ACTA BIOLOGICA CRACOVIENSIA Series Botanica 50/1: 27–34, 2008

HISTOPATHOLOGY OF *DAUCUS CAROTA* L. ROOT CELLS TREATED WITH TOXIC METABOLITES PRODUCED BY *ALTERNARIA RADICINA* AND *A. ALTERNATA*

KRYSTYNA TYLKOWSKA[1*], AGNIESZKA BAGNIEWSKA-ZADWORNA[2], JADWIGA GRABARKIEWICZ-SZCZĘSNA[3], DOROTA SZOPIŃSKA[1], HANNA DORNA[1] , AND ELŻBIETA ZENKTELER[2]

[1]*Department of Seed Science and Technology, Poznan University of Life Sciences, Baranowo, ul. Szamotulska 28, 62–081 Przeźmierowo, Poland*
[2]*Department of General Botany, Adam Mickiewicz University, ul. Umultowska 89, 61–614 Poznań, Poland*
[3]*Department of Chemistry, Poznan University of Life Sciences, ul. Wojska Polskiego 75, 60–625 Poznań, Poland*

Received September 4, 2007; revision accepted May 25, 2008

Vascular storage parenchyma cells of carrot roots were treated with methanol solutions of radicinin and *epi*-radicinol produced by *Alternaria radicina* and with alternariol and alternariol methyl ether produced by *A. alternata* at concentrations of 25 μg/ml and 250 μg/ml, as well as culture filtrates of both fungi. Cell ultrastructure was observed by TEM. No visible changes were noted after treatment with 25 μg/ml toxin solutions. The most extensive plication of cell membranes, and sometimes also cell walls, and the formation of numerous vesicles in the cytoplasm, was observed in cells treated with the higher concentration of toxins. Plasma membrane withdrawal and vesiculation, microvacuole formation, and accumulation of plastoglobuli in chromoplasts also occurred. No changes in the structure of endoplasmic reticulum and dictyosomes were noted. The responses of cell structures to particular toxins were nonspecific. Treatment with culture filtrates from *A. radicina* resulted in the occurrence of osmiophilic, electron-dense substance in the cytoplasm and plastoglobuli. All alterations induced by filtrates were more extensive than those resulting from toxin solutions, but membrane integrity was not disturbed after any of the treatments.

Keywords: Alternariol, alternariol methyl ether, *epi*-radicinol, radicinin, ultrastructure, carrot root.

INTRODUCTION

Alternaria radicina Meier, Drechsler & Eddy [syn. *Stemphylium radicinum* (Meier, Drechsler et Eddy) Neerg.] is a pathogen causing a range of symptoms in carrot, including pre- and post-emergence damping-off, necrosis of the crown and root, foliar, flower- and seed-stalks, and umbel and seed blight. The greatest damage occurs post-harvest, during root storage (Meier et al., 1922; Neergaard, 1945; Maude, 1966; Pryor et al., 1994, 1998; Pryor and Gilbertson, 2002). *A. alternata* (Fr.) Keissler is one of the most common saprotrophs or facultative parasites associated with various parts of plants (Scheffer, 1992). Up to 68% of carrot root samples collected in several European countries were found to be contaminated with the fungus (Solfrizzo et al., 2005). As much as 70% of mature carrots can be rendered unmarketable if heavily infested or infected by *A. radicina* and *A. alternata* (Solfrizzo et al., 2005).

Alternaria species produce both host-specific and non-host-specific toxic secondary metabolites (Montemurro and Visconti, 1992; Visconti et al., 1992; Bottalico and Logrieco, 1998; Solfrizzo et al., 2004b). *A. radicina* was capable of producing primarily radicinin (RAD, syn. stemphylone) followed by radicinols (ROHs), i.e. radicinol (ROH) and *epi*-radicinol (*epi*-ROH) when grown on carrot root discs. The latter have also been found in carrot roots either naturally contaminated or inoculated with

*e-mail: kwtylk@poczta.onet.pl

Abbreviations: AOH – alternariol; AME – alternariol methyl ether; Ch – chromoplast; CW – cell wall; D – dictyosome; *epi*-ROH – *epi*-radicinol; M – mitochondrion; N – nucleus; RAD – radicinin; ROHs – radicinols; V – vacuole.

PL ISSN 0001-5296

A. radicina and showing symptoms of black rot (Solfrizzo et al., 2004b, 2005; Tylkowska et al., 2005). Both RAD and ROHs are non-host-specific toxins. In addition to *A. radicina*, RAD is produced by *A. chrysanthemi, A. helianthi, Bipolaris coicis, Cochliobolus lunata* and *Phoma andina*, ROH by *A. chrysanthemi* and *C. lunata*, and *epi*-ROH by *A. chrysanthemi* (Hansen, 1954; Robeson et al., 1982; Tal et al., 1985; Nakajima et al., 1997; Solfrizzo et al., 2004b).

From among a number of toxins produced by *A. alternata*, alternariol (AOH) and alternariol methyl ether (AME) were mainly detected on carrot root discs inoculated with the fungus isolated from carrot seeds (Solfrizzo et al., 2005). Both are also produced by a number of other *Alternaria* species, including *A. brassicae, A. brassicicola, A. capsiciannui, A. cheiranthi, A. citri, A. cucumerina, A. dauci, A. kikuchiana, A. longipes, A. porri, A. raphani, A. solani, A. tenuissima* and *A. tomato* (Bottalico and Logrieco, 1998).

The phytotoxic effects caused by *A. radicina* and *A. alternata* toxins on carrot seedlings and seeds have been described (Tylkowska et al., 1998, 2003; Solfrizzo et al., 2004b). Phytotoxicity of RAD and ROH to other plant species was previously reported by Hansen (1954), Nakajima et al. (1997) and Robeson et al. (1982). Park et al. (1977), Kohmoto et al. (1993) and Shabana et al. (1997) showed that toxins produced by *Alternaria* spp. caused changes in plant cell structures. No information has been found on such an effect of toxic metabolites produced by *A. radicina* and *A. alternata*. The main goal of this study was to determine whether they are capable of inducing changes in carrot root cells at the ultrastructural level.

MATERIALS AND METHODS

SOLUTIONS OF TOXINS

Radicinin (RAD) and *epi*-radicinol (*epi*-ROH) produced by *Alternaria radicina* and alternariol (AOH) and alternariol methyl ether (AME) produced by *A. alternata* were used in the study. Standards of RAD, AOH and AME (> 95% purity) were purchased from Sigma-Aldrich (Poznań, Poland), and *epi*-ROH was obtained from Dr. M. Solfrizzo of CNR, Institute of Sciences of Food Production, Italy. Solutions of the toxins were made by dissolving them in 5% methanol at concentrations of 25 μg/ml and 250 μg/ml.

CULTURE FILTRATES OF FUNGI

Single-spore cultures of *Alternaria radicina* and *A. alternata* were maintained on potato-dextrose-agar (Merck, Darmstadt, Germany) plates in four replicates at 20°C in the dark for 14 days. Five plugs ~0.5 cm in diameter were cut from the margins of colonies and transferred to 500 ml flasks containing 150 ml standard Fries' medium (Dhingra and Sinclair, 1986) and then incubated at 20°C in the dark for 14 days. Occasionally the flasks were vigorously shaken. The culture filtrates were obtained by vacuum filtration (Sartorius, Goettingen, Germany).

DETERMINATION OF *ALTERNARIA* TOXINS IN CULTURE FILTRATES

The culture filtrates were filtered through a 0.45 μm membrane filter (LCR – Millipore) and analyzed by reversed-phase liquid chromatography (Waters 2695) with a UV diode array detector set at 256 nm for *A. alternata* and 345 nm for *A. radicina*. AOH and AME were determined as described by Solfrizzo et al. (2004a), using two consecutive isocratic mixtures of acetonitrile/sodium dihydrogen phosphate (Sigma-Aldrich, Poznań, Poland) solution. RAD and *epi*-ROH were determined as described by Solfrizzo et al. (2004b), using a 10–30% linear gradient of acetonitrile in water for 22 min at a flow rate of 1 ml/min. Sodium dihydrogen phosphate and all solvents were of HPLC grade.

TREATMENT OF TEST ROOTS, INCUBATION CONDITIONS AND TISSUE SAMPLING

Storage roots of Nantes cv. type were purchased at a local market. After washing under tap water and surface drying, they were dipped in 70% ethanol for 3 min, wiped off, dipped in 1.5% solution of sodium hypochlorite for 3 min, washed under running tap water twice for 10 min, and left for surface drying. Hollows 3 mm in diameter and 6 cm depth from the crown were made in the central part of the root xylem with a flame-sterilized cork-bore.

The root hollows were filled with 5% methanol solutions of RAD, *epi*-ROH, AOH and AME at concentrations of 25 μg/ml and 250 μg/ml, as well as with culture filtrates of either fungi, and incubated in the dark at 20°C and 95% RH for 7 days. The control roots were either not treated or treated with 5% methanol or Fries' medium.

Pieces of carrot roots (2 mm × 5 mm) containing storage parenchyma cells were selected under a stereoscopic microscope and fixed with 4% glutaraldehyde and 4% paraformaldehyde mixture (1:1, pH 6.8; Polysciences, U.S.A.) for 2 h and post-fixed with 1% osmium tetroxide for 2 h at room temperature. The fixed material was counterstained for 1 h with 2% uranyl acetate (pH 5.0; Polysciences). Samples were dehydrated in a 10–100% graded acetone series and embedded in low-viscosity Spurr's resin (Spurr, 1969). Ultrathin sections (0.1 μm) cut with an Ultracut S ultramicrotome (Leica-Reichert,

Germany) were collected on Formvar-coated copper grids and stained with uranyl acetate and lead citrate (Reynolds, 1963). Results were averaged from five samples from three different roots per sampling. For each sample, 10 ultrathin sections were examined under a JEM 1200 EX II (Jeol, Japan) transmission electron microscope operating at 80 kV.

RESULTS

PRODUCTION OF *ALTERNARIA* TOXINS IN CULTURE FILTRATES

Neither AOH and AME nor *epi*-ROH were detected (detection limits: 0.1 μg/ml for AOH and AME, 0.2 μg/ml for *epi*-ROH) in culture filtrates from the isolates tested, whereas *A. radicina* isolate produced RAD at 19.3 ± 2.6 μg/ml.

CONTROL ROOT CELLS

In transverse section, storage vascular parenchyma cells of carrot root were isodiametric in shape and had large central vacuoles. Electron-dense cytoplasm was located along the cell wall. Cell wall thickness varied, and middle lamellae were pronounced.

Visible next to the cell wall was the plasma membrane, endoplasmic reticulum and chromoplasts (Figs. 1–4). Irregularly shaped nuclei near the cell wall were surrounded by an inner and outer membrane and contained weakly condensed chromatin and a single nucleolus (Fig. 1). Exceptionally, two nucleoli of fibrous type were present. Occasionally, electron-light regions were observed in the nucleolus. Only a few elements of endoplasmic reticulum were present, but there were many vesicles, cisternae, and derivatives of Golgi bodies (Fig. 2). Dictyosomes of the Golgi apparatus were rare and usually consisted of 4–6 cisternae together with a few vesicles in their margins (Fig. 2). There were many polyribosomes and cytoplasmic granules in the cytoplasm.

In cell transverse section, round, ellipsoidal or elongated mitochondria were surrounded by double membrane envelopes with distinct cristae. In general, the lumen of the cristae was fairly narrow (Fig. 3). The endoplasmic reticulum carried numerous ribosomes on the outer face; therefore we describe it as rough. Rough cisternae of the reticulum were more frequent than smooth ones. Plastids were spherical, spindle-form/fusiform or amoeboid, and had a double membrane envelope. Among them, crystalline (usually) and crystalline-globular (rarely) chromoplasts were present (Fig. 4); only very few proplastids occurred (not shown). Chromoplasts usually contained very few plastoglobuli. Clear zones were visible in many chromoplasts, likely representing crystals of β-carotene. They were electron-transparent after chemical fixation, and some of the crystals were expelled from the chromoplasts during the preparation procedure (Fig. 4). Treatment with methanol or Fries' medium did not induce ultrastructural alterations in any of the cellular components, including cytoplasmic membranes (Figs 5, 6).

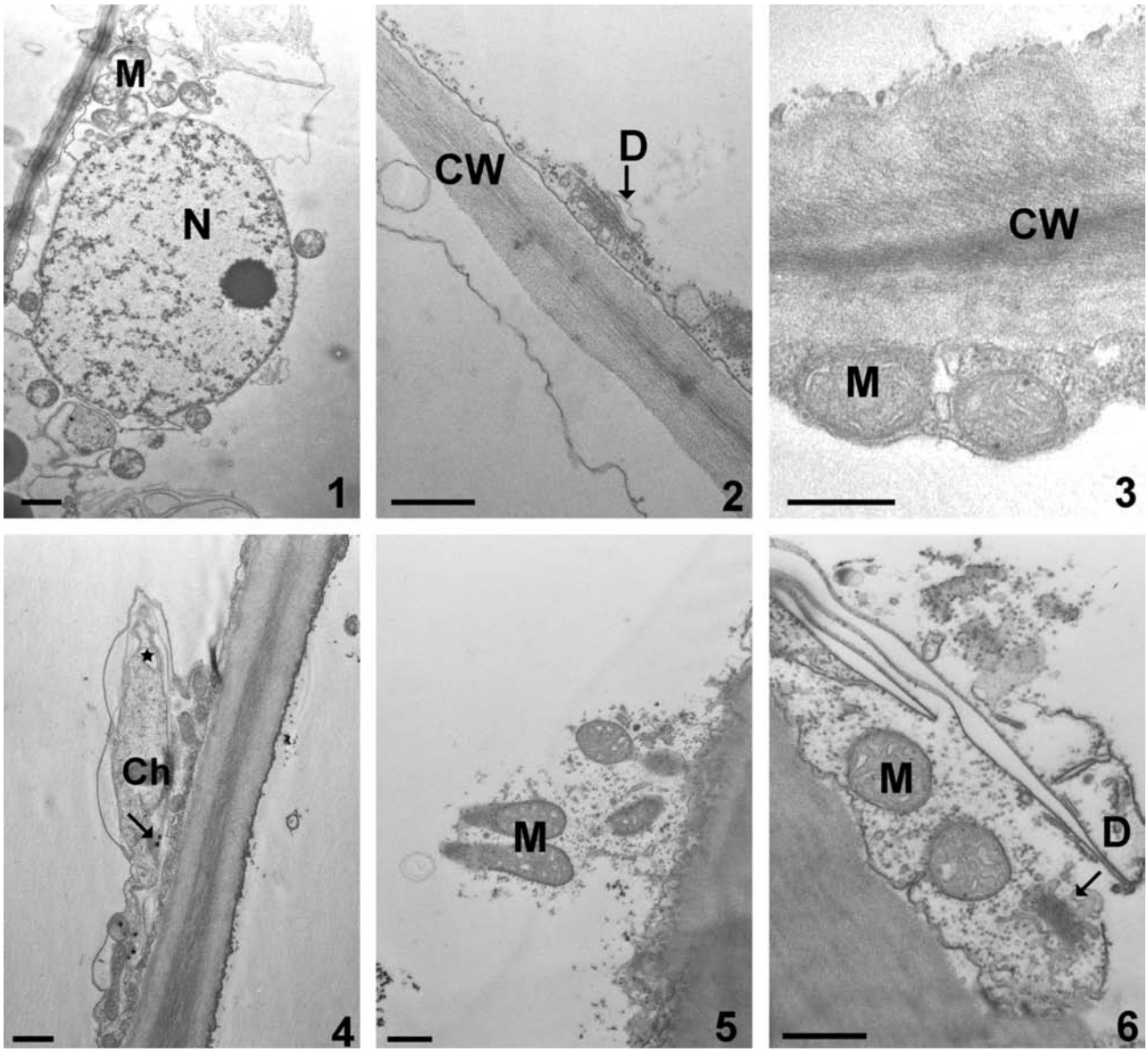
ROOT STORAGE PARENCHYMA CELLS TREATED WITH TOXINS OR CULTURE FILTRATES

Transmission electron microscopy (TEM) showed no visible changes in cell ultrastructure in parenchymatic cells from roots treated with 25 μg/ml toxin extracts. These cells still possessed cytoplasm of medium electron density, occurring as a narrow band rich in organelles. Nevertheless, after treatment with AME and *epi*-ROH we noted withdrawal of some parts of the plasma membrane (Figs. 7, 8) as well as accumulation of small vesicles and vacuoles (Figs. 7, 9). Under TEM, small vesicles appeared as uninterrupted circular outlines.

Changes in the cell structure and alterations of the cytoplasm were discernible after root treatment with toxin solutions at the higher concentration (250 μg/ml). The cells appeared turgid, but with very extensive plication of cell membranes and part of the cell walls, and having numerous vesicles in the cytoplasm. Plasma membrane withdrawal was observed along the entire circumference of the cell as well as that of the cell wall after treatment with *epi*-ROH (Fig. 10) and AME (Fig. 11). Microvacuole formation also occurred, marked by increased numbers of small vacuoles and secretory vesicles (Fig. 11). Distension of mitochondrial cristae was seen, but most of the mitochondria resembled those observed in the control material (cf. Figs. 12 and 3). Responses of carrot root cells to treatments with particular toxins were nonspecific. No changes in the structure of dictyosomes (Fig. 13) and endoplasmic reticulum were seen. Nuclear chromatin was not condensed (not shown). Treatment with toxins at 250 μg/ml resulted in accumulation of plastoglobuli in the chromoplasts (Fig. 13).

After treatment with culture filtrates from *A. radicina*, an osmiophilic, electron-dense substance was deposited in the cytoplasm, and microvacuoles (Fig. 14) or plastoglobuli (Fig. 15) accumulated. In cells exposed to this experimental treatment, electron-light zones were frequent in the chromoplasts, along with large numbers of small vesicles and vacuoles (Fig. 16).

Treatment of root parenchyma cells with culture filtrates from *A. alternata* resulted in accumulation of osmiophilic granules in the cytoplasm, and plastoglobuli in chromoplasts (Figs. 17–19). Chromatin condensation in the nucleus did not occur (Fig. 19). In some chromoplasts there were lighter areas (Figs. 18, 19). These alterations were more extensive than those developing after treat-



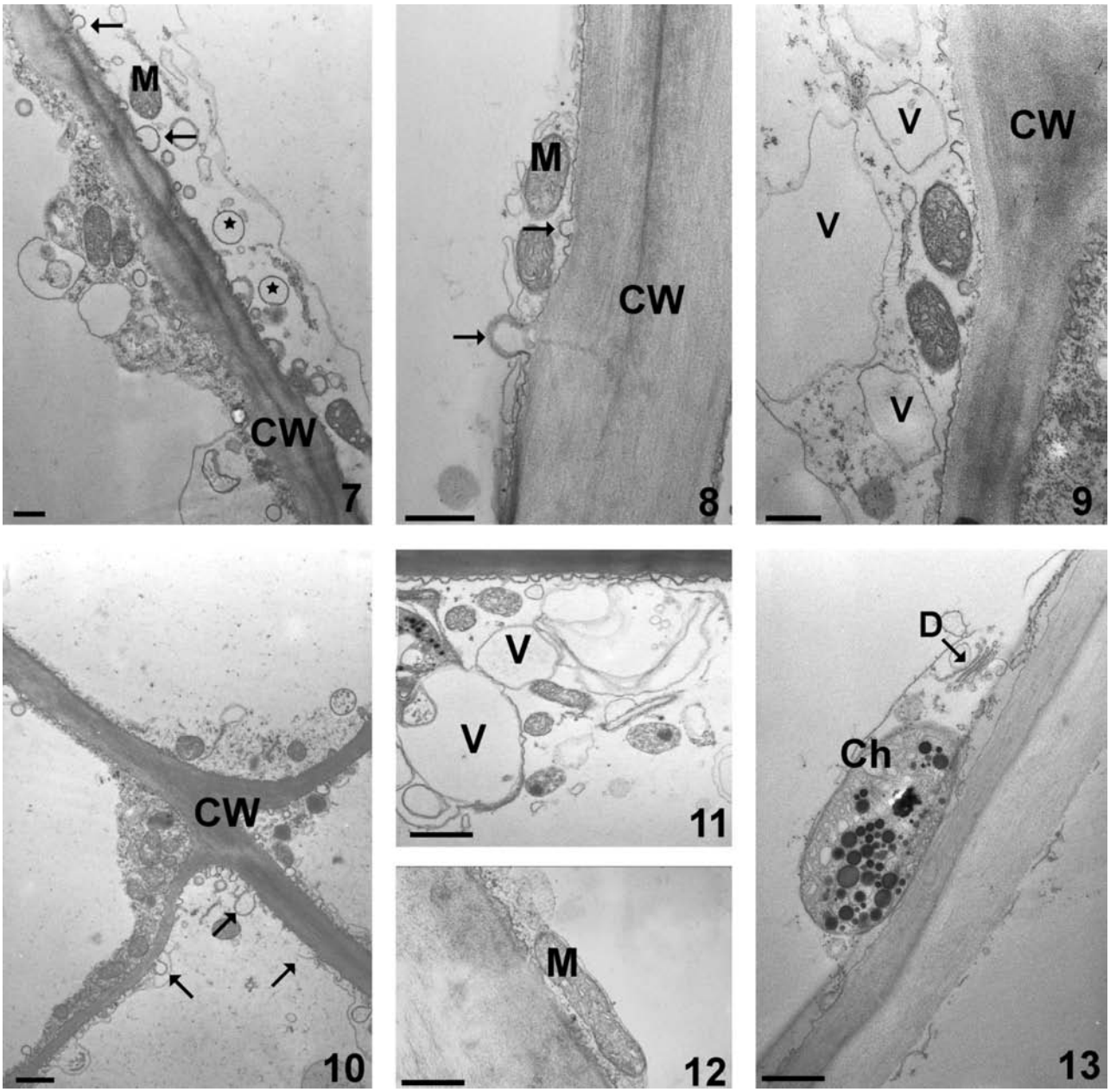
Figs. 1–6. Ultrastructure of vascular storage parenchyma cells of untreated carrot roots (Figs. 1–4) and roots treated with methanol or Fries' medium (Figs. 5,6). **Fig. 1**. Subcellular organization of nucleus and cytoplasm, with other organelles characteristically located along cell wall. Bar = 1μm. **Figs. 2,3**. Dictyosome of Golgi apparatus (Fig. 2) and mitochondria (Fig. 3) in vascular parenchyma cell. Bars = 1 μm in Fig. 2 and 500 nm in Fig. 3. **Fig. 4**. Chromoplast with very few plastoglobuli (arrow) and some clear zones (star) likely representing crystals of β-carotene. Bar = 1μm. **Figs. 5,6**. Subcellular organization of root cells after methanol treatment. Note the lack of changes after treatment. Bars = 500 nm. CW – cell wall; N – nucleus; M – mitochondrion; D – dictyosome; Ch – chromoplast.

ment with toxin extracts, but in neither case was membrane integrity compromised.

DISCUSSION

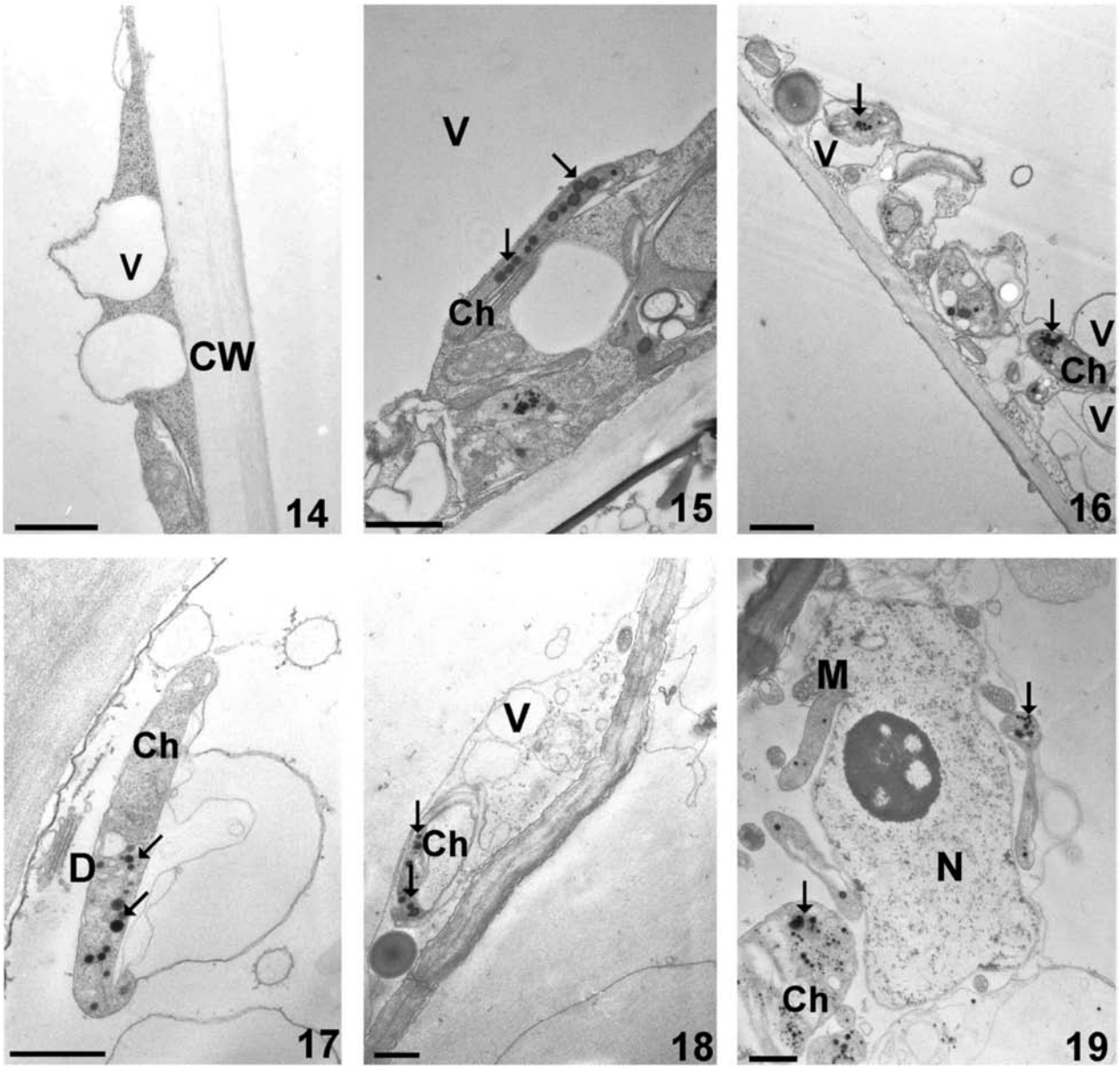
Fungal metabolites produced by necrotrophs causing necrosis of a plant are termed toxins (Švabová and Lebeda, 2005). *A. radicina* is a necrotrophic pathogen of carrot, whereas *A. alternata* is considered a saprotroph or facultative parasite associated with this plant (Tylkowska, 1991). Phytotoxins, that is, mycotoxins that are toxic to the cells of their plant hosts, may play various roles in plant pathogenesis (Švabová and Lebeda, 2005).

Both *A. radicina* and *A. alternata* are capable of producing toxins on carrot root discs in vitro, including RAD and *epi*-ROH in the case of the for-



Figs. 7–9. Ultrastructure of root vascular parenchyma cells treated with toxins at concentration of 25 μg/ml. Vesicle formation (Fig. 7 – stars), withdrawal of plasma membrane (arrows), after treatment with AME (Fig. 7) and *epi*-ROH (Fig. 8) and microvacuolation after AME treatment (Fig. 9). Bars = 500 nm. **Figs. 10–13**. Ultrastructure of root vascular parenchyma cells treated with toxins at concentration of 250 μg/ml. Note plasma membrane withdrawal (arrows) after treatment with ROH (Fig. 10), formation of small vacuoles after AME treatment (Fig. 11) and structure of mitochondrial cristae (Fig. 12) after RAD treatment. Bars = 1μm. Fig. 13. Dictyosome and chromoplast ultrastructure after *epi*-ROH treatment. Note large number of plastoglobuli inside chromoplast. Bar = 1μm. CW – cell wall; M – mitochondrion; V – vacuole; D – dictyosome; Ch – chromoplast.

mer species and AOH and AME in the latter (Solfrizzo et al., 2005). Based on the presence of RAD and ROHs, including *epi*-ROH, in diseased roots, especially in cultivars reported to be more sensitive to *A. radicina*, Solfrizzo et al. (2004b) concluded that the toxins played a role in the pathogenicity of this species. In contrast, in spite of the widespread occurrence of *A. alternata*, its toxins were not detected in roots of umbelliferous plants (Solfrizzo et al., 2005).



Figs. 14–19. Ultrastructure of root vascular parenchyma cells treated with culture filtrates. **Fig. 14.** Formation of small vacuoles along plasma membrane after treatment with *A. radicina* culture filtrates. Bar = 1μm. **Figs. 15, 16.** Vesicle formation and accumulation of plastoglobuli (arrows) in chromoplasts in root cells treated with *A. radicina* culture filtrates. Bar = 1μm. **Figs. 17–19**. Membrane vesiculation, vacuole formation and accumulation of plastoglobuli (arrows) in chromoplasts of cells in root treated with *A. alternata* culture filtrates. Bars = 1μm. CW – cell wall; N – nucleus; M – mitochondrion; V – vacuole; D – dictyosome; Ch – chromoplast.

The present study demonstrated alterations induced by solutions of *A. radicina* and *A. alternata* toxins, and by culture filtrates of both fungi, in some cellular structures of carrot root xylem. The extent of changes was much greater after treatment with 250 μg/ml of either the toxins or fungal cultures than after treatment at a tenfold lower concentration. This may suggest that although metabolites of *A. radicina* and *A. alternata* are capable of inducing alterations in cell structure, their effect strengthens with the concentration. What is the lowest level of toxin that leads to the observed alterations? There is considerable variation of the occurrence of RAD and ROHs in naturally infected carrot roots and inoculated carrot discs, depending on the cultivar and the *A. radicina* isolate used. The recorded ranges of RAD and ROHs are 0.20–9.48 μg/g in naturally infected and 213–40,964 μg/g in artificially inoculat-

ed carrot roots, and they have been detected at 2.4–2944 μg/g in inoculated carrot root discs (Solfrizzo et al., 2004b, 2005; unpublished data). The production of AOH and AME in inoculated carrot discs ranges from 10 μg/g to 922 μg/g (Solfrizzo et al., 2005; unpublished data). These data do not permit a conclusive statement about the local concentrations of the toxins in carrot tissue, due to differences in the methodologies of the cited and current works. The toxins applied in the present study are known as non-host-specific. According to Rotem (1998), non-host-specific toxins are less damaging to plants than host-specific ones, and are not a prerequisite for infection although both could be involved at any or all stages of infection. Thomma (2003) pointed out that although non-host-specific toxins had a relatively mild phytotoxic effect, they might act as a factor predisposing the host to disease.

No differences were noted in the responses of cellular structures to the toxins produced by either of the *Alternaria* species tested, although they are known to differ in their pathogenicity to carrot. Could wounding, and thus weakening of the root tissue, have played a role in enhancing the activity of AOH and AME?

The phytotoxicity of a number of secondary metabolites present in culture filtrates of pathogenic fungi has been demonstrated (Švabová and Lebeda, 2005). In the present study, alterations observed after treatment with fungal culture filtrates were more extensive than after treatment with toxin solutions. Culture filtrate from *A. radicina* contained RAD at 19.3 ± 2.6 μg/ml, whereas neither AOH nor AME were detected in culture filtrate of *A. alternata*. Based on our results, the concentration of RAD in the culture filtrate apparently was too low to induce visible changes in cell structures. These data suggest the involvement of other metabolites, and probably enzymes, in the processes that led to the alterations observed in the cell structures of carrot root xylem. According to Thomma (2003), enzymatic processes in *Alternaria* infections are generally similar to those in other diseases. Polygalacturonase, pectin methylesterase and cellulase were detected in liquid cultures of *A. radicina* (Curren, 1969). The involvement of enzymes in induction of cellular alterations has been described in carrot roots infected by *Mycocentrospora acerina* (Le Cam et al., 1997) and *Pythium violae* (Guérin et al., 1998).

The Golgi apparatus in the analyzed cells produced a large number of small vesicles, which became incorporated in the plasma membrane. This kind of vesiculation, especially when associated with the plasma membrane, may indicate the occurrence of exocytotic processes that could remove and restore possible damage in membranes. A similar response was described earlier in a dehydration-tolerant plant (Corbineau et al., 2004). It is an important finding that membrane integrity was not compromised after either toxin or culture filtrate treatments, protecting these cells from electrolyte leakage. Interestingly, plasma membrane modifications, including vesicle formation, were reported in *Pyrus pyrifolia* treated with AK-I, a toxin produced by *Alternaria alternata* Japanese pear pathotype (Shimizu et al., 2006). These authors suggested that lipid peroxidation in membranes was induced by reactive oxygen species generated as a result of the activity of this host-specific toxin.

Our results have shown the involvement of some toxic metabolites in the formation of ultrastructural alterations in carrot root cells, but this phenomenon apparently is more complex, and awaits elucidation.

ACKNOWLEDGMENTS

We thank Dr. Michele Solfrizzo of CNR, Institute of Sciences of Food Production (Bari, Italy) for kindly providing *epi*-radicinol, Dr. Małgorzata Jędryczka of the Institute of Plant Genetics, Polish Academy of Sciences (Poznan, Poland) for her valuable help in preparing culture extracts, and Dr. Rund W. van den Bulk of Plant Research International (Wageningen, The Netherlands) for his valuable suggestions. This work was supported by the European Commission, Quality of Life and Management of Living Resources Programme (contract No QLK1–1999–0986), Key Action 1 on Food, Nutrition and Health, and by the Polish State Committee for Scientific Research (KBN).

REFERENCES

Bottalico A, and Logrieco A. 1998. Toxigenic *Alternaria* species of economic importance. In: Sinha HK, and Bhatnagar D [eds], *Mycotoxins in agriculture and food safety*, 65–108. Marcel Dekker, New York.

Corbineau F, Berjak P, Pammenter N, Vinel D, Picard MA, and Come D. 2004. Reversible cellular and metabolic changes induced by dehydration in desiccation-tolerant wheat seedling shoots. *Physiologia Plantarum* 122: 28–38.

Curren T. 1969. Pectic and cellulolytic enzymes of *Stemphylium radicinum*. *Canadian Journal of Microbiology* 15: 1241–1243.

Dhingra OD, and Sinclair JB. 1986. *Basic plant pathology methods*, 355. CRC Press, Boca Raton, FL.

Guérin L, Benhamou N, and Rouxel F. 1998. Ultrastructural and cytochemical investigations of pathogen development and host reactions in susceptible and partially-resistant carrot roots infected by *Pythium violae*, the major causal agent for cavity spot. *European Journal of Plant Pathology* 104: 653–665.

Hansen RO. 1954. Stemphylone, a root-killing substance from *Stemphylium radicinum*. *Acta Chemica Scandinavica* 8: 1332–1334.

Kohmoto K, Itoh Y, Shimomura N, Kondoh Y, Otani H, Kodama M, Nishimura S, and Nakatsuka S. 1993. Isolation and biological activities of two host-specific toxins from tangerine pathotype of *Alternaria alternata*. *Phytopathology* 83: 495–502.

Le Cam B, Leberton L, Massiot P, and Rouxel F. 1997. Production of cell-wall polysaccharide degrading enzymes in carrot root tissues infected by *Mycocentrospora acerina*. *Plant Pathology* 46: 276–281.

Maude RB. 1966. Studies on the etiology of black rot, *Stemphylium radicinum* (Meier, Drechsler et Eddy) Neerg., and leaf blight, *Alternaria dauci* (Kühn) Groves et Skolko, on carrot crops and on fungicide control of their seed-borne infection phases. *Annals of Applied Biology* 57: 83–93.

Meier FC, Drechsler C, and Eddy ED. 1922. Black rot of carrots caused by *Alternaria radicina* N. sp. *Phytopathology* 12: 157–168.

Montemurro N, and Visconti A. 1992. *Alternaria* metabolites – chemical and biological data. In: Chełkowski J, and Visconti A [eds], *Alternaria*: *Biology, plant diseases and metabolites*, 449–557. Elsevier Sciences Publisher, Amsterdam, London, New York, Tokyo.

Nakajima H, Ishida T, Otsuka Y, Hamasaki T, and Ichinoe M. 1997. Phytotoxins and related metabolites produced by *Bipolaris coicis*, the pathogen of job's tears. *Phytochemistry* 45: 41–45.

Neergaard P. 1945. *Danish species of Alternaria and Stemphylium*. Oxford University Press, London.

Park P, Tsuda M, Hayashi Y, and Ueno T. 1977. Effect of a host-specific toxin (AM-toxin I) produced by *Alternaria mali*, an apple pathogen, on the ultrastructure of plasma membrane of cells in apple and Japanese pear leaves. *Canadian Journal of Botany* 55: 2383–2393.

Pryor BM, Davis RM, and Gilbertson RL. 1994. Detection and eradication of *Alternaria radicina* on carrot seed. *Plant Disease* 78: 452–456.

Pryor BM, Davis RM, and Gilbertson RL. 1998. Detection of soilborne *Alternaria radicina* and its occurrence in California carrot fields. *Plant Disease* 82: 891–895.

Pryor BM, and Gilbertson RL. 2002. Black Rot. In: Davis RM, and Raid RN [eds], *Compendium of Umbelliferous crops*, 25–27. APS Press, St. Paul, Minnesota.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron microscopy. *Journal of Cell Biology* 17: 208–212.

Robeson DJ, Gray GR, and Strobel GA. 1982. Production of the phytotoxins radicinin and radicinols by *Alternaria chrysanthemi*. *Phytochemistry* 21: 2359–2362.

Rotem J. 1998. *The genus Alternaria. Biology, epidemiology, and pathogenicity*. APS Press, St. Paul, Minnesota.

Scheffer RP. 1992. Ecological and evolutionary roles of toxins from *Alternaria* species pathogenic to plants. In: Chełkowski J, and Visconti A [eds], *Alternaria*: *Biology, plant diseases and metabolites,* 101–122. Elsevier Sciences Publisher, Amsterdam, London, New York, Tokyo.

Shabana YM, Baka ZAM, and Abdel-Fattah GM. 1997. *Alternaria eichhorniae*, a biological control agent for waterhyacinth: mycoherbicidal formulation and physiological and ultrastructural host responses. *European Journal of Plant Pathology* 103: 99–111.

Shimizu N, Hosogi N, Hyon GS, Jiang S, Inoue K, and Park P. 2006. Reactive oxygen species (ROS) generation and ROS-induced lipid peroxidation are associated with plasma membrane modifications in host cells in response to AK-toxin I from *Alternaria alternata* Japanese pear pathotype. *Journal of General Plant Pathology* 72: 6–15.

Solfrizzo M, de Girolamo A, Vitti C, Tylkowska K, Grabarkiewicz-Szczęsna J, Szopińska D, and Dorna H. 2005. Toxigenic profile of *Alternaria alternata* and *Alternaria radicina* occurring on umbelliferous plants. *Food Additives and Contaminants* 22: 302–308.

Solfrizzo M, de Girolamo A, Vitti C, Visconti A, and van den Bulk R. 2004a. Liquid chromatographic determination of *Alternaria* toxins in carrots. *Journal of AOAC International* 87: 101–106.

Solfrizzo M, Vitti C, de Girolamo A, Visconti A, Logrieco A, and Fanizzi FP. 2004b. Radicinols and radicinin phytotoxins produced by *Alternaria radicina* on carrots. *Journal of Agriculture and Food Chemistry* 52: 3655–3660.

Spurr AR. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* 26: 31–43.

Švabová L, and Lebeda A. 2005. In Vitro selection for improved plant resistance to toxin-producing pathogens. *Journal of Phytopathology* 153: 52–64.

Tal B, Robeson DJ, and Aasen AJ. 1985. Phytotoxins from *Alternaria helianthi*: Radicinin and the structures of deoxyradicinol and radianthin. *Phytochemistry* 24: 729–731.

Thomma BPHJ. 2003. Pathogen profile: *Alternaria* spp.: from general saprophyte to specific parasite. *Molecular Plant Pathology* 4: 225–236.

Tylkowska K. 1991. *Alternaria radicina* M.D. et E. w materiale siewnym marchwi. *Roczniki AR Poznań, Rozprawy* 220: 1–69.

Tylkowska K, Grabarkiewicz-Szczęsna J, and Iwanowska H. 1998. Wpływ radicininy, alternariolu oraz eteru metylowego alternariolu na kiełkowanie nasion marchwi. *Materiały z VIII Krajowej Konferencji "Grzyby mikroskopowe – patogeny roślin i ich toksyczne metabolity"*: 59–62. Instytut Genetyki Roślin Polskiej Akademii Nauk, Poznań.

Tylkowska K, Grabarkiewicz-Szczęsna J, and Iwanowska H. 2003. Production of toxins by *Alternaria alternata* and *A. radicina* and their effects on germination of carrot seeds. *Seed Science and Technology* 31: 309–316.

Tylkowska K, Grabarkiewicz-Szczęsna J, Szopińska D, Dorna H, Solfrizzo M, and de Girolamo A. 2005. Wpływ temperatury i okresu inkubacji na tworzenie toksycznych metabolitów przez *Alternaria radicina* i *A. alternata*. *Acta Agrobotanica* 59: 7–18.

Visconti A, Sibilia A, and Sabia C. 1992. *Alternaria alternata* from oilseed rape: mycotoxin production, and toxicity to *Artemia salina* larvae and rape seedlings. *Mycotoxin Research* 8: 9–16.