Neth. J. Pl. Path. 96 (1990) 65-74

Acute ozone exposure predispose Phaseolus vulgaris beans to Botrytis cinerea

G. LEONE and A.E.G. TONNEIJCK

Research Institute for Plant Protection (IPO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands

Accepted 12 February 1990

Abstract

The impact of ozone in predisposing *Phaseolus vulgaris* to *Botrytis cinerea* has been investigated. One day after 8 h exposures to 0, 120, 180 and 270 μg ozone m^{-3}, primary and trifoliate leaves of four bean cultivars were detached and inoculated with conidia suspended in water or in an inorganic phosphate (Pi) solution. Visible ozone injury increased with increasing ozone concentrations in all cultivars. Primary leaves were more sensitive than trifoliate leaves. Conidia suspended in Pi solution caused lesions on healthy leaves, whereas conidia in water did not. Ozone-injured leaves of all cultivars showed lesions by *B. cinerea* after inoculations in water. The number of lesions was significantly correlated with ozone injury for primary leaves. After Pi inoculations, the number of lesions on the ozone-sensitive cultivars also increased with increasing ozone concentrations. However, the ozone-tolerant cultivar Groffy showed first a decrease in the Pi-stimulated infection at the lowest ozone dosages. The trifoliate leaves of all cultivars were less predisposed to the fungus than the primary leaves. The results indicate that realistic concentrations of ozone enhance the predisposition of bean leaves to *B. cinerea*. The rate of enhancement depends on the level of ozone-induced injury which was influenced by cultivar, leaf and ozone concentration.

Additional keywords: air pollution, foliar injury, interaction, predisposition, weak pathogen.

Introduction

Ozone is the major component of the photochemical air pollution complex. It is well documented now that increased ambient ozone concentrations adversely affect the vegetation throughout the most industrialized countries (Krupa and Manning, 1988; Tonneijck, 1989). Foliar symptoms on field grown crops such as bean have been recorded also in the Netherlands (Tonneijck, 1983). In a study to assess the effect of air pollutants on crop productivity in this country, ozone has been estimated to cause 70% of the total air pollution-induced crop loss (Van der Eerden et al., 1988). Legumes appeared to be especially sensitive to ozone.

Besides primary effects such as foliar injury and yield reduction, ozone can also cause secondary effects to plants by interacting with other stress factors. Although the impact of ozone on the interaction between plants and pathogens has not yet been extensively investigated, ozone has been reported to affect the predisposition of plants to diseases and to enhance infections by weak pathogens (Manning, 1976; Heagle, 1982; Kvist, 1986). According to Fehrmann et al. (1986), leaf attack of cereals by weak pathogens has become much more serious in Central Europe during recent years. They suggested that the preinfectional influence of air pollutants such as ozone and sulphur dioxide might play a role.

Therefore, a research program was started to examine the potential for ozone to predispose bean plants (*Phaseolus vulgaris* L.) to infections by the weak pathogen *Botrytis cinerea* Pers.: Fr.. Bean was chosen because injury on leaves by ambient ozone has been observed in the field and because of existing information on the ozone sensitivity of a number of cultivars (Tonneijck, 1983). The aim of the present study was to determine the effect of acute exposures to realistic concentrations of ozone on the susceptibility of bean to *B. cinerea*. Four bean cultivars differing in ozone sensitivity were used to assess whether variation in ozone sensitivity could affect the degree of predisposition.

Materials and methods

Cultivation. Seeds of four bean cultivars, the ozone-sensitive cultivars Pros and Stratego, the moderately sensitive cultivar Lit and the ozone-tolerant cultivar Groffy, were planted in 12-cm diameter plastic pots filled with a high fertility peat-clay potting mixture (Triomf no. 17, modified, Trio BV, Westerhaar) and grown in a glasshouse with charcoal-filtered air at a 16-h photoperiod, a 20/18 °C day/night temperature regime and 75% relative air humidity. Pots were watered daily with tap water. Fourteen days after planting beans were thinned to two plants per pot. All plants were 21 or 25 days old when fumigated.

Fumigations. Plants were exposed to 0, 120, 180 or 270 μg ozone m^{-3} for 8 h. All exposures to ozone were conducted in 3.3 m^3 environmentally controlled fumigation chambers. Plants were placed in the chambers ca 16 h before initiation of the exposure and maintained at 75% relative air humidity, 20/18 °C day/night temperature and at 22 000 lux (ca 75 W m^{-2}) from 5.00 till 21.00 hours. Charcoal-filtered air flowed through the chambers at a rate of 4000 m^3 h^{-1}, resulting in a wind velocity of at least 50 cm sec^{-1}. Ozone was generated by UV irradiation of pure oxygen and added to the air stream of three chambers. Plants in the fourth chamber served as control. Concentrations of ozone were monitored with a Dasibi 1003 AH analyzer, that was regularly calibrated with air of known ozone content from an UV generator.

Inoculum production, inoculation and incubation of the leaves. *B. cinerea* was isolated from naturally infected bean plants and maintained on potato dextrose agar (PDA) slants at 4 °C. Sporulating cultures were obtained by transferring the fungus on slants of synthetic medium X (Last and Hamley, 1956) and by incubating it for 11 days at 20 °C in the dark. Conidial suspensions for inoculation were prepared in water or in a 62.5 mM KH_2PO_4 (Pi) solution as previously reported (Leone and Tonneijck, 1990), at a concentration of 2×10^6 conidia ml^{-1}. For each ozone treatment four detached leaves per cultivar were inoculated 24 h after the start of the exposure period with 20-μl drops (ten drops per leaf) of the conidial suspension in water. Similarly, four other leaves were inoculated with the Pi suspension. The inoculated leaves were subsequently incubated in transparent plastic trays in an environmentally controlled chamber as reported elsewhere (Leone and Tonneijck, 1990). The procedure for inoculation and incubation was performed separately for primary and trifoliate leaves.

Statistics. The number of successful lesions and the percentage of ozone injury were determined two days after inoculation. Ozone-induced visible injury on primary and trifoliate leaves was assessed using a modified Horsfall-Barratt scale (Hofstra and Ormrod, 1977) ranging from 0 (no injury) to 12 (100% injury). Data of percentage successful lesions and of foliar injury were subjected to regression analysis with a generalized linear model using the statistical package Genstat V. Data from primary and trifoliate leaves were analyzed separately because their sensitivity to ozone differed. The methods of maximum likelihood and of maximum quasi-likelihood were applied to the percentage of successful lesions and to the percentage of foliar injury, respectively (Mc Cullagh and Nelder, 1983). The logit function was used as link function, which implies a sigmoidal dependence of fungal lesions and foliar injury on ozone concentration. The analysis of deviance obtained was used to perform an approximate F test, in analogy with the usual analysis of variance. Main effects were assessed eliminating the effect of the nuisance factor 'experiment'.

Results

Development of Botrytis cinerea lesions on primary leaves after ozone exposures. Twentyone-day-old plants of the four bean cultivars Groffy, Lit, Pros and Stratego were exposed to ozone, inoculated with *B. cinerea* and incubated as described. At the end of the exposures, some foliar injury was already visible on the ozone-sensitive cultivars at the highest ozone concentration. The level of foliar injury by ozone depended significantly on the ozone concentration ($P < 0.001$) and the cultivar ($P < 0.005$) used.

The results of the foliar injury assessments for the four cultivars are presented in Fig. 1. The primary leaves of cultivar Groffy exhibited clearly less injury than the other cultivars whereas the leaves of cultivar Pros showed the highest level of injury. Foliar injury of all cultivars increased with an increase in ozone concentration.

The results of the analysis of deviance on the percentage of successful lesions by *B. cinerea* are reported in Table 1. The three factor interaction was not significant and therefore was included in the residual. Compared with inoculation in water, addition of Pi to the inoculum significantly stimulated the fungal infection on all cultivars (Table 1 and Fig. 2). On healthy non-exposed leaves water-inoculated conidia hardly caused infection, whereas the number of lesions on leaves of exposed plants was significantly higher than that on leaves of non-exposed plants (Fig. 2A). The increase in percentage of successful lesions was found to be dependent on the increase in ozone concentration

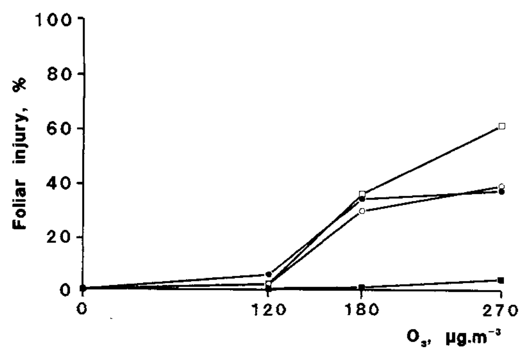

Fig. 1. Foliar injury (%) on primary leaves of four bean cultivars after 8 h exposures to different ozone concentrations. Groffy (■); Lit (○); Pros (□); Stratego (●).

Table 1. Analysis of deviance on successful lesions by *Botrytis cinerea* on primary leaves of four bean cultivars after acute exposures to different ozone concentrations.

Source of variation	d.f.	Deviance	Mean deviance	F test	*P*
Experiment	3	55.657	18.552		
Cultivar	3	19.153	6.384	5.20	0.005
Inoculum composition	1	431.195	431.195	351.31	<0.001
Ozone concentration	3	191.850	63.950	52.10	<0.001
Cultivar × Inoculum	3	24.742	8.247	6.72	0.001
Cultivar × Ozone	9	19.185	2.132	1.74	0.050
Inoculum × Ozone	3	11.272	3.757	3.06	0.025
Residual	94	115.376	1.227		
Total	119	868.429	7.298		

for both inoculation methods. Cultivar Pros was significantly more infected by *B. cinerea* than all other cultivars after the ozone exposures. Compared to the other cultivars, Groffy showed a retarded increase in infection. The trends observed for the cultivars Lit and Stratego were intermediate between those of cvs Pros and Groffy. When infections originated from inoculum drops containing Pi, cvs Groffy and Pros showed first a slight, although not significant, decrease in successful lesions on leaves of plants exposed to 120 μg ozone m^{-3} (Fig. 2B). A positive, highly significant correlation between percentage of successful lesions and percentage of ozone injury was always found on leaves of exposed plants inoculated with conidia suspended in water (Table 2).

Development of Botrytis cinerea lesions on primary and trifoliate leaves after ozone exposures. Twentyfive-day-old plants of the above mentioned four bean cultivars were exposed to ozone, and detached primary and trifoliate leaves were inoculated with *B. cinerea* and incubated as already described. As in the previous experiments, the level

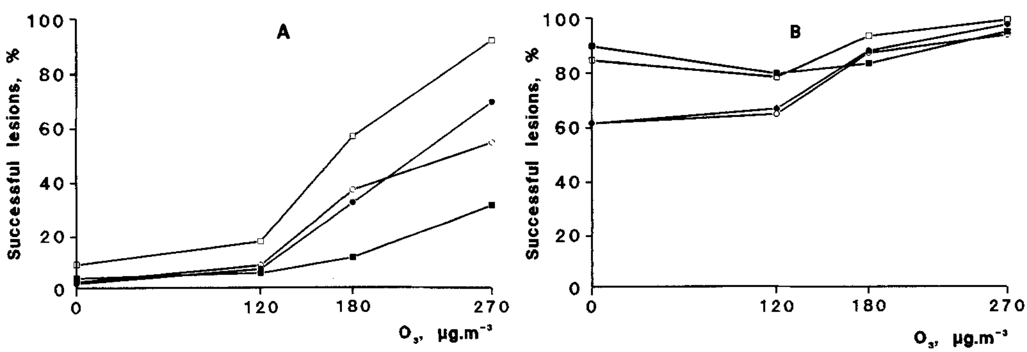

Fig. 2. Successful lesions by *Botrytis cinerea* (in percentage of the total number of inoculation drops) 48 h after conidial inoculation (spore concentration 2×10^6 conidia ml^{-1}) in water (A) or in a Pi solution (B) of primary leaves of four bean cultivars, exposed for 8 h to different ozone concentrations. Groffy (■); Lit (○); Pros (□); Stratego (●).

Table 2. Correlation coefficients for the relationship between the percentage of successful lesions by *Botrytis cinerea* and the percentage of ozone injury on primary leaves of four bean cultivars after acute exposures to different ozone concentrations.

Cultivar	Inoculum composition	Correlation coefficient
Groffy	Water	0.673**[a]
	Pi	0.617*
Lit	Water	0.914**
	Pi	0.418
Pros	Water	0.848**
	Pi	0.759**
Stratego	Water	0.811**
	Pi	0.437

[a] Significant at $P < 0.05$ (*) or at $P < 0.01$ (**).

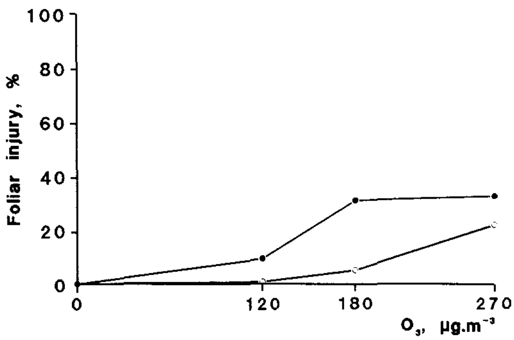

Fig. 3. Foliar injury (%), averaged for all cultivars, on primary (●) and trifoliate leaves (o) of four bean cultivars after 8 h exposures to different ozone concentrations.

of foliar injury by ozone depended significantly on the ozone concentration ($P < 0.001$) and the cultivar ($P < 0.001$) used. Cultivar Groffy was clearly more tolerant to ozone than the other cultivars. Trifoliate leaves appeared to be significantly more ozone-tolerant than primary leaves (Fig. 3).

Results of the analysis of deviance of the percentage of successful lesions by *B. cinerea* are shown in Table 3. Also in this case, three and four factor interactions were not significant and were included in the residual. All main factors were highly significant. Ozone significantly influenced the number of lesions. For all ozone-exposed plants, the number of lesions present on trifoliate leaves was significantly less than that on primary leaves, regardless of the inoculation method (Figs. 4-5). As in the previous experiments, more lesions originated both on Pi-inoculated leaves, compared with water-inoculated leaves, and on water-inoculated leaves of ozone-exposed plants, compared with non-exposed plants. The increase in the number of lesions for each cultivar was similar to that already observed. After water inoculations, a clear increase in successful lesions was observed at increased ozone concentrations for the cultivars Lit, Pros and Stratego, whereas leaves of the cultivar Groffy showed a slower increase (Fig. 4A, B). After Pi inoculations, primary leaves of cv. Groffy showed first a decline in infection after exposures to the lowest ozone concentrations (Fig. 5A). A significant decrease in rate of infection also

Table 3. Analysis of deviance on successful lesions by *Botrytis cinerea* on primary and trifoliate leaves of four bean cultivars after acute exposures to different ozone concentrations.

Source of variation	d.f.	Deviance	Mean deviance	F test	*P*
Experiment	2	28.275	14.138		
Cultivar	3	26.156	8.719	5.00	0.005
Inoculum composition	1	514.034	514.034	294.92	<0.001
Ozone concentration	3	108.816	36.272	20.81	<0.001
Leaf	1	45.744	45.744	26.25	<0.001
Cultivar × Inoculum	3	34.639	11.546	6.62	0.001
Cultivar × Ozone	9	37.506	4.167	2.39	0.050
Cultivar × Leaf	3	27.276	9.092	5.22	0.005
Inoculum × Ozone	3	29.598	9.866	5.66	0.005
Inoculum × Leaf	1	0.000	0.000	0.00	—
Ozone × Leaf	3	52.986	17.662	10.13	<0.001
Residual	159	277.131	1.743		
Total	191	1182.162	6.189		

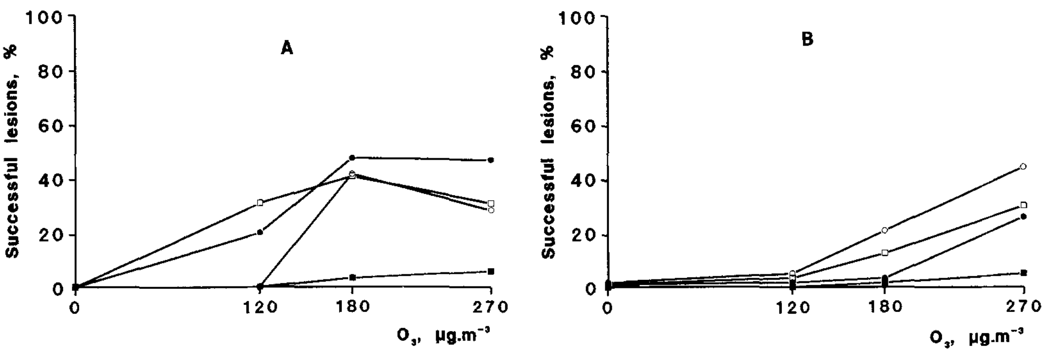

Fig. 4. Successful lesions by *Botrytis cinerea* (in percentage of the total number of inoculation drops) 48 h after conidial inoculation in water (spore concentration 2×10^6 conidia ml^{-1}) of primary (A) and trifoliate leaves (B) of four bean cultivars, exposed for 8 h to different ozone concentrations. Groffy (■); Lit (○); Pros (□); Stratego (●).

occurred on the trifoliate leaves of this cultivar (Fig. 5B). Trifoliate leaves of cv. Pros also showed a decrease in infection rate at the lowest ozone concentration. A significant correlation between the percentage of successful lesions and the percentage of ozone injury was found for the primary leaves of all cultivars, particularly for lesions originating from water-inoculated conidia (Table 4). With the trifoliate leaves, only those of cultivar Lit that were inoculated with spores in water showed a significant correlation between percentage of lesions and ozone injury.

Discussion

Measurements of ambient ozone in the Netherlands from 1980 till 1985 showed that the maximum values of the maximum daily 8 h average concentrations amounted to

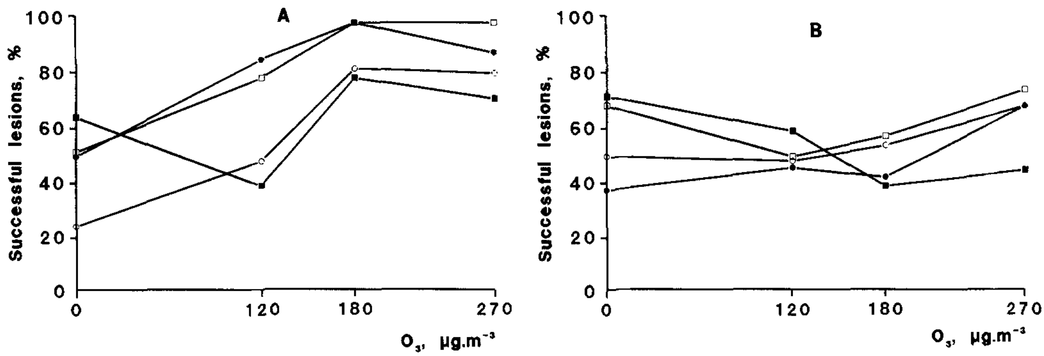

Fig. 5. Successful lesions by *Botrytis cinerea* (in percentage of the total number of inoculation drops) 48 h after conidial inoculation in a Pi solution (spore concentration 2×10^6 conidia ml^{-1}) of primary (A) and trifoliate leaves (B) of four bean cultivars, exposed for 8 h to different ozone concentrations. Groffy (■); Lit (○); Pros (□); Stratego (●).

Table 4. Correlation coefficients for the relationship between the percentage of successful lesions by *Botrytis cinerea* and the percentage of ozone injury on primary and trifoliate leaves of four bean cultivars, after acute exposures to different ozone concentrations.

Cultivar	Inoculum composition	Type of leaf	
		primary	trifoliate
Groffy	Water	0.906**[a]	0.356
	Pi	0.415	0.498
Lit	Water	0.972**	0.769**
	Pi	0.673*	0.428
Pros	Water	0.744**	0.508
	Pi	0.755**	0.207
Stratego	Water	0.890**	0.174
	Pi	0.683*	−0.091

[a] Significant at $P < 0.05$ (*) or at $P < 0.01$ (**).

191-350 μg ozone m^{-3}, depending on the measuring location (Slooff et al., 1989). Therefore, the ozone concentrations used in this study for 8 h exposures are realistic.

The ozone sensitivity of the four bean cultivars at the ozone dosages used in the experiments confirmed the earlier findings of Tonneijck (1983). Clear differences in sensitivity between the cultivars were observed at 180 and 270 μg ozone m^{-3}. At these concentrations, cultivar Groffy was the most ozone-tolerant, whereas cultivar Pros exhibited the highest level of ozone injury. The cultivars Lit and Stratego appeared to be slightly less sensitive than the latter one. Furthermore, the type of leaves affected their response to ozone, as the younger trifoliate leaves were less sensitive than the older primary ones, regardless of the cultivar.

Conidia of *B. cinerea* inoculated in water are known to be generally unable to cause infections on healthy leaves (Blakeman, 1980). Thus, it was decided to inoculate leaves with spores suspended both in a Pi solution and in water, as previous studies had shown that additions of Pi to the inoculum stimulated fungal pathogenicity on healthy bean leaves without the need of a carbon-nitrogen source (Leone and Tonneijck, 1990). However, in the presence of visible ozone injury, bean plants also became highly susceptible to *B. cinerea* after water inoculations, irrespective of the cultivar or of the type of leaf under examination. On primary leaves, a highly significant correlation between the number of fungal lesions and the degree of ozone injury was found. The ozone-induced predisposition of bean leaves to infection by *B. cinerea* is in agreement with the ozone-induced predisposition to the same fungus reported for geranium, onion, and potato leaves (Manning et al., 1969; 1970; Rist and Lorbeer, 1984a). Necrosis induced by ozone, serving as an infection court, has been suggested to be the cause of the change in susceptibility to *B. cinerea* of geranium and potato leaves (Manning et al., 1969; 1970). An alternative hypothesis, based on leakage of nutrients to the leaf surface as a result of ozone-induced injury on cell membranes, has been developed for onion leaves (Rist and Lorbeer, 1984b). Whether the same or other biochemical and/or structural changes are involved in the ozone-induced predisposition of bean leaves to *B. cinerea*, will be object of further investigations.

Conidial inoculations in a Pi solution resulted in the development of more lesions than inoculations in water. However, after Pi inoculations the ozone-tolerant cultivar Groffy exhibited constantly a decrease in the number of lesions at the lowest ozone dosages. A comparable decrease has been observed also on the more ozone-tolerant trifoliate leaves of the ozone-sensitive cultivars. It is difficult to explain why this effect appears when fungal infectivity is stimulated by Pi. However, it is known that the phenomenon of plant immunization to diseases seems to be induced by a low-level and persistent stress (Kuć, 1987). Ozone at the lowest dosage could have induced such a plant response on the ozone-tolerant plant material. A triggering of the resistance mechanisms by ozone in ozone-tolerant plant material appears to be necessary, since susceptibility to *B. cinerea* and ozone sensitivity of the bean cultivars are not directly related (Leone and Tonneijck, 1990).

Ambient air pollution is likely to affect adversely plants as well as pathogens. However, there are reasons to suppose that the potential for ambient ozone to predispose field-grown bean plants to infections by *B. cinerea* is greater than its possible adverse effect on the fungus itself. It is known that pathogenicity of *B. cinerea* on geranium plants is reduced when conidia are ozonized for two 6-h periods at 299 μg ozone m^{-3} (Krause and Weidensaul, 1978), a dosage higher than that used in our experiments. Rist and Lorbeer (1984a) reported that chronic exposures of onion plants to 274 μg ozone m^{-3} for 5 h per day for 5 days had no effect on the expansion of pre-established lesions of *B. cinerea*. Besides, foliar injury enhances the risk of attack of the plant by the fungus in a later period of the season, by the provision of weakened or dead plant material. It is well-known that *B. cinerea* is a primary saprophyte and, as a pathogen, it usually becomes established first on dead or dying vegetable matter (Mansfield, 1980). For the above mentioned reasons and in view of ozone abatement strategies, in this investigation it was decided to study the interaction between *B. cinerea* and bean plants only with preinoculative exposures of the plants to ozone.

The relative age of the leaves and the cultivar used were reported to influence the response of onion plants to the ozone-induced predisposition to infection by *B. cinerea* (Rist and Lorbeer, 1984a). In our study too, after ozone exposures both the ozone-tolerant bean cultivar and the younger trifoliate leaves became less susceptible to the fungus than the ozone-sensitive cultivars or the older primary leaves. Therefore, it can be concluded that many factors regulate the change in predisposition of bean plants to *B. cinerea* such as cultivar, leaf age, inoculum composition and ozone level. However, it is likely that under ambient ozone levels equal or exceeding the ozone concentrations used in this study, field-grown ozone-sensitive plants would be injured by the pollutant, this eventually giving a primary negative effect on production. Fungal infections, stimulated by the ozone-induced predisposition of the leaves under favorable conditions for *B. cinerea*, could give rise to a subsequent, additional yield reduction. As the fungus is not a major pathogen of bean leaves, these ozone-enhanced infections should have limited economic significance but the epidemiology of *B. cinerea* in the interested areas could be strongly affected.

Acknowledgements

The authors are indebted to Mr C. van Dijk and Ms I. van de Linde for skillful technical assistance and to drs J. de Bree for statistical aid. The research program was partly funded by the contract EV4V-0027-NL of the 4th Environment Research Program of the European Community.

Samenvatting

Acute blootstelling aan ozon verhoogt de vatbaarheid van boon (Phaseolus vulgaris) voor Botrytis cinerea

De invloed van ozon op de vatbaarheid van boon voor *Botrytis cinerea* werd onderzocht. Een dag na de blootstelling van vier bonecultivars gedurende 8 uur aan 0, 120, 180 en 270 μg ozon m^{-3}, werden primaire en drietallige bladeren geïnoculeerd met conidiën in water of in een anorganische fosfaatoplossing (Pi). Zichtbare beschadiging door ozon nam met de concentratie toe in alle cultivars. De primaire bladeren waren gevoeliger dan de drietallige. In tegenstelling tot conidiën in water, veroorzaakten conidiën in de Pi-oplossing lesies op gezonde bladeren. Bij ozonbeschadiging vertoonden bladeren van alle cultivars lesies door *B. cinerea* na inoculatie in water. Voor primaire bladeren was het aantal lesies significant gecorreleerd met de bladbeschadiging. Na Pi-inoculatie nam bij de ozongevoelige cultivars het aantal lesies ook toe met de concentratie ozon. Echter, de ozontolerante cultivar Groffy vertoonde eerst een afname in de door Pi gestimuleerde infectie bij de laagste ozonconcentratie. De door ozon verhoogde vatbaarheid van drietallige bladeren was minder dan die van primaire bladeren. De resultaten tonen aan dat realistische concentraties van ozon boon vatbaarder maken voor *B. cinerea*. Deze stijging in vatbaarheid is afhankelijk van het niveau van de ozonbeschadiging die wordt beïnvloed door cultivar, soort blad en ozonconcentratie.

References

Blakeman, J.P., 1980. Behaviour of conidia on aerial plant surfaces. In: Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R. (Eds), The biology of *Botrytis*. Academic Press, New York, London, p. 115-152.

Eerden, L.J., van der, Tonneijck, A.E.G. & Wijnands, J.H.M., 1988. Crop loss due to air pollution in the Netherlands. Environmental Pollution 53: 365-376.

Fehrmann, H., Tiedemann, A., von & Fabian, P., 1986. Predisposition of wheat and barley to fungal leaf attack by preinoculative treatment with ozone and sulphur dioxide. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 93: 313-318.

Heagle, A.S., 1982. Interactions between air pollutants and parasitic plant diseases. In: Unsworth, M.H. & Ormrod, D.P. (Eds), Effects of gaseous air pollutants in agriculture and horticulture. Butterworths, London, p. 333-348.

Hofstra, G. & Ormrod, D.P., 1977. Ozone and sulphur dioxide interaction in white bean and soybean. Canadian Journal of Plant Science 57: 1193-1198.

Krause, C.R. & Weidensaul, T.C., 1978. Effects of ozone on the sporulation, germination and pathogenicity of *Botrytis cinerea*. Phytopathology 68: 195-198.

Krupa, V. & Manning, W.J., 1988. Atmospheric ozone: formation and effects on vegetation. Environmental Pollution 50: 101-137.

Kuć, J., 1987. Plant immunization and its applicability for disease control. In: Chet, I. (Ed.), Innovative approaches to plant disease control. John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore, p. 255-274.

Kvist, K., 1986. Fungal pathogens interacting with air pollutants in agricultural crop production. In: Directorate-General for Science, Research and Development Environment Research Programme of the Commission of the European Communities (Ed.), How are the effects of air pollutants on agricultural crops influenced by the interaction with other limiting factors? Proceedings Workshop at Riso National Laboratory, Denmark 23-25 March 1986, p. 67-78.

Last, F.T. & Hamley, R.E., 1956. A local-lesion technique for measuring the infectivity of conidia of *Botrytis fabae* Sardiña. Annals of Applied Biology 44: 410-418.

Leone, G. & Tonneijck, A.E.G., 1990. A rapid procedure for screening the resistance of bean cultivars (*Phaseolus vulgaris* L.) to *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Euphytica, in press.

Manning, W.J., 1976. The influence of ozone on plant surface microfloras. In: Dickinson, C.H. & Preace, T.C. (Eds), Microbiology of aerial plant surfaces. Academic Press, New York, London, p. 159-172.

Manning, W.J., Feder, W.A., Perkins, I. & Glickman, M., 1969. Ozone injury and infection of potato leaves by *Botrytis cinerea*. Plant Disease Reporter 53: 691-693.

Manning, W.J., Feder, W.A. & Perkins, I., 1970. Ozone injury increases infections on geranium leaves by *Botrytis cinerea*. Phytopathology 60: 669-670.

Mansfield, J.W., 1980. Mechanisms of resistance to *Botrytis*. In: Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R. (Eds), The biology of *Botrytis*. Academic Press, New York, London, p. 181-218.

Mc Cullagh, P. & Nelder, J.A., 1983. Generalized linear models. University Press, Cambridge.

Rist, D.L. & Lorbeer, J.W., 1984a. Moderate dosages of ozone enhance infection of onion leaves by *Botrytis cinerea* but not by *Botrytis squamosa*. Phytopathology 74: 761-767.

Rist, D.L. & Lorbeer, J.W., 1984b. Ozone-enhanced leaching of onion foliage in relation to lesion production by *Botrytis cinerea*. Phytopathology 74: 1217-1220.

Slooff, W., Aalst, R.M. van, Heijna-Merkus, E. & Thomas, R., 1989. Integrated criteria document ozone. Report no. 758474002, National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands.

Tonneijck, A.E.G., 1983. Foliar injury responses of 24 bean cultivars (*Phaseolus vulgaris*) to various concentrations of ozone. Netherlands Journal of Plant Pathology 89: 99-104.

Tonneijck, A.E.G., 1989. Evaluation of ozone effects on vegetation in the Netherlands. In: Schneider, T., Lee, S.D., Wolters, G.J.R. & Grant, L.D. (Eds), Atmospheric ozone research and its policy implications. Elsevier Science Publishers B.V., Amsterdam, p. 251-260.