

Assessing effects of ambient ozone on crops in The Netherlands with ethylenediurea (EDU): spatial and temporal variation

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ABSTRACT

As part of UN/ECE ICP-Crops program, plants of clover (Trifolium subterraneum cv. Geraldton) and bean (Phaseolus vulgaris cv. Lit) were exposed to ambient air at four rural sites in the growing seasons of 1994 through 1996. The influence of ozone on visible injury, growth and yield was determined by comparing the response of plants treated with ethylene diurea (EDU) to that of untreated plants. EDU is an antioxidant that protects plants from ozone damage. A considerable degree of ozone-induced injury in both crop species has been observed at all sites. Although injury in each species varied between years and sites, the site-dependent pattern was similar for each year. Adverse effects of ozone on pod yield in bean were observed each year but did not vary between sites. Plant responses were not related to the measured ozone levels (AOT40) and environmental factors such as VPD seem to have a strong influence on the ozone sensitivity of plants in terms of foliar injury. Evidence suggests that the short-term critical level to protect plants against ozone injury should be lower than those that have been proposed recently.

1. INTRODUCTION

It has been recognized over the past two decades that levels of air pollutants within Europe are sufficiently high to cause adverse effects on sensitive crops. Thus, policies to reduce these levels and their associated impacts on the environment need to be developed on an international basis. For Europe, these policies are being formulated mainly within the Convention on Long Range Transboundary Air Pollution (LRTAP Convention) of the United Nations/Economic Commission of Europe (UN/ECE). In 1988, the International Cooperative Program on effects of air pollution and other stresses on crops and non-woody plants (ICP-Crops) came into force under this convention. The aim of this program is to provide quantitative information regarding the responses of crops and non-woody plants to air pollution.

Ozone is the pollutant of primary concern to the ICP-Crops which aims to verify the long-term critical level of ozone for yield reduction and the short-term critical level for protection of crops against visible injury (Benton et al., 1995). Critical levels have been defined as the concentrations of pollutants in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge (UN/ECE, 1988). Recently, short-term and long-term critical levels to protect crops against significant effects by ambient ozone have been proposed (Kärenlampi and Skärby, 1996). These critical levels are expressed as cumulative exposures over the threshold concentration of 40 ppb ozone during daylight hours and are referred to as AOT40.

Ozone-induced foliar injury has been frequently observed after episodes with elevated concentrations and growing seasonal mean concentrations of ambient ozone are considered to be sufficiently high to adversely affect yield of sensitive crops in the Netherlands (Tonneijck, 1989). However, data on effects of ozone in ambient air on crop productivity were lacking for our country. Furthermore, information on temporal and spatial variation of plant responses to ambient ozone and data to determine relationships between exposure and plant response were hardly available.

As part of the UN/ECE ICP-Crops program, a three-year project was started in the Netherlands in 1994 to (1) quantitatively assess the degree of ozone injury, (2) to assess

growth and yield reductions resulting from chronic exposures, (3) to study the spatial and temporal variations of ozone-induced effects and (4) to develop relationships between exposure and plant response. In this project, the antioxidant ethylenediurea (EDU) was used to investigate the responses of ozone sensitive plant species such as clover (Trifolium subterraneum) and bean (Phaseolus vulgaris) to ambient air at four sites in rural areas. EDU is currently the best known systemic antioxidant and has been used extensively to study the effects of ambient ozone on crops and trees (Manning and Krupa, 1992). Some results concerning plant responses at the various sites during the growing seasons of 1994 through 1996 are presented in this paper and discussed briefly in relation to exposure levels and the proposed critical levels for ozone.

2. EXPERIMENTAL PROCEDURES

Experimental procedures were performed according to the UN/ECE ICP-Crops protocol (UN/ECE, 1994) with minor adjustments. For the growing seasons of 1994 through 1996, potted plants of clover (Trifolium subterraneum cv. Geraldton) and bean (Phaseolus vulgaris cv. Lit) were exposed to ambient air at four sites in rural areas: Westmaas (51°47' N, 04°27' E), Schipluiden (51°59' N, 04°16' E), Zegveld (52°08' N, 04°50' E), and Wageningen (51°58' N, 05°38' E). For experimental details see Table 1. The influence of ambient ozone on visible injury (proportion of leaves injured) was assessed and the effects on growth and yield were determined by comparing the responses of plants treated with EDU to those of untreated plants.

Table 1.

Timetable of events for the experiments with bean and clover in the growing seasons of 1994 through 1996

Year	Experiment	Start of exposure	Harvest 1	Harvest 2
Clover				
1994	1	June 21	July 19	August 16
	2	August 23	September 20	October 18
1995	1	June 20	July 18	August 15
	2	August 15	September 12	October 10
1996	1	June 18	July 16	August 13
	2	August 13	September 10	October 8
Bean				
1994	1	June 28	August 9	September 13
1995	1	June 27	August 8	September 5
1996	1	June 25	August 20	October 2

Two successive eight-week experiments with clover were performed each year. Three-week-old plants (three plants per pot) were transported to the sites and the first application with EDU was carried out. EDU was applied at 2-week intervals as a soil drench using 100 ml per pot (10x10 cm) of a 150 mg l⁻¹ solution. Control plants received an equal volume of distilled water. After four weeks (Harvest 1), fully expanded leaves were removed to determine the amount of foliar injury and biomass, and the plants were then left to regrow. The final harvest was performed after another four weeks and the amount of foliar injury and leaf dry weight were assessed (Harvest 2).

One experiment with bean was performed each year. Three-week-old plants (one plant per pot) were transported to the sites and the first application with EDU was carried out. EDU was applied as a soil drench using 200 ml per pot (5 liter) of a 100 mg l⁻¹ solution. Additional applications of EDU were given at 2-week intervals with 100, 150, 200 and 250 mg l⁻¹ solutions, respectively. To assess the amount of visible injury and dry weight of green pods, an intermediate harvest (Harvest 1) was performed when the green pods

were ready for market. A dry harvest (Harvest 2) was carried out when the pods were mature and contained loose seeds, and pod dry weight was determined.

Concentrations of O₃, NO_x and SO₂ were recorded at monitoring stations of the National Air Quality Monitoring Network (RIVM, 1989), that were located within 500 m of the biomonitoring sites. Exposures to ozone are expressed as AOT40 (Accumulated exposure Over a Threshold of 40 ppb). This exposure index is calculated as the sum of the differences between the hourly ozone concentrations in ppb and 40 ppb for each hour when the concentration exceeds 40 ppb. The AOT40 for each site is calculated only for daylight hours with a mean global radiation 50 W m⁻² (Kärenlampi and Skärby, 1996) at Wageningen.

3. RESULTS AND DISCUSSION

3.1. Foliar injury

The first symptoms in non-EDU-treated beans were observed two (in 1994 and 1995) and five weeks (in 1996) after the start of the exposures. Symptoms characteristic of O₃ stress consisted of dark brown blemishes and occurred mainly on the adaxial leaf surfaces. These ozone-induced symptoms have been recorded on field-grown beans in the Netherlands since the early eighties (Tonneijck, 1983) and in many other European countries (Sanders and Benton, 1995). The degree of injury varied between sites and years (Figure 1). On average, injury amounted to 27% in 1994, to 8% in 1995 and to 1% in 1996. In the last year, symptoms were observed at one site (Schipluiden) only. The greatest amount of injury (35%) was recorded at Wageningen in 1994. Injury differed significantly between the sites each year and was greatest at Schipluiden in two of the three growing seasons.

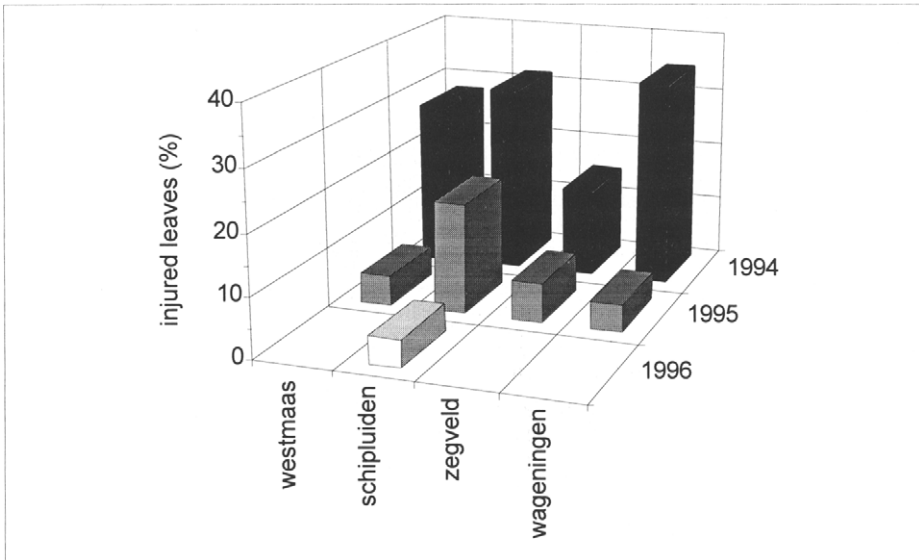


Figure 1. Ozone injury (% leaves injured) in bean after exposure to ambient air at four rural sites in the growing seasons of 1994 through 1996 (Harvest 1).

Subterranean clover was sensitive to air pollution by ozone as it displayed the characteristic symptoms of chlorotic or necrotic lesions until Harvest 1 of Experiment 2 each year. Thus, concentrations of ambient ozone were sufficiently high to cause foliar injury in this species until the end of the summer. As in bean, the degree of injury in clover also varied between sites and years (Figure 2). On average, injury as assessed at Harvest 1 of Experiment 1, amounted to 9% in 1994, to 22% in 1995 and to 1% in 1996. Injury generally differed between the sites each year and was greatest at Schipluiden. The greatest amount of injury (31 %) was recorded at this site after four weeks of exposure (Harvest 1 of Experiment 1) in 1995.

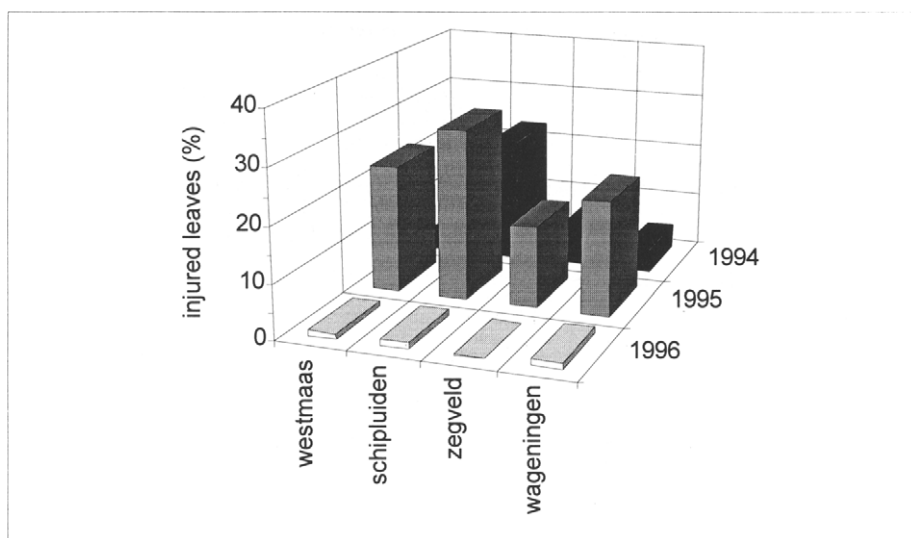


Figure 2. Ozone injury (% leaves injured) in clover after exposure to ambient air at four rural sites in the growing seasons of 1994 through 1996. Data relate to Harvest 1 of Experiment 1.

3.2. Growth and yield

Pod yield in non-EDU-treated plants of bean was reduced each year in comparison to EDU-treated plants (Table 2). Although EDU itself may reduce or increase pod yield in bean (eg Kostka-Rick and Manning, 1993), the observed reduction of pod yield in non-EDU-treated bean plants could be attributed to enhanced concentrations of ambient ozone (Tonnejck and Van Dijk, 1997b) and was observed especially when mature pods were harvested. Yield reduction of mature pods was greatest in 1994 and amounted to 17%, on average. Analyses indicated that the adverse effects of ozone on pod yield did not differ between sites for each year, which contrasted with the site-dependent pattern of ozone injury in this species. Furthermore, interannual variation concerning ozone-induced yield reduction appeared to differ from that concerning visible injury, thereby indicating that the degree of ozone injury is not linked to proportional yield reduction by ozone in bean.

The effect of ambient ozone on leaf biomass production in clover is not yet clear. Leaf

biomass in non-EDU-treated clover plants was generally lower than in EDU-treated plants when ozone concentrations were high. Differential responses in leaf biomass between EDU and non-EDU-treated plants were variable when concentrations of ambient ozone were relatively low (Tonnejck and Van Dijk, 1997a). Results showed that proportional reduction in leaf biomass production in non-EDU-treated plants as compared to EDU-treated plants generally did not depend on the site and was smaller than 10 % on average for each year (data not shown).

Table 2

Proportional decrease (%) in yield of green and mature pods of non-EDU-treated plants of bean as compared to EDU-treated plants (adverse effect of ozone), after exposure to ambient air at four rural sites in the growing seasons of 1994 through 1996.

Plant stage	Reduction pod weight (%)		
	1994	1995	1996
Green pods	-0.5	10.7	2.7
Mature pods	16.6	3.0	13.5

3.3. Plant response in relation to ozone exposure

Research is currently being performed to study various aspects of the relationships between ozone exposure and plant response and to verify the short-term and long-term critical levels for ozone as defined in Kärenlampi and Skärby (1996). Analyses of the data of 1994 have shown that observed responses concerning injury, biomass production and yield in bean and subterranean clover were not related to ozone exposures (AOT40) measured at the sites (Tonnejck and Van Dijk, 1997a,b). This may indicate that AOT40 is not a good exposure index to describe the response of bean and clover to ambient ozone under Dutch conditions. Environmental factors such as temperature, relative humidity and the presence of other air pollutants can influence the sensitivity of plants to ozone (Guderian et al., 1985). The lack of correlation between ozone exposure and

response may also be due to environmental conditions that are likely to vary between sites and exposure periods. Sulphur dioxide and nitrogen oxides do not seem to be relevant since the measured concentrations appeared to be low and similar among the sites (Tonneijck and Van Dijk, 1997a,b).

To elucidate various aspects of plant response in relation to ambient ozone, some data of our study are presented concerning visible injury in clover. According to Balls et al. (1996), vapor pressure deficit (VPD) has a relatively strong influence on the degree of ozone injury in this species by influencing pollutant uptake of the leaves; the lower the VPD the greater the injury response to ozone. In 1994 (Figure 3), daily mean values of VPD and daily maximum temperatures in Wageningen were lower in the second experiment with clover (August 23-October 18) than in the first experiment (June 21-August 16). Daily mean values of VPD were generally below 1.5 kPa, a limit in the proposed short-term critical levels (Benton et al., 1996), throughout the growing season. Daily concentrations of ambient ozone (AOT40) were relatively high up to and including August 4 and declined strongly after this date. However, the degree of ozone injury after four weeks of exposure in Experiment 2 was about equal to the observed injury in Experiment 1. Thus, clover was more sensitive to ambient ozone in the second experiment than in the first experiment. These results indicate that injury response in clover may not only depend on ozone exposure (AOT40). Furthermore, many observations within the UN/ECE ICP-Crops program have shown that sensitivity of clover to ambient ozone increased with decreasing VPD (Benton et al., 1996).

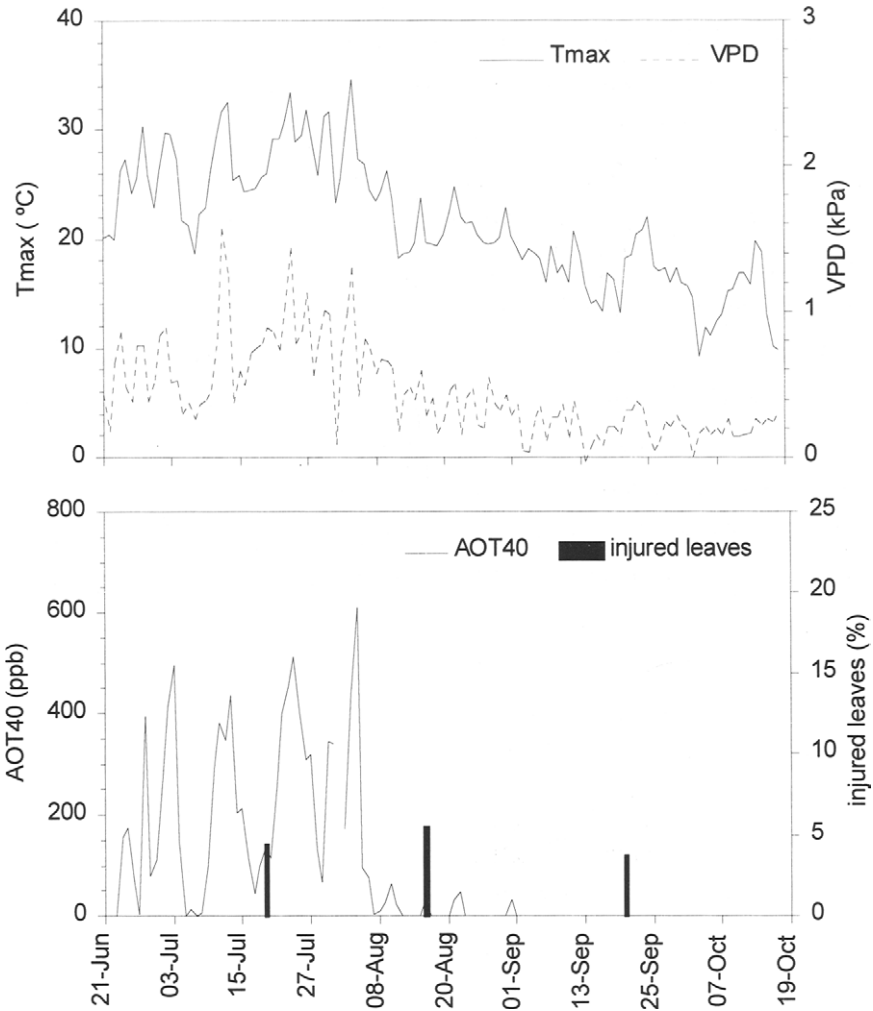


Figure 3. Ozone injury (% leaves injured) in clover after different exposures of four weeks to ambient air and day-by-day variation in maximum temperature (Tmax), vapor pressure deficit (VPD) and concentrations of ambient ozone (AOT40) at Wageningen in 1994.

Recently, Benton et al. (1996) proposed two short-term critical levels (AOT40 accumulated over five consecutive days) for injury development by ozone depending on

the level of VPD: an AOT40 of 500 ppb(h when mean VPD (9.30-16.30 h) exceeds 1.5 kPa and an AOT40 of 200 ppb(h when mean VPD is below 1.5 kPa. Calculations for 1994 and 1995 (Table 3) have shown that the AOT40 accumulated over five consecutive days prior to ozone injury in clover generally exceeded the proposed short-term critical levels at all sites each year except for the second experiment in 1994. In this experiment, foliar injury was observed at all sites whereas the maximal five-days AOT40 ranged from 1 to 81 ppb(h. The mean VPD was rather low during the corresponding five-days period at Wageningen and amounted to 0.16 kPa. The occurrence of such low levels of VPD may be an important reason that injury was observed at exposures below the short-term critical level (AOT40) of 200 ppb(h ozone. The results suggest that the short-term critical level to protect plants against ozone injury should be lower than those that have been proposed recently. Since injury in clover was observed once after a five-days exposure (AOT40) to 1 ppb(h ozone, the threshold value of 40 ppb used to calculate the accumulative exposures may be too high and should also be reconsidered.

Table 3

Maximal AOT40 (ppb(h) at different sites accumulated for five consecutive days in the week prior to the first observation of injury in Trifolium subterraneum cv. Geraldton during the growing seasons of 1994 and 1995.

Site	Experiment 1		Experiment 2	
	Before	After	Before	After
	Harvest 1	Harvest 1	Harvest 1	Harvest 1 ^a
1994				
Schipluiden	924	1724	81	0
Zegveld	963	1297	1	2
Wageningen	1102	2033	33	0
Westmaas	947	1251	27	0
1995				
Schipluiden	697	781	781	22
Zegveld	836	670	710	3
Wageningen	1252	704	1011	11
Westmaas	912	1048	1008	26

^a Since no injury has been observed, data relate to the maximal AOT40 accumulated for five consecutive days during the four weeks exposure period between Harvest 1 and Harvest 2.

4. CONCLUDING REMARKS

The concentrations of ambient ozone in the Netherlands are sufficiently high to cause adverse effects on sensitive crop species such as clover and bean. Foliar injury has been observed at all sites till the middle of September in each year. The degree of injury differed between sites and the site-dependent variation generally was similar for both

species each year. A year-to-year variation in the degree of injury was observed for both crop species. Studies with the antioxidant EDU led to the conclusion that ambient ozone can adversely affect pod yield in bean in the Netherlands while the effects of ozone on biomass production in clover are not yet clear. In contrast to injury, effects on growth and yield did not vary between sites.

Knowledge concerning spatial variation of plant responses is necessary when attempting to evaluate the risk from exposures to ambient ozone. Short-term exposures to high concentrations generally result in visible injury while chronic exposures to low concentrations can cause physiological alterations that ultimately result in growth and yield reductions. These physiological alterations are probably not linked to visible symptoms. Our results indicate that the occurrence of spatial variation depends on the nature of plant response (injury versus growth and yield) and, thus, possibly on the type of exposure to ambient ozone (acute versus chronic).

Guidelines or critical levels to protect crops against adverse effects of ozone are generally deduced from artificial fumigation experiments in open-top chambers. This also applies to the AOT40 concept. At present, there is an ongoing debate about the relevance of results from this type of experiments to explain the response of crops to ambient ozone in the field (eg Grünhage and Jäger, 1996). Key issues relate to ozone exposure dynamics such as the importance of ozone peaks versus mid-range hourly values of ozone and to factors influencing pollutant uptake such as atmospheric transport properties and VPD. Our results underline this problem since the observed plant responses could not as yet be related to AOT40 values. Clover appeared to be relatively sensitive to ambient ozone at the end of the growing season under conditions of low VPD. Evidence concerning the occurrence of injury in this species suggests that a threshold value of 40 ppb ozone may be too high to calculate cumulative exposures. To extend our knowledge on the risk of exposures to ambient ozone, more research is needed to study the relation between plant responses and exposures under 'real world' conditions.

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