UNIVERSITÀ DEGLI STUDI FIRENZE

FLORE
Repository istituzionale dell'Università degli Studi di Firenze

Cytological and ultrastructural responses of Platanus acerifolia(Ait.) Willd. leaves to cerato-platanin, a protein fromCeratocystis fimbriata

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Cytological and ultrastructural responses of Platanus acerifolia(Ait.) Willd. leaves to cerato-platanin, a protein fromCeratocystis fimbriata f. sp. platani / A. Bennici, R. Calamassi, L. Pazzagli, C. Comparini, S. Schiff, R. Bovelli, B. Mori, C. Tani, A. Scala. - In: PHYTOPATHOLOGIA MEDITERRANEA. - ISSN 0031-9465. - STAMPA. - 44:(2005), pp. 153-161.

Availability:

The webpage https://hdl.handle.net/2158/326580 of the repository was last updated on

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

07 September 2026

Phytopathol. Mediterr. (2005) 44, 153–161

Cytological and ultrastructural responses of *Platanus acerifolia* (Ait.) Willd. leaves to cerato-platanin, a protein from *Ceratocystis fimbriata* f. sp. *platani*

Andrea Bennici[1], Roberto Calamassi[1], Luigia Pazzagli[2], Cecilia Comparini[3], Silvia Schiff[1], Ruggero Bovelli[1], Bruno Mori[1], Corrado Tani[1] and Aniello Scala[3]

[1]Dipartimento di Biologia Vegetale, Laboratori di Botanica Agraria e Forestale, Università, P.le delle Cascine 28, 50144 Firenze, Italy
[2] Dipartimento di Scienze Biochimiche, Università, V.le Morgagni 50, 50134 Firenze, Italy
[3] Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università, Via della Lastruccia 10, 50019 Sesto Fiorentino, Firenze, Italy

Summary. Cerato-platanin (CP) is a purified protein isolated from the culture filtrate of the ascomycete *Ceratocystis fimbriata* f. sp. *platani* (*Cfp*), the causal agent of canker stain disease of plane. The responses of cells/tissues of plane leaves to CP was studied by light microscopy (LM) and by transmission electron microscopy (TEM) using two experimental procedures. The most significant responses occurred already at 24 h after treatments, and were also visible at 48 h. The main effects of CP were to cause a great increase in primary starch and a certain degree of intercellular and intracellular disorganization of the spongy parenchyma cells and plasmolysis processes. In addition, an increase of intracellular phenolic compounds was observed in the palisade cells. The effects of *Cfp* were similar but less evident than those of CP.

Key words: plane tree, canker stain, phytotoxicity, cytology, primary starch.

Introduction

The ascomycete *Ceratocystis fimbriata* (Ell. and Halst.) Davidson f. sp. *platani* Walter (*Cfp*) is the causal agent of canker stain, a severe disease of *Platanus* species in the Mediterranean part of Europe. *Cfp* attacks *Platanus acerifolia* (Ait.) Willd., a common ornamental urban tree (Anselmi *et al*., 1994; Vigouroux *et al*., 1997; Panconesi, 1999). Other forms of *Cfp* are pathogenic to many other hosts, such as coffee, cocoa, aspen, etc. (Webster and Butler, 1976; Baker *et al*., 2003). *Cfp* reproduces sexually by ascospores and asexually through three different conidial forms produced by endoconidiophore hyphae (Panconesi, 1981). *Cfp* can penetrate into the branches and trunks of trees only through wounds caused by pruning and through root anastomosis (Mutto Accordi *et al*., 1978; Panconesi, 1981). Unlike the typical vascular wilt pathogens, *Cfp* colonizes not only the xylematic vessels, but also the sapwood and the deeper wood tissues. As the disease progresses, infected bark becomes necrotic. The only way to control canker stain is still by prevention (Panconesi, 1999). Currently *P. acerifolia* lines resistant to the pathogen are not commercially available in Europe,

Corresponding author: A. Bennici
Fax: +39 055 360137
E-mail: andrea.bennici@unifi.it

although some clones of the American species *P. occidentalis* L., a direct progenitor of *P. acerifolia* (Vigouroux *et al*., 1997), have been found to be naturally resistant to *Cfp*. However, *P. occidentalis* is not adapted to the European climate (El Modafar *et al*., 1995). In this connection, the first hybrid plane clone showing complete canker stain resistance has recently been obtained in France (Vigouroux and Olivier, 2004).

Recently, Pazzagli *et al*. (1999) purified and characterized a new 12.4 kDa protein from the culture filtrate of *Cfp*. This protein, named cerato-platanin (CP), is 120 amino acids in length, contains 4 cysteines forming two S-S bridges, Cys20-57 and Cys60-115, and has a high percentage (40%) of hydrophobic residues. According to the data banks, CP is the founder member of the cerato-platanin family, and its N-terminal region is very similar to cerato-ulmin, a class II hydrophobin involved in the pathogenesis of Dutch elm disease (Del Sorbo *et al*., 2002). CP self-assembles, is located in the *Cfp* cell walls, is secreted early in *Cfp* culture filtrates, and elicits phytoalexin and/or cell necrosis in host and non-host tissues (Pazzagli *et al*. 1999, 2001, 2004; Boddi *et al*. 2004; Scala *et al*., 2004).

In the present work we investigated and characterized the *P. acerifolia* leaf tissue response to CP by light microscopy (LM) and transmission electron microscopy (TEM), and compared the changes caused by CP with those presumed to be caused by *Cfp*.

Materials and methods

Fungal culture and CP purification

The origin and long-term maintainance of *Cfp* strain Cf AF 100 was described by Pazzagli *et al*. (1999). Conidia of this strain were obtained by growing *Cfp* in potato dextrose broth (PDB) on a rotary shaker at 90 rpm at 24°C in the dark. Conidia were collected from 3-day-old PDB cultures, washed three times with sterile distilled water, and adjusted to a concentration of 4×10^4 conidia ml^{-1} for leaf treatment.

CP was purified according to Pazzagli *et al*. (1999).

Leaf treatment

Two experimental procedures were used: either the CP solution was applied as droplets to the leaf surface, or the leaf petiole was dipped in the CP solution.

With the first procedure newly matured leaves were collected in May–June 2002 from two *P. acerifolia* trees. Twenty-eight leaves (14 per tree) of the same age were cut from two branches (7 leaves per branch). The leaves were carefully washed with distilled water, dried on both sides with filter paper, and placed in glass Petri dishes each containing five moist Whatman filter paper sheets (1 leaf per dish). Ten 10 μl droplets of water each containing 1 nmol of CP were applied to one half of the lower surface of the leaf blade. Control drops containing sterile distilled water and 4×10^2 conidia were applied to the other half of the same leaf surface. Droplets were evenly distributed over the leaf surface. Treated leaves were maintained in Petri dishes at 25°C under continuous light from Sylvania Daylight fluorescent tubes F36W/154-ST (35 μmol m^{-2} s^{-1}) for 24, 48 and 72 h. Starting from 0 h, 6–7 leaves (3–4 per tree) were taken each day for examination.

In the second experimental procedure, the leaf petioles were dipped in 100 μM CP or in distilled water (control), and maintained for 24 and 48 h at the same temperature and light conditions as above. During the treatment periods, these leaves were kept in a moist chamber. At each testing date 4 leaves were taken (2 leaves per tree, 12 leaves in total). Portions of foliar lamina were excised from the basal (near the petiole), the middle and the upper leaf zones and processed for LM examination as described below.

Cyto-histological procedures

For LM examination, all leaf fragments from both types of treatment and controls, and including a surrounding area (0.5 cm wide) immediately adjacent to the treated part, were excised, washed 4 times with distilled water, and fixed in a 2.5% glutataraldehyde solution buffered in 0.1 M sodium phosphate, pH 7.4. This material was dehydrated in an ethanol series (from 40 to 100%) and embedded in LR Gold resin. Serial sections (2 μm thick) were cut with a Reichert OM-U3 microtome using glass knives. These sections were then stained with the following stains: blue astra fucsin (for the cell wall), fucsin (wall and phenols), Lugol (starch), periodic acid Schiff (PAS) (starch), aniline blue black plus blue astra and fucsin (pro-

teins, phenols, wall), Schiff reagent plus calcofluor (cellulose and modified walls) (Hughes and McCully, 1975; O'Brien and McCully, 1981; Mori and Bellani, 1996; Kraus *et al*., 1998).

For TEM examination, both treated and control leaf fragments, excised and washed as above, were prefixed overnight in a solution of 2.5% glutaraldehyde and 4% paraformaldehyde, adjusted to pH 7.2 with 0.1 M phosphate buffer, then washed with the same buffer and fixed in 2% osmium tetroxide. After dehydration through a gradual alcohol series from 40 to 100%, the samples were embedded in Spurr's (1969) epoxy resin. Ultrathin sections obtained with a Reichert ultramicrotome (OM U3-S) were stained with uranyl acetate (Gibbons and Grimstone, 1960) and lead citrate (Reynolds, 1963), and examined in a Philips TEM 300 operating at 80 KV.

Results

Foliar lamina treatments

Cyto-hystological responses to the direct application of CP and *Cfp* conidia to the leaf surface were visible as early as 24 h after treatment. Changes caused by CP were the same as those caused by *Cfp* but were stronger. *Cfp* never penetrated into the leaf mesophyll, even when conidia produced abundant mycelium in the drops. After the first 24 h the chloroplasts of the palisade cells in the treated areas, especially those treated with CP, exhibited a great number of large starch grains; starch grains were less abundant in spongy tissue cells. After another 24 h the spongy cells, in particular those near palisade tissue, also appeared rich in starch reserves. At the start of the experiment the number of starch grains in the test leaves was small, and in the control leaves it remained small throughout. The results on the treated leaves after 72 h were not considered significant because of injuries to both treated and control leaves due to prolonged maintenance under artificial conditions.

After 24 h the leaf palisade and spongy cells of both CP and *Cfp* treated leaves had a more intense colour than did the same cells of the water-treated leaves: this was associated with an increased phenol content of the cytoplasm. These changes were however accompanied by the loss of the strong reddish colour initially observed in the cell walls (Fig. 1A–D). Disorganization of spongy parenchyma tissue in the form of a general loosening was also seen after 24 h. In addition, plasmolysis phenomena were observed in both palisade and spongy tissue cells especially after 24 h in response to CP. The mesophyll zones treated with CP or *Cfp* became thinner than the surrounding tissues or than the mesophyll zones in the water controls (Fig. 1E, F and Fig. 2A, B). Fig. 2C and D show that *Cfp* was less effective in reducing leaf thickness than CP.

CP absorption through the petiole

The absorption of CP through the petiole caused an increase in the number of starch grains in all the leaf tissues (palisade and spongy parenchyma cells) from the base to the tip of the foliar lamina, between 24 and 48 h of treatment as compared with the water control. The starch grains also appeared to be larger. After 48 h of treatment the starch, especially in the spongy tissue, appeared in part hydrolysed. Moreover, numerous starch grains of a greater size were observed in the cells near the principal leaf veins (Fig. 2E, F). No other effect of CP absorption through the petioles was seen.

The higher starch levels in leaf tissues treated with CP, observed under the LM, were confirmed with the TEM. Chloroplasts rich in large starch grains were very numerous in the palisade cells but less numerous in the spongy tissue (Fig. 3A–C). Moreover, in the spongy cells the tylacoidal system of the chloroplasts was disorganized and the cytoplasm presented abnormal vesicular structures; the tonoplast and other membrane systems displayed breaks at many points. In particular, the plasmalemma and chloroplast membranes were sometimes indistinct or fragmented (Fig. 3 E). Another symptom induced by CP was an alteration of the outer epidermis and guard cell walls, which showed the inclusion of a material of a crystalline nature (Fig. 3D, F, G).

Discussion

The present study provided evidence that CP is perceived by plane leaf cells both when it is applied in solution to the leaf surface and when it is absorbed through the petiole, and causes serious tissue and cell alterations. Similar leaf damage was caused also by *Cfp* when applied to the leaf surface, though it was less severe than with CP. How-

ever, C*fp,* which is not a foliar pathogen, never entered the leaves. Therefore, to explain the damage observed we should assume that the fungus was able to produce in the inoculum liquid (a 10 μl droplet of distilled water) and in a short period of time (24 h or less) enough toxin to be adsorbed by the leaf tissues and to produce the alterations observed. On the other hand, we should also consider that CP is located in the *Cfp* cell walls (Boddi *et al*., 2004) and it is abundantly released from the

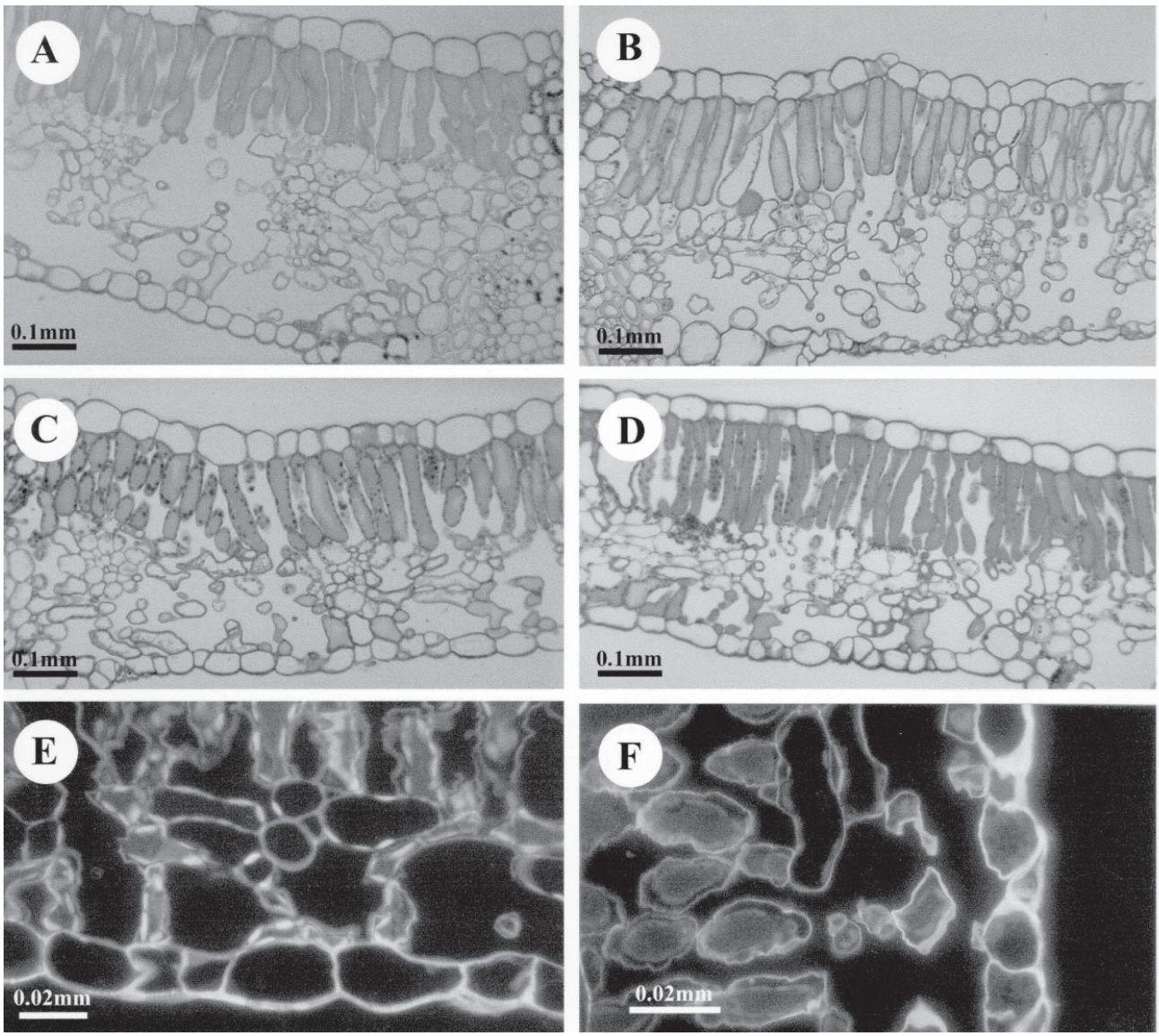

Fig. 1. Light microscopy sections of *Platanus acerifolia* leaves after the first experiment. (A, B) Half leaves treated with distilled water droplets after 24 and 48 h: the leaf anatomy is completely normal; note the absence of starch grains in all cells. Bar=0.1 mm. (C, D) The same half-leaves treated with pure cerato-platanin (CP) after 24 and 48 h: the palisade and spongy cells. The latter, especially after 48 h, show numerous starch grains in the cytoplasm. Moreover, the palisade cell cytoplasm has a brighter colour than the control(s) due to higher levels of phenols. Bar=0.1 mm. (E, F) Intercellular and intracellular disorganization in spongy leaf tissue caused by treatment with *Ceratocystis fimbriata* f. sp. *platani* and CP respectively. Cell plasmolysis processes are particularly evident in the CP-treated leaf. Bar=0.02 mm.

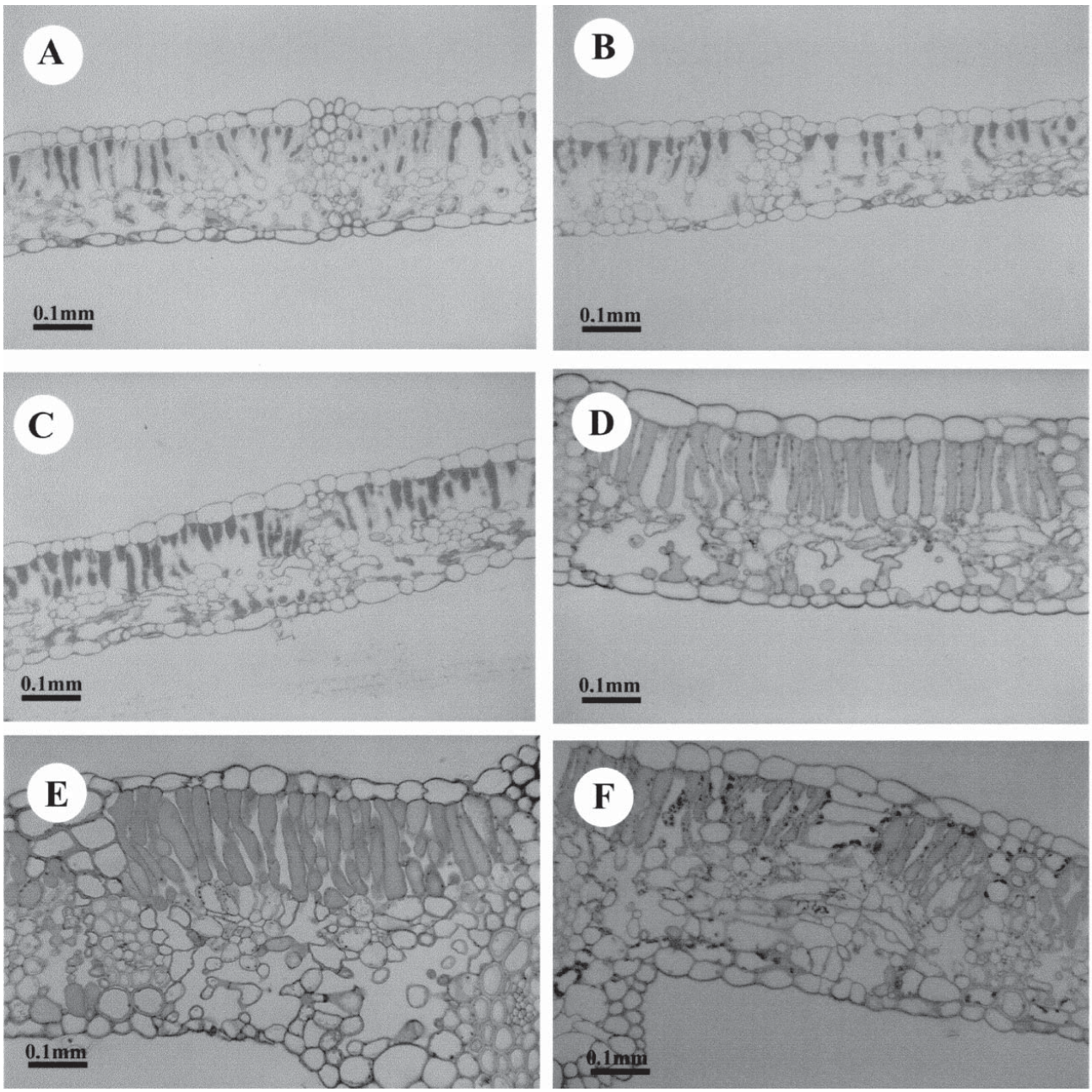

Fig. 2. Light microscopy sections of *Platanus acerifolia* leaves after the first (A, B, C, D) and second (E, F) experimental procedure. (A, B) Leaves treated with cerato-platanin (CP) and control leaves after 24 h: note the reduced leaf thickness due to plasmolysis and loosening of the spongy cells in the treated leaf as compared with the control. (C, D) Effects of *Ceratocystis fimbriata* f. sp. *platani* (*Cfp*) and CP after 48 h. More starch grains are formed with CP than with *Cfp*. (E, F) Control and CP treated leaves after 48 h. The higher starch levels in all leaf cells with CP applied to the leaf surface are clearly visible: in the control leaf starch remains very low. All pictures bar=0.1 mm.

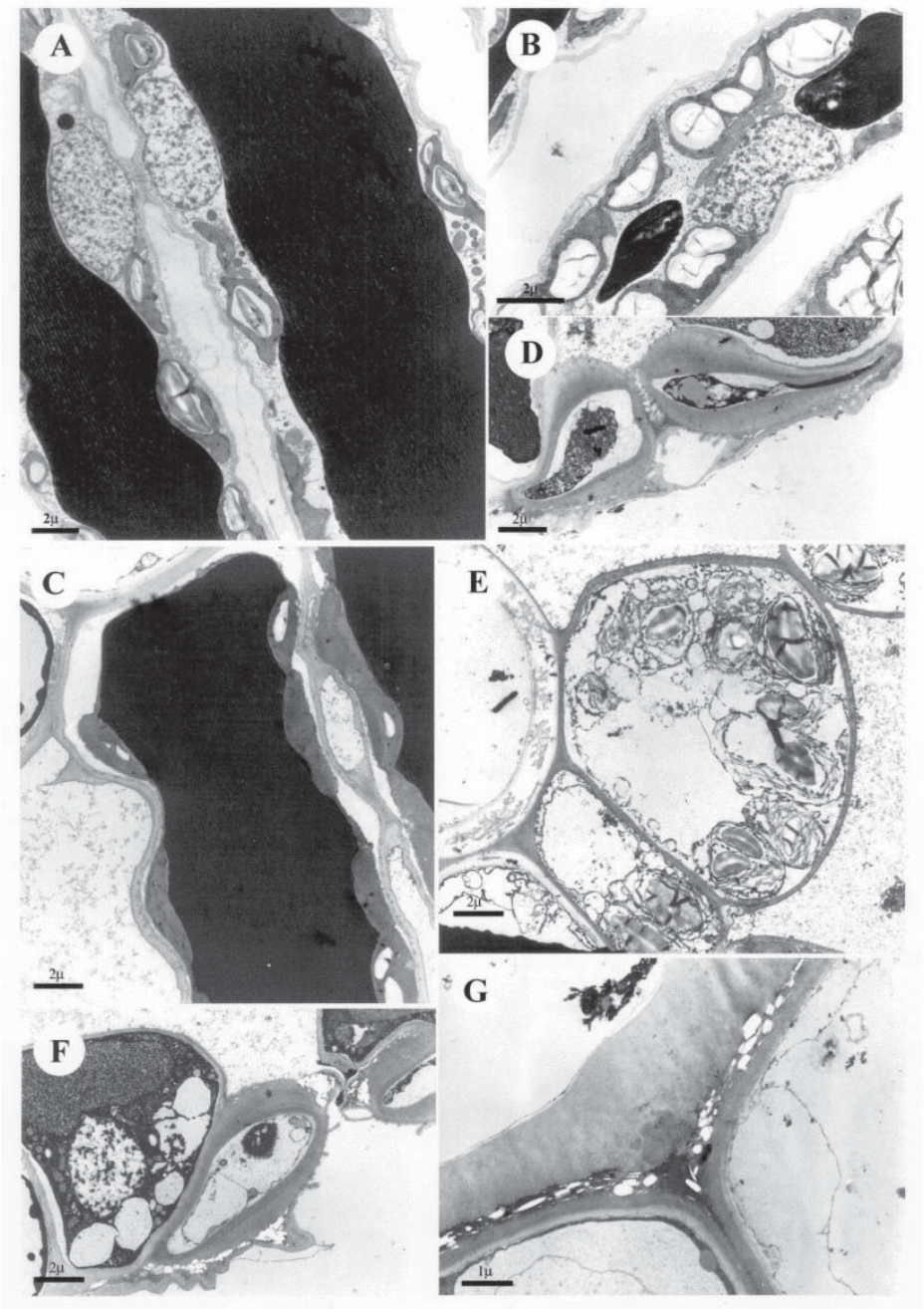

Fig. 3. Transmission electron microscopy (TEM) sections of *Platanus acerifolia* leaves. (A) Palisade cells of a control leaf: particular showing chloroplasts containing starch grains. Bar=2μm. (B) Cerato-platanin (CP) treated leaf: palisade cell(s) presenting chloroplasts with large starch reserves. Bar=2μm. (C) Some spongy cells of CP treated leaf: note the low starch content, detachment of cytoplasm from the walls and plasmolysis phenomena. Moreover, the chloroplast structure is not clearly distinguishable. Bar=2μm. (D) Guard cells of control leaf exhibiting normal structure. Bar=2μm. (E) Spongy cells of the leaf under the CP-treated zone: the chloroplasts show a disorganized tylacoidal system as well as starch grains, and the cytoplasm has a degenerated appearance with fragmented membranes and vesicular parts, no nuclei are visible, and plasmolysis processes are evident. Bar=2μm. (F) A particular of a stoma in a CP-treated leaf showing crystalline material, cytoplasm and nucleus alterations in the outer cell walls. Bar=2μm. (G) Particular of a CP-treated leaf epidermis: abundant crystalline material occurs in the middle part of the cell walls. Bar=1μm.

first day of growth in liquid (PDB) shake culture (Pazzagli *et al*., 2001; Scala *et al*., 2004).

The present work showed that plane leaves are a simple and practical means to study CP biological activity on plane. CP caused a number of cytological and histological changes. One major change was an accumulation of starch in the chloroplasts of the palisade and spongy tissue cells. This change was well visible at 24 h with both types of treatment and with both LM and TEM examination. The starch grains were significantly larger and more numerous in the treated leaves than in the controls. Significant increases in the starch content of leaves are commonly reported to be caused by abiotic (de Groot *et al*., 2003; Kim and Wetzstein, 2003; Kivimäenpää *et al*., 2003; Widodo *et al*., 2003; Zaidi *et al*., 2003; Liu *et al*., 2004) as well as by biotic stress. For example, various leguminous plants show higher starch levels in those of their cells that are infected with *Rhyzobium* spp., whereas in *Anadenanthera peregrina* var. falcata, a Brazilian woody leguminous, starch accumulation occurred throughout the infected tissue and inner cortex, but only in uninfected cells (Gross *et al*., 2002). Moreover, the starch metabolism of cabbage hypocotyls was impaired when they were infected with *Plasmodiophora brassicae* (Williams *et al*., 1968) and fluctuations in starch levels were found in rust-infected wheat leaves (Mac Donalds *et al*., 1970) and in various other plants (Mirocha and Zaki, 1966) invaded by rust and mildew fungi. Recently, apicidin, a cyclic tetrapeptide toxin produced by the plant pathogens *Fusarium sambucinum* and *F. semitectum*, has been reported to cause excess starch in the frond cells of duckweed (*Lemna pausicostata* L.), and to enhance host cell electrolyte leakage and chloroplast deterioration (Abbas *et al*., 2001; 2002). This higher starch content and larger granule size could be due to an increase of photosynthesis and/or a greater activity of enzymes involved in the starch biosynthetic pathway, or to reduced starch hydrolysis. It is generally accepted that, unlike what occurs in the storage organs, starch in the leaves is remobilized each night and the enzymes responsible for starch degradation are also present by day, when they could be active during starch synthesis in the chloroplast. In our case CP could be considered a primary signal in the plane leaf cells indicating a rise in photosynthesis levels as part of the wider metabolic context in which certain plant defence compounds or their precursor substances are formed. The partial starch hydrolysis observed in the leaves 48 h after absorption of the CP solution strengthens this hypothesis. El Modafar *et al*. (1993; 1995) reported that independently of the degree of resistance/susceptibility to *Cfp*, plane leaves when appropriately stimulated produced defence-related phenolic compounds such as scopoletin and umbelliferone, two phytoalexins with known antifungal properties (Lusso and Kuć, 1999). Phytoalexin (umbelliferone) synthesis was elicited in *P. acerifola* leaves by leaf treatment with CP (Pazzagli *et al*., 1999; Scala *et al*, 2004). Phytoalexins formed only after the primary metabolic precursors were diverted into novel secondary metabolic pathways (Hammond-Kosack and Jones, 2000). In this connection, the high phenol levels observed under the light microscope in the plane cell cytoplasm of leaves treated with CP suggested that CP stimulated defence-related reactions in the plane leaves, even though it also strongly reduced the size of the cell walls.

Another type of leaf response to CP consisted in various cell alterations such as cell plasmolysis and the fragmentation of the plasmalemma, the tonoplast, and the membranes of the chloroplasts and cytoplasmatic vesicles. Taken as a whole these alterations showed the strong effect of CP on leaf tissues and resembled the type of damage caused by many phytotoxins (Daly, 1981; Scheffer, 1983). These CP-induced structural or functional changes, especially at the cell plasma membrane level, may stimulate ion effluxes, which may in turn be a prerequisite for the activation of defence mechanisms (Hammond-Kosack and Jones, 2000). In this case both the plasmolysis processes and the partial loosening of the spongy tissue that was observed would be only secondary consequences.

The results provide new information about the biological activity of CP. This protein was known to elicit phenolic phytoalexin synthesis in plane leaves and soya cotyledons, and an apparently hypersensitive cell necrosis in tobacco leaves and soya cotyledons. CP behaved like a typical fungal non-host specific elicitor. Moreover, the localization of CP in the *Cfp* cell walls and its abundant early release in *in vitro* culture suggested that CP might be one of the first *Cfp* molecules to be perceived by the host. The use of plane leaves as sampling ma-

terial was justified by El Modafar (1995) and finds further justification in the fact that many other elicitors also act on susceptible hosts. The present study confirmed that CP strongly increased phenol production, whereas starch accumulation alone is not a certain sign that CP behaves as an elicitor. The strong activity of CP in altering cell structure and functions suggests that it has a toxin-like ability to damage plane leaf tissues and cells. Wolpert *et al*. (2002) gave some examples of the activity of host-selective toxins against plant tissues, which is sometimes similar to the activity of avirulence determinants. A comparison of some toxins and elicitors showed that it was not always easy to define the markers of disease resistance and separate them from those indicating susceptibility. The outcome of a micro-organism-plant interaction depends on many factors, which together determine the activity of the pathogen and the defence response of the host. Interestingly, the foliar pathogen *Pyrenophora tritici-repens* produces the 13.2 kDa Ptr Tox A protein, a host-selective necrosis toxin that causes disturbances in the functioning of plasma membranes in susceptible wheat. Ptr Tox A requires the active involvement of host processes, including de novo mRNA and protein synthesis (Kwon *et al*., 1998; Manning *et al*., 2004).

Further studies must investigate the manner of CP penetration and migration through the leaf, how the CP signal is transmitted to the cell nucleus, and whether CP has a role in the natural *Cfp* infection of plane trees.

Acknowledgements

The work was supported by the Ministero dell'Università e della Ricerca Scientifica.

Literature cited

Abbas H.K., J.W. Gronwald, K.L. Plaisance, R.N. Paul and Y.W. Lee, 2001. Histone deacetylase activity and phytotoxic effects following exposure of duckweed (*Lemna pausicostata* L.) to apicidin and HC-toxin. *Phytopathology* 91, 1141–1148.

Abbas H.K., W.T. Shier, J.W. Gronwald and Y.W. Lee, 2002. Comparison of phytotoxicity and mammalian cytotoxicity of nontrichothecene mycotoxins. *Journal of Natural Toxins* 11, 173–186.

Anselmi N., L. Cardin and G. Nicolotti, 1994. Plane decline in European and Mediterranean countries: associated pests and their interactions. *EPPO Bulletin* 24, 159–171.

Boddi S., C. Comparini, R. Calamassi, L. Pazzagli, G. Cappugi and A. Scala, 2004. Cerato-platanin protein is located in the cell walls of ascospores, conidia and hyphae of *Ceratocystis fimbriata* f. sp. *platani*. *FEMS Microbiology Letters* 233, 341–346.

Daly J.M., 1981. Mechanisms of actions. In: *Toxins in Plant Disease* (R.B. Durbin, ed.), Academic Press, New York, NY, USA, 331–394.

de Groot C.C., R. den Boogaard, L.F.M. Marcelis, J. Harbinson and H. Lambers, 2003. Contrasting effects of N and P deprivation on the regulation of photosynthesis in tomato plants in relation to feedback limitation. *Journal of Experimental Botany* 54, 1957–1967.

Del Sorbo G., A. Scala, F. Scala and S. Tegli, 2002. Ceratoulmin, a toxin produced by the pathogens of the Dutch elm disease. In: *Advances in Microbial Toxin Research and its Biotechnological Exploitation* (R.K. Upadhyay, ed.), Kluwer Academic Plenum Publishers, New York, NY, USA, 93–104.

El Modafar C., A. Clérivet, A. Fleuriet and J.J. Macheix, 1993. Inoculation of *Platanus acerifolia* with *Ceratocystis fimbriata* f. sp. *platani* induces scopoletin and umbelliferone accumulation. *Phytochemistry* 34, 1272–1276.

El Modafar C., A. Clérivet, A. Vigouroux and J.J. Macheix, 1995. Accumulation of phytoalexins of plane tree (*Platanus* spp.) expressing susceptibility or resistance to *Ceratocystis fimbriata* f. sp. *platani*. *European Journal of Plant Pathology* 101, 503–509.

Gibbons I.R. and A.V. Grimstone, 1960. On the flagellate structure in certain flagellates. *Journal of Biophysical and Biochemical Cytology* 7, 697–7116.

Gross E., L. Cordeiro and F.H. Caetano, 2002. Nodule ultrastructure and initial growth of *Anadenanthera peregrina* (L.) Speg. var. *falcata* (Benth.) Altschul plants infected with *Rhizobia*. *Annals of Botany* 90, 175–183.

Hammond-Kosack K. and J.D.G. Jones, 2000. Responses to plant pathogens. In: *Biochemistry & Molecular Biology of Plants*. (B.B. Buchanan, W. Gruissen, R.L. Jones, ed.), American Society of Plant Physiologists, Rockville, MD, USA, 1102–1156.

Hughes J. and M.E. McCully, 1975. The use of an optical brightener in the study of plant structure. *Stain Technology* 50, 319–329.

Kim T. and H.Y. Wetzstein, 2003. Cytological and ultrastructural evalutions of zinc deficiency in leaves. *Journal of the American Society for Horticultural Science* 128, 171–751.

Kivimäenpää M., S. Sutiven, P.E. Karlsson and G. Selldén, 2003. Cell structural changes in the needles of Norway Spruce exposed to long-term ozone and drought. *Annals of Botany* 92, 779–793.

Kraus J.E., H.C. de Sousa, M.H. Rezende, N.M. Castro, C. Vecchi and R. Luque, 1998. Astra blue and basic fuchsin double staining of plant materials. *Biotechnic & Histochemistry* 73(5), 235–243.

Kwon C.Y., J.B. Rasmussen and S.W. Meinhardt, 1998. Activity of Ptr ToxA from *Pyrenophora tritici-repentis* requires host metabolism. *Physiological and Molecular Plant Pathology* 52, 201–212.

Liu F., C.R. Jensen and M.N. Andersen, 2004. Drought stress effect on carbohydrate concentration in soybean leaves and pods during early reproductive development: its implication in altering pod set. *Field Crops Research* 86, 1–13.

Lusso M. and J. Kuć, 1999. Plant Responses to Pathogens. In: *Plant responses to Environmental Stresses*. (H.R. Lerner, ed.), Marcel Dekker, Inc., New York, NY, USA, 683–706.

Mac Donalds P.W. and G.A. Strobel, 1970. Adenosine diphosphate-glucose pyrophosphorylase control of starch accumulation in rust-infected wheat leaves. *Plant Physiology* 46, 126–135.

Manning V.A., R.M. Andrie, A.F. Trippe and L.M. Ciuffetti, 2004. Ptr ToxA requires multiple motifs for complete activity. *Molecular Plant-Microbe Interactions* 17, 491–501.

Mirocha C.J. and A.I. Zaki, 1966. Fluctuations in amount of starch in host plants invaded by rust and mildew fungi. *Phytopathology* 56, 1220–24.

Mori B. and L. Bellani, 1996. Differential staining for cellulosic and modified plant cell walls. *Biotechnic and Histochemistry* 71(2), 71–72.

Mutto Accordi S., M. Ferrara and V. D'Ambra, 1978. Ricerche al microscopio elettronico sul legno di platano invaso da *Ceratocystis fimbriata* f. sp. *platani*. *Phytopathologische Zeitschrift* 91, 39–51.

O'Brien T.P. and M.E. McCully, 1981. *The Study of Plant Structure. Principles and Selected Methods*. Thermarcaphi Pty. Ltd., Melbourne, Australia.

Panconesi A., 1981. *Ceratocystis fimbriata* of plane trees in Italy: biological aspect and control possibility. *European Journal of Forest Pathology* 11, 385–395.

Panconesi A., 1999. Canker stain of plane trees: a serious danger to urban plantings in Europe. *Journal of Plant Pathology* 81(1), 3–15.

Pazzagli L., G. Cappugi, G. Manao, G. Camici, A. Santini and A. Scala, 1999. Purification, characterization, and amino acid sequence of cerato-platanin, a new phytotoxic protein from *Ceratocystis fimbriata* f. sp. *platani*. *The Journal of Biological Chemistry* 274, 24959–24964.

Pazzagli L., G. Cappugi, G. Manao, G. Camici, A. Santini, C. Comparini and A. Scala, 2001. Cerato-platanin, a hydrophobin-like protein, is rapidly released in culture by *Ceratocystis fimbriata* f. sp. *platani*. In: *Proceedings of the 5th Congress of the European Foundation for Plant Pathology*, September 18–22, 2000, Taormina-Giardini Naxos, Italy, 280–282.

Pazzagli L., L. Carresi, B. Pantera, C. Zoppi, G. Cappugi, C. Comparini, B. Tiribilli, A. Spisni and A. Scala, 2004. Cerato-platanin, a phytotoxic protein from *Ceratocystis fimbriata* f. sp. *platani:* cloning, expression and functional characterization. *Proceedings of the 7th National Biotechnology Congress*, September 8–10, 2004, Catania, Italy, 280–282 (abstract).

Reynolds E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 117, 208–212.

Scala A., L. Pazzagli, C. Comparini, A. Santini, S. Tegli and G. Cappugi, 2004. Cerato-platanin, an early-produced protein by *Ceratocystis fimbriata* f. sp. *platani*, elicit phytoalexin synthesis in host and non-host plants. *Journal of Plant Pathology* 86, 23–29.

Scheffer R.P., 1983. Toxins as chemical determinants of plant disease. In: *Toxins and Plant Pathogenesis* (J.M. Daly, B.J. Deverall, ed.), Academic Press Australia, North Ride, NSW, Australia, 1–40.

Spurr A.R., 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26, 31–43.

Vigouroux A., 1979. Les "dépérissements" des platanes: causes, importance, mesures envisageables. *Revue Forestière Francaise*, 31, 28–39.

Vigouroux A., G. Besnard, K. Sossey-Alaoui, M. Tersac and A. Bervillè, 1997. Le statut d'hybride de *Platanus acerifolia* confirmé et celui de *P. densicoma* mis en évidence à l'aide de marqueurs génétiques moléculaires; conséquences. *Acta Botanica Gallica* 144, 243–251.

Vigouroux A. and R. Olivier, 2004. First hybrid plane trees to show resistance against canker stain (*Ceratocystis fimbriata* f. sp. *platani*). *Forest Pathology* 34, 307–319.

Widodo W., J.C.V. Vu, K.J. Boote, J.T. Baker and L.H. Allen, Jr., 2003. Elevated growth CO_2 delays drought stress and accelerates recovery of rice leaf photosynthesis. *Environmental and Experimental Botany* 49, 259–272.

Williams P.H., N.T. Keen, J.D. Strandberg and S.S. McNabola, 1968. Metabolite synthesis and degradation during clubroot development in cabbage hypocotyls. *Phytopathology* 58, 921–928.

Wolpert T.M., 2002. Host-selective toxins and avirulence determinants: what's in a name? *Annual Review in Phytopathology* 40, 251–285.

Zaidi P.H., S. Rafique and N.N. Singh, 2003. Response of maize (*Zea mays* L.) genotypes to excess soil moisture stress: morpho-physiological effects and basis of tolerance. *European Journal of Agronomy* 19, 383–399.

Accepted for publication: April 4, 2005