



## Ultrastructural features of *Mimulus aurantiacus* (Scrophulariaceae) pollen tubes *in vivo*

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### ABSTRACT

The aim of this study is to give information on ultrastructure of *in vivo* pollen tubes of *Mimulus aurantiacus* which were collected from the Botanical Garden of the University of California at Berkeley. Materials were prepared according to electron microscopy methods and examined under Zeiss electron microscope. Four zones were examined in the pollen tubes of *Mimulus aurantiacus*. **Apical zone:** Mitochondria, smooth endoplasmic reticulum, rough endoplasmic reticulum, dictyosomes and secretory vesicles were observed. **Subapical zone:** This area contained abundant rough endoplasmic reticulum and occasionally some smooth endoplasmic reticulum. The polysomes, mitochondria, proplastids that contain starch, small vacuoles and a few lipid bodies were detected. **Nuclear zone:** Both generative and vegetative cell nuclei lie in this zone. The vegetative cell nucleus was large and long. Rough endoplasmic reticulum, mitochondria, ribosomes, dictyosomes, and amyloplasts that are rich of starch were observed. **Vacuolation and plug formation zone:** Cytoplasm of the tubes was full of large vacuoles. Few organelles such as mitochondria, dictyosome and rough endoplasmic reticulum were detected along their periphery.

**Key words:** *in vivo*, *Mimulus*, pollen tube, ultrastructure.

### INTRODUCTION

Pollen, the male gametophyte of higher plants, is a biological system playing a central role in sexual plant reproduction (Cresti et al. 1992, Moscatelli and Cresti 2001). Interactions between pollen and the stigma surface initiate pollen germination, which involves an asymmetric extrusion of the pollen cytoplasm through a germination pore to initiate the outgrowth of a pollen tube. Various studies have been done about pollen tubes mostly concerning molecular (Graaf et al. 2005), cytochemical (Georgieva 1987), biochemical (Zonia et al. 2002) physiological; *in vitro* (Dane et al. 2004), *in vivo* (Unal 1986), *semivivo* (Bergamini Mulcahy and Mul-

cahy 1985) and *in situ* (Dane 2000) aspects and pollen-pistil interactions (Ferrari et al. 1985). The influences of various external factors; such as some chemicals (Kandasamy and Kristen 1987, Kim et al. 2003, Röderer and Reis 1988, Sawidisand and Reiss 1995) heat shock (Kandasamy and Kristen 1989), caffeine (Lancelle et al. 1997) and also pistil (Herrero and Arbeloa 1989) on pollen tube growth have been investigated. But ultrastructural studies about pollen tubes are very rare. Initial studies have been done in *Petunia* (Solanaceae) (Sassen 1964), (Cresti and Van Went 1976) and *Lilium longiflorum* (Liliaceae) (Rosen et al. 1964). Few reports have appeared in 1980s, concerning the fine structure of the pollen tubes in *Lycopersicum peruvianum* (Solanaceae) (Cresti et al. 1977), (Cresti et al. 1980), *Prunus avium* (Rosaceae) (Cresti et al. 1979), (Uwate and Lin 1980),

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*Prunus* sp. (Rosaceae) (Ciampolini et al. 1982), *Nicotiana glauca* (Solanaceae) (Lancelle et al. 1987). In recent years, ultrastructural studies about pollen tubes have continued with *Hosta ventricosa* (Liliaceae) (Shi-Yi et al. 1992), *Lilium longiflorum* (Liliaceae) (Pierson et al. 1990), (Lancelle and Hepler 1992), *Nicotiana tabacum* (Solanaceae) (Yu and Russell 1993, Rutten 1993), *Asclepias exaltata* (Asclepiadaceae) (Sage and Williams 1995), *Arabidopsis thaliana* (Brassicaceae) (Lennon et al. 1998), and *Conospermum* species (Magnoliaceae) (Stone et al. 2004).

Those studies were generally on the ultrastructure of *in vitro* pollen tubes except the studies with *Prunus avium* (Cresti et al. 1979), (Uwate and Lin 1980). It is too hard to perform and to follow the development of *in vivo* pollen tubes, so these studies are very important and restricted. Therefore, the number of those studies should be increased.

Some electron microscope observations on pollen grains have been done in some members of subtribe *Castilleiinae* (Jensen et al. 1974) and in the genus *Mimulus* of subtribe *Gratiroleae* Scrophulariaceae (Argue 1980). Olgun has done embryological researches on *Digitalis* sp. (Scrophulariaceae) with light microscope (Olgun 1979), (Yakar and Olgun 1984). She continued her studies with TEM such as ultrastructures of embryo sac (Olgun and Jensen 1987) and endothelium (Dane et al. 2007) in *Penstemon gentianoides* which belongs to the same family. This study can be regarded as the follow-up of those studies.

The ultrastructure of *in vivo* pollen tubes of *Mimulus aurantiacus* (Scrophulariaceae) which is naturally grown in North America, and possible similarities related to the ultrastructure of *in vitro* and *in vivo* pollen tubes in other angiosperms (Moscatelli and Cresti 2001) were emphasized in this study.

Ultrastructural studies were generally realized with the families Liliaceae, Solonaceae, Rosaceae and in recent years with some members of Asclepiadeceae, and Magnoliaceae. However, no studies have been found on ultrastructural level with the pollen tubes of Scrophulariaceae family members neither *in vivo* nor *in vitro*. This will be the first ultrastructural study on *in vivo* pollen tubes of *Mimulus aurantiacus* and also in Scrophulariaceae family.

## MATERIALS AND METHODS

*Mimulus aurantiacus* (Scrophulariaceae) flowers were collected from the Botanical Garden of the University of California at Berkeley. Gynoecia were removed from flowers and they were fixed in 4% glutaraldehyde in 0.1M cacodylate buffer pH 6.8, for 2 hours. Then, the pollinated pistils were washed several times in buffer, fixed overnight with 2% buffered OsO<sub>4</sub>; dehydration was applied with gradually increasing acetone-propyleneoxide series with staining in 70% acetone containing 1% uranyl nitrate overnight. The material was embedded in Spurr's medium. 0.5-1 m thick semi-thin sections were stained with methylene blue (1%) using 0.02 M NaOH to control pH and observed by light microscopy. Thin sections were cut on a Porter-Blum ultramicrotome with a glass or diamond knife. Sections were stained on grids with lead citrate (Reynolds 1963) for one minute and observed by a Zeiss EM 9A electron microscope.

## RESULTS

In this study, four zones were examined *in vivo* pollen tubes of *Mimulus aurantiacus* as similar in the previous studies.

### APICAL ZONE

The cytoplasm of that zone was rich of organelles. Many electron dense dictyosomes and spherical mitochondria were visible. Small vacuoles, abundant rough endoplasmic reticulum (RER), occasionally some smooth endoplasmic reticulum (SER) and electron dense lipid bodies were also seen (Fig. 1a). A well-developed RER (Fig. 1b) and dictyosomes were observed. Many vesicles budded out from the active dictyosomes were seen, in various sizes around cis and trans regions of dictyosomes, in the apical zone (Fig. 1c). Cell wall and cell membrane were also clearly visible in the electron micrographs of apical zone (Figs. 1a, b).

### SUBAPICAL ZONE

Proplastids containing starch granules, small vacuoles and spherical shaped mitochondria can be seen in this zone like apical zone (Fig. 2a). This zone also contained a few electron dense lipid bodies and abundant RER (Figs. 2a, b). The ribosomes were frequently aggregated

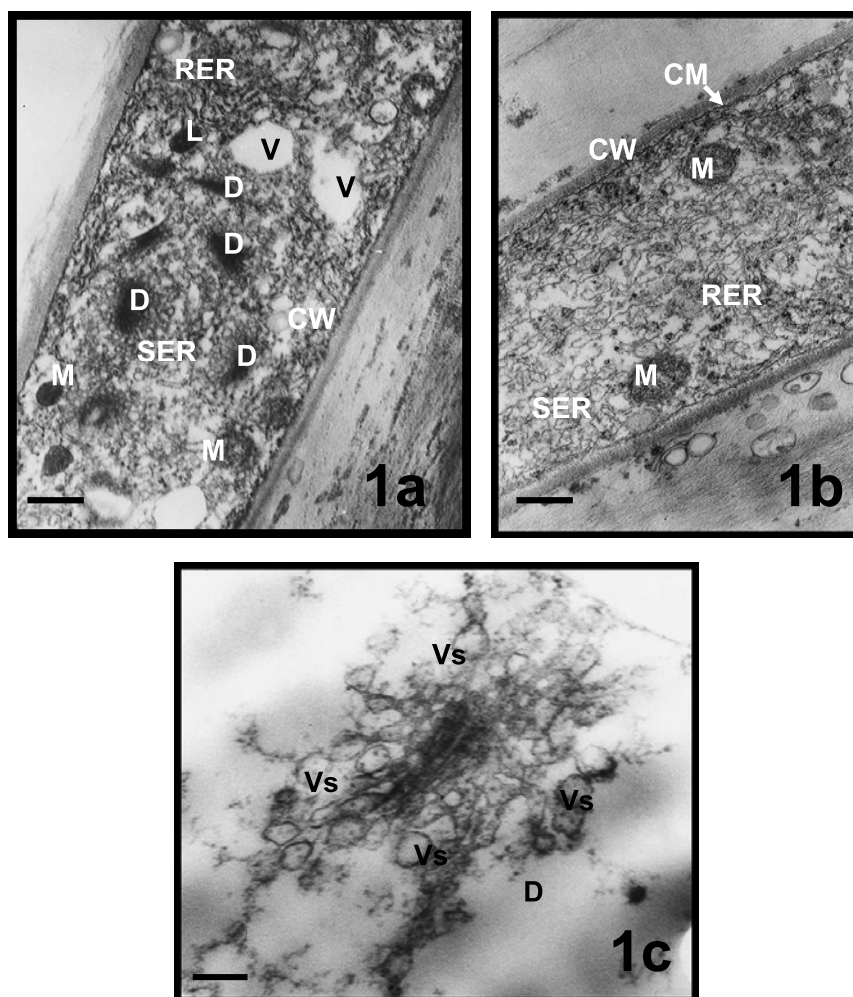


Fig. 1 – Apical zones of pollen tubes in *Mimulus aurantiacus*. a, b, views from apical zone; c, well-developed dictyosome in the apical zone. a, bar = 1  $\mu$ m; b, bar = 0, 5  $\mu$ m; c, bar = 150 nm. (CM, cell membrane; CW, cell wall; D, dictyosome; L, lipid; M, mitochondria; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; Vs, vesicle).

as polysomes. Well-developed active dictyosomes and budding vesicles were also found in the subapical zone (Fig. 2c).

#### NUCLEAR ZONE

Vegetative and generative nuclei were located in this zone (Figs. 3, 4a, b). The vegetative cell nucleus was large and long. It contained one nucleolus and several weakly-stained chromatin aggregations. It was rich of euchromatin material. Vacuoles in different sizes, mitochondria and dictyosomes were observed around vegetative nucleus (Figs. 3a, b). There were 2, 3 amyloplasts

containing starch grains and spherical shaped mitochondria around generative nucleus (Figs. 4a, b). Electron dense lipid bodies were seen near the amyloplast full of starch grains (Fig. 4b).

#### VACUOLATION AND PLUG FORMATION ZONE

Cytoplasm of the tubes was full of large and old vacuoles (Fig. 5a). Mitochondria, few ribosomes, polysomes and dictyosomes were seen along their periphery (Figs. 5a, b). There were also autophagic vacuoles exactly in the same size as others. They contained fibrillar materials (Fig. 5b).

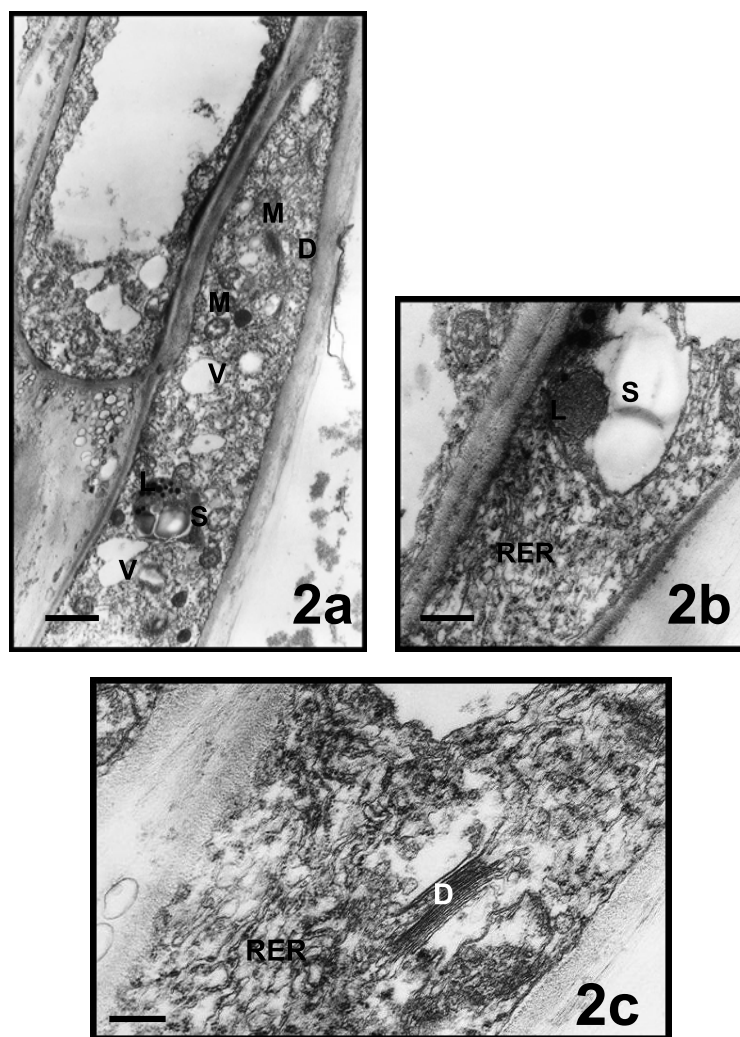


Fig. 2 – Subapical zones of pollen tubes in *Mimulus aurantiacus*. a, bar = 1, 5  $\mu$ m; b, bar = 0, 5  $\mu$ m; c, bar = 0, 2  $\mu$ m; (D, dictyosome; L, lipid; M, mitochondria; RER, rough endoplasmic reticulum; S, starch; V, vacuol).

#### DISCUSSION

The term “zonation” was introduced to describe the functional cytoplasmic distribution in the pollen tubes of *Lycopersicum peruvianum* (Cresti et al. 1977); in which four different areas were identified: (i) the apical zone, rich in SVs (Secretory Vesicles); (ii) the subapical area, containing organelles; (iii) the nuclear zone, comprising the MGU (Male Germ Unite); and (iv) the zone of vacuolization and callose plug formation (Moscatelli and Cresti 2001), (Bhojwani and Soh 2001). The presence of four zones was detected in the *in vivo* growing pollen tubes of *Mimulus aurantiacus* as reported by

Cresti et al. (1977), Ciampolini et al. (1982) in their *in vitro* and by Cresti et al. (1979, 1980), Uwate and Lin's (1980) *in vivo* studies. In general, the constitution of organelles in various zones *in vitro* and *in vivo* are quite similar as also seen in *Lycopersicum* (Cresti et al. 1977), (Cresti et al. 1980), in *Malus* (Speranza et al. 1982), in *Prunus avium* (Cresti et al. 1979), in cherry (Ciampolini et al. 1982), and in *Conospermum* (Stone et al. 2004).

It has been hypothesized that the apical zonation – applicable to most Angiosperm pollen tubes – is maintained by cytoplasmic movements within the growing tube and by the presence of a “capture mechanism”

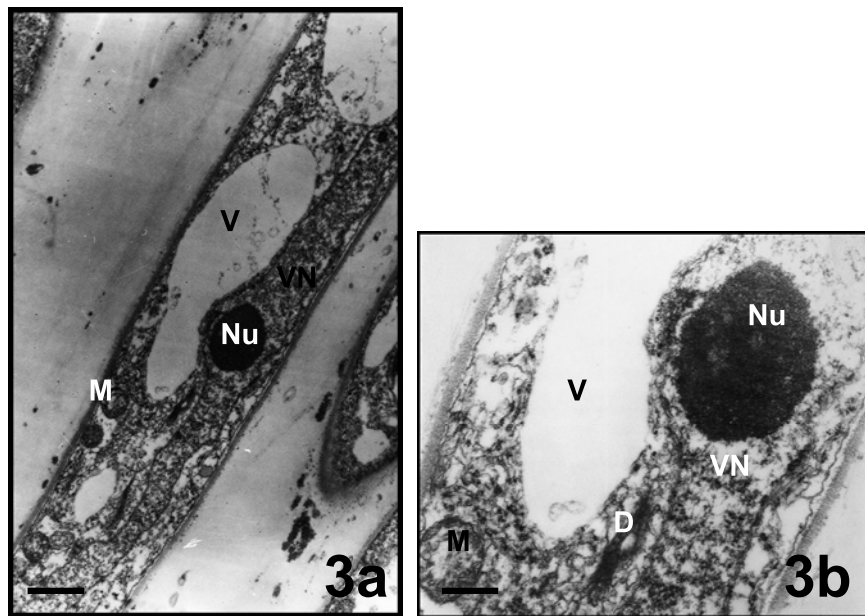


Fig. 3 – Nuclear zones (vegetative) of pollen tubes in *Mimulus aurantiacus*. a, bar = 1  $\mu$ m; b, bar = 100 nm. (D, dictyosome; M, mitochondria; Nu, nucleolus; V, vacuol VN, vegetative nucleus).

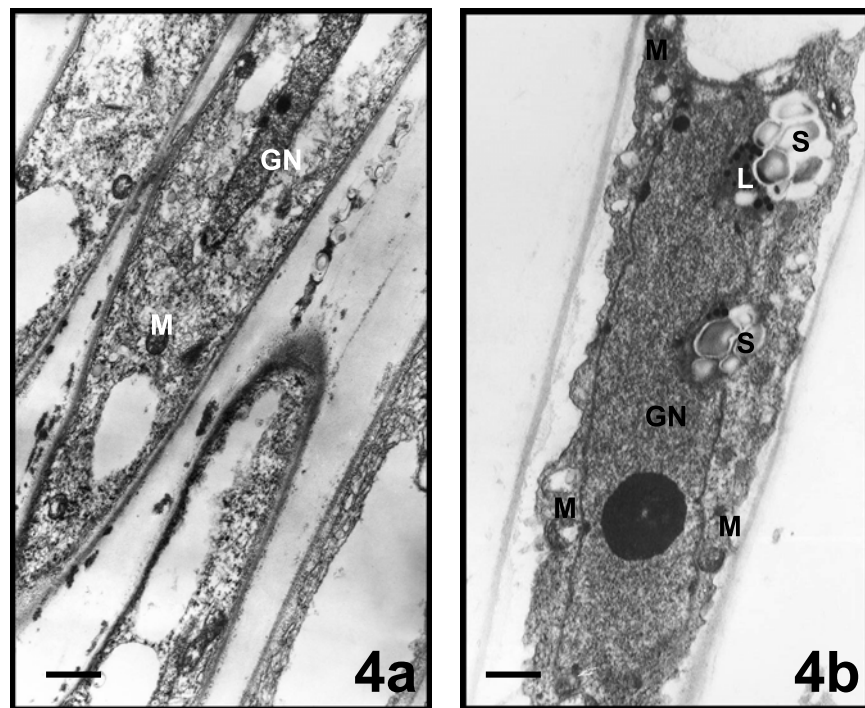


Fig. 4 – Nuclear zones (generative) of pollen tubes in *Mimulus aurantiacus*. a, bar = 1  $\mu$ m; b, bar = 750 nm. (GN, generative nucleus; L, lipid; M, mitochondria; S, starch).

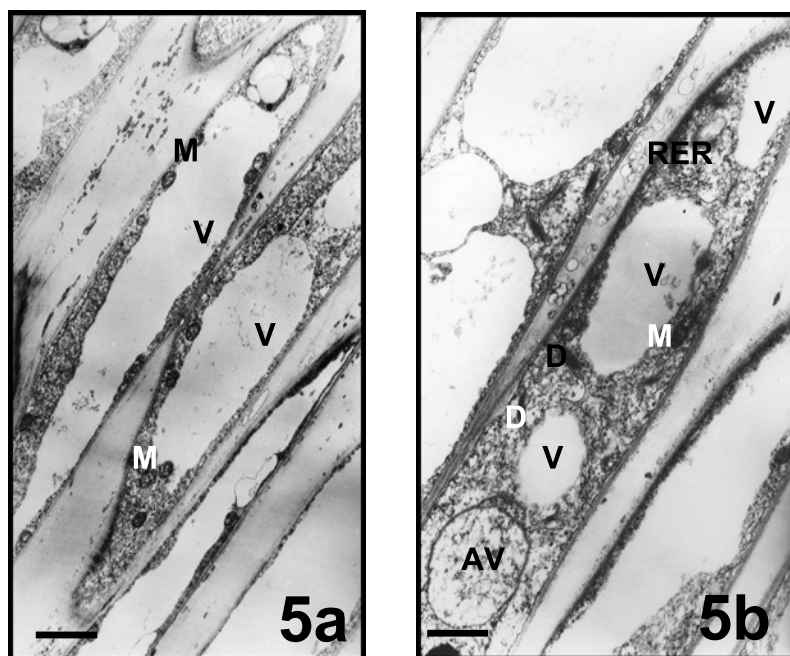


Fig. 5 – Vacuolation and plug formation zones of pollen tubes in *Mimulus aurantiacus*. a,b bar = 1, 5  $\mu$ m; (AV, autophagic vacuole; D, dictyosome; M, mitochondria; RER, rough endoplasmic reticulum; V, vacuol).

which concentrates Golgi-derived SVs at the tip (Heslop-Harrison and Heslop-Harrison 1989) where they fuse with the plasma membrane. This process of exocytosis provides wall polysaccharides and new plasma membrane for tube elongation (Moscatelli and Cresti 2001). Apical and subapical zones are active zones because they are rich of organelles such as: mitochondria, well-developed dictyosomes and lipid bodies. We also observed areas which would be designated as smooth and rough endoplasmic reticulum zones (Uwate and Lin 1980). Our micrographs showed small vesicles arising from the well-developed dictyosome cisternae. Besides playing a major role in the carbohydrate metabolism, dictyosomes have functioned as sorting and dispatching station for the ER products (Dupree and Sherrier 1998). According to Uwate and Lin (1980), these vesicles have been arisen from smooth endoplasmic reticulum cisternae, and rough endoplasmic reticulum circumscription of vacuoles. Lipid bodies were visible in various zones in our study. This was also mentioned by Uwate and Lin (1980).

Since pollen tubes go into the stigma and stylus, they need glycoproteins and glycolipids to build pollen tube

wall. These molecules are synthesized in these well-developed dictyosomes. Amiloplasts detected *in vivo* growing pollen tubes of *Mimulus aurantiacus* are less than *in vitro* ones of *Prunus avium* (Ciampolini et al. 1982). *In vivo* growing pollen tubes can absorb nutrients from the stylar tissue surrounding them. However, this may also occur *in vitro* studies due to high concentration of sucrose used in culture mediums.

Vacuolization zone exhibits autolysis or any other symptoms of degradation in pollen tubes of *M. aurantiacus* *in vivo* because this zone contains a lot of autophagic vacuoles in various sizes. Ciampolini et al. (1982) have specially mentioned the origin, ultrastructure and the role of secretory vesicles. These vesicles are generated by the dictyosomes, and seen in all zones of the pollen tubes. The older and larger vesicles are more visible in the vacuolization zone than in other zones. This is similar to the smaller vesicles in the apical zone due to participation in the pollen wall construction. Our *in vivo* electron micrographs of *Mimulus aurantiacus* pollen tubes also exhibit autophagic vacuoles. They have also been observed in pollen tubes of *Lycopersicum peruvianum* *in vitro* (Cresti et al. 1977).

According to Cresti and Van Went (1976), there are two ways of callose deposition in *Petunia* pollen tubes growing in the style. The first one is callose deposition outside the plasma membrane; the second one is callose deposition within the cytoplasm as distinct callose grains, leading to the formation of callose plugs. Both pollen tube elongation and organelle distribution must be highly coordinated, so that pollen tube growth makes the oldest part of the vegetative cytoplasm move forward, and it becomes isolated by callose plug formation. The mechanism of growth in pollen tubes as well as in other cell types, requires the integrity of the secretory system, namely ER and Golgi apparatus. The secretory system involves the secretion of protein and polysaccharide components. In this study, the elements of RER, SER and Golgi apparatus dispersed through the pollen tube of *Mimulus aurantiacus*.

In conclusion, the ultrastructure of zonation in pollen tubes of *Mimulus aurantiacus in vivo* had similarities with the ultrastructure of *in vivo* pollen tubes in *Prunus avium* (Cresti et al. 1979, Uwate and Lin 1980). In addition, it was seen that callose deposition occurred within the cytoplasm of pollen tubes in *Mimulus aurantiacus*.

#### RESUMO

O objetivo deste estudo é informar sobre a ultraestrutura de tubos de pólen de *Mimulus aurantiacus in vivo* coletados no “Botanical Garden” da Universidade da Califórnia em Berkeley. O material foi preparado de acordo com os métodos de microscopia eletrônica e examinado em microscópio eletrônico Zeiss. Quatro zonas dos tubos de pólen de *Mimulus aurantiacus* foram examinadas. **Zona apical:** foram observados mitocôndrias, retículo endoplasmático liso; retículo endoplasmático rugoso, dictiossomos e vesículas secretoras. **Zona sub-apical:** esta área continha retículo endoplasmático rugoso em abundância e, ocasionalmente, algum retículo endoplasmático liso. Foram detectados polissomos, mitocôndrias, proplastídeos que contêm amido, pequenos vacúolos e alguns corpos lipídicos. **Zona nuclear:** nesta área, existem tanto núcleos de células geradoras como vegetativas. O núcleo de célula vegetativa é grande e longo. Foram observados retículo endoplasmático rugoso, mitocôndria, ribossomos, dictiossomos e amiloplastos ricos em amido. **Zona de vacuolização e de formação de “plug”:** o citoplasma dos tubos estava cheio de grandes vacúolos. Algumas organelas como mitocôndria,

dictiossomo e retículo endoplasmático rugoso foram detectadas em toda a periferia desta área.

**Palavras-chave:** *in vivo*, *Mimulus*, tubo de pólen, ultraestrutura.

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