The effect of aldicarb on certain aspects of the ultrastructural organization in leaves and roots of *Nicotiana tabacum*

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Treatment of young tobacco plants with the insecticide aldicarb (2-methyl-2-(methylthio) propionaldehyde 0-(methylcarbomoyl)oxime) resulted in ultrastructural changes usually associated with senescence of plant tissues.

Starch grains and osmiophilic globules accumulated in the chloroplasts and some of the globules were released into the ground plasm and vacuole as the outer membranes of the chloroplasts ruptured. Deformation of mitochondrial cristae of leaf and root cells was accompanied by partial disintegration of the outer mitochondrial membrane, implying a decrease in respiratory efficiency. Disintegration of the plasmalemma, tonoplast and cytoplasm was observed after treatment with aldicarb.

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Behandeling van jong tabakplante met die insekdoder aldikarb (2-metiel-2-(metieltio)propionaldehied 0-(metielkarbamoïel)oksiem) veroorsaak ultrastrukturele veranderinge wat normaalweg met verouderingsverval geassosieer word.

Styselkorrels en osmiofiliese globule het in die chloroplaste geakkumuleer. Sommige van die osmiofiliese globule is in die grondplasma en vakuole vrygestel nadat die chloroplasmembrane op plekke gedisintegreer het. Vervorming van die kristas van mitochondrions van loofblaar- en wortelselle het gepaard gegaan met die gedeeltelike disintegrering van die buitenste mitochondrionmembrane. Hierdie veranderinge dui op 'n vermindering in die respiratoriese effektiwiteit van die selle. Disintegrering van die plasmalemma, tonoplas en sitoplasma is na behandeling met aldikarb waargeneem.

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Introduction

Aldicarb, a broad spectrum systemic pesticide, has been used to control several major insect and mite pests on a wide range of plants. It has, however, become apparent that aldicarb has a direct effect on the development of some of these plants. According to Coosemans & Van Assche (1981) aldicarb had no effect on mungbean and a slightly positive effect on the growth of lettuce. The growth of wheat and maize was, however, inhibited by aldicarb. The purpose of this study was to determine the effect of aldicarb on the fine structure of tobacco leaves and roots.

Materials and Methods

Nicotiana tabacum L.cv.TL 33 plants were grown in steam sterilized soil in 15 cm pots in a temperature controlled growth room. Three-week-old plants were carefully removed from the pots and placed in 1/4 strength Hoagland's solution. After two weeks different plants were placed in containers with 0; 1,0; 3,0; 6,0 and 9,0 mg aldicarb dm^{-3} in 1/4 strength Hoagland solution respectively, for three weeks. Aldicarb was extracted from Temik 15G® (®Registered trademark of Union Carbide Corporation U.S.A. for aldicarb insecticide) and purified by recrystallization from acetone-water solutions (Ashton *et al.* 1977). Plants were kept in a growth room at 25°C ± 2°C with a 12-h photoperiod and an intensity of 2 800 lux at the level of the primary leaves.

Tissue samples taken from the lamina of the oldest leaves and from root tips were fixed in 3 % glutaraldehyde and 1 % osmium tetroxide in cacodylate buffer, pH 7,2, dehydrated in an acetone series and embedded in an epoxy resin (Spurr 1969). Sections were cut with glass knives on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with Philips 301 and Siemens Elmiskop 101 electron microscopes.

Results and Discussion

The mature mesophyll cells of freshly harvested tobacco leaves from untreated plants had large central vacuoles within a peripheral layer of cytoplasm. The cytoplasm was delineated on the inside by the tonoplast and on the outside by the plasmalemma (Figures 1a & 1b). The ribosomes appeared to be grouped together in the cytoplasm (Figure 1b). Chloroplasts were surrounded by double membranes and contained well developed thylakoid systems with prominent grana and few small osmiophilic globules (Figure 1a). The oval, elongated or circular mitochondrial profiles were

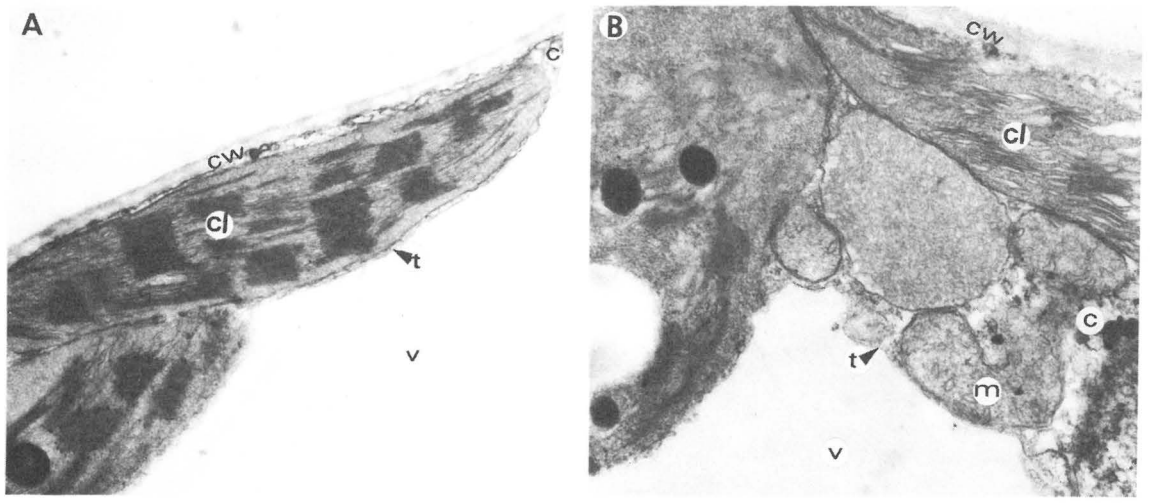

Figure 1 Electron micrograph of mesophyll cells of a control plant. Cytoplasm (c), chloroplasts (cl), cell wall (cw), mitochondria (m), tonoplast (t) and vacuole (v). 1A: × 22100; 1B: × 37400.

surrounded by a double membrane and contained clearly discernible cristae (Figure 1b).

Cells of leaves from plants treated with aldicarb showed marked ultrastructural changes usually associated with senescence of plant tissue (Butler & Simon 1971). Large starch grains and osmiophilic globules accumulated in the chloroplasts (Figures 2a, 2b, 3a, 3b & 5a) and the grana decreased in size and number, while the stroma thylakoids became less clearly discernible. The outer membrane of the chloroplasts ruptured in places and some of the globules were released into the ground plasm and vacuole (Figure 2a arrow).

Harris & Arnott (1973) reported a loss of grana and thylakoids and an increase in the number of osmiophilic globules in chloroplasts of senescing tobacco leaves. Treatment of radish seedlings with the herbicide bentazon resulted in similar increases in the number and size of osmiophilic globules in the chloroplasts, indicating an early chloroplast senescence (Meier & Lichtenthaler 1981).

Figure 3a shows that the formation of osmiophilic globules may be attributed to the breakdown of grana. According to Dodge (1970) the lipid framework of the thylakoid membranes dissolves during leaf senescence and the lipids then pass into the osmiophilic globules. Therefore, it may be assumed that the osmiophilic globules in senescent chloroplasts represent membrane breakdown products (Barton 1966) as well as a general store of insoluble lipid material not necessarily connected with membrane formation or breakdown (Butler 1967).

The two single membrane components of the double membrane surrounding the chloroplasts separated and vesicles appeared between the two layers (Figure 4c). This observation corresponds to that of Shaw & Manocha (1965) in senescing wheat leaves and to the results of Harris & Arnott (1973) for senescing tobacco leaves. The stroma lamellar structure of the chloroplast appeared tubular and vesicles were formed (Figures 4a & 5a). According to Harnischfeger (1973) such changes may be attributed to cellular disorganization which may lead to complete disintegration of the membranes. The remains of partly disintegrated chloroplasts were observed in the vacuole (Figure 3b) while intravacuolar membrane-bound vesicles, which may represent the final phase of chloroplast breakdown, were also observed (Figure 3a).

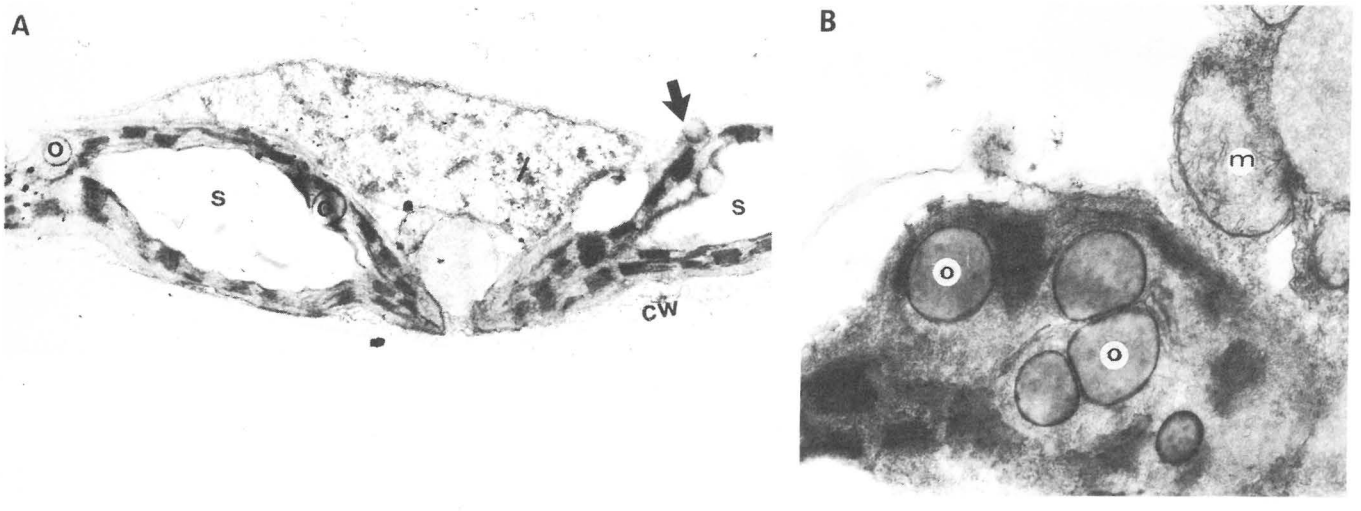

Figure 2 Electron micrograph of mesophyll cells of a plant treated with 1 mg aldicarb dm^{-3} nutrient solution. Ruptured membrane of chloroplasts (arrow), cell wall (cw), mitochondrion (m), osmiophilic globules (o) and starch grains (s) in chloroplasts. 2A: × 14250; 2B: × 30600.

Although the chloroplasts showed marked changes at relatively low concentrations of aldicarb, the mitochondria still appeared intact (Figures 2b & 3a) and it may be presumed that respiration could still occur unimpeded. Harris & Arnott (1973) made similar observations on senescing tobacco leaves. However, at higher concentrations of aldicarb, swelling accompanied by deformation of the cristae was observed (Figures 4b & 5b), and the surrounding outer membranes became ill defined owing to partial disintegration. Decompartmentation of the mito-

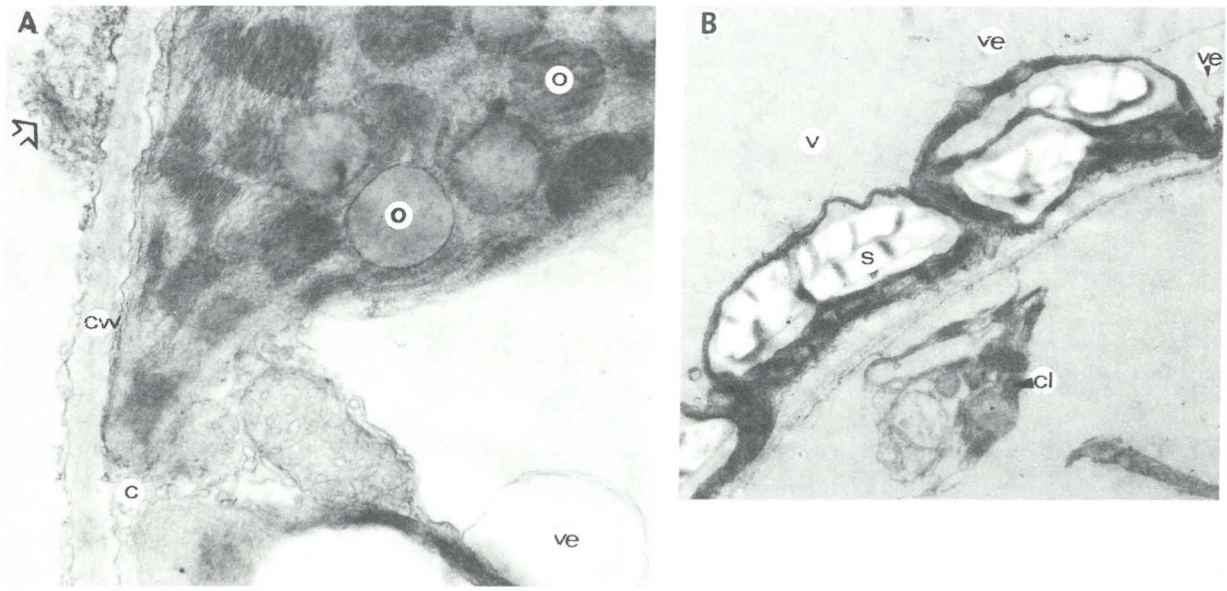

Figure 3 Electron micrograph of mesophyll cells of a plant treated with 3 mg aldicarb dm^{-3} nutrient solution. Remains of cytoplasm (arrow), cytoplasm (c), chloroplast (cl), cell wall (cw), osmiophilic globules (o) and starch grains (s) in chloroplasts, vacuole (v) and vesicles (ve). 3A: × 37400; 3B: × 10320.

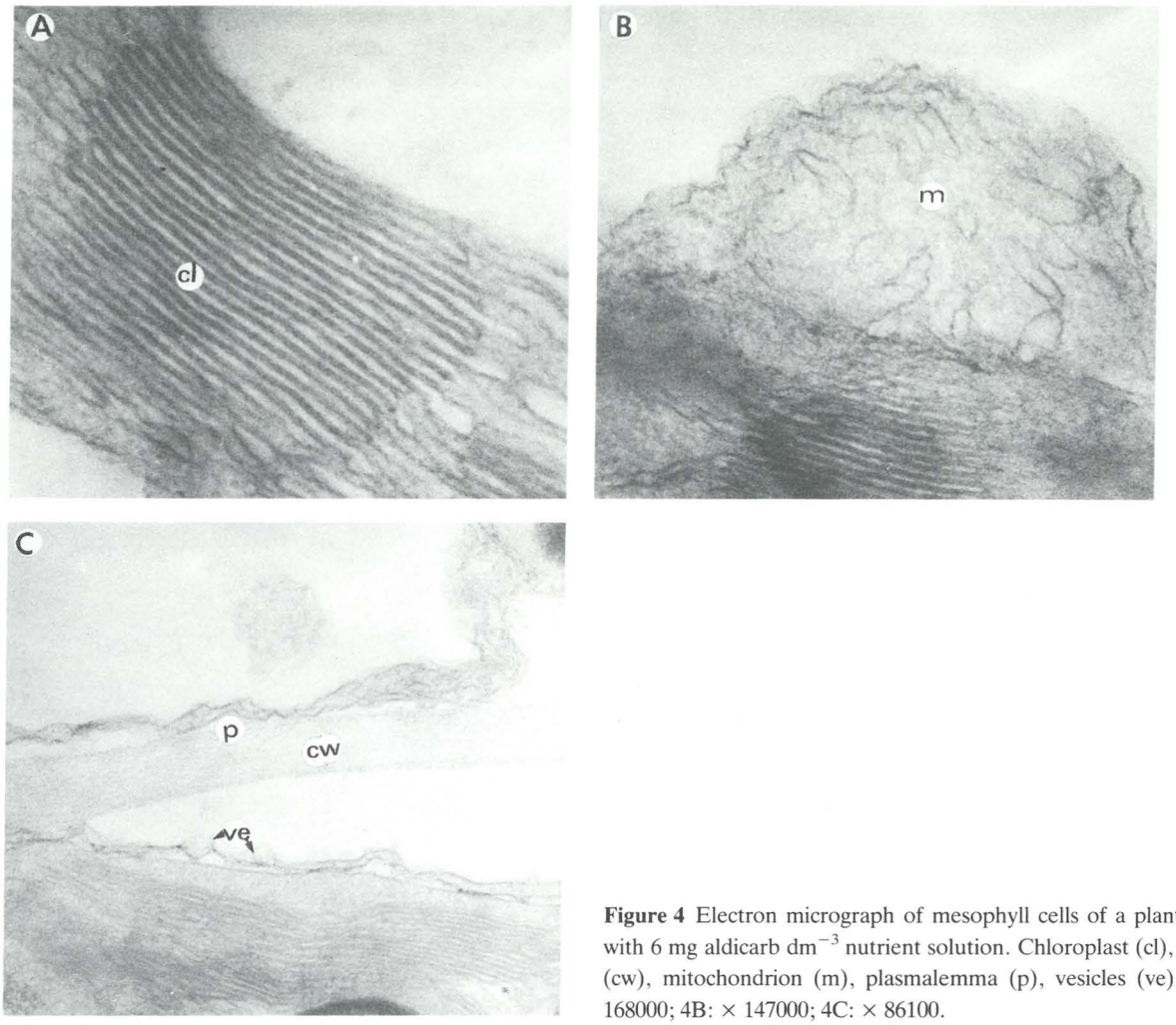

Figure 4 Electron micrograph of mesophyll cells of a plant treated with 6 mg aldicarb dm^{-3} nutrient solution. Chloroplast (cl), cell wall (cw), mitochondrion (m), plasmalemma (p), vesicles (ve). 4A: × 168000; 4B: × 147000; 4C: × 86100.

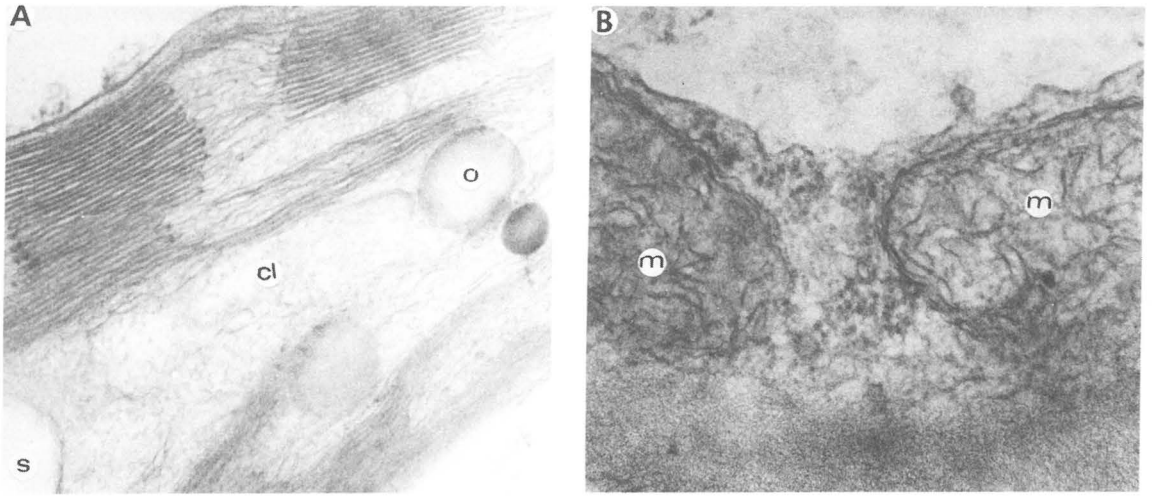

Figure 5 Electron micrograph of mesophyll cells of a plant treated with 9 mg aldicarb dm^{-3} nutrient solution. Chloroplast (cl) with osmiophilic globules (o) and mitochondria (m). 5A: × 81900; 5B: × 105000.

chondria and the loss of cristae imply a decrease in both respiratory rate and the ratio of ATP produced for each oxygen atom utilized (P/O ratio) (Shaw & Manocha 1965). Butler (1967) regards changes in the mitochondrial structure as another consequence of senescence. According to Matile & Winkenbach (1971) disintegration of organelles may be due to the breakdown of the tonoplast. The release of lytic enzymes and other possibly toxic substances on tonoplast dissolution would result in cell death and disintegration.

The tonoplast was clearly visible as a single membrane in mesophyll cells of leaves from untreated plants. In leaf cells from treated plants the tonoplast broke down and the vacuolar content came into contact with the cytoplasm (Figures 2b & 3a). The breakdown of the tonoplast is a characteristic feature of senescence (Butler 1967; Shaw & Monocha 1965; Matile & Wiemken 1976). The vacuolar content contains relatively high concentrations of substances which may exert toxic effects on the cytoplasm and may hasten the degeneration of other cellular organelles. According to Shaw & Manocha (1965), the leakage of vacuolar content through the tonoplast even before the latter disintegrates is an important feature in senescence. A likely cause of tonoplast rupture is the cessation of metabolic activity and thus cessation of the supply of membrane building material or energy necessary for the maintenance of membrane integrity (Matile & Wiemken 1976).

Disintegration of the plasmalemma as well as complete disintegration of the cytoplasm occurred in the leaf cells of aldicarb treated plants (Figure 3a). The plasmalemma became detached from the cell wall and the membrane was vesiculating (Figures 3a, 3b & 4c). Dodge (1970) observed similar separation of the plasmalemma from the cell wall in senescing *Betula* leaves. Splitting of the plasmalemma and the cell wall was accompanied by degeneration of the cytoplasm and a loss of ribosomes in plants treated with aldicarb (Figures 3a & 3b). Hernández-Gil & Schaedle (1973) reported a reduction of the cytoplasm to a thin layer around the periphery of the cell. Similar observations were made on tobacco leaf cells treated with aldicarb (Figure 3b).

Meristem cells of roots from untreated plants contained clearly distinguishable mitochondria and plastids which appeared to be dividing. Few vacuoles were seen in these cells. The mitochondria and nuclei of these cells were surrounded by double membranes, while the cytoplasm and nucleoplasm appeared dense (Figure 6).

The nucleoplasm of cells from roots of plants treated with aldicarb appeared less dense. Vesicles and vacuoles were formed in the cytoplasm (Figures 7, 8 & 10b) and the endoplasmic reticulum became distended (Figures 8, 9, 10a & 10b). A similar formation of vesiculate material was also observed by Dodge (1970) in senescing *Betula* leaves. According to Shaw & Manocha (1965) the degeneration of the endoplasmic reticulum is the first change to occur in senescing wheat leaves. The vesicles were probably derived from the distended endoplasmic reticulum.

The mitochondria in apical meristem cells of treated roots showed rapid degeneration. The cristae became swollen (Figure 9) and the internal membrane structure became disorganized (Figures 9 & 10b). Complete disintegration of the inner and outer membranes could be observed (Figures 9 & 10b). Mitochondrial decompartmentation again implies decrease in respiratory rate. Butler & Simon (1971) regard mitochondrial disorganization as one of the final results of senescence.

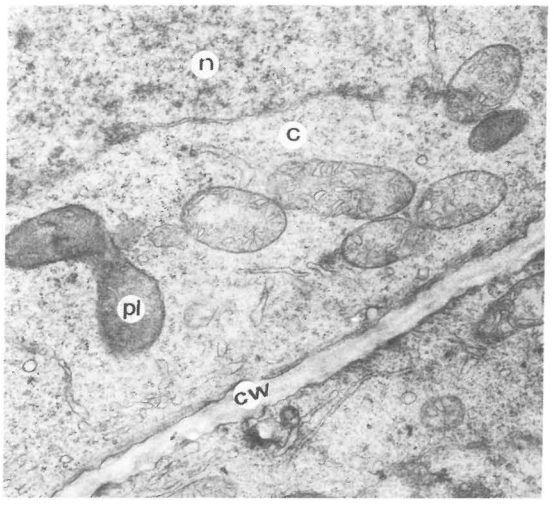

Figure 6 Electron micrograph of root cells of a control plant. Cytoplasm (c), cell wall (cw), nucleus (n) and plastid (pl). × 73100.

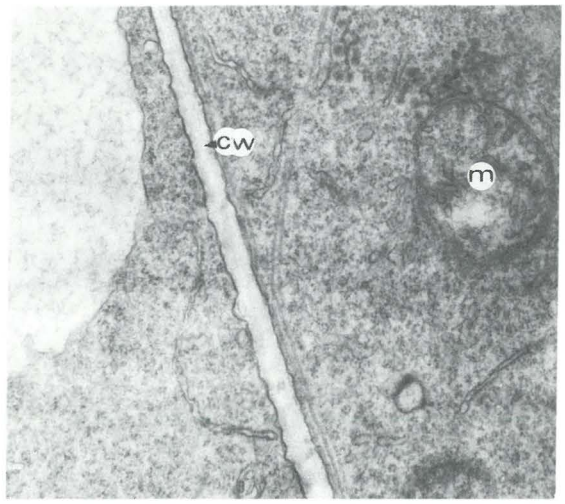

Figure 7 Electron micrograph of root cells of a plant treated with 1 mg aldicarb dm^{-3} nutrient solution. Cell wall (cw) and mitochondrion (m). × 37400.

Conclusion

The ultrastructural observations made on cells treated with aldicarb showed a marked similarity to observations made by Chia *et al.* (1981) and Harris & Dodge (1972) on cells treated with the herbicide paraquat.

At low concentrations of aldicarb the tonoplasts and chloroplasts of treated cells showed signs of deterioration, while the mitochondria still appeared intact. Chloroplasts showed an increase in size and number of osmiophilic globules and eventual disintegration of the grana and chloroplast envelope. Vesiculation of membranes was also a characteristic feature of organelles in treated plants. Higher concentrations of aldicarb caused the swelling of cristae, the formation of myelin-like membrane whorls and the eventual disintegration of the outer membranes of mitochondria.

Harris & Dodge (1972) attributed the swelling and rupture of the cytoplasmic organelles to the disturbance of the osmotic balance of the cytoplasm, resulting from the rupture of the tonoplast. The volume of the contents of the cells

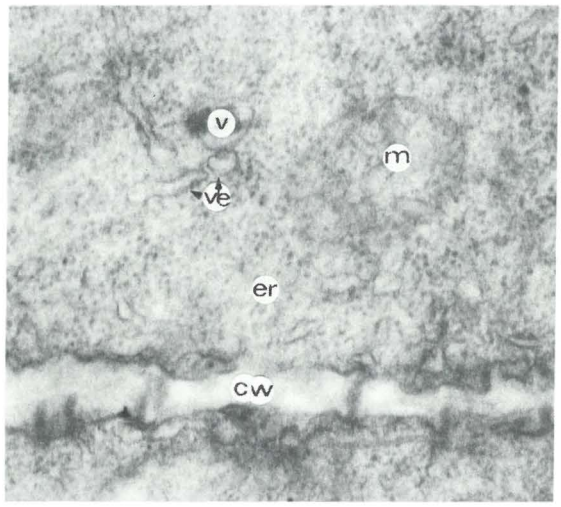

Figure 8 Electron micrograph of root cells of a plant treated with 3 mg aldicarb dm^{-3} nutrient solution. Cell wall (cw), endoplasmic reticulum (er), mitochondrion (m), vacuole (v) and vesicle (ve). × 73000.

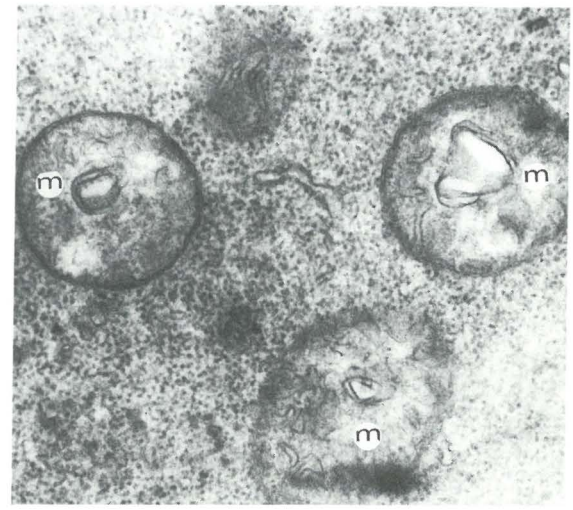

Figure 9 Electron micrograph of mitochondria (m) in root cells of a plant treated with 6 mg aldicarb dm^{-3} nutrient solution. Disorganization of cristae is shown while the outer membranes are still intact. × 61200.

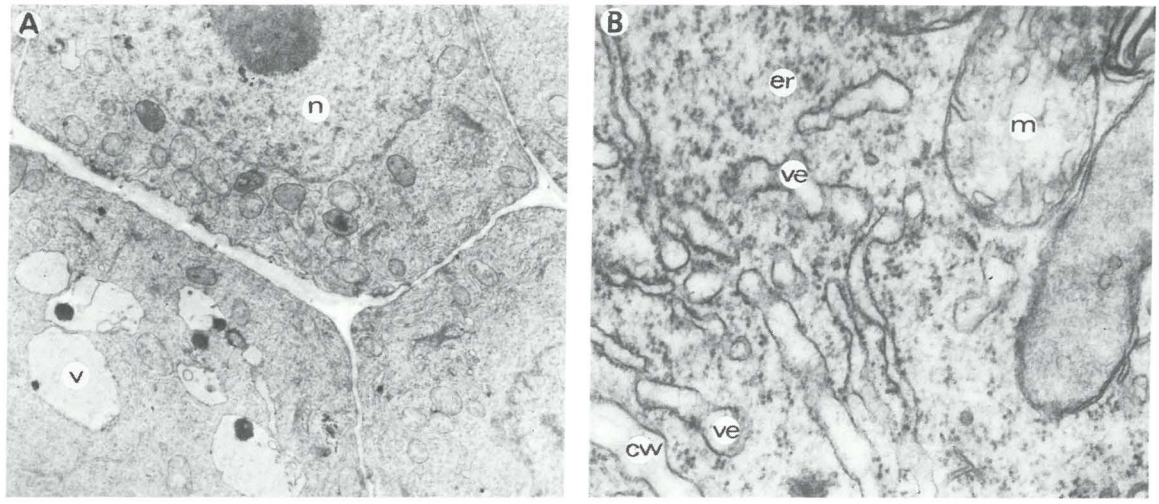

Figure 10 Electron micrographs of root cells of a plant treated with 9 mg aldicarb dm^{-3} nutrient solution. Endoplasmic reticulum (er), mitochondrion (m), nucleus (n), vacuole (v) and vesicle (ve). 10A: × 10030; 10B: × 61200.

treated with aldicarb decreased and the plasmalemma separated from the cell wall.

Similar ultrastructural changes have been widely reported during the course of senescence in plants (Butler & Simon 1971; Balagué *et al.* 1983; D'Agostino *et al.* 1982). It may therefore be concluded that aldicarb accelerates normal senescence in tobacco plants.

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