DENDROBIOLOGY
2005, vol. 53, 27–33

Jacek Grzebyta[1], *Piotr Karolewski*[2], *Roma Żytkowiak*[2], *Marian J. Giertych*[2], *Antoni Werner*[2], *Marcin Zadworny*[2], *Jacek Oleksyn*[2]

Effects of elevated temperature and fluorine pollution on relations between the pedunculate oak (*Quercus robur*) and oak powdery mildew (*Microsphaera alphitoides*)

Received: 14 March 2005, Accepted: 5 May 2005

Abstract: Effects of elevated temperature and soil pollution with fluorine on host-pathogen relations were studied in seedlings of the pedunculate oak (*Quercus robur* L.) inoculated with oak powdery mildew (*Microsphaera alphitoides* Griff. et Maubl.) and control seedlings. The plants were grown for 1 month in elevated temperature (on average by 1.6°C) and soil pollution with sodium fluoride (330 ppm F). The above factors did not have any significant effect on nitrogen content of leaves or on concentrations of metabolites favourable to growth and development of the fungal pathogen (total non-structural carbohydrates, including soluble carbohydrates and starch) and those unfavourable to fungi (soluble phenols, condensed tannins and lignins). The elevated temperature and fluorine pollution did not affect the leaf infection rate. However, a significant temperature × pollution interaction was observed in inoculated seedlings. At the elevated temperature, fluorine caused a less severe infection by powdery mildew. This could be due to a direct toxic effect of fluorine on the pathogen or by an indirect influence, resulting from changes in levels of other metabolites, which were not analysed in this study. The inoculation of oak seedlings with powdery mildew caused a decline in the carbohydrate content of leaves but did not have any significant effect on levels of other analysed metabolites. However, it significantly affected the distribution of phenols and lignins in oak leaves. Those compounds accumulated within necrotic lesions and in adjacent cells. Our results do not enable drawing definite conclusions on effects of a slight rise in temperature and a relatively low level of fluorine pollution of the soil on relations between the pedunculate oak and oak powdery mildew. Lower values of the leaf infection rate in seedlings growing in elevated temperature and fluorine pollution suggest that in warmer years a lower level of infection by *M. alphitoides* may be expected in areas affected by fluorine pollution.

Additional key words: environmental pollution, phenols, tannins, lignins, carbohydrates, starch, nitrogen.

Addresses: [1]University of Warmia and Mazury, Faculty of Biology, Department of Genetics, Pl. Łódzki 3, PL 10-727 Olsztyn, jgrzebyta@gazeta.pl
[2]Institute of Dendrology, Parkowa 5, PL 62-035 Kórnik, pkarolew@man.poznan.pl

Introduction

The Intergovernmental Panel on Climate Change (IPCC) has published a report confirming a marked increase in temperature. Between the late 19th century and the year 2000 mean annual temperature over lands increased on average by 0.4–0.8°C (Houghton et al. 2001). IPCC also forecasts that between 2000 and 2100, a further global rise in temperature will take place, ranging from 1 to 5.5°C. Consequently, this will probably exert a considerable influence on ecosystems (McKone et al. 1998), including disturbances in relations between host plants and fungal pathogens (Ayres and Lombardero 2000).

Another important ecological phenomenon is environmental pollution with fluorine compounds. This does not result from large amounts of emitted substances (as fluorine emissions have decreased) but from the high chemical activity of fluorine. The toxicity of fluorine compounds is much higher than that of nitrogen oxides, chlorine (Cl_2 and Cl^-) and sulfur dioxide (Kluczyński 1989; Weinstein and Davison 2003).

The oak powdery mildew (*Microsphaera alphitoides* Griff. et Maubl.) is a widespread pathogen in woodlands, not only in Poland but also all over Europe (Butin 1995). It causes the greatest losses in forest nurseries, as it attacks mainly young seedlings, but it also infects leaves of older trees, sometimes resulting in serious damage to forest stands. Because of the widespread distribution of this pathogen, as early as the 1970s a research program was initiated to determine its distribution in areas subject to environmental pollution. The results showed that the oak powdery mildew is sensitive to sulfur dioxide (Grzywacz and Ważny 1973) as well as to nitrogen oxides and carbon monoxide (Flückiger and Oertli 1978). So far, no published information has been available on *M. alphitoides* sensitivity to fluorine compounds. Our preliminary observations indicated that leaves of trees growing in areas polluted with fluorine compounds were much more strongly infected by the pathogen than leaves from a control, unpolluted area. This may indicate a low susceptibility of this pathogen to fluorine.

The aim of this study was to assess the influence of elevated temperature and soil pollution with fluorine on the scope of infection of oak leaves by powdery mildew. More precisely, we attempted to answer the following questions: (1) Do the elevated temperature and soil pollution affect the leaf infection rate? (2) To what extent do the above factors affect the concentration and distribution of metabolites in leaves? (3) Can changes in concentrations of metabolites determine the susceptibility of oak seedlings to infection by *M. alphitoides*, as was suggested by some previous studies (Johnson and Schaal 1957a; Paul and Scharman 2002)? (4) How does inoculation of oak leaves with powdery mildew affect the concentrations and distribution of metabolites in the leaves?

Material and methods

Plant material

Three-year-old seedlings of the pedunculate oak (*Quercus robur* L.) were planted in pots of 13 cm in diameter and 11 cm in height, in a mixture of forest soil (under oak trees) and acid peat (1:1, v:v). The dry weight of the soil in each pot was about 820 g. Until the beginning of the experiment, the seedlings were watered with tap water and fertilized with a standard commercial fertilizer ("Florovit", Biovita, Poland), containing nitrogen (3% N, including 2.3% $N\text{-}NO_3$ and 0.7% $N\text{-}NH_4$), potassium (2%), calcium (0.064%), magnesium (1%), copper (70 mg/l), iron (Fe 400 mg/l), manganese (170 mg/l), molybdenum (20 mg/l), zinc (150 mg/l), sulfur, boron, and other substances. The fertilizer was applied weekly as a solution (7.5 ml of medium per 1 liter of tap water), at a dose of 100 ml per seedling.

In the autumn of the preceding year, oak leaves infected by the *Microsphaera alphitoides*, with perithecia containing ascospores on the underside, were collected. This material was stored at +5°C and later used for inoculation. The usefulness of such material was confirmed earlier, by means of preliminary inoculation of oak seedlings under controlled conditions.

The experiment was conducted for one month (between May 23 and June 24, 2002). Three variables were tested: temperature (T), fluorine pollution (P), and inoculation with the fungus (I). Each treatment was represented by 10 seedlings grouped in two blocks. The leaf infection rate was assessed for individual seedlings (n=10), while chemical analyses were performed for pooled samples from each block (n=2).

On the starting date of the experiment, all oak seedlings were placed under a shading screen, reducing sunlight by about 50%. Irradiation was measured with a phytophotometer (FF-01, SONOPAN, Poland). One half of the seedlings grew at the natural ambient temperature (lower temperature, LT), while the other half grew at an elevated temperature (higher temperature, HT). The rise in temperature was achieved by means of polyethylene tents, which had no significant effect on photosynthetic irradiation. Air temperature was measured with Hobo data logger sensors (Onset Computer Corp., USA), located at half the height of the seedlings. Temperature was recorded every 5 minutes throughout the experiment, and mean values for day, night and 24-hour periods are shown in Table 1.

At the beginning of the experiment, half of the seedlings were inoculated with oak powdery mildew, by attaching some infected oak leaves (collected ear-

Table 1. Minimum, mean and maximum air temperatures during the experiment, calculated separately for day (04.00–21.00 h), night (21.00–04.00 h), and 24-hour periods

Temperature treatment	Time of day	Temperature (°C)		
		minimal	mean	maximal
control	day	6.22	20.09	38.77
	night	6.22	13.44	24.01
	day+night	6.22	18.40	38.77
elevated	day	6.62	22.02	42.46
	night	7.03	14.08	23.63
	day+night	6.62	20.02	42.46

lier) to the main stem at the height of leaves (treatment +I). In the fluorine pollution treatment (+F), sodium fluoride was added to the soil at a concentration of 19.27 mg NaF (8.72 mg F), dissolved in distilled water in a volume of 25 or 50 ml, depending on the moisture of the soil in pots. As a result, the final dose per seedling was 0.6 g NaF (330 ppm F). Control seedlings (–F) were watered with distilled water (the same volume). Earlier trials showed that the applied level of soil pollution does not cause necroses on oak leaves. By contrast, the same dose caused necroses on needles of 2-year-old Scots pine seedlings (Grzebyta, unpublished data).

On the last day of the experiment, for each seedling separately, the leaf infection rate was assessed visually, i.e. the mean percentage of total leaf area infected by the pathogen. On the same day, leaves were collected for chemical and histochemical analyses.

Methods of chemical analysis

In leaves of oak seedlings, there were assessed concentrations of compounds favorable to the growth of *M. alphitoides*: starch, soluble carbohydrates (SC), and nitrogen (N); as well as compounds unfavorable to fungal growth: total phenols (TPh), condensed tannins, and lignins. The sum of starch and soluble carbohydrates was a synthetic variable, i.e. total non-structural carbohydrates (TNC). Fluorine (F) concentration was also measured.

Plant material was dried at 65°C for 48 h. Only the material for tannin assays was dried at 40°C for one week, to prevent the decomposition of those compounds at a high temperature and their hydrolysis by enzymes (Wina et al. 2000).

The TNC concentrations were determined using a modification of the method described by Haissig and Dickson (1979) and Hansen and Møller (1975). Sugars were extracted from oven-dried leaf tissue powdered in methanol-chloroform-water, and tissue residuals were used for starch content determination. Soluble sugars were determined colorimetrically with anthrone-reagent at 625 nm within 30 min. Starch in the tissue residue was then gelled and converted to glucose with amyloglucosidase. Glucose concentrations were measured with glucose oxidase by mixing the sample with peroxidase-glucose oxidase-*o*-dianisidine dihydrochloride reagent. Absorbance was measured at 450 nm after 30 min incubation at 25°C are. Glucose concentrations were calculated from standard curvilinear regression equations.

The N concentration was measured on ground dried leaves digested by the Kjeldahl method. Soluble sugars, starch and N concentration were expressed as percent of dry mass.

The content of phenolic compounds (TPh) was determined in 0.1 g of foliage after double extraction for 15 and 10 min in boiling 95 and 80% ethanol, respectively. Analyses were performed using the spectrophotometric method described by Johnson and Shaal (1957b) and modified by Singleton and Rossi (1965) using the Folin & Ciocalteu's Phenol Reagent. The content of total phenols was expressed in μmol of chlorogenic acid per gram of dry mass.

For the assays of condensed tannins, by the method described by Price et al. (1978), 0.025 g of tissue was used. Tannins were extracted from tissue samples with absolute methanol at room temperature for 20 min. The color reaction was performed with the use of 0.5% vanillin solution in 4% HCl solution in absolute methanol. Absorption was measured at λ=500 nm. Catechin was used as a standard, and the results are expressed as μM catechin per 1 g of dry weight (DW).

Lignin content of leaf tissue was assessed using the method of Moore and Johnson (1967), which is based on removal of all substances other than lignins. The lignin content (% of DW) was calculated on the basis of the initial sample mass and the mass of the residue after extractions.

The total fluorine content was measured by the potentiometric method (Roost and Sigg 1978). The ground tissue (1 g) was extracted with the TISAB buffer. The measurements were made using ion meter (716 DMS Titrino, Metrohm, Switzerland), and the ion-selective fluoride electrode (cat. no. 6.0502.150 Fluoride I.S.E., Metrohm), while an Ag/AgCl electrode (cat. no. 6.0726.110, Metrohm) was used as a standard. Concentrations of fluorine compounds were expressed in ppm F (= mg F kg^{-1} DW). The assays of this element were performed in the Veterinary and Agrochemical Research Centre CODA – CERVA in Tervuren, Belgium.

Histochemical methods

Fresh, hand-made sections were used for histochemical analyses. The distribution of selected compounds in oak leaves was examined by using toluidine blue (Feder and O'Brian 1968) for phenols, floroglucin in HCl for lignins, 0.5–1% $Fe_2(SO_4)_3$ or $FeCl_3$ for tannins, and the nitrose reaction (Jensen 1974) for catechol tannins.

Statistical analysis

For all variables, statistical differences among treatments were calculated by analysis of variance (GLM procedures) using JMP software (version 4.0.4, SAS Institute, Cary, NC, U.S.A.).

To assess the significance of elevated temperature and fluorine pollution on concentrations of chemical compounds in oak leaves, calculations were made only for non-inoculated seedlings (Table 2). To assess effects of elevated temperature and fluorine pollution on the leaf infection rate, calculations were made only for inoculated seedlings (Fig. 1). To assess the influence of the pathogen on concentration of metabolites and fluorine in leaves, ANOVA was made for the inoculated and non-inoculated plants (Table 3).

For the statistical analysis, all percentages were transformed according to C.I. Bliss's formula to achieve a normal distribution (Snedecor and Cochran 1976).

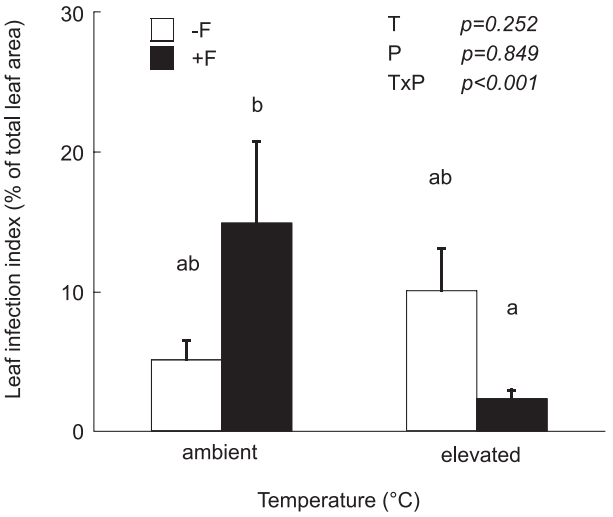

Fig. 1. Leaf infection rate of oak seedlings inoculated with powdery mildew and grown in an unpolluted soil (open bars) and a fluorine-polluted soil (shaded bars) at an ambient and elevated temperature. Statistical significance of effects of temperature (T), soil pollution (P) and T×P interaction on the leaf infection rate is given in the upper right corner. Bars marked by the same letters do not differ significantly (Tukey test, $p \leq 0.05$)

Table 2. Effect of elevated temperature (low: LT; high: HT) and fluorine pollution on concentrations of starch, soluble carbohydrates (SC), total non-structural carbohydrates (TNC), total phenols (TPh), condensed tannins, lignins, nitrogen (N) and fluorine (F) in leaves of oak seedlings (unpolluted seedlings: –F; polluted seedlings: +F). Only seedlings from non-inoculated treatments of the experiment were included in the statistical analyses. Mean values are given with standard errors in brackets. The right side of the table presents results of ANOVA. Significant values ($p = 0.05$) are in bold.

Metabolites and elements	Temperature (T)		Pollution (P)		ANOVA effects $P > F$		
	control (LT)	elevated (HT)	control (– F)	Fluoride (+ F)	T	P	T x P
Starch (%)	0.68 (0.10)	0.56 (0.15)	0.50 (0.06)	0.78 (0.10)	*0.49*	*0.18*	*0.81*
SC (%)	7.6 (0.5)	7.3 (0.2)	7.0 (0.3)	7.9 (0.3)	*0.56*	*0.11*	*0.28*
TNC (%)	8.3 (0.6)	7.9 (0.5)	7.5(0.6)	9.5 (0.3)	*0.43*	*0.15*	*0.48*
N (%)	1.9 (0.33)	1.4 (0.19)	1.6 (0.40)	1.8 (0.22)	*0.43*	*0.65*	*0.58*
TPh (μM g^{-1})	330 (15)	285 (21)	287 (12)	327 (19)	*0.13*	*0.18*	*0.95*
Tannins (μM g^{-1})	611 (73)	463 (66)	473 (52)	600 (89)	*0.22*	*0.28*	*0.89*
Lignins (%)	24.1 (0.2)	22.9 (1.0)	23.2 (1.0)	23.7(0.5)	*0.32*	*0.61*	*0.37*
F (ppm)	12.3 (6.3)	11.4 (5.5)	3.2 (0.4)	20.5 (4.6)	*0.87*	***0.04***	*0.74*

Table 3. Effect of infection of oak seedlings with powdery mildew (non-infected seedlings: -I; infected seedlings: +I) on concentrations of starch, soluble carbohydrates (SC), total non-structural carbohydrates (TNC), total phenols (TPh), condensed tannins, lignins, nitrogen (N) and fluorine (F) in oak leaves. Mean values are given with standard errors in brackets. The right side of the table presents results of ANOVA. Significant values ($p = 0.05$) are in bold

Metabolites and elements	Inoculation (I)		ANOVA effects $P > F$
	control (– I)	inoculated (+ I)	
Starch (%)	0.64 (0.08)	0.56 (0.05)	*0.51*
SC (%)	7.5 (0.3)	6.9 (0.2)	*0.07*
TNC (%)	8.1 (0.4)	7.5 (0.2)	***0.02***
N (%)	1.7 (0.2)	1.8 (0.1)	*0.56*
TPh (μM g^{-1})	307 (15)	332 (17)	*0.18*
Tannins (μM g^{-1})	537 (53)	598 (53)	*0.90*
Lignins (%)	23.5 (0.5)	22.9 (0.7)	*0.55*
Fluor (ppm)	11.9 (3.9)	11.2 (3.9)	*0.87*

Results and discussion

The statistical analyses showed that the elevated temperature and soil pollution did not affect the leaf infection rate (Fig. 1). This may be due to the small temperature increase (by 1.6°C). By contrast, Lovell et al. (2004) reports that leaf infection of wheat by *Mycosphaerella graminicola* was significantly affected by raised temperature. More intensive sporulation on leaves in elevated temperatures were also observed by Pfister et al. (2004) on rhododendrons and by Su et al. (2004) on lettuce. However, those authors studied effects of a much higher range of temperatures (from 15 to 30°C and from 5 to 25°C, respectively).

Fluorine pollution at the level applied in our study did not cause any significant changes in concentrations of metabolites and nitrogen in oak leaves (Table 2). This was probably the reason for the lack of differences in the leaf infection rate (Fig. 1).

Although each of the studied abiotic factors analysed separately had no significant effect on the leaf infection rate, there was observed a significant temperature × pollution interaction in inoculated seedlings (Fig. 1). A rise in air temperature resulted in a decrease in the leaf infection rate of seedlings polluted with fluorine. In unpolluted seedlings, the elevated temperature caused an increase in the leaf infection rate. An analysis of all studied metabolites did not reveal any significant temperature × pollution interaction. Thus it seems that the lower leaf infection rate at the elevated temperature was probably due to a direct, more toxic influence of fluorine on the fungus. However, it is also possible that some other metabolites, not analysed in this study, significantly affected the fungal growth.

In contrast to the studied abiotic factors, inoculation with the *M. alphitoides* caused changes in concentrations of some metabolites (Table 3). Levels of soluble carbohydrates and total non-structural carbohydrates (TNC) decreased. However, no increase in defence metabolites was observed in response to inoculation with the pathogen. This does not confirm the widely believed opinion that phenols and lignins play an important role in plant defense against fungal pathogens (Bennett and Wallsgrove 1994). The observed decrease in TNC under the influence of *M. alphitoides* is probably associated with a reduction of photosynthetic rate in infected leaves (Gomes-Laranjo et al. 2004; Rollof et al. 2004; Maust et al. 2003). Although some authors report on accumulation of carbohydrates in infected leaves of various plant species (Eleftheriou and Tsekos 1991; Aboot and Losel 2003), they consider it a result of inhibition of the transport of carbohydrates to roots, rather than intensified synthesis of those compounds.

Results of histochemical analyses indicate that the distribution of the studied metabolites in leaves of oak seedlings inoculated with powdery mildew was affected by fluorine pollution but not by the elevated temperature. In polluted seedlings, fungal infection

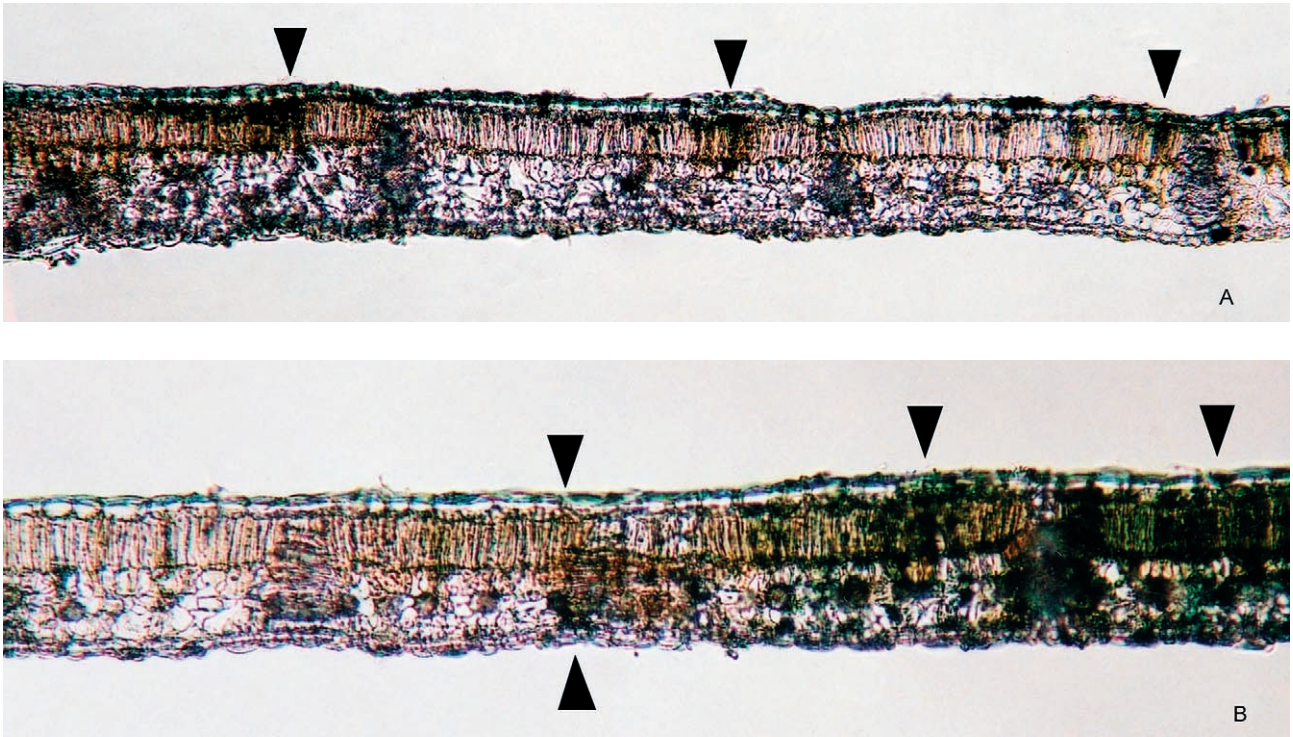

Fig. 2. Colour reaction of toluidine blue with phenols in leaves of oak seedlings inoculated with powdery mildew and grown in pots with an unpolluted soil (A) and a fluorine-polluted soil (B). Arrows indicate products of the colour reaction, i.e. the brown or greenish-brown filling of some cells of palisade mesophyll (A) or of both palisade and spongy mesophyll (B)

Fig. 3. Colour reaction of lignins with floroglucin in necrotic lesions on leaves of oak seedlings inoculated with powdery mildew. Arrows indicate products of the colour reaction, i.e. the reddish-brown staining of cell walls and cell interior

caused an accumulation of phenols in the epidermis and in both spongy and palisade mesophyll of leaves (Fig. 2A, B). In the control (seedlings inoculated with the fungus but not polluted with fluorine), accumulation of phenols was noticed only in the epidermis and in single cells of palisade mesophyll. In addition to accumulation of phenols, also lignification – another defense response – was observed in necrotic lesions and adjacent cells (Fig. 3). The stronger defense response may indicate that the sensitivity of oak seedlings to fungal infection is higher under conditions of soil pollution.

In conclusion, our results indicated that under the influence of elevated temperature and realistic range of F pollution, no significant effect on concentrations of metabolites in oak leaves was observed. This most likely resulted in the lack of significant differences in the leaf infection rate. However, for simultaneous elevation of temperature and fluorides, oak leaves were less infected by powdery mildew (significant temperature × pollution interaction). This may indicate that under conditions of fluorine pollution, climatic warming will not increase the infection of *Quercus robur* trees by *Microsphaera alphitoides*.

Acknowledgements

We thank Dr. Ludwig De Temmerman, Lesław Rachwał and Alicja Bukowska for help during the experiment and chemical analyses. This study was supported by the State Committee for Scientific Research, Poland (KBN grant no. 6 P04G 022 21).

References

Aboot J.K., Losel D.M. 2003. Changes in carbohydrate composition of cucumber leaves during the development of powdery mildew infection. Plant Pathology 52: 256–265.

Ayres M.P., Lombardero M.J. 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. The Science of the Total Environment 262: 263–286.

Bennett R.N., Wallsgrove R.M. 1994. Secondary metabolites in plant defense mechanisms. New Phytologist 127: 617–633.

Butin H. 1995. Tree diseases and disorders. Causes, biology and control in forest amenity trees. Oxford University Press, Oxford. pp. 262.

Eleftheriou E.P., Tsekos I. 1991. Fluoride effects on leaf cell ultrastructure of olive trees growing in the vicinity of the Aluminum Factory of Greece. Trees 5: 83–89.

Feder N., O'Brien T.P. 1968. Plant microtechnique: Some principles and new methods. American Journal of Botany 55: 123–142.

Flückiger W., Oertli J.J. 1978. Der Einfluss verkehrsbedingter Luftverunreinigungen auf den Befall der Eiche durch *Microsphaera alphitoides*. Phytopathologische Zeitschrift 93: 363–366.

Gomes-Laranjo J., Araujo-Alves J., Ferreira-Cardoso J., Pimentel-Pereira M., Abreu C.G., Torres-Pereira J. 2004. Effect of chestnut ink disease on photosynthetic performance. Journal of Phytopathology 152: 138–144.

Grzywacz A., Ważny J. 1973. The impact of industrial air pollutants on the occurrence of several important pathogenic fungi of forest trees in Poland. European Journal of Forest Pathology 3: 129–141.

Haissig B.E., Dickson R.E. 1979. Starch measurement in plant tissue using enzymatic hydrolysis. Physiologia Plantarum 47: 151–157.

Hansen J., Møller I. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical Biochemistry 68: 87–94.

Houghton J.T., Ding Y., Griggs D.J., Noguer M., van der Linden P.J., Dai X., Maskell K., Johnson C.A. 2001. Climate change 2001: The Scientific Basis. IPCC 2001. Cambridge University Press. (link was checked on 14th March 2005)

Jensen W.A. 1974. Botanical histochemistry: Principles and practice. Freeman W.H. and Company, San Francisco. 408. pp.

Johnson G., Schaal L.A. 1957 a. Chlorogenic acid and other orthodihydricphenols in scab-resistant russet burbank and scab-susceptible triumph potato tubers of different maturities. Phytopathology 47: 253–255.

Johnson G., Schaal L.A. 1957 b. Accumulation of phenolic substances and ascorbic acid in potato tuber tissue upon injury and their possible role in disease resistance. American Potato Journal 34: 200–209.

Kluczyński B. 1989. Wpływ związków fluoru na rośliny drzewiaste. In: Białobok S. (ed.) Życie drzew w skażonym środowisku, Nasze drzewa leśne. PWN, Warszawa–Poznań 105–128.

Lovell D.J., Hunter T., Powers S.J., Parker S.R., Van den Bosch F. 2004. Effect of temperature on latent period of septoria leaf blotch on winter wheat under outdoor conditions. Plant Pathology 53: 170–181.

Maust B.E., Espadas F., Talavera C., Aguilar M., Santamaria J.M., Oropeza C. 2003. Changes in carbohydrate metabolism in coconut palms infected with the lethal yellowing phytoplasma. Phytopathology 93: 976–981.

McKone M.J., Kelly D., Lee W.G. 1998. Effect of climate change on mast-seeding species: frequency of mass insect seed predators. Global Change Biology 4: 591–596.

Moore W.E., Johnson D.B. 1967. Acid insoluble lignin in pulp – Modified hydrolysis method. In: Procedures for the chemical analysis of wood and products. Forest Products Laboratory. forest Service U.S. Department of Agriculture.

Paul P.K., Sharma P.D. 2002. *Azadirachta indica* leaf extract induces resistance in barley against leaf stripe disease. Physiological and Molecular Plant Pathology 61: 3–13.

Pfister S.E., Halik S., Bergdahl D.R. 2004. Effect of temperature on *Thekopsora minima* urediniospores and uredinia. Plant Disease 88: 359–362.

Price M.L., van Scoyoc S., Butler L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry 26: 1214–1218.

Roost F., Sigg A. 1978. Erfahrungen mit einer potentiometrischen Fluor-Bestimmungsmethode für biologische Materialen. Staub-Reinhalt Luft 38: 363–366.

Roloff I., Scherm H., van Iersel M.W. 2004. Photosynthesis of blueberry leaves as affected by septoria leaf spot and abiotic leaf damage. Plant Disease 88: 3397–3401.

Singleton V.I., Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagent. American Journal for Enology and Viticulture 16: 144–158.

Snedecor W., Cochran W.G. 1976. Statistical methods. 6th edition. Ames, Iowa, USA. The Iowa State University Press, pp. 327–329.

Su H., van Bruggen A.H.C., Subbarao K.V., Scherm H. 2004. Sporulation of *Bremia lactucae* affected by temperature, relative humidity, and wind in controlled conditions. Phytopathology 94: 396–401.

Weinstein L.H., Davison A.W. 2003. Native plant species suitable as bioindicators and biomonitors for airborne fluoride. Environmental Pollution 125: 3–11.

Wina E., Tangedjaja B., Palmer B. 2000. The effect of drying conditions on condensed tannin estimates (CT) in Calliandra calothytsus. In: Brooker J.D. (ed.) Tannins in Livestock and Human Nutrition. Australian Centre for International Agricultural Research (ACIAR) Proceedings 92: 116–120. http://www.aciar.gov.au/(link was checked on 14th March 2005).