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Some Effects of Chlorsulfuron on the Ultrastructure of Root and Leaf Cells in Pea Plants

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Abstract. Roots of intact pea plants were treated with 2.8×10^{-4} M Chlorsulfuron (CS). Meristematic root cells and leaf mesophyll cells were studied. Mitochondria, nucleoli, and chloroplasts were the first cell compartments to show ultrastructural disturbances. Mitochondria in treated plants had a visibly translucent matrix. The nucleoli were in the process of segregation of their fibrillar and granular components and reduction of their volume. The structural disturbances of the chloroplasts were similar to those observed during senescence. The results support the hypothesis that CS inhibits cell growth through the accumulation of toxic intermediates.

Key Words. Chlorsulfuron—*Pisum sativum*—Ultrastructure—Mitochondria—Nucleolus—Chloroplast

The mode of action of sulfonylurea herbicides is not fully understood (Cobb 1992). The sulfonylurea herbicide Chlorsulfuron (CS) inhibits plant growth by blocking some processes necessary for cell division. The DNA synthesis (S) and mitosis (M) phases are not affected directly, whereas the transition steps (G_2 to M and G_1 to S) are affected (Rost 1984). It has been shown that CS inhibits RNA synthesis but not protein synthesis in pea (*Pisum sativum*) roots (Rost 1984). The original model for the sulfonylurea herbicides was that the herbicide inhibits the enzyme acetolactate synthase which in turn blocks the biosynthesis of the branched amino acids (BAA) (valine, isoleucine, and leucine) (Ray 1984). Thus depletion of the BAA pool results in a decrease of cell cycle-specific proteins or nucleic acids (Rost 1984, Shaner and Reider 1986). It has also been proposed that accumulation of the intermediate 2-ketobutyrate rather than depletion of BAA inhibits growth events (La Rossa et al. 1987, Rhodes et al. 1987). This toxic substance might inhibit growth by inhibition of acetyl-CoA synthesis (La Rossa et al. 1987) or by a subsequent building up of α-amino-*n*-butyrate, another toxic BAA intermediate (Rhodes et al. 1987). There is evidence that α-amino-*n*-butyrate can inhibit cell division in *Allium* root tips (Langzagorta et al. 1988), acetylate histones in *Physarum* (Loidl et al. 1984), and stop cell division in *Hordeum* roots tips by acidifying the cytoplasm (Reid et al. 1985).

This study was aimed at describing ultrastructural changes in cell compartments after CS treatment of plants rich in amino acids. Ultrastructural changes consistent with the synthesis of toxic BAA intermediates could be observed long before those due to amino acid starvation.

Materials and Methods

Pea seeds (*P. sativum* cv. Ran 1) were surface sterilized in 5% NaOCl, soaked for 24 h in distilled water, and placed on moistened filter paper for 48 h at 24°C. Pea seedlings were grown in hydroponic culture on half-strength aerated Hoagland's solution in a growth chamber at a temperature of 25 ± 1°C, 12-h photoperiod, and relative humidity of about 50%. Daylight fluorescent tubes provided irradiance of about 60 μmol m^{-2} s^{-1} at plant height. Four days later CS (2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]-benzene sulfonamide; Glean, E. I. du Pont de Nemours and Co., Wilmington, DE, USA) at a concentration of 2.8×10^{-4} M was added to the nutrient solution of some plants. The samples were taken 2 mm from the root tip 24 h after herbicide treatment and from the mesophyllous tissue of the third leaf 8 days (192 h) after treatment. The samples were fixed with 3% glutaraldehyde in phosphate buffer (pH 7.2), postfixed in 2% buffered OsO_4 solution, and embedded in Spurr's resin (Spurr 1969). Thin sections were stained with uranyl acetate and lead citrate. Ten

Abbreviations: CS, Chlorsulfuron; S phase, DNA synthesis phase; M phase, mitosis phase; BAA, branched amino acid(s); CoA, coenzyme A.
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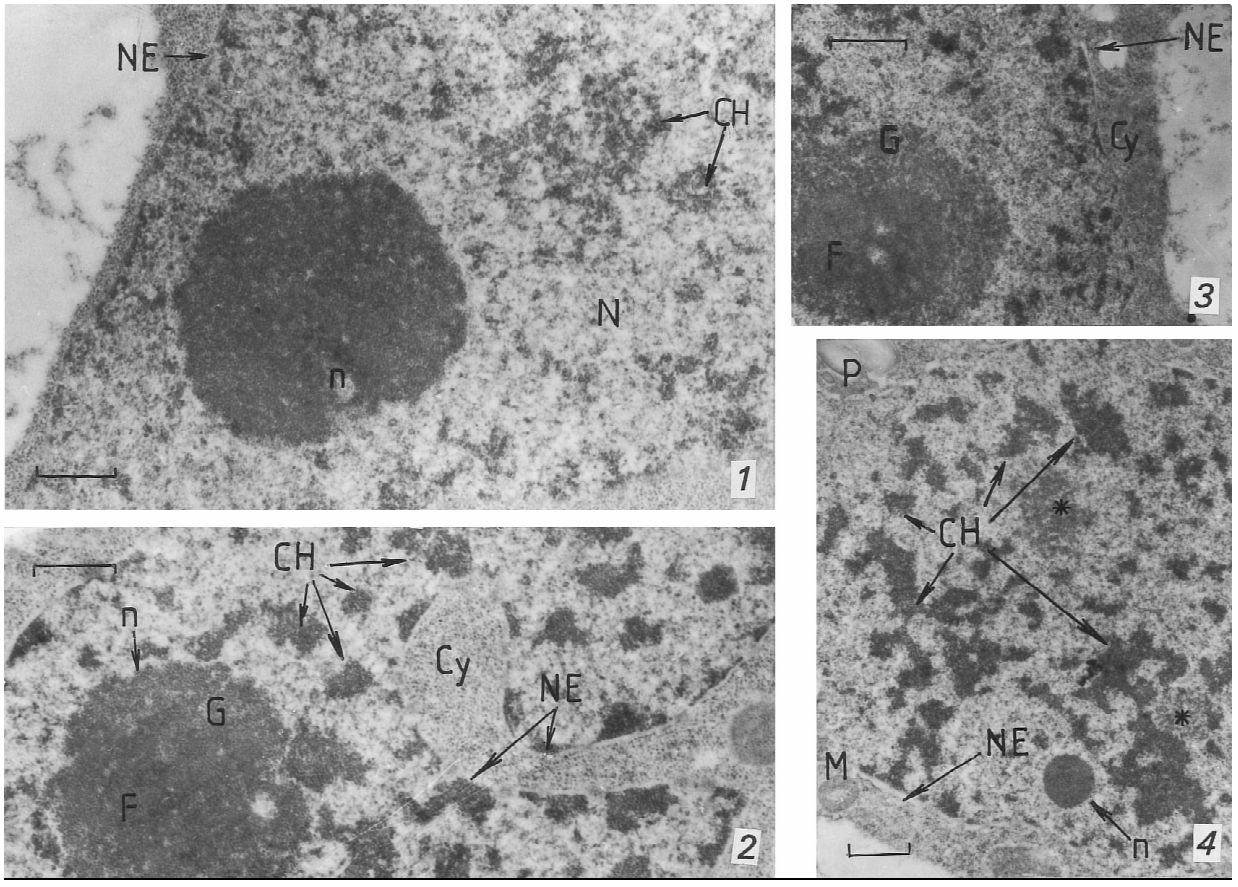


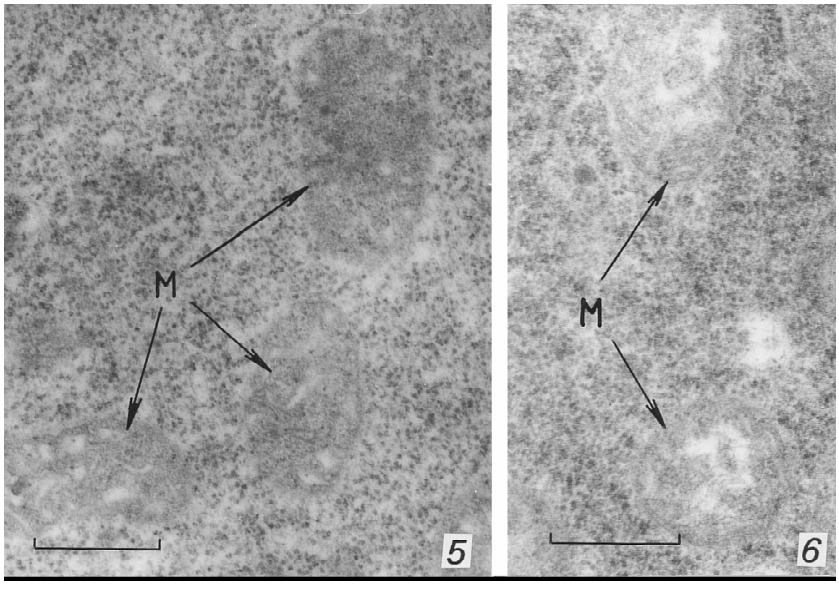
Figs. 1–4. Effects of CS on the ultrastructure of the nucleus in meristematic root cells 24 h after CS treatment. **Fig. 1,** control plant. The nucleolus contains intermingled fibrillar and granular material. *Bar,* 0.1 μm. **Fig. 2,** initial phases of nucleolar segregation. *Bar,* 0.1μm. **Fig. 3,** the segregated nucleolus. *Bar,* 0.1 μm. **Fig. 4,** a small and compact nucleolus. *Bar,* 1 μm. *N,* nucleus; *n,* nucleolus; *F,* fibrillar part; *G,* granular part; *CH,* condensed chromatin; *Cy,* cytoplasm; *M,* mitochondrion; *P,* amiloplast; *asterisk,* chromatin puff.

cells/section, two sections/tissue block, three blocks/variant were examined. To estimate the changes in the organization of the cell organelles and the surface densities and distribution of the ribosome particles we analyzed the electron micrographs obtained by the electron microscope (Zeiss EM-10).

Results and Discussion

The morphology of the root tips of pea plants was visibly unchanged 24 h after the herbicide action, but the structure of the nuclei (especially nucleoli) and the mitochondria in root cells (see Figs. 2–4) differed from those of control plants (Fig. 1). The granular and fibrillar parts of the cell nucleolus of control plants were mixed. The nucleoli of treated plant cells were in the process of segregation into granular and fibrillar zones. At the onset of nucleolus segregation invaginations (cytoplasmatic channels) directed toward the nucleoli (Fig. 2) were observed. Besides the differentiation of the structural components of the nucleolus in the granular (outer) and fibrillar (inner) zones (Fig. 3), a general tendency for compaction and a decrease in the nucleolar volume was established. As a result of this process about 20% of the nucleoli became very small and compact (Fig. 4). In the nuclei with compact nucleoli large clusters of condensed chromatin appeared, and unusual chromatin formations like puffs (shown by the *asterisk* on Fig. 4) were also found. The mitochondrial matrix in CS-treated plants became translucent with a loss of membrane cristae and ribosomes mainly in the center of mitochondria (Figs. 5 and 6). At the same time the ribosome density in the cytoplasm of treated root cells was high enough to allow for an unchanged level of protein synthesis in them (Figs. 5 and 6).

Investigation of the leaf mesophyllous cells 8 days after treatment (Figs. 7–9) showed changes in the ultrastructure of the nuclei and mitochondria similar to those observed in the root cells. The majority of nucleoli were either segregated or small and very compact (Fig. 8). Numerous mitochondria were concentrated close to the

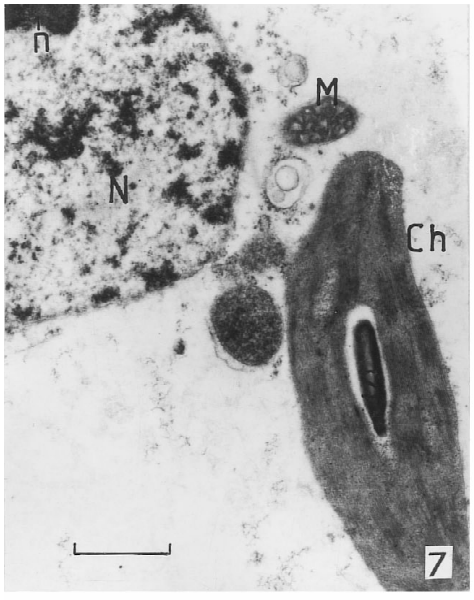


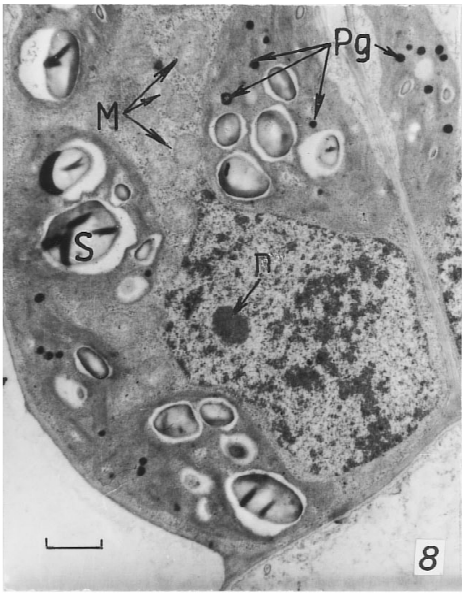
Figs. 5 and 6. Effect of CS on the ultrastructure of mitochondria in meristematic root cells. **Fig. 5,** control plant mitochondria with well developed inner systems of cristae. *Bar,* 0.1 μm. **Fig. 6,** 24 h after CS treatment. Mitochondria with translucent matrix and reduced number of cristae and ribosomes. *Bar,* 0.1 μm.

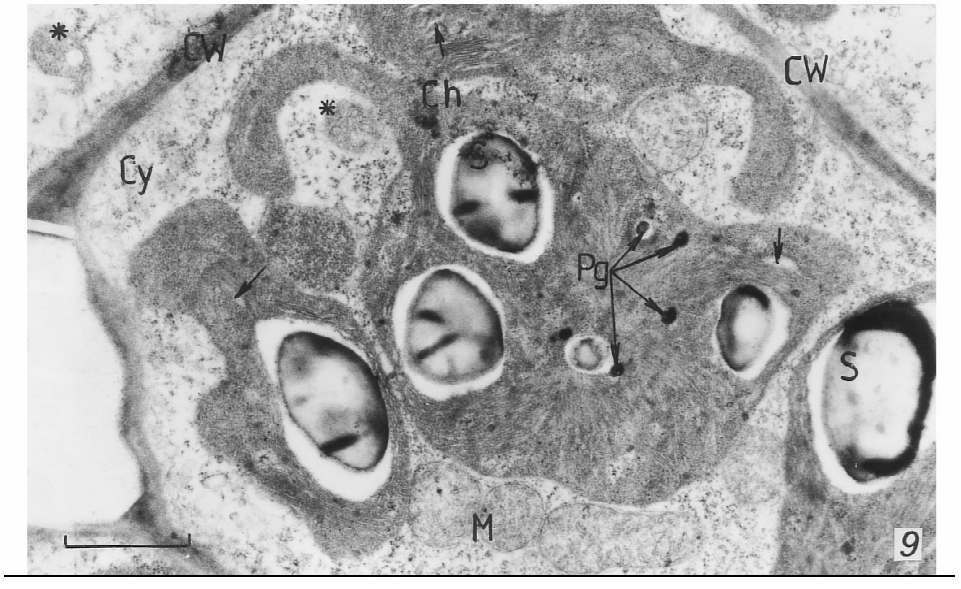
chloroplasts, their matrix being visibly translucent. Some of the mitochondria were in an advanced process of destruction (shown by the *asterisk* in Fig. 8). Structural disturbances in the chloroplasts after CS treatment were similar to those observed during senescence. In addition to the decrease of chloroplast ribosome density, swelling of some thylakoids, an increased number of plastoglobules, loss of the regular spindle shape, large starch grain formation, and a large number of different protrusions (Figs. 8 and 9) were seen. The lighter electron contrast of the chloroplast stroma as well as of the mitochondrial matrix could account for the lower concentration of enzymes and ribosomes in them after treatment. The ultrastructural responses to CS were similar in the spongy and palisade cells but were most pronounced in spongy mesophyllous cells. The abnormal shapes of the chloroplasts and a decreased electron density of their stroma were associated with some yellowing of the leaves. We failed to find a significant difference between the controls and the treated plants with respect to the protein synthesizing apparatus in the cytoplasm of the leaf cells (data not shown). This might be because the ribo- and polysome density/μm^2 of the cytoplasm, even of two adjacent mesophyllous cells, was quite variable.

The seed is a rich source of amino acids for 8-day-old plants, which is why it is unlikely that the disturbances in the root meristematic cells of CS-treated plants were a result of BAA deficit. Furthermore, the reduction of bulk proteins in plants is usually due to an inhibition of protein synthesis rather than to amino acid limitation (Osborne 1962); that is, RNA synthesis plays a key role in the regulation of protein metabolism. The changes in the nucleolar structure confirmed the rapid inhibition of RNA synthesis shown by Rost (1984) after CS treatment. The ultramicrographs (Figs. 2–4) reflect gradual alterations in the nucleus ultrastructural organization caused by CS. As the chromatin is condensed, nucleolar size diminishes, and the granular zone segregates from the fibrillar one and eventually disappears, leaving a body consisting essentially of fibrils. The changes in the nucleolus ultrastructural organization under the effect of CS (reduction of its volume, segregation of its fibrillar and granular components) resembled those caused by actinomycin D (Semeshin 1975). Almost all substances causing nucleolar segregation (Simard 1970) were part of a group of compounds binding directly to the DNA molecules and interfering with its template activity; that is, segregation is a reflection or a response to some specific alteration of DNA rather than the consequence of the inhibition of RNA synthesis (Simard 1970).

The ultrastructure of the nuclei (Gimenez-Martin et al. 1977, Simard 1970), mitochondria, and chloroplasts showed a decrease of transcriptional and translational activity. These ultrastructural changes and the lack of distinct changes in the cytoplasm ribosome density in the present study are in accordance with the data of Spenser (1973), indicating that the reduction in the bulk of cell proteins occurs first in the cell organelles, especially in the chloroplasts, but not in the cytoplasm. The concentration of a great number of mitochondria around the chloroplasts and the formation of perturbances on the body of chloroplasts in the proximity of mitochondria simultaneously with the disturbances in the inner structures of these organelles suggest that the exchange of energy and metabolites between organelles is inhibited. The formation of chloroplast protrusions, swelling of thylakoids, changes in starch grains, plastoglobuls, etc. are nonspecific symptoms of stresses such as drought,







Figs. 7–9. Effect of CS on the ultrastructure of spongy mesophyllous cells. **Fig. 7,** control plant. Mitochondrion with electron-dense matrix and well developed system of cristae. Chloroplast with dense stroma and well developed system of thylakoids. *Bar,* 0.1 μm. **Fig. 8,** 8 days after CS treatment. Numerous mitochondria in close proximity to the chloroplasts with a large number of starch grain and plastoglobules. *Bar,* 1 μm. **Fig. 9,** 8 days after CS treatment. Chloroplasts with irregular shapes and abnormalities in their organization. *Bar,* 1 μm. *Ch,* chloroplast; *S,* starch grain; *Pg,* plastoglobules; *Cw,* cell wall; *arrows,* swelling of thylakoids; *Pr,* protrusion.

mineral deficiencies, acid rains, etc. (Holopainen et al. 1992). These symptoms detected after CS can be regarded as signs of premature senescence.

To summarize generally, the inhibition of the syntheses both of RNA and some proteins after CS treatment of pea plants (Rost 1984, Rost et al. 1990) may be a result of a specific inhibition of transcription or a secondary result of disrupted mitochondrial functions. The rapidly occurring changes in the structural organization of both nuclei and mitochondria in plants rich in amino acids support the hypothesis (La Rossa et al. 1987, Rhodes et al. 1987, Rost et al. 1990) of the effect of these herbicides mediated through the formation of toxic intermediates. Mitochondria and chloroplasts are the first cell compartments with altered structure, suggesting a reduction of protein synthesis.

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