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Effect of cadmium on pollen germination and tube growth in *Lilium longiflorum* and *Nicotiana tabacum*

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Dedicated to the memory of Dr. Hans-Dieter Reiss, colleague and friend

Summary. Cadmium had a highly toxic effect on pollen germination and tube growth, which were greatly inhibited as metal concentrations increased. Cadmium concentrations up to 10^{-2} M completely stopped pollen germination and pollen showed an increasing tendency to burst within 1 h. At low concentrations, the metal caused a slight stimulation of pollen germination, growth rate and tube elongation at the initial stages of tube development. Comparing the two plants studied, cadmium was more toxic for *Nicotiana tabacum* than for *Lilium longiflorum* pollen. Pollen tubes showed a range of strong morphological abnormalities, characterized by uneven or aberrant growth, including apical branching or swelling at the tip of the pollen tube. Cell wall intrusions at or near the tip were evident on the inner side, whereas a loose network formed from fibrillar material was observed on the outer layers. After prolonged cadmium exposure, round (ball-like) aggregates were embedded in a fine fibrillar network. Increased cadmium concentrations (10^{-3} –10^{-2} M) decreased or completely paralyzed cytoplasmic streaming. No typical cytoplasmic zonation existed, while cell organelles (plastids, lipid droplets) were relocated toward the tip. The vesicular apical zone was drastically reduced, with vesicles dispersed into the subapical region. Mitochondria were distributed throughout the subapical region and among the vesicles of the tube apex. Visible ultrastructural changes in cell organelles were not observed.

Keywords: *Lilium longiflorum*; *Nicotiana tabacum*; Cadmium; Pollen; Germination; Ultrastructure.

Introduction

During the last decades, environmental contamination with heavy metals has increased drastically, causing hazardous effects on human, animal, and plant organisms (Noodelkoska and Doran 2000). Among them, cadmium has attracted the most attention due to its potential toxicity to man and relatively high mobility into the plant system (McLaughlin and Singh 1999). Its widespread distribution and high toxicity make it a potential contaminant and a threat to a wide number of natural environments and biota (Traina 1999). It has been reported that cadmium has the most toxic effect on wheat growth, followed by the metals Cu > Ni > Zn > Pb > Cr (Athar and Masood 2002). Other studies have demonstrated that cadmium is the second most deleterious metal (within a set comprising Hg, Cd, Co, Pb, and Zn) for seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus* (Munzuroglu and Gekil 2002).

Cadmium is generally considered to be readily available to plants from both air and soil sources, mainly in areas with high anthropogenic pressure (Fusconi et al. 2007). Therefore, it is possible that plants could be subjected to the adverse effects of cadmium taken up through both leaves and roots. On the other hand, cadmium has no beneficial effects on plants and minimizing its content in biological systems is desirable. Cadmium neither is an essential microelement for plant growth nor does it participate in the process of cell metabolism, and thus, it is toxic even at very low concentrations. The uptake and subcellular effects of the so-called toxic metals have been investigated in animal and human cell cultures (McKinney 1993).

Among plant cells or vegetative parts of plants, pollen is considered to be extremely sensitive to various pollutants. Pollen germination and growth of the tubes are inhibited in the presence of various heavy metals, especially cadmium. Thus, the in vitro culture of pollen tubes can provide a sensitive standard system with which the biological activity of various toxic metals at the cellular level can be investigated (Kristen et al. 1993, Gur and Topdemir 2005). This

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is facilitated by simple and inexpensive cultivation which does not need sterile conditions, providing fast germination and growth of the tube. In addition, the absence of chloroplasts allows the pollen tubes to be used as a suitable model for the toxicological assessment of compounds harmful to animals and humans (Heslop-Harrison 1987, Steer and Steer 1989, Kristen and Kappler 1995).

During the past two decades, pollen tubes of various plant species have been used to determine the cytotoxic effects of environmental pollutants (Gentile et al. 1973, Masaru et al. 1980, Pfahler 1981, Cox 1988, Röderer and Reiss 1988, Xiong and Peng 2001, Gur and Topdemir 2005). The idea that in vitro culture of pollen tubes can serve as a sensitive system for the evaluation of the toxicity of various compounds in plants is very convincing and worth testing. In previous studies (Sawidis and Reiss 1995, Sawidis 1997), the influence of various heavy metals on the pollen germination and tube growth of *Lilium longiflorum* was investigated and the general mechanism of heavy-metal action on pollen tube growth was discussed. Among them, cadmium showed the strongest effects and this was the reason for a more detailed study extending the metal concentration range, the pollen source, and the method of treatment.

Material and methods

Flower bud treatment

A series of standard solutions of $CdCl_2$ ($10^{-1\,2}$–10^{-2} M) was prepared in distilled water. Flower buds of *Lilium longiflorum* Thunb. and *Nicotiana tabacum* Speg. and Comes were arranged with their pedicel in the above solutions for 24, 36, and 48 h before anthesis. Cadmium solutions were fed through the pedicel to represent the natural uptake of toxic substances. Controls were carried out by putting flower buds of the same age in distilled water for the same time. After the above treatment, pollen grains were sown in a normal aqueous culture medium containing 10% (w/v) sucrose and 1.6×10^{-4} M (10 ppm) boric acid and were allowed to germinate at room temperature. The pollen grains from control and treated flowers were separated from the dry anthers by shaking and with a small brush and were tested for germination rate. The experiment was repeated 3 times and about 100 pollen grains per culture were evaluated.

Pollen germination

Pollen grains of *L. longiflorum* and *N. tabacum* were sown in an aqueous culture medium containing 10% (w/v) sucrose and 1.6×10^{-4} M (10 ppm) boric acid. Boron is believed to promote pollen germination by affecting H^+-ATPase activity, which initiates pollen germination and tube growth (Obermeyer and Blatt 1995). To investigate the metal influence on germination rate, the medium also contained 0 (control) or 10^{-12}–10^{-2} M cadmium, applied as chloride salt ($CdCl_2$). In a series of pre-experiments, the effect of adding nitrate and/or carbonate salts was also investigated. Since no differences could be observed, the chloride salt was used for further studies. Germination rate and pollen tube length were determined under a dissecting microscope at 1 h intervals starting 1 h and ending 4 h after the start of cultivation.

Pollen tube growth pattern and ultrastructure

In another experiment, pollen tubes were first grown in a normal aqueous medium containing 10% sucrose and 1.6×10^{-4} M (10 ppm) boric acid. After the onset of tube emergence, the pollen were incubated for 1–4 h at room temperature in different concentrations of cadmium (10^{-5}–10^{-4} M $CdCl_2$). Effects on growth pattern, tip organization, and organelle movement were observed with a Zeiss Axioplan or an inverted light microscope (ICM 405 Zeiss) using differential interference contrast Nomarski optics. Staining with 1% pianese mixture and acetic carmine dye was used for preliminary light microscopy observations. For ultrastructural study, control and cadmium-treated (10^{-4} M $CdCl_2$) tubes were fixed with 2% glutaraldehyde buffered in 0.1 M cacodylate (pH 7.2) and postfixed with 2% OsO_4. After dehydration through an acetone series, the specimens were embedded in Spurr resin. Ultrathin sections were investigated with a Philips EM-400 or a Zeiss 9 S-2 electron microscope.

Results

Flower bud treatment

The pollen germination rates of *L. longiflorum* and *N. tabacum* flower buds after treatment with cadmium ions before anthesis are shown in Figs. 1 and 2, respectively. The first impressive observation was that the anthers of the treated flower buds became shorter than those in the

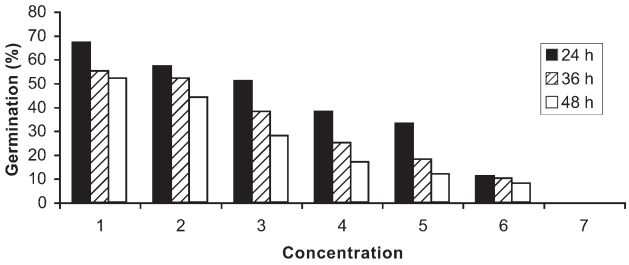


Fig. 1. Germination rate (%) of *L. longiflorum* pollen grains after flower bud treatment with cadmium for 24, 36, and 48 h. *1* 0 (control), *2* 10^{-12}, *3* 10^{-10}, *4* 10^{-8}, *5* 10^{-6}, *6* 10^{-4}, *7* 10^{-2} M $CdCl_2$. Absence of columns indicates that pollen germination was totally inhibited at that cadmium concentration

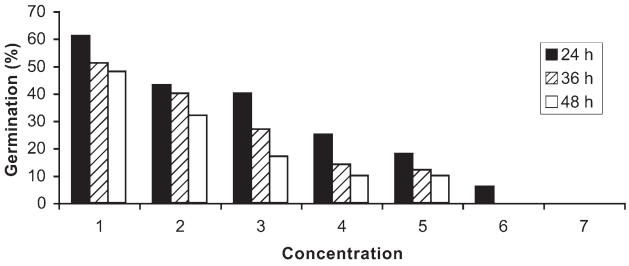


Fig. 2. Germination rate (%) of *N. tabacum* pollen grains after flower bud treatment with cadmium for 24, 36, and 48 h. *1* 0 (control), *2* 10^{-12}, *3* 10^{-10}, *4* 10^{-8}, *5* 10^{-6}, *6* 10^{-4}, *7* 10^{-2} M $CdCl_2$. Absence of columns indicates that pollen germination was totally inhibited at that cadmium concentration

control and did not fully open. This was most obvious at high metal concentrations (10^{-6}, 10^{-4} M). In 10^{-2} M, anthers did not open at all. Increasing the time of cadmium treatment to 32 or 48 h strongly inhibited anther growth and lengthening and, therefore, the pollen germination rate was decreased. Consequently, very few pollen grains were available in each case. The germination percentage also decreased significantly in the control as the time of pedicel immersion in pure water increased. Comparing the two plants, the pollen of *N. tabacum* appeared to be more affected after the above treatment than that of *L. longiflorum.*

Pollen germination in medium containing cadmium

The influence of cadmium ions on pollen germination and tube growth of *N. tabacum* and *L. longiflorum* was investigated by light microscopy. In this study, pollen grains were left to germinate in culture medium containing cadmium. Pollen germination and tube growth rate depended strongly on pollen quality. Pollen from flowers which opened on the day of the experiment showed the highest rate. No differences could be observed between pollen from the same flower. Germination rates of fresh untreated pollen (control) could differ by 30–60%. Therefore, for each experiment into drug effects, a control culture with the same material was analyzed too. Generally, there was a reduction in pollen germination as metal concentrations increased (Fig. 3). For both plants, pollen germination was stimulated by the presence of cadmium at low concentrations (10^{-12} M). The effects of this metal on pollen germination demonstrated that cadmium is more toxic for *N. tabacum* than for *L. longiflorum* pollen. To calculate germination frequency, about 200 pollen grains per culture were evaluated.

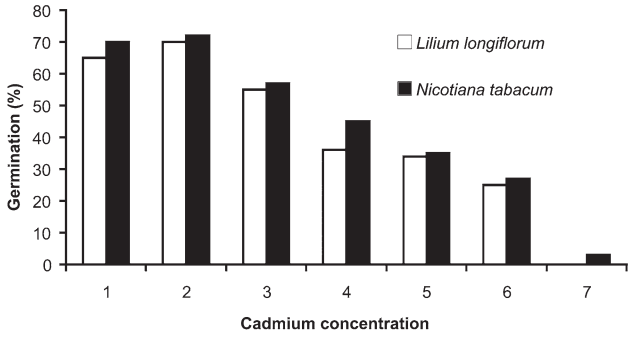


Fig. 3. Germination rate (%) of *L. longiflorum* and *N. tabacum* pollen grains in cadmium. *1* 0 (control), *2* 10^{-12}, *3* 10^{-10}, *4* 10^{-8}, *5* 10^{-6}, *6* 10^{-4}, *7* 10^{-2} M $CdCl_2$. Absence of columns indicates that pollen germination was totally inhibited at that cadmium concentration

Figures 4 and 5 show that the tube length of *L. longiflorum* and *N. tabacum* pollen depended on cadmium concentration and time of application. Pollen tube elongation was greatly inhibited by high concentrations. Cadmium reduced the average tube length drastically in both plants. At low concentrations (10^{-12}–10^{-10} M), cadmium caused a slight stimulation in growth rate and tube elongation (compared with the control tubes) at the initial stages of tube development. About 100 tubes were used for each calculation. Due to the fact that the tubes showed an increasing tendency to burst after about 4 h of cultivation in the presence of the metal, measurements were not extended beyond that time. After about 10 h, tubes were completely destroyed and no further germination could be observed.

At light microscopy level, tobacco pollen tubes cultured in standard medium were generally straight, uniformly cylindrical, about 10 μm in diameter (Figs. 6 and 7), and differed greatly from the pollen tubes cultured in medium containing cadmium. Control tubes exhibited the typical

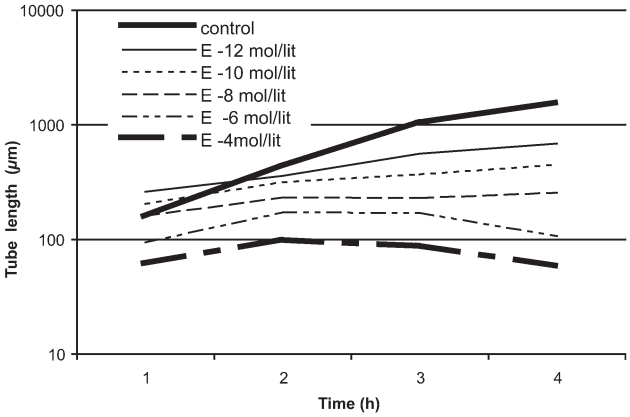


Fig. 4. Influence of cadmium application for 4 h on *L. longiflorum* pollen tube length. The expressions E-12, E-10, E-8, E-6, and E-4 mean 10^{-12}, 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M, respectively

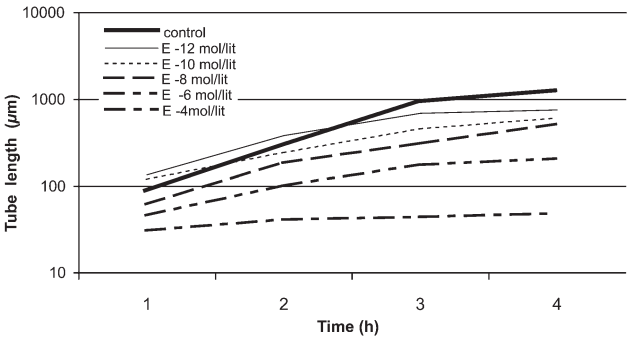
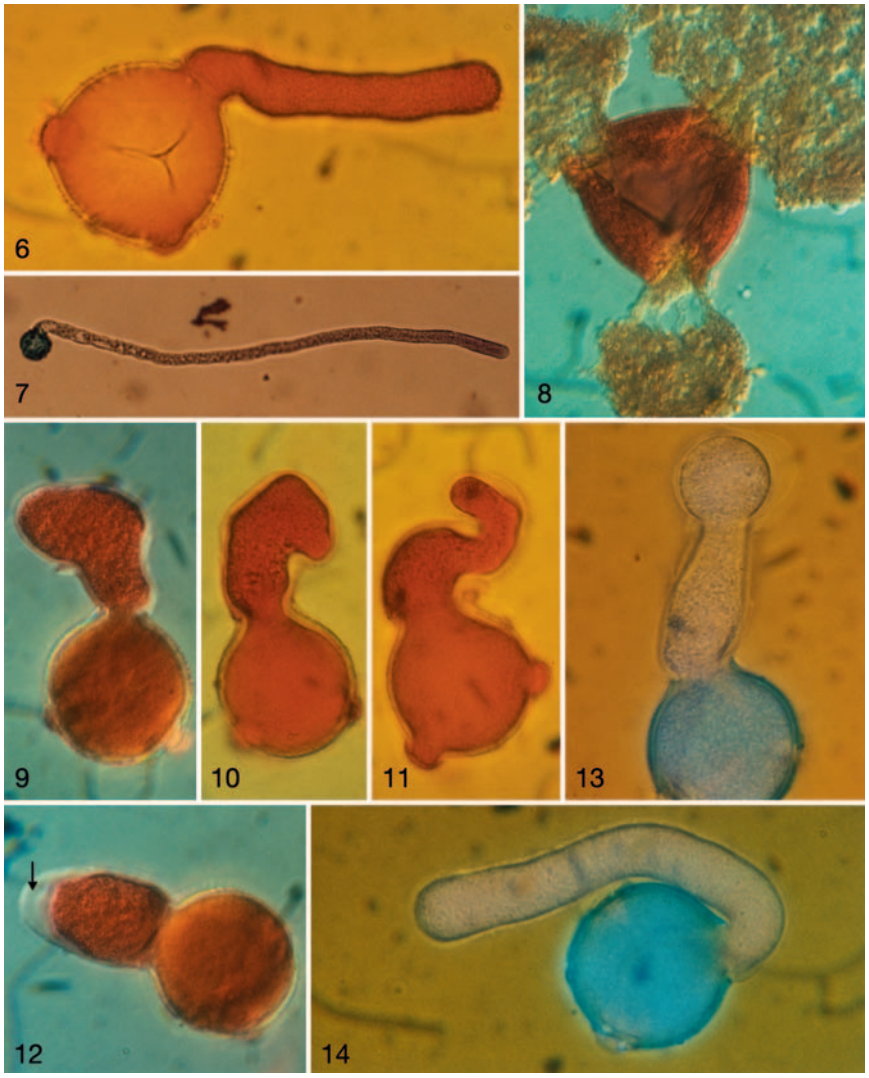


Fig. 5. Influence of cadmium application for 4 h on *N. tabacum* pollen tube length. The expressions E-12, E-10, E-8, E-6, and E-4 mean 10^{-12}, 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M, respectively



Figs. 6–14. Effects of cadmium application on *N. tabacum* pollen

Fig. 6. Two-hour-old control of tobacco pollen germinated in medium without metal. Stained with acetic carmine. ×4000

Fig. 7. Three-hour-old control of tobacco pollen germinated in medium without metal. Stained with pianese mixture. ×500

Fig. 8. Pollen grain cultivated in high (10^{-2} M) cadmium concentration for 1 h, burst on all three sides. Stained with acetic carmine. ×4000

Figs. 9–11. Various patterns of uneven pollen tube growth, after 2 h germination in 10^{-4} M cadmium. Tube growth has almost stopped and tubes show varying morphological responses. Stained with acetic carmine. ×3000

Fig. 12. Wall thickening in the tip region of a pollen tube after 2 h in 10^{-4} M cadmium (arrow). Stained with acetic carmine. ×3000

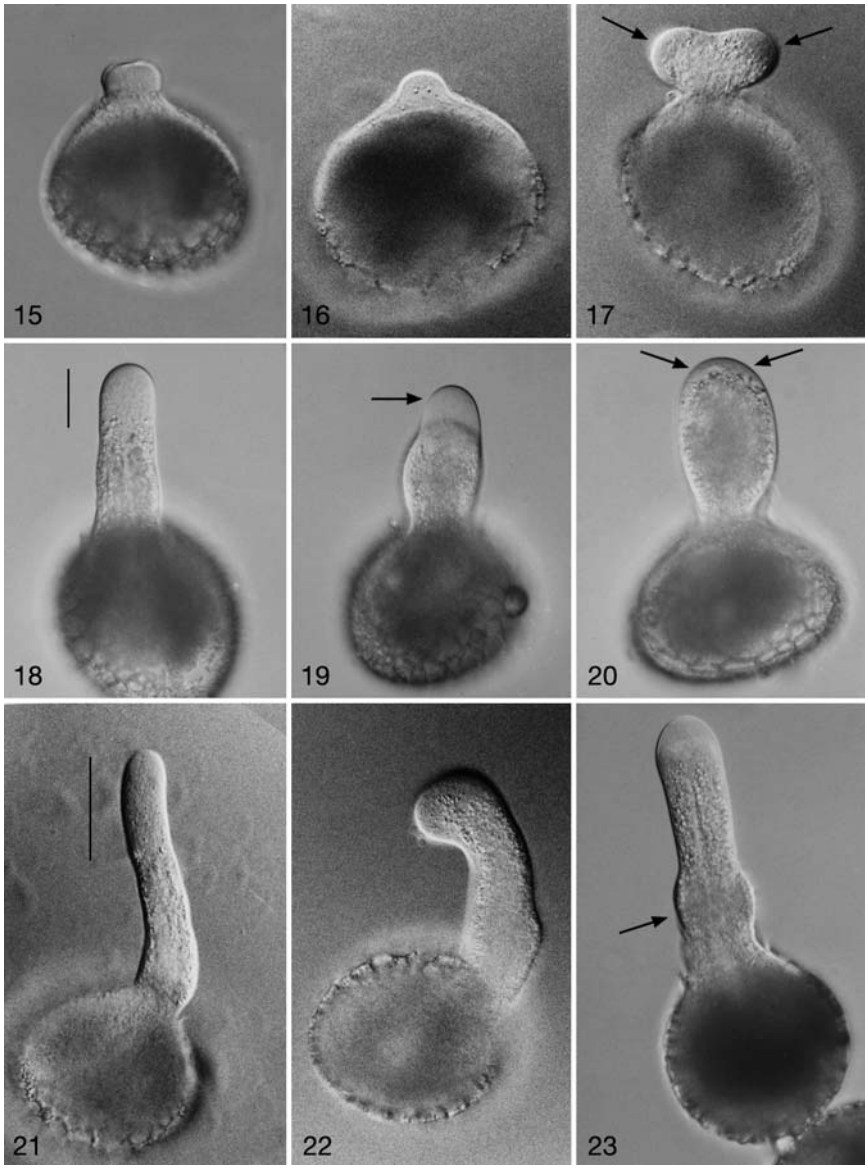
Fig. 13. Tip and basal region swelling after 2.5 h application of 10^{-5} M cadmium in culture medium. Stained with pianese mixture. ×3000

Fig. 14. Damage located at the side where the pollen grain has stretched over the pollen tube, after a 3 h application of 10^{-5} M cadmium in the culture medium. Stained with pianese mixture. ×3500

"clear cap" at the tube tip, an area lacking microscopically visible organelles and oriented cytoplasmic streaming. High cadmium concentration (10^{-2} M) in the culture medium completely stopped pollen germination, and in many cases, the pollen showed an increasing tendency to burst within 1 h of cultivation (Fig. 8). On the other hand, pollen grains cultured in 10^{-4} M cadmium showed a range of strong morphological abnormalities which were characterized by uneven or aberrant growth. However, the pollen tube growth pattern differed among tubes, revealing a great variability in growth pattern (Figs. 9–11). These abnormalities led to slowing down or complete cessation of pollen tube elongation.

After 2 h, the tube length decreased drastically, whereas the diameter increased abnormally up to 40 μm (the control cells measured were about 10 μm). Generally, tobacco pollen tube growth was drastically inhibited at this concentration (10^{-4} M) from the first stages. A free cap frequently occurred in the uneven tubes, probably derived from the formation of wall thickenings in the tip region (Fig. 12). On the other hand, pollen tubes cultured in the presence of 10^{-5} M cadmium showed drastic swelling of the tips and of the base of the tube (Fig. 13). In some cases, the pollen tube doubled up over the pollen grain (Fig. 14).

Lilium longiflorum pollen grains cultured in medium containing 10^{-5} M cadmium showed a range of effects on pollen tube growth pattern. Cadmium treatment reduced pollen germination and tube growth and caused morphological abnormalities, including cell wall thickening and, in some cases (about 5%), apical branching (Figs. 15–17). In the control tubes, a clear tip region lacking any organelles was visible, characterized as a clear cap (Fig. 18). This region was greatly reduced in most (about 70%) of the treated tubes (Fig. 19). Cell wall thickening could be detected at or near the tip followed by the relocation of



Figs. 15–23. Effect of cadmium on succeeding stages of pollen germination of *L. longiflorum*

Fig. 15. One-hour-old control pollen grain. Pollen tube growth has started. ×700

Fig. 16. Pollen grain cultured for 1 h and germinated in the presence of 10^{-5} M cadmium. Thick cell wall. Pollen tube growth is retarded. ×700

Fig. 17. Pollen cultured for 2.5 h and germinated in the presence of 10^{-5} M cadmium. Apical branching of a pollen tube (arrows). ×700

Fig. 18. Three-hour-old control showing a granule-free region of about 20 μm (bar). ×500

Fig. 19. Pollen grain cultured for 3.5 h and germinated in the presence of 10^{-5} M cadmium. An irregularly enlarged clear cap lacking organelles is visible in the tip region (arrow). ×500

Fig. 20. Pollen grain cultured for 3.5 h and germinated in the presence of 10^{-5} M cadmium. An extremely swollen tip region without the typical internal tube organization. Cell wall thickening (arrows). Cell organelles are not visible in the central area of the tube. ×500

Fig. 21. Four-hour-old control showing a granule-free region of about 30 μm (bar). ×500

Fig. 22. Pollen grain cultured for 4 h and germinated in the presence of 10^{-5} M cadmium. Uneven growth pattern of pollen tube (occurred in about 40% of the studied tubes), without a clear cap and swollen at the base region. ×500

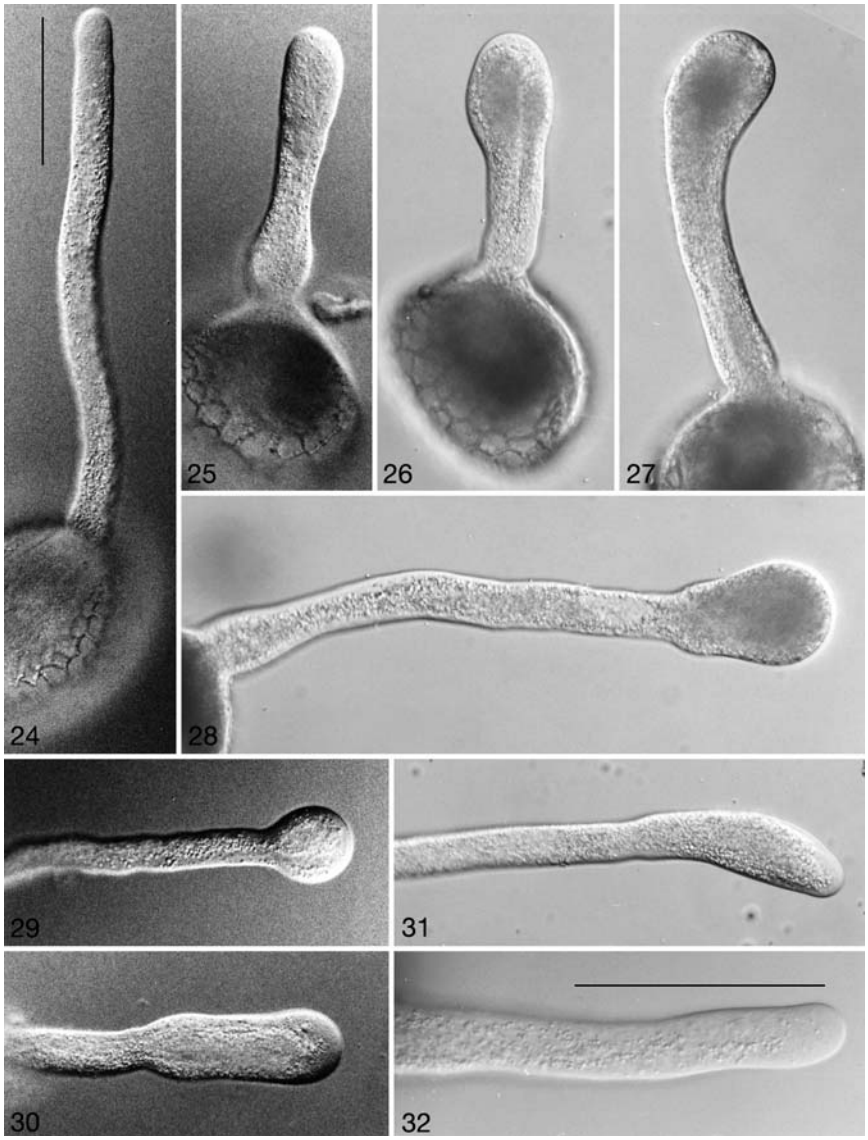
Fig. 23. Pollen grain cultured for 4 h and germinated in the presence of 10^{-5} M cadmium. A short clear cap is visible (occurred in about 60% of the studied tubes), and a slow plasma streaming can be observed. The basal area is swollen (arrow). ×500

light microscopically visible cell organelles (plastids, lipid droplets) toward the tip (Fig. 20). In many cases, cadmium-treated tubes formed swollen tips and stopped elongating, unlike the control (Fig. 21). Swelling also occurred progressively at the basal region of the tube (Figs. 22 and 23).

Cadmium impact on germinated pollen

In another experiment, the drug was added to the culture medium after 2, 3, and 4 h of normal germination. Untreated lily pollen tubes were generally straight, uniformly cylindrical, and 12–15 μm in diameter. Light microscopy revealed a well defined, granule-free clear cap of about 20 to 40 μm at the tube tip (Fig. 24) and intense protoplasmic streaming throughout the tube. Treatment of the germinated pollen with cadmium for 3 h resulted in a retarded tube growth rate, and with increasing cadmium concentrations, the tube growth successively decreased or completely stopped.

Tubes treated with cadmium at lower concentrations (10^{-5} M) showed slightly swollen tips with a reduced tip region (Fig. 25). Those at higher cadmium concentrations (10^{-4} M) showed reduction in the tube length and highly swollen tips up to 20 μm in diameter (Fig. 26), with thickened walls and an uneven growth pattern (Fig. 27). In tubes grown for 3.5 h, cadmium treatment (10^{-4} M) caused a granule-free region at the central area of the swollen tip (Fig. 28). As exposure time to the metal increased, the length of the tip region was drastically re-



Figs. 24–32. Progressive effects on *L. longiflorum* pollen treated with 10^{-5} and 10^{-4} M cadmium after 2–4 h of germination in control medium

Fig. 24. Three-hour-old control pollen tube, showing a granule-free region (clear cap) about 40 μm (bar) from the tip. ×500

Fig. 25. Application of 10^{-5} M cadmium for 1 h after 2 h of normal germination. ×500

Fig. 26. Application of 10^{-4} M cadmium for 1 h after 2 h of germination. A granule-free region is visible in the central area of the swollen tip. ×500

Fig. 27. Application of 10^{-4} M cadmium for 1 h after 3 h of germination. A swollen tip region without visible organelles is present in the central area. ×500

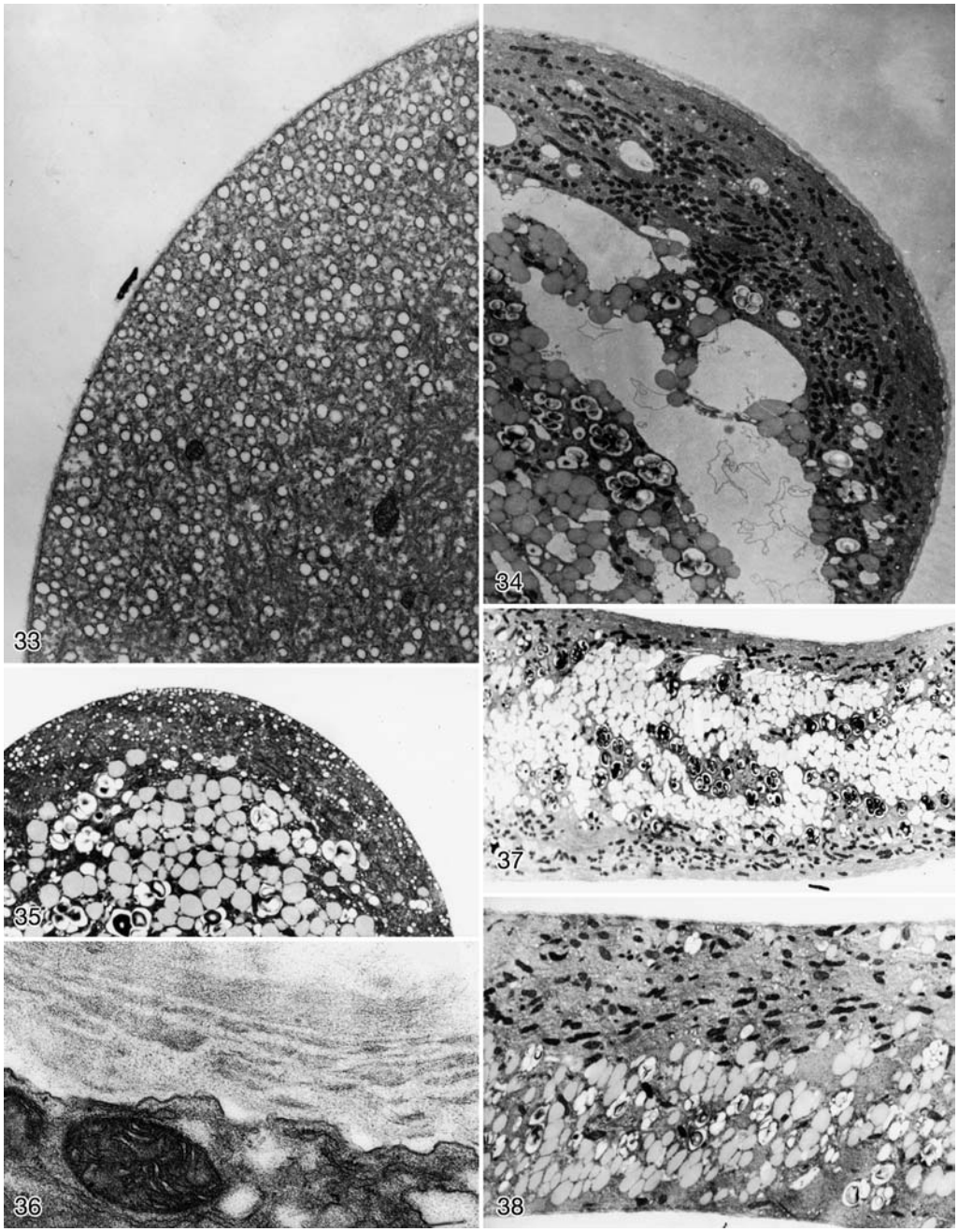
Fig. 28. Application of 10^{-4} M cadmium for 1 h after 3.5 h of germination. A granule-free region remains in the central area of the swollen tip. ×500

Fig. 29. Application of 10^{-5} M cadmium for 1 h after 4 h of germination. A round swollen tip is visible. ×400

Fig. 30. Application of 10^{-5} M cadmium for 1 h after 4 h of germination. Swelling continues from the tip, forming a distinct subapical collar

Fig. 31. Application of 10^{-5} M cadmium for 1 h after 4 h of germination. An irregular subapical collar is present. ×400

Fig. 32. Five-hour-old control showing a granule-free region of about 100 μm (bar). ×400



Figs. 33–38. Effects of cadmium on *L. longiflorum* ultrastructure

Fig. 33. Tip region of control pollen tubes. The cytoplasm shows the typical polar organization of the cell organelles within the very tip. Pollen tubes have a characteristic polar distribution of organelles. The apical zone is rich in secretory vesicles and few smooth ER cisternae. ×12,000

Fig. 34. Treatment with 3×10^{-4} M cadmium applied for 2 h after onset of tube germination. A swollen tube tip with abnormal internal organization is visible. The vesicle zone is missing and a great number of mitochondria are present. Plastids and lipid droplets have entered the tip and even larger irregularly shaped vacuoles occur. ×8000

Fig. 35. Longitudinal median section of a pollen tube treated with 3×10^{-4} M cadmium. A pollen tube with accumulated plastids at the tip is visible. The vesicular apical zone is drastically limited. ×2500

Fig. 36. Mitochondrion, the dominant organelle, close to the subapical region near the thick cell wall. ×30,000

Fig. 37. Subapical zone with mitochondria in the periphery and large organelles (vacuoles) located centrally. Peroxisomes appear to be more frequent in the periphery. ×3000

Fig. 38. Prolonged cadmium application leads to large organelles being arranged on one side of the pollen tube and smaller ones on the other. ×3500

duced by the relocation of light microscopically visible cell organelles (plastids, lipid droplets) toward the tip. After 4 h of growth, the application of cadmium mainly affected the tip of the pollen tubes. The first tube irregularity to be observed was the round, swollen tip (Fig. 29), which progressively extended to the subapical area, forming a distinct subapical collar (Figs. 30 and 31), in contrast to the cylindrical shape of the control (Fig. 32). Increased cadmium concentration (10^{-2} M) stopped pollen tube growth and decreased or completely paralyzed cytoplasmic streaming within 20 min of application.

Cadmium impact on tube ultrastructure

Following treatment with cadmium, even at low concentrations (e.g., 10^{-5} M), the glutaraldehyde-fixed pollen tubes became highly fragile and tended to burst during very gentle handling while performing the electron microscopy observation. The effect of cadmium exposure on the intracellular organelle organization was a disturbance of the typical cytoplasmic zonation. A large number of secretory vesicles were found in the tube apex of the control tubes (Fig. 33). In the corresponding region of the cadmium-treated tubes, a number of organelles (mitochondria, plastids) and lipid droplets were present. Cadmium caused a drastic disturbance of the organelle zonation, reducing the number of secretory vesicles within the very tip.

When the clear cap region at the tip was reduced, plastids and lipid bodies and, in some instances, large vacuoles were found in the swollen tip area (Fig. 34). The vesicular apical zone was drastically reduced (Fig. 35) and no longer distinct, with vesicles dispersed into the subapical region. Mitochondria were found distributed throughout the subapical region near the thick cell wall (Fig. 36). Cadmium concentrations above 10^{-5} M caused total loss of cytoplasmic structural integrity. Organelle streaming was facilitated by concentrating lipid bodies or large organelles (e.g., amyloplasts) in the central area and the smallest (e.g. mitochondria) at the peripheral region (Fig. 37). About 20 min after the application of cadmium, the above organelle arrangement no longer existed (Fig. 38) and the streaming stopped.

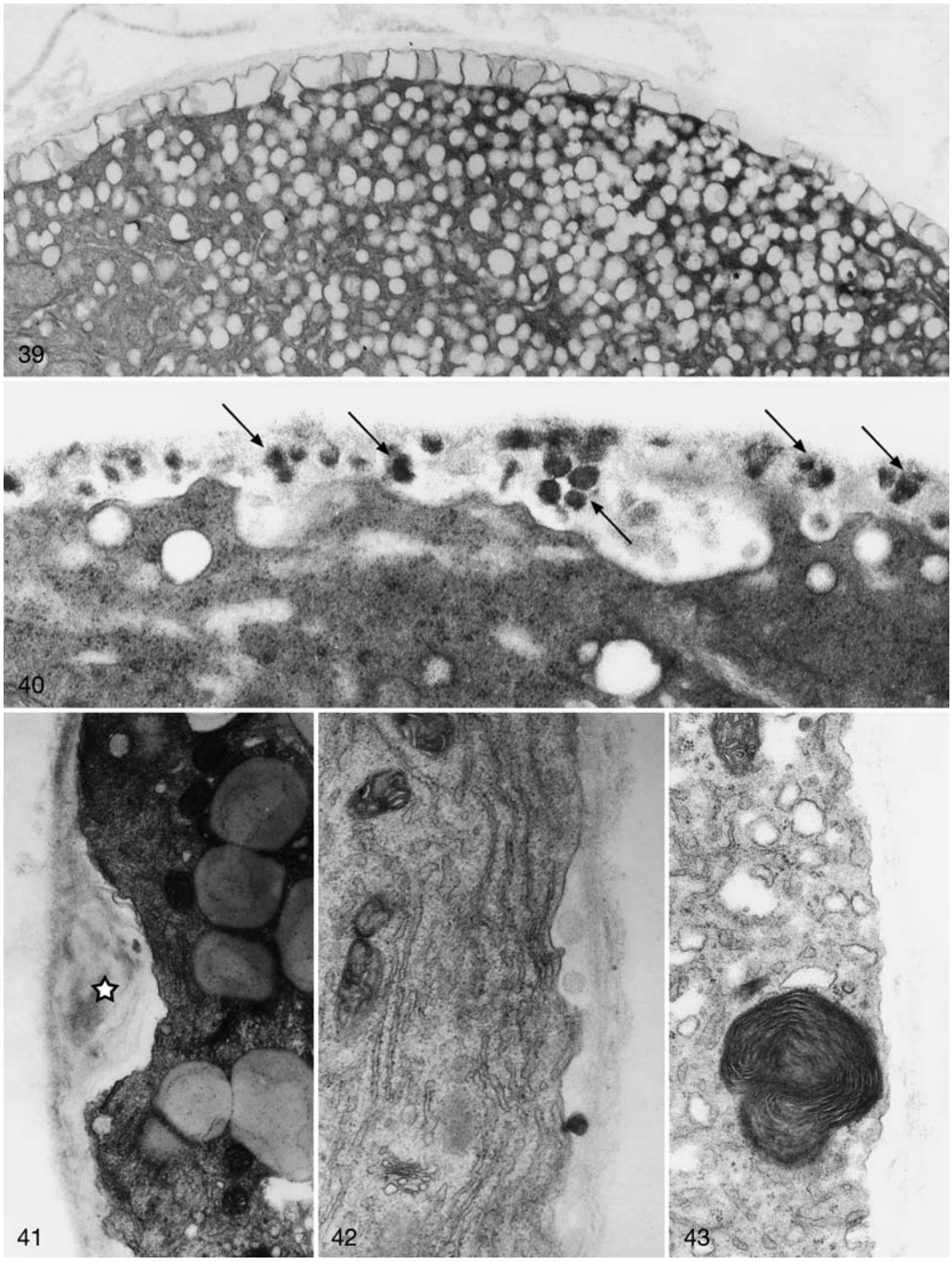
The main effect of cadmium on pollen tubes was an abnormal cell wall organization, mostly at the tip. In contrast to the control (Fig. 39), cell wall intrusions were evident on the inner side, whereas a loose network of fibrillar material was formed on the outer layers. After prolonged cadmium exposure, spherical (ball-like) aggregates were embedded in a fine fibrillar network. These globules resembled, in shape and size, the secretory vesicles of the dictyosomes near the plasma membrane of the tip. Some of the clumped cell wall material from the outer wall layers was released into the surrounding medium, whereas the inner layers contained single wall globules, thus forming a labyrinth-like structure (Fig. 40). These wall globules decreased in number, size, and density with increasing distance from the tube tip. The wall material was more or less compact (Fig. 41), where in some cases, electron-dense material was included in the massive wall intrusions. Visible ultrastructural changes in cell organelles were not observed at the above cadmium concentrations. The Golgi vesicle accumulation was not restricted to the tip itself but observed even at a distance from it (Fig. 42). On the other hand, cadmium treatment did not disturb dictyosomal ultrastructure. The rough endoplasmic reticulum (ER) cisternae were often present near the inner cell wall of the subapical area, at a distance from the tip. Large myelin-like structures of membranous material were frequently observed in close contact with the plasmalemma (Fig. 43).

Discussion

Effects of cadmium on pollen germination and tube growth

The present observations demonstrate that cadmium adversely affects semivivo pollen germination and tube growth, indicating that it may disturb an important event in both of these processes. In general, the pollen viability decreased gradually even at low concentrations. Germinated *N. tabacum* pollen were more sensitive to cadmium compared with those of *L. longiflorum*, confirming literature data showing that dicotyledons are generally more sensitive than monocotyledons (Wojcik and Tukendorf 1999, Ünyayar et al. 2006, Fusconi et al. 2007). Xiong and Peng (2001) revealed that cadmium inhibited pollen germination at 2.51 mg/ml or higher.

Tube growth was inhibited at concentrations of 1.58 mg/ml or higher, while lower concentrations stimulated further pollen tube growth. However, in our study, stimulation was observed below 10^{-10} M cadmium for both plants during the initial (2–3 h) stages of pollen tube growth. Many authors have admitted that low concentrations of heavy metals could stimulate pollen germination and tube growth (Searcy and Mulcahy 1985, Sawidis and Reiss 1995). Low metal concentrations can stimulate enzymatic activities, whereas high concentrations inhibit these processes. In our experiments, an increase in the germination rate was observed at low (10^{-12} M) cadmium concentrations. This phenomenon, termed hormesis (from



Figs. 39–43. Effects of cadmium on *L. longiflorum* ultrastructure

Fig. 39. Lateral region of a control pollen tube with a thick wall. The pollen tube cytoplasm contains numerous secretory vesicles in close contact with smooth ER. 40,000

Fig. 40. Thickened cell wall at the tip, showing structural details of the electron-dense wall material, after 1 h treatment with 10^{-4} M cadmium. Clumps of wall fibrils occur in the cell wall (arrows). ×45,000

Fig. 41. Irregular cell wall thickening with cell wall intrusions in the subapical zone (star). ×12,000

Fig. 42. Subapical zone with concentrated ER. Secretory vesicles are completely absent. ×10,000

Fig. 43. Membranous accumulations forming myelin-like structures. ×15,000

the Greek word horme, impulse), has been defined as a stimulatory effect of a subinhibitory dose of any toxic substance to any organism (Stebbing 1998). Hormesis in pollen germination and growth has been found in other plant species, such as *Acer pseudoplatanus* (Turner et al. 1991) and *Plantago depresa* (Xiong and Peng 2001).

When the metal was not applied directly to the culture medium but through the pedicel via a translocation process, no stimulation effect was observed (Figs. 1 and 2). Anthers might afford pollen some protection, allowing only a small proportion of the applied metal to affect the pollen. The reduction in anther lengthening and final opening is strongly correlated with the mitotic activity of anther tissues, as observed in other meristems, such as root apices (Fusconi et al. 2007). Cadmium, like other heavy metals such as lead, induces the lengthening of the cell cycle in root apices, as has been demonstrated for *Allium cepa* (Borboa and De la Torre 1996, Wierzbicka 1999).

The presence of cadmium reduces cell wall plastic extensibility and impairs normal cell elongation in growing pollen tubes, causing morphological and structural alterations. The microscopic observations demonstrate that cadmium acts primarily on cell wall development in germinating and growing pollen tubes. This can be explained by the interaction of metal ions with the anionic contents of secretory vesicles and the fact that pollen tube cell walls contain large quantities of pectins and callose, but less cellulose (Herth et al. 1974, Röderer and Reiss 1988, Sawidis and Reiss 1995). This special feature of pollen tube cell walls may result in a reaction different from that of other plant cells that possess a normal cellulosic cell wall. Consequently, normal growth is inhibited, the cell diameter increases, and walls become thicker.

Wall anomalies caused by cadmium have also been described for some algae, such as *Chara vulgaris* cells, where local wall thickenings or protuberances, consisting of disordered microfibrils, were observed after cadmium treatment (Heumann 1987). In the brown alga *Cystoseira barbata*, cadmium caused dense deposits in the cell wall (Pellegrini et al. 1991). In animal organisms, such as in the slug *Deroceras reticulatum*, cadmium treatment resulted in electron-dense inclusions in the vacuoles of digestive cells, throughout the cytoplasm (McGrath 1999).

A growing pollen tube is a highly active secreting cell, exhibiting oriented exocytosis at the tube tip, including vesicle transport and membrane fusion. The most obvious feature of a normally growing pollen tube is the accumulation of vesicles at the tip region, causing the formation of the characteristic vesicle zone (Fig. 33). This may be related to the accumulation of smooth ER at the tip of the pollen tubes (Fig. 39). The inhibition or disappearance of this zone that follows the increase in cadmium concentration results in a decrease in pollen tube growth. The loosening of the tube wall structure, concomitantly with the increase in wall thickness, might result directly from an interference of the cadmium with wall polysaccharides or from an influence on the secretory mechanism.

The formation of uneven pollen tubes deviating from the normal, straight growth would usually implicate changes in the cytoskeletal pattern, and actin filaments are known to be involved in the regular tip growth of pollen tubes (Rao and Kristen 1990). Oriented pollen tube growth depends on the presence of cortical microtubules, but in our experiment, cadmium treatment did not seem to affect this system. Thus, it is more likely that the reason for deviated tube growth is not the breakdown of the actin filament system but the inactivation of vesicle material originating from the Golgi apparatus.

Pollen tubes have a characteristic polar distribution of organelles. The disorganization of the polar zonation of cell organelles within the tube tip region (Reiss and Herth 1979), observed clearly after cadmium application, may indicate a possible intracellular action of the metal, but could also indicate secondary effects of the treatment. Cadmium concentrations of 10^{-6}–10^{-4} M showed no distinct effects on the cell organelles of the pollen tubes. It is, therefore, possible that the cadmium applied to the pollen culture was almost all bound to the cell wall, and only small amounts, if any, were taken up into the cytosol. If some metal was accumulated intracellularly, the time until the tubes stopped growing and burst might have been too short to allow manifestation of visible intracellular effects.

The apical zone of control pollen tubes is rich in secretory vesicles and a few smooth ER cisternae. The formation of complexes of concentrically arranged and stacked rough ER cisternae in the cadmium-treated tubes is a common feature of inactive or dormant cells. It has been suggested either to be related to enhanced protein synthesis and accumulation or to indicate resting stages or even inhibition of protein synthesis (Rao and Kristen 1990). It may be the result of utilization of existing membrane material under conditions of decreased growth as has also been suggested for *L. longiflorum* pollen tubes treated with chlorotetracycline (Reiss and Herth 1979).

The environmental importance of the present results is not difficult to determine. Since cadmium is present in the environment, e.g., as vapor from smelters or high-temperature industrial operations (Prasad 1995), it is likely to come into contact with the anthers and stigmas of plants.

By accumulation in the stigmatic secretion, concentrations can be reached that directly impair the germination and growth of pollen tubes. Cultures of higher-plant cells may be less tolerant to increased concentrations of heavy metals, but plants may accumulate these trace elements and survive on contaminated soils (Chukwuma 1993). This may be the case for plant cells which possess a "regular" cellulosic cell wall unlike the pectin- and callose-rich pollen tubes. Although the special features of pollen tube walls may result in a different reaction to heavy metals, this could play a decisive role for the whole plant because pollen tubes are part of the plant reproductive system.

Although most of the available evidence of cadmium toxicity to various organisms comes from laboratory experiments (McGrath 1999), contamination of air, soil, and water by this metal could lead to a similar reaction in vivo. Toxic effects were observed at metal concentrations of 10^{-4} M. Such concentrations can be measured in contaminated urban and industrial areas. Concentrations of cadmium in ambient air normally fall within the range of 0.1–4 ng/m^3 in rural areas and 2–150 ng/m^3 in urban and industrial areas (OECD 1994), taking into account that deposition of cadmium from the atmosphere has increased in recent decades (Alloway and Steinnes 1999).

Conclusion

We conclude that cadmium toxicity at least plays a decisive role in pollen grain germination and pollen tube growth. In accordance, even at low concentrations, cadmium may adversely affect both processes. As an air pollutant and soil contaminant, in elevated concentrations in plants, it might contribute to the disappearance of some sensitive plant species from regions of high pollution by reducing their reproductive ability and could also influence higher-plant reproduction.

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