BIOLOGIA PLANTARUM 50 (4): 675-682, 2006

Peroxidase, catalase, amine oxidase and acid phosphatase activities in *Pisum sativum* during infection with *Fusarium oxysporum* and *F. solani*

L. LUHOVÁ*, A. LEBEDA**[1], E. KUTROVÁ*, D. HEDERER OVÁ*,** and P. PEČ*

Department of Biochemistry and Department of Botany**, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic*

Abstract

Two genotypes (cv. Smaragd and line DP1059) of *Pisum sativum* with different susceptibility to *Fusarium oxysporum* and *F. solani* and influence of pathogenesis on enzyme activities were studied. The increase of activity of studied enzymes was mostly observed in both roots and shoots during pathogenesis. Only activity of acid phosphatase decreased in the root and increased in shoots. The correlation between enzyme activity change and susceptibility of pea cultivars to *F. oxysporum* or *F. solani* was observed.

Additional key words: defence response, degree of susceptibility, enzymes, fusarium wilt, pea, reactive oxygen species, root and stem rot, vascular wilt of pea.

Introduction

Defence mechanisms of plants against pathogens include especially the production of reactive oxygen species, the synthesis of antimicrobial phytoalexins, the induction of hydrolytic enzymes (*e.g.*, chitinase, glucanase), the construction of defensive barriers (*e.g.*, lignin, suberin), and the hypersensitive reaction (Hammond-Kosack and Jones 1996, Mellersh *et al.* 2002, Šindelář and Šindelářová 2005). Produced ROS participate in the damage of the attacking pathogen (Peng and Kuc 1992), cross-link proteins into the plant cell wall (Bradley *et al.* 1992), lignification of cell wall (Olson and Varner 1993), and induction of expression of a variety of defence-related genes. A final result of the induced oxidative burst may be its participation in the hypersensitive response (Hückelhoven and Kogel 2003, Levine *et al.* 1994). The role of enzymes in plant-fungal pathogen interactions was summarised by Lebeda *et al.* (2001).

Peroxidase (POX) and catalase (CAT) belong to the important enzymes removing active oxygen species in plants. Plant peroxidases (EC 1.11.1.7) [donor: hydrogen peroxide oxidoreductase] have been studied for: *1*) important role in lignification (Gaspar *et al.* 1991) and suberization (Mohan and Kolattudy 1990); *2*) active participation in the formation of diphenyl bridges (Pena *et al.* 1996); *3*) the cross-linking of hydroxyproline-rich proteins (extensins) in the cell wall matrix (Brownleader *et al.* 1994); *4*) control function of redox state in apoplast (Takahama 1993). The catalase (EC 1.11.1.6) [hydrogen-peroxide:hydrogen-peroxide oxidoreductase] is present as multiple isoforms in plants (Scandalios 1994). The catalases play the role in the protection of cells from the toxic effects of hydrogen peroxide. The participation of copper-containing amine oxidase (AO) (EC 1.4.3.6.) [amine:O_2 oxidoreductase (deaminating)] in defence response was confirmed (Angelini *et al.* 1993). It has been suggested that H_2O_2, generated by amine oxidation, is important in lignification both in normal and stress conditions (Angelini *et al.* 1993, Rea *et al.* 1998). Acid phosphatases (AP) (EC 3.1.3.2) [orthophosphoric-monoester phosphohydrolase (acid optimum)] hydrolysing a number of phosphomonoesters and phosphoproteins are widely distributed in plants. The expression of some isoforms with this activity seems to be under hormonal control. It is known that acid phosphatase isoforms are induced by phosphate starvation (Duff *et al.* 1994).

Received 3 March 2005, *accepted* 22 September 2005.

Abbreviations: AO - amine oxidase; AP - acid phosphatase; CAT - catalase; CDA - Czapek-Dox agar; dai - days after inoculation; DI - degree of infection; DS - degree of susceptibility; hai - hours after inoculation; POX - peroxidase; ROS - reactive oxygen species.

Acknowledgements: Authors express the sincere thanks to Mr. Ian McLenaghan for comments to the English. This research was supported by grants: "Stress and pathological biology, biochemistry and bioenergetics of plants" (MSM 153100010) and "Variability of components and interactions in plant pathosystem and impact of environmental factors on their expression " (MSM 6198959215) of the Czech Ministry of Education.

[1] Corresponding author; fax: (+420) 585634800; e-mail: lebeda@prfholnt.upol.cz

Fusarium spp. are important pathogens of agricultural crops (Zemánková and Lebeda 2001). *F. oxysporum* Schlecht. f. sp. *pisi* (Van Hall) Snyd. *et* Hans. causes vascular wilt of pea, because of its mycelium plugs in the tracheas. *F. solani* (Mart.) App. *et* Wr. causes root and stem rot following by necrosis and death of leaves (Kraft 1994). Both pathogens occur very often simultaneously. Pathogenic forms of *Fusarium* spp. penetrate a host root either through wounds or directly through root apices and the fungus moves into the vascular tissue. The pathogen is spread throughout the plant by means of mycelia growth or conidia, primarily microconidia, produced in infected xylem vessel elements. One of the earliest responses to *Fusarium* spp. infection is the deposition within contact cells of additional wall callose material (papillae). The vascular plugging was found to seal off xylem elements of resistant pea cultivars, the same as physical barriers (lignification) could retard or prevent vascular invasion (Kraft 1994).

The aim of this work was the study of some enzymes, mainly those participating in the metabolism of ROS during pathogenesis of *Fusarium* spp. on *Pisum sativum* with different degree of susceptibility of roots and above-ground parts of plants.

Materials and methods

Plants and cultivation: The seeds of field pea (*Pisum sativum* L.) cv. Smaragd and line DP1059, were obtained from *Agritec Ltd*. (Šumperk, Czech Republic). Seeds were sterilised in 5 % *Chloramin* (*Bochemie Ltd.*, Bohumín, Czech Republic) for 20 min before the germination. Eight-day-old seedlings grown on wet filter paper were transplanted into glass tubes containing Czapek-Dox agar (CDA) with Knop's solution (Lebeda and Buczkowski 1986). The plants were cultivated for 28 d at 20/22 ^{o}C day/night temperature, 12-h photoperiod with an irradiance of 100 μmol m^{-2} s^{-1} in a growth chamber. The roots and above-ground parts of plants were harvested in two day intervals and stored at -50 °C or used fresh for histochemical study.

Pathogen isolates and growth conditions: *Fusarium oxysporum* Schlecht. f. sp. *pisi* (isolate 576/1997) and *F. solani* (Mart.) App. *et* Wr. (isolate 769/1997) originate from naturally infected peas grown in the Czech Republic. Cultures of both pathogens (isolates) are maintained at the Department of Botany, Palacký University in Olomouc. The isolates were multiplied on CDA in Petri dishes in dark at laboratory temperature.

Plant inoculation, incubation and disease assessment: An adapted tube resistance screening test according to Lebeda and Buczkowski (1986) was used. Isolates of *F. solani* and *F. oxysporum* were cultivated on the surface of CDA in glass tubes (diameter 16 mm) in the dark at 23 °C. Glass tubes with 7-d-old pathogen culture were used for pea seedlings growing. The plants for control experiment were grown in glass tube without pathogen. At least 10 pea plants were used for degree of infection (DI) evaluation and material sampling at each time period of the experiment. Growing of infected and control plants was realised in three repetitions. The symptoms on roots and shoots (stems and leaves) were assessed on a 0 - 3 scale (Lebeda and Buczkowski 1986). Roots: 0 - roots free of any visual symptoms; 1 - limited occurrence of local necrosis, discoloration (browning) on the main and lateral roots; 2 - mild necrotisation and reduced development of main and lateral roots; 3 - severe necrotisation and growth depression of main root, no development of lateral roots, complete collapse of roots. Shoots: 0 - stems and leaves free of any visual symptoms; 1 - limited growth depression and wilting, plant is more or less turgid; 2 - mild growth depression, wilting and chlorosis of leaves; 3 - severe wilting and chlorosis, complete collapse of plant. The final degree of infection (DI) was counted according to Lebeda and Buczkowski (1986):

$$DI\ [\%] = [\Sigma\ (n \times v)/3N] \times 100$$

where DI - of total sample, n - number of plants with certain degree of infection, v - degree of infection (0 - 3), N - total number of plants in sample.

Preparation of plant extracts and assays: Plant material was homogenised in 0.1 M Tris/HCl, pH 7.0 (ratio 1:2 (m/v)) by sea sand in a porcelain mortar. Extracts were filtered through nylon cloth and centrifuged at 12 000 g, for 30 min at 4 ^{o}C.

Enzyme activities were assayed by spectrophotometric (UV/VIS spectrophotometer *Beckman DU 7500*, Fullerton, USA) methods. AO activity was measured by method with *E*-2-butene-1,4-diamine as a substrate (Macholán *et al.* 1975). The method with guaiacol was used for determination of the POX activity (Angelini *et al.* 1990). AP activity was detected by the method with *p*-nitrophenyl phosphate used as substrate (Bergmeyer *et al.* 1974). The CAT activity was measured by assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdenate (Góth 1991).

Protein content was determined according to Bradford (1976) with bovine serum albumin as a standard.

Histochemical study: Using the hand slicing microtome, transverse sections (40 - 60 μm thick) were obtained from root and stem segments. AO was localised by the method based on tissue staining due to the oxidation of an artificial peroxidase substrate (4-chloro-1-naphthol) upon putrescine addition, as was previously reported (Angelini and Federico 1989). The same substrate was used for histochemical visualisation POX activities. The detection of lignin was performed by phloroglucinol/HCl staining

(Bate *et al*. 1994). Microscopy was carried out using a *Model BX50* light system microscope (*Olympus Optical Co*., Tokyo, Japan) equipped with a *Cool SNAP Camera System* (*Roper Scientific*, Tuscon, AR, USA) connected to a PC. Image analysis was performed using the *Cool SNAP* software (*Roper Scientific*).

Statistics: Growing and treatment of plants was done in three independent experiments (see Plant inoculation, incubation and disease assessment). The data reported in graphs are means of three repetitions. The vertical bars in the graphs represent standard errors of mean.

Results and discussion

The interaction between *Pisum sativum* and two *Fusarium* spp. was used as a model system to study some of the plant defence responses to these fungi. An intensive defence response would be expected mainly in

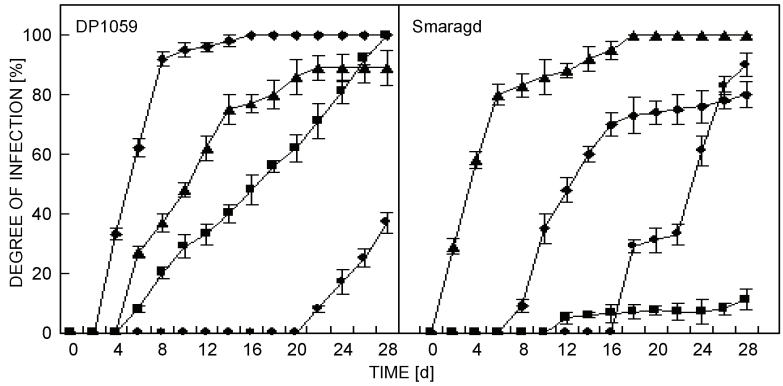

Fig. 1. Infection degree by pea genotypes DP1059 and Smaragd after inoculation by *F. oxysporum* and *F. solani* [above-ground part of plants infected by *F. oxysporum* (*circles*), *F. solani* (*squares*); roots infected by *F. oxysporum* (*triangles*), *F. solani* (*rhombs*)].

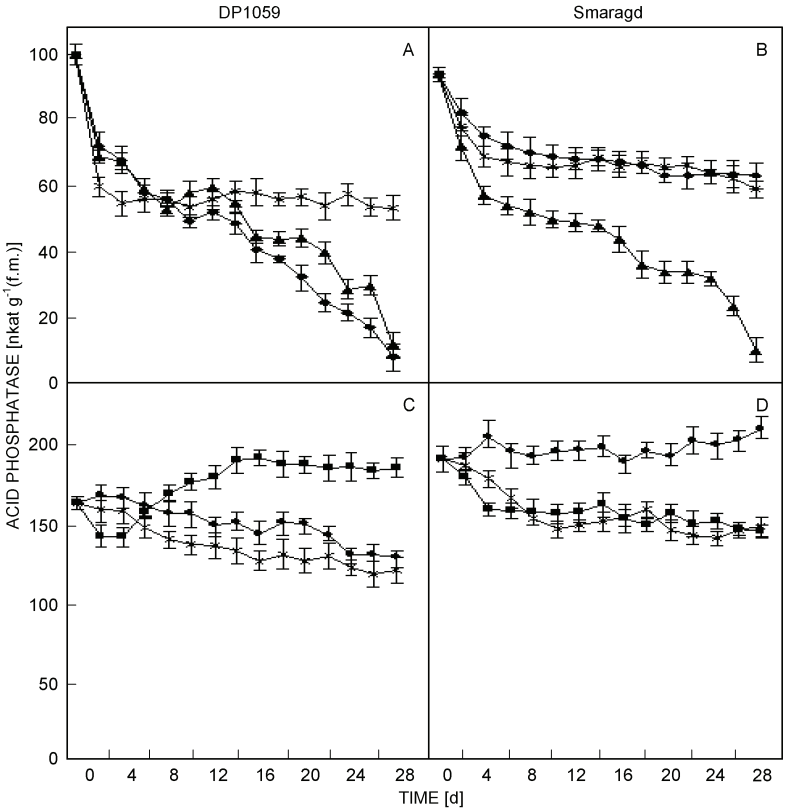

Fig. 2. Acid phosphatase activity after inoculation of pea genotypes DP1059 and Smaragd by *F. oxysporum* and *F. solani* in roots (*A*, *B*) infected by *F. oxysporum* (*triangles*) or *F. solani* (*rhombs*) and in above-ground part of plants (*C*, *D*) infected by *F. oxysporum* (*circles*) or *F. solani* (*squares*), control - noninfected (*crosses*).

the roots after primary infection by root pathogens. However, for a more comprehensive understanding of defence mechanisms we studied the subsequent influence of pathogen attack on enzyme activities in above-ground parts of the plants as well. This work adds to previous data on altered enzyme activity after infection by *F. solani* in twelve field cultivars of *P. sativum* and one accession of wild *P. sativum* ssp. *transcaucasicum* (Luhová *et al*. 2002). The enzyme activities were studied during 4 weeks after inoculation. This period is sufficient for monitoring changes in enzyme activities both during the process of pathogenesis, and also at later stages of symptom expression (Luhová *et al*. 2002).

Smaragd in comparison with DP1059 is more susceptible to *F. oxysporum* (Fig. 1). The first symptoms of root infection were recorded 2 d after inoculation (dai) on Smaragd and 6 dai on DP1059. Maximum DI (100 %) was observed 18 dai on roots of Smaragd, DP1059 was characterized by DI 89 % in this time. The first disease symptoms (wilting of plants) on shoots were obvious 18 dai in Smaragd and 22 dai in DP1059. The first symptoms of *F. solani* infection of line DP1059 occurred 4 dai in roots and 6 dai in shoots (Fig. 1). However, in case of Smaragd a presence of symptoms was found 8 dai in roots, and 12 dai in shoots. Maximum DI was recorded 16 dai in roots and 28 dai in shoots of DP1059. Pea line DP1059 shows medium degree of susceptibility (DS) to *F. oxysporum* and very high DS to *F. solani*, while Smaragd has high DS to *F. oxysporum* and low DS to *F. solani*.

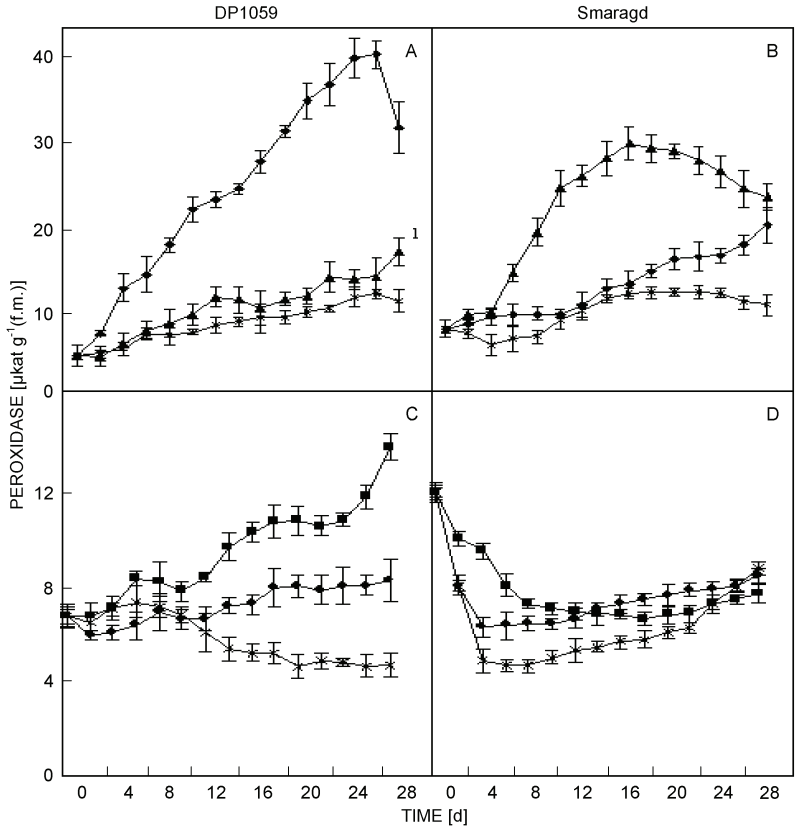

Fig. 3. Peroxidase activity after inoculation of pea genotypes DP1059 and Smaragd by *F. oxysporum* and *F. solani* in roots (*A*, *B*) infected by *F. oxysporum* (*triangles*) or *F. solani* (*rhombs*) and in above-ground part of plants (*C*, *D*) infected by *F. oxysporum* (*circles*) or *F. solani* (*squares*), control - noninfected (*crosses*).

The observed trend of changes (increased activity) in both parts of the pea plants was the same for the majority of enzymes studied during the process of pathogenesis. An exception was AP activity, which decreased in the roots and increased in the shoots (Fig. 2). The roots of Smaragd infected by *F. solani* showed similar development of AP activity like control plants. More visible changes were in DP1059 infected by *F. solani* than by *F. oxysporum*, and in Smaragd infected by *F. oxysporum* than by *F. solani*. A relationship between the AP activity changes and susceptibility of pea genotypes to *F. solani* or *F. oxysporum* was observed. AP activity in the both parts of Smaragd, with a low degree of susceptibility (highly tolerant) to *F. solani*, was the same in infected and control plants. It is known that enzymes with phosphatase activity play a role in regulating numerous enzymes. One of them is plasma membrane NADPH-dependent O_2-generating oxidase, which is active in its phosphorylated form (Low and Merida 1996). The activation of the oxidative burst is governed by a phosphorylation/dephosphorylation balance (Levine *et al.* 1994, Tenhaken *et al.* 1995). In our host-pathogen system, a substantial decrease of AP activity was observed to coincide with total damage of

the infected roots. The biochemical process associated with increased AP activity in the above-ground part of infected pea plants is not known. A relationship between changes of POX and CAT activity in infected plants and their degree of susceptibility to the fungi studied is evident. The increase of activity of both enzymes was observed in all inoculation experiments in comparison with control plants. The considerable increase of POX activity was detected in roots of DP1059 after infection by *F. solani* and in Smaragd infected by *F. oxysporum* (Fig. 3*A*). The decrease of POX activity was recorded 26th dai in DP1059 and 18th dai in Smaragd. As well as in roots, high increase of POX activity was recorded in shoots of DP1059 infected by *F. solani* (Fig. 3*B*). A more than a thirty-fold increase of CAT activity was recorded 12th dai in DP1059 infected by *F. solani*, however only three-fold after infected by *F. oxysporum* (Fig. 4*A*). Mild increase of CAT activity was found in Smaragd after infection by *F. oxysporum* in comparison with plants infected by *F. solani*. Very fast and substantial increase of CAT activity was detected in shoots of both cultivars infected by both *Fusarium* spp. followed by various levels of decrease (Fig. 4*B*). Variation in the dynamics of enzyme activity during pathogenesis was more marked after infection by *F. solani* than by *F. oxysporum*. This observation correlates with differences in degree of resistance of pea genotypes to *Fusarium* spp. The relationship between the changes of POX and CAT activities and the degree of infection was similar, but less intensive, in shoots of pea plants. The interesting dynamics of CAT activity in this part of the plants merits to the research to elucidate the role of CAT during pathogenesis. Enhanced antioxidant capacity may protect plants but it will also interfere with the signalling cascade involved in plant adaptation process. CAT contributes towards removing toxic hydrogen peroxide, production of which is typical as a non-specific defence mechanism in plant-pathogen interactions (Lamb and Dixon 1997, Low and Merida 1996). The decline of CAT activity at 12 dai (DI = 100 % in the root), resp. POX activity 28 dai, may be caused by complete necrosis of plant root tissue.

Higher POX activity in infected plants was also confirmed by histochemical methods. Transverse sections, obtained from root and stem, were stained for AO and POX activity (Fig. 5). An intensive black precipitate locates this enzyme mainly along the cell wall of root cells (Fig. 5*C*). The observed loss of shape of parenchyma cells was a typical consequence of infection. The increase of POX activity in cells of the shoots is also accompanied by intensive black colouring along cell walls (Fig. 5*F*). Peroxidases had long been postulated to produce H_2O_2, which was originally interpreted to play a

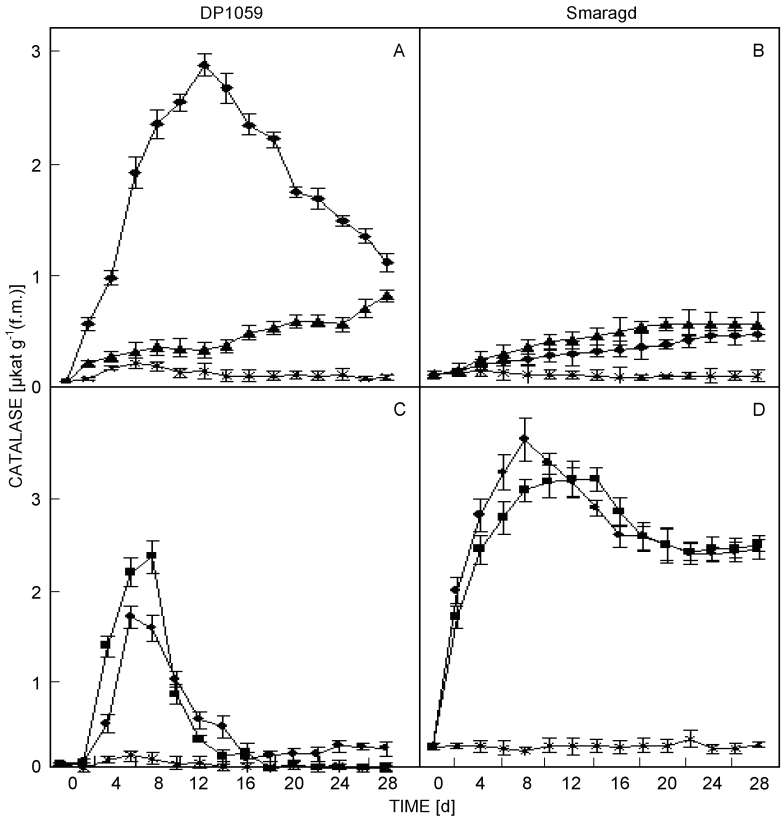

Fig. 4. Catalase activity after inoculation of pea genotypes DP1059 and Smaragd by *F. oxysporum* and *F. solani* in roots (*A*, *B*) infected by *F. oxysporum* (*triangles*) or *F. solani* (*rhombs*) and in above-ground part of plants (*C*, *D*) infected by *F. oxysporum* (*circles*) or *F. solani* (*squares*), control - noninfected (*crosses*).

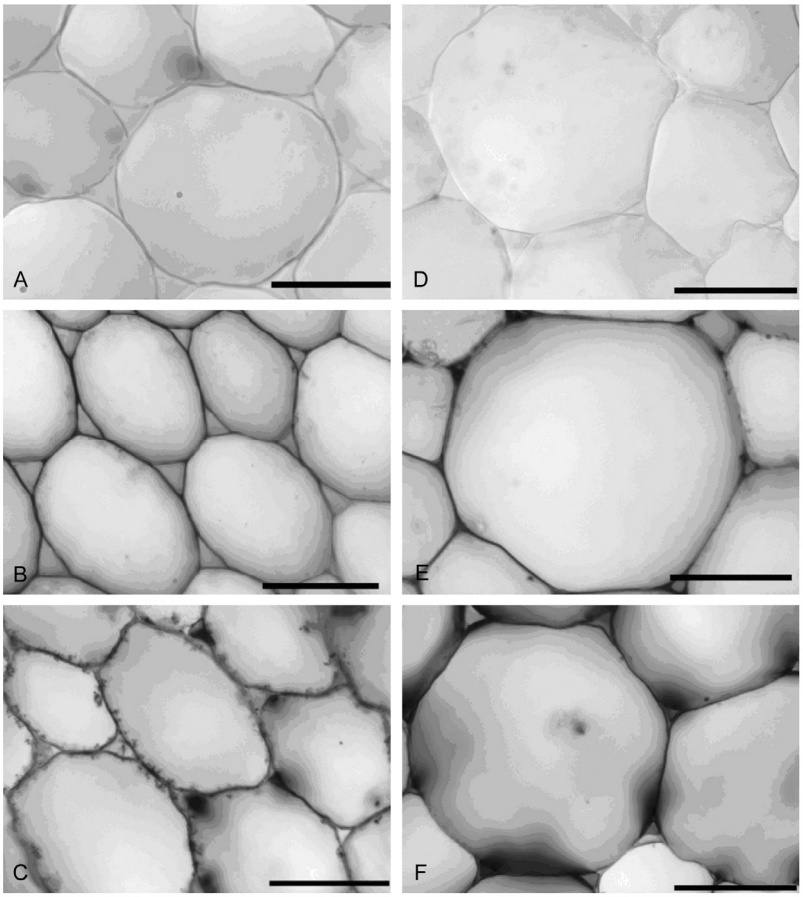

Fig. 5. Histochemical detection of POX activity in healthy and infected pea plants (cv. Smaragd infected by *F. solani*). Fresh transverse sections (approx. 40 μm thick) obtained from 28-d-old plants (20 d after inoculation of 8-d-old seedlings) were incubated in a medium containing 4-chloro-1-naphthol and H_2O_2 for histochemical determination of POX activity. Control sections were put on reaction solution without H_2O_2 (*A*, *D*). Peroxidase activity was detected in the sections of healthy plants (*B*, *E*) and infected plants (*C*, *F*). The cells of cortical parenchyma tissues from roots are in *A* - *C* and from stems in *D* - *F*.

role confined to lignification (Campa 1991). This phenomenon has been confirmed in pea plants under light stress conditions (*e.g.*, Luhová *et al*. 2003). More recently, cell wall peroxidases have been thought to be responsible for some, if not all, of the H_2O_2 production in the oxidative burst (Bestwick *et al.* 1997, Blokhina *et al.* 2003, Bolwell 1999, Montesano *et al.* 2003). Higher peroxidase activity after the infection of pea by *Fusarium* spp. may participate in removal of ROS and their generation. The second eventuality could be encouraged by the localization of these enzymes near the cell wall as was shown also in our histochemical study (an increased POX activity). However, following detailed study was aimed on behaviour of cell-wall-bound peroxidases during pathogenesis, because they belong to the potential sources of ROS in plants (Mittler 2002).

Amino oxidases are enzymes belonging to the newly recognised (Medda *et al.* 1995) group of quinoproteins, and have been subjected to intensive study since they are believed to play a key role in essential metabolic pathways in plants (Bilková *et al.* 2005). At the molecular level these enzymes degrade of biogenic amines, which are important in cell division and proliferation, apoptosis, senescence, wound healing, and mechanisms of defence against stress and pathogen infection. In our experiments, decline of AO activity during the first 8 dai was typical for physiological development of AO in plants (Fig. 6). Substantial changes of AO activity during pathogenesis were not detected. A slow decrease of AO activity occurred in inoculated plants in comparison with control plants, although an increase on the 20 dai (DI = 100 %) was detected in roots of Smaragd infected by *F. oxysporum*. The trend of a slight increase of activity was observed in the shoots of infected plants in the end of the experiment. The participation of AO (increase of activity) in defence responses was reported after mechanical injury of chick-pea seedlings (Rea *et al.* 1998, Scalet *et al.* 1991) and also after infection of pea by *Ascochyta rabiei* (Angelini *et al.* 1993). It has been hypothesised that the increase of endogenous putrescine may play specific protective roles in plants adapted to extreme environments (Angelini *et al.* 1993, Kuehn *et al.* 1990). This fact agrees with the observed increase of AO activity and subsequently with

the production of H_2O_2, a recognised plant signal molecule and substrate for peroxidases. Participation of enhanced POX activity in the cross-linking process after infection of roots was confirmed by histochemical methods (Angelini *et al.* 1993). Lignified and suberinized barriers in infected rhizodermis may have a role in blocking pathogen invasion or diffusion of toxins from it. In our case, the increased production of lignin in the rhizodermis was observed mainly after infection by *F. solani*.

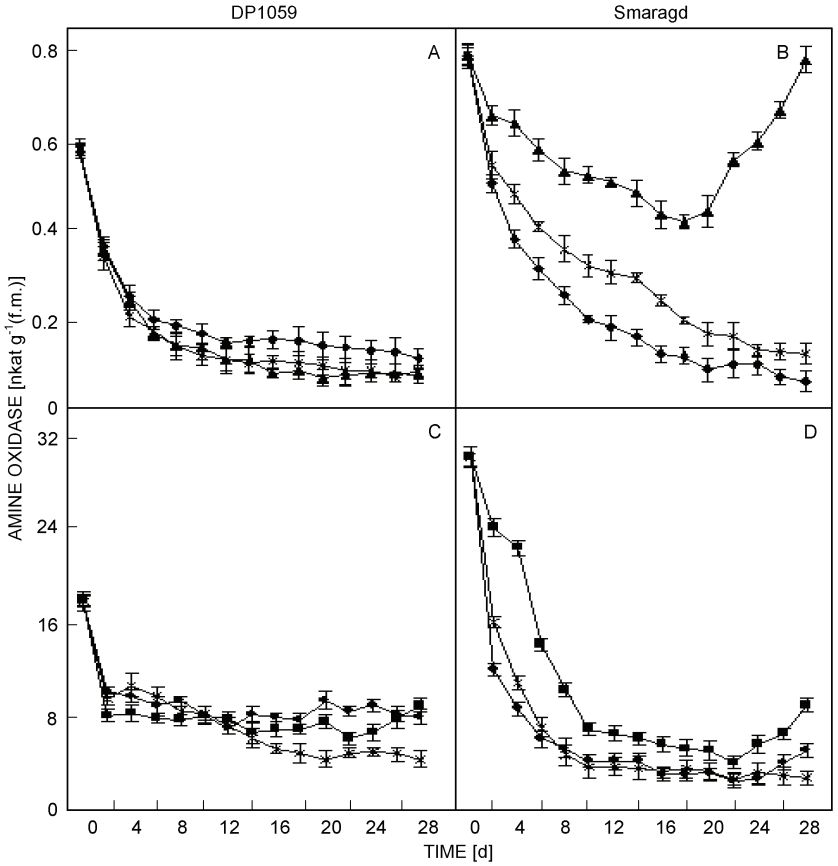

Fig. 6. Amine oxidase activity after inoculation of pea genotypes DP1059 and Smaragd by *F. oxysporum* and *F. solani* in roots (*A*, *B*) infected by *F. oxysporum* (*triangles*) or *F. solani* (*rhombs*) and in above-ground part of plants (*C*, *D*) infected by *F. oxysporum* (*circles*) or *F. solani* (*squares*), control - noninfected (*crosses*).

References

Angelini, R., Bragaloni, M., Federico, R., Infantino, A., Portapuglia, A.: Involvement of polyamines, diamine oxidase and peroxidase in resistance of chickpea to *Ascochyta rabiei.* - J. Plant Physiol. **142**: 704-709, 1993.

Angelini, R., Federico, R.: Histochemical evidence of polyamine oxidation and hydrogen peroxide production in the cell wall. - J. Plant Physiol. **135**: 212-217, 1989.

Angelini, R., Manes, F., Federico, R.: Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. - Planta **182**: 89-96, 1990.

Bate, N.J., Orr, J., Ni, W.T., Meromi, A., Nadlerhassar, T., Doerner, P.W., Dixon, R.A., Lamb, C.J., Elkind, Y.: Quantitative relationship between phenylalanine ammonia-lyase levels and phenylpropanoid accumulation in transgenic tobacco identifies a rate-determing step in natural product synthesis. - Proc. nat. Acad. Sci. USA **91**: 7608-7612, 1994.

Bergmeyer, H.U., Gawehn, K., Grassl, M.: Enzymes as biochemical reagents. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Vol. 1. Pp. 496-497. Academic Press, New York 1974.

Bestwick, C.S., Brown, I.R., Bennett, M.H., Mansfield, J.W.: Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv. *phaseolicola*. - Plant Cell **9**: 209-221, 1997.

Bilková, A., Bezáková, L., Bilka, F., Pšenák, M.: An amine oxidase in seedlings of *Papaver somniferum* L. - Biol. Plant. **49**: 389-394, 2005.

Blokhina, O., Virolainen, E., Fagerstedt, K.V.: Antioxidants, oxidative damage and oxygen deprivation stress: a review. - Ann. Bot. **91**:179-194, 2003.

Bolwell, G.P., Blee, K.A., Butt, V.S., Davies, D.R., Gardner, S.L., Gerrish, C., Minibayeva, F., Rowntree, E.G., Wojtaszek, P.: Recent advances in understanding the origin

of the apoplastic oxidative burst in plant cells. - Free Radical Res. **31**: 137-145, 1999.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Bradley, D.J., Kjellbom, P., Lamb, C.J.: Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cellwall protein: a novel, rapid defence response. - Cell **70**: 21-30, 1992.

Brownleader, M.D., Ahmed, N., Trevan, M., Chaplin, M., Dey, P.M.: A study of extensin and extensin peroxidases. - Plant Peroxidase Newslett. **4**: 3-15, 1994.

Campa, A.: Biological Roles of Plant Peroxidases: Known and Potential Function. Vol. 2. - CRC Press, Boca Raton 1991.

Duff, S.M.G., Sarath, G., Plaxton, W.C.: The role of acid plant phosphorus metabolism. - Physiol. Plant. **90**: 791-800, 1994.

Gaspar, T., Penel, C., Hagege, D., Greppin, H.: Peroxidases in plant growth, differentiation, and developmental processes. - In: Lobarzewski, J., Greppin, H., Penel, C., Gaspar, T. (ed.): Biochemical, Molecular and Physiological Aspects of Plant Peroxidases. Pp. 249-281. Université de Genève, Genève 1991.

Góth, L.: A simple method for determination of serum catalase and revision of reference range. - Clin. chim. Acta **196**: 143-152, 1991.

Hammond-Kosack, K.E., Jones, D.G.: Resistance gene-dependent plant defence response. - Plant Cell **8**: 1773-1791, 1996.

Kraft, J.M.: Fusarium wilt of peas (a review). - Agronomie **14**: 561-567, 1994.

Kuehn, G.D., Bagga, S., Rodriguez-Garay, B., Philips G.: Biosynthesis of Uncommon Polyamines in Higher Plants and Their Relationship to a Biotic Stress Responses. - American Society of Plant Physiologists, Rockville 1990.

Lamb, C., Dixon, R.A.: The oxidative burst in plant disease resistance. - Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 251-275, 1997.

Lebeda, A., Buczkowski, J.: *Fusarium oxysporum, Fusarium solani* - tube test. - In: Lebeda, A. (ed.): Methods of Testing Vegetable Crops for Resistance to Plant Pathogens. Pp. 247-249. VHJ Sempra, Research and Breeding Institute for Vegetable Crops, Olomouc 1986.

Lebeda, A., Luhová, L., Sedlářová, M., Jančová, D.: The role of enzymes in plant - fungal pathogens interactions. - J. Plant Dis. Protect. **108**: 89-111, 2001.

Levine, A., Tenhaken, R., Dixon, R., Lamb, C.: H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. - Cell **79**: 583-593, 1994.

Low, P.S., Merida, J.R.: The oxidative burst in plant defence: Function and signal transduction. - Physiol. Plant. **96**: 532-542, 1996.

Luhová, L., Hedererová, D., Lebeda, A., Peč, P.: The influence of *Fusarium solani* to enzyme activity of *Pisum sativum* cultivars. - J. Plant Dis. Protect. **109**: 113-128, 2002.

Luhová, L., Lebeda, A., Hedererová, D., Peč, P.: Activities of amine oxidase, peroxidase and catalase in seedlings of *Pisum sativum* L. under different light conditions. - Plant Soil Environ. **49**: 151-157, 2003.

Macholán, L., Hubálek, F., Šubová, H.: Oxidation of 1,4-diamino-2-butene to pyrrol, a sensitive test of diamine oxidase activity. - Collect. Czech. Chem. Commun. **40**: 1247-1265, 1975.

Medda, R., Padiglia, A., Floris G.: Plant copper-amine oxidases. - Phytochemistry **39**: 1-9, 1995.

Mellersh, D.G., Foulds, I.V, Higgins, V.J, Heath, M.C.: H_2O_2 plays different roles in determining penetration failure in three diverse plant- fungal interactions. - Plant J. **29**: 257-268, 2002.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. - Trends Plant Sci. **7**: 405-410, 2002.

Mohan, R., Kolattudy, P.E.: Differential activation of expression of a suberization-associated anionic peroxidase gene in near-isogenic resistant and susceptible tomato lines by elicitors of *Verticillium albo-atrum*. - Plant Physiol. **92**: 276-280, 1990.

Montesano, M., Brader, G., Palva, E.T.: Pathogen derived elicitors: searching for the receptors in plants. - Mol. Plant Pathol. 4: 73-79, 2003.

Olson, P.D., Varner, J.E.: Hydrogen peroxide and lignification. - Plant J. **4**: 887-892, 1993.

Pena, M.J., Revilla, G., Zarra, I.: Changes in dehydroferulic acids and peroxidase activity against ferulic acid associated with cell walls during growth of *Pisum pinaster* hypocotyl. - Plant Physiol. **111**: 941-946, 1996.

Peng, M., Kuc, J.: Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf discs. - Phytopathology **82**: 696-699, 1992.

Rea, G., Laurenzi, M., Tranquilli, E., D'Ovidio, R., Federico, R., Angelini, R.: Developmentally and wound-regulated expression of the gene encoding a cell wall copper amine oxidase in chickpea seedlings. - FEBS Lett. **437**: 177-182, 1998.

Scalet, M., Federico, R., Angelini, R.: Time courses of diamine oxidase and peroxidase activities, and polyamine changes after mechanical injury of chick-pea seedlings. - J. Plant Physiol. **137**: 571-575, 1991.

Scandalios, J.G.: Regulation and Properties of Plant Catalases. - CRC Press, Boca Raton 1994.

Šindelář, L., Šindelářová, M.: Regulation of metabolic pathways PVY-RNA biosynthesis in tobacco: host´s RNA degradation. - Biol. Plant. **49**: 309-312, 2005.

Takahama, U.: Regulation of peroxidase dependent oxidation of phenols by ascorbic acid: different effects of ascorbic acid on the oxidation of coniferyl alcohol by apoplastic soluble and cell wall bound peroxidases from epycotyls of *Vigna angularis*. - Plant Cell Physiol. **34**: 809-817, 1993.

Tenhaken, R., Levine, A., Brisson, L.F., Dixon, R.A., Lamb, C.: Function of the oxidative burst in hypersensitive disease resistance. - Proc. nat. Acad. Sci. USA **92**: 4158-4163, 1995.

Zemánková, M., Lebeda, A.: *Fusarium* species, their taxonomy, variability and significance in plant pathology. - Plant Protect. Sci. **37**: 25-42, 2001.