THE EFFECT OF CADMIUM ON IAA AND FC-INDUCED GROWTH AND PROTON SECRETION IN CORN COLEOPTILE SEGMENTS

W. Karcz[1], E. Malkowski[1], R. Kurtyka[1], J.M. Kuperberg[2]

[1]Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia, 28 Jagiellonska St, 40-032 Katowice, Poland
e-mail: karcz@us.edu.pl

[2]Institute for International Cooperative Environmental Research, Florida State University, 226 Morgan Building, 2035 E. Paul Dirac Drive, Tallahassee, Florida, USA

Abstract
Interrelations between growth regulators auxin (IAA) or fusicoccin (FC) and cadmium were investigated. The experiments were conducted with 10-mm long maize coleoptile segments obtained from 96-h old etiolated seedlings. Cadmium (0.1 mM) was added to the incubation medium before, simultaneously or after application of growth regulators. We found that cadmium caused different effects depending on the time of application. Cadmium applied to the incubation medium before addition of growth regulators had an enhanced inhibitory effect on elongation growth of maize coleoptile segments as compared to cadmium applied after addition of IAA or FC. These effects also differed from those when cadmium was applied together with IAA or FC. The addition of cadmium did not significantly change media pH.

Introduction
Cadmium is one of the most toxic metals found in air, water and soil and is not essential for plant growth. Plants can easily absorb cadmium, mainly through the root system. Intensity of the absorption process is proportional to the concentration of this metal in solution or in soil. Cd has adverse effects on plants including growth and development limitations (1). Plant growth and development are tightly regulated by plant growth regulators, among which indole-3-acetic acid (IAA) plays a key role (2). IAA stimulates elongation growth in specific regions of plants, e.g., in parts of dicot hypocotyls of a certain developmental stage, and in parts of grass coleoptiles. Excised coleoptile or hypocotyl segments respond not only to IAA, but also to other growth substances (e.g., fusicoccin - FC), with an enhancement of elongation and acidification of incubation medium. FC, the fungal phytotoxin, can also stimulate growth and acidification (better than IAA), although these two substances have different mechanisms of action (3). It has been proposed that IAA enhances elongation growth by induction of transcription, translation and exocytosis with a lag-phase of 10-20 min, whereas FC stimulates elongation growth by induction of enzymes located in the plasma-membrane without a lag-phase (4).

Little research has been published (5, 6) concerning the mechanism of IAA and FC-induced growth in the presence of heavy metals. The goal of the present study was to investigate interrelations between the action of these growth regulators (IAA or FC) and cadmium on plant growth.

Material and methods

Plant material
Seeds of maize (*Zea mays* L.) cv. Koka were soaked in tap water for 2 h, then placed on wet wood shavings in plastic boxes and placed in an incubator. The experiments were conducted with 10-mm long maize coleoptile segments obtained from 96-h-old etiolated seedlings grown in the dark, at 27±1°C. The coleoptile segments with the first leaves removed were excised 3 mm below the tip and collected in APW incubation medium (1.0 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$).

Chemicals
Indole-3-acetic acid (IAA or auxin) (Merck, Germany) was used as potassium salt (dissolved in water) at the final concentration 10 μM. Fusiccocin (FC) (Sigma, Germany) was dissolved in ethanol and

added to the incubation medium (APW) for a final concentration of 1 μM. Growth regulators (IAA or FC) were introduced into the incubation medium at 120 min of the experiment. $CdCl_2$ (Fluka, Switzerland) dissolved in APW was added to the incubation medium (APW) to get a final concentration 0.1 mM. $CdCl_2$ was introduced into the incubation medium at 0 (start of the experimeny), 60, 120 min (simultaneously with IAA or FC) and 240 min. The initial pH of the all incubation solutions was adjusted to 5.8-6.0 with 0.1 N NaOH or 0.1 N HCl.

Growth measurements

The growth experiments were carried out in two, independent, elongation-measuring systems. In the first, high resolution measurements of growth rate were performed with an angular position transducer (TWK Electronic, Germany) which resulted in a precise record of the growth kinetics (7, 8). In this system six unabraded coleoptile segments were strung on a stainless steel needle and inserted vertically into an intensively aerated APW solution (30 ml).

In the second system an apparatus for simultaneous measurements of maize coleoptile segment growth and incubation medium pH was used (9, 10). An optical system was used for growth measurements (shadow graph method) that recorded the longitudinal extension of a stack of 21 segments. The volume of the incubation medium (solution with the same composition as that used in the first system) in the elongation and pH-measuring apparatus was 6.3 ml (0.3 ml segment^{-1}). In this apparatus the incubation medium also flowed through the lumen of the coleoptile cylinders (10). All manipulations and growth experiments were conducted under dim green light. The temperature of all solutions in the elongation-measuring systems was thermostatically controlled at 25±0.5°C.

Measurements of pH

Incubation medium pH was measured simultaneously with growth using the same tissue sample. Measurements of pH were performed with a pH-meter (type CI-316, Elmetron, Poland) and pH electrode (OSH 10-10, Metron, Poland).

Statistical analysis

Data were analyzed using the computer software Statistica for Windows, version 6.0, StatSoft Inc., Tulsa, Oklahoma, USA (Licence SN: AXXPO116660424 NET11).

Results

Application of IAA at 120 min of experiment rapidly enhanced the growth rate of maize coleoptile segments (Fig. 1a). Addition of Cd at 0 min strongly inhibited growth rate induced by IAA, whereas Cd added at 60 min only partially inhibited the IAA-induced growth response (Fig. 1a). The introduction of Cd simultaneously with IAA had little effect on elongation growth (Fig. 1b). Cadmium added after IAA-induced acceleration of growth (at 240 min) slightly enhanced IAA-induced growth (Fig. 1b).

Addition of FC to the incubation medium stimulated endogenous growth of maize coleoptile sections (Fig. 2) in a similar way as for IAA (data not shown). Cadmium applied at 0 and 60 min reduced not only endogenous growth but also FC-induced growth (Fig. 2). Administration of Cd at 120 min had an intermediate effect on FC-induced elongation growth, whereas Cd added at 240 min did not change elongation growth significantly (Fig. 2).

Acidification of the incubation medium, observed within endogenous growth (APW), of coleoptile sections was enhanced by administration of IAA and FC at 120 min (Fig. 3). “Neutral peak” (highest pH) was observed earlier when Cd was added at 0 and 60 min compared to pH changes within endogenous growth (Fig. 3a). In general, administration of Cd caused higher rate of acidification of the incubation medium of coleoptile sections (Fig. 3). FC introduced into the incubation medium was much more effective in the stimulation of H^+ extrusion than IAA (Fig. 3a and 3b). The initial FC-induced acidification of the incubation medium was much more accelerated in the presence of Cd as compared to the effect of FC (Fig. 3b). Steady state level of pH observed in the presence of Cd did not differ significantly among all treatments.

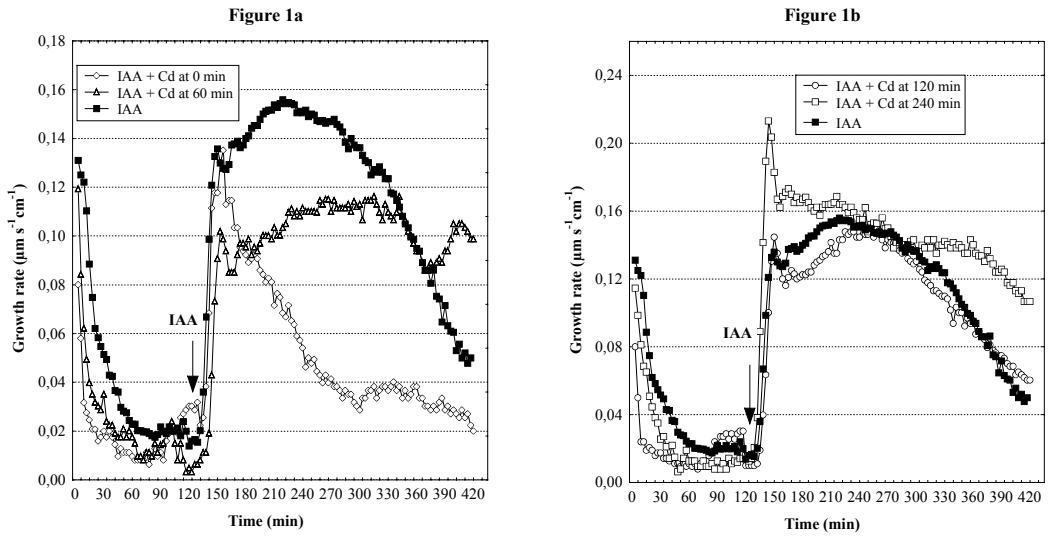

Fig. 1 (a-b). Growth of maize coleoptile segments in the presence of IAA and Cd. IAA was added to the incubation medium at 120 min. Cd was applied at 0 and 60 min (Figure 1a) or at 120 and 240 min (Figure 1b).

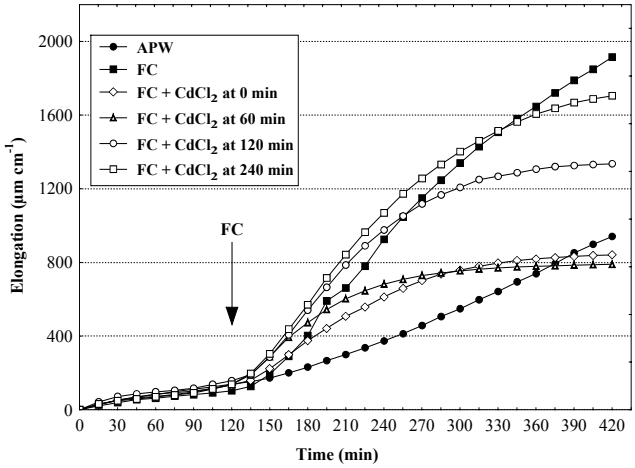

Fig. 2 – Effect of cadmium on FC-induced elongation growth. Cadmium was added to the incubation medium of maize coleoptile segments at 0, 60, 120 and 240 min.

Discussion

The growth experiments performed simultaneously with measurements of media pH showed that IAA and FC cause acceleration of elongation growth of maize coleoptile segments (Fig. 1 and 2). It was shown that FC, in comparison to IAA, was much more effective in stimulation of H^+ extrusion (Fig. 3). These results are in agreement with our previous data (8, 10) and with data obtained earlier by Kutschera and Schopfer (11, 12).

It was found in the present study that cadmium caused different effects on the elongation growth of maize coleoptile segments depending on the time of application. Addition of Cd at 0 min strongly affected growth rate induced by IAA (Fig. 1a). Karcz et al. (13) observed similar effects of Cd on growth rate for another maize cultivar (*Zea mays* cv. K33xF2). Cadmium introduced simultaneously with IAA into the incubation medium had practically no effect on elongation growth of coleoptile segments of maize cv. Koka (Fig. 1b), whereas Kurtyka et al. (5) found significant inhibition of growth of segments of maize cv. K33xF2. FC-induced growth (Fig. 2) was inhibited in a manner similar to that of IAA (Fig. 1a) by Cd applied at 0 and 60 min. In contrast to IAA-induced growth in the presence of Cd (Fig.1b), addition of Cd at 120 min had an inhibitory effect on FC-induced elongation growth (Fig.2).

In the current study pH changes of the incubation medium were measured simultaneously with growth using the same tissue sample. The IAA-induced acidification of the incubation medium of maize coleoptile segments was greater in the presence of Cd as compared to the experiment in which only IAA was present (Fig. 3a).

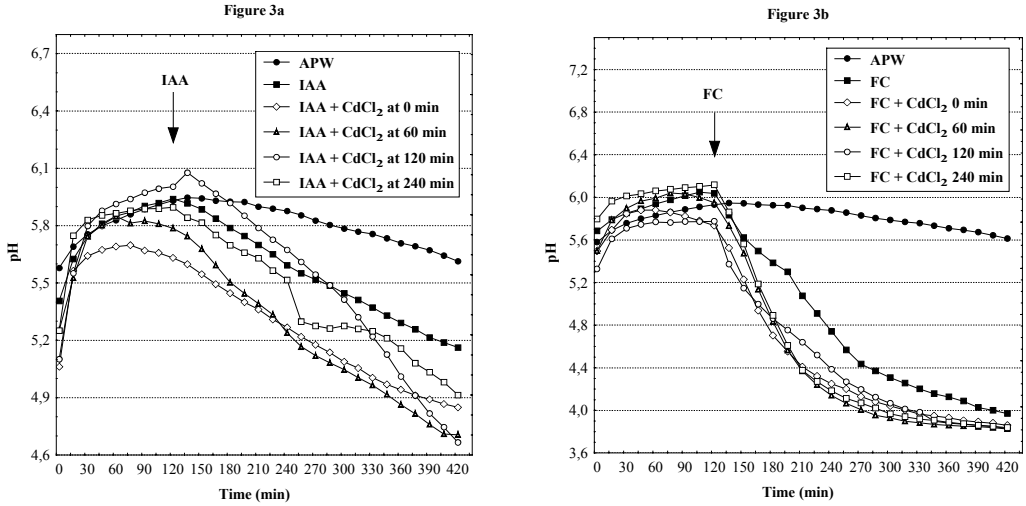

Fig. 3 (a-b). Effect of Cd on IAA-induced (Figure 3a) or FC-induced (Figure 3b) pH changes in incubation medium. Cd was added to the incubation medium of maize coleoptile segments at 0, 60, 120 or 240 min.

The initial phase of FC-induced medium acidification was significantly faster in the presence of Cd, whereas the steady state level of pH observed in the presence of the metal did not differ significantly among all treatments (Fig. 3b). There is a lack of literature on the effect of Cd on IAA- or FC-induced pH changes of the incubation medium of plant tissue. Kennedy and Gonsalves (14) investigated the influence of Cd on H^+ efflux from excised maize roots. They observed alkalinization of culture solution by maize roots after administration of Cd, however plant growth regulators were not investigated in their experiments.

It is suggested that Cd is much more toxic to plant growth if it is applied before the initiation of growth by growth effectors. This observation, in turn, could suggest that retardation of growth by Cd is caused, in part, by its action on mechanisms involved in cell-wall-loosening.

Conclusions

1. Auxin (IAA) and fusicoccin (FC) stimulated growth and acidification of the incubation medium of maize coleoptile sections.
2. Cadmium applied to the incubation medium before addition of growth regulators (IAA, FC) significantly inhibited elongation growth of maize coleoptile segments as compared to cadmium administrated after addition of IAA or FC.
3. IAA or FC-induced acidification of the incubation medium was slightly enhanced in the presence of cadmium.
4. It is suggested that retardation of maize coleoptile segments growth by Cd is caused by its effect on mechanisms involved in cell-wall-loosening.

References

1. A. STROINSKI, Acta Physiol.Plant., **21**, 175-188 (1999).
2. T.S. MOORE, Biochemistry and physiology of plant hormones, 2nd edn. Springer-Verlag Inc., New York 28-85 (1989).
3. U. KUTSCHERA, New Phytol., **126**, 549-569 (1994).
4. A. HAGER, G. DEBUS, H.-G. EDEL, H. STRANSKY, R. SERRANO, Planta, **185**, 527-537 (1991).
5. R. KURTYKA, J. STOLAREK, W. KARCZ, Biol. Plant., **36(suppl.)**, S28 (1994).
6. W. KARCZ, R. KURTYKA, E. MALKOWSKI, 153/P1-71, III Conference On Trace Metals Effects On Organisms And Environment, Sopot, Poland, 6-8 June (2000).
7. W. KARCZ, H.LUTHEN, M. BOTTGER, Acta Physiol. Plant., **21**, 133-139 (1999).

8. W. KARCZ, Z. BURDACH, J. Exp. Bot., **53**, 1089-1098 (2002).
9. W. KARCZ, J. STOLAREK, M. PIETRUSZKA, E. MALKOWSKI, Physiol. Plant., **80**, 257-261 (1990).
10. W. KARCZ, J. STOLAREK, H. LEKACH, R. KURTYKA, Z. BURDACH, Acta Physiol. Plant. **17**, 3-8 (1995).
11. U. KUTSCHERA and P, SCHOPFER, Planta, **163**, 483-493 (1985a).
12. U. KUTSCHERA and P, SCHOPFER, Planta, **163**, 494-499 (1985b).
13. W. KARCZ, R. KURTYKA, E. MALKOWSKI, M. KEDZIORSKA, **488**, Zeszyty Problemowe Postepow Nauk Rolniczych, 679-685. (2002).
14. C.D. KENNEDY and F.A.N. GONSALVES, J. Exp. Bot., **38**, 800-817 (1987).