ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 1—2: 29—32
1981

Microsporogenesis and pollen grains in *Silene dioica* (L.) Cl. and alterations in its anthers parasited by *Ustilago violacea* (Pers.) Rouss. (*Ustilaginales*)

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Abstract

Healthy and infected anthers are comparatively studied with optical and electron microscopic techniques. The fungus stops the stamen histogenesis at an early stage and destroys specifically the sporogenous tissue.

INTRODUCTION

Silene dioica (*Caryophyllaceae*), a dioeicous plant growing in fresh and humid forest, is the common host of *Ustilago violacea* (*Ustilaginales*). This fungus is responsible for the female (Batcho et al., 1980) and male (Audran, Batcho, 1980a, b; Batcho, Audran, 1980) structure destruction leading to the castrations of *Silene dioica*: anther and ovary contents are occupied by a powdery black mass of spores (the smut).

The processes occurring in the healthy and infected anthers will be successively analysed.

MATERIAL AND METHODS

Healthy and infected floral buds of male *Silene dioica* were fixed with glutaraldehyde for the electron microscope, post-fixed with osmium tetroxyde and embedded in araldite (Glauert, 1978). Ultra-thin sections were usually contrasted by uranyl acetate and lead citrate (Berlyn, Miksche, 1976), or stained by the PATAg for the carbohydrates detection (Thiery, 1967). At the light microscope, the cytochemical colorations used for the detection of lipids, carbohydrates and proteins (Lison, 1960; Jensen 1962) have been performed either on material

Fig. 1 A-D. Microsporogenesis in healthy anther
A-C — M. O., Glut/OsO_4 1 µm plastic sections, basic touidine blue; D — T.E.M. Glut/OsO_4, ultra-thin section — PATAg).

A — microsporangium structure at the end of its histogenesis phase. Uninucleated tapetal cells and pollen mother cells are distinguishable. The sporogenous tissue is still coherent (× 800).

B — tetrads. Ectexine is in course of formation around each microspore (× 1 200).
C — bicellular pollen grains into a predehiscent microsporangium (× 1 100).
D — sporoderm structure in extra apertural zone and heterogeneous and massive orbicule (× 17 000).

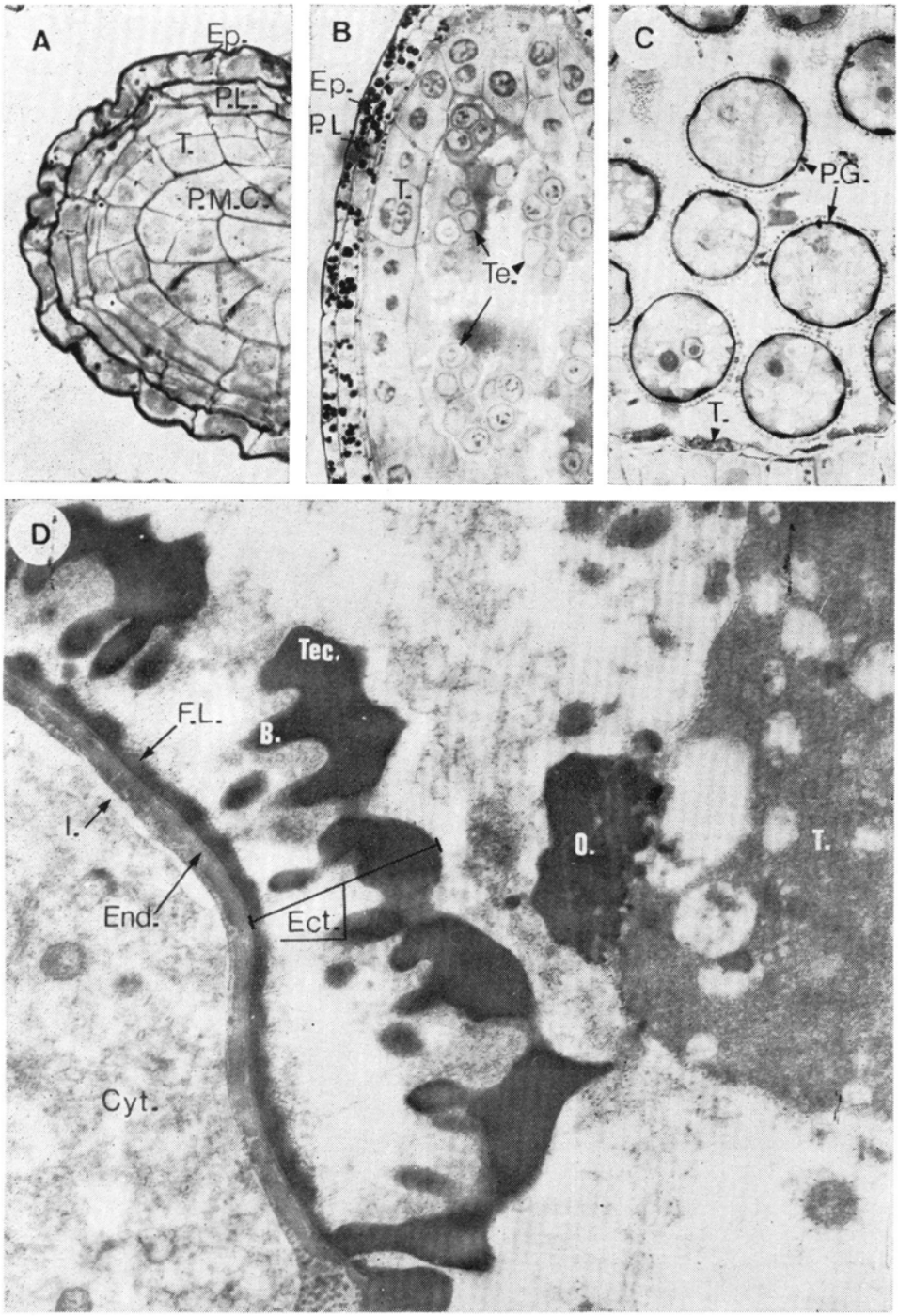
Key to labelling: B. — bacule; Cyt. — cytoplasm; Ect. — ectexine; End. — endexine; Ep. — epidermis; F.L. — foot-layer; H.Sp.C. — hypertrophied sporogenous cell; I. — intine; M. — mitochondria; My. — mycelium; N. — nucleus; n. — nucleolus; N.Sp.C. — necrosed sporogenous cell; O. — orbicule; P.G. — pollen grain; P.L. — parietal layers; P.M.C. — pollen mother cell; T. — tapetum; Te. — tetrad; Tec. — tectum; V. — vacuole.

fixed with Carnoy, embedded in paraffin and cut at 7 µm or on material fixed and embedded according to usual methods for electron microscopy and cut at 1 µm.

RESULTS

Pollen and tapetum ontogeneses in healthy anthers

Fig. 1A shows partial view of an anther section at the end of histogenesis. The following are recognized from outward: 1) an epidermis; 2) three parietal cell layers; 3) two tapetal layers of uninucleated cells and 4) the sporogenous tissue constituted by pollen mother-cells (P. M. C. s) just before meiosis. That latter is normal and leads to the formation of tetrads simultaneously septated (Fig. 1B). The ectexine composed of an interrupted tectum, a columnar infratectal layer and an irregular foot-layer is underlining the callosic special wall, wrapping each tetrad, and within a polysaccharidic primexine fibrillar in texture. Later the endexine is formed after the tetrad dissociation due to the callose wall dissolution. During microspore vacuolisation and enlargment, the exine structure and chemical composition are modified (Fig. 1D). After the sporal mitosis, bicellular pollen grains are constitued (Fig. 1C). The tapetum pertains to the secretory type. Its cell walls are destroyed as soon as the tetrad stage is over. Orbicules of large size and irregular in shape are elaborated in the periplasmic space (Fig. 1D). Tapetum cells are damaged, liberating in the anther locule an important quantity of tryphine, which is deposited in the exine cavities. Sporogenous cells,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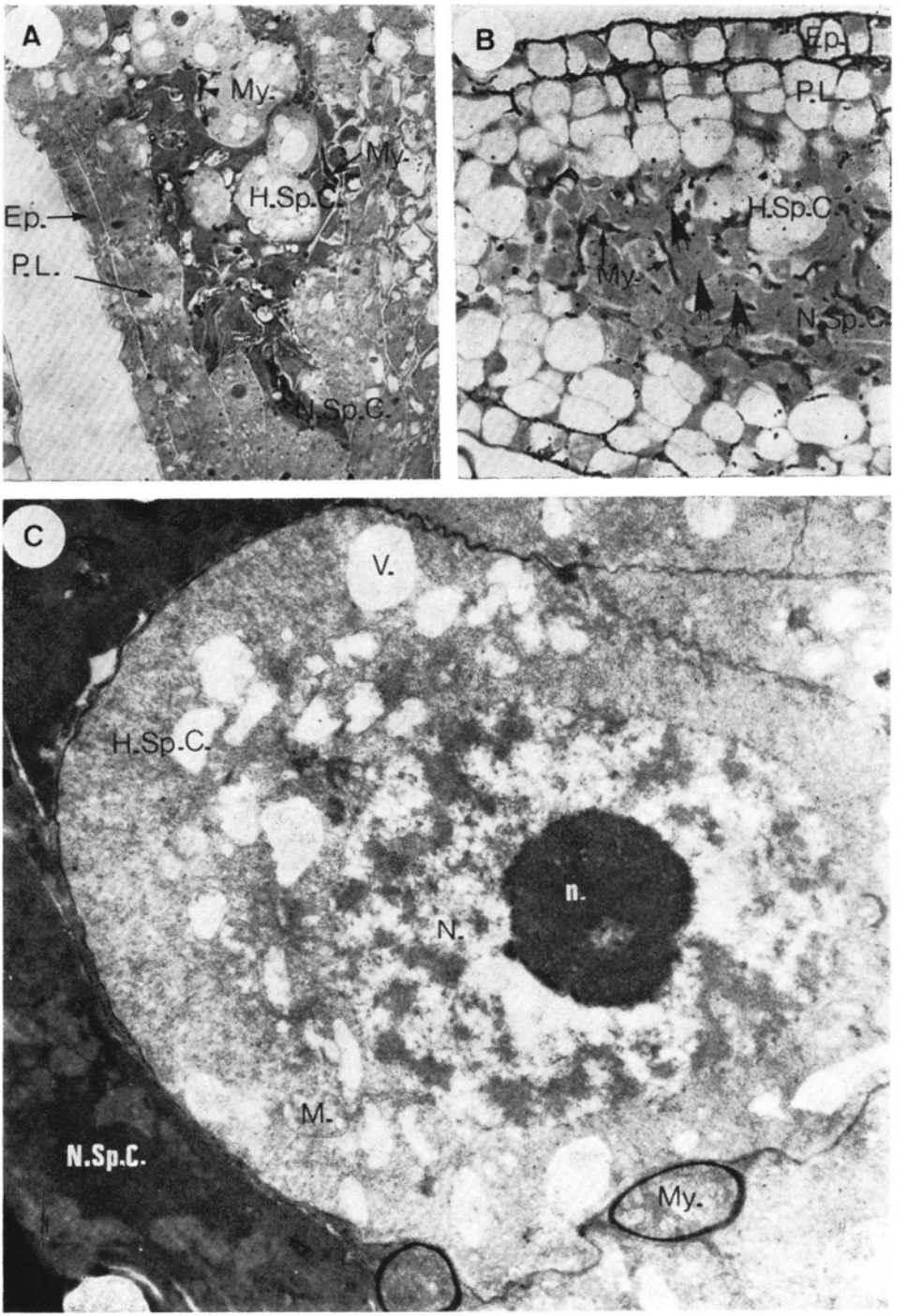

Fig. 2 A-C. Microsporogenesis in infected anther
(M.O., Glut/OsO_4, 1 µm plastic section; A — Sudan black; B — A.P.S.; C — T.E.M., Glut/OsO_4, ultra-thin section, PATAg).

A — necrosed sporogenous cells and hyphae are colored by the lipidic test .(× 1 100)

B — many starch granules appear in the necrosed cells (arrows) (× 1 500).

C — detailed aspect of necrosed and hypertrophied cells (× 10 500).

Key to labelling as in Fig. 1

microspores and pollen grains never contain starch; polyholosid is essentially localized in epidermic and parietal cells of anther; they contain lipidic globules the amount of which is progressively decreasing.

Alterations in infected anthers

The dikaryotic mycelium grows between the host plant cells (Fig. 2C) but rarely penetrates them. The fungus inhibits the stamen histogenesis at an early stage destroying specifically the sporogenous cells. They are necrotized according to two different modalities: 1) only some sporogenous cells undergo lysis and are transformed into compact masses; 2) then necrosis extends throughout the sporogenous tissue following hypertrophy ,caused by extensive vacuolisation (Fig. 2C). Simultaneously, hyphae grow abundantly within the necrotic sporogenous mass and the fungus sporulates. The teliospores fill up the anther locule. In the necrosed sporogenous cells, the lipidic amount lightly increases (Fig. 2A) and starch is produced in plastids (Fig. 2B). Then, these substances decrease whereas lipidic and glycogenic masses appear in the hyphea. Cytoplasmic proteins are unmasked at the time when sporogenous cells are necrosed .

DISCUSSION

The data obtained in the ultrastructural and cytochemical enquiry of the microsporogenesis in the healthy anthers are in accordance with the general opinion given on that subject by Heslop-Harrison (1963a, b) in some *Caryophyllaceae*. Thus, two major events contribute to the exine genesis: the formation of an exinic matrix, with the control of haploid genome of the spore, and the growth of the exine by the deposition of sporopollenin originating mainly from the sporophytic tapetum.

In the infected anthers, microsporogenesis is inhibited. From structural point of view, the fungus stops the stamen histogenesis at an early stage before destroying its content. Thus, the cells—the vocation of which is to produce microspores—remain in juvenile aspect of sporogenous cells; tapetum is not differentiated and meiosis does not occur. From biochemical point of view, the fungus presence within the anther goes along with an accumulation of glucidic and lipidic reserves in the sporogenous tissue. The fungus develops its hyphae in the anther tissues and produces teliospores at the place where pollen grains would have been produced.

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