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Effect of oxygen concentration on plant growth, lipidperoxidation, and receptivity of tomato roots to *Pythium* F under hydroponic conditions

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Abstract

The effects of the nutrient solution oxygenation on the growth of tomato plants and colonization of plant roots by *Pythium* F707, an isolate with filamentous non-inflated sporangia, were investigated under hydroponic conditions. Lipoperoxidation was also estimated determining lipoxygenase activity and conjugated dienes. Tomato plants were grown under either a high (11–14%; Air treatment), a moderate (5.8–7%; Control) or a low (0.8–1.5%; Nitrogen treatment) oxygen concentration and inoculated or not with the pathogen. The high oxygen treatment resulted in a marked increase in plant growth, as measured by shoot and root weights. Root and top weights were about the same in the nitrogen-treated plants and the controls. In these treatments, plants started showing typical symptoms of root decay and infection within 6 days after inoculation with *Pythium* F, while highly oxygenated plants remained healthy throughout the experiment and showed a significant decrease in root colonization by the pathogen, as estimated by the immunoenzymatic staining procedure and isolation of thalles on selective medium. Nitrogen-treated plants and controls produced higher amounts of conjugated dienes and revealed increased lipoxygenase activities in comparison with highly oxygenated plants. These differences were more pronounced after inoculation with the pathogen. Our data suggest that increases in lipoxygenase activity detected in the present study in tomato roots grown under oxygen stress and inoculated with *Pythium F* may lead to degradation and disorganization of membrane lipids. That disorganization may facilitate root colonization by the pathogen and appearance of decay.

Introduction

Root rots caused by *Pythium* spp. resulting in stunting and reduced yields on a wide range of greenhouse-grown crops, including tomato (*Lycopersicon esculentum*), are fairly common (Chérif et al., 1994; Zinnen, 1988). Losses from *Pythium* infections are particularly important under hydroponic conditions which favor proliferation and spread of these pathogens (Chérif and Bélanger, 1992). While *Pythium ultimum* Trow and *P. aphanidermatum* (Edson) Fitzp. have been reported to be extremely virulent under hydroponic conditions, potentially destroying a crop within few days (Chérif et al., 1994), the majority of the other *Pythium* spp. are generally considered as 'minor' root pathogens that reduce plant growth without causing obvious symptoms (Cook and Papendick, 1972; Drew and Lynch, 1980). Recent epidemiological studies (Rafin, 1993) revealed that *Pythium* isolates of the group F, characterized by their filamentous non-inflated sporangia, were frequently associated with tomato roots in soilless cultures resulting in yield losses even in absence of root rot symptoms. *Pythium* F spp. are considered to be non-pathogenic or moderately pathogenic in soil (Rafin, 1993; Rey et al., 1996). The frequent infections observed under hydroponic conditions may indicate a higher receptivity of tomato roots to these microorganisms. Therefore, our attention has focused on the role of environment in the development of these diseases in the hope of obtaining information useful in reducing

damages related to *Pythium* F infections in hydroponic culture systems.

Environmental factors such as nutrition, temperature and light can be closely monitored in hydroponic cultures to provide optimal growth conditions and minimal stress for the crop. On the other hand, monitoring of oxygen concentration in the nutrient solution immediatly around plant roots is difficult to achieve. Plants grown in hydroponic systems can quickly deplete the dissolved oxygen in the nutrient solution resulting in poor root aeration especially when greenhouse temperatures are high (Zinnen, 1988). Such conditions are quite similar to those occurring in the field when soil is subject to prolonged periods of water saturation associated with poor drainage and improper irrigation (Drew and Lynch, 1980).

The effects of poor aeration of soil on root function and plant growth have been extensively studied in literature (Jackson et al., 1991). Many reports revealed that root diseases caused by fungal pathogens, especially those belonging to the genera of *Pythium* and *Phytophthora*, are favored by water saturated soil conditions (Drew and Lynch, 1980; Kuan and Erwin, 1980). Some investigators have studied the direct effect of oxygen concentration on the development of disease symptoms and a correlation has been usually established between inadequate oxygenation and aggravation of disease symptoms (Eldon Brown and Kennedy, 1966; Kuan and Erwin, 1980). Nevertheless, to our knowledge, no studies were performed to evaluate the effects of low levels of oxygen tension on infection of plant roots by plant pathogenic fungi under hydroponic conditions. Poor aeration of the nutrient solution under these conditions may affect the physiology and function of plant roots and these deleterious conditions may be more favorable to infection by the typical 'minor' root pathogens *Pythium* F spp.

The causes of aggravation of disease symptoms under poor aeration conditions remain obscure, probably because of the multiplicity of mechanisms. Studies associating physiological or biochemical explanations to this phenomenon are very rare (Drew and Lynch, 1980). Nevertheless, it is known that stress conditions and/or pathogen infections result in the peroxidation of unsaturated fatty acids and degradation of cell membranes, which are characteristic symptoms of oxidative plant damage (Baker and Orland, 1995; Croft et al., 1990; Todd et al., 1990). Oxidative damage is generally associated with the induction of biological processes in plant tissues, including the formation of active oxygen species and free radicals that are deleterious to the host cells and could contribute to damage caused by pathogens as well as serve as antimicrobial agents (Edreva, 1989). Free radicals result from the peroxidation of membrane lipids which occurs when levels of active oxygen species and/or lipoxygenase (LOX) activity are increased (Croft et al., 1990). Growing plants under conditions of poor root aeration could affect peroxidation of plant membrane lipids and consequently affect the plant-pathogen interaction.

The objectives of the present studies were: i) to determine the effects of oxygenation on tomato plants growth, on fungal colonization of the roots and on development of *Pythium* root rot under hydroponic conditions; and ii) to estimate lipid peroxidation, as determined by conjugated dienes titre and lipoxygenase activity, in the tomato-*Pythium* F interaction under different oxygen concentrations.

Materials and methods

Plant material. Tomato seeds cv. Typico were surface sterilized by immersion in 70% ethanol for 1 min, soaked in 2% aqueous sodium hypoclorite for 10 min, then thoroughly rinsed and soaked overnight in distilled water. They were then sown in rockwool cylinders (3×5 cm) (Grodan) and fertilized daily with a nutrient solution for 4 wk under greenhouse conditions. The nutrient solution was prepared with tap water; its final pH was adjusted to 5.8 and electrical conductivity ranged from 2 to 2.5 mS/cm. The plants were then transferred to 2.5-L plastic containers filled with nutrient solution, and supported with styrofoam flotation boards. One board holding 2 plants spaced 12 cm apart was floated on top of the nutrient solution in each container and covered with a black plastic. Plants were kept in the greenhouse at 22–25 °C and were supplemented with 16 h of cool -white fluorescent light (145 μE m^{-2} s^{-1}).

Inoculations. Inoculum was prepared from the isolate *Pythium* F707 obtained from tomato roots by Rafin from soilless cultures during an epidemiological study conducted in 1990 in Brittany (France). *Pythium* F707 was chosen as a representative of *Pythium* spp. with filamentous non-inflated sporangia. The isolate was grown on potato-dextrose-agar (PDA) on 9-cm petri dishes for 7 days. Zoospores were produced by placing ten 7-mm-diameter plugs of PDA culture of the fungus in petri dishes containing 20 ml of sterile distilled water. The dishes were incubated overnight in the dark

at room temperature. For each container to be inoculated, 15 ml of a 10^5/ml suspension of zoospores were added to the nutrient solution. Sterile distilled water was used for the controls.

Effect of oxygenation on plant growth and fungal development. Once tomato plants had been transferred into plastic containers, they received one of the three following treatments: (A) high oxygen supply ranging from 11 to 14% (5–7 ppm), obtained by bubbling of the nutrient solution with compressed air; (B) low oxygen supply ranging from 0.8 to 1.5% (0.4–0.7 ppm), obtained by bubbling with compressed nitrogen instead of air; and (C) moderate oxygen supply ranging from 5.8 to 7% (2.5–3 ppm), where the nutrient solution was not treated (control). Eighteen containers were used for each treatment, for a total of 108 plants per experiment. For the first two treatments (A and B), the flow rate of oxygen was monitored in order to maintain approximatively the same oxygen concentration in every container of the same series. Percentage oxygen was measured with an MG2-PC analyser (Biocyber, St. Philbert du Peuple, France). Two weeks after being transferred to the containers, the plants were inoculated by adding *Pythium* F707 zoospores. The experiment was carried out for 12 days after the plant inoculation by the pathogen. By that time, roots of inoculated plants which had been treated according to A or C procedures exhibited advanced stages of decay. Roots and aerial parts were collected from three replicate plants per treatment every 2 days after inoculation and weighed. A portion of the root system was processed to estimate root colonization by the pathogen, and the remaining part was frozen at -70 °C until used for biochemical analysis. The experiment was performed three times.

Effect of oxygenation on root colonization by Pythium *F707.* Two methods were used for assessing root colonization by *Pythium* F707. For each sampling day and treatment, root samples were segregated into two sets: the first was used to isolate *Pythium* F707 on selective medium by applying the culture plate technique and the other one was used to detect fungal hyphae on tomato roots by using the immunoenzymatic staining procedure.

Culture plate technique. Tomato roots (3 g) were rinsed 3 times in distilled water and blended for 30 s in 100 ml of distilled water. Successive dilutions 1, 1/10, 1/100, 1/1000 were made from the suspension, and 200 μl of each dilution were added to a *Pythium*-selective medium coded CMA-PARP (Jeffers and Martin, 1986). Three replicates of 4 plates were used for each dilution, and after 48-72 h of incubation in the dark at 22 °C, *Pythium* thalles were counted. Results were expressed as number of propagules per g of fresh roots.

Immunoenzymatic staining procedure. Whole roots were cut into 5-mm segments and were stained immunoenzymatically according to the method previously described by Rafin et al. (1994). Root segments were incubated in a blocking buffer for 1 h in wells of a 96-well assay plate (Microwell 96U, Nunc A/S, Kamstrup, Denmark). They were then incubated for 2 h at room temperature in antiserum prepared against *Pythium* F (1/500 dilution in the blocking buffer) (Rafin et al., 1994). After incubation, the root segments were washed three times with PBS-Tween, then incubated for 1 h at room temperature in goat antirabbit IgG (1/500) conjugated with alkaline phosphatase. The roots were then washed as described above, and incubated in naphtol-AS-biphosphate (Sigma) plus fast blue BB substrate solution for 5 min at room temperature. The root pieces were rinsed in distilled water before examination with a light microscope. *Pythium* hyphae were counted on 10 cm of root segments. Three replicates per sampling day and per treatment were performed.

Effect of oxygenation on lipidperoxidation. Lipidperoxidation of membrane lipids was estimated by determining lipoxygenase activity and analysing conjugated dienes.

Lipoxygenase activity. Root tissues (500 mg fresh weight of whole roots) were ground with mortar and pestle in liquid nitrogen to a fine powder, further ground in 0.1 M potassium buffer (pH 7.5) containing 10 mM $Na_2S_2O_5$, 1 mM EDTA and 1% insoluble polyvinylpyrrolidone (PVP, Sigma). Homogenates were centrifuged (4 °C, 25 min, 20 000 g) and supernatants were saved for determining LOX activity. LOX activity was spectrophotometrically assayed using linoleic acid as substrate, according to Grossman and Zakut (1979). The assay solution, freshly prepared because of substrate instability, contained 0.25 mM linoleic acid (Sigma) and 0.25% Tween 20 in 0.2 M citrate-phosphate buffer (pH 7). The reaction mixture contained 2.4 ml of assay solution and 0.1 ml of plant extract. Absorbance was read at 234 nm. Activity was expressed in ΔA. min^{-1}.mg prot^{-1}. Proteins were

determined according to Bradford (1976), using serum albumin as standard.

Conjugated dienes. Detection of conjugated dienes was spectrophotometrically assayed according to refs. (Chérif et al., 1996, Engelmann-Sylvestre, 1988). Root tissues (500 mg fresh weight of whole roots) were ground with a mortar and pestle in liquid nitrogen to a fine powder, then further ground in 3 ml methanol, 100 mg EDTA (Sigma), 3 ml chloroform, and 3 ml of a solution containing 5 mM EDTA and 1% NaCl. The mixture was centrifuged (4 °C, 10 min, 4000 g) in glass tubes. The chloroformic phase was evaporated under nitrogen. The residue was dissolved in 500 μl chloroform. Fifty μl were again dried under nitrogen and dissolved in 800 μl of absolute ethanol. Absorbance was read at 234 nm in black quartz cuvette (Ref: 108. 002B-QS) in Shimatzu UV-160 A spectrophotometer. Results were expressed in absorbance per g of fresh material.

Data analysis. Data were analysed and means were separated by Duncan's multiple ranged test ($p \leq 0.05$). The software superANOVA (Abacus Concepts, Berkeley, CA) was used for statistical analysis.

Results

Effect of oxygenation on plant growth and fungal development

Aeration of the nutrient solution with compressed air resulted in a significant increase in the top (Figures 1, 3) and root growth (Figures 2, 4) of tomato plants as compared with nitrogen and control treatments, regardless of the presence of *Pythium*. In fact, air-treated plants showed more than a two fold increase in their aerial weight when compared to non-aerated plants, at the end of the sampling period (Figure 3A, B; see also Figure 1). While aerated roots continued to develop, nitrogen-treated roots and the control nearly stopped growing until the end of the experiment (Figure 4A, B). Root and top weights were about the same in the nitrogen-treated plants and the controls (Figures 3, 4). Inoculation of tomato plants with *Pythium* F did not result in a significant decrease in plant growth over the short experimental period (Figures 3, 4) but control and nitrogen-treated plants started showing typical symptoms of root decay and infection within 6 days after inoculation, while aerated plants remained healthy throughout the experiment (Figure 1). By day 10, control and nitrogen-treated plants had reached an advanced stage of root decay (Figure 2). To ascertain whether the observed damage was due to *Pythium* F, isolations from diseased plants were carried out on PDA. Only *Pythium* was isolated from infected roots and this isolate was identical to that used for inoculation. Root decay observed in infected control and nitrogen-treated plants was never associated with appearence of wilting symptoms or plant mortality.

The presence of *Pythium* F on and within infected tomato roots was confirmed by the immunoenzymatic staining procedure and isolation of thalles on selective medium. Hyphae of *Pythium* F on infected roots stained densely after incubation with the antiserum and were easily detected and counted under light microscopy. When these results were used to compare root colonization among treatments, it was unequivocal that aeration was beneficial against infection with *Pythium* F (Figure 5A). Similar results were obtained when *Pythium* presence was quantified by the isolation of thalles on selective medium (Figure 5B). While tomato root colonization occurred very early in the low and moderate oxygen treatments, *Pythium* thalles were not observed on highly oxygenated roots until 10 days after inoculation (Figure 5A). Interestingly, despite the healthy appearance of the aerated roots at days 10 and 12, *Pythium* F was observed on and reisolated from root samples taken from that treatment (Figure 5). Initially, a higher level of root colonization was observed in plants supplied with the moderate oxygen concentration as compared to roots grown in presence of nitrogen (Figure 5). Nevertheless, by day 12, similar levels of root contamination by *Pythium* F were observed in both treatments (Figure 5). In addition to providing a quantitative estimation of root colonization immunological method was very useful in the spacial localization of the fungus on the roots. At the initial stages of infection, the fungus was preferentially localized at the elongation zone and at the zone of lateral root emergence. Nevertheless, at later stages of infection, fungal hyphae were present in all areas of tomato roots.

Effect of oxygenation on lipoperoxidation

Lipoxygenase activity. As shown in Figure 6, LOX activity was low throughout the experimental period when oxygen concentration was the highest in the nutrient solution. This activity was only moderately increased by decreasing oxygen concentration (Figure

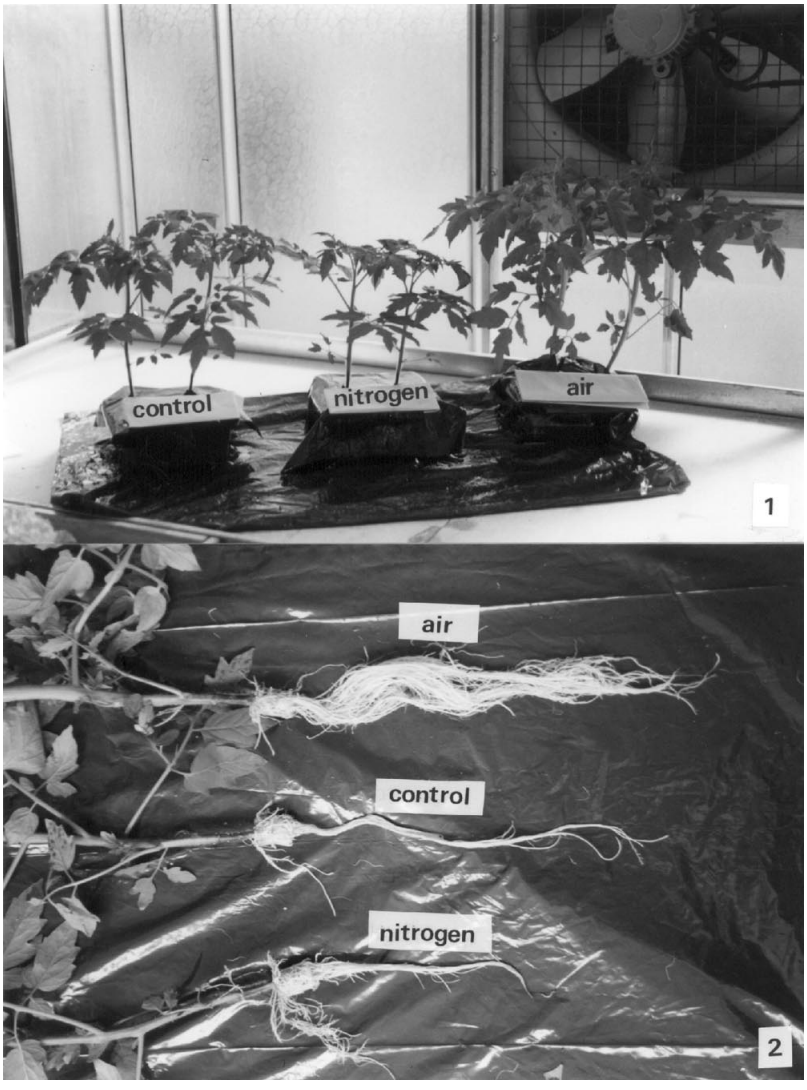

Figure 1. Effect of oxygenation on aerial parts and roots growth of tomato plants cv. Typico 6 days after inoculation with *Pythium* F707.

6A). After inoculation with *Pythium* F, no major changes in LOX activity were observed in the air treatment while marked increases occurred in the nitrogen-treated plants and the controls (Figure 6B). Incidently, under these treatments, infection with *Pythium* F resulted in a rapid initial 3-4 fold increase in LOX activity, followed by a slowly declining activity during the remaining period of the experiment (Figure 6B).

Conjugated dienes. One possible criterion for detecting lipid peroxidation is the formation of conjugated dienes which are the first products of oxidized polyunsaturated fatty acids. In absence of *Pythium* F, the amounts of conjugated dienes were lower in aerated roots than those measured in nitrogen-treated and control plants, regardless of the sampling day (Figure 7A). Results were quite similar in the latter two treatments (Figure 7A). In *Pythium*-infected plants, relatively stable levels of conjugated dienes were detected in highly oxygenated plants while a significant increase was observed in the other two treatments (Figure 7B). Such increase was noticed two days after inoculation with *Pythium* F and reached a maximum level two days later to remain almost constant up to the end of the experiment (Figure 7B). After infection, levels of conjugated dienes were significantly higher in nitrogen-treated plants at any time when compared to infected controls (Figure 7B). In fact, an increasing gradation was observed from

Figure 2. Effect of oxygenation on roots growth of tomato plants cv. Typico 10 days after inoculation with *Pythium* F707.

air-treated plants to control and nitrogen-treated ones (Figure 7).

Discussion

Results from this study clearly demonstrate that oxygen concentration in the nutrient solution has a significant effect upon the development of *Pythium* F root rot of hydroponically grown tomato plants and that colonization of roots by the fungus could be presumably reduced by aeration of the nutrient solution. The culture plate and immunoenzymatic staining techniques were simultaneously used in the present work to estimate *Pythium* F infection of tomato roots. In our experiments, the two methods gave similar results. The nitrogen treatment, with low oxygen concentration, and the control treatment, with moderate oxygen concentration (within the range found to naturally occur around plant roots in the commercial hydroponic system, i.e. 6-8%) (Zinnen, 1988) were both conducive to fungal colonization of tomato roots by *Pythium* F. This effect appears to be more related to root physiology than to the pathogen activity since denser fungal growth and root colonization were observed during the first 10 days under moderate oxygen concentration when compared to low oxygenation. The lower fungal development observed in the nitrogen treatment is not surprising if one considers a previous study (Eldon Brown and Kennedy, 1966), which showed that despite the exceeding tolerance of *Pythium* to low oxygen

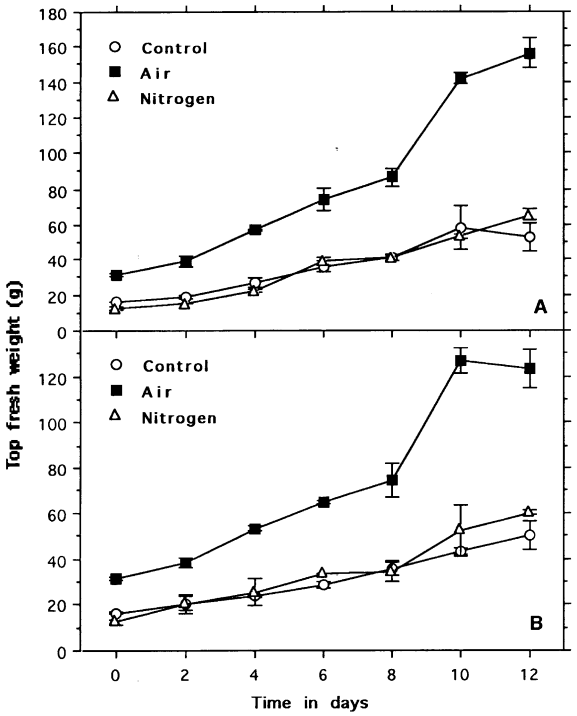

Figure 3. Fresh aerial weight of tomato plants cv. Typico submitted to three different oxygenation levels (Control: 2.5–3 ppm; Air: 5–7 ppm; Nitrogen: 0.4–0.7 ppm) and non-inoculated (A) or inoculated (B) with *Pythium* F707 over time. Values represent the means from three independent experiments; bars indicate standard deviation (SD).

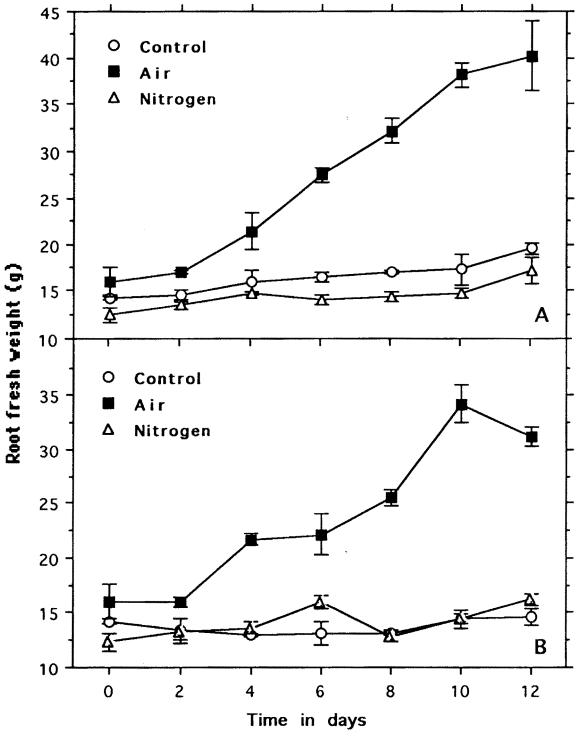

Figure 4. Fresh root weight of tomato plants cv. Typico submitted to three different oxygenation levels (Control: 2.5–3 ppm; Air: 5–7 ppm; Nitrogen: 0.4–0.7 ppm) and non-inoculated (A) or inoculated (B) with *Pythium* F707 over time. Values represent the means from three independent experiments; bars indicate SD.

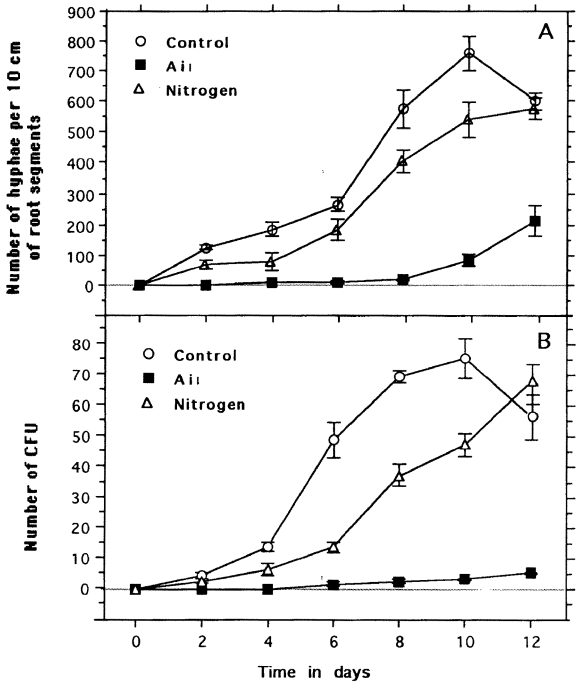

Figure 5. Colonization over time of tomato roots of cv. Typico, grown under three different oxygenation levels (Control: 2.5–3 ppm; Air: 5–7 ppm; Nitrogen: 0.4–0.7 ppm), by *Pythium* F707 using (A) the immunoenzymatic staining technique and (B) isolation of thalles on selective medium. Values represent the means from three independent experiments; bars indicate SD.

concentration, its growth was significantly reduced at oxygen concentration of 1.3%. In that study, fungal growth was not affected by oxygen concentrations of 4% or more. Several reports dealing with the effect of soil water saturation on fungal root rot of various plants revealed that the increased severity observed under these conditions is more likely the result of root tissue predisposition under oxygen stress and not a direct result of flooding (Bateman, 1961; Kuan and Erwin, 1980).

In addition to increased severity of root rot, the low and moderate oxygen treatments resulted in a significant reduction of root and shoot growth of tomato plants, regardless of *Pythium* infection. Retarded shoot growth, restricted and often damaged root system, mineral deficiency, and premature senescence were often reported as results of poor root aeration (Drew and Lynch, 1980; Hook and Crowford, 1978). According to previous studies (Drew and Lynch, 1980; Givan, 1968), the primary cause of such deleterious effects is the impairment of root metabolism because of inadequate generation of ATP in anaerobic respiration, loss of cell membrane integrity (Hiatt and Lowe, 1967), and production of autotoxic substances by anaerobic metabolism in the roots (Drew and Lynch, 1980).

Although advanced stages of root decay were observed in the non-aerated plants, symptoms of leaf wilting or plant mortality were not observed after inoculation with *Pythium* F. This is in agreement with the recent cytological and ultrastructural studies reported by Rey et al. (1996), who working with the same isolate of *Pythium* F, have incidently shown that fungal invasion was restricted to outer root tissues and cortical areas. Moreover, these authors showed that *Pythium* F, conversely to *P. aphanidermatum*, induced important defense reactions of the plant, including formation of papillae and deposition of wall apposition and phenolic compounds (Rey et al., 1996). Nevertheless, whether these defense mechanisms are still effective under poor aeration conditions, named as hypoxia, need to be further investigated, especially if we know that oxygen deficiency may impair resistance mechanisms of the host such as phytoalexin biosynthesis and polyphe-

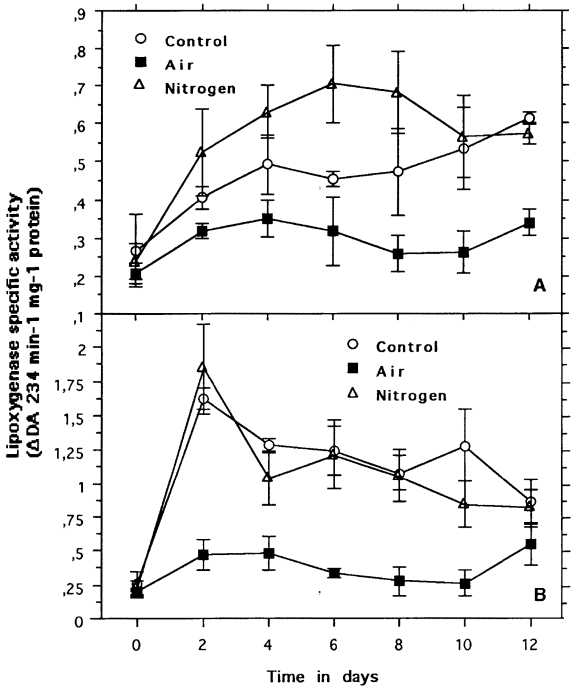

Figure 6. Variation over time of lipoxygenase activity in *Pythium* F707-non-inoculated (A) and – inoculated (B) tomato roots cv. Typico grown under three different oxygenation levels (Control: 2.5–3 ppm; Air: 5–7 ppm; Nitrogen: 0.4–0.7 ppm). Values represent the means from three independent experiments; bars indicate SD.

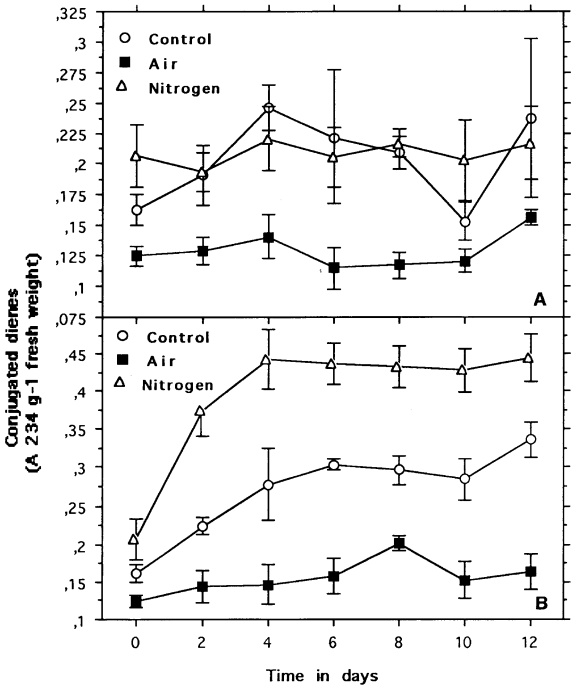

Figure 7. Variation over time of conjugated dienes in *Pythium* F707-non-inoculated (A) and -inoculated (B) tomato roots cv. Typico grown under three different oxygenation levels (Control: 2.5–3 ppm; Air: 5–7 ppm; Nitrogen: 0.4–0.7 ppm). Values represent the means from three independent experiments; bars indicate SD.

noloxidases activity (Cruickshank and Perrin, 1967; Drew and Lynch, 1980).

From the present study it is evident that major metabolic changes occur in plants grown under oxygen deficiency conditions. Such plants revealed an increased LOX activity and produced higher amounts of conjugated dienes in comparison with well aerated plants. The differences became more conspicuous after inoculation with *Pythium* F. Although no clearly defined physiological role has been demonstrated for LOX, there has been increasing evidence that this enzyme plays an important role in plant growth and development, senescence, wound responses, and resistance against pathogens and pests (Koch et al., 1992). To our knowledge this is the first report indicating the increase of LOX activity in plant tissues under hypoxia. Since lipid peroxydation is oxygen dependent, a possible explanation of the increased LOX activity could be that, under hypoxia, oxygen is still not a limitating factor for peroxydation in root tissues; but oxygen deficiency induced a stress and consequently an increased LOX activity. LOX catalyzes the oxidation of free fatty acids such as linoleic and linolenic acids forming the conjugated diene hydroperoxides. In our studies, the increases in LOX activity under low oxygen content and *Pythium* infection coincided with increases in the accumulation of conjugated dienes, which are the first products of oxidized polyunsaturated fatty acids. In plant tissues, the fatty acid hydroperoxides formed by the lipoxygenase reaction are further metabolized through one of two major pathways leading to the formation of different compounds, such as traumatin, a wound hormone, and jasmonic acid, which play important roles as signalling molecules within the plant (Todd et al., 1990). Nevertheless, many reports showed that LOX could be involved either positively, by the formation of signalling molecules which might induce resistance against stress, or negatively, by playing a deleterious role similar to that played in senescence (Hildebrand, 1989; Todd et al., 1990). As a matter of fact, LOX hydroperoxide-products can cause senescence by several mechanisms including inactivation of protein synthesis, and deterioration of cellular membranes (Hildebrand, 1989; Avdiushko et al., 1993).

The high increases in LOX activity 2 days after inoculation with *Pythium* F in the oxygen stressed

plants could be interpreted as an attempt to resist fungal agression. However, although LOX activity remained relatively high after that time, such attempt failed since it was followed with appearance of root decay and aggravation of disease symptoms. Such increases in LOX activity were not observed in the highly oxygenated roots. This perhaps could be explained by our observations: during at least the first eight days of the experiment, it seems that the well-oxygenated roots were not colonized by the pathogen. Under such conditions there is apparently no need in the high oxygen plants for attempted resistance. On the other hand, we should keep in mind that our host-fungus system involves a minor root pathogen. Oxidative stress under oxygen deficiency conditions is not well documented. Conversely, most of the studies dealing with oxidative stress were reported in the case of hypersensitive resistance (HR) (Doke et al., 1987; Gönner and Schlosser, 1993; Keppler and Novacky, 1986). In most of these host-parasite systems the HR has been associated with lipid peroxidation and LOX activity was, in contrast to our observations, causally linked to resistance expression (Keppler and Novacky, 1989; Rojas et al., 1993; Yamamoto et al., 1988). A correlation between LOX activity and systemically induced resistance has also been reported in cucumber plants after pathogen infection or chemical treatment (Avdiushko et al., 1993). Incidently, a recent study dealing with oxidative stress in a system comparable to ours, which involved oat plants and the perthotrophic pathogen *Drechslera* spp., revealed, in concordance with our findings, a marked increase in lipid peroxidation accompanied with high LOX activity and malondialdehyde accumulation in leaves of plants infected with the highly virulent species of the pathogen, whereas there were no such changes after inoculation with the weakly virulent species (Gönner and Schlosser, 1993). Interestingly, in the *Drechslera* system as well as in most studies dealing with HR and systemically induced resistance (Avdiushko et al., 1993; Gönner and Schlosser, 1993; Keppler and Novacky, 1989; Rojas et al., 1993; Yamamoto et al., 1988), the authors supposed that LOX could be involved in the process of cell necroses. This assumption speaks in favor of the idea that increases in LOX activity detected in the present study in tomato roots grown under hypoxia and inoculated with *Pythium* F may lead to degradation and disorganization of membrane lipids, and this disorganization may finally result in root decay (Adam et al., 1989; Anderson et al., 1991; Gönner and Schlosser, 1993). Nevertheless, other more detailed studies dealing with lipid peroxidation in the tomato-*Pythium* F-oxygen system and in other host-fungus systems involving minor pathogens are needed to validate this hypothesis. Further investigations measuring active oxygen species, free radicals, and antioxydants in these systems could be of paramount importance to understand the causes of aggravation of disease symptoms under poor aeration conditions.

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