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Nickel effect on sunflower leaf cell membranes

R. B. SLIVINSKAYA

Komarov Botanical Institute, 197376 Leningrad, U.S.S.R.

SUMMARY

The toxic effect of nickel on lipid content and cell membrane structure in leaves of sunflower seedlings (*Helianthus annuus*) was studied. Addition of 2·5 mg l^{-1} nickel sulphate to the nutrient medium resulted in a decrease in the total lipid and phospholipid contents of the seedlings. The ultrastructural study of mesophyll cells showed nickel to cause destruction to the tonoplast membranes, as well as other changes to the cell ultrastructure. The response of plants to harsh environmental factors is discussed.

Key-words: Helianthus annuus L., leaves, lipids, nickel, ultrastructure.

INTRODUCTION

The global monitoring programme, adopted by the UNO in 1980, characterized nickel as one of the most dangerous environmental pollutants, with severe toxic and carcinogenic potential. In plants nickel accumulates in the roots, as well as in vegetative and reproductive organs (Cataldo *et al.* 1978; Berzina 1980). A more comprehensive account of the effect of nickel on plant growth and metabolism is given in the review by Mishra & Car (1974). The mechanism of its damaging effect on plants has, until recently, remained obscure.

Various harsh environmental factors are known to primarily affect the structure and composition of plant biomembranes. Disturbances in membrane structure result in the alteration of organelle structure, which in turn, interferes with cellular functions and plant metabolism.

For normal functioning of biomembranes the condition of the phospholipid layer is of special significance as it affects membrane permeability, the activity of membrane-binding enzymes, photosynthesis and respiration (Lyons 1973; Dubacq & Tremolières 1983; Gounaris *et al.* 1983).

The present work was undertaken to investigate the effect of toxic nickel concentration on plant-cell lipid content and on cell ultrastructure.

MATERIALS AND METHODS

Helianthus annuus L. plants were cultivated in a greenhouse, on Knop nutrient solution. Experimental plants were transferred to the nutrient medium and supplied with 2·5 mg l^{-1} nickel in the form of nickel sulphate for 2–5 days.

For biochemical and electron microscopy studies, 14–20 day-old seedlings, which by this time had two leaf pairs, were fixed at different stages in the manifestation of nickel toxicity in the leaves. These were as follows: (a) on the second day of exposure to nickel, i.e.

Table 1. Lipid and phospholipid content in leaf homogenates of the sunflower, grown at 2·5 mg l^{-1} nickel. The data represent mean values from three experiments. $P = 0{\cdot}05$

Exposure time	Treatment	Lipid (mg g^{-1} dry weight)	Lipid content as percentage of control	Phospholipid (mg P g^{-1})	Phospholipid content as percentage of control
2 days	Control	110·7 ± 4·6	100·0	1·385 ± 0·08	100·0
	Nickel	80·6 ± 2·6	72·8	0·954 ± 0·01	68·9
3 days	Control	117·5 ± 7·8	100·0	0·938 ± 0·04	100·0
	Nickel	78·2 ± 1·6	66·6	0·568 ± 0·03	60·6
4 days	Control	130·4 ± 9·8	100·0	1·200 ± 0·04	100·0
	Nickel	78·3 ± 0·9	60·0	0·726 ± 0·03	60·5

before the emergence of the visual signs of plant damage, (b) on the third day, i.e. on the appearance of slight chlorosis, and (c) on the fourth and fifth days, when there is formation of localized necroses on the chlorosis spots in the mesophyllic areas between the vascular bundles.

To determine the lipid content the plants were fixed using the Kates & Eberhardt (1957) method; extraction of total lipids was performed using the Folch technique, modified by Bligh & Dayer (1959). Quantitative evaluation of the phospholipid contents was analysed according to a colorimetric ascorbate–molybdate method (Chen *et al.* 1958).

For ultrastructural studies the central part of the second leaf was used. The leaves were fixed in glutaraldehyde and post-fixed with osmium tetroxide according to conventional techniques. Ultrathin sections were cut on a Reichart ultratome, stained with Reynolds stain and examined with a Tesla electron microscope.

Three growth experiments were performed, followed by five biochemical tests. The results were subjected to statistical analysis.

RESULTS

Nickel-induced changes in membrane lipid components

The effect of nickel exposure to total lipid and phospholipid levels is presented in Table 1. While the lipid content in control plants increased with time, exposure to nickel caused it to fall markedly. As early as day 2, before nickel toxicity became visible, total lipid content in the nickel treated plants had decreased by 27%; on day 3 the decrease was by 33%. By day 4, when there was pronounced signs of damage, the total lipid content of the treated plants had decreased by a further 7%. Along with this decrease, there was a steady fall in the phospholipid level. On the second day of nickel exposure, the difference between the control and the nickel treatment already amounted to 31% and reached 39% on day 3 and 4.

Nickel-induced changes in cell ultrastructure

The ultrathin sections revealed particularly dramatic cell changes occurring by the second day of exposure, before the appearance of visual signs of nickel toxicity (Fig. 1a). The cell area of the exposed plants appeared to be significantly enlarged, while its length-width ratio also changed (Fig. 1f and g). The cells became longer and narrower. Flake-like

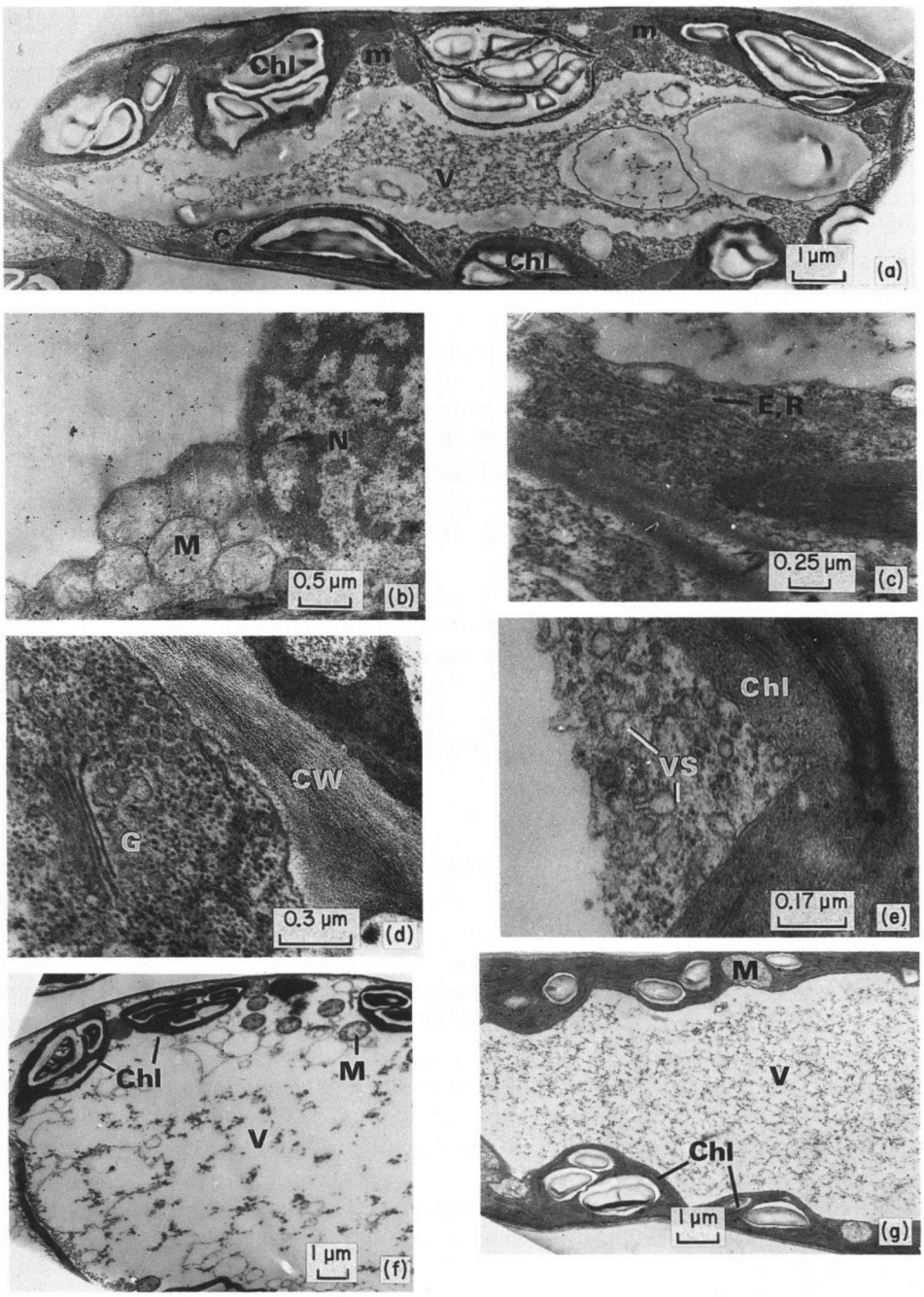

Fig. 1. Nickel-induced changes in the ultrastructure of sunflower mesophyll cells. (a) Accumulation of large amounts of starch in chloroplasts; increase in the chloroplast size and the cytoplasmic volume; (b) arrangement of mitochondria in groups; (c) proliferation of endoplasmic reticulum; (d) activation of Golgi apparatus; (e) accumulation of vesicles in the cytoplasm; (f) destruction of tonoplast membranes; (g) the cell of control plants. Abbreviations: C = cytoplasm, chl = chloroplasts, CW = cell wall, ER = endoplasmic reticulum, G = Golgi body, M = mitochondria, N = nucleus, S = starch grains, V = vacuoles, Vs = vesicles.

osmiophilic material began to disappear from the vacuoles. Tonoplasts appeared to be irregular in outline in the early stages of nickel toxicity.

Mitochondria had lighter matrices (Fig. 1b). Their regular arrangement was disrupted. While in the control plants, mitochondria occurred isolated along the cell periphery, in the nickel-exposed plants they were associated in groups.

A pronounced ultrastructural change, brought about by exposure to nickel, was the increase in the partial volume of the cytoplasm. The cytoplasm was abundant in free, heavily contrasted ribosomes. Endoplasmic reticulum was significantly more abundant in nickel-treated plants where the cisternae were dilated, than in the control plants (Fig. 1c).

The number of dictyosomes rose dramatically; Golgi and other vesicles were observed accumulating in the cytoplasm, particularly near the cell walls (Fig. 1d and e). At this stage of nickel toxicity, many membranes were characterized by reduced contrast, mainly in the chloroplast envelopes and intergranal thylakoids, nuclear membrane and tonoplast. The tonoplast was completely disintegrated upon the appearance of chlorotic spots between the vascular bundles in the leaves (Fig. 1f). The vacuoles became electron transparent. Endoplasmic reticulum and Golgi bodies disappeared. Considerable changes occurred in the cell photosynthetic apparatus. Chloroplasts became greatly enlarged due to the accumulation of large amounts of starch. This is accompanied by a reduction of the thylakoid system (Fig. 1d).

To sum up, nickel excess leads to the following changes in the cell membrane apparatus: in the early stages of exposure—there was an attenuation of the contrasts of the chloroplast envelope, the intergranal thylakoids, and of the nuclear membrane, furthermore a proliferation and expansion of the ER cisternae, and activation of Golgi apparatus. Prolonged nickel exposure results in tonoplast destruction and degradation of the chloroplast membrane system, the Golgi complex and the endoplasmic reticulum.

DISCUSSION

We have established that nickel causes biochemical changes in cell membranes, which is manifested in a decrease in lipid and phospholipid content. These changes correlate with destructive processes in membrane morphology.

Disturbance of lipid metabolism caused by various harsh environmental factors were observed in flax and maize with a boron deficiency (Alexeeva 1971), in potato leaves grown at low temperatures (Rodionov *et al.* 1973) and in sunflower seedlings grown in high saline conditions (Gharsally & Cherif 1984).

Kuiper (1984) reported that the lipid content and composition in higher plants may vary under the influence of environmental conditions. These changes indicate stress-induced processes of biodegradation. In our experiment, lipid degradation was due to nickel-induced stress.

Our data on cell-ultrastructural changes in plants grown in conditions of nickel excess are in agreement with the results by other authors. Subcellular changes on the second day of nickel exposure, i.e. stimulation of Golgi bodies, swelling of ER and the formation of numerous vesicles, have also been reported for the green alga *Cosmarium lundellii* in hypertonic solution (Ghardard 1970), for flax and maize at the occurrence of visible symptoms of boron deficiency (Alexeeva 1971), and also for sunflower and maize under boron and molybdenum excess (Herich *et al.* 1980). The above authors also observed overall reduction of ER membrane system after prolonged application of high molybdenum concentrations.

Our experiments showed considerable nickel-induced changes in the photosynthetic apparatus of mesophyll cells. Excessive amounts of molybdenum and boron also led to an increase in the number of starch grains in the amyloplasts, and the destruction of the chloroplast internal membrane system, simultaneously with the destruction of the chloroplast envelope (Herich *et al.* 1980). Vazquez *et al.* (1987) observed the appearance of amyloplasts with hyperdeveloped starch grains, as well as the destruction of membrane structures in bean leaves under chromium influence. In this case, the presence of chromium inhibited the development of chloroplast lamellae causing indistinct separation of grana and the swelling of thylakoid membranes and their confluence with internal membrane invaginations. Similar manifestations of structural disturbance of chloroplasts were observed by Alexeeva (1971). The disappearance of the plastid envelope, with the internal membrane system (grana and lamellae) remaining intact, was observed at stages of cell degradation in flax at acute boron deficiency.

Herich *et al.* (1980) reported general decrease of membrane contrast at higher molybdenum concentrations. The authors assumed this to be due to membrane changes at the molecular level. We observed the same phenomenon with sunflower plants which had been exposed to a nickel excess and were able to confirm, this assumption by showing that nickel excess, at any rate, affects membrane lipid components.

In summary, the results of our simultaneous biochemical and ultrastructural research support the assumption that exposure to harsh environmental factors causes functional, as well as structural changes in the cell membrane apparatus.

The above material leads us to conclude that the plant's first response to harsh environmental factors is activation of metabolic processes in cells. This is manifested in the activation of Golgi bodies, the proliferation of endoplasmic reticulum and the accumulation of starch in chloroplasts. Prolonged exposure to the stress-causing environmental agent results in the degradation of the cell membrane system, leading to the disturbance of fundamental biochemical and physiological processes in plants.

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