Ultrastructural Changes in Pollen Exposed to Ozone

B. H. Harrison and W. A. Feder

Assistant Professor of Biology, University of Massachusetts, Boston 02116; and Professor of Plant Pathology, University of Massachusetts, Waltham 02154, respectively.

The authors appreciate the technical assistance of Mrs. C. Downing.

ABSTRACT

Pollen of ozone-sensitive and ozone-tolerant cultivars of petunia exposed to ozone showed lowered germination rates. Pollen of both cultivars were prepared for electron microscopy using three standard methods. Controls had uniformly electron-opaque cell walls and normal organelles. Over 50% of the ozonated pollen of the ozone-sensitive cultivar had a peripheral band of cytoplasm which was free of all organelles except ribosomes. Fewer grains of the ozone-tolerant pollen showed the cytoplasmic change. It is suggested that the organelles migrate away from the plasma membrane in response to ozone and that this, in turn, affects germination and cell wall development of the pollen tube.

Phytopathology 64:257-258

Additional key word: air pollution.

Several air pollutants have been shown to affect plant structure. Ozone reduced both germination rate and tube length in pollen of sensitive cultivars of tobacco and petunia (1, 2). In vitro studies of germinating pollen with time-lapse photography and light microscopy showed that brief exposure to ozone interrupted normal tube elongation; this effect was reversible, although the structure of the tube wall was permanently changed (W. A. Feder, *unpublished*). At the ultrastructural level, Thompson et al. (7) showed damage to the chloroplasts of pinto beans resulting from treatment with peroxyacetyl nitrate.

The effects of ozone on pollen ultrastructure have not been reported, and the work described here was undertaken specifically to study structural changes in ozonated pollen which might relate to reduced germination rate.

MATERIALS AND METHODS.—Petunia cultivars 'Blue Lagoon' and 'White Bountiful' were grown in a greenhouse in charcoal-filtered, ozone-free air. Pollen samples were left as nontreated controls or were ozonated at 0.50 ppm for 3 hr at 25 C, and germination rates were determined for all specimens (1, 2).

Pollen samples for electron microscopy were selected at random from control and ozonated groups of each cultivar immediately after treatment with ozone or air, and were promptly fixed. Three fixation methods were used to detect artifacts due to any one fixation method. These were: (i) 4% buffered glutaraldehyde followed by post-fixation in 1% buffered osmium tetroxide; (ii) glutaraldehyde-acrolein fixative in phosphate buffer, followed by buffer wash and post-fixation in 1% buffered osmium tetroxide, according to the method of Van Der Woude et al. (8) and (iii) 1% osmium tetroxide in Millonig's buffer.

Following fixation, all samples were washed in buffer, suspended in 1% melted agar, and centrifuged at low speed for 1 min in order to pellet the pollen grains. Blocks of the pollen-agar pellets were dehydrated in graded ethanols, transferred to propylene oxide, and embedded in Epon 812. Silver sections were mounted on bare copper grids and stained with uranyl acetate and lead citrate.

RESULTS AND DISCUSSION.—The germination rate was reduced 80% for White Bountiful pollen, an ozone-sensitive cultivar, and 15% for pollen of Blue Lagoon which is ozone-tolerant (2).

The ultrastructure of the nontreated pollen was similar to normal nongerminated and germinated pollen described by others (3, 5, 6). The pollen wall was sculptured, with an electron opaque exine and fibrillar intine. The cytoplasm was packed with mitochondria, rough endoplasmic reticulum, free ribosomes, Golgi apparatus, and leucoplasts; and the vegetative and generative nuclei were frequently seen (Fig. 1-3).

In ozonated pollen also, the exine was electron opaque and the intine fibrillar. Ozonated samples fixed only in 1% osmium tetroxide showed nexine 2 to be more electron opaque than nexine 1; however, this change may be due only to the difference in the basic structure of nexines 1 and 2, which was reported by Heslop-Harrison (3). In any case, in our samples, the change was not consistent enough with all methods of fixation to be considered a certain result of ozonation.

There was one change in pollen ultrastructure which was found only in the ozonated samples and was identical with all three fixation methods. More than 50% of the White Bountiful pollen, and less than 20% of the Blue Lagoon pollen, had a band of cytoplasm next to the plasma membrane which was entirely free of all organelles except free ribosomes (Fig. 2 and 4). This band appeared to be continuous throughout the entire periphery of the cytoplasm inside the plasma membrane. The remainder of the cytoplasm appeared normal and in no instance was there evidence of significant numbers of damaged or disintegrating organelles (Fig. 2). The majority of the Blue Lagoon pollen grains were not affected by the ozone, and had the same appearance as the nontreated controls.

Certain species of plants have ozone-sensitive and ozone-resistant cultivars, indicated by reduced germination rates in the sensitive cultivars, coupled with the failure of pollen tubes to elongate properly (2). Studies of germinating pollen in vitro with time-lapse photography showed that brief exposure to ozone interrupted normal tube elongation and changed the structure of the tube wall, indicating an intimate association between ozonation and certain structural characteristics. In addition, ozone stress has been reported to alter the free amino acid and peptide pools of pollen grains (4).

Our study relates ultrastructural changes to observed reduction of germination and tube growth in ozonated pollen. The difference in the percentage of structurally altered pollen grains, and the percentage reduction of germination is probably related to the nonstatistical nature of electron microscopy. An effort was made to select samples randomly, and to examine sections from several blocks of any one specimen. The apparent absence of disintegrating organelles in ozonated pollen, and the

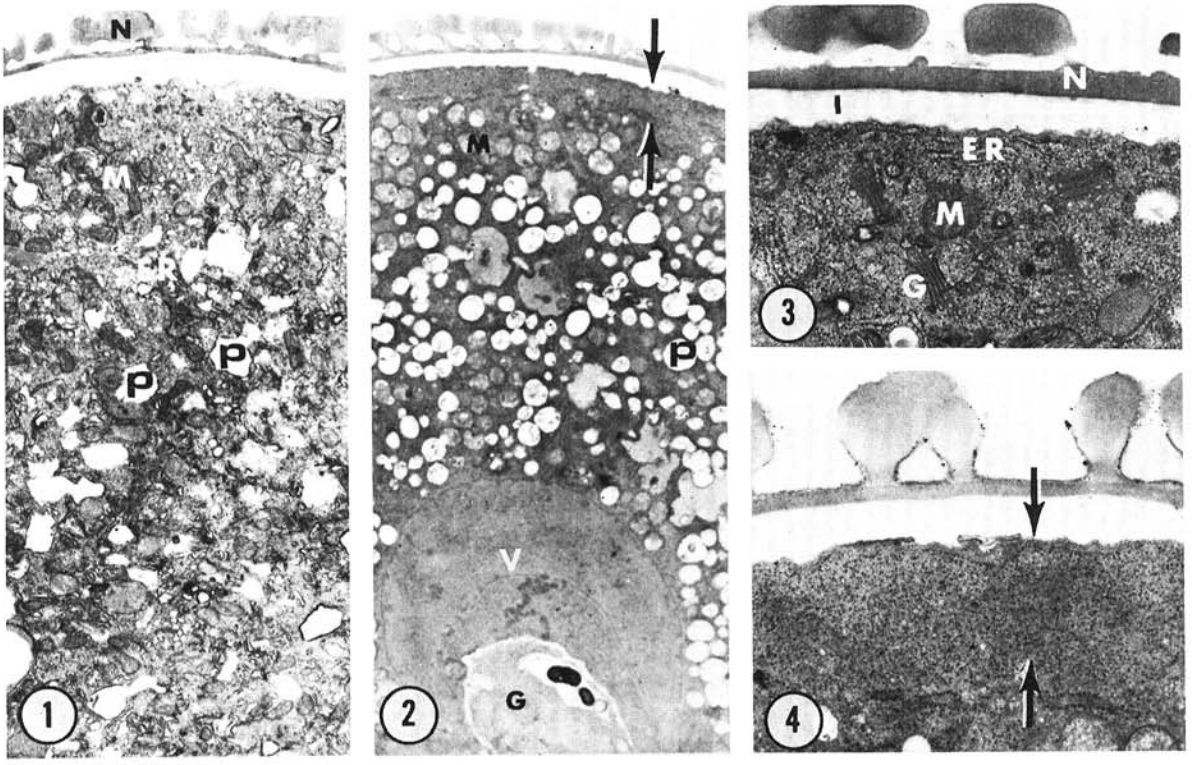

Fig. 1-4. Petunia ('White Bountiful') pollen. **1).** Control; fixed in glutaraldehyde and acrolein followed by osmium tetroxide. The nexine (N) is sculptured and mitochondria (M), rough endoplasmic reticulum (ER), and plastids (P) are present (× 6,150). **2).** Ozonated; fixed as in Fig. 1. Note the band of cytoplasm (between arrows) in which there are only ribosomes. Both vegetative (V) and generative (G) nuclei are visible in this section (× 6,150). **3).** Detail of pollen in Fig. 1. Sculptured nexine (N) and fibrillar intine (I) are visible. Endoplasmic reticulum (ER), mitochondria (M) and Golgi bodies (G) are close to plasma membrane (× 21,600). **4).** Detail of pollen in Fig. 2 showing band of cytoplasm which is free of all organelles except ribosomes (between arrows) (× 21,600).

reversibility of the effect of ozone on growing pollen tubes, suggest that the organelles may migrate away from the plasma membrane in response to ozone. It seems likely that any movement of organelles away from the plasma membrane could affect the synthesis of cell wall materials, and this, in turn, would account for reduced germination and tube growth.

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