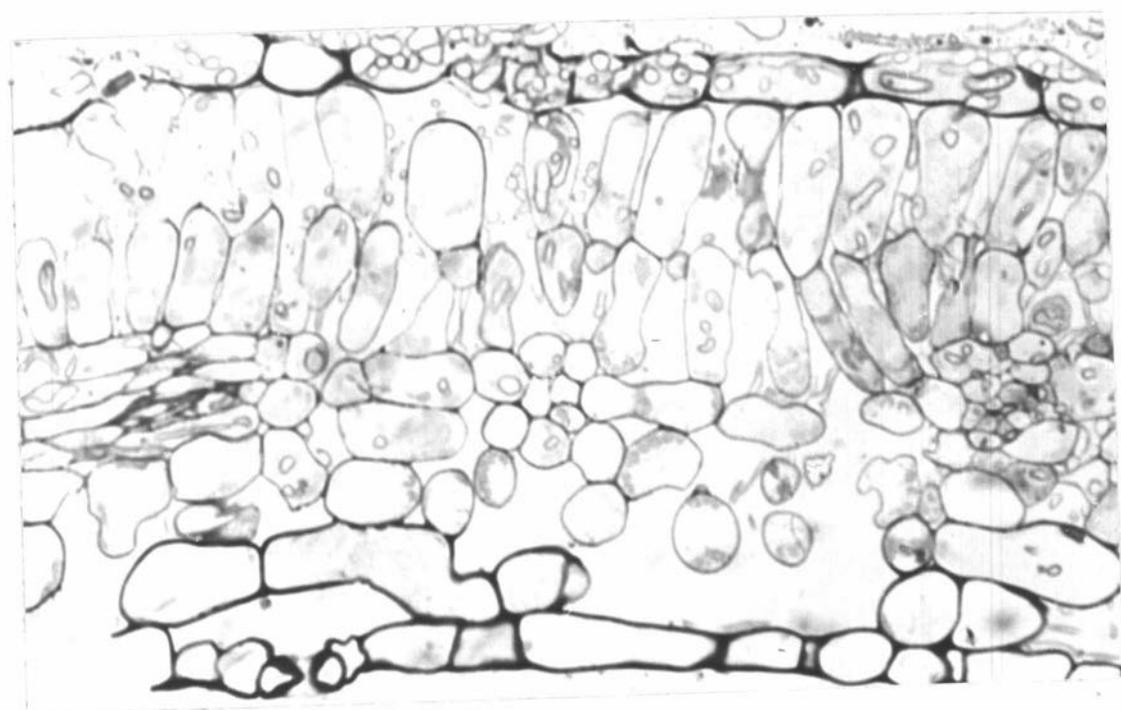


FIG. 148: Infection of *P. nigra* cv. Italica 'Aurea' by *M. populi* 6 days following inoculation.

Hyphae have ramified extensively throughout palisade and mesophyll cells. X600.

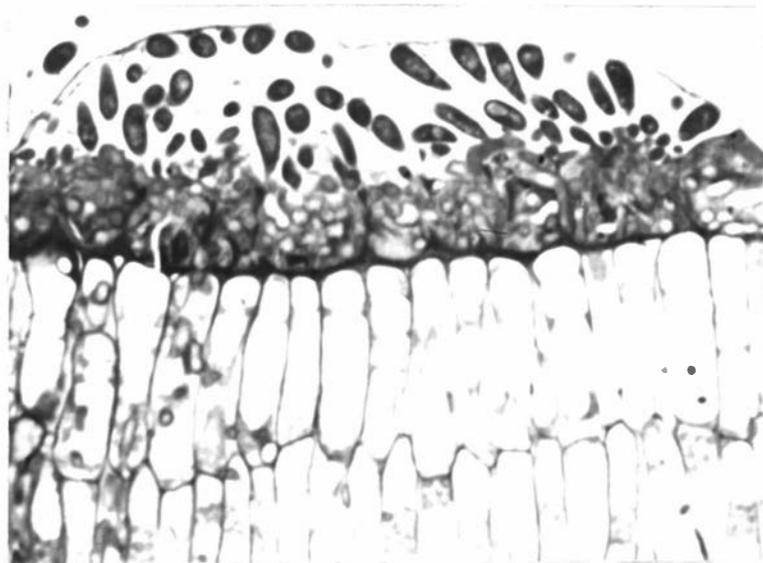
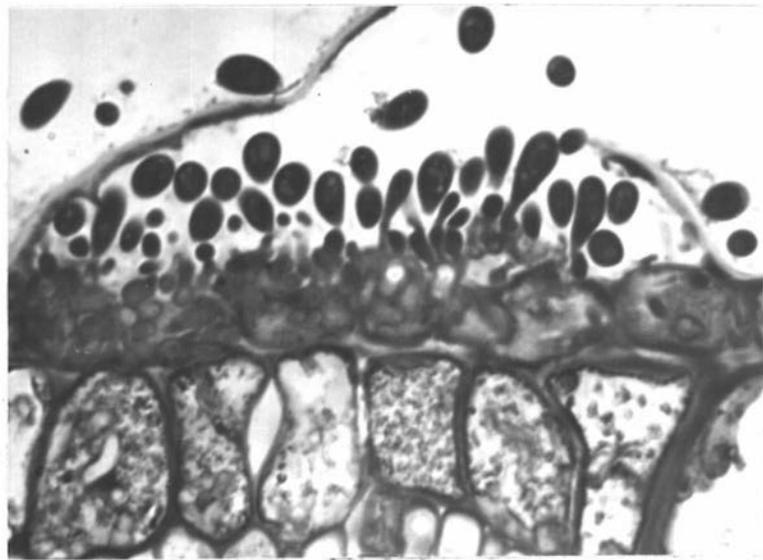


FIG. 149: Vertical sections through acervuli of *M. populi* formed on host tissue.

TOP: *P. x euramericana* cv. Robusta. X750.

BOTTOM: *P. nigra* cv. Vert de Garonne. X550.

Note that on these hosts acervuli were always formed intraepidermally.

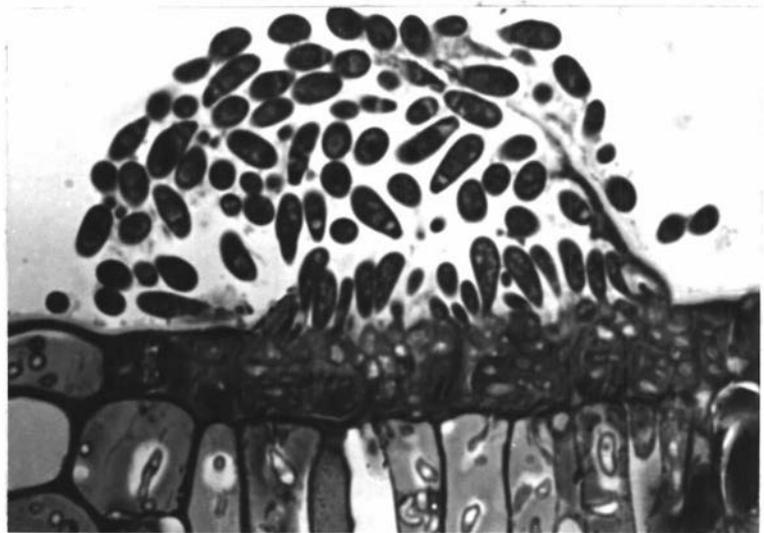
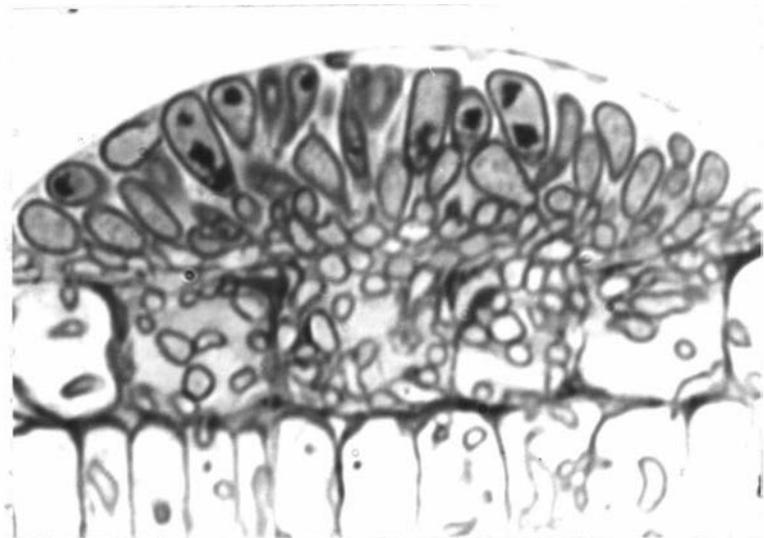
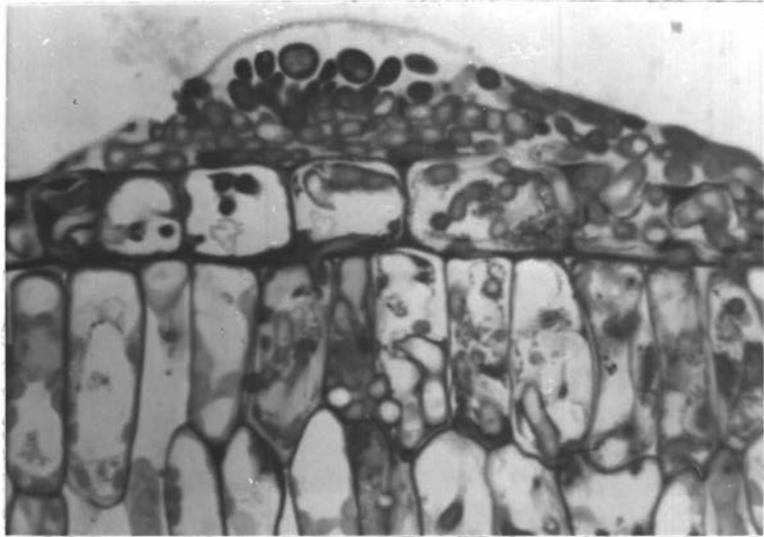


FIG. 150: Vertical sections through acervuli of *M. populi* formed on *P. nigra* cv. Italica 'Aurea'.

TOP: Development of hyphae intraepidermally and subcuticularly 6 days following inoculation, X750.

CENTRE: Formation of conidia subcuticularly 12 days following inoculation. X750.

BOTTOM: Formation of conidia intraepidermally 12 days following inoculation. X480.

Note that on this host acervuli were formed both subcuticularly and intraepidermally.



FIG. 151: Subcuticular infection of *P. nigra* cv. Italica 'Aurea' by *M. populi*.

Hyphae within epidermal cells showing detachment of cuticle from the epidermal cell wall as hyphae ramify subcuticularly. Note the direct penetration of the epidermal cell wall by hyphae. The contents of both cells are moribund. X4,050.

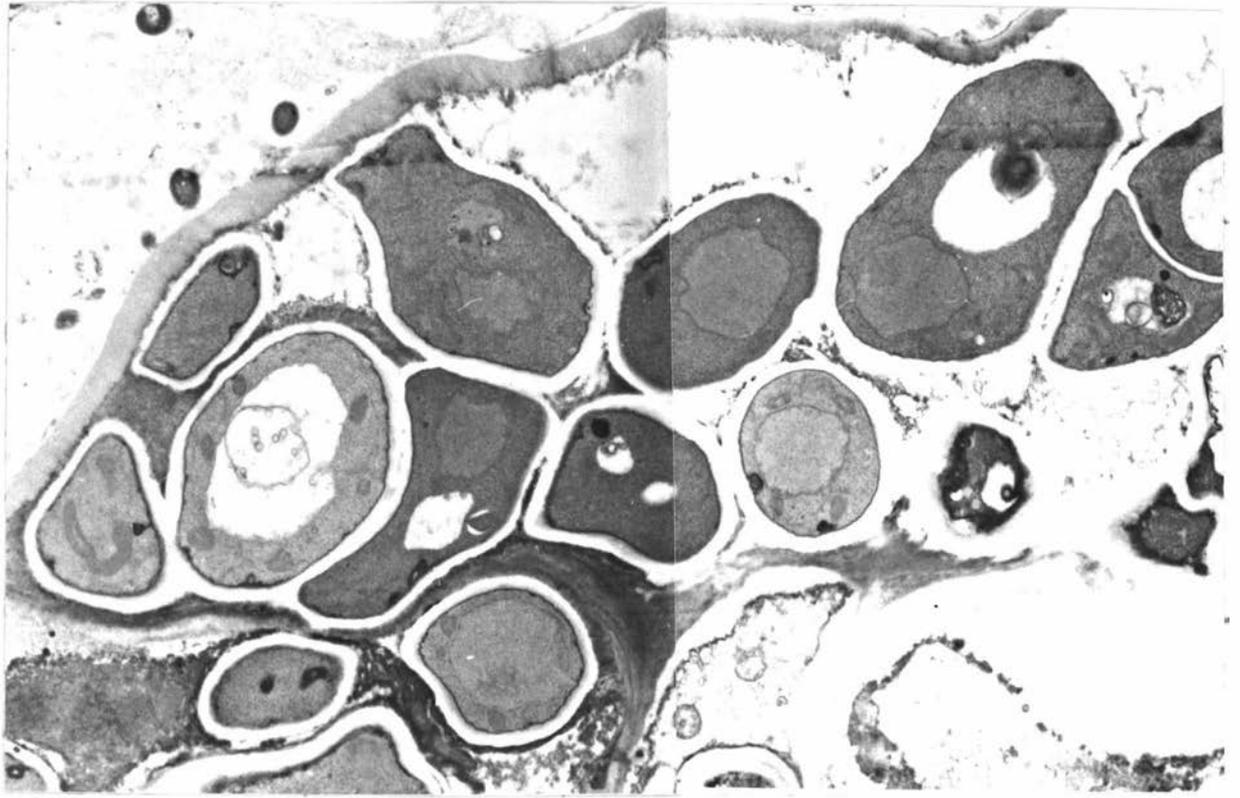


FIG. 152: Subcuticular infection of *P. nigra* cv. Italica
'Aurea' by *M. populi*.

TOP: Hyphae ramifying subcuticularly 6 days following
inoculation. Note that hyphae are morphologically
similar to those of *M. brunnea*. X8,100.

BOTTOM: Formation of conidia subcuticularly. Note the
abundant lipid globules within hyphae and conidia.
X2,300.

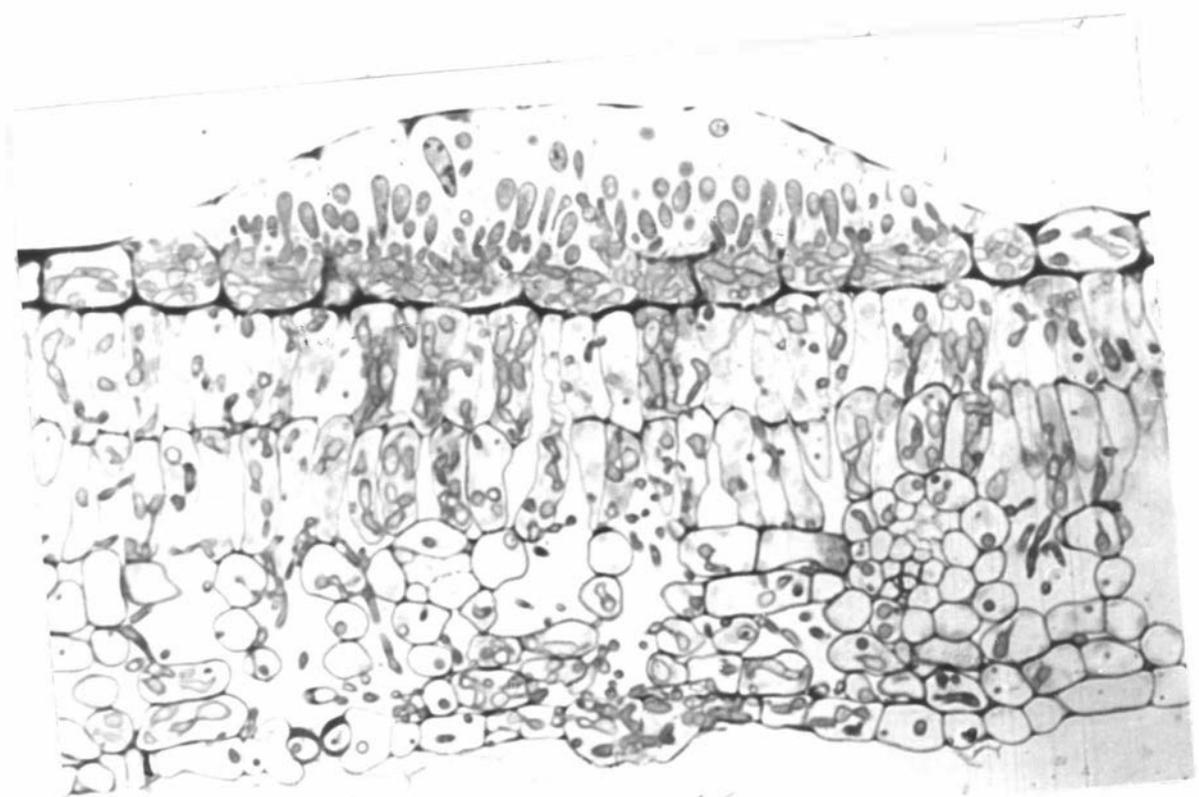


FIG. 153: Infection of *P. alba* by *M. castagnei* 6 days following inoculation. Hyphae have ramified extensively throughout palisade and mesophyll tissue and also have infected cells of the lower epidermis. X600.



FIG. 154: Reaction of healthy cells to hyphal penetration.

M. castagnei, *P. alba*. X11,500.

TOP: Formation of two electron opaque deposits concurrently following simultaneous penetration of a palisade cell. Note the septum formed in the hypha following penetration.

BOTTOM: The infection hypha has breached the electron opaque deposit and has expanded when clear of the deposit suggesting that these structures offer some resistance to hyphal penetration. Note the direct penetration of the cell wall by the infection hypha and the septum formed following penetration. The cellular contents are moribund with only the remains of the chloroplasts being discernable.

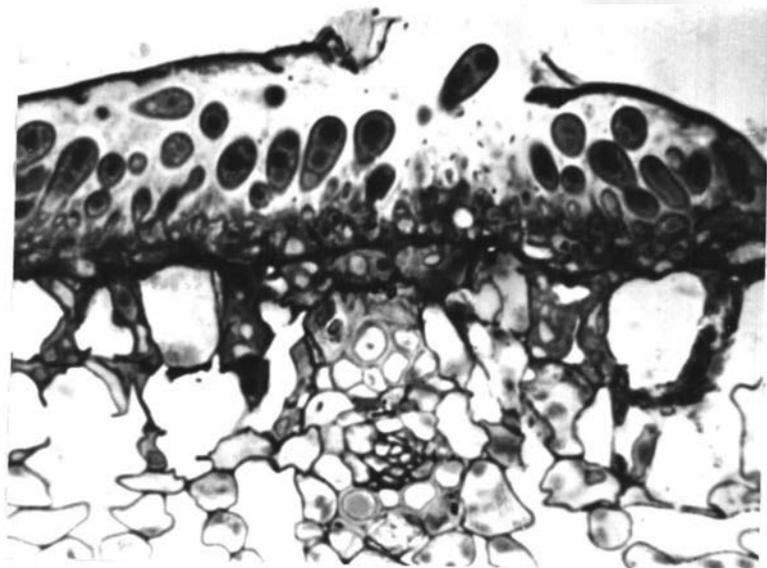
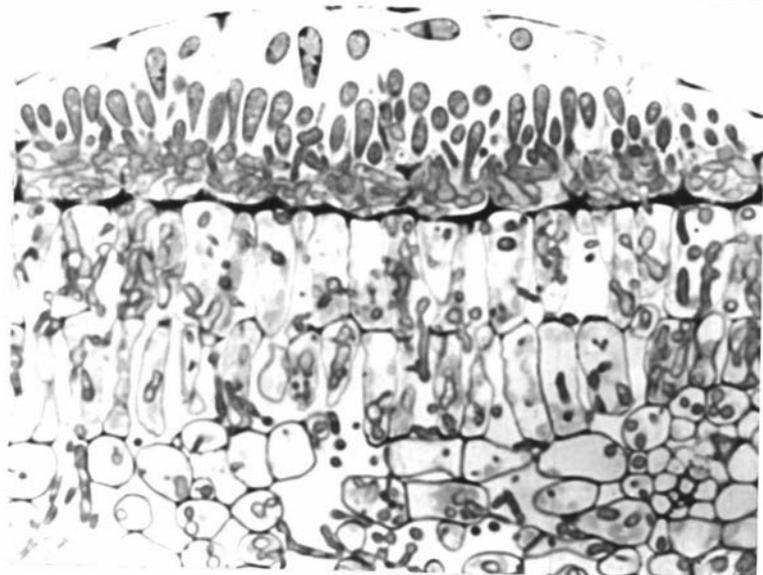


FIG. 155: Vertical sections through acervuli of *M. castagnei* on host tissue.

TOP: *P. alba* cv. NZ old clone. X360,

BOTTOM: *P. alba* x *P. nigra* Sempervirens cv. Mareg 2. X700.

Note that acervuli are formed intraepidermally.

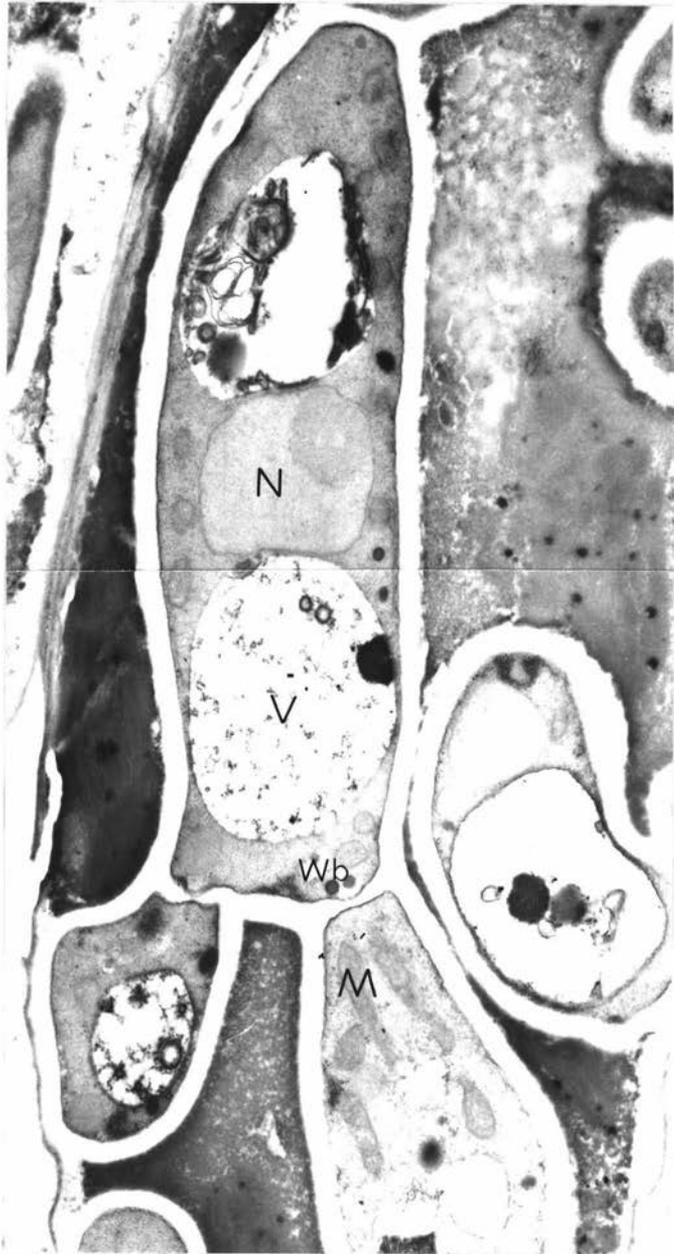


FIG. 156: Hyphae of *M. castagnei* within *P. alba*.

Hypha within palisade cell showing typically observed ultrastructure. Note the single nucleus (N), vacuoles (V), mitochondria (M), simple septa and Woronin bodies (Wb). Hyphal cell walls are electron opaque and inconspicuously bilayered. X11,600.

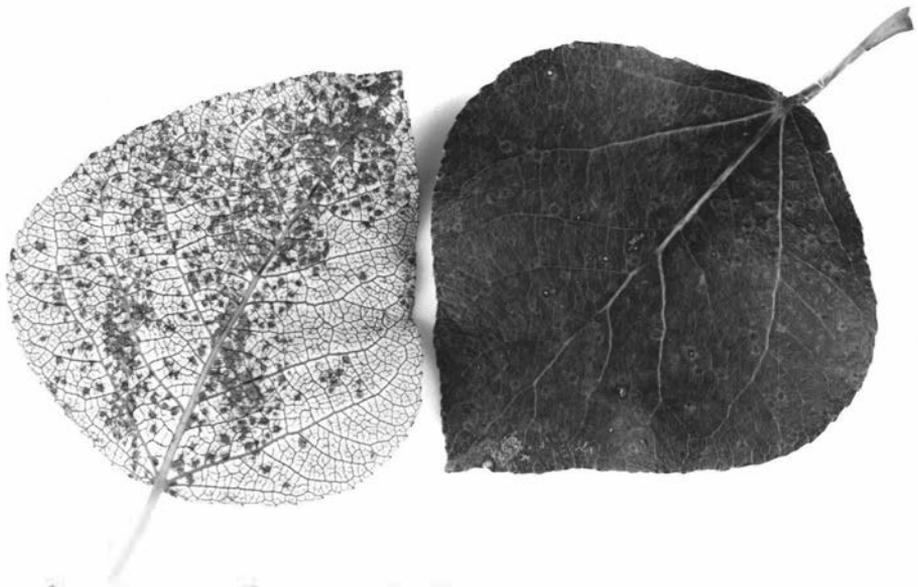
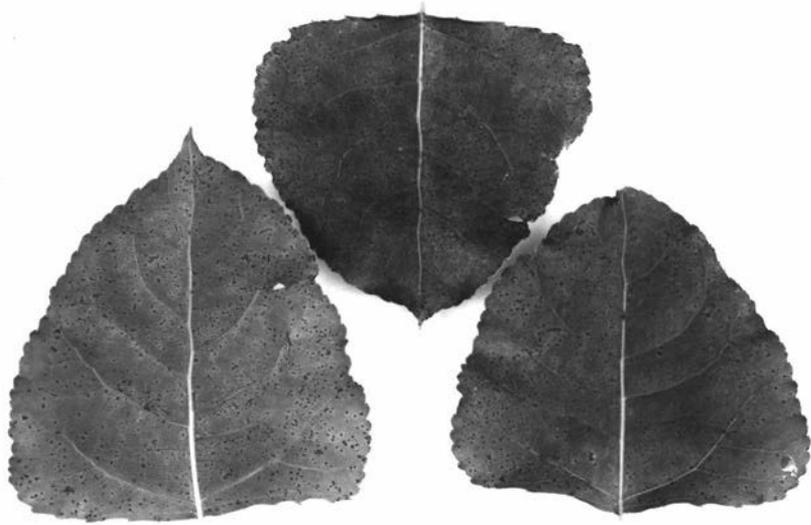


FIG. 157: Influence of host on the level of infection recorded on the adaxial and abaxial leaf surface.

TOP: *P. x euramericana* cv. Eugenei PU. Note the comparable levels of infection on the adaxial and abaxial leaf surfaces.

BOTTOM: *P. trichocarpa* seedling. Note much higher level of infection on the abaxial leaf surface.

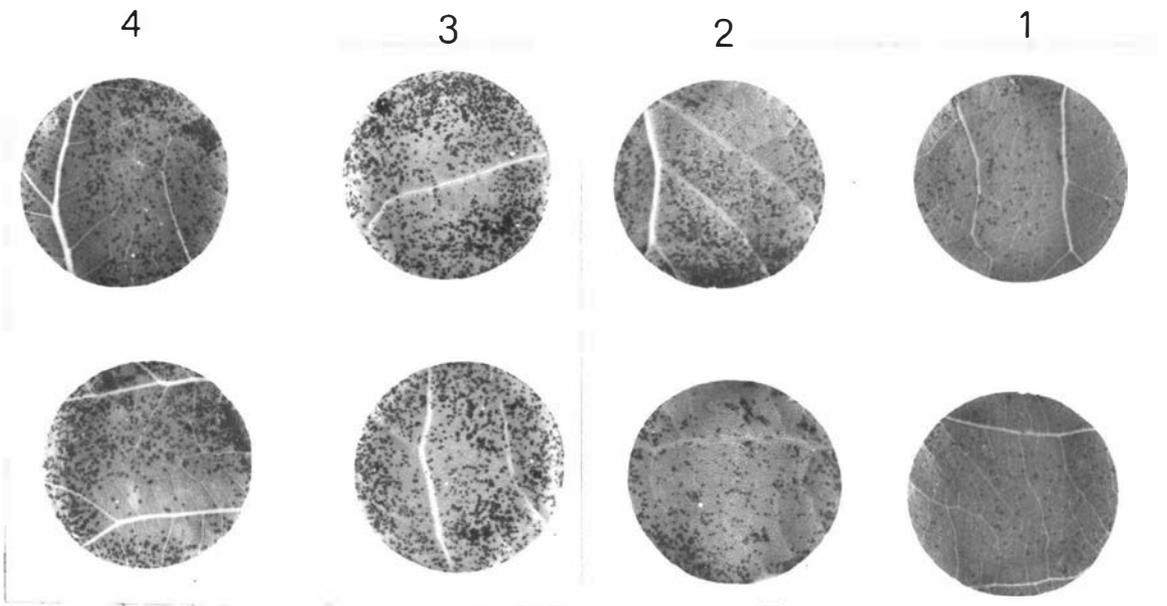
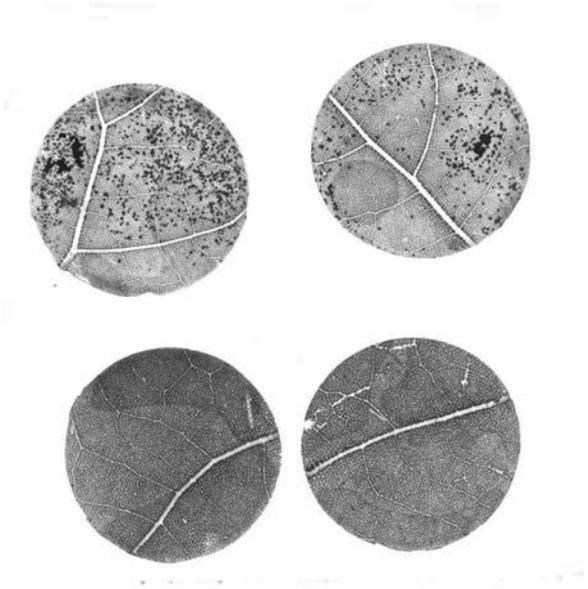


FIG. 158: *P. deltoides* cv. ANU A60/129 influence of leaf age and surface on the level of infection.

TOP:	Left abaxial surface	soft expanding leaf tissue.
	Right adaxial surface	" " " "
BOTTOM:	Left abaxial surface	mature leaf tissue
	Right adaxial surface	" " "

Note the uniformly high levels of infection established on both leaf surfaces of soft expanding leaves compared to virtually no infection established on either leaf surface of mature leaves.

FIG. 159: *P. x euramericana* cv. Eugenei PU: influence of leaf age and surface on the level of infection.

1.	Adaxial surface	mature leaf tissue
2.	Abaxial surface	" " "
3.	Abaxial surface	soft expanding leaf tissue
4.	Adaxial surface	" " " "

Note the comparable infection levels established on both surfaces within each age class and the much higher levels of infection established on soft expanding leaves.

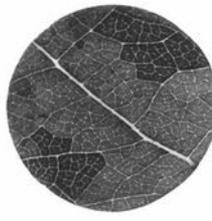
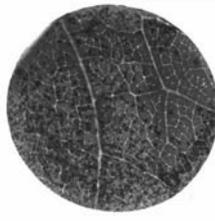
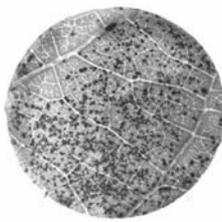
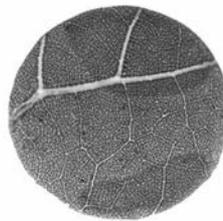
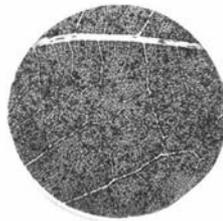
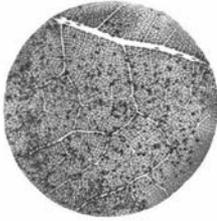


FIG. 160: *P. x euramericana* cv. NL 1603: influence of leaf age and surface on the level of infection.

TOP: Left adaxial surface soft expanding tissue.
 Right abaxial surface " " "

BOTTOM: Left adaxial surface mature leaf tissue.
 Right abaxial surface " " "

Note the much higher levels of infection established on soft expanding leaf tissue.

FIG. 161: *P. candicans*: influence of leaf age and surface on the level of infection.

TOP: Left abaxial surface young expanding leaf tissue.
 Right abaxial surface mature leaf tissue,

BOTTOM: Left adaxial surface young expanding leaf tissue.
 Right adaxial surface mature leaf tissue.

Note the increased resistance of the adaxial leaf surface of mature leaf tissue to infection whereas the abaxial leaf surface (of mature leaf tissue) remained highly susceptible.

FIG. 162: *P. x euramericana* cv. I154: influence of leaf surface on the level of infection.

TOP: Left and right adaxial surface soft expanding leaf tissue.

BOTTOM: Left and right abaxial surface soft expanding leaf tissue.

Note the comparable levels of infection established between leaf surfaces within the same age class.

FIG. 163: *P. candicans*: influence of leaf surface on the level of infection. Mature leaf tissue.

TOP: Left and right adaxial surface.

BOTTOM: Left and right abaxial surface.

Note the significantly higher levels of infection established on the abaxial leaf surface.

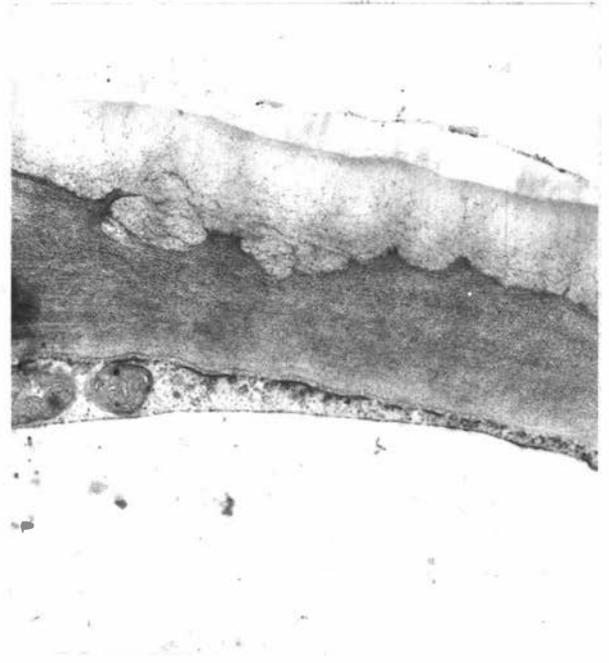
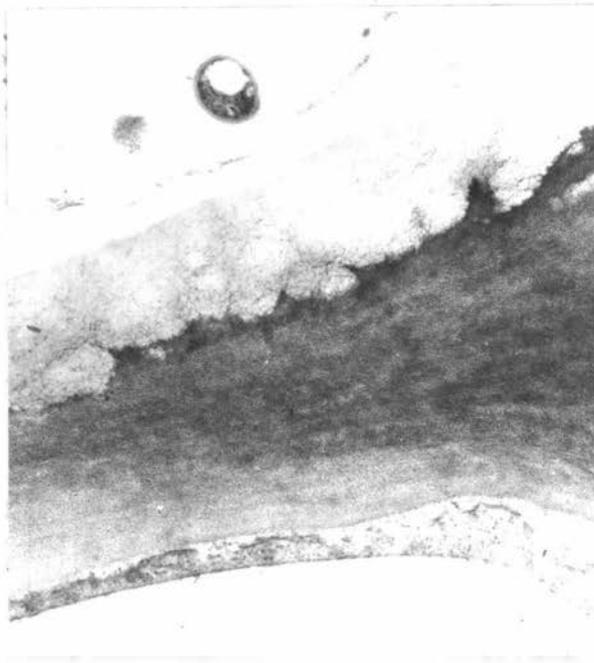
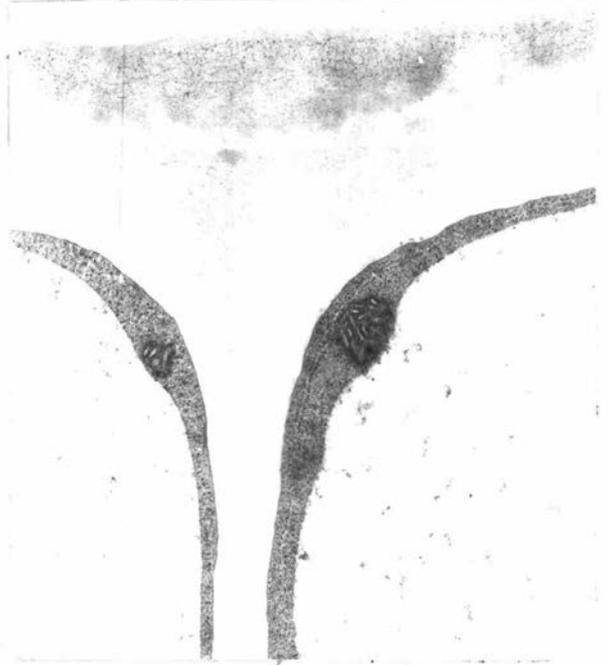


FIG. 164: Ultrastructure of epidermal cell walls of
P. x euramericana cv. I154.

TOP LEFT: Adaxial surface soft expanding leaf tissue. X20,500.

TOP RIGHT: Abaxial surface " " " " X20,200.

BOTTOM LEFT: Adaxial surface mature leaf tissue. X18,400.

BOTTOM RIGHT: Adaxial surface " " " X17,400.

Note within each age class the comparable morphology of the epidermal cell walls of the two leaf surfaces. Note also the conspicuous microfibrils and well developed cuticles of epidermal cell walls of mature leaves.

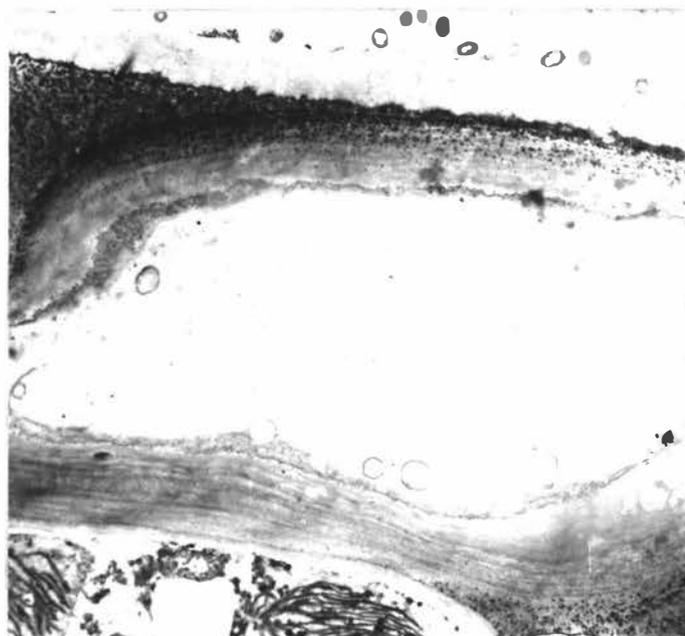
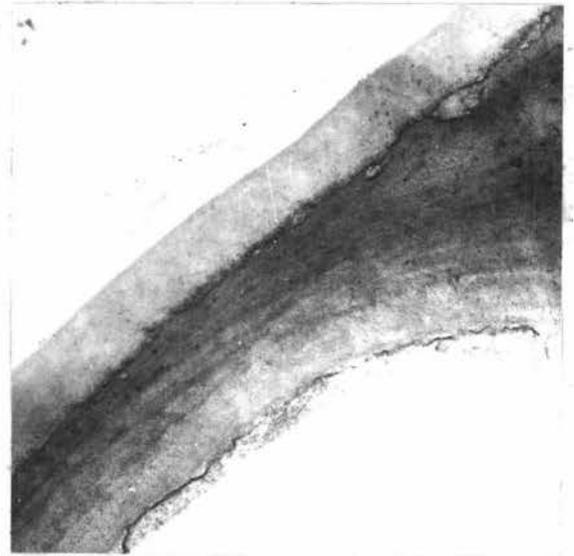
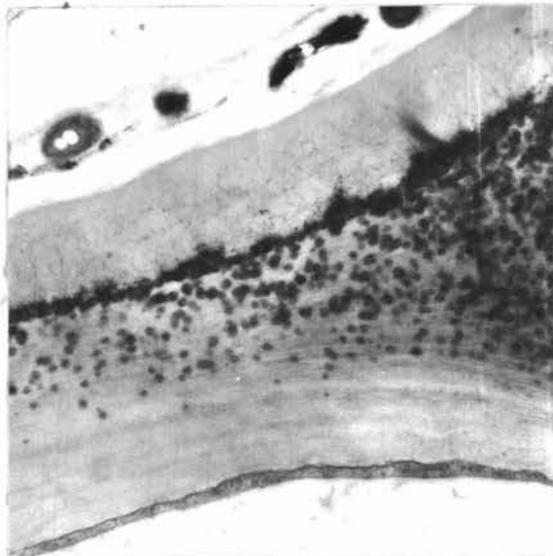
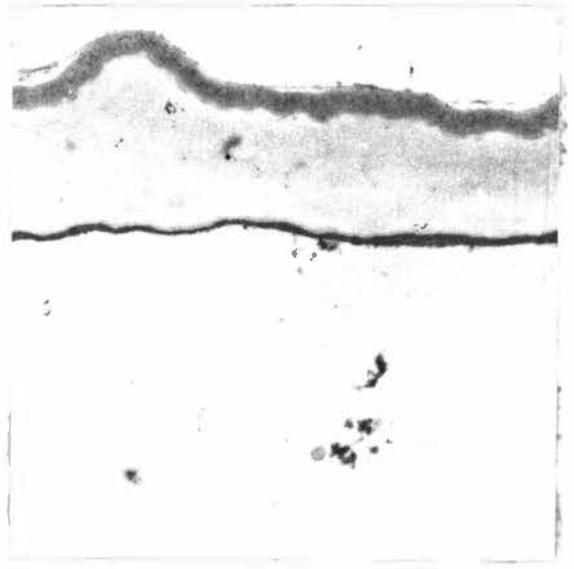
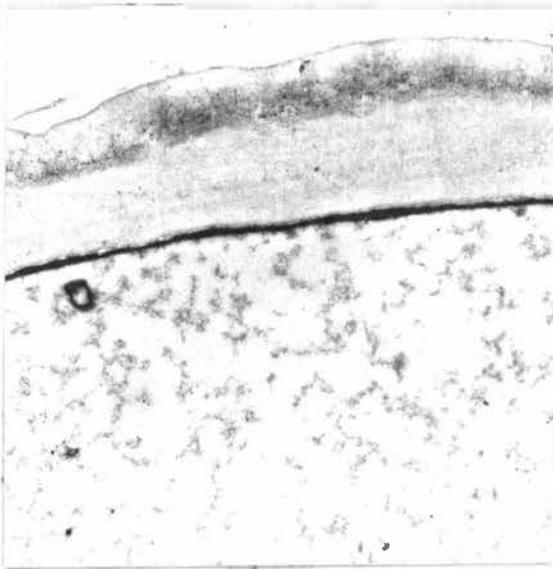


FIG. 165: Ultrastructure of epidermal cell walls of young expanding leaves of *P. candicans*. X5,000.

LEFT: Adaxial leaf surface.

RIGHT: Abaxial leaf surface.

Note the morphological similarity of cell walls from both leaf surfaces, the poorly developed cuticles and amorphous appearance.

FIG. 166: Ultrastructure of epidermal cell walls of mature leaves of *P. candicans*.

TOP LEFT: Adaxial leaf surface. X14,100.

TOP RIGHT: Abaxial leaf surface. X12,800.

Note the well developed cuticles and conspicuous microfibrils. Note also the darkly stained wall of the adaxial leaf surface.

BOTTOM: Epidermal cell (adaxial surface). Note the darkly stained cell wall under the cuticle.

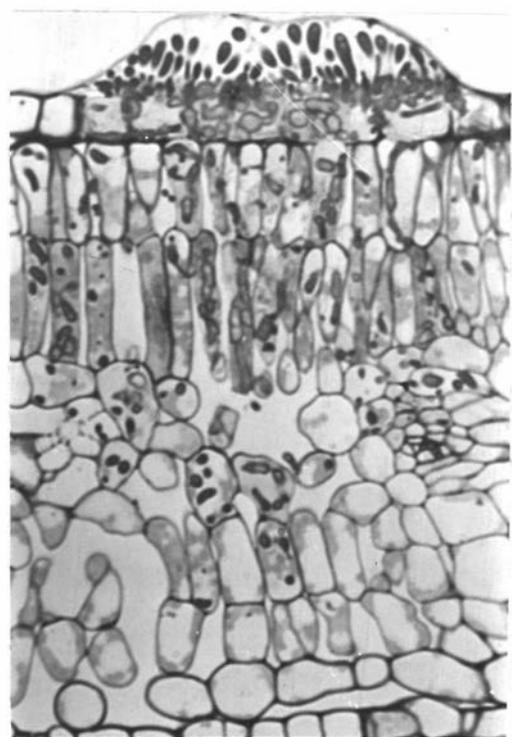
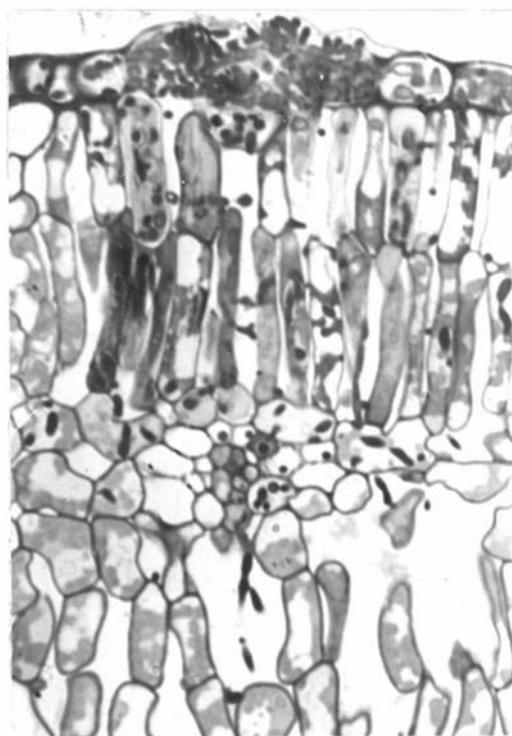


FIG. 167: Comparative spread of infection throughout soft expanding and mature leaf tissue of *P. x euramericana* cv. I154 8 days following inoculation.

TOP: Mature leaf.

BOTTOM: Soft expanding leaf.

Note the comparable spread of hyphae throughout the two leaves and the formation of conidia on the soft expanding leaf. X500.

FIG. 168: Comparative spread of hyphae throughout expanding and mature leaf tissue of *P. x euramericana* I154.

TOP: Epidermal cells of soft expanding leaf tissue infected with *M. brunnea*. X2,800.

BOTTOM: Epidermal cells of mature leaf tissue infected with *M. brunnea*. X3,000.

Note deformation of the elastic cell walls of soft expanding leaves by extensively ramifying hyphae. By contrast cells of mature leaves retain their shape and the walls offer more resistance to hyphal penetration.

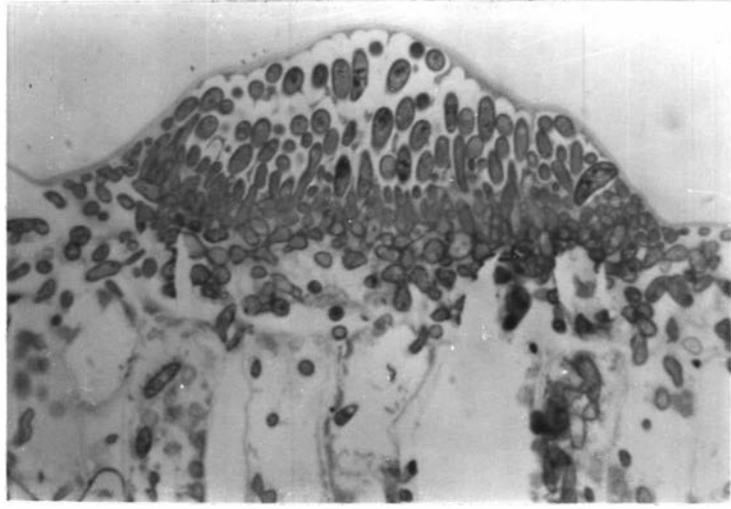


FIG. 169: Infection of soft expanding leaves of *P. candicans*.

TOP: Vertical section through acervulus.
Note that hyphae have ramified extensively throughout the epidermal cells which are no longer discernable. X600.

BOTTOM: Electron micrograph showing the slight resistance offered to hyphae by soft expanding cell walls. X2,800.