Bull. Environm. Contam. Toxicol. 22,379-385 (1979)

Ozone and Nickel Effects on Pea Leaf Cell Ultrastructure

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Increased tissue concentrations of the heavy metals cadmium and zinc have been shown to accentuate ozone-induced visible leaf injury (CZUBA and ORMROD 1974). Nickel additions to the rooting medium also enhance ozone injury at nickel concentrations which do not decrease growth (ORMROD 1977). The role of heavy metals in this response of plant foliage to ozone remains unknown. The objective of this study was to determine the effect of ozone on the cell ultrastructure of pea leaves with elevated nickel content using an ozone-sensitive cultivar 'Dark Skin Perfection' (ORMROD 1976).

Ozone is an air pollutant which can affect the cell ultrastructure of susceptible plants (SWANSON et al. 1973; THOMSON et al. 1966, 1974). General disruption of cellular membranes and organelles, and their subsequent aggregation in the cells is one form of injury. This is seen in petunia pollen where organelles, except ribosomes, tend to be found away from the plasma membrane (HARRISON and FEDER 1974). Injury takes place in two phases in bean palisade parenchyma (THOMSON et al. 1966). First, granulation and increased electron density of the chloroplast occur, with ordered fibrillation and granulation, and second, aggregation of the remains of the chloroplast occurs. THOMSON et al. (1966) suggest that this is a reaction of oxidative agents in general, as results were similar with peroxyacetyl nitrate. In further studies with bean THOMSON et al. (1974) observed that ozone damage was progressive, that membrane degradation was general, and that the first affected and most severely injured parts were the chloroplasts. Crystalloids and fibrils occurred near localized disruptions of the chloroplast envelope. RUFNER et al. (1975) also noted chloroplast disruptions and concluded that ozone affected the chloroplast envelope first, then the tonoplast and plasmalemma.

EXPERIMENTAL

Pea plants, Pisum sativum L., cv 'Dark Skin Perfection', were grown in perlite wetted daily with an excess of complete nutrient solution (HOAGLAND and ARNON 1950), in a controlled

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0007-4861/79/0022-0379 $01.40

environment at 22 ± 2°C, 10 klux of cool white fluorescent light, 16 h photoperiod, and 55 ± 20% relative humidity. Addition of 100 µM $NiSO_4$ to the nutrient solution provided plants with elevated tissue nickel levels. At 22 to 24 days after seeding, plants with 5 to 7 fully developed leaves were transferred to a plexiglass treatment chamber. Under the same environmental conditions, plants were exposed to ozone in the middle of the photoperiod. The ozone was generated by an Elcar Viva ozonator and monitored with a Mast ozone meter. Plants grown with nickel were indistinguishable in appearance from control plants and had tissue nickel concentration of about 40 µg/g dry weight (ORMROD 1977).

Detailed examination was made of young fully expanded leaves and older leaves grown with or without 100 µM $NiSO_4$ and sampled after a 4 h exposure to 50 pphm ozone. Leaf samples were immediately fixed at 0°C in 4% glutaraldehyde in Millonig's phosphate buffer (HAYAT 1972) pH 7.0, for 1 h, rinsed in ice cold buffer and post-fixed in buffered 1% osmium tetraoxide for 1 h. Samples were then rinsed in cold buffer again, dehydrated in a graded acetone series, and embedded in Spurr's low viscosity epoxy resin medium (SPURR 1969). Thin sections prepared on a Reichert OmU2 ultramicrotome were stained on grids with aqueous 2% uranyl acetate for 1.5 h and lead citrate for 7 min (REYNOLDS 1963), then observed in a Phillips EM 300.

RESULTS

Ultrastructure of untreated pea leaf mesophyll cells of cv 'Dark Skin Perfection' showed typical development of organelles (Fig. 1, 2, 3). Chloroplasts were regular in shape and the chloroplast envelope was intact (Fig. 1). The stroma was uniform and contained some ribosomes, while grana and lamellae were well organized (Fig. 3). Mitochondria exhibited round to oval shapes and had intact membranes (Fig. 2).

In leaves of plants grown in nutrient solution with 100 µM $NiSO_4$ the chloroplasts were regular and the envelope intact (Fig. 4). The stroma was homogeneous with ribosomes, and the grana and lamellae exhibited normal membrane structure (Fig. 6). Mitochondria exhibited round to oval shapes, with both the inner and outer membranes intact (Fig. 5).

Young leaf tissue exposed to 50 pphm ozone for 4 h was injured. Some cells were injured while others were unchanged (Fig. 7). Plasmolysis and/or alteration of the endoplasmic reticulum (ER) were apparent before any visible injury to the chloroplast (Fig. 8). The chloroplasts were eventually affected (Fig. 10) and disintegration of the grana occurred near localized areas where the chloroplast envelope had been disrupted. Mitochondria appeared normal but may have been swollen to some degree (Fig. 9).

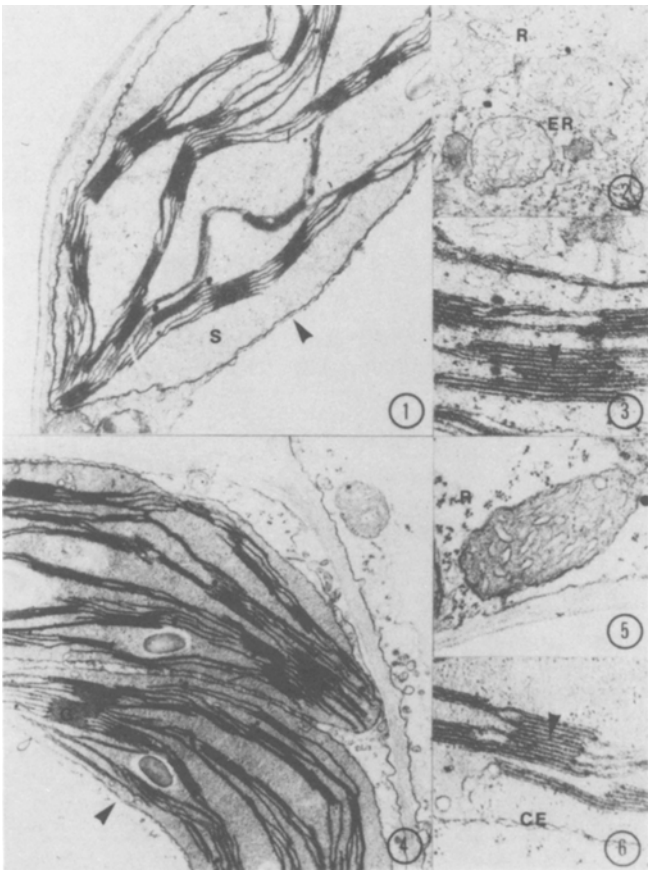
Older leaves of similarly treated plants had all the symptoms of progressive leaf injury seen in young leaf tissue. Severe plasmolysis was apparent in cells containing normal appearing chloroplasts (Fig. 12). In cells containing injured chloroplasts, the chloroplasts were reduced to fibrous masses bounded by electron dense precipitates (Fig. 11, 14). Mitochondria were also changed, although the effect did not appear to be on the membrane integrity. The osmotic balance of the organelle seemed to be changed, causing the cristae to be swollen, and the inner and outer membranes to be separated (Fig. 13).

Young leaf tissue of plants grown in 100 μM $NiSO_4$ and exposed to ozone had membrane alterations similar to those in tissue treated with ozone alone but the injury was more severe and extensive. Cells were slightly plasmolyzed, but the ER was intact and ribosomes were evident (Fig. 15). Chloroplasts exhibited apparent progressive injury, with some displaying disintegration of the grana in areas near localized membrane disruption (Fig. 15), and others with fibrillation and loss of shape (Fig. 16). The mitochondrial membranes remained intact and the shape unaltered (Fig. 17).

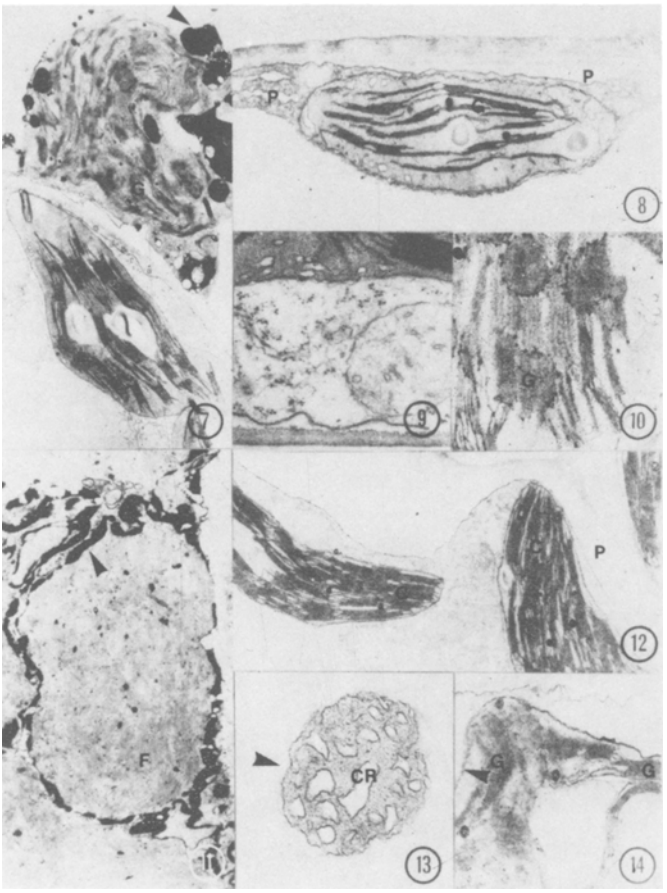
Older leaves treated with both nickel and ozone had the same kind of progressive injury. Plasmolysis and some loss of chloroplast integrity marked more tolerant cells (Fig. 18), while fibrillation and the precipitation of electron dense material in the area of the chloroplast envelope occurred in other cells (Fig. 19). The mitochondria appeared to be unchanged (Fig. 20). In these older leaf cells there was an absence of ER, and the cytoplasm was granular and lacked ribosomes (Fig. 18, 19).

DISCUSSION

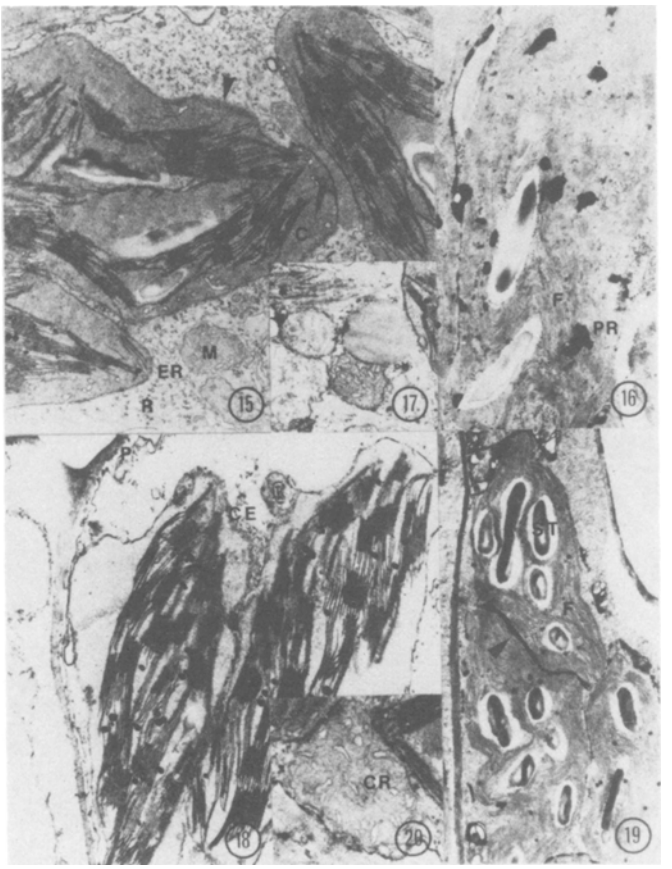
Plants exposed to 50 pphm ozone had apparent progressive cellular injury when sampled 4 h after treatment. Ozone toxicity was manifested particularly in the chloroplasts. The loss of integrity in the grana near areas of localized membrane disruptions apparently continued until the stroma was filled with a fabrillar mass, presumably the remains of the grana and lamellae. This mass was bounded by an accumulation of electron dense precipitates in the area of the chloroplast envelope, perhaps between the inner and outer membranes (THOMSON et al. 1974). Young tissue appeared to be more tolerant than old, but did exhibit the earlier stages of disruption found in older leaf tissue. Plasmolysis in cells of young leaves was not as severe as in cells of older leaves. The mitochondria of young mesophyll cells were apparently less susceptible to osmotic imbalance and subsequent swelling than those of older cells. The disruption of the chloroplast membranes, and the lack of similar mitochondrial disruptions, suggest that the site of ozone action is the chloroplast, and specifically the inner membrane systems,



FIGS. 1-6. Leaf cells from control and Ni-treated 'Dark Skin Perfection' plants. Fig. 1. Control mesophyll cell. There is no plasmolysis, the chloroplast is intact, the envelope is undamaged (➤), the grana are normal, and the stroma uniform. X27,000. Fig. 2. Control plant mitochondria. Membranes are intact and endoplasmic reticulum and ribosomes are present in the cytoplasm. X34,000. Fig. 3. Control plant chloroplast. Grana show the integrity of the membranes making up the organelle (➤). X34,000. Fig. 4. Mesophyll cells of Ni-treated plants showing the integrity of the chloroplast membrane (➤). There is no plasmolysis and the grana are intact. X18,000. Fig. 5. Mitochondrion of Ni-treated plant. The membrane is intact and ribosomes are present in the cytoplasm. X48,000. Fig. 6. Mitochondrion of Ni-treated plant. The envelopes and membranes of the grana stacks (➤) are well defined. X34,000.



FIGS. 7-14. Cells from young and old 'Dark Skin Perfection' leaves exposed to ozone. Fig. 7. Mesophyll tissue of a young leaf showing two adjacent cells. One cell is uninjured and the chloroplast shows well-defined inner membrane systems. The damaged cell is characterized by electron dense precipitates at the periphery (►), and loss of integrity of inner membrane systems. X13,000. Fig. 8. Cell from ozone-treated young leaf showing plasmolysis of the ER and plasmalemma. X19,000. Fig. 9. Mitochondrion in young leaf cell showing membranes intact. X33,000. Fig. 10. Chloroplast showing disintegration of some grana while other grana remain intact. X19,000. Fig. 11. Mesophyll tissue of an old leaf showing the chloroplasts of a severely injured cell. The stroma is fibrillar and there is an accumulation of electron dense precipitate at the envelope (►). X12,000. Fig. 12. Old leaf cell exposed to ozone showing the periphery which is plasmolyzed in contrast to the chloroplasts which are uninjured. X12,000. Fig. 13. A single mitochondrion from old leaf tissue exposed to ozone. Osmotic injury is exhibited characterized by separation of the membrane (►) and swelling of cristae. X25,000. Fig. 14. Portion of a chloroplast showing progressive injury involving loss of integrity in grana near membrane disruptions (►), while grana near intact membranes are sound. X15,000.



FIGS. 15-20. Cells from young and old 'Dark Skin Perfection' leaves on Ni-treated plants exposed to ozone. Fig. 15. Young leaf cell with chloroplasts showing progressive injury with grana losing integrity near localized disruptions of the envelope (►). Some areas are unaffected and membrane structure is intact. X9,000. Fig. 16. Young leaf tissue showing the complete disruption of the chloroplast, fibrillation of the stroma, and accumulation of electron dense precipitate in the vicinity of the chloroplast envelope. X18,000. Fig. 17. Young leaf cell showing partial section of a mitochondrion with cristae and outer membrane intact. X19,000. Fig. 18. Old leaf mesophyll cells with plasmolysis evident and loss of integrity of chloroplast envelope. X15,000. Fig. 19. Old leaf cells with chloroplast disruption characterized by fibrillation and accumulation of electron dense precipitate (►) near the envelope. X8,000. Fig. 20. Old leaf cell showing a mitochondrion with cristae and membranes intact. X23,000.

the grana, which are the sites of photosynthesis. This is consistent with the results of CHANG and HEGGESTAD (1974) who found ozone to impair photosystem II.

The presence of elevated nickel in the tissue did not have any apparent effect on the nature of ozone injury at the ultrastructural level. Cells from plants grown with 100 μM $NiSO_4$ and exposed to ozone had the same pattern of ozone injury as those treated with ozone alone. In samples of leaf mesophyll from both young and old leaves, the chloroplast membranes were progressively injured, as for ozone alone. The mitochondria were unchanged in structure, but showed some osmotic effects. The only observable effect differentiating ozone-treated plants with nickel from those without, was more extensive degradation in nickel-treated tissue. The presence of the heavy metal at concentrations without apparent effect alone, predisposes the tissue to injury by ozone (ORMROD 1977), but this is apparently not due to distinctive electron sensitive changes in ultrastructure.

ACKNOWLEDGEMENT

Support was from the National Research Council of Canada Grant A5951.

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ABBREVIATIONS USED IN FIG. 1-20: C-chloroplast, CE-chloroplast envelope, CR-cristae, ER-endoplasmic reticulum, F-fibrillar stroma, G-grana, M-mitochondrion, P-plasmolysis, PR-electron dense precipitate, R-ribosomes, S-stroma, ST-starch grain.