The mechanism of NH_3 and SO_2 uptake by leaves and its physiological effects

Het mechanisme van de NH_3 en SO_2 opname door bladeren en hierdoor veroorzaakte fysiologische effecten

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The mechanism of NH_3 and SO_2 uptake by leaves and its physiological effects

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H. C. van der Plas,
in het openbaar te verdedigen
op woensdag 18 oktober 1989
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

STELLINGEN

1. De bestaande modellen voor de gasuitwisseling van bladeren met de aangrenzende atmosfeer zijn ontoereikend voor het berekenen van NH_3 en SO_2 opname door bladeren.

   Dit proefschrift

2. Bij planten die onder optimale condities opgroeien, veroorzaken de gemiddelde NH_3 en SO_2 concentraties welke in Nederland in de atmosfeer worden gemeten, geen direkt waarneembaar effekt op de fotosynthese en stomataire geleiding.

   Dit proefschrift

3. De aanwezigheid van 'water' op het bladoppervlak, in de vorm van een waterfilm en/of gebonden aan de polaire groepen in de cuticula, speelt een cruciale rol bij de adsorptie van NH_3 en SO_2 aan het bladoppervlak.

   Dit proefschrift.

4. De stikstof verliezen van een gewas ten gevolge NH_3 emissie door de bladeren zijn te verwaarlozen.

   Dit proefschrift

5. De conclusie van Ivens *et al.* dat uit concentratie-metingen van NH_4^+ en SO_4^{2-} in "doorvalwater" een goede schatting van de droge depositie van NH_3 en SO_2 op een vegetatie-oppervlak kan worden gemaakt, is onjuist.

   Ivens, W.P.M.F., Draaijers, G.P.J., Bos, M.M. and Bleuten, W. 1988. Dutch forests as air pollutant sinks in agricultural areas. Dutch Priority Programme on Acidification, Report 37-09, RIVM Bilthoven.

   Dit proefschrift

6. De door Genty *et al.* geintroduceerde procedure voor het non-destructief meten van de fotosynthese aan bladeren van planten met behulp van chlorofyl fluorescentie munt uit door eenvoud en snelheid en is derhalve bij uitstek geschikt voor toepassing binnen ecofysiologisch onderzoek.

   Genty, B., Briantais, J.M. and Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990: 87-92.

7. De economische groeifilosofie welke het Nationaal Milieubeleidsplan hanteert om het milieu te redden, leidt niet tot een serieuze terugdringing van de uitstoot van gassen verantwoordelijk voor het broeikaseffect.

   RIVM-rapport "Zorgen voor morgen, nationale milieuverkenning 1985-2010".

8. Het leven in dichtbevolkte, goed georganiseerde steden is uit een oogpunt van energiebesparing en milieubehoud meer verantwoord dan het leven op het platteland.

Paehlke R.C. 1989. Environmentalism and the future of progressive politics. Yale University Press.

9. Het verdient aanbeveling om in economische beschouwingen over het tropische regenwoud meer de nadruk te leggen op de commerciële waarde van het regenwoud als leverancier van niet-hout produkten.

Peter, C.M., Gentry, A.H. and Mendelsohn, R.O. 1989. Valuation of an Amazonian rainforest. Nature 339: 655-656.

10. De plantenveredeling in de laatste decennia heeft onbedoeld geleid tot cultuurrassen met een grotere tolerantie tegen luchtverontreiniging.

Gould, R.P. and Mansfield, T.A.. 1989. The sensitivity of early 20th century cultivars of wheat to air pollution. Environmental Pollution 56: 31-37.

11. De resultaten van Terashima et al. tonen duidelijk aan, dat uit de relatie tussen de interne CO_2-concentratie en CO_2-assimilatie een onjuiste conclusie kan worden getrokken ten aanzien van niet-stomataire of stomataire remming van de fotosynthese ten gevolge van b.v. 'stress' door droogte, indien de opening van de stomata van het blad ongelijk is.

Terashima, I., Wong, S.-C., Osmond, C.B. and Farquhar, G.D. (1988). Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. Plant Cell Physiology 29: 385-394.

12. Gezien de moeilijkheid om stikstofoxiden ($NO+NO_2$) uit de lucht m.b.v. koolstoffilters te verwijderen, moet het waarschijnlijk worden geacht, dat veel in de literatuur beschreven langdurige begassingen met ozon, zwaveldioxide of ammoniak in feite mengbegassingen met stikstofoxiden zijn geweest.

Dit proefschrift.

13. Gezien de traagheid waarmee veranderingen tot behoud van het milieu worden gerealiseerd, vormt de milieuproblematiek nog een schier onuitputtelijke bron van stellingen voor toekomstige generaties promovendi.

Stellingen behorende bij het proefschrift: "*The mechanism of NH_3 and SO_2 uptake by leaves and its physiological effects*", van L.W.A. van Hove.

Wageningen, 18 oktober 1989.

NN08201, 1305

ERRATA

Page v	line 16 from bottom:	.... prof. dr. W.J. Vredenberg....
	line 4 from bottom:	.....duryen....
Page 99	line 6 from top :	.....Louwerse (1980)....
Page 110	line 2 from bottom:	.....(Grace 1981)....
Page 111	line 8 from bottom:	.....Grace (1981)....
Page 123	line 12 from bottom:	.....Saxe (1983)....
Page 133	line 7 from bottom:	.....nutriënten....
Page 140	line 12 from top :	.....(1979a).....

The following references should be added:

Black, C.R. and Black, V.J. (1979a). The effects of low concentrations of sulphur dioxide on stomatal conductance and epidermal cell survival in field bean (*Vicia faba L.*). Journal of Experimental Botany 30 (115): 291-298.

Black, C.R. and Black, V.J. (1979b). Light and scanning electron microscopy of SO_2-induced injury to leaf surfaces of field bean (*Vicia faba L.*). Plant, Cell & Environment 2: 329-333.

Boxman, A.W., Van Dijk H.F.G. and Roelofs J.G.M. (1987). Some effects of ammonium sulphate deposition of pine and deciduous forests in the Netherlands. In: Acid Rain; Scientific and Technical Advances (eds. Perry, R., Harrison, R.M., Bell, J.N.B. and Lestor, J.N.): 680-687. Selper London.

Page 141	line 7 from bottom:	Erisman, J.W., ... (1989).....

"*The mechanism of NH_3 and SO_2 uptake by leaves and its physiological effects*" by L.W.A. van Hove.

Wageningen, september 25, 1989.

VOORWOORD

Het in dit proefschrift beschreven onderzoek werd in de periode van begin 1984 tot begin 1989 uitgevoerd. Het onderzoek gebeurde in een samenwerkingsverband tussen de vakgroep *Luchthygiëne en -verontreiniging* en de vakgroep *Plantenfysiologisch Onderzoek* van de Landbouwuniversiteit te Wageningen. Aanvankelijk werd het onderzoek gefinancierd door het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer (VROM) (afd. Bestuurszaken, sectie externe veiligheid). Sinds juni 1986 maakt het onderzoek deel uit van het Nationaal Programma Verzuringsonderzoek, dat wordt gecoördineerd door het Rijks Instituut voor Volksgezondheid en Milieuhygiëne (RIVM) te Bilthoven. In de periode van medio 1988 tot mei 1990 wordt in een vervolgprojekt (projekt 110) eenzelfde soort onderzoek gedaan bij de Douglas spar (*Pseudotsuga menziesii*).

Graag wil ik de vertegenwoordigers van VROM en het Nationaal Programma Verzuringsonderzoek bedanken voor hun financiële ondersteuning van het onderzoek.

Echter, "*geld alleen maakt niet gelukkig*". Het onderzoek zou niet tot stand zijn gekomen zonder de inzet van velen. Graag wil ik van de gelegenheid gebruik maken om hen hiervoor te bedanken.

In de eerste plaats gaat mijn dank uit naar mijn beide promotoren, prof. dr. E.H. Adema en prof. W.J. Vredenberg, voor hun steun en raad tijdens het verloop van het onderzoek en het kritisch bekijken van de manuscripten. In dit verband ben ik ook dr. G.A. Pieters (PFO) erkentelijk. Veel dank ben ik ook verschuldigd aan Olaf van Kooten. Olaf was voor mij de "goeroe" op het gebied van de chlorofyl fluorescentie. Samen deden we vanaf 1986 "zure regen"-onderzoek onder de paraplu van het Nationaal Programma Verzuringsonderzoek, hetgeen zijn neerslag vond in gezamenlijke publikaties op dit gebied.

Het onderzoek vereiste een omvangrijke proefopstelling. De inzet van Wim Tonk is van onschatbare waarde voor de ontwikkeling hiervan geweest. Als belangrijkste "wapenfeiten" dienen genoemd te worden: het ontwerp van de bladkamer of bladcuvette, en de begassingskamers waarin planten gedurende een lange tijd kunnen worden blootgesteld aan verschillende gassen. Zijn inventiviteit, deskundigheid, inspanning en het "durfen nemen van risico's" hebben er mede toe bijgedragen dat ideeën werkelijkheid zijn geworden. In dit verband ben ik ook de medewerkers van de Stichting Technische Fysische Dienst voor de Landbouw (TFDL) erkentelijk.

Met name gaat mijn dank uit naar Rinus van Ginkel, degene die uiteindelijk de bladcuvette heeft gemaakt.

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Tevens wil ik de grote groep medewerkers van de vakgroep Luchthygiëne en -verontreiniging bedanken. De assistentie gegeven door Wim Braun, Pieter Versloot, Joop Willems, Hillion Wegh, Jan van Tongeren, Eric Molenaar en Paul Heeres is van groot belang geweest bij de uitvoering van het onderzoek.

Mijn dank gaat ook uit naar de medewerkers en medewerksters van de service afdeling, de electro/electronica afdeling, de tekenkamer, de afdeling fotografie, het apparaten- en het chemisch magazijn van het Biotechnion.

Veel dank ben ik ook verschuldigd aan Rob Scholtens, Andries Koops, Gerald Laheij, Klaas Jan van Wijk, Daniël Veenhuysen en Martin Stempher. In het kader van hun doctoraalstudie of afstudeeropdracht hebben zij een waardevolle bijdrage aan het onderzoek geleverd.

Erkentelijk ben ik ook voor de adviezen en steun gegeven door medewerkers van het Instituut voor Plantenziektenkundig Onderzoek (IPO), werkzaam op het gebied van de effekten van luchtverontreiniging op planten.

Vermeld dient ook de inzet van Manon Mensink te worden. Na haar aanstelling in februari 1989 heeft zij zich in korte tijd het onderzoek eigen gemaakt, hetgeen een grote prestatie genoemd kan worden. Hierdoor kon ik mij "onbekommerd" aan het schrijven van het manuscript wijden.

Verder wil ik Andrea Velthuyzen van Zanten uit mijn geboortedorp Hillegom bedanken voor het ontwerp van de omslag van het proefschrift.

Mijn ouders hebben mij gestimuleerd en de mogelijkheden geboden om te gaan studeren. Ik ben hen daar zeer dankbaar voor.

CONTENTS

List of abbreviations and symbols

A	projected leaf area
C	capacity representing surface sorption of NH_3
C_{in}	inlet gas concentration of the leaf chamber
C_{out}	outlet gas concentration of the leaf chamber
C_{ss}	steady state gas concentration in the leaf chamber
C_e	gas concentration immediately adjacent to the leaf
C_i	gas concentration in the substomatal cavity
C_s	gas concentration at the leaf surface
D	diffusion coefficient of a gas in air ($m^2.s^{-1}$)
D_v	diffusion coefficient of water vapour in air
D_H	diffusion coefficient of heat in air
e_s	saturated water vapour pressure
e_a	actual water vapour pressure
E	transpiration rate of a leaf
f	air flow through the leaf chamber
F	flux density of a gas towards of away from the leaf
F_{max}	maximal fluorescence
F_v	variable fluorescence
$(F_v)_s$	saturated variable fluorescence
F_o	initial fluorescence
g_s	stomatal conductance
g_m	mesophyll conductance
$g_{t,c}$	leaf conductance for CO_2
GS	glutamine synthetase
GOGAT	glutamine: 2-oxoglutarate-aminotransferase
I	irradiance
PAR	photosynthetically active radiation (400-800 nm)
P_{max}	CO_2 assimilation at light saturation
P_n	net CO_2 assimilation
PFD	photon flux density ($\mu mol.m^{-2}.s^{-1}$).
PTFE	polytetrafluorethylene
PVDF	polyvinylidene fluoride
QY	quantum yield for CO_2 fixation
q_Q	photochemical quenching
q_E	non-photochemical quenching

r_b	boundary layer resistance
$r_{b,h}$	boundary layer resistance for heat
$r_{b,v}$	boundary layer resistance for water vapour
r_c	cuticular resistance
r_{ic}	resistance for gas transport after passage of the cuticle
r_i	internal resistance
r_s	stomatal resistance
$r_{s,v}$	stomatal resistance for H_2O
R_d	dark respiration
R.H.	relative humidity
R_n	net radiation
Re_{crit}	critical Reynolds number
RUBISCO	ribulose-1,5-biphosphate-carboxylase-oxygenase
u	wind velocity
VPD	vapour pressure deficit
w	length of leaf parallel to wind speed
γ	psychrometric constant (Pa $°C^{-1}$)
τ	time constant
λ	heat of vaporization of H_2O ($J.g^{-1}$)
ρC_p	volumetric heat capacity of the air ($J.m^{-3}.°C^{-1}$)
Φ_{Po}	quantum yield in the non-energized state (q_E=0 and all traps open)

ABSTRACT

The relation between uptake of atmospheric ammonia (NH_3) and sulphur dioxide (SO_2) by individual leaves, photosynthesis and stomatal conductance was examined. The experiments were carried out with bean plants (*Phaseolus vulgaris L.*) and poplar shoots (*Populus euramericana L.*). The method of analysis was derived from methods used in photosynthetic research. The uptake of NH_3 or SO_2 was experimentally determined by using a leaf chamber specially developed for this research. Simultaneously, transpiration and carbondioxide (CO_2) assimilation of leaves were measured.

The adsorption of NH_3 and SO_2 strongly increased with increasing air humidity, indicating a major role of water in the adsorption process. A descriptive model for the adsorption in the cuticle-water-system is proposed. The affinity of SO_2 for the leaf surface was found to be approximately twice that of NH_3. A mixture of these gases in the air mutually stimulated their adsorption on the leaf surface. No significant desorption or transport of these gases through the cuticle could be detected.

The uptake of NH_3 into leaves appeared to be dependent on the leaf boundary layer and stomatal resistance and NH_3 concentration at the leaf surface. In contrast, a less clear relation between SO_2 uptake and stomatal resistance was found, in particular at a low vapor pressure deficit (VPD). The measured flux was larger than can be calculated from the boundary layer and stomatal resistance for H_2O, suggesting a lower resistance of the diffusion pathway. The same was observed for NH_3 at a low temperature and VPD. It is postulated that this discrepancy is due to a difference in path length.

Under the conditions of the present research the physiological effects caused by a prolonged exposure to NH_3 or SO_2 became notable at concentrations of about 100 $\mu g.m^{-3}$. The NH_3 exposure had a positive effect on photosynthesis, stomatal conductance and NH_3 uptake, whereas a small irreversible inhibition of photosynthesis and stomatal conductance was induced by the SO_2 exposure.

The relations assessed in this study can be used to construct a descriptive model for NH_3 and SO_2 transfer into leaves as a function of wind velocity, light intensity, air temperature and humidity.

Key word index: Air pollution, NH_3-uptake, SO_2-uptake, photosynthesis, stomatal conductance, *Phaseolus vulgaris L.*, *Populus euramericana L.*, leaves.

CHAPTER 1

GENERAL INTRODUCTION

Emission and deposition of acidifying substances in the Netherlands

Atmospheric deposition of acidifying substances in the Netherlands differs markedly from that in other northern and western European countries. In addition to deposition of sulphur dioxide (SO_2) and nitrogen oxides (NO_x) also a large deposition of ammonia/ammonium (NH_3/NH_4^+) occurs (table 1.1).

Table 1.1. The deposition of acidifying substances in The Netherlands (in eq. $H^+.ha^{-1}.year^{-1}$).

	1980 wet	dry	total	1986 wet	dry	total
sulphur oxides	740	2100	2840	560	1400	1960
nitrogen oxides	400	1250	1650	330	1240	1570
ammonia/ammonium	630	720	1350	620	760	1380
total acid	1770	4070	5840	1510	3400	4910

(Schneider and Bresser 1987)

About one third of the total deposition of acidifying substances is by wet deposition; the remaining part occurs in gaseous form as so called dry deposition. The deposition of SO_2 and NO_x originates to a large extent from emissions in neighbouring countries. Important antrophogenic sources are industry and road traffic. The large decrease of SO_2 deposition in the period from 1980-1986 is mainly a result of a reduced emission in these countries. The SO_2 emission in the Netherlands in this period has been reduced with about 40%. In contrast no reduction of NO_x emission has taken place. The largest part (about 80%) of the locally emitted SO_2 and NO_x is transported abroad (Erisman et al. 1989).

The annual mean SO_2 concentration in the Netherlands is about 25 $\mu g.m^{-3}$. Occasionally maximum hourly-mean concentrations up to 100 $\mu g.m^{-3}$ have been measured (Anonymous 1986; Vermetten 1989).

By comparison the annual mean concentration in urbanized and industrialized areas in the United States and Western Europe may exceed 100 $\mu g.m^{-3}$ (Fowler and Cape 1982; Krause 1988).

The annual NO_x concentration in the Netherlands is in the same order of magnitude as the SO_2 concentrations (Anonymous 1986). However, maximum hourly-mean concentrations far above 100 $\mu g.m^{-3}$ may be reached.

The relatively high deposition of NH_3 originates almost entirely from emission sources in The Netherlands itself. The emissions in the period from 1950-1980 have doubled (table 1.2), while for Europe as a whole the NH_3 emissions have increased with 50% (ApSimon et al. 1987). The dominant emission of NH_3 (about 90%) arises from agricultural sources, mainly livestock wastes. The total NH_3 emission in the Netherlands amounts to 240 x 10^3 tons per year, of which a large part (50-60%) is emitted in areas where intensive livestock breeding is concentrated (LEI-report, in prep.). Maximum monthly-mean NH_3 concentrations of 35 $\mu g.m^{-3}$ have been measured in these areas (Erisman et al. 1987). In particular when manure is spread out on the fields, hourly-mean NH_3 concentrations can reach a maximum of 60 $\mu g.m^{-3}$ (Vermetten 1989). These concentrations are far above the concentrations measured in other parts of the country (<5 $\mu g.m^{-3}$). A large part of the emitted NH_3 is also deposited in the emission areas, as a result of which the total amount deposited nitrogen in these areas may be 10-20 times the natural nitrogen deposition of 5-10 $kg.ha^{-1}.y^{-1}$ (Anonymous 1987).

Effects on vegetation

In 1983 a first national survey about the health condition of the Dutch forests was carried out by the Dutch National Forest service. This survey showed a 'critical' condition of many forest stands (SBB 1983). In the period 1983-1987 the percentage non vital or sick increased from 9.5 to 21% (SBB 1987). The most striking symptoms are premature loss and yellowing of needles or leaves. Serious forest decline is found in areas where intensive livestock breeding is concentrated. In these areas more than 50% of all trees has been classified as less vigorous. Another obvious phenomenon during the last decades is the change of heathlands into grasslands. In particular grass species as *Molinia caerulea (L.)* and *Moench and Deschampsia flexuosa (L.) Trin.* have strongly expanded at the expense of *Calluna vulgaris (L.)* and other heathland species (Heil and Diemont, 1983; Roelofs et al. 1984; Roelofs 1986).

Table 1.2. Trends in emissions of NH_3 from livestock (1950-1980)

Country	area ($x10^3$ km^2)	emission ($x10^3$ t) 1950	1960	1970	1980	% change	Emissions 1980 ($t.km^{-2}$)
Austria	83	49	53	56	61	24	0.73
Belgium	30.5	40	57	67	75	88	2.46
Bulgaria	110	-	55	54	74	(34)	0.67
Czechoslovakia	128	-	101	98	119	(18)	0.93
Denmark	42.5	68	83	79	84	24	1.98
Finland	333	40	39	39	37	-7	0.11
France	544	337	409	449	527	56	0.97
F.R.G.	250	243	286	333	357	47	1.43
G.D.R.	108	78	115	131	148	90	1.37
Greece	129	32	47	40	43	34	0.33
Hungary	92	60	59	62	69	15	0.75
Ireland	68	91	105	129	146	60	2.14
Italy	298	199	215	232	218	9	0.73
Netherlands	*41*	*54*	*77*	*100*	*127*	*135*	*3.10*
Norway	324	29	28	25	26	-10	0.08
Poland	313	159	213	256	310	95	0.99
Spain	499	-	148	153	160	(8)	0.32
Sweden	444	55	54	44	46	-16	0.10
Switzerland	41	32	38	42	46	43	1.12
U.K.	244	236	321	336	366	55	1.49
Yugoslavia	253	140	149	137	132	-6	0.52
Total		2360	2850	3110	3450	46	

(ApSimon et al. 1987)

Numerous reports have shown that the high deposition of NH_3 is to a large extent responsible for the observed effects (Van Breemen et al. 1982; Den Boer and Van den Tweel 1985; Roelofs et al. 1985 and 1987; Van Dijk and Roelofs 1988; Boxman et al. 1987; Draaijers et al. 1987; Ivens et al. 1987; Heil et al. 1987; Van der Eerden 1982; Roelofs 1986). The effects are mainly attributed to effects of the NH_3 deposition on the soil. Most forests in the Netherlands are located on nutrient poor soils with a limited buffering capacity.

Throughfall measurements indicate that vegetation may act as an important sink for atmospheric NH_3. Moreover these measurements indicate that SO_2 may play an important role in this deposition and consequently may be responsible for the observed effects as well. It was shown that both gases mutually stimulate their deposition on the vegetation which can be attributed to the formation of ammonium sulphate on the canopy surface (Van Breemen et al. 1982, Roelofs et al. 1985, Heil et al. 1987 and 1988, Ivens et al. 1988). In nitrifying soils the ammonium sulphate reaching the soil after leaching by rainwater oxidizes rapidly to nitric and sulfuric acid, causing acidification in non-calcareous soils. As a result of this acidification large amounts of aluminum may be released from the soil complex (Van Grinsven 1988). Also needles and leaves show an excess in nitrogen content and a relative shortage of other nutrient elements such as potassium, phosphorus and magnesium. This may be a result of disturbed nutrient balance in the soil due to high inputs of acids or nitrogen compounds, as well as of uptake of atmospheric nitrogen compounds by the needles or leaves (Van Dijk and Roelofs 1988). In addition, these needles or leaves show an accumulation of amino acids such as arginine, glutamine or asparagine, which may be an indication of a severe nitrogen overload.

Objectives and approach

In the present research the uptake of NH_3 and SO_2 by leaves and the relation between uptake, photosynthesis and stomatal conductance has been examined. The research project started in 1984. Since june 1986 it is carried out as part of the Dutch Acidification Program.

The objective was to get a better understanding of:

1. the relative importance of leaf surface adsorption and transfer of these gases through the cuticle and stomata into leaves,
2. the relationship between concentration, transport resistances and flux densities,
3. the influence of climatic variables (wind velocity, air humidity and temperature) and plant properties on the uptake of these gases by leaves,
4. the influence of SO_2 on the uptake of NH_3 by leaves,
5. the relationship between uptake of these gases into leaves, stomatal conductance and photosynthesis at a short term exposure and after a long term exposure to low and moderate concentrations of these gases.

The results contribute to a better understanding of the (dry) deposition of NH_3 and SO_2 on vegetation and physiological processes induced by these gases. Data on the actual amount of uptake may, for instance, be used in dose-response relationships for the assessment of threshold levels for these gases. The results can also be used for the development of physiologically based simulation models which are a valuable tool to obtain understanding of vegetation responses to air pollutants.

The uptake of NH_3 and SO_2 by leaves was experimentally determined in the laboratory. The method of analysis is similar to that commonly used in photosynthetic research where the gas exchange of leaves is analyzed by using electrical resistance analogues, with the total resistance to transfer being partioned into gas-phase and liquid-phase components (Jarvis, 1971; Sharkey 1985). The uptake of NH_3 and SO_2 by leaves was experimentally determined by using a leaf chamber, specially developed to meet our requirements.

The uptake was measured simultaneously with transpiration and photosynthesis of the leaf. In this way the uptake of NH_3 and SO_2 could be directly related to stomatal behaviour and photosynthesis of leaves.

The measurements were performed with bean plants (*Phaseolus vulgaris L.*) and poplar shoots (*Populus euramericana L.*).

Outline of the thesis

Sofar only a limited number of leaf chambers have been designed for measuring photosynthetic responses and transpiration of leaves during fumigation with a pollutant gas. Moreover, these designs were less suited for analyzing the uptake of low concentrations of pollutant gases at different environmental conditions. Therefore, a leaf chamber was developed to meet our requirements. *Chapter 2* reports on the performance and properties of the newly developed leaf chamber.

The research first concentrated on the uptake of NH_3 by leaves. *Chapter 3* presents the results of a study in which the relation between concentration and uptake of NH_3 into leaves during a short term exposure has been studied. A continuous measuring method for NH_3 was modified and applied. This method is now used in other projects of the Acidification Program. The NH_3 uptake was analysed using a resistance analogue presented in this chapter.

Relatively little was known about the quantities of NH_3 and SO_2 that are adsorbed on the external leaf surface, as compared to the quantities transported into leaves.

Therefore, the adsorption of these gases has been extensively examined. The results are presented in *chapter 4*.

Only a limited number of papers deals with the physiological effects of atmospheric NH_3 taken up by the leaves. Papers on this subject mainly report on visible effects, for example as result of the release of high NH_3 concentrations at accidents. *Chapter 5* reports on a study in which the effects of a long term exposure to low NH_3 concentrations on the relationship between NH_3 uptake, stomatal conductance and photosynthesis was examined. The experiments were carried out with poplar shoots. Also chlorophyll fluorescence measurements were performed to obtain more information about the effects of this NH_3 exposure on the photosynthesis process at the chloroplast level. It was examined whether the fluorescence method and analysis can be used for the diagnosis of plant stress induced by air pollutants.

Despite the extensive literature on the effects of SO_2 many uncertainties still exist with respect to the physiological effects caused by a long term exposure to low concentrations of this gas. *Chapter 6* reports on a study in which the effects of a long term exposure to low SO_2 concentrations on the relationship between SO_2 uptake, stomatal conductance and photosythesis was examined. Furthermore, the effects of a long term exposure to a combination of SO_2 and NH_3 was studied. Also in this study poplar shoots have been used. *Chapter 7* presents the results of a study in which the influence of wind velocity, air humidity and temperature on the uptake of these gases into leaves was examined.

CHAPTER 2

A leaf chamber for measuring the uptake of pollutant gases at low concentrations by leaves, transpiration and carbon dioxide assimilation

L.W.A. van Hove, W.J.M. Tonk, G.A. Pieters, E.H. Adema and
W.J. Vredenberg

(published in Atmospheric Environment Vol. 22, No 11, pp. 2515-2523, 1988)

Abstract

A leaf chamber has been developed for analyzing the uptake of pollutant gases (NO, NO_2, O_3, SO_2, NH_3), in ambient concentrations, into leaves and the effects there of on stomatal behaviour and photosynthesis.

Performance studies showed a negligible permanent reaction of these pollutant gases with the internal surfaces. With SO_2 and NH_3, however, a considerable time was necessary until the desired concentration within the leaf chamber was reached. This adsorption of NH_3 and SO_2 might be related to the presence of a thin waterfilm on the internal surfaces of the leaf chamber. A wide range of air temperatures and humidities can be applied in the leaf chamber. The wind velocity across both leaf surfaces is homogenuous and can be varied, up to a maximum of about 3 $m.s^{-1}$. Consequently, the relation between the boundary layer resistance and uptake of a pollutant gas can be studied properly. Measurements with a thermovision camera have shown that the control of leaf temperature distribution at the leaf surface is improved. This enables a higher accuracy in the determination of the stomatal resistance.

Introduction

The responses of plants to pollutant gases vary with pollutant concentration and exposure time, with climatic and soil variables and with biological variables. Due to the large number of variables it is hard to assess the share of the pollutant gases in the observed effects and to predict the responses of plant to specific exposures.

Valuable tools to obtain understanding of the effects of air pollutants on vegetation are physiologically based simulation models. These models are based on existing crop ecological models, which already contain environmental variables and their effects on photosynthesis and other physiological processes, e.g. stomatal opening. In modelling plant responses to air pollutants a better understanding of the uptake of pollutant gases by leaves is essential. Data on the actual amount of uptake may, for instance, be used in dose response relationships for the assessment of threshold levels for different pollutant gases. The results may also contribute to a better understanding of the (dry) deposition of pollutant gases on vegetations and physiological processes induced by pollutant gases.

The uptake of pollutant gases by leaves includes surface adsorption, cuticular absorption, and transfer via the stomata. Understanding of the uptake process requires knowledge about the relationships between stomatal opening, photosynthesis, rates and extents of uptake, as well as the biotic and abiotic factors affecting these rates.

These relations can only be assessed and studied in detail under defined steady-state environmental conditions. Hence measurements in a controlled environment are required. Once the different relations have been assessed and integrated in a mechanistic model, field measurements are necessary for verification.

The uptake of a pollutant gas can be experimentally determined and analyzed in the laboratory according to methods used in photosynthetic research (Unsworth et al., 1976). The CO_2 assimilation of a leaf is analyzed by using electrical resistance analogues, with the total resistance to CO_2 uptake being partitioned into gas-phase and liquid-phase components (Jarvis, 1971; Sharkey, 1985).

For assessing these resistances at different environmental conditions leaf chambers have been developed, which allow precise control of leaf environment and continuous monitoring of CO_2 and H_2O fluxes (Jarvis et al., 1971; Schulze, 1972; ; Field, Berry and Mooney, 1982).

Only a limited number of leaf chambers have been designed for measuring photosynthetic responses and transpiration of leaves during fumigation with a pollutant gas (Legge et al. 1979, Black and Unsworth, 1979; Noble and Jensen, 1983; Atkinson et al., 1986). These designs are however less suited for analyzing the uptake of low concentrations of pollutant gases at different environmental conditions (air temperature, humidity and wind velocity). In general, the main difficulty is, that materials have been used with which the pollutant gases may react. In addition, the control of some critical environmental factors within the leaf chamber, such as wind velocity, can only be varied in a limited range.

This paper reports on the properties and performance of a new leaf chamber, which has been developed to meet our requirements. The leaf chamber is part of a gas exchange fumigation system described in chapter 3.

Method, design and performance

Criteria

A leaf chamber was required for processing low concentrations of pollutant gases, applied individually or in combination. A first requirement to be met was therefore the prevention of pollutant gases being adsorbed on or reacting at the internal surfaces of the leaf chamber.

Another important requirement is a precise control of air temperature within the leaf chamber for studying the influence of different air temperatures. It is necessary to avoid large fluctuations in temperature of the cooling or heating element to minimize fluctuations in the concentrations of pollutant gases and in relative air humidity. In addition, condensation of water vapour on internal surfaces, in which the pollutant gases may dissolve, should be prevented.

The leaf chamber is part of an open system (Jarvis and Catsky, 1971), where uptake of a pollutant gas, transpiration and CO_2-assimilation of a leaf is determined by measuring the difference between inlet and outlet concentration of the leaf chamber. However, differences between inlet and outlet concentration are only detectable, if flow rates of air passing the leaf chamber are small (5 to 6 $l.min^{-1}$). As a consequence mechanical mixing within the leaf chamber is necessary in order to prevent space variability of gas concentrations. However, mixing of the chamber air is also important with regard to the boundary layer resistance of the leaf (r_b). The value of r_b, which determines the heat and mass exchange between the leaf and the

surrounding air, is closely related to wind speed across the leaf surface. In the absence of mechanical mixing, r_b may exceed the stomatal resistance of a leaf. Consequently, rates of photosynthesis, transpiration and uptake of a pollutant gas are primarily determined by this physical resistance and less affected by physiological changes. The project reported was an attempt to develop a leaf chamber with a homogeneous wind speed across both leaf surfaces, which could be varied up to a maximum of at least 1 m s^{-1}. In this way the influence of r_b on the gas exchange between the leaf and the surrounding air can be studied properly. In addition, this may improve the determination of r_b and control of leaf temperature, which is of primary importance for the calculation of the stomatal resistance of a leaf (Pieters 1972).

Because of the small flow rates of the air passing the leaf chamber, the internal volume of the leaf chamber has to be as small as possible for fast detection of changes in gas exchange. Furthermore, it was desirable that the leaf chamber has transparent windows on both sides. This offers the opportunity to control the irradiation on the abaxial side of the leaf and to perform other types of measurements, such as fluorescence measurements, simultaneously with the gas exchange measurements.

Further requirements of the design were: airtight, convenient operation, ready access to the leaf studied and an easy cleaning.

Construction

Glass and teflon have been used for all internal construction parts. In comparison with other materials such as plexiglass, these materials have a small absorptive capacity for pollutant gases and are not affected by e.g. O_3. A block of PTFE (polytetrafluorethylene) teflon (dimensions 450 x 300 x 50 mm) has been used as starting material (figure 2.1).

Spaces for the leaf, the recirculating ducting and two centrifugal fans have been made in this block. The total internal air volume is 2.6 l, giving a residence time of 25 to 30 s at an air flow rate passing the leaf chamber of 5 to 6 l.min^{-1}. To avoid deformation the teflon block has been sandwiched between two aluminum plates. Because no contact with the pollutant gases is allowed, teflon sheets (3 mm thick), alkali etched on one side, have been glued on the inner side of these plates. The leaf space (dimensions 200 x 250 x 21 mm) is closed at the upper and lower side with an identical window of double, tempered glass.

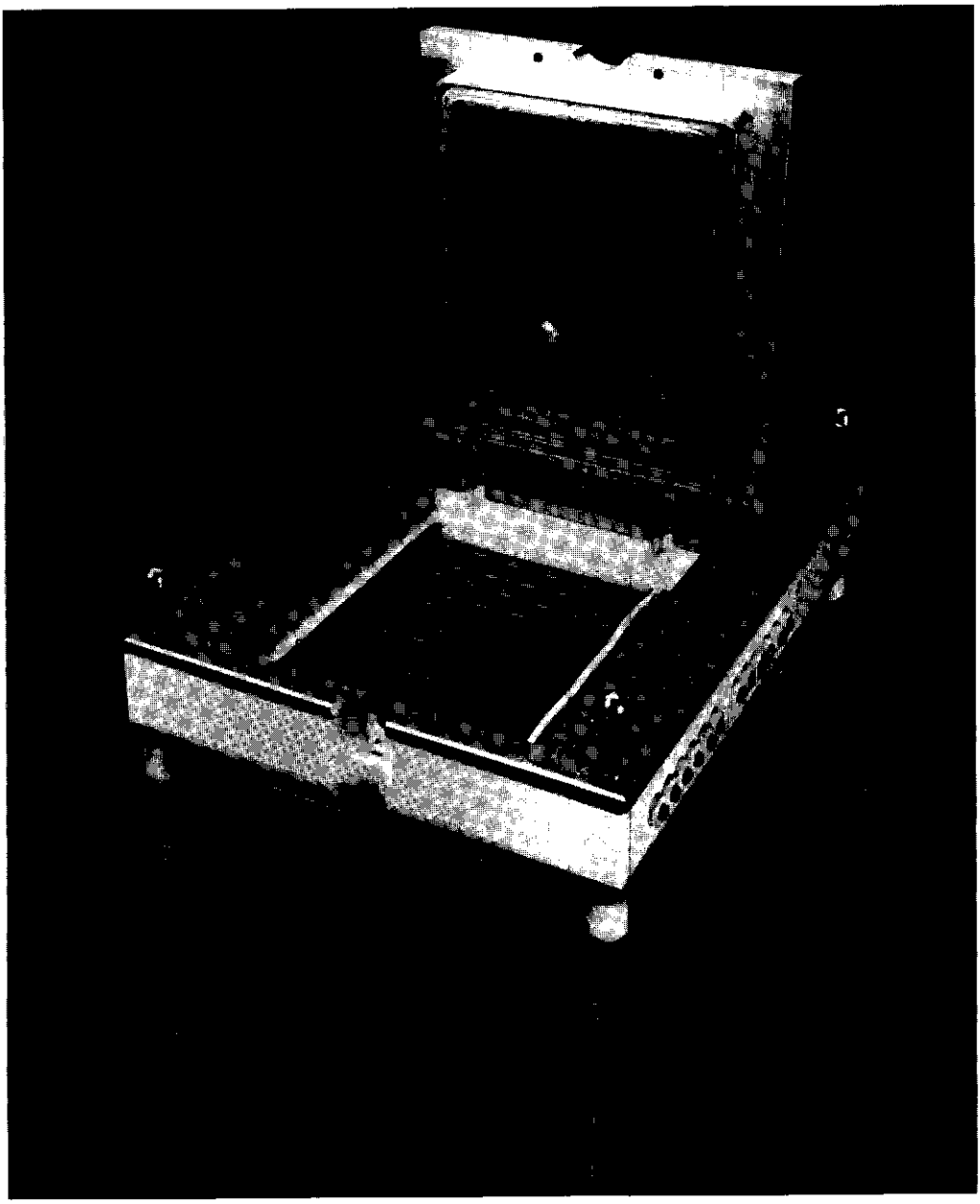

Figure 2.1. A side view of the gas-exchange cuvette or leaf chamber showing the teflonblock, the upper window and the leaf space. A slot in the end wall of the cuvette provides for stem or petiole entry into the chamber. The leaf is held in the middle of the leaf chamber by thin teflon wiring.

The upper window is removed routinely to enclose the leaf of the plant. A petiole can be inserted into a slot in one end wall of the leaf chamber. When the petiole is inside, a small teflon block with an opening for the stem is pushed into place, and the opening around the stem is sealed with a silicone based precision impression material (Xantopren, Bayer Dental). This material was found to have no effect on the plant. The leaf is held in the middle of the leaf chamber by thin teflon wiring.

To prevent dilution of the chamber air with room air the leaf chamber has a small overpressure of 98 Pa (10 mm water column).

Air is continuously kept recirculating inside the leaf chamber by two fans, made of PVDF (polyvinylidene fluoride). To gain a maximum of pressure the fans are placed in series, with the second fan rotating about 6% faster than the first one. The motor and drive mechanism of both fans are situated outside, under the leaf chamber. The motor is a DC motor-tacho-combination (Mattke). Rotation speed of the motor can be set precisely with a maximum of 10,000 r.p.m.. Leakage at the bearings was prevented by the use of teflon seals, which have a low friction with the driving shaft. A detailed drawing of a fan bearing is shown in figure 2.2. Wind velocities across the leaf surface of up to 3 $m.s^{-1}$ can be obtained using the fans. The distribution of wind velocity across the leaf surface can be adjusted by vanes situated in the supply channel of the recirculating ducting.

The low thermal conductivity of the teflon material provided the operation of the leaf chamber to be independent of temperature variations of the room, in which the leaf chamber is used. Particularly when the leaf chamber is operated in a room with unsufficient possibilities of regulating the air temperature, this is an important advantage. The possibility of using peltier modules or heating and cooling fins within the leaf chamber had to be rejected, because mechanical obstructions lowered the maximum wind velocity to be obtained. In addition, internal heating and cooling elements introduce materials with which pollutant gases may react. Therefore, both glass windows of the leaf chamber, which have a relatively large surface, are used as heat exchanger. Between the double glass of the windows water is recirculating, of which temperature is controlled - with feed back control - by a modified thermostatic waterbath. The recirculating water is not connected directly to the thermostatic waterbath, but by means of a heat exchanger in order to reduce contamination between the double glass and the risk of breaking of the glass by varying water pressures.

The leaf chamber is mounted on a support consisting of 4 spindles, which can be used to adjust the distance between the leaf chamber and the lights. For cleaning purposes the leaf chamber can be taken apart.

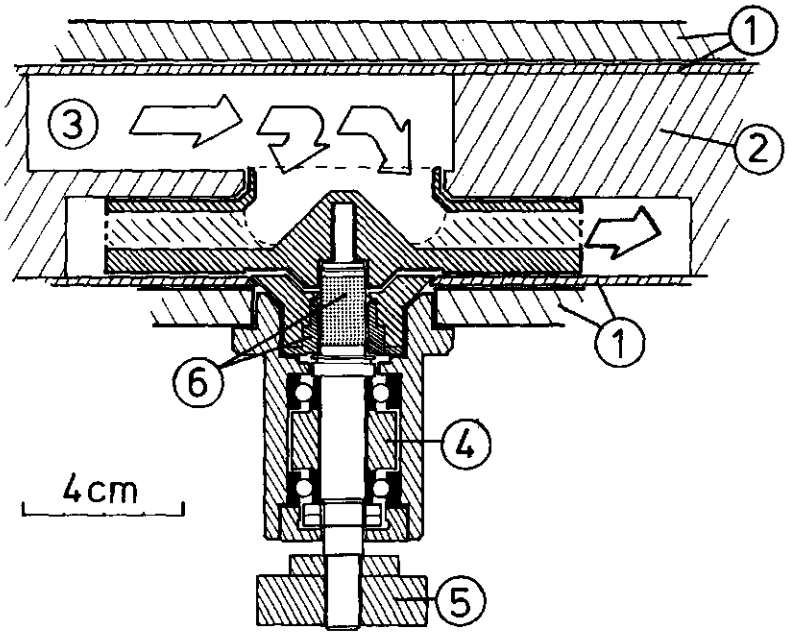

Figure 2.2. Fan bearings. 1. 51 ST aluminum plate (10 mm) with teflon sheet (3 mm). 2. PTFE-teflon block. 3. recirculating ducting. arrows=wind direction. 4. compartment with a set of stainless-steel chromium angular contact bearings. 5. pulley (convex). 6. sealing compartment; stainless-steel shaft coated with chromium dioxide (0.2 micron); PTFE seal with aluminum pressure ring.

Performance

Air temperature and humidity

Under moderate light conditions (up to 80 $W.m^{-2}$ PAR) temperature control was sufficient to keep the air in the leaf chamber at a constant temperature regardless of heat loading from the light sources. However, at a high light intensity (290 $W.m^{-2}$ PAR) air temperature within the leaf chamber was several degrees above the preselected value. Although air temperatures from 10 to 40 °C can be obtained, measurements are usually performed at a temperature between 20 and 25 °C. When equilibrium was reached at a preselected value, temperature did not deviate more than 0.2 °C. Temperature differences between windows and chamber air are small under moderate environmental conditions. As a result relative humidities of up to 90% can be maintained without bulk condensation on the windows and internal surfaces taking place.

Wind velocity

The distribution of wind velocity in the leaf chamber was measured using a hot wire anemometer. The handle of the anemometer could be moved through the slot for the petiole of the leaf chamber. The sensorhead was held sheer at the wind direction. Wind velocity was measured every 2 cm along the imaginary middle line of the leaf space.

Figure 2.3 shows that there were only small differences in wind velocity, in particular in the area where the leaf is situated (0-16 cm).

Air mixing and ventilation resistance

It can be calculated that e.g. at a wind velocity of 1 $m.s^{-1}$ the amount of recirculated air within the leaf chamber is about 5.25 $l.s^{-1}$. So, the time needed for mixing the leaf chamber air is far less than the average residence time of the flow passing the leaf chamber. As a consequence concentration gradients within the leaf chamber cannot exist long enough to influence measurably the rate of process under study. Due to the uniform mixing the gas concentrations within the leaf chamber can be obtained by measuring the concentrations in the outgoing air of the leaf chamber (C_{out}).

In our gas fumigation system the concentration of the ingoing air (C_{in}), and not that of the leaf chamber air is kept constant.

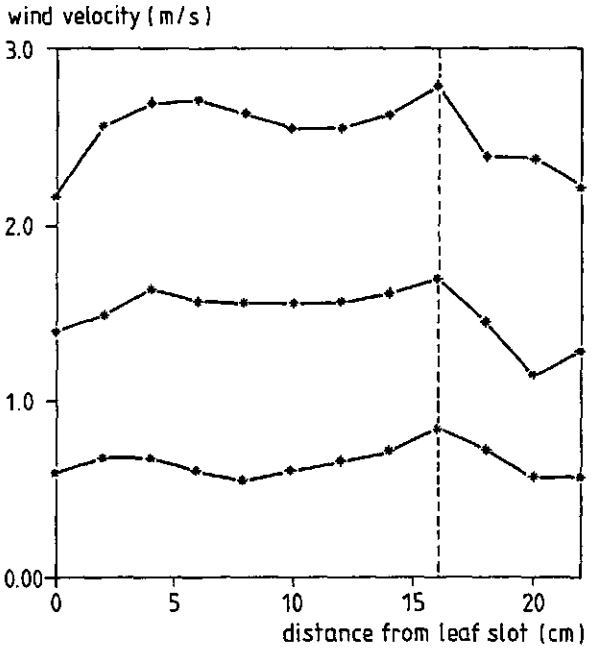

Figure 2.3. Distribution of wind velocity within the leaf chamber, measured every 2 cm along the imaginary middle line connecting the shortest sides of the leaf space.

According to Unsworth (1982) C_{in} and the concentration of the leaf chamber air ($=C_{out}$) are related by the equation (2.1):

$$C_{out} = C_{in} \cdot Fx(A/f) \qquad (2.1)$$

where A is leaf area (m^2), f the air flow through the leaf chamber ($m^3.s^{-1}$) and F the flux density of a gas towards or away from the leaf. In this equation the ratio A/f is defined the leaf ventilation resistance (r_v) in the chamber. The value of A/f ranges between 50 and 150 $s.m^{-1}$. If r_v is large (low ventilation rates), uptake of a pollutant gas by the leaf results in a substantial depletion of its concentration in the leaf chamber.

The boundary layer resistance

The relation between wind velocity in the leaf chamber and the boundary layer resistance (r_b) was assessed for leaves with different widths.

r_b was determined by measuring the evaporation rate (E in $g.m^{-2}.s^{-1}$) from a model leaf consisting of a water saturated double layer of filter paper.

Two methods of calculating r_b for water vapour ($r_{b,v}$) were compared:

1. a direct method which uses the 'leaf' temperature (Jarvis, 1971),
2. an indirect method in which r_b is determined by using the energy balance of the model leaf (Monteith 1973; Parkinson, 1985).

Besides these experimental methods there are quantitative approximations derived from theoretical considerations for determining r_b (Goudriaan, 1977; Grace, 1981). An example is equation (2.2), describing the relation between the boundary layer resistance for heat ($r_{b,h}$) for a leaf surface, wind velocity (u in m s^{-1}) and length of the leaf parallel to the wind speed (w):

$$r_{b,h} = 0.5 \times 1.8 \times 10^2 \times (w/u)^{0.5} \qquad (2.2)$$

The multiplication factor 0.5 accounts for the resistance of the two sides of a leaf being connected in parallel. Equation (2.2) is derived from expressions describing $r_{b,h}$ for isothermal surfaces, such as metal plates (Goudriaan 1977). From $r_{b,h}$ the boundary layer for water vapour can be calculated by multiplying $r_{b,h}$ with $(D_v/D_H)^{-2/3}$ (D is diffusion coefficient). Figure 2.4 shows the results of the experimentally determined values of $r_{b,v}$ (for one side of the leaf) and of the calculated values applying equation (2.2). $r_{b,v}$ decreases exponentially with wind velocity and approaches its minimal value at a wind velocity of 2 $m.s^{-1}$. This means that a further increase in wind velocity in the leaf chamber hardly has any influence on heat and mass exchange of the leaf. Except for wind velocities below 0.3 $m.s^{-1}$ it appears, that for a leaf with a flat, smooth surface equation (2.2) gives a good approximation of r_b.

Leaf temperature

An accurate determination of the stomatal resistance of a leaf (r_s) is of crucial importance for analyzing the transport resistances determining the uptake of a pollutant gas into the leaf. Apart from the transpiration rate the leaf temperature is a critical parameter in the determination of r_s (Jarvis 1971, Pieters 1972, Monteith 1973, Goudriaan 1977). So, to get an impression of the reliability of r_s determined with the leaf chamber it is necessary to determine the actual temperature of a leaf in the leaf chamber under a variety of experimental conditions.

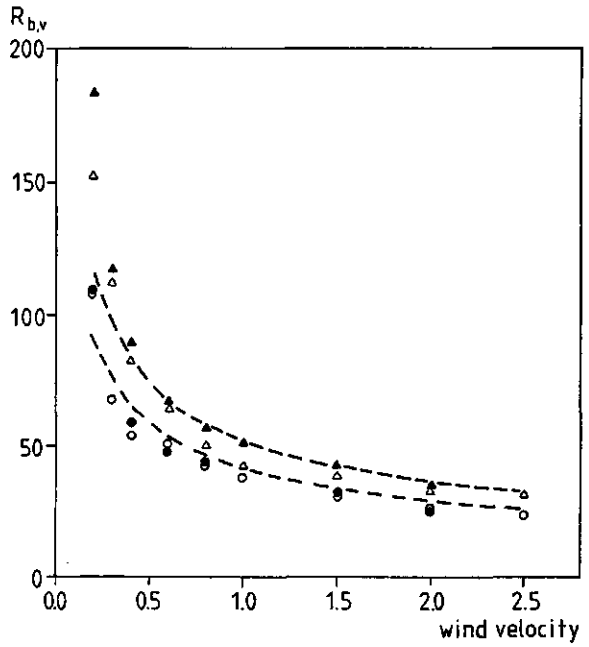

Figure 2.4. Boundary layer resistance for one side of the leaf, as a function of wind velocity and leaf width. Open symbols: conventional method. Closed symbols: energy balance method. Dashed lines: calculated values according to Goudriaan (1977). o ● = leaf width 6 cm. Δ ▲ = leaf width 10 cm.

For this purpose the temperature of a bean leaf (*Phaseolus vulgaris L.*) enclosed in the leaf chamber was determined with an infrared thermovision camera (AGA thermovision 782), situated under the leaf chamber. To accommodate infrared thermometry the double glass in the lower window had to be replaced by a frame closed with polyethylene film. Because this may modify the heat balance of the leaf, the measurements were carried out in a controlled environment room kept at the same temperature as that of the cooling water in the upper window (i.e. 19.5 °C).

The measurements were carried out at different light intensities and wind velocities. Simultaneously, leaf temperature was measured with three thermocouples (copper-constantan) pressed against the abaxial surface of the leaf, while air temperature within the leaf chamber was measured with a thermolinear resistor (Pt 100 element). The thermovision pictures (in black and white) could be stored on a videotape and processed by a computer (AGA TC 800) to obtain (colour) pictures of the temperature distribution across the leaf surface, the average temperature and standard deviation.

In figure 2.5 the influence of light intensity and wind speed on leaf temperature distribution is shown. The figure was obtained by overdrawing the thermovision (colour) pictures of the leaf. Particularly at a low wind speed and high light intensity there were large temperature differences across the leaf surface.

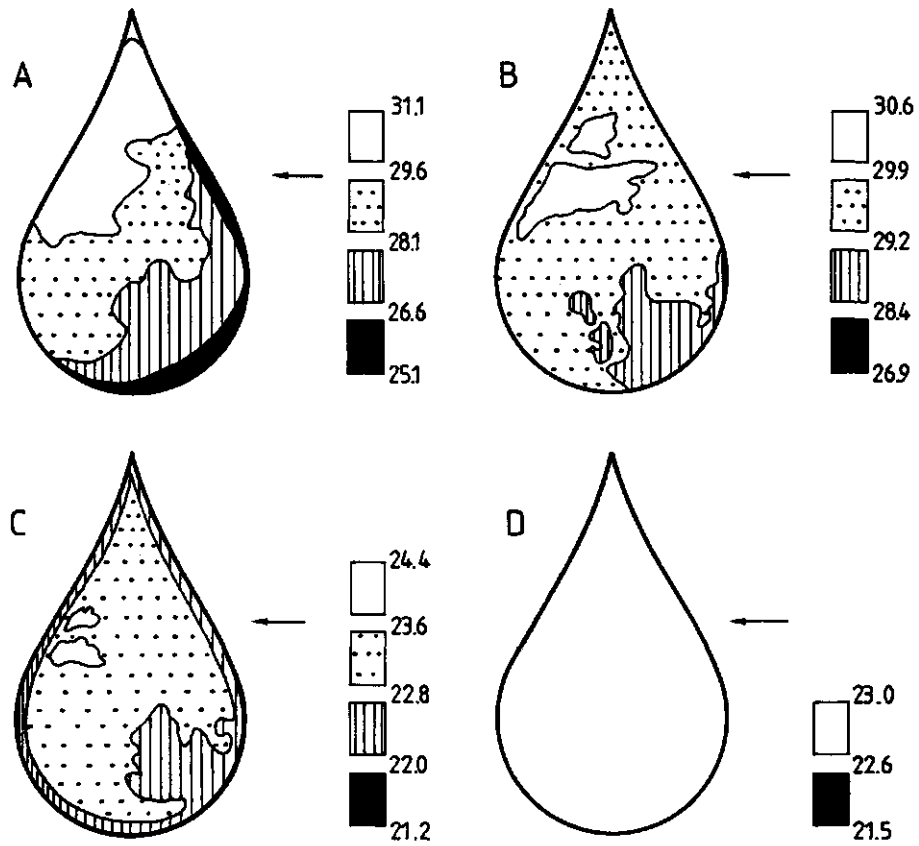

Figure 2.5. The influence of wind velocity (u) and light intensity on temperature distribution of a leaf enclosed in the leaf chamber. arrows = wind direction. A: u = 0.25 m.s^{-1}, 290 W.m^{-2} (PAR); B: u = 2.5 m.s^{-1}, 290 W.m^{-2} (PAR); C: u = 0.25 m.s^{-1}, 84 W.m^{-2} (PAR); D: u = 1 m.s^{-1}, 84 W.m^{-2} (PAR).

Table 2.1. Leaf temperatures measured with an infrared thermovision camera and with thermocouples.

PAR $W.m^{-2}$	u $m.s^{-1}$	T_a	IR	std	TC	std
290	0.25	24.4	30.9	1.1	30.3	0.8
	0.50	24.0	28.0	0.9	28.2	0.7
	1.00	23.2	27.2	0.6	26.9	0.4
	1.50	23.4	27.0	0.7	26.8	0.6
	2.00	24.7	27.1	0.6	27.2	0.5
	2.50	28.2	30.3	0.5	30.0	0.2
84	0.25	22.6	23.7	0.3	23.4	0.2
	0.50	22.0	22.9	0.2	23.0	0.2
	1.00	21.9	22.8	0.2	22.6	0.3
	1.50	22.0	22.8	0.2	23.0	0.2
	2.00	23.1	23.8	0.2	23.9	0.2
	2.50	26.4	27.5	0.2	27.2	0.2
DARK	0.25	22.0	22.0	0.3	21.9	0.0
	0.50	21.9	22.3	0.2	21.9	0.0
	1.00	21.8	22.6	0.2	22.7	0.1

PAR= photosynthetically active radiation; u=wind velocity; T_a, IR, TC = leaf chamber air temperature, infrared thermovision temperature and thermocouple temperature (°C), respectively; std=standard deviation.

Table 2.1 shows that at a high light intensity the average leaf temperature decreased rapidly with increasing wind velocity. Also temperature gradients across the leaf surface decreased rapidly, as is shown by the standard deviation. A wind velocity larger than 2 m/s had however no longer an influence on leaf temperature distribution. These results confirm the results of the boundary layer measurements showing that r_b is no longer influenced by a further increase in wind velocity.

At a higher wind velocity air temperature within the leaf chamber increased considerably which is probably due to friction of the recirculating air. Although temperature differences across the leaf surface decreased rapidly with increasing wind speed, they still remained significant. In addition, the average leaf temperature was significantly higher than the average air temperature. In the dark or at a moderate light intensity a homogeneous temperature across the leaf surface was attained at a wind velocity of about 1 m/s. There were only small differences between actual leaf temperatures and leaf temperatures measured with thermocouples. Also differences between average leaf temperatures and chamber air temperatures were small under these conditions.

Reaction with NO, NO_2, O_3, SO_2 and NH_3

The adsorption or reaction of NO, NO_2, O_3, SO_2 and NH_3 at the internal surfaces of the leaf chamber was assessed for different gas concentrations and at different air humidities.

NO and NO_2 were supplied by a permeation tube system (Verhees and Adema, 1985), and the concentrations were monitored with a chemiluminescence analyzer (Monitor Labs 8840). NH_3 and SO_2 were supplied from a cylinder containing a calibrated mixture of the gas of 1000 ppm in nitrogen. NH_3 was measured with a NO_x analyzer, equipped with a catalytic converter to oxidize NH_3 to NO (Aneja et al.,1978). The SO_2 concentration measurements were carried out with a UV pulsed fluorescent analyzer (Monitor Labs 8850). O_3 was generated by passing pure oxygen or purified air through a quartz tube beneath an ultra violet lamp. The concentration was measured with a gas phase chemiluminescence analyzer (Bendix 8002).

After the whole system had been equilibrated at a specific air humidity for 3 hours, a pollutant gas was injected into the flow of conditioned air passing the leaf chamber. The outlet concentration was then recorded until a steady state concentration was attained. Subsequently, the gas injection was terminated and desorption of the leaf chamber to a zero concentration was measured. The experiment was repeated for the system without leaf chamber in order to correct for a contribution of the in- and outlet tubings of the leaf chamber.

Because mixing of the leaf chamber air is uniform, the accumulation of the internal concentration of the pollutant gas after starting of the gas injection can be measured by measuring the outlet concentration of the leaf chamber (C_{out}). C_{out} follows a exponential time course according to equation (2.3):

$$C_{out} = C_{ss} \times (1 - e^{-t/\tau}) \qquad (2.3)$$

The curve is characterized by the time constant τ (relaxation time). In absence of adsorption or absorption on the internal surfaces τ is the same as the residence time, i.e. the ratio between leaf chamber volume and flow rate (V/f). τ increases with the adsorptive capacity of the internal surfaces for a pollutant gas. The steady state concentration, C_{ss}, in equation (2.3) equals C_{in}, when no permanent reaction of the pollutant gas at the internal surfaces takes place.

When C_{ss} differs from C_{in}, a rate constant (k_o in s^{-1}) for this reaction can be calculated according to equation (2.4) (Verhees, 1986):

$$k_o = ((C_{in} - C_{out})xf/V)/C_{out} \qquad (2.4)$$

The curve for C_{out} after termination of the gas injection can, in principle, be described by the expression:

$$C_{out} = C_{ss} \times e^{-t/\tau} \qquad (2.5)$$

The NO and NO_2 concentrations at the inlet of the leaf chamber were 50 and 70 ppb. Both gases had no adsorption on or a permanent reaction at the internal surfaces of the leaf chamber, neither in dry air nor in humid air (R.H. 90%). τ was found to be equal the residence time. This also indicates that mixing within the leaf chamber is uniform (ideal).

A similar result was obtained, when SO_2 was injected into dry air (R.H.; 0-20%) passing the leaf chamber. However, in humid air τ was found to increase considerably depending on relative humidity of the air (figure 2.6). τ also increased slightly with the chamber air temperature and decreased with increasing C_{in} (figure 2.7). After termination of the SO_2 injection there was a rapid decrease in C_{out} initially, after which it took 2 to 3 hours before the concentration was completely zero. The calculated t values for this last part of the recorded curve were slightly dependent on relative air humidity.

NH_3 also showed a strong adsorption on the internal surfaces of the leaf chamber in presence of water vapour and a tailing of the recorded C_{out} after termination of the NH_3 injection. However, in comparison with SO_2 the calculated τ values showed a less clear relation with air humidity (figure 2.8). There were two other differences as compared to SO_2: First, τ decreased strongly with increasing C_{in} and second, there was also NH_3 adsorption in dry air (τ was 6.25×10^2 s).

The inlet concentrations of O_3 were 40 and 80 ppb. In the first experiments O_3 showed a strong permanent reaction with the internal surfaces of the leaf chamber. The steady state concentration was about 20% less than C_{in}. The average value for k_o in these experiments was 8×10^{-3} s^{-1}. Probably this loss was due to O_3 scavenging impurities on the internal surfaces of the leaf chamber (Grosjean, 1985).

In order to react away these impurities the leaf chamber was treated with a high O_3 concentration (1 - 2 ppm) for 72 h. After this treatment k_o had been reduced to an average value of 1.29×10^{-3} s^{-1}. This value is larger than those mentioned for teflon and glass by Grosjean (1985) and Verhees (1986). However, at the flow rates used in our experiments these loss rates give only small differences between inlet and outlet O_3 concentration ($\leq$ 3%). Nevertheless, a correction for this loss rate have to be made, when the uptake of O_3 by a leaf is measured.

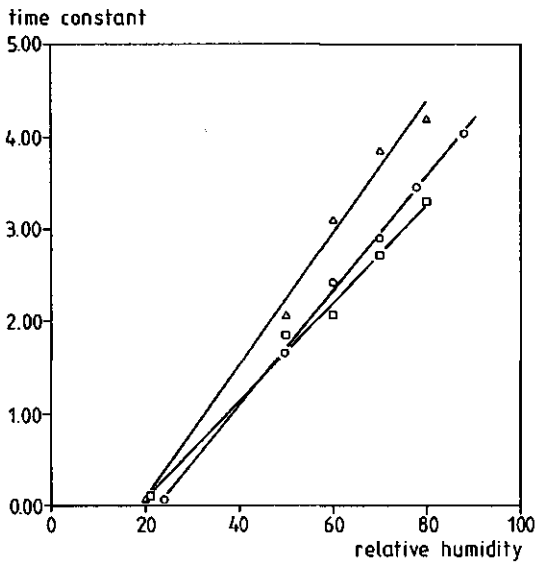

Figure 2.6. The time constant (τ in $x10^3$ s) for the adsorption of SO_2 on the internal surfaces of the leaf chamber, as a function of relative air humidity and temperature in the leaf chamber.
□ = 14.5 °C; o = 20 °C; Δ = 24.5 °C. The points are averages of two or three measurements, at a concentration in the range of 30 to 100 $\mu g.m^{-3}$.

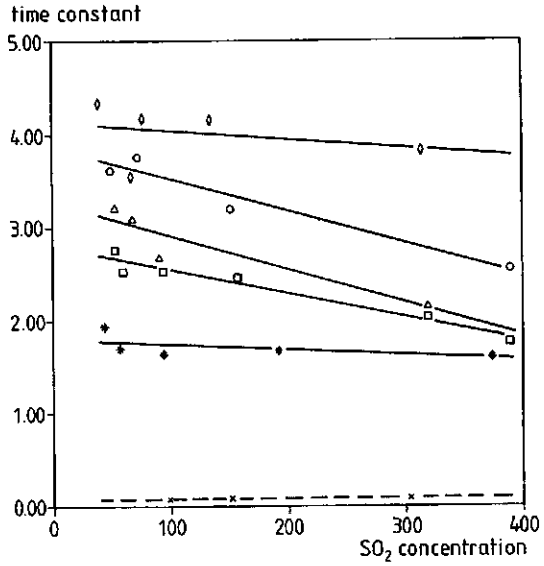

Figure 2.7. The time constant (τ in 10^3 s) for the adsorption of SO_2 on the internal surfaces of the leaf chamber as a function of the inlet concentration (C_{in}) and relative air humidity in the leaf chamber (at 20 °C). x = ≤20% R.H.; * = 50% R.H.; □ = 60% R.H.; Δ = 70% R.H.; o = 80% R.H.; ◇ = 90% R.H.

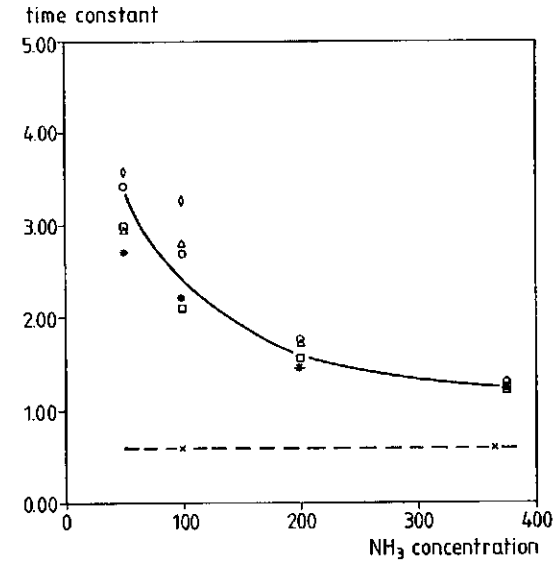

Figure 2.8. The time constant (τ in x10^3 s) for the adsorption of NH_3 on the internal surfaces of the leaf chamber, as a function of the inlet concentration (C_{in}) and relative air humidity in the leaf chamber (at 20 °C). x = 0% R.H.; * = 50% R.H.; □ = 60% R.H.; Δ = 70% R.H.; o = 80% R.H.; ◇ = 90% R.H..

Discussion

Due to the low permanent reaction of the pollutant gases with the internal surfaces and the precise control of environmental variables the present leaf chamber has the capability of accurately analyzing the transfer of pollutant gases into leaves. Because the uptake of CO_2 and transpiration of a leaf can be measured simultaneously, this transfer can be related directly to physiological processes such as photosynthesis and stomatal behaviour. Preliminary results for NH_3 have been published in a previous paper (Van Hove et al., 1987).

The present leaf chamber has a homogeneous wind velocity across both leaf surfaces, which can be varied up to 3 $m.s^{-1}$. In this way, not only the relation between wind velocity, r_b and the transfer of a pollutant gas into the leaf can be studied properly, but also the heat balance and with that leaf temperature can be influenced. With r_b, the leaf temperature is critical parameter in the calculation of r_s. Measurements with the infrared thermovision camera showed, that under moderate light intensities a wind velocity above 1 $m.s^{-1}$ causes only small temperature gradients across the leaf surface. Also differences between the actual leaf temperature and the leaf temperature measured with thermocouples are small. Hence a correct determination of leaf temperature is possible and consequently the calculation of r_s from the water vapour deficit between the substomatal cavity and the surrounding atmosphere of the leaf is much more accurate (Jarvis et al. 1971, Pieters 1972). However, at a high light intensity this method of determining r_s is less accurate, because temperature gradients across the leaf surface appeared to be too large, even at a relatively high wind velocity of 2.5 $m.s^{-1}$. These temperature gradients are more likely the result of differences in transpiration, due to differences in aperture of the stomata of the leaf, than a result of too a low a wind velocity (Hashimoto et al., 1981). So, a further increase in wind velocity above 2.5 $m.s^{-1}$ will probably not result in a decrease of the temperature gradients at this light inten-sity. The energy balance approach gives a better approximation of r_s under these conditions (Monteith 1973, Goudriaan 1977).

Rather surprising was the considerable adsorption of SO_2 and NH_3 on the internal surfaces in humid air, despite the fact that inert materials for the internal construction parts have been used. On the other hand no adsorption with NO, NO_2 and O_3 occurred, which is consistent with literature data (Grosjean, 1985, Verhees, 1986).

SO_2 and NH_3 are more soluble in water than NO, NO_2 and O_3. This suggests that adsorption of SO_2 and NH_3 is a result of a reaction of these gases with water, rather than a direct reaction with teflon or glass. The absence of SO_2 adsorption in relatively 'dry' leaf chamber air ($\leq$ 20% R.H.) supports this conclusion. As no visible condensation had taken place, it is assumed that this 'water' is present as a thin waterlayer or waterfilm on the internal surfaces of the leaf chamber. In this waterfilm aqueous SO_2 may react with H_2O producing sulphite, that subsequently can be oxidized to sulphate (Cox and Penkett, 1983; Adema et al. 1986). Indications that these reactions may indeed have taken place, are: i) the large SO_2 adsorption as compared to NH_3 despite the fact that SO_2 is less soluble in water than NH_3, and ii) the fact that SO_2 adsorption slightly increased at a higher temperature instead of decreasing as would be expected according to Henry's law for the dissolving of gases in solutions.

More difficult to explain is the loss of NH_3 in the leaf chamber at a zero internal relative humidity. Presumably this is caused by, either specific surface adsorption, or a relatively high permeability of PTFE-teflon for NH_3.

The slow accumulation of the NH_3 and SO_2 concentration in the empty leaf chamber was found to be reproducible at a set air temperature and humidity. Consequently, the adsorption of these gases on the leaf surface can be determined from the difference between the accumulation curves of these gases in the empty leaf chamber and in the leaf chamber containing a leaf.

However, fast kinetic measurements with varying inlet concentrations of NH_3 or SO_2 are not easily possible at humidities in the physiological range. This would require a further reduction of the adsorption of these and other soluble gases on the internal surfaces of the leaf chamber. The presence of a waterfilm sets a limit to this reduction.

CHAPTER 3

Analysis of the uptake of atmospheric ammonia by leaves of Phaseolus vulgaris L.

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(published in Atmospheric Environment Vol.21, No.8, pp.1759-1763, 1987).

Abstract

Individual leaves of *Phaseolus vulgaris L.* were exposed for 9 h in a leaf chamber to different NH_3 concentrations at different light intensities. The rates of NH_3-uptake, transpiration and photosynthesis were measured simultaneously. The flux density of NH_3 increased linearly with concentration in the range of 4-400 $\mu g\ m^{-3}$. Flux densities also increased with light intensity. Resistance analysis indicated that NH_3 transport into the leaf is via the stomata: transport via the cuticle is negligible under the experimental conditions. There is no internal resistance against NH_3 transport. The NH_3 flux was found not to influence the photosynthesis.

Introduction

Intensive livestock breeding in the Netherlands is concentrated in areas with predominantly sandy soils. The emission of ammonia (NH_3) in these areas, caused by NH_3 volatilization of animal manure, amounts to 76 x 10^3 tons NH_3 per year or 53% of the total NH_3 emission in the Netherlands (Buijsman et al., 1985). In particular when manure is spread out on the fields the monthly average concentrations of NH_3 can reach up to 35 $\mu g\ m^{-3}$ (Vermetten et al., 1985). There is increasing evidence that this high NH_3 emission contributes significantly to the serious dieback of forests and to the strong decline of plant species in these areas (den Boer and Bastiaens, 1984; Roelofs et al., 1984, 1985).

This impact of NH_3 may be a result of soil acidification. It is postulated that NH_3 interacts with SO_2 (from fossil fuels) on the surfaces of the vegetation. The resultant ammonium sulfate is washed off by rainwater. In the soil ammonium sulfate oxidizes rapidly to nitric acid by nitrification and sulfuric acid, giving rise to extremely low pH values in the range between 2.8 and 3.5 (Van Breemen et al., 1982).

The deposition of NH_3 may also lead to a change in the composition of a vegetation in favour of nitrophylic plant species. An example of this phenomenon is the substantial increase of grass species such as *Molinia caerulea (L.)* and *Deschampsia flexuosa (L.)* in heathlands. Another effect of the extra input of nitrogen to the vegetation may be an increased susceptibility of plants to other pollutants and secondary stress factors such as frost or phytopathogens. Furthermore the absorption of NH_3 by leaves may lead to an increased leaching of essential mineral nutrients such as K and Mg out off the leaves causing deficiency symptoms (Nihlgård, 1985; Roelofs et al.1984,1985).

Besides indirect effects NH_3 may also have direct toxic effects on leaf tissue. A comprehensive evaluation of the toxicity of NH_3 for a great number of plant species is given by Van der Eerden (1982). So far only visible effects on growth have been reported, whereas physiological effects of NH_3, for example on photosynthesis, have not been studied yet.

It is estimated that about two-third of the total deposition of NH_3 (and other gaseous pollutants) in the Netherlands is via dry deposition (van Aalst, 1984). Many aspects of the dry deposition of NH_3 on vegetation are not well understood. In particular there is a lack of information about the deposition of NH_3 to vegetation surfaces and the quantities sorbed under different environmental conditions.

This information is required for a better understanding of the mechanisms and effects of NH_3 deposition on vegetation.

In this context the process of NH_3 uptake from the air by leaves was studied, that is: the capture of NH_3 at the external surface of the leaf and transport into the leaf interior. It has already been established that leaves can be an important sink for atmospheric NH_3 (Porter et al., 1972; Hutchinson et al., 1972; Faller, 1972; Meyer, 1973; Rogers and Aneja, 1980). The objective of this study was to get a better understanding of: (i) the relationship between concentration, transport resistances and flux densities, and (ii) the influence of environmental factors and plant properties on this relationship.

The method of analysis is similar to that commonly used in the analysis of H_2O and CO_2 fluxes. These fluxes are known to be linearly related to concentration differences and conductances (i.e. inverse of resistances) (Jarvis, 1971).

This paper reports on studies in which leaves of *Phaseolus vulgaris L.* were exposed for a short time to different concentrations of NH_3 using a leaf chamber. The rates of NH_3 uptake, transpiration and photosynthesis were measured simultaneously.

Materials and Methods

Plant material

Plants of *Phaseolus vulgaris L. cv. 'witte zonder draad'* were grown in 12 cm pots containing a mixture of sterilized peat and sand (2:3), to which lime was added. The plants were grown in a controlled environment room at a temperature of 20°C (24 h), a relative humidity of 60-70% and a light period of 14 h at an intensity of 80 $W.m^{-2}$ (PAR, Philips fluorescent tubes TLMF 140 W/33 RS). Plant age at time of exposure was 3 weeks.

Experimental procedure

The top leaf of the first trifoliate leaf attached to the plant was enclosed in a leaf chamber and, after a recovery period in the dark of 9 h exposed for about 9 h to NH_3 in the light. The temperature was 23-25°C and relative humidity 60% (± 5%).

A continuous flow of purified air at a rate of 5-6 $l.min^{-1}$ containing known amounts of NH_3, CO_2 and H_2O, was led through the leaf chamber. Air within the leaf chamber was changed by this flow at a rate of about 4 min^{-1}.

At the inlet and outlet of the chamber the concentrations of NH_3, H_2O and CO_2 were measured. The rates of NH_3 uptake, transpiration and photosynthesis were calculated from the difference between inlet and outlet concentration.

After the beginning of the experiment it took 1-2 h before the NH_3, H_2O, and CO_2 concentrations in the leaf chamber were at a stationary level. The flux densities were calculated from data obtained after the steady-state situation was reached. The flux densities of NH_3 to the leaf were obtained after correction for the NH_3-sorption by the internal walls of the leaf chamber. This was determined by measuring the uptake of the empty chamber at each NH_3 concentration. During these adsorption measurements the humidity was adjusted to that during the measurements of the leaves.

Leaf chamber

Both the plant and the leaf chamber were housed in a controlled environment room and illuminated by two high pressure iodine vapour lights (Philips HPLI 400 W). A water bath containing running water was placed between the lights and the chamber to reduce heating of the chamber interior.

Air within the leaf chamber was continuously kept recirculating in order to reduce space variability of gas concentrations and to reduce the boundary layer resistance of the leaf.

Air supply system[1]

Purified air free from CO_2 and ambient contaminants was obtained by passing ambient air through a series of filters containing soda lime, molecular sieve 5a, granular charcoal, hopcalite and molecular sieve 5a, respectively, and a dust filter.

The humidity and temperature were controlled by passing the air through three water filled glass vessels in series. The first vessel was placed in a thermostatic water bath set at 30°C for moistening the air, the two others were placed in a second thermostatic water bath set at a lower temperature to ensure saturation of the air. Finally the temperature of the air was adjusted by passing it through copper coils immersed in a third thermostatic water bath. The conditioned air was mixed with CO_2 and NH_3 using thermal mass flow controllers (Brooks instruments). NH_3 was supplied to the system from a

[1] a schematic representation is given in the appendix of this chapter

cylinder with a calibrated gas mixture of 0.1% NH_3 in N_2.

All tubing in contact with NH_3 were of Teflon, glass or stainless steel. Tubing to the NO_x-analyser (for NH_3 measurement) was heated to prevent water vapour condensation and dissolving of NH_3 in the condensed water.

Measurements

The NH_3-concentrations at the inlet and outlet of the chamber were continuously measured with a modified NO_x-analyser (Mon Labs 8840) equipped with a catalytic converter to oxidize NH_3 to NO (accuracy ± 2 ppb, converter efficiency ≥ 85%). The converter consists of a stainless steel tube (Chrompack, st st 304, pretreated) maintained at 800°C by a tube furnace, through which the sample stream to the analyser was passed (Anèja et al., 1978). The efficiency of the NH_3-converter was thoroughly checked at different humidities of the air. An increase of the moisture content of 1 $g.m^{-3}$ caused a decrease in efficiency of only 0.6%.

The NO_x analyser was calibrated regularly with two cylinders filled with calibrated gas mixtures of 200 ppb (1 ppb = 1 part in 10^9 by volume) and 900 ppb NO (nitrogen oxide) in N_2 (BOC, Hy-line standard).

An infrared gas analyser (ADC 225 Mk 3) was used to measure the CO_2 concentration at the inlet of the chamber. A second infrared gas analyser using two channels measured the difference between inlet and outlet concentration.

The H_2O concentration at the inlet of the chamber was measured with a katharometer (Pieters, 1971), while for the measurement of the difference between inlet and outlet concentration an infrared gas analyser (ADC 225 Mk3) was used. Both the CO_2 and H_2O analysers were calibrated with the air supply system.

Using copper constantan thermocouples the temperature of the air within the chamber, the lower and upper window temperature of the leaf chamber and the 'leaf' temperature were measured. For the measurement of the irradiance on the leaf a UDT-80-X-optometer (350-1100 nm) was used. The leaf area was measured with a leaf area meter described by Pieters (1984).

Mathematical analysis

The results were analysed according to a resistance analogue shown in figure 3.1.

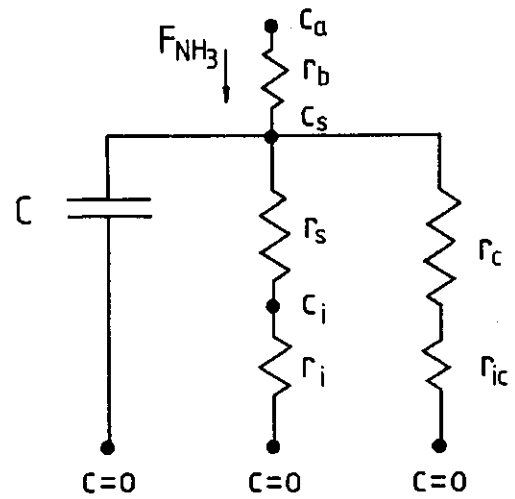


Fig. 3.1. A resistance analogue for the uptake of NH_3 by one side of a leaf. $F(NH_3)$ is the total flux density towards the leaf. c_a, c_s and c_i are NH_3 concentrations in the leaf chamber, at the leaf surface and in the substomatal cavity of the leaf, respectively. r_b is the boundary layer resistance of the leaf, r_s the stomatal resistance and r_c the cuticular resistance. r_i and r_{ic} are internal resistances. The capacity (C) represents surface sorption of NH_3.

In the transport of NH_3 from the surrounding air into the leaf several regions will be passed. Each region can be characterized by a resistance against transport. The first resistance to be met is due to the presence of the boundary layer of the leaf (r_b).

From the leaf surface there are two parallel pathways into the leaf interior; (i) through the stomata with resistance r_s or (ii) through the cuticle (resistance r_c). Within the substomatal cavity, where NH_3 concentration is c_i, NH_3 is likely to dissolve in the waterfilm coating the walls of the mesophyll cells. Subsequently, NH_3 may be transported into the mesophyll cells, where it may take part in the metabolism of the cells. In the figure this air-liquid diffusion path of NH_3 into its sink is represented by resistance r_i. By definition the concentration in this sink is zero. A similar resistance, r_{ic} is given for NH_3 transport after passage of the cuticle.

Besides transport into the leaf NH_3 is adsorbed on the external surface of the cuticle. This adsorption is represented in figure 3.1 by a capacity (C). In this study we do not use this capacity.

In the case of a steady-state situation in the leaf chamber (see experimental procedure) the total flux density of NH_3 (in $g.m^{-2}.s^{-1}$) towards the leaf can be written as:

$$F = (C_a - 0)/r_t \qquad (3.1)$$

r_t (in $s.m^{-1}$) is the sum of the boundary layer resistance and the resultant of the resistances of the two pathways into the leaf:

$$r_t = r_b + \{(r_s+r_i)^{-1} + (r_c+r_{ic})^{-1}\}^{-1} \qquad (3.2)$$

The terms in equation (3.2) are determined as follows: r_t is obtained from equation (3.1) in which the flux density is calculated from the difference between inlet and outlet concentration of the leaf chamber (ΔC in $g.m^{-3}$), the gas flow (f in $m^3.s^{-1}$) and the leaf area (A in m^2). In formula:

$$F = (f \times \Delta C)/A \qquad (3.3)$$

A similar formula is used to calculate the rate of transpiration and photosynthesis. From the rate of transpiration the stomatal resistance for H_2O transport out of the substomatal cavity (r_s) was obtained. The stomatal resistance for NH_3 (r_s) was calculated according to: $r_s = r_{s,v} \times (D_v/D_{NH_3})$, in which D are diffusion coefficients.

Since accurate measurements of leaf temperature are difficult to make, $r_{s,v}$ was determined by using a modified Penman equation for a hypostomatous leaf (Monteith, 1973).

$$\lambda E = \frac{sR_n + (\rho C_p(e_s - e_a)/\ \frac{1}{2}r_{b,h})}{s + (\gamma(r_{b,v}+r_{s,v})/\ \frac{1}{2}r_{b,h})} \qquad (3.4)$$

where λ is the heat of vaporization of H_2O ($J.g^{-1}$), E the rate of transpiration ($g.m^{-2}.s^{-1}$), R_n the net radiation ($W.m^{-2}$), ρC_p the volumetric heat capacity of the air ($J.m^{-3}.°C^{-1}$), e_s the saturated water vapour pressure at the temperature

(Pa), e_a the actual water vapour pressure (Pa), s the slope of the curve of saturated water vapour against air temperature ($Pa.°C^{-1}$), γ the psychrometric constant ($Pa.°C^{-1}$), $r_{b,h}$ the boundary layer resistance for heat ($s.m^{-1}$) and $r_{b,v}$ the boundary layer resistance for H_2O.

R_n in equation (3.4) was estimated as;

$$R_n = 0.85 \times I \times (1-0.2), \qquad (3.5)$$

where I is the measured irradiance ($W.m^{-2}$) on the leaf and the numerical factors being the fraction of irradiance transmitted by the window of the leaf chamber (0.85) and the fraction of the irradiance transmitted and reflected by the leaf (0.2).

The boundary layer resistance $r_{b,v}$ was determined by measuring the evaporation of a water saturated piece of filter paper of the same shape and size as the leaf. Obtained values for $r_{b,v}$ were 70-80 $s.m^{-1}$ (for one side of the leaf). From $r_{b,v}$ the values for $r_{b,h}$ and r_b can be calculated by multiplying $r_{b,v}$ with $(D_H/D_v)^{-2/3}$ and $(D_{NH_3}/D_v)^{-2/3}$, respectively (Thom,1968).

The resistances r_i and (r_c+r_{ic}) were determined indirectly using a graphical method applied by Black and Unsworth (1979) for SO_2. In this method equation (3.2) is transformed to:

$$(r_t-r_b)^{-1} = (r_s+r_i)^{-1} + (r_c+r_{ic})^{-1} \qquad (3.6)$$

If $(r_t-r_b)^{-1}$ is plotted against $(r_s+r_i)^{-1}$ a straight line should result with slope unity and intercept $(r_c+r_{ic})^{-1}$. By fitting the determined experimental relationship between $(r_t-r_b)^{-1}$ with equation (3.6), values of r_i and (r_c+r_{ic}) become known (see also figure 3.3).

Results

Rates of NH_3-uptake, transpiration and photosynthesis were determined at a light intensity of 11 and 70 $W.m^{-2}$ (PAR). Figure 3.2 shows that at both light intensities the flux density of NH_3 increased linearly with the NH_3-concentration in the range of 4-400 μgm^{-3}. Up to a concentration of about 5 μgm^{-3} no measurable NH_3-uptake was found. In view of this result it was attempted to measure the emission of NH_3 by passing NH_3-free air through the leaf chamber. However, in all cases no emission of NH_3 could be detected.

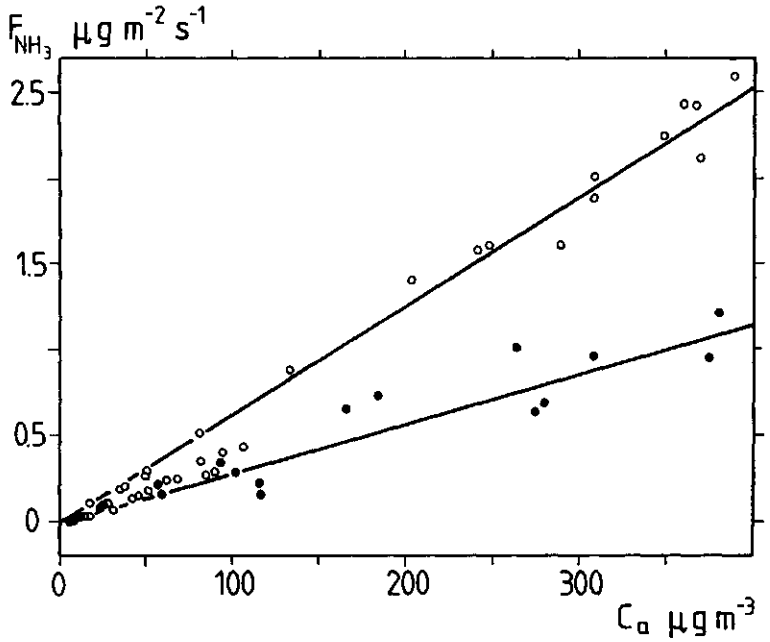


Fig. 3.2. The rate of NH_3 uptake ($F(NH_3)$) by leaves of *Phaseolus vulgaris L.* at a light intensity of 11 (•) and 70 W.m^{-2} (o) (PAR) as a function of NH_3 concentration at the leaf surface (C_a). Temperature: 22-25 °C; relative humidity: 60% ± 5.

The higher flux densities of NH_3 observed at a light intensity of 70 W.m^{-2} were found to correspond closely with higher transpiration rates of the leaves. This indicates that the stomata are an important controlling factor for the uptake process.

To determine the resistance r_i the values for the flux density of NH_3 and those for the rate of transpiration were analysed according to the method of Black and Unsworth (1979). The result is illustrated in figure 3.3 for the situation at which r_i is assumed to be zero.

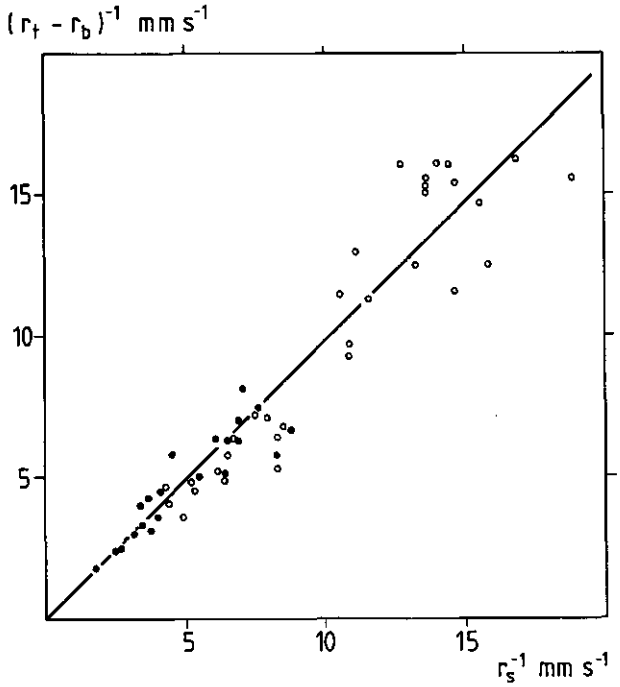


Fig. 3.3. Values of $(r_t - r_b)^{-1}$ obtained from NH_3 uptake measurements plotted against values of r_s^{-1}, obtained from transpiration measurements. The meaning of the symbols is the same as in figures 3.1 and 3.2.

Regression analysis shows that the points are well fitted ($r^2 = 0.93$) by a straight line with slope 0.98 (± 0.09, $P \leq 0.05$). The small value of the intercept (0.188 m.ms^{-1}) indicates that transport through the cuticle can be neglected, i.e. that $(r_c + r_{ic})$ is large compared to r_s. The values for the flux density of NH_3 were related to those for the photosynthesis. It appears that in these short term experiments there is no measurable influence of NH_3 on the rate of photosynthesis.

Discussion

Leaves of *Phaseolus vulgaris L.* appear to have a high affinity for NH_3 in the light. Even at high ambient NH_3-concentrations the uptake rate increased linearly with the NH_3-concentration in the leaf chamber.

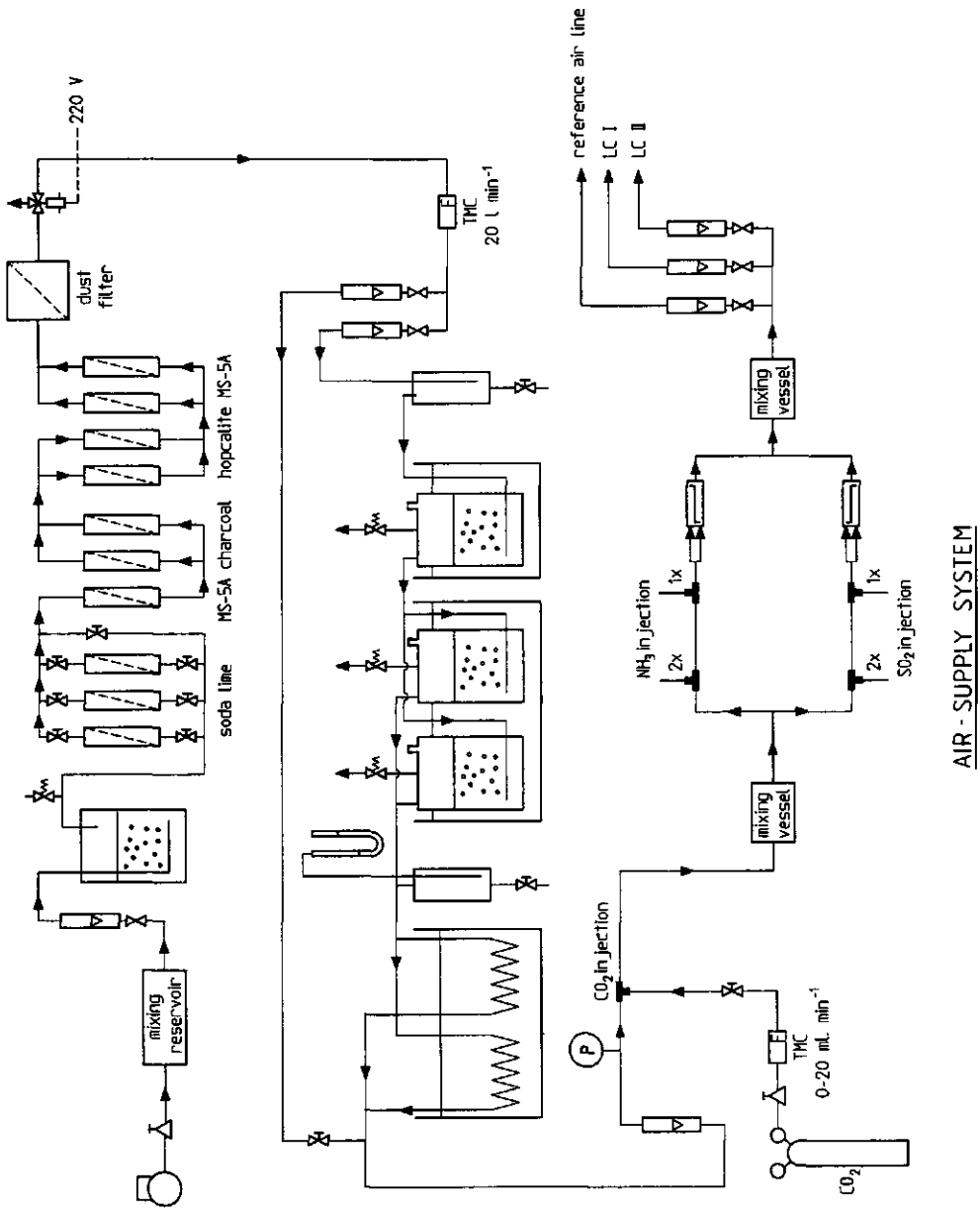
The same has been observed for a number of other plant species (Porter et al., 1972; Meyer, 1973). In those experiments plants were exposed for a short time (up to 5 days) to concentrations of up to 10 mg.m^{-3}.

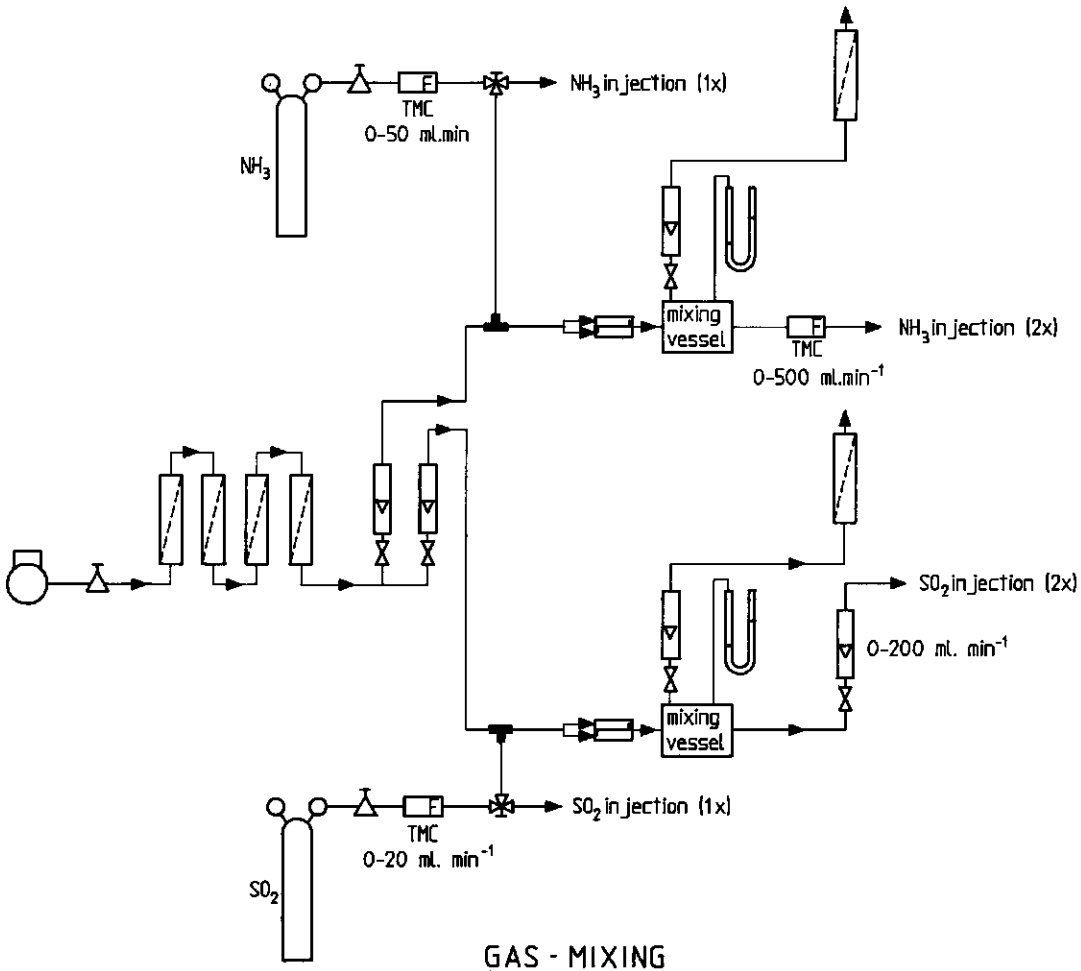
The linear relation between NH_3-uptake and NH_3-concentration suggests that uptake of NH_3 by a leaf is mainly a passive diffusion process, independent of the metabolism of the plant. This presumption is confirmed by the analysis in figure 3.3, showing a negligible small internal resistance. In addition figure 3.3 indicates that at a 65% relative humidity of the air transport of NH_3 through the cuticle can be neglected. So, the NH_3 flux into the leaf can be predicted by data on the boundary layer resistance, stomatal conductance for H_2O and NH_3-concentration at the leaf surface. However, it should be noted that this conclusion is only valid for short term exposures. Until now the effect of a long term exposure on NH_3-uptake by leaves has not been studied yet. The same restriction has to be made for the results of the photosynthesis measurements.

Once past the stomatal pore and cavity, NH_3 is likely to dissolve in the waterfilm of the mesophyll cells in the substomatal cavity. This waterfilm may act as an infinite sink for NH_3. Experiments with $^{15}NH_3$ showed that in the cell ^{15}N is incorporated in amino acids and proteins and is also transported to the roots (Porter et al., 1972). Van der Eerden (1982) observed a sharp increase of the glutamine content in exposed plants, indicating that cellular NH_3-fixation is probably via the enzyme glutamitesynthetase. In vitro experiments showed a high affinity of this enzyme for NH_3 (Anderson and Done, 1977a,b).

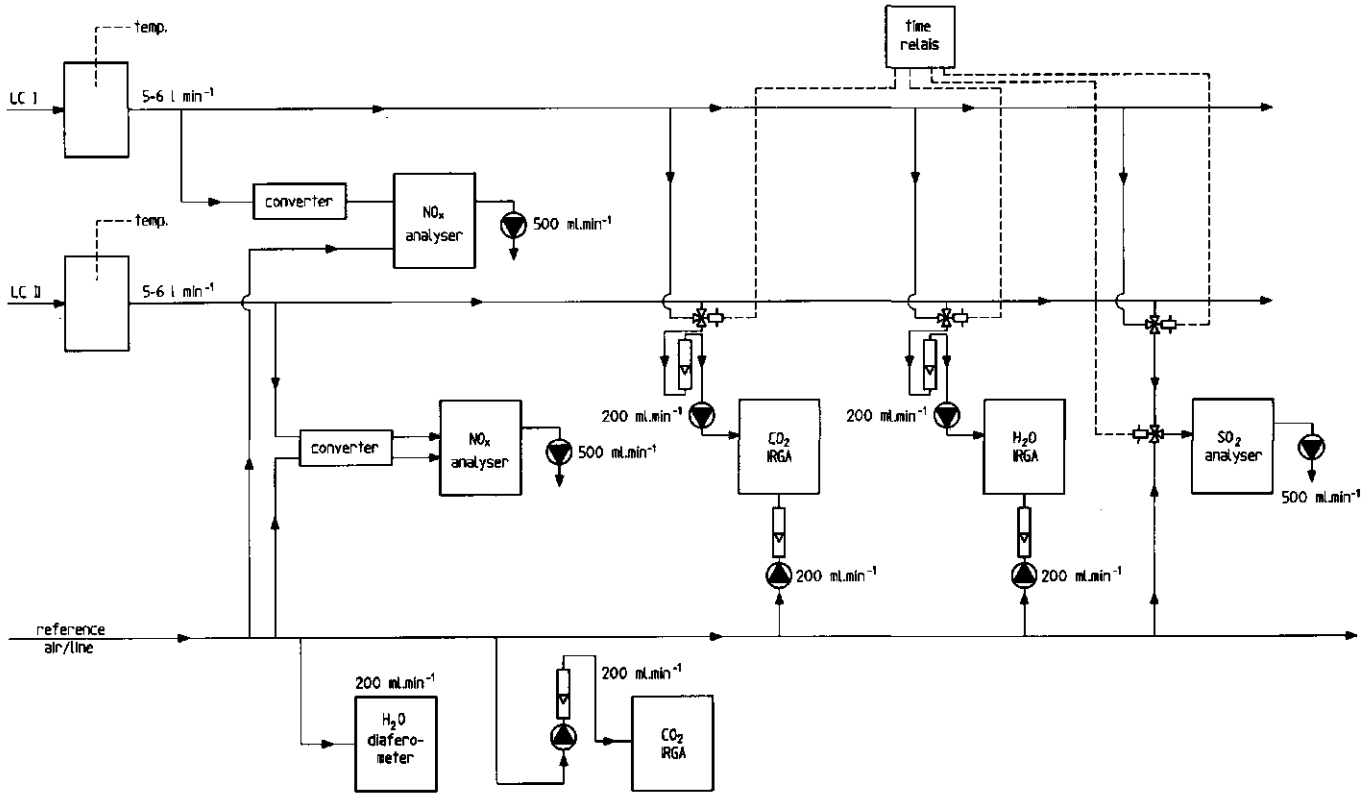
Besides a transport into the leaf an emission of NH_3 has been reported for a number of plant species (Porter et al., 1972; Meyer, 1973; Denmead et al., 1978; Stutte and Weiland, 1978; Stutte et al., 1979). For Phaseolus vulgaris Farquhar et al. (1980) obtained a compensation point for an ambient concentration range of 1.5-4.2 $\mu g.m^{-3}$, depending on temperature. Although our results indicate a compensation point in the same range, no clear evidence for NH_3-emission could be obtained.

Appendix. Schematic representation of the gas exchange fumigation system.




NH3 injection (1x)
TMC
0-50 ml.min
NH3
mixing vessel
NH3 injection (2x)
TMC
0-500 ml.min-1
SO2 injection (2x)
0-200 ml. min-1
mixing vessel
SO2 injection (1x)
TMC
0-20 ml. min-1
SO2

GAS - MIXING



MONITORING SYSTEM

CHAPTER 4

A study of the adsorption of NH_3 and SO_2 on leaf surfaces

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Abstract

The adsorption of NH_3 and SO_2 on the external leaf surface of bean (*Phaseolus vulgaris L.*) and poplar (*Populus euramericana L.*) was studied. The adsorbed quantities increased strongly with increasing air humidity, indicating that water on the leaf surface plays a major role in the interaction of these gases with the leaf surface. On the other hand temperature in the range between 15 and 26 °C had no significant influence. The adsorbed quantities of NH_3 at a specific air humidity appeared to be proportional to NH_3 concentration. This proportionality was less clear for SO_2. The affinity of SO_2 for the leaf surface was found to be approximately twice that of NH_3. A mixture of these gases in the air mutually stimulated their adsorption on the leaf. No significant desorption or uptake of these gases through the cuticle could be detected, indicating that the bulk of the adsorbed gases remains associated with the cuticle.

Introduction

In areas with intensive livestock breeding in the Netherlands high concentrations of ammonium (NH_4^+) and sulphate ions (SO_4^{2-}) have been measured in canopy throughfall (rainwater falling through the canopy) and stemflow (Van Breemen et al. 1982, Roelofs et al. 1985, Heil et al. 1987 and 1988, Ivens et al. 1988).

The high NH_4^+-concentrations result from dry deposition on vegetation surfaces of NH_3, volatilized from liquid domestic animal manure. It is assumed that on the leaf surface NH_3 reacts with sulfur dioxide from fossil fuels producing ammonium sulphite or ammonium sulphate. Van Breemen et al. (1982) have reported that the ammonium sulphate reaching the soil after leaching by rainwater oxidizes rapidly to nitric and sulfuric acid causing acidification and disturbances in the ionic balance of the soil. As a result growth and development of the vegetation may be seriously affected (Roelofs et al. 1985).

The results from throughfall measurements suggest that large quantities of NH_3 and SO_2 are adsorbed on the external surfaces of leaves and stems. However, only a limited amount of data are available sustaining these observations. (Garsed and Read, 1977a, 1977b, Fowler and Unsworth 1979, Black and Unsworth 1979, Taylor and Tingey, 1983). Moreover, most information refers to SO_2, whereas no information is available for NH_3 or a combination of these gases. Consequently, it is not possible to make a satisfactory integrated and unified model for this aspect of dry deposition and its environmental impact.

Therefore, in the present study the adsorption of NH_3 and SO_2 on the external leaf surface was studied in some detail. The consequences of this adsorption are discussed in relation to uptake of these gases via the stomata or cuticle of the leaf. In a related study the physical and chemical parameters governing the dry deposition of SO_2 and NH_3 in ambient air on thin water layers have been determined (Adema et al. 1986, Heeres and Adema 1989). These both studies will provide basic information for the dry deposition of SO_2 and NH_3 on vegetation.

Materials and methods

Plant material

The experiments were carried out with leaves of bean plants (*Phaseolus vulgaris L. cv. 'witte zonder draad'*) and with poplar shoots (*Populus euramericana L. cv. 'Flevo'*) obtained from cuttings. The bean plants and poplar shoots were planted in 12 cm pots containing a soil mixture of peat and sand (3:1) to which lime was added. The plants were grown in a controlled environment room of 20 °C (24 h), a relative humidity of 60-70%, and a light period of 14 h at an intensity of 70 $W.m^{-2}$ (PAR, Philips fluorescent tubes TLMF 140 W/33 RS). The bean plants at time of exposure were three weeks old, the poplar shoots 6 weeks, after sprouting of the axle bud.

Experimental procedure

The adsorption of NH_3 and SO_2 on the external leaf surface was measured using a leaf chamber. The leaf chamber and the gas exchange fumigation system have been described in previous papers (Van Hove et al., 1987, 1988). The adsorption was determined for top leaves of the first trifoliate leaf of the bean plants and leaves situated at the 10th internodium of the poplar shoots.

The adsorption of NH_3 or SO_2 on the leaf surface could only be determined in the absence of uptake of these gases via the stomata of the leaf. Therefore, to assure complete stomatal closure the leaf was cut off from the plant, after which the petiole was held in a solution of abscisic acid (ABA) of 60 $\mu mol.l^{-1}$ (Kriedemann et al. 1972). Furthermore, the leaf was kept in the dark after enclosure in the leaf chamber. The absence of stomatal uptake was verified by measuring the transpiration of the leaf using an infrared gas analyzer (ADC 225 Mk3).

After the whole system had been equilibrated at a specific air humidity for 3 hours NH_3, SO_2 or a mixture of both gases was injected into the flow of conditioned air passing the leaf chamber. NH_3 and SO_2 were supplied from a cylinder containing a calibrated mixture of the gas of 1000 ppm in nitrogen. NH_3 concentrations were 3.29 and 5.88 $\mu mol.m^{-3}$, SO_2 concentrations 0.84 and 1.32 $\mu mol.m^{-3}$. The outlet concentration (C_{out}) was then recorded until a steady state concentration in the leaf chamber was reached. Subsequently, the gas injection was terminated and the decay of C_{out} till a zero concentration was recorded.

The experiment was repeated for the empty leaf chamber in order to correct for the adsorption of the gases at the internal surfaces of the leaf chamber.

The amount of adsorbed NH_3 or SO_2 on the leaf surface was calcu-lated from the difference in area between the two curves for C_{out} (see figure 4.1).

Measurements

NH_3 was measured with a modified NO_x analyzer (Monitor Labs 8840) equipped with a catalytic converter to oxidize NH_3 to NO (accuracy ± 2 ppb, converter efficiency > 85%). The converter consisted of a stainless steel tube (Chrom-pack st.st. 304, pretreated), maintained at 800 °C by a tube furnace, through which the sample stream to the analyser was passed (Aneja et al.,1978).

The SO_2 concentration measurements were carried out with an UV pulsed fluorescent analyzer (Monitor Labs 8850). To enable measurements in the low concentration range the sensitivity of the analyser had been enhanced by increasing the high-voltage power supply of the photomultiplier tube and by changing the instrument electrical outputs.

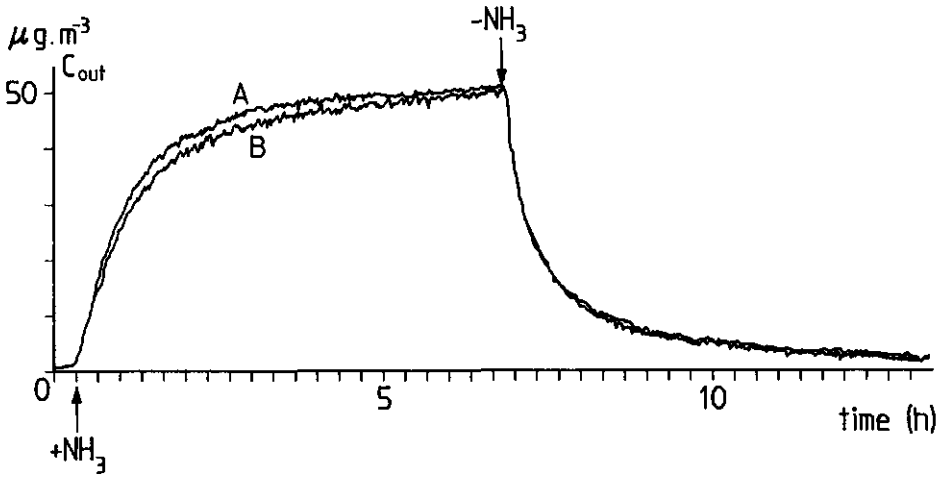

Figure 4.1. An example of an experimental run for NH_3 showing the outlet concentration of the leaf chamber (C_{out}) (in $\mu g.m^{-3}$) as function of time (t)(in hr). A = empty leaf chamber. B = leaf chamber+leaf. $+NH_3$ and $-NH_3$ (arrows); respectively, starting and termination of the gas injection.

Results

Figure 4.1 gives an example of an experimental run for NH_3 showing the recorded outlet concentrations (C_{out}) of the leaf chamber in the absence and presence of a leaf (curve a and b respectively). Similar curves were obtained for SO_2 or a combination of both gases.

The recorded curves for C_{out} as a function of time (t) after starting of the gas injection could be described according to equation (4.1) ($r^2>0.985$):

$$C_{out} = C_{ss} \times (1-e^{-t/\tau}) \qquad (4.1)$$

where τ is the time constant or relaxation time and C_{ss} the steady state concentration. In the experiments with NH_3 and SO_2 an apparent C_{ss} was usually reached after more than 5 hr (fig. 4.1).

The amount of gas adsorbed on the leaf surface was calculated from the difference in area between both curves. The area of each curve was either calculated by integration of equation (4.1) describing the recorded curve, or determined directly using a planimeter.

The curve for the decay of C_{out} after termination of the gas injection was more complex showing a fast and slow decay phase. The fast decay phase can be explained by the small residence time of the air in the leaf chamber (25-30 s), as a consequence of which the polluted air was quickly replaced with clean air after termination of the gas injection. The large tailing of C_{out} hereafter was probably due to the slow release of the adsorbed gases into clean air. No clear differences in the decay curve of C_{out} between the empty leaf chamber and that containing a leaf were measured.

Also no clear differences in C_{ss} in the absence and presence of a leaf were measured indicating that saturation of NH_3 and SO_2 adsorption on the leaf surface has been completed. Furthermore, this suggests that transport of these gases into the leaf interior, under the experimental conditions, is negligible. However, a minor transpiration (about 2% of full scale) was always measured, indicating that the leaf interior is not completely closed from the atmosphere. These apparently contradictory results are likely the result of a difference in accuracy between the NO_x-and SO_2 analyzer on the one side and the infrared analyzer for water vapour on the other side. This can also be demonstrated by calculating the expected difference between C_{in} and C_{out} as a result of this transport using the leaf resistance obtained from the transpiration measurement. A description of this calculation has been given in chapter 3.

A leaf resistance of 4000-5000 $s.m^{-1}$ could be calculated from the transpiration rate. At the concentrations used in our experiments this resistance would give differences between C_{in} and C_{out} at a steady state situation of less than 1 ppb. This is in the range of noise level of both the NH_3 and SO_2 analyzer.

It was verified whether this transpiration was due to an incomplete stomatal closure or to permeability of the cuticle. For that purpose a number of leaf samples were taken, of which microscopic slides of the epidermis were made. A technique was used, with which an instantaneous impression of the leaf epidermis can be made (Pieters and Van den Noort, in prep.). Open stomata were not observed and consequently it is assumed that this small transpiration was largely due to cuticular transpiration. If NH_3 and SO_2 follow the same transport pathway as water, this would imply that the cuticle is also permeable for these gases and that a small error is made in determining the absorbed quantities.

The adsorption of NH_3 and SO_2 at the leaf surface was determined at relative air humidities in the range from 50 to 90%. The adsorption of these gases at relative air humidities below 50% was too small for an accurate determination with our measuring method. Figure 4.2a and 4.2b show that the adsorbed quantities of NH_3 and SO_2 on the leaf surface of bean strongly increased with increasing air humidity. The adsorbed quantities of NH_3 at a specific air humidity appeared to be proportional with their concentration in the air. This proportionality was less clear for SO_2 adsorption.

The air humidity in these and following figures has been expressed in vapour pressure deficit in stead of relative air humidity or water activity. The reason for this follows from figure 4.3. In this figure the results of an experiment have been plotted, in which the influence of air humidity on NH_3 and SO_2 adsorption was analyzed at different air temperatures (14.5, 20 and 26 °C). It appeared that the effect of temperature on the adsorption of both gases comes mainly - if not exclusively - into expression via the vapour pressure deficit of the air. From figure 4.2 and 4.3 it can also be derived that per $\mu mol.m^{-3}$ the apparent affinity of SO_2 for the leaf surface is approximately twice that of NH_3.

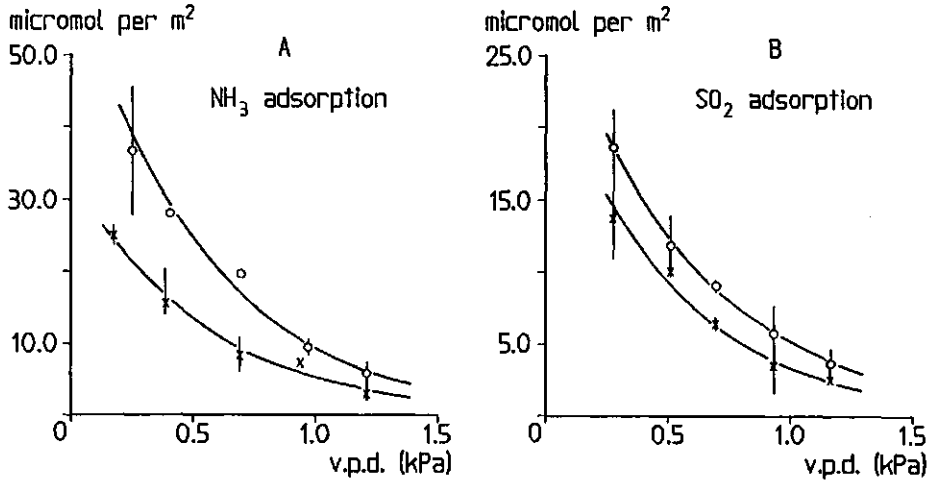

Figure 4.2. The amount of NH_3 and SO_2 adsorption (in μmol per m^2 projected leaf area) as a function of vapour pressure deficit of the air (kPa) and concentration. A. NH_3 concentrations: 3.29 (x) and 5.88 (o) $\mu mol.m^{-3}$. B. SO_2 concentrations: 0.84 (x) and 1.32 (o) $\mu mol.m^{-3}$. Air temperature was 20 °C. Points represent averages of 3 determinations. Standard errors given as vertical bars. The curves were obtained by regression analysis (exponential).

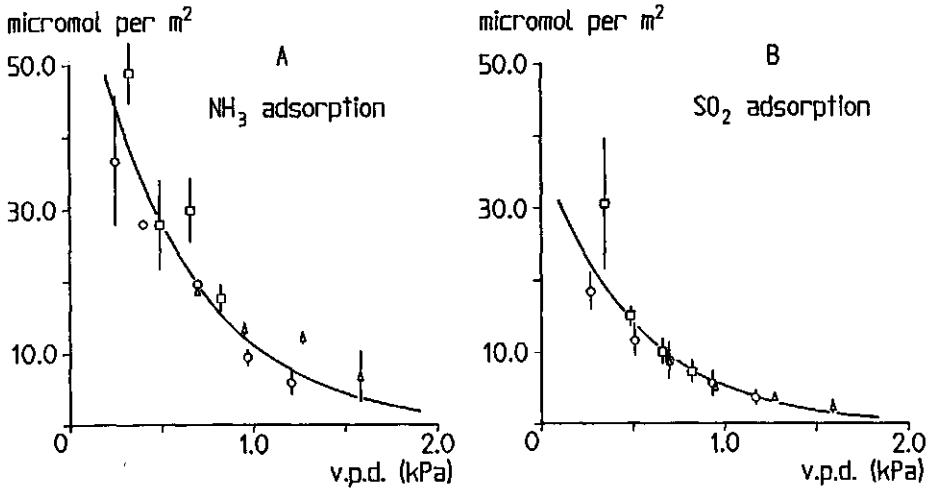

Figure 4.3. The amount of NH_3 and SO_2 adsorption (in μmol per m^2 projected leaf area) as a function of vapour pressure deficit (kPa) and temperature of the air. □ = 14.5 °C; o = 20 °C; Δ = 26 °C. A. NH_3 concentration was 5.88 $\mu mol.m^{-3}$, B. SO_2 concentration was 1.32 $\mu mol.m^{-3}$. Points represent averages of 3 determinations. Standard errors given as vertical bars. The curves were obtained by regression analysis (exponential).

The influence of air humidity was also studied for poplar leaves. Figure 4.4 shows that the quantities adsorbed by poplar leaves did not differ significantly from those adsorbed by bean leaves.

A mixture of NH_3 and SO_2 in the air mutually stimulated their adsorption on the leaf surface, as is shown in figure 4.5. The amount of NH_3-adsorption increased approximately twice as much as compared with the amount of SO_2-adsorption, indicating that NH_3 reacted with SO_2 on the leaf surfaces producing ammonium sulphite and ammonium sulphate.

Discussion

The strong increase of NH_3 and SO_2 adsorption on the leaf surface with increasing air humidity indicates that 'water' on the leaf surface plays a major role in the interaction of these gases with the leaf surface. Moreover, the adsorbed quantities of NH_3 and, to a less extent, those of SO_2 appeared to be proportional with their concentrations in the air suggesting that the adsorption of NH_3 and SO_2 on the external leaf surface has similarities with the dissolving of these gases in a liquid phase. This process is described by Henry's law. However, leaf surfaces, with the exception of the stomatal openings, have been shown to have a high water repellency. Particularly the presence of waxy substances deposited on the leaf surface and embedded within the cutin matrix of the cuticle markedly affects the efficiency of the cuticle as a water barrier (Schönherr 1976b). This raises the question, in which way water molecules interact with the hydrophobic cuticle, thereby influencing the adsorption of NH_3 and SO_2.

It has been shown that the amount and composition of waxes of a leaf varies with age and environmental conditions, including air pollution. Also differences in density, e.g.between the ad- and abaxial side and near veins, may occur, allowing the penetration of water molecules and other water soluble substances (Baker and Hunt 1981, Baker 1982, Reed and Tukey 1982, Shelvey and Koziol 1986). In addition, it has been shown that the cutin matrix not only has lipophilic properties, but also may have hydrophilic properties. This is due to the presence of -COOH, -OH and NH_3^+ groups contributed by cutin and compounds covalently bound to the cutin matrix, such as polyuronic acids, proteins, and phenolic compounds (Schönherr 1982).

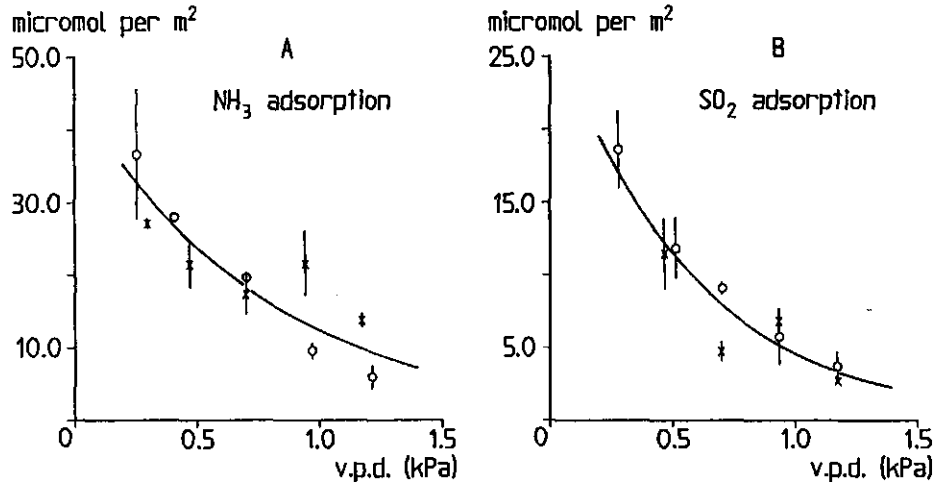

Figure 4.4. The amount of NH_3 and SO_2 adsorption (in μmol per m^2 projected leaf area) on bean leaves (o) and poplar leaves (x) as a function of vapour pressure deficit of the air (kPa). A. NH_3 concentration was 5.88 μmol.m^{-3}, B. SO_2 concentration was 1.32 μmol.m^{-3}. Air temperature was 20 °C. Points represent averages of 3 determinations. Standard errors given as vertical bars. The curves were obtained by regression analysis (exponential).

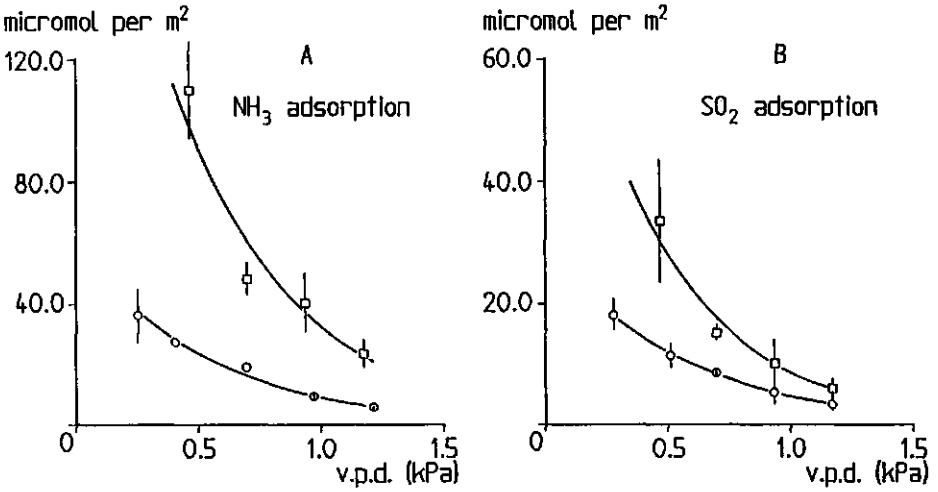

Figure 4.5. The amount of NH_3 and SO_2 adsorption (in μmol per m^2 projected leaf area), as a function of vapour pressure deficit of the air (kPa) at a combination of both gases. NH_3 concentration was 5.88 μmol.m^{-3} and SO_2 concentration 1.32 μmol.m^{-3}. A. NH_3 adsorption; o = applied individually, □ = in combination with SO_2. B. SO_2 adsorption; o = applied individually, □ = in combination with NH_3. Air temperature was 20 °C. Points represent averages of 3 determinations. Standard errors given as vertical bars. The curves were obtained by regression analysis (exponential).

It is postulated that due to the presence of these polar groups the properties of the cutin matrix can be compared with those of a porous membrane, that is: the cutin matrix contains water-filled pores (with a diameter of approximately three water molecules), which cross the membrane in a tortuous path and develop only upon hydration of the polar functional groups. With increasing vapour pressure the number of water molecules associated with each polar group increases and as a consequence both diffusion and permeability coefficients usually increase with increasing water content of the cutin matrix. One could imagine that the cuticle behaves as a sponge, its cavities impregnated with wax, which expands under humid conditions and contracts under dry (Schönherr and Schmidt 1979, Schönherr 1982). Thus, the strong increase in water content and water mobility of the cuticle with increasing air humidity might explain the strong increase in NH_3 and SO_2 adsorption with air humidity.

Because the cutin matrix carries both positive and negative charges, it behaves like a polyelectrolyte with an isoelectric point at about 3 (Schönherr 1976a, Schönherr and Huber 1977). As a consequence pH and counterions (Na^+ or Ca^{2+}) also may have an effect on the water content and water mobility of the cuticle, thereby influencing the adsorption of NH_3 or SO_2.

In table 4.1 a compilation of the chemical reactions and equilibria constants for NH_3 and SO_2 in the water-air system (at 25 °C) have been given. Temperature has an opposite effect on the solubility and dissociation constants of the gases. For instance, the solubility of SO_2 declines with increasing temperature, whereas its reaction with water increases. This might explain the relatively small effect of temperature on the adsorption of NH_3 and SO_2 in the range studied, except for its effect on the vapour pressure deficit of the air. Despite the fact that SO_2 is less soluble in water than NH_3, a higher affinity of SO_2 for the leaf surface was observed. This phenomenon may presumably be explained by the irreversible formation of sulphate (Cox and Penkett, 1983; Adema et al. 1986). The relatively large amounts of ammonium sulphate found in throughfall and stemflow is in accordance with this assumption (Van Breemen et al. 1982, Roelofs et al. 1985, Heil et al. 1987 and 1988, Ivens et al. 1988).

Table 4.1 also shows the strong pH dependency of the reactions of NH_3 and SO_2 with water, which may be of crucial importance for the absorption of NH_3 and SO_2. For instance, when SO_2 dissolves in water, the resultant decrease in pH value reduces the capacity to retain SO_2 in solution.

Table 4.1. Compilation of the chemical reactions, equilibria (K) and rate (k) coefficients for NH_3, SO_2 and CO_2 in the water-air-system (at 25 °C).

1.	$H_2O \rightleftharpoons OH^- + H^+$	K_w	$= 1 \times 10^{-14}$ M^2
2.	$(SO_2)_{aq} \rightleftharpoons HSO_3^- + H^+$	K_2	$= 1.27 \times 10^{-2}$ M
3.	$HSO_3^- \rightleftharpoons SO_3^{--} + H^+$	K_3	$= 6.24 \times 10^{-5}$ M
4.	$H_2SO_4 \rightleftharpoons HSO_4^- + H^+$	K_4	$= 10^4$ (arbitrary)
5.	$HSO_4^- \rightleftharpoons SO_4^{--} + H^+$	K_5	$= 1.54 \times 10^{-2}$ M
6.	$(SO_2)_g \rightleftharpoons (SO_2)_{aq}$	H_6	$= 1.24$ $M.atm^{-1}$
7.	$(NH_3)_g \rightleftharpoons (NH_3)_{aq}$	H_7	$= 57$ $M.atm^{-1}$
8.	$(NH_3)_{aq} + H_2O \rightleftharpoons OH^- + NH_4^+$	K_8	$= 1.77 \times 10^{-5}$ M
9.	$(CO_2)_g \rightleftharpoons (CO_2)_{aq}$	H_9	$= 3.4 \times 10^{-2}$ $M.atm^{-1}$
10.	$(CO_2)_{aq} \rightleftharpoons HCO_3^- + H^+$	K_{10}	$= 4.45 \times 10^{-7}$ M
11.	$HCO_3^- \rightleftharpoons CO_3^{--} + H^+$	K_{11}	$= 4.68 \times 10^{-11}$ M
12.	$SO_3^{--} + \frac{1}{2}O_2 \rightarrow SO_4^{--}$	k_{12}	$= 1.2 \times 10^{-4} [H^+]^{-0.16}$
13.	$HSO_3^- + \{O\} \rightarrow SO_4^{--}$	k_{13}	$= 2.76 \times 10^{-5}$ s^{-1}
14.	$SO_3^{--} + \{O\} \rightarrow SO_4^{--}$	k_{14}	$= 1.2 \times 10^{-4}$ s^{-1}

(Adema et al. 1986)

This might explain that no measurable uptake of SO_2 has been found in a steady state situation. The same was observed for NH_3, which may also be contributed to a reduced capacity, in this case as result of a pH-increase of the liquid phase. The dissolving of NH_3 in the liquid phase of the cuticle may be accompanied with an enhanced dissolving of CO_2 from the air, resulting in a partial prevention of the pH-increase of the liquid phase. Experiments studying the deposition of NH_3 on wet surfaces using a small scale windtunnel showed that this may significantly stimulate the dissolving of NH_3 (Adema et al. 1986). Indications that this reaction indeed takes place, were obtained by Van Breemen et al. (1982), who measured the occasional presence of NH_4HCO_3 in throughfall and stemflow.

In addition, the windtunnel experiments and throughfall measurements indicated that the opposite pH-dependent behavior of NH_3 and SO_2 mutually stimulates their adsorption on the leaf surface. This was also clearly demonstrated in our experiments. The fact that the amount of NH_3 adsorption increased approximately twice as much as compared with the amount of SO_2 adsorption, sustains the assumption that ammonium sulphate is formed on the canopy surface when both gases are present in the atmosphere.

The similarities between the interaction of NH_3 and SO_2 with the external leaf surface and their dissolving in water suggest, that the cuticle-water-system might behave like a free waterlayer. In particular for modelling purposes this would offer interesting opportunities, because the amount of NH_3 and SO_2 adsorption on the leaf surface could then be estimated using calculating models for the dry deposition on thin waterlayers (Adema et al. 1986, Heeres and Adema 1989).

To verify whether this comparison is allowed, the amount of available water in the cuticle-water-system of the leaf as a function of air humidity was calculated using the equilibria constants in table 4.1. The thickness of the apparent waterlayer was derived from the amount of the adsorbed NH_3. Due to the irreversible reaction of the sulphite oxidation to sulphate, it is not possible to use results of SO_2 adsorption for these calculations.

The total amount of NH_3 dissolving in water depends on the following equilibria:

$$(NH_3)_g \overset{H_7}{\rightleftharpoons} (NH_3)_{aq} \qquad (4.2)$$

$$(NH_3)_{aq} + H_2O \overset{H_8}{\rightleftharpoons} NH_4^+ + OH^- \qquad (4.3)$$

From equation (4.2) and (4.3) the following equation for $[NH_4^+]$ can be derived:

$$[NH_4^+] = (K_8 \times H_7 \times p_{NH_3} \times [H^+])/K_w \qquad (4.4)$$

With equation (4.2) and (4.4) the concentrations of NH_3+NH_4^+ in water (further defined as $[NH_3]_T$) for different equilibrium pH values were calculated. The calculations were made for a NH_3 concentration in the air of 5.88 $\mu mol.m^{-3}$ (p_{NH_3}=143 x 10^{-4} Pa)(at 25 °C). The quantities adsorbed NH_3 measured at the highest and lowest v.p.d. were about 6 and 50 $\mu mol.m^{-2}$ respectively (see fig.4.3a). By dividing these values by $[NH_3]_T$ the range for the thickness of the free waterlayer equivalent was obtained. The results of these calculations are shown in figure 4.6.

Experiments studying the deposition of NH_3 in ambient air on thin water layers have shown that the pH of the solution rises to a value of about 8 in a steady state situation (Adema et al. 1986). Figure 4.6 shows that the thickness of the waterfilm at pH=8 would be far larger than that of the leaf cuticle which is in the range of 0.5-15 μm.

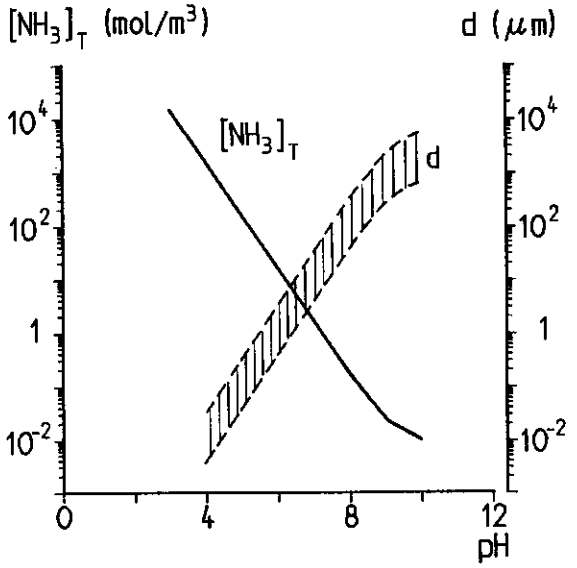

Figure 4.6. The calculated concentrations of (NH_3+NH_4^+) in water ($[NH_3]_T$ in $mol.m^{-3}$) as a function of pH (solid line), at a NH_3 concentration in the air of 5.88 $\mu mol.m^{-3}$ (at 25 °C). The area between the dashed lines represents the range for the thickness of the apparent waterlayer (d in μm), calculated from the amounts of NH_3 found to be adsorbed on the leaf surface at a v.p.d-range of 0.2-1.5 kPa (see fig. 4.3a). Details of the calculations are given in the text.

However, water of the cuticle-water-system does not necessarily behave like distilled water, because of the presence of substances excreted by the leaves such as metabolites, cations and anions (Evans, 1984). These substances may partly neutralize the absorbed NH_3, thereby preventing the rise in pH value. From figure 4.6 it follows that this has a dramatic effect on the calculated $[NH_3]_T$ and thickness of the waterfilm. For instance, if the pH of the leaf surface is the same as the 'mean' leaf pH of 6.1 (Pfanz and Heber, 1986), the thickness of the waterfilm would be in the same range as that of the leaf cuticle.

Another uncertain factor is the amount of NH_3 dissolving in the lipid phase of the cuticle. That this factor may be of importance too, has been demonstrated by Lendzian (1984) for SO_2. He found that SO_2 is much more soluble in isolated cuticular membranes than in distilled water indicating that this gas dissolves in the lipid phase of the cuticle as well.

So, despite the similarities between the interaction of NH_3 and SO_2 with the leaf surface and their dissolving in water, one has to be careful in extrapolating values for the solubility of NH_3 and SO_2 in a undisturbed, free waterlayer to their interaction with the leaf surface. More data about the pH of the cuticle-water-system and the dissolving of NH_3 and SO_2 in the lipid phase of the leaf cuticle are needed first.

It has been shown that stomata play a dominant role in determining the rate of uptake of SO_2 and NH_3 (Black and Unsworth 1979, Taylor and Tingey 1983, Van Hove et al. 1987). An impression of the relative importance of NH_3 and SO_2 adsorption can be obtained by calculating the uptake of these gases by a leaf at a typical value for the stomatal resistance, assuming that the internal resistance is negligible (for details see Van Hove et al. 1987). With a minimal boundary layer plus stomatal resistance (for H_2O) of 150 $s.m^{-1}$ and an external concentration of 5.88 $\mu mol\ m^{-3}$ uptake of NH_3 via the stomata is about 4.0×10^{-8} $mol.m^{-2}.s^{-1}$. When the stomata have been open during 12 hr, the leaves would have taken up a quantity of 1.7×10^{-3} $mol.m^{-2}$. The largest quantity that was found to be absorbed at the same NH_3 concentration was about 50 $\mu mol\ m^{-2}$ (fig.4.3a) or 3% of the quantity taken up via the stomata. A similar calculation for SO_2 at the concentration of 1.32 $\mu mol.m^{-3}$ can be made, showing that the largest quantity adsorbed SO_2 (about 30 $\mu mol.m^{-2}$, see fig.4.3b) would be about 16% of the quantity taken up by the stomata during a 12 h photoperiod.

These percentages for NH_3 and SO_2 adsorption may increase with a factor 4 and 2 respectively, when both gases are present in the atmosphere (figure 4.5). From these calculations it follows that under certain environmental conditions the quantities of NH_3 and, in particular, those of SO_2 adsorbed on the external leaf surface may be considerable as compared to the daily uptake via the stomata.

Large percentages for the adsorption are also mentioned in literature. For instance, Fowler and Unsworth (1979) estimated for wheat (*Triticum aestivum L.*), that during the day 70% of the SO_2 was taken up through the stomata and most of the remaining 30% by the cuticular surface. Similar results have been obtained with other techniques and plants (Garsed and Read 1977a, 1977b), and other pollutant gases (Elkiey and Ormrod, 1981). More in agreement with our results are those of Black and Unsworth (1979). From their results it can be calculated that at a typical value of stomatal resistance of 300 $s.m^{-1}$ about 90% is taken up by the leaf via the stomata. The remaining 10% of the SO_2 is

considered to be deposited on the surface and/or diffused through the cuticle.

Furthermore, our measurements indicated that the permeability of the cuticle for NH_3 and SO_2 is small. The cuticular resistance for these gases is probably in the same range as that for water vapour ($(2\text{-}40)\times10^3$ $s.m^{-1}$) (Schönherr and Schmidt, 1979). Also no significant emission of the gases out off the liquid phase of the cuticle into the air could be detected. Apparently the bulk of the deposited NH_3 and SO_2 on the external leaf surface remains associated with the cuticle and can only be removed by (rain) water, after which a renewed adsorption of these gases on the plant surfaces may occur.

This cleaning effect of rain water followed by a renewed adsorption might explain the large quantities found in throughfall and stemflow water (Van Breemen et al. 1982, Roelofs et al. 1985, Heil et al. 1987 and 1988, Ivens et al. 1988).

CHAPTER 5

Physiological effects of long term exposure to low and moderate concentrations of atmospheric NH_3 on poplar leaves

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W.J. Vredenberg, G.A.Pieters

(Plant, Cell & Environment; in press)

Abstract

Poplar shoots (*Populus euramericana L.*) obtained from cuttings were exposed for 6 or 8 weeks to NH_3 concentrations of 50 and 100 $\mu g.m^{-3}$ or filtered air in fumigation chambers. After this exposure the rates of NH_3 uptake, transpiration, CO_2 assimilation and respiration of leaves were measured using a leaf chamber. During the long term exposure also modulated chlorophyll fluorescence measurements were carried out to obtain information about the photosynthetic performance of individual leaves. Both fluorescence and leaf chamber measurements showed a higher photosynthetic activity of leaves exposed to 100 μg $NH_3.m^{-3}$. These leaves showed also a larger leaf conductance and a larger uptake rate of NH_3 than leaves exposed to lower concentrations of NH_3. The long term NH_3 exposure did not induce an internal resistance against NH_3 transport in the leaf, nor did it affect the leaf cuticle. So, not only at a short time exposure, but also at a long term exposure uptake of NH_3 by leaves can be calculated from data on the boundary layer and stomatal resistance for H_2O and NH_3-concentration at the leaf surface. Furthermore, the NH_3 exposure had no effect on the relation between CO_2-assimilation and stomatal conductance, indicating that NH_3 in concentrations up to 100 $\mu g.m^{-3}$ has no direct effect on stomatal behavior, e.g. by affecting the guard or contiguous cells of the stomata

Introduction

Serious dieback of forests and decline in number of plant species are observed in areas in the Netherlands where intensive animal breeding is concentrated (Roelofs et al, 1985; Roelofs 1986; Den Boer, 1986). Evidence is accumulating that the high emission of ammonia (NH_3) in these areas, as a result of volatilization from animal manure, is one of the major causes for the observed effects.

So far particular emphasis has been given to the way in which the deposited NH_3 influences the vegetation via the soil. Only a limited number of papers deals with the effects of atmospheric NH_3 taken up by the leaves. Papers on this subject mainly report on visible effects, for example as result of the release of high NH_3 concentrations at accidents (Temple et al, 1979; De Temmerman 1982; Van der Eerden 1982). Furthermore the uptake of NH_3 by leaves and the contribution of this uptake to the total nitrogen supply and plant growth has been studied (Faller 1972; Porter, Viets and Hutchinson, 1972; Rogers and Aneja 1980; Cowling and Lockyer 1981; Lockyer and Whitehead, 1986).

However, little is known about the physiological effects of atmospheric NH_3 on plants. In a previous study we observed that leaves have a high affinity for NH_3 in the light. Even at concentrations up to 400 $\mu g.m^{-3}$ the NH_3 flux into leaves appeared to be linearly related with the concentration. In addition no effect on the CO_2 assimilation rate of the leaves was observed (Van Hove et al., 1987). However, the leaves in that study were exposed to NH_3 for a relatively short time. Therefore, a major objective of the present study was to examine whether a long term exposure to low and moderate NH_3 concentrations may have an effect on the uptake of this gas into leaves and on photosynthesis.

The experiments were carried out with poplar shoots (*Populus euramericana L.*) which were exposed to NH_3 concentrations of 50 and 100 $\mu g.m^{-3}$ during a period of 6 or 8 weeks. To approach the outdoor situation plants were grown in a soil medium with a sufficient nitrogen supply. After this exposure the NH_3 uptake into leaves, transpiration and CO_2 assimilation were measured using a leaf chamber. During the long term exposure also modulated chlorophyll fluorescence measurements on individual leaves were carried out. The purpose of these measurements was twofold: In the first place information about the influence of NH_3 taken up by the leaf on the photosynthesis process at the chloroplast level can be obtained with this method.

Secondly, the possibility was examined whether this method can be applied for the diagnosis of plant 'stress' induced by air pollutants.

Materials and methods

Plant material

The poplar shoots (*Populus euramericana L. cv. 'Flevo'*) were obtained from cuttings which had been kept at a temperature of -1 to -4 °C before planting. The cuttings were planted in 1 l pots containing a soil mixture of peat and sand (3:1). The pots were standing in a waterlayer of 1 cm to which a slow working NPK-fertilizer (0.5 g per pot) had been added, containing (in weight percentages): 7.25% NH_4^+, 7.75% NO_3^-, 11% P_2O_5, 13% K_2O and 2% MgO. After sprouting of the axillary bud also a Fe-EDTA solution and a solution with micro-elements were added.

The cuttings were transferred to the fumigation chambers after the appearance of the 10th leaf. At that time also the first roots became visible. Only a limited number of leaves could be measured every week with the leaf chamber. Therefore the cuttings were transferred in groups of six per week to the fumigation chambers.

The fumigation chambers

The fumigation chambers consisted of a framework of dexion (0.6 x 0.8 x 0.6 m) and sheets of transparent F.E.P. film stretched across the inside of the framework to form the walls. The door was made of plexiglass and situated on the front wall. Air exchange in the fumigation chamber was 2 to 3 times per minute. The ingoing air was passed through a granular charcoal filter treated with H_3PO_4 to remove NH_3. The air entered the fumigation chamber on one side, through a perforated stainless steel sheet to reduce space variability of gas concentrations.

The fumigation chambers were in a controlled environment room of the phytotron. The conditions in the fumigation chambers were: temperature 22 °C during the day and 18 °C at night, relative humidity 60-80%, the light intensity at 50 cm height 280 $\mu mol.m^{-2}.s^{-1}$ (Philips fluorescent tubes TLMF 140 W/33 RS) and a photoperiod of 16 h.

NH_3 was generated by adding an ammonia solution (25%) with a minipuls pump into a heated round bottomed vessel. The generated NH_3 was taken away by a controlled flow of air passing through the vessel and injected into the air entering the fumigation chamber.

The NH_3 concentrations were measured with a modified NO_x analyzer (Monitor Labs 8840), equipped with a catalytic converter to oxidize NH_3 to NO (accuracy ± 2 ppb, converter efficiency > 85%). The converter consisted of a stainless steel tube (Chrompack st.st. 304, pretreated), maintained at 800 °C by a tube furnace, through which the sample stream to the analyser was passed (Aneja et al.,1978).

The average NH_3 concentrations in the fumigation chambers were: 50 and 100 $\mu g.m^{-3}$ (±10). The deviations in concentrations were a result of variations in air temperature and humidity in the fumigation chambers and uptake of NH_3 by the leaves. Probably as a result of this uptake lower concentrations were usually measured during the light period. Always a low NH_3 concentration with an average of 15 $\mu g.m^{-3}$ (±5) was measured in the fumigation chamber with filtered air. Furthermore, the air in each fumigation chamber contained a low NO_x concentration of 20 $\mu g.m^{-3}$ (±10).

The leaf chamber measurements

After the exposure period of 6 or 8 weeks plants were taken from the fumigation chambers. Immediately thereafter NH_3 uptake, transpiration, CO_2 assimilation and respiration of leaves were measured in the leaf chamber.

The leaf chamber is part of an open system (Jarvis and Catsky 1971) where uptake of a pollutant gas, transpiration and CO_2 assimilation of a leaf are determined by measuring the difference between inlet and outlet concentration of the leaf chamber. The gas exchange fumigation system and leaf chamber have been described in detail in previous papers (Van Hove et al., 1987, 1988).

Leaves positioned at the 10th internode (counted from below) were measured. The NH_3 concentrations at the inlet of the leaf chamber were 50 or 100 $\mu g.m^{-3}$, depending on the concentrations the leaves had received in the fumigation chambers. Leaves exposed to filtered air in the fumigation chamber were exposed to 100 $\mu g.m^{-3}$ NH_3.

The measurements were carried out for 9 h in the dark and for 9 h in the light. For each leaf a light response curve was assessed at light intensities ranging from 0 to 360 $\mu mol.m^{-2}.s^{-1}$ (PFD). Temperature in the leaf chamber was about 20 °C, relative humidity 70% (± 5%), CO_2 concentration 330 (±10) $\mu l.l^{-1}$ and wind velocity across both leaf surfaces 1 $m.s^{-1}$. The boundary layer resistance for one side of the leaf at this wind velocity was about 30 $s.m^{-1}$.

Fluorescence measurements

Fluorescence of a leaf was determined with a pulse amplitude modulation chlorophyll fluorometer (Schreiber, Schliwa and Bilger, 1986, Schreiber 1986). The leaf is repetitively excited by 1 μsec pulses of light from a light emitting diode (LED) passing through a short-pass filter (λ<670 nm) (L_1). The fluorescence response is monitored by a photodiode detector protected by a long-pass filter (λ>700 nm). The fiberoptics consists of four separate bundles, which merge into one joint bundle, over a 400 mm pathway. Two bundles are used for the measuring light source (pulsed LED) and the fluorescence detector (photodiode). The other two provided the possibility to add actinic light with an intensity of about 35 $\mu mol.m^{-2}.s^{-1}$ from a LED light source (peak wavelength 650 nm) (L_2) and saturating light pulses with an intensity of 7000 $\mu mol.m^{-2}.s^{-1}$ from a Tungsten flash lamp (white light, λ<700 nm) (L_3).

The fluorescence measurements were started during the second week of the exposure. Leaves positioned at the 10th internode of the plant were measured. The measurements were performed with a small cuvette in which a part of the leaf could be clamped without damaging the leaf surface. The cuvette provided access on the upper side to the fiberoptics bundle which could be positioned at 3 mm from the leaf surface. During the measurement a continuous flow of a moistened gas mixture of 320 ppm CO_2 and 2% O_2 in N_2 at a rate of 80 $ml.min^{-1}$ was passed through the cuvette. The low O_2 concen-tration was meant to minimize corrections for O_2-reducing reactions. Before the actual measurement took place, the plant was dark adapted for 30 min in order to determine both the minimal (F_o) and maximal fluorescence (F_{max}) level. The photochemical efficiency of photosystem II (PSII) at F_o is maximal because all centers are "open", i.e. all primary acceptors, Q_A, are oxidized. At the start the leaf was illuminated with actinic light (L_2) causing a fast rise (in fractions of a second) in fluorescence due to a lowering of the quantum yield for photo-chemistry. This new changing level of fluorescence intensity is denoted by F_v and the obtained curve is known as the 'Kautsky' curve (Kautsky 1931). The change in fluorescence intensity, exclusively associated with the oxidation-reduction state of Q_A, is caused by photochemically quenching or Q-quenching. After 1 s a short pulse (about 700 ms in length) of saturating white light (L_3) was added. This causes a complete reduction of the quinone acceptors Q_A and Q_B of photosystem II (PSII), including all plastoquinones between PSII and PSI, and a complete saturation of the electron transport chains in the

thylakoids of the chloroplasts. As a consequence PSII efficiency is minimal (all centers are "closed") and a maximal fluorescence is obtained (F_{max}). After this saturating pulse the fluorescence will fall off to the level of F_v induced by L_2. The short pulse with L_3 was repeated every 3 s during the first minute. As the time of actinic illumination proceeds the fluorescence level induced by L_3 reaches a maximum level below that of F_{max}. This lower level, denoted by $(F_v)_s$, is a result of non-photochemical or 'energy' dependent quenching (E-quenching) which is thought to be related to the "energization" of the thylakoid membrane (Krause, Brantais and Vernotte 1982, 1983; Krause and Weis 1988). So, by adding saturating pulses of white light during the actinic illumination of the leaf with L_2, quenching of the fluorescence signal can be related to a photochemical (Q-quenching) and non-photochemical (E-quenching) component. After 1 min the pulses with L_3 were added with a frequency of 1 pulse per 10 s, until both F_v and F_{vs} reached a steady state level. Subsequently, L_2 was turned off and the time course of F_v and F_{vs} in the dark was monitored during 1 minute, with saturating pulses every 20 s.

Analyses of data

Leaf chamber measurements

The NH_3 flux ($F(NH_3)$) into a leaf was calculated from the difference between inlet and outlet concentration of the leaf chamber (ΔC in $g.m^{-3}$), the gas flow rate passing the leaf chamber (f in $m^3\ s^{-1}$) and the projected leaf area (A in m^2):

$$F(NH_3) = (f \times \Delta C)/A \qquad (5.1)$$

In a similar way the transpiration rate, net CO_2 assimilation (P_n in $\mu mol.m^{-2}.s^{-1}$) and dark respiration (R_d in $\mu mol.m^{-2}.s^{-1}$) were assessed. From the transpiration rate the stomatal conductance for the H_2O flux out off the leaf was calculated using a modified Penmann equation for a hypostomatous leaf (Monteith 1973). The H_2O flux only depends on the boundary layer and stomatal resistance of the leaf. Therefore, this flux was compared with the NH_3 flux into the leaf in order to determine the internal resistance for NH_3 transport in the leaf and the resistance for cuticular transport. This analysis has been described in detail in a previous paper (Van Hove et al. 1987) (see also figure 5.5).

From the CO_2 assimilation at light saturation (P_{max}) a mesophyll conductance for CO_2 (g_m in $mm.s^{-1}$) was derived according to equation (5.2):

$$g_m = [r_{t.c} - (1.39 \times r_b + 1.65 \times r_s)]^{-1} \qquad (5.2)$$

where $r_{t.c}$ the total resistance for CO_2 uptake, r_b and r_s the boundary layer and stomatal resistance for H_2O respectively. The constants in equation (5.2) are the conversion factors for the differences in diffusion coefficients between CO_2 and H_2O (Goudriaan and Van Laar 1978). $r_{t.c}$ was calculated by dividing the CO_2 concentration in the leaf chamber by the sum of P_{max} and R_d, assuming that the CO_2 concentration at the carboxylation sites is zero.

The CO_2 assimilation light response curve for individual leaves can be described by an asymptotic exponential function (Goudriaan 1982):

$$P_n = (P_{max}+R_d)(1-\exp(-PFD \times QY/(P_{max}+R_d)))-R_d, \quad (5.3)$$

where PFD is the photon flux density in $\mu mol.m^{-2}.s^{-1}$ and QY the initial efficiency or quantum yield for CO_2 fixation (=dimensionless). P_{max} and QY were determined by fitting the obtained data to equation (5.3) using an optimization program.

The relation between P_n and stomatal conductance was assessed by plotting values for P_n against those for the leaf conductance for CO_2 ($g_{t.c}$). This gives a linear relationship described by:

$$P_n = g_{t.c} \times (C_a - C_i) \qquad (5.4)$$

$g_{t.c}$ was calculated from the stomatal and boundary layer resistance for H_2O taking into account the differences in diffusion coefficients between CO_2 and H_2O. From the slope of the line ($=(C_a-C_i)$) and the CO_2 concentration in the leaf chamber air (C_a) the internal CO_2 concentration (C_i) was calculated (Goudriaan and Van Laar 1978).

Fluorescence measurements

The photochemical and non-photochemical quenching, q_Q and q_E respectively, were determined according to the following equations (see Schreiber and Bilger 1987):

$$q_Q = (F_{vs}-F_v)/(F_{vs}-F_o) \qquad (5.5)$$

$$q_E = (F_{max} - F_{vs})/(F_{max} - F_o) \qquad (5.6)$$

To get an impression of the photochemical efficiency of PSII the value Φ_{Po} was calculated:

$$\Phi_{Po} = (F_{max}-F_o)/F_{max} = F_v/F_{max} \qquad (5.7)$$

Φ_{Po} represents the potential yield of the photochemical reaction of a non-energized thylakoid membrane (i.e. in a dark adapted state or $q_E = 0$) with all acceptors oxidized. Φ_{Po} has been found to be very uniform (0.832 ± 0.004) among leaves of many vascular plant species and ecotypes (Björkman and Demmig 1987). Furthermore, it has been shown that environmental stress factors affecting PSII cause a decrease in Φ_{Po} (Krause and Weis 1988).

Results

CO2 assimilation and stomatal conductance

Figure 5.1 shows the light response curve for net CO_2 assimilation (P_n) of the leaves after an NH_3 exposure period of 6 or 8 weeks. The calculated values for maximum photosynthesis (P_{max}), quantum yield (QY) as well as the measured dark respiration (R_d) are presented in table 5.1. It appeared that QY and P_{max} declined considerably in the period between 6 and 8 weeks. This is probably related to leaf senescence. Leaves exposed to 100 $\mu g.m^{-3}$ NH_3 showed a larger P_{max} than leaves exposed to a concentration of 50 $\mu g.m^{-3}$ or filtered air. These leaves showed also a smaller decline in P_{max} in the period between 6 and 8 weeks. However, there were no significant differences in QY and R_d (P=0.05).

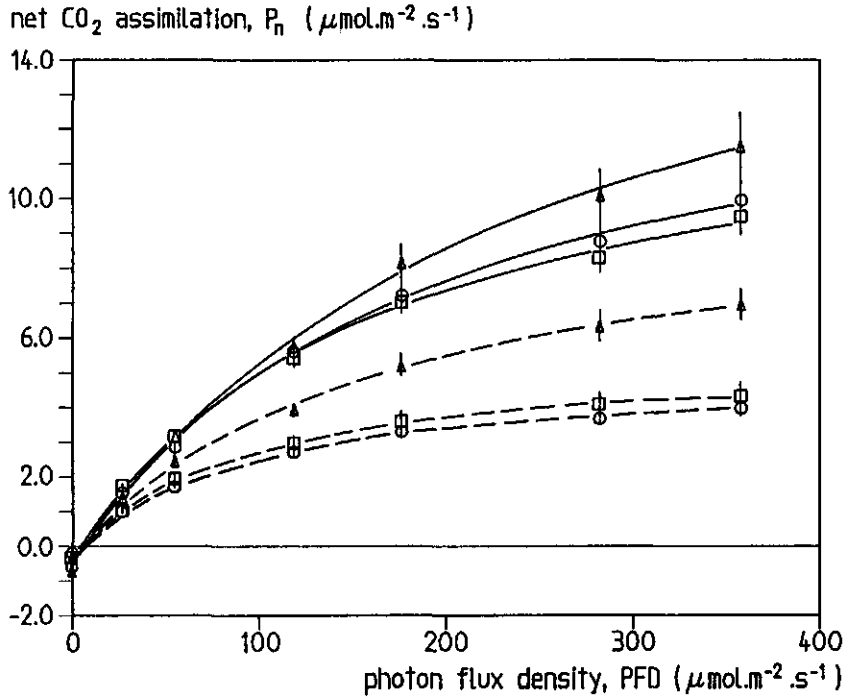

Figure 5.1. Net CO_2 assimilation (in μmol.m^{-2}.s^{-1}) as function of photon flux density (PFD in μmol.m^{-2}.s^{-1}) of poplar leaves (*Populus euramericana L.*) exposed to 15 (filtered air) (o), 50 (□) and 100 (Δ) μg.m^{-3} NH_3 in fumigation chambers. Solid lines: leaves exposed for 42 days (n=9). Dashed lines: leaves exposed for 56 days (n=5). Vertical bars represent standard errors of the mean.

Only minor differences in P_{max} between leaves exposed to 50 μg.m^{-3} NH_3 and those exposed to 15 μg.m^{-3} in the filtered air were observed. This would imply that the effect of NH_3 on CO_2 assimilation was not proportional with the concentration in the air, suggesting the existence of a threshold level for the effect of NH_3.

Similar results were obtained for the stomatal conductance (fig. 5.2). The higher CO_2-assimilation rate of leaves exposed to 100 μg.m^{-3} NH_3 corresponded to a larger stomatal conductance. These leaves showed also a smaller decline in stomatal conductance after the exposure period of 8 weeks, as well as a smaller decline in g_m (table 5.2).

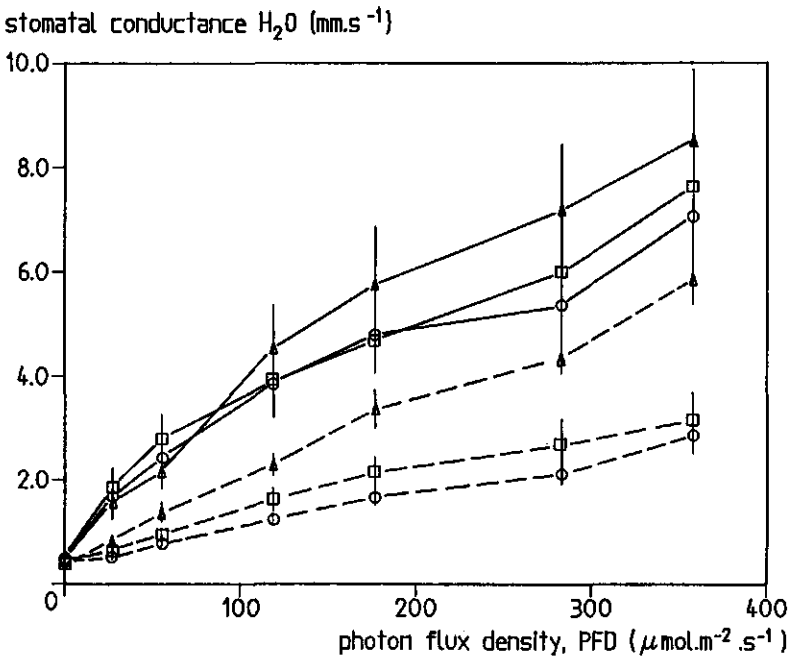

Figure 5.2. Stomatal conductance for H_2O (in mm.s^{-1}) as function of photon flux density (PFD in μmol.m^{-2}.s^{-1}) of poplar leaves (*Populus euramericana L.*) exposed to 15 (filtered air) (o), 50 (□) and 100 (Δ) μg.m^{-3} NH_3 in fumigation chambers. Solid lines: leaves exposed for 42 days (n=9). Dashed lines: leaves exposed for 56 days (n=5). Vertical bars represent standard errors of the mean.

The response of stomata to a change in light intensity was found to be much slower than that of CO_2 assimilation. Nevertheless, the relationship between stomatal opening and P_n was always restored after some time. Figure 5.3 shows an almost linear relationship between P_n and the leaf conductance for CO_2 ($g_{t,c}$) in the steady state situation. This was observed for 6 weeks as well as for 8 weeks old leaves. The proportionality between P_n and $g_{t,c}$ was not influenced by the NH3 exposure. However, on the other hand, a small influence of leaf age was observed, reflected in the slope of the regression line. Table 5.2 shows that the decline in the slope of the regression line in the period between 6 and 8 weeks was related to a rise in internal CO_2 concentration. Furthermore it should be noted that the assimilation rate of in particular 8 weeks old leaves increased only marginally with leaf conductance when light intensity became saturating. According to Von Caemmerer and Farquhar (1981) this might be related to a reduced activity of ribulose bisphosphate carboxylase/oxygenase (RUBISCO).

Table 5.1. Estimated values (± standard error of the mean) for CO_2 assimilation at light saturation (P_{max}) and quantum yield (QY), and the measured dark respiration (R_d) of poplar leaves (*Populus euramericana L.*), exposed for 42 and 56 days to different NH_3 concentrations in fumigation chambers.

		42 days (n=9)	
NH_3 ($\mu g.m^{-3}$)	15*	50	100
P_{max} ($\mu mol.m^{-2}.s^{-1}$)	10.4 ± 1.2	9.8 ± 0.6	12.8 ± 1.4[a]
QY	0.089 ± 0.005	0.089 ± 0.004	0.088 ± 0.004
R_d ($\mu mol.m^{-2}.s^{-1}$)	0.62 ± 0.08	0.55 ± 0.07	0.68 ± 0.10
		56 days (n=5)	
P_{max} ($\mu mol.m^{-2}.s^{-1}$)	3.8 ± 0.3	4.3 ± 0.4	7.1 ± 0.5[b]
QY	0.057 ± 0.006	0.055 ± 0.005	0.061 ± 0.003
R_d ($\mu mol.m^{-2}.s^{-1}$)	0.23 ± 0.05	0.22 ± 0.02	0.32 ± 0.05

*: filtered air. a and b : significantly different at P=0.10 and P=0.01 respectively.

Table 5.2. Estimated values for internal CO_2 concentration (C_i) and mesophyll conductance for CO_2 (g_m) of poplar leaves (*Populus euramericana L.*), exposed for 42 and 56 days to different NH_3 concentrations in fumigation chambers.

		42 days (n=9)	
NH_3 ($\mu g.m^{-3}$	15*	50	100
C_i ($g.m^{-3}$)	0.43 ± 0.01	0.44 ± 0.02	0.44 ± 0.02
g_m ($mm.s^{-1}$)	0.91 ± 0.10	0.88 ± 0.07	1.14 ± 0.08
		56 days (n=5)	
C_i ($g.m^{-3}$)	0.51 ± 0.01	0.49 ± 0.01	0.50 ± 0.01
g_m ($mm.s^{-1}$)	0.39 ± 0.02	0.41 ± 0.03	0.69 ± 0.04[a]

*: filtered air. a: significantly different at P=0.01.

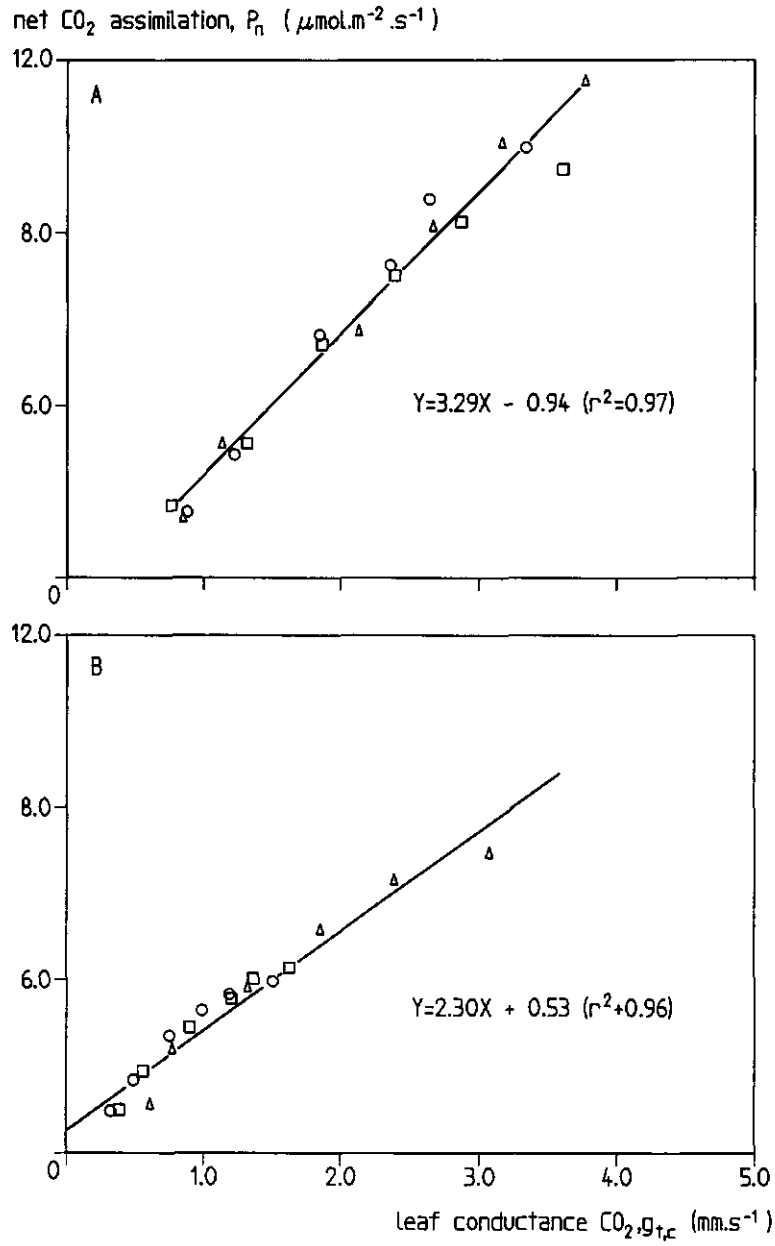

Figure 5.3. The relation between leaf conductance for CO_2 ($g_{t,c}$ in $mm.s^{-1}$) and net CO_2 assimilation (in $\mu mol.m^{-2}.s^{-1}$) of poplar leaves (*Populus euramericana L.*) exposed to 15 (filtered air) (o), 50 (□) and 100 (Δ) $\mu g.m^{-3}$ NH_3 in fumigation chambers. A, B: after an exposure period of 42 (n=9) and 56 (n=5) days, respectively.

NH_3 transfer into the leaf

Leaves exposed to 100 $\mu g.m^{-3}$ NH_3 in the fumigation chamber showed also a larger uptake rate of this gas than leaves exposed to filtered air (figure 5.4). Considering the difference in concentration at the inlet of the leaf chamber the flux of these leaves was also larger than that of leaves exposed to 50 $\mu g.m^{-3}$ NH_3.

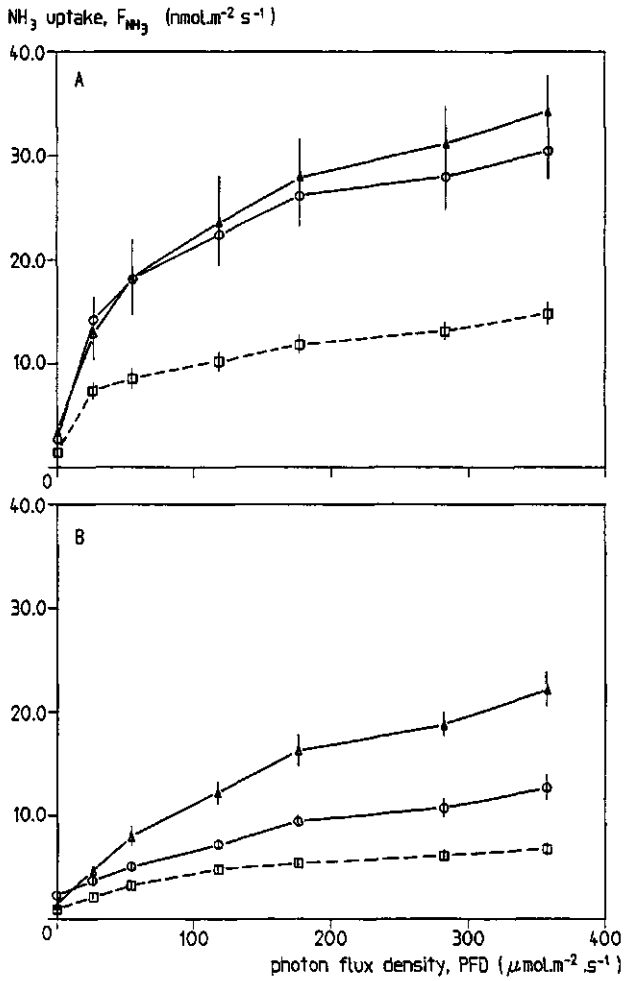

Figure 5.4. Uptake of NH_3 ($F(NH_3)$ in $nmol.m^{-2}.s^{-1}$) as function of photon flux density (PFD in $\mu mol.m^{-2}.s^{-1}$) of leaves exposed to 15 (filtered air)(o), 50 (□) and 100 (Δ) $\mu g.m^{-3}$ NH_3 in fumigation chambers. A, B: after an exposure period of 42 (n=9) and 56 (n=5) days, respectively. The NH_3 concentrations at the inlet of the leaf chamber were 50 (---) or 100 (———)$\mu g.m^{-3}$, depending on the concentrations the leaves had received in the fumigation chambers. For the leaves exposed to filtered air the inlet concentration was 100 $\mu g.m^{-3}$ NH_3. Vertical bars represent standard errors of the mean.

NH_3 transfer into the leaf was analyzed according to a method described in a previous paper (Van Hove et al. 1987). The result is shown in fig. 5.5. The internal resistance against NH_3 transport in the leaf (r_i) was assumed to be zero in this analysis. No significant differences between the different treatments were observed; all points could be well fitted by one straight line. The slope of the regression line (1.11 ± 0.09, $P<0.05$) indicates that r_i was negligible small or slightly negative. The small value of the intercept (0.21 $mm.s^{-1}$) indicates that transport through the leaf cuticle was negligible as compared to transport via the stomata. This implies that the larger NH_3 flux into leaves which had been exposed to 100 $\mu g.m^{-3}$ NH_3, was solely due to a larger stomatal conductance.

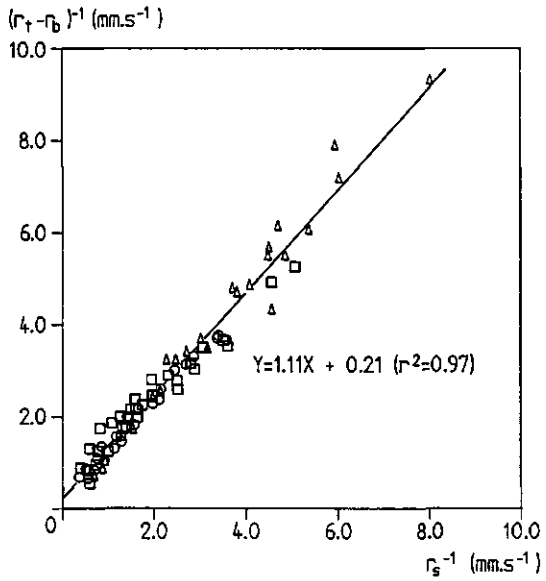

Figure 5.5. Analysis of NH_3 transfer into the leaf. Values for the NH_3 flux were compared with those obtained for the H_2O flux according to the equation (Van Hove et al. 1987):

$$(r_t-r_b)^{-1} = (r_s+r_i)^{-1} + (r_c+r_{ic})^{-1}.$$

r_t = total resistance against NH_3 transfer into the leaf calculated from NH_3 uptake measurements; r_b = boundary layer resistance of the leaf; r_s = stomatal resistance for NH_3 calculated from the r_s for H_2O taking into account the differences in diffusion coefficients between NH_3 and H_2O; r_i = internal resistance against NH_3 transport in the leaf (=assumed to be zero in this analysis); $(r_c+r_{ic})^{-1}$ = resistances determining transport of NH_3 through the leaf cuticle. o,□,Δ; NH_3 concentration of 15 (filtered air), 50 and 100 $\mu g.m^{-3}$, respectively.

Chlorophyll fluorescence

Also the fluorescence of leaves exposed to 100 $\mu g.m^{-3}$ NH_3 was different from that of leaves exposed to 50 $\mu g.m^{-3}$ NH_3 or filtered air. After an exposure period of two weeks differences were observed during the first minute of illumination (fig. 5.6a). Figure 5.6b shows that leaves exposed to 100 $\mu g.m^{-3}$ NH_3 had a lower q_Q in the first seconds and a lower q_E after 30 s. From this it can be derived that these leaves had a lower electron transport rate in the first seconds than leaves exposed to 50 $\mu g.m^{-3}$ NH_3 or filtered air. A high photochemical quenching level coupled to a relatively low non-photochemical quenching level in the first minute in the light is usually measured for juvenile leaves (Van Kooten, unpublished results). This would imply that during the first weeks of exposure the leaves exposed to 100 $\mu g.m^{-3}$ NH_3 were somewhat faster in their development. However, more research is required to find evidence for this assumption.

The differences during the first minute disappeared during the further development of the leaves. In stead differences were observed in the steady state situation. After an exposure period of 7 weeks leaves exposed to 100 $\mu g.m^{-3}$ NH_3 showed a significantly lower q_E (fig. 5.6d) in the steady state situation and a small, but significantly (P=0.05) higher Φ_{Po} (table 5.3) as compared to leaves exposed to 50 $\mu g.m^{-3}$ NH_3 or filtered air. The lower q_E indicates an increased electron transport rate correlated to a higher activity of the Calvin cycle under the experimental conditions of the present study (Weis and Berry 1987). The higher Φ_{Po} is related to an increased photochemical efficiency of PSII (Björkman and Demmig 1987). Φ_{Po} remained unaffected in the period between 6 and 8 weeks, in contrast to the QY derived from the gas exchange measurements.

Table 5.3. Calculated values (± standard error of the mean) for the efficiency of PSII photosystem (Φ_{Po})** of poplar leaves (*Populus euramericana L.*), exposed for 14, 35 and 49 days to different NH_3 concentrations in fumigation chambers (n=7).

	NH_3 ($\mu g.m^{-3}$)		
days	15*	50	100
14	0.766 ± 0.017	0.788 ± 0.011	0.813 ± 0.009
35	0.809 ± 0.008	0.801 ± 0.013	0.818 ± 0.005
49	0.786 ± 0.008	0.793 ± 0.005	0.821 ± 0.004

*: filtered air. **: according to Björkman and Demmig (1987)

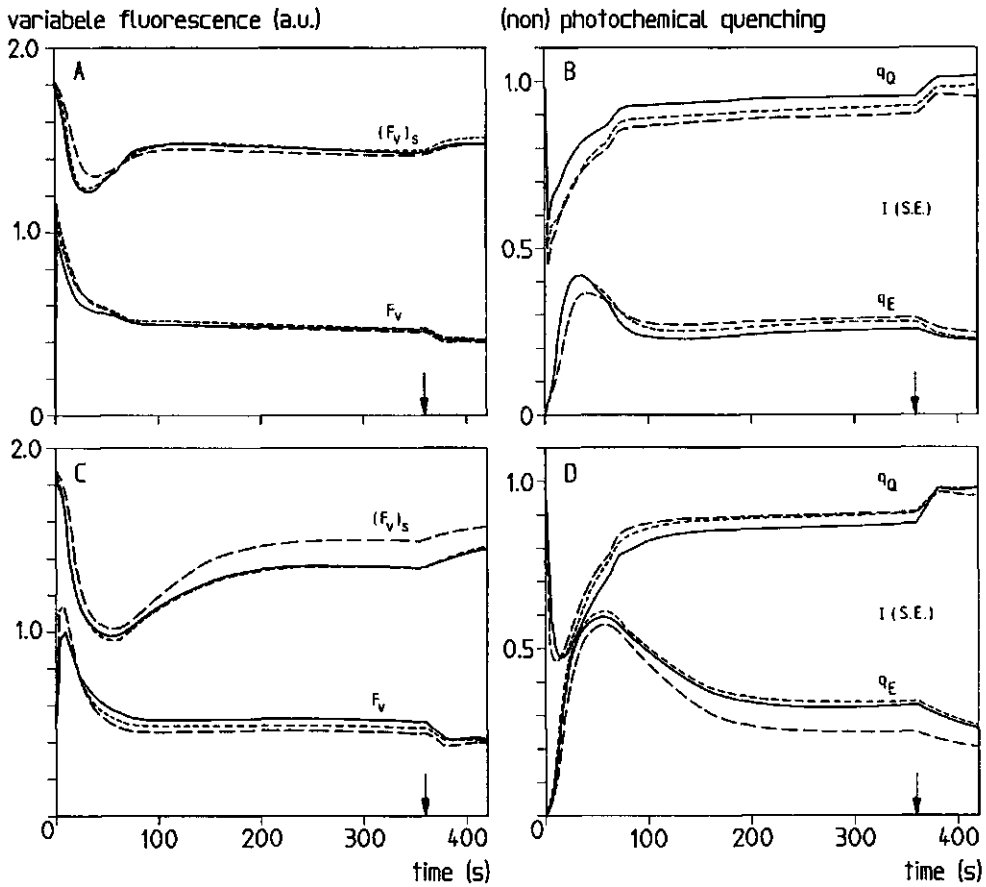

Figure 5.6. The variable (F_v) and saturated (F_{vs}) fluorescence (in arbitrary units) (figures on the left), and the calculated photochemical (q_Q) and non-photochemical (q_E) quenching (figures on the right) of poplar leaves (*Populus euramericana L.*) after transition from dark to light. The leaves had been exposed to NH_3 concentrations of 15 $\mu g.m^{-3}$ (filtered air) (——), 50 $\mu g.m^{-3}$ (- - -), or 100 $\mu g.m^{-3}$ (— — —) in fumigation chambers. A and B: after an exposure period of 14 days (n=7). C and D: after an exposure period of 49 days (n=5). Before the actual measurement took place, the leaves were dark adapted for 30 min. The actinic light source was turned on at t=0 and switched off at 360 s (arrow).

Discussion

The results of the present study indicate that exposure to a moderate NH_3 concentration may have a positive effect on photosynthesis and stomatal conductance of leaves. In addition our results indicate that exposure to this concentration also may have a positive effect on NH_3 uptake into leaves. The NH_3 exposure was found to have no effect on the leaf cuticle. This would imply that not only at a short term exposure, but also at a long term exposure the NH_3 flux into leaves can be calculated from data on the boundary layer and stomatal resistance for H_2O and NH_3 concentration at the leaf surface.

The effect of the NH_3 exposure of 100 $\mu g.m^{-3}$ on photosynthesis is in accordance with literature data showing a positive correlation between leaf-N content and photosynthetic capacity (Evans 1983; Wong, Cowan and Farquhar, 1985; Hirose and Werger, 1987). This suggests that the positive effect on photosynthesis was a result of a substantial contribution of this NH_3 exposure to the total N-content in the leaf.

An impression of the magnitude of this contribution can be obtained by comparing the NH_3 flux into the leaf with the 'N-need' on a leaf area basis which can be estimated from the (net) CO_2 assimilation. For this calculation the light intensity in the fumigation chambers has been taken which was, expressed in PFD, about 200 $\mu mol.m^{-2}.s^{-1}$. According to figure 5.1 the CO_2 assimilation of 6 weeks old leaves at this light intensity was 8 $\mu mol.m^{-2}.s^{-1}$ which corresponds to a C-supply of 96 $\mu g.m^{-2}.s^{-1}$. Results of growth experiments with poplar shoots show that about 60% of the acquired C is used for leaf growth (Pieters, unpublished results).

The nitrogen and carbon contents of leaf dry matter are typically 2 and 40% by weight, respectively. So, a N-flux of 2.9 $\mu g.m^{-2}.s^{-1}$ is required to maintain the C:N ratio in the leaf. The uptake of NH_3 by the leaves at this light intensity and a concentration in the air of 100 $\mu g.m^{-3}$ was 28 $nmol.m^{-2}.s^{-1}$ (figure 5.4). This corresponds to a N-supply of 3.9×10^{-7} $g.m^{-2}.s^{-1}$ or 13.5% of the total 'N-need' of the leaves.

This percentage is close to the percentages assessed by Lockyer and Whitehead (1986) for Italian ryegrass. They found that a NH_3-concentration of 118 $\mu g.m^{-3}$ supplied about 20% of total plant N when the plants were grown at a low dosage of N in the root-medium, and about 10% of total plant N when plants were grown at a higher dosage.

Although only a rough estimation could be made here, it clearly demonstrates that a moderate of NH_3 concentration in the air may substantially contribute to the total N needed by the leaves.

In particular the maximum CO_2 assimilation (P_{max}) was influenced by the NH_3 exposure of 100 $\mu g.m^{-3}$. This could be attributed to a higher activity of the Calvin cycle. In contrast no NH_3 effect on the quantum yield for CO_2 fixation (QY) was observed. The smaller decline in P_{max} between 6 and 8 weeks in leaves from this treatment suggests a delay in the rate of ageing in these leaves as compared to leaves exposed to 50 $\mu g.m^{-3}$ or filtered air. It can be assumed that this was a result of an inhibition of the decline in RUBISCO activity. The close correlation found between leaf-N content, the activity of this enzyme and CO_2 assimilation may provide evidence for this assumption (Huffaker 1982; Evans 1983; Von Caemmerer and Farquhar 1981; Wong et al. 1985; Makino, Mae and Ohira 1988). However, on the other hand, all treatments showed a decline in QY in the same period (table 5.1). The fluorescence measurements indicated that this decline was not a result of a decrease in Φ_{Po}, i.e. the maximum photochemical efficiency of PSII (table 5.3). From this it can be concluded that PSII remained unaffected and that the decline in QY, as measured with the leaf chamber, was related to a decrease in the number of PSII's per m^2 leaf area, i.e. the chlorophyll content per m^2 leaf area must have decreased. However, caution is needed in the interpretation of these results because the determination of QY from the light response curve for CO_2 assimilation is less accurate than the determination of Φ_{Po}.

The (net) CO_2 assimilation rate (P_n) was found to be linearly related with the leaf conductance for CO_2 ($g_{t.c}$) (fig.5.3) which has also been observed for other plant species (Goudriaan and Van Laar 1978; Louwerse 1980; Bell 1982; Farquhar and Sharkey 1982). The predominant effect on this relationship appeared to be that of leaf age. No influence of the NH_3 exposure was observed indicating that NH_3 has no direct effect on stomatal behaviour, e.g. by affecting the guard or contiguous cells of the stoma. This is in accordance with results reported by Wong et al. (1985). For Zea mays L. and Gossypium hirsutum L. they found that the proportionality between $g_{t.c}$ and P_n was not influenced by N or P nutrition. Neither an effect of light during growth or of natural variation among plants was observed. The fact that this relationship is not influenced by NH_3, may be of particular importance for modelling NH_3 deposition on vegetation. The relationship can be used to estimate the NH_3 flux into leaves from CO_2 assimilation data.

The absence of an internal resistance against NH_3 transport into the leaf indicates that NH_3 assimilation in the leaf is very efficient under the environmental conditions of the present study. A possible regulation mechanism of the plant is proposed in figure 5.7. In leaf cells NH_3 is converted into the α-amino group of glutamate via the glutamine/glutamate cycle (GS/GOGAT-cycle) (Mitchell and Stocking 1975; Anderson and Cone 1977; Hirel et al.1982). To keep the GS/GOGAT-cycle going α-ketoglutarate is required which is derived from the tricarboxylic-acid (TCA) cycle or Krebs cycle. It has been shown that addition of NH_3 to a suspension of photosynthesizing isolated mesophyll cells may stimulate the reaction catalyzed by glutamine synthetase (GS). Of particular importance is that also a stimulation of the reactions catalyzed by pyruvate kinase and phospho-enol-pyruvate carboxylase has been observed (Platt, Plaut and Bassham, 1977; Paul, Cornwell and Bassham, 1978). The increased activities of these reactions led to a higher activity of the Krebs-cycle and subsequently a rapid rise in the concentration of α-keto-glutarate in the cells. The increased flux of carbon in this direction appeared to come mainly at the expense of glucose synthesis. So, NH_3 taken up by the leaf may act as an important regulatory agent in cell leafs; the net effect might be that the newly fixed carbon in photosynthesis is redistributed away from carbohydrates and into amino acids. Evidence for this assumption might be the large accumulation of amino acids such as asparagine, arginine and glutamine, observed in leaves exposed to atmospheric NH_3 (Van der Eerden 1982; Van Dijk and Roelofs, 1988; Zedler, Plarre and Rothe 1986).

The results of this study are at variance with those based on field observations. However, it should be noted that the plants in this study were grown under rather 'optimal' conditions. There are indications that NH_3 may affect plants when they endure stress from other environmental factors. For instance, Van der Eerden (1982) observed that conifers are much more sensitive to NH_3 in winter than in the summer. This can be explained by the low metabolic activity and carbohydrate supply under these conditions, as a result of which the ability to assimilate NH_3 is strongly reduced. Also plants exposed to NH_3 showed a higher frost sensitivity (Van der Eerden 1982; De Temmerman et al. 1988). This might be due to a reduced formation of carbohydrates as result of NH_3 uptake, as well as the prolonged activity of the leaves.

Furthermore there are indications that the extra input of N causes relative shortages of other mineral nutrients in leaves, such as potassium, phosphorus and magnesium (De Temmerman et al. 1988; Van Dijk and Roelofs, 1988).
The acquisition of N by shoots may also have implications for the acid-base regulation in plants. Raven (1988) calculated that NH_3 taken up by the leaves may give an excess of some 0.22 mol H^+ per mol N assimilated. Plants can dispose of this excess by root excretion. So, according to these calculations NH_3 acquisition by shoots may have an acidifying effect on the root environment. This effect may be enhanced by a reduced uptake of NO_3^- by the roots, as was observed by Lockyer and Whitehead (1982) for Italian ryegrass.

In areas with a high deposition of NH_3 also a substantial deposition of SO_2 on vegetation has been measured, which may be partly responsible for the observed effects in the field as well (Van Breemen et al. 1982; Heil et al. 1988). Therefore, sequel to the present study the physiological effects of a combination of NH_3 and SO_2 were examined. The results thereof will be reported in a forthcoming paper.

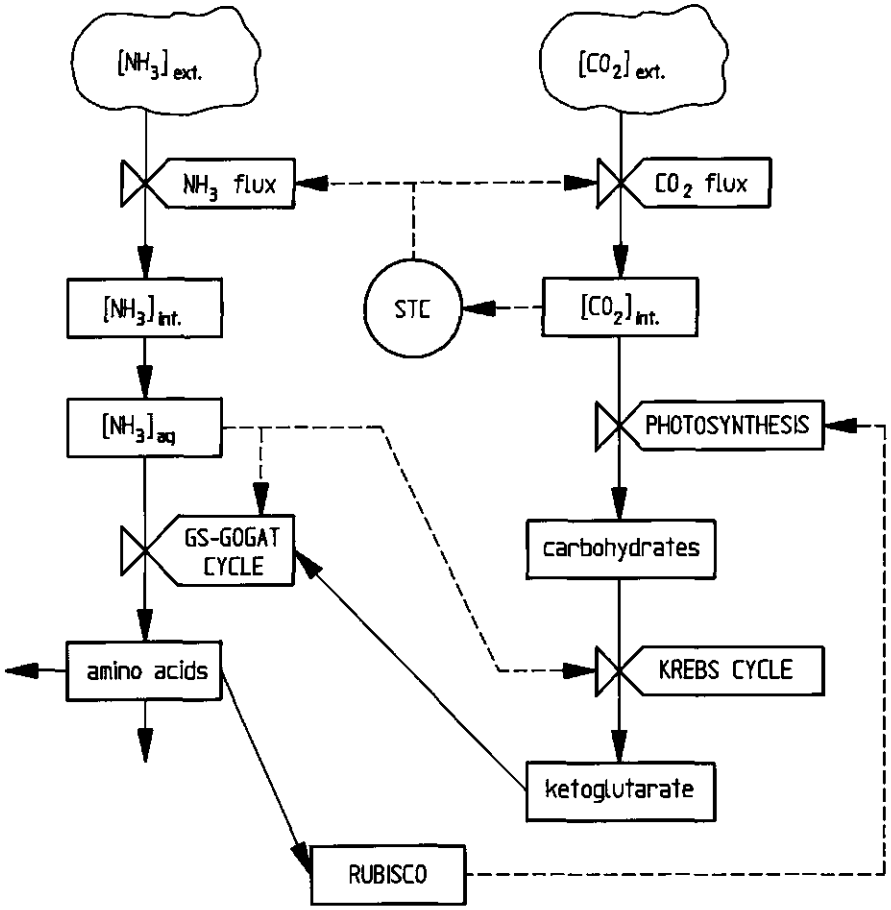

Figure 5.7. Relational diagram for the assimilation of atmospheric NH_3 by the leaves. The diagram was drawn according to Penning de Vries and Van Laar (1982). Rectangles represent state variables, circles intermediate or auxiliary variables, and the valve symbols represent rates of change of the state variables. Flows and direction of 'material' are presented by solid arrows and the dashed arrows show the direct or indirect effects of variables. $[NH_3]$ext. and $[CO_2]$ext.: external concentrations. $[NH_3]$int. and $[CO_2]$int.: concentrations in the substomatal cavity; $[NH_3]$aq.: NH_3 in the liquid phase; STC: stomatal conductance; GS/GOGAT-cycle: glutamine-glutamate cycle; RUBISCO: ribulose-1,5-bisphosphate-carboxylase-oxygenase.

CHAPTER 6

Physiological effects of long term exposure to low concentrations of SO_2 and NH_3 on poplar leaves

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Abstract

Poplar shoots (*Populus euramericana L.*) were exposed to filtered air, SO_2, NH_3 or a mixture of both gases during a period of 7 weeks in fumigation chambers. After this exposure gas exchange measurements were carried out using a leaf chamber. Leaves exposed to 112 $\mu g.m^{-3}$ SO_2 showed a small reduction in maximum CO_2 assimilation rate and stomatal conductance as compared to leaves exposed to filtered air. These leaves showed also a slightly higher quantum yield and dark respiration. In addition, the fluorescence measurements indicated that the Calvin cycle of these leaves was more rapidly activated after transition from dark to light. An exposure to 65 $\mu g.m^{-3}$ NH_3 was found to have a positive effect on CO_2 assimilation, stomatal conductance and NH_3 uptake of the leaves. This positive effect was counteracted by a SO_2 concentration of 45 $\mu g.m^{-3}$. The exposure treatments appeared to have no effect on stomatal behaviour which was confirmed by microscopic examination. Neither an effect on SO_2 or NH_3 transfer into the leaf was observed. Resistance analysis showed that NH_3 transfer into the leaf can be estimated from data on the boundary layer and stomatal resistance for H_2O transfer and ambient NH_3 concentration, irrespective of whether the leaves are exposed during a short or long time to NH_3 or to a mixture of NH_3 and SO_2. In contrast SO_2 uptake into the leaves was only partly correlated to the stomatal resistance. The results suggest a lower resistance for the uptake of this gas into leaves. The possibility of a difference in path length between SO_2 and H_2O molecules is proposed.

Introduction

Despite the extensive literature on the effects of SO_2 on vegetation many uncertainties still exist with respect to the physiological effects caused by this gas (Mudd, 1975; Hälgren 1978, 1984; Heath, 1980; Black, 1982). In particular very few experiments have been performed in which concentrations characteristic of rural areas, away from point sources, have been employed. Moreover, conflicting results have been obtained. For instance, a reversible reduction as well as an irreversible reduction of photosynthesis have been reported, even for SO_2 concentrations in the same range (Black and Unsworth 1979b; Hällgren and Gezelius 1982; Saxe 1983). A similar controversy exists with respect to the effect of low SO_2 concentration on dark respiration and stomatal opening (Black and Unsworth 1979b and 1980; Black and Black 1979a; Saxe 1983).

In the present study the physiological effects of a long term exposure to low SO_2 concentrations on poplar leaves (*Populus euramericana L.*) were examined. A major objective was to assess the relationships between stomatal opening, rates and extents of SO_2 uptake into leaves and photosynthesis, both at a short and long term exposure to this gas.

In areas in the Netherlands where intensive animal husbandry is concentrated, moderate concentrations of NH_3 are measured. It has been shown that a mixture of NH_3 and SO_2 in the atmosphere may seriously affect the vegetation in these areas. The observed effects are mainly attributed to effects of the deposited gases via the soil (Den Boer, 1986; Roelofs 1986; Roelofs et al. 1985; Van Dijk and Roelofs 1988; Van Breemen et al. 1982). However, little is known about the physiological effects of a mixture of both gases on the upper parts of the vegetation. Therefore, also the effects of a long term exposure to a mixture of SO_2 and NH_3 have been examined in the present study.

The experiments were carried out with poplar shoots which were exposed to SO_2 concentrations of 45 and 112 $\mu g.m^{-3}$, a NH_3 concentration of 64 $\mu g.m^{-3}$ and a mixture of 45 $\mu g.m^{-3}$ SO_2 and 69 $\mu g.m^{-3}$ NH_3 during a period of 7 weeks in fumigation chambers. The shoots were grown under favourable conditions in order to exclude the possible interference of other 'stress' factors. After this exposure the uptake of SO_2 and NH_3 into leaves, transpiration, CO_2 assimilation and dark respiration were measured using a leaf chamber. During the long term exposure also modulated chlorophyll fluorescence measurements on individual leaves were carried out to obtain more information on the action of SO_2 and NH_3 on the photosynthesis process at the chloroplast level.

Furthermore, samples of leaf tissue were taken at the end of the exposure period for microscopic examination in order to determine whether the epidermal cells and cells of the stomatal complex were affected by the exposure treatments.

Material and methods

Plant material

Cuttings of poplar (*Populus euramericana L. cv. 'Flevo'*) were planted in small rockwool blocks (3 x 3 x 5 cm, Grodan), mounted in 5 cm long PVC tubes with a diameter of 3 cm. The tubes were inserted into covers of 1.5 l polyethylene containers filled with a 10% aerated Hoagland solution modified after Steiner (1968). After emergence of the first roots the concentration of the nutrient solution was gradually increased to a final concentration of 30% during a two weeks period. The nutrient solution was refreshed every week during the exposure period. The cuttings were transferred to the fumigation chambers when the shoots had a length of about 5 cm.

The fumigation chambers

The fumigation chambers are of tempered glass fastened in a rectilinear aluminum framework (0.85 x 1.00 x 0.90 m). The bottom plate consists of a perforated PVC plate through which air enters the chamber. The air exhaust is a transparent 80 cm long PVC tube with a diameter of 6 cm, situated in one of the corners of the chamber. Before entering the chamber the air is passed through granular charcoal filters and a humidifier. The air exchange of each fumigation chamber is 0.5 min^{-1}. In addition, air within the fumigation chamber is continuously kept recirculating at a rate of 4.0 $m^3.min^{-1}$ in order to reduce space variability of gas concentrations and to improve gas exchange between the fumigation chamber air and plant leaves. The fumigation chambers were in a controlled environment room of the phytotron. The conditions in the in the fumigation chambers were: temperature of 23 °C during the day and 20 °C at night, wind velocity (at bottom plate) 0.3 $m.s^{-1}$, relative humidity 60-80%, a light intensity at 50 cm height 280 $\mu mol.m^{-2}.s^{-1}$ (PAR, Philips fluorescent tubes TLMF 140 W/33 RS) and a photoperiod of 16h. SO_2 was supplied from a calibrated mixture of the gas of 10,000 ppm in nitrogen (v/v). The SO_2 concentrations in the fumigation chambers were mea-sured with an UV pulsed fluorescent analyzer (Mon. Labs 8850). The analyser had been modified to enable measurement of SO_2 concentrations lower than 100 $\mu g.m^{-3}$.

The SO_2 concentrations were 45 (± 15) and 112 (± 44) $\mu g.m^{-3}$ and in combination with NH_3: 46 (± 22) $\mu g.m^{-3}$ (values between brackets are standard deviations).

NH_3 was generated by adding an ammonia solution (25%) with a minipuls pump into a heated round bottomed vessel. The generated NH_3 was taken away by a controlled flow of air passing through the vessel and injected into the air entering the fumigation chamber. The NH_3 concentrations in the fumigation chambers were measured with a modified NO_x analyzer (Monitor Labs 8840), equipped with a catalytic converter to oxidize NH_3 to NO (accuracy ± 2 ppb, converter efficiency > 85%) (Aneja et al.,1978). The NH_3 concentrations were 64 (± 33) and in combination with SO_2: 69 (± 33) $\mu g.m^{-3}$. The deviations in SO_2 and NH_3 concentrations were a result of variations in air temperature and humidity in the fumigation chambers and uptake of these gases by the leaves. Probably as a result of this uptake lower concentrations were usually measured during the light period. Despite the use of charcoal filters the air entering the fumigation chambers always contained low concentrations of NH_3 and NO_x of 15 (±5) and 40 (± 10) $\mu g.m^{-3}$, respectively.

The leaf chamber measurements

After an exposure period of 7 weeks plants were taken from the fumigation chambers. Immediately thereafter SO_2 and NH_3 uptake, transpiration, CO_2-assimilation and respiration of leaves were measured in the leaf chamber. The leaf chamber is part of an open system (Jarvis and Catsky 1971) where uptake of a pollutant gas, transpiration and CO_2 assimilation of a leaf are determined by measuring the difference between inlet and outlet concentration of the leaf chamber. The gas exchange fumigation system and leaf chamber have been described in detail in previous papers (Van Hove et al., 1987, 1988). The mixing part of the system was modified to be able to expose leaves to a mixture of SO_2 and NH_3. Both gases were supplied from calibrated gas mixtures of 1000 ppm (v/v) in nitrogen. Before mixing each gas was diluted first to several ppm's in order to reduce reactions between both gases on internal surfaces of the fumigation system.

The SO_2 concentrations at the inlet of the leaf chamber were 50 and 100 $\mu g.m^{-3}$, the NH_3 concentration was 75 $\mu g.m^{-3}$. These concentrations were also given to leaves which had been exposed to filtered air in the fumigation chamber. The gas exchange measurements were carried out for 9 h in the dark and for 9 h in the light.

The leaf positioned at the 10th internode (counted from below) was measured. For each leaf a light response curve was determined at light intensities ranging from 0 to 485 $\mu mol.m^{-2}.s^{-1}$ (PFD). Temperature was about 20 °C, relative humidity 70% (± 5%), CO_2 concentration 330±10 $\mu l.l^{-1}$ and wind velocity across both leaf surfaces 1 $m.s^{-1}$. The boundary layer resistance at this windspeed for one side of the leaf was about 30 $s.m^{-1}$.

The calculation of the SO_2 or NH_3 flux into the leaf, transpiration and CO_2-assimilation and further analysis of the results have been described in detail elsewhere (Unsworth et al. 1976; Black and Unsworth 1979a; Van Hove et al., 1987 and 1989).

Fluorescence measurements

The fluorescence measurements were started in the second week of exposure and performed once a week during the exposure period. The leaf positioned at the 10th internode of the shoot was measured. The measurements were conducted with a pulse amplitude modulation chlorophyll fluorometer (Schreiber et al. 1986; Schreiber 1986). Chlorophyll fluorescence is excited with a low intensity modulated light (L_1) and subsequently detected with a fluorometer selective for this modulated fluorescence. This permits monotoring of the yield of fluorescence independent of the intensity of background illumination. A specially developed cuvette was used, in which a part of the leaf could be clamped. The cuvette provided access on the upper side to the fiberoptics bundle of the measuring system. A gas mixture containing 350 $\mu l.l^{-1}$ CO_2 and 2% O_2 (v/v) was led through the cuvette. The low O_2 concentration was meant to minimize corrections for O_2-reducing reactions.

The experimental procedure have been described in detail in a previous paper (Van Hove et al. 1989). Before the actual measurement took place, the plant was dark adapted for 30 min in order to determine both the minimal (F_o) and maximal (F_{max}) fluorescence level. After this treatment the enclosed part of the leaf was continuously illuminated with an actinic light source with an intensity of 35 $\mu mol.m^{-2}.s^{-1}$ (L_2). The change in fluorescence level was followed until a steady state situation was reached. During the illumination with L_2 also saturating pulses of white actinic light (L_3) were added, which causes a complete closure of the reaction center of PSII (pulse duration is 700 ms, pulse intensity is 8000 $\mu mol.m^{-2}.s^{-1}$). During the pulse the efficiency of PSII is minimal and fluorescence is maximal (F_{max}).

After this saturating pulse the fluorescence will fall off to the lower fluorescence level induced by L_2 (F_v). As the actinic illumination proceeds the fluorescence level induced by L_3 reaches a level below that of F_{max}. This lower level ($(F_v)_s$) is a result of 'non-photochemical' quenching (E-quenching), which is thought to be related to the "energization" of the thylakoid membrane (Krause et al. 1982 and 1983). The redox level of the PSII acceptor responds very rapidly to changes in the light regime whereas factors causing a change in energization of the thylakoid membrane are much slower. So, by adding short saturating pulses with L_3 during illumination of the leaf with L_2, quenching of the fluorescence signal can be related to a photochemical (Q-quenching) and non-photochemical (E-quenching) component.

By multiplying the efficiency of the excitation energy capture of PSII, i.e. $((F_v)_s-F_o)/(F_v)_s$, with the fraction of "open" reaction centres $q_Q = ((F_v)_s-F_v)/((F_v)_s-F_o)$, the quantum yield of non-cyclic electron transport Φ_{PSII} can be determined (Genty et al. 1989):

$$\Phi_{PSII} = ((F_v)_s - F_v)/(F_v)_s \qquad (6.1)$$

This quantum yield is equal to the number of photochemical events in PSII per photon absorbed by PSII complexes. Φ_{PSII} is proportional to QY when photo-respiratory processes are negligible, as is the case at 2% O_2 (v/v).

Microscopy

At the end of the exposure period leaf samples were taken for microscopic examination. For this epidermal strips of the lower and upper leaf surface were prepared using a quick-hardening silicone based precision impression material (Xanthopren, Bayer Dental) which was spread on the leaf surface and peeled off after hardening. In a similar way a transparant copy of the imprint was made using polystyrene toluene, which could be examined by light microscopy.

Also samples of leaf tissue were taken for examination by scanning electron microscopy. Preparation of the leaf sample occurred according to the so called cryo-technique (Cryo-SEM) at which the sample is quickly frozen at a temperature of -200 °C. Subsequently, the surface was coated with gold before microscopy.

Results

Carbon dioxide assimilation and stomatal conductance

Figure 6.1 shows the light response curve for net CO_2 assimilation (P_n) and stomatal conductance for H_2O (g_s) after the 7 weeks of exposure. The calculated values for maximum photosynthesis (P_{max}), apparent quantum yield (QY) as well as the measured dark respiration (R_d) are presented in table 6.1.

A small reduction in P_{max} was observed for leaves which had been exposed to 112 $\mu g.m^{-3}$ SO_2 in the fumigation chamber. However, on the other hand these leaves showed a higher QY and R_d as compared to leaves exposed to filtered air (table 6.1). The leaves exposed to NH_3 appeared to have a higher P_{max} which corresponded to a higher g_s and mesophyll conductance for CO_2 (g_m) (table 6.2). The positive effect of NH_3 on P_{max} and g_s was counteracted by the low SO_2 concentration.

A short term exposure to similar concentrations of SO_2, NH_3 or a mixture of both gases in the leaf chamber had no effect on P_{max}. Furthermore, leaves exposed to 112 $\mu g.m^{-3}$ SO_2 in the fumigation chamber showed no recovery in P_{max} when they were subsequently exposed to filtered air in the leaf chamber during a period of 72 hours. These results indicate that the effects of a long term SO_2 or NH_3 exposure on P_{max} were related to structural changes, rather than to a short term reversible effect as result of e.g. a competitive inhibition between sulfite and CO_2 for RUBISCO.

The average values of P_n of each treatment were plotted against those for the leaf conductance for CO_2 ($g_{t,c}$) calculated from the boundary layer and stomatal conductance for H_2O. Figure 6.2 shows an almost linear relationship between P_n and $g_{t,c}$. It appeared that the proportionality between P_n and $g_{t,c}$ was not influenced by the exposure the shoots had received. From this it can be derived that the long term exposure to SO_2 and NH_3 had no direct influence on stomatal behaviour, e.g. by affecting the cells of the stomatal complex.

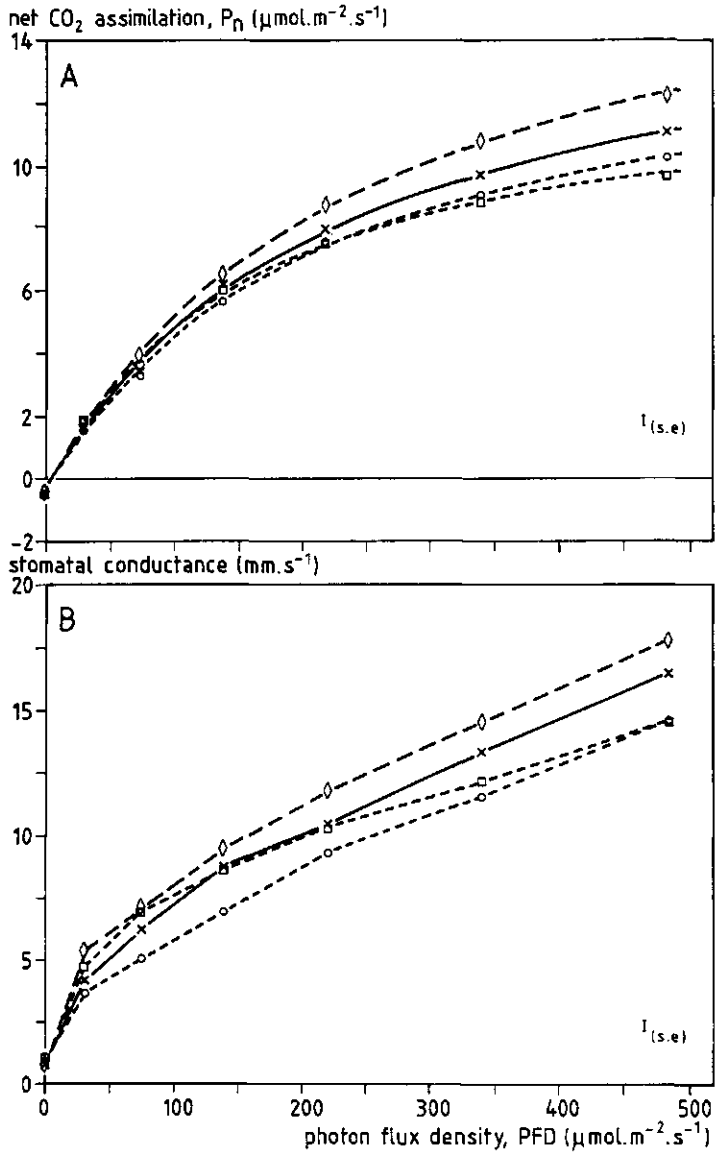

Figure 6.1. Net CO_2 assimilation (in $\mu mol.m^{-2}.s^{-1}$) (A) and stomatal conductance for H_2O (in $mm.s^{-1}$) (B) as function of photon flux density (PFD in $\mu mol.m^{-2}.s^{-1}$) of poplar leaves (*Populus euramericana L.*). (——) filtered air and 46 $\mu g.m^{-3}$ SO_2+69 $\mu g.m^{-3}$ NH_3; (- - - - -) 45 $\mu g.m^{-3}$ SO_2 (o) and 112 $\mu g.m^{-3}$ SO_2 (□); (— — —) 64 $\mu g.m^{-3}$ NH_3. The leaves had been exposed for 49 days in fumigation chambers. The curves are the averaged results of 7-13 measurements on different plants (see also table 6.1). The standard error of the mean (S.E.) is given in the right hand down corner.

Table 6.1. Estimated values (± standard error of the mean) for CO_2 assimilation at light saturation (P_{max}) and quantum yield (QY), and the measured dark respiration (R_d) of poplar leaves (*Populus euramericana L.*), exposed for 49 days to filtered air (FA), 45 $\mu g.m^{-3}$ SO_2 (SL), 112 $\mu g.m^{-3}$ SO_2 (SH), 46 $\mu g.m^{-3}$ SO_2 + 69 $\mu g.m^{-3}$ NH_3 (SL+N) and 64 $\mu g.m^{-3}$ NH_3 (N) in fumigation chambers. Numbers followed by a different letter (between brackets) are significantly different (P=0.05).

	n	P_{max}	QY ($\mu mol.m^{-2}.s^{-1}$)	R_d ($\mu mol.m^{-2}.s^{-1}$)
FA	13	11.3 ± 0.5 (ab)	0.076 ± 0.005 (a)	0.38 ± 0.03 (a)
SL	7	10.4 ± 0.9 (a)	0.072 ± 0.003 (a)	0.32 ± 0.03 (a)
SH	8	9.5 ± 0.3 (a)	0.086 ± 0.004 (b)	0.44 ± 0.02 (b)
SL+N	9	11.3 ± 0.4 (ab)	0.075 ± 0.004 (a)	0.35 ± 0.02 (a)
N	8	13.1 ± 1.2 (b)	0.078 ± 0.007 (a)	0.34 ± 0.02 (a)

Table 6.2. Estimated values (± standard error of the mean) for internal CO_2 concentration (C_i in $g.m^{-3}$) and mesophyll conductance for CO_2 (g_m in $mm.s^{-1}$) of poplar leaves (*Populus euramericana L.*), exposed for 49 days to filtered air (FA), 45 $\mu g.m^{-3}$ SO_2 (SL), 112 $\mu g.m^{-3}$ SO_2 (SH), 46 $\mu g.m^{-3}$ SO_2 + 69 $\mu g.m^{-3}$ NH_3 (SL+N) and 64 $\mu g.m^{-3}$ NH_3 (N) in fumigation chambers.

	n	C_i	g_m
FA	13	0.52 ± 0.01	0.90 ± 0.04
SL	7	0.52 ± 0.01	0.85 ± 0.07
SH	8	0.52 ± 0.01	0.83 ± 0.04
SL+N	9	0.51 ± 0.01	0.98 ± 0.07 (a)
N	8	0.51 ± 0.01	1.04 ± 0.06 (a)

(a): significantly different (P=0.05).

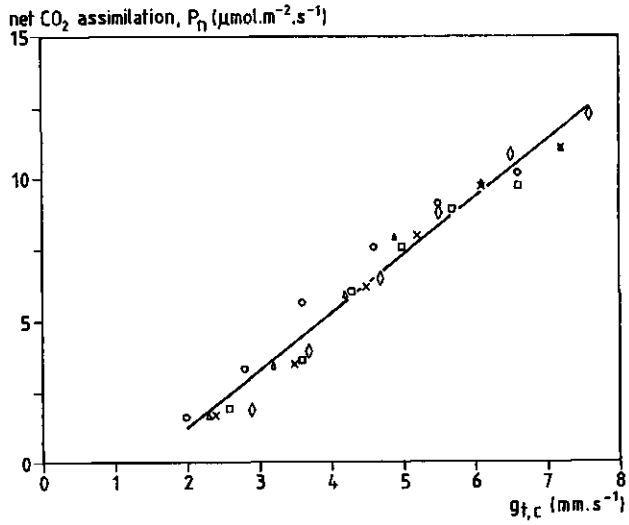

Figure 6.2. The relation between leaf conductance for CO_2 ($g_{t,c}$ in $mm.s^{-1}$) and net CO_2 assimilation (in $\mu mol.m^{-2}.s^{-1}$) of poplar leaves (*Populus euramericana L. cv. 'Flevo'*). The leaves had been exposed for 49 days in fumigation chambers. (x) filtered air; (o) 45 and (□) 112 $\mu g.m^{-3}$ SO_2; (◇)64 $\mu g.m^{-3}$ NH_3; (△) 46 $\mu g.m^{-3}$ SO_2+ 69 $\mu g.m^{-3}$ NH_3.

Transfer of SO_2 and NH_3 into the leaf

Leaves exposed to NH_3 in the fumigation chamber showed also a larger uptake rate of this gas than leaves exposed to filtered air. However, the NH_3 uptake of leaves exposed to a mixture of NH_3 and SO_2 was not different from that of leaves exposed to filtered air. Further, no differences in SO_2 uptake between leaves exposed to SO_2 or a mixture of this gas with NH_3 and those exposed to filtered air were measured. The measured SO_2 flux was found to be proportional to the external concentration.

Transfer of SO_2 or NH_3 into leaves was analyzed by comparing the leaf resistance (r_t) of these gases calculated from the measured fluxes with the stomatal resistance (r_s) for SO_2 or NH_3 transfer calculated from the stomatal resistance for leaf transpiration ($r_{s,v}$). For this the following equation was applied (Black and Unsworth 1979; Van Hove et al. 1987):

$$(r_t - r_b)^{-1} = (r_s + r_i)^{-1} + (r_c + r_{ic})^{-1} \qquad (6.2)$$

r_b in equation (6.2) is the leaf boundary layer resistance. r_s for SO_2 or NH_3 transfer was calculated according to: $r_s = r_{s,v} x (D_v/D_g)$. D_v and D_g are diffusion coefficients of water vapour and gas respectively. r_i is the internal resis-

tance encountered by the gas in the leaf, whereas $(r_c+r_{lc})^{-1}$ represents the pathway for gas transfer into the leaf via the cuticle. In fig. 6.3 the values for $(r_t-r_b)^{-1}$ and r_s^{-1} determined for each leaf were plotted against each other assuming that r_l=0. Figure 6.3a shows a large difference between these both values for SO_2 transfer. It can be derived that the real SO_2 flux was larger than the flux that can be calculated from $r_{s,v}$. In contrast, no clear discrepancy between $[r_t-r_b]^{-1}$ and r_s^{-1} was observed for NH_3 transfer (fig. 6.3b). The slope of the regression line had a value of 1 indicating that the stomatal conductance was the sole factor controlling NH_3 transfer into the leaf. It can also be derived from figure 6.3b that transport via the cuticle was negligible, irrespective of whether the leaves had been exposed to filtered air, NH_3 or a mixture of NH_3+SO_2.

Chlorophyll fluorescence

During the exposure in the fumigation chambers a change in the minimal (F_o) and maximal (F_{max}) fluorescence was observed. Both values slightly declined during the first weeks of exposure, followed by an increase at the end of the exposure period. However, apart from effects related to leaf develop-ment, no significant effects of SO_2 or NH_3 exposure on chlorophyll fluorescence could be detected in most cases. However, leaves which had been exposed to 112 $\mu g.m^{-3}$ SO_2 for 6 weeks in the fumigation chamber behaved different. Figure 6.4a shows that the non photochemical quenching (q_E) of these leaves reached a steady state level more rapidly after the onset of illumination. In figure 6.4b the changes in quantum efficiency of non-cyclic electron transport Φ_{PSII} are shown. The decrease in the first 20 s of illumination is caused by a diminution of electron acceptors and a build up of fotosynthetic membrane energization. The consecutive rise is related to an activation of the enzymic CO_2 fixation processes of the Calvin cycle. The different behaviour of leaves exposed to 112 $\mu g.m^{-3}$ SO_2 was only evident in the transition from dark to light. After 150 s at an irradiance of 35 $\mu mol.m^{-2}.s^{-1}$ the difference between the treatments was negligible.

Microscopy

Leaves exposed to SO_2, NH_3 or a mixture of NH_3+SO_2 showed no injury of the leaf cuticle. Neither an effect on the guard and subsidiary cells of the stomata was observed, nor on the epidermal cells adjacent to the stomatal pores.

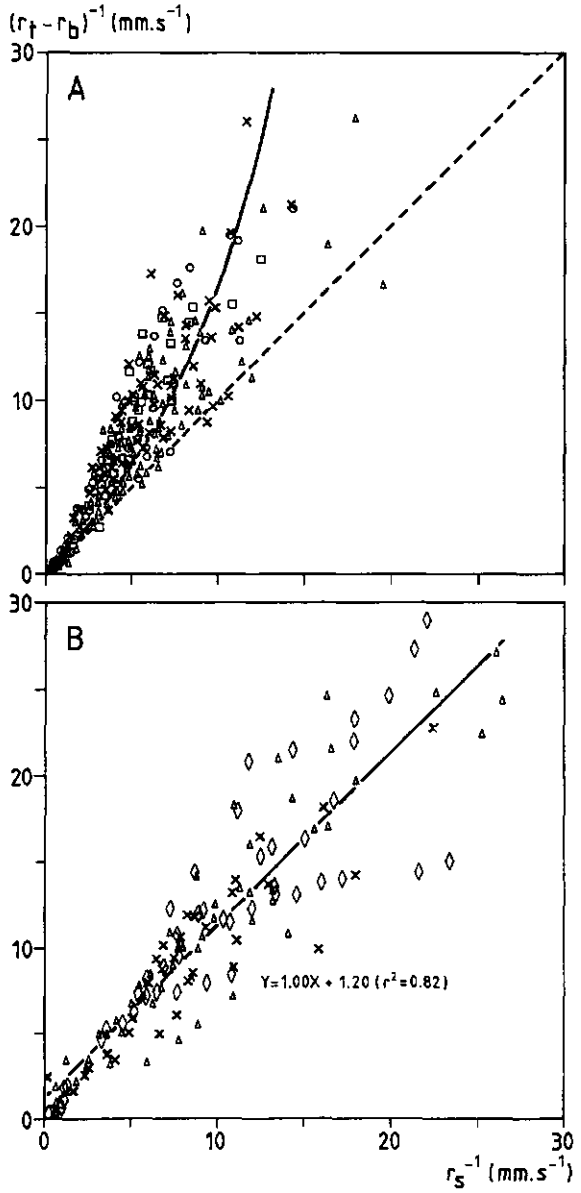

Figure 6.3. Reciprocal values of the leaf resistance for SO_2 (A) and NH_3 transfer (B) into the leaf minus the boundary layer resistance ($[r_t - r_b]^{-1}$) plotted against values for the stomatal conductance (r_s^{-1}) for these gases (for an explanation see text). (x) filtered air; 45 (o) and 112 (□) $\mu g.m^{-3}$ SO_2 respectively; (Δ) 46 $\mu g.m^{-3}$ SO_2+69 $\mu g.m^{-3}$ NH_3; (◇) 64 $\mu g.m^{-3}$ NH_3. The leaves had been exposed for 49 days in fumigation chambers.

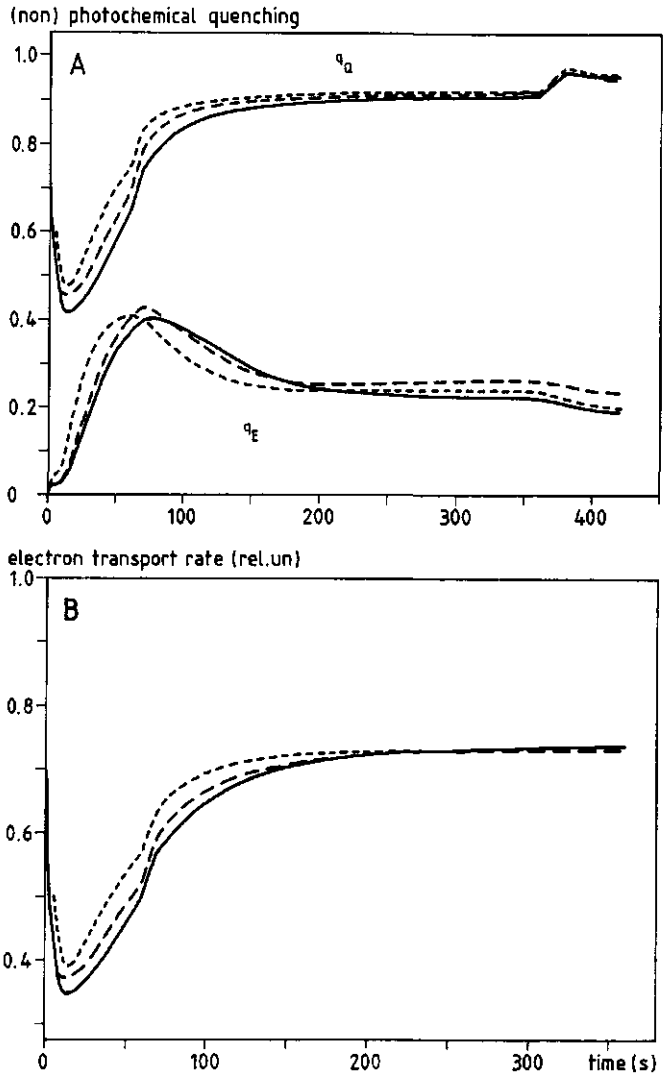

Figure 6.4. A calculated photochemical (q_Q) and non-photochemical (q_E) quenching of poplar leaves (*P. euramericana L.*) after transition at t=0 from dark to light (35μmol.m^{-2}.s^{-1}, 650nm). At t=360 s the light is turned off. (____) filtered air (- - - -) 112 μg.m^{-3} SO_2; (— — —) 64 μg.m^{-3} NH_3. The leaves had been exposed for 42 days in the fumigation chambers. The curves are the averaged results of 7 measurements on different plants.
B. calculated quantum yield Φ_{PSII} of averaged fluorescence response curves (see materials and methods).

Discussion

The positive effect of the long term NH_3 exposure on CO_2 assimilation, stomatal conductance and NH_3 uptake by the leaves is in accordance with previous experimental results (Van Hove et al. 1989). However, the results on chlorophyll fluorescence were somewhat different from reported before. In contrast to earlier results the higher P_{max} of NH_3 exposed leaves as compared to leaves exposed to filtered air is not correlated with a lower non-photochemical quenching (q_E) in the steady state situation (fig 3a). However, the lower q_E measured previously was correlated with a larger difference in P_{max} than that of the present study.

It appeared that the NH_3 concentration in the atmosphere counteracts the negative effect of SO_2 on the CO_2 assimilation of leaves. This can be explained by the opposite effect of these gases on processes involved in photosynthesis. However, also a reaction between NH_4^+ and S(IV) species ($[SO_2]_{aq}+HSO_3^-+SO_3^{2-}$) in leaf cells might have caused this neutralizing effect. It has been shown that NH_3 promotes the oxidation of S(IV) to sulphate (Adema et al. 1986). Sulphate may be transported into the vacuole, reduced to sulfide (Hällgren, J.E. and Fredriksson, S. 1982; Rennenberg and Filner 1982), or incorporated into organic compounds.

Our results indicate that NH_3 transfer into leaves is mainly controlled by the stomatal resistance. This implies that the NH_3 flux into the leaf can be estimated from data on the boundary layer and stomatal resistance for H_2O transfer and NH_3-concentration at the leaf surface, irrespective of whether the leaves are exposed during a short or long time to NH_3 or to a mixture of NH_3 and SO_2.

In contrast, the SO_2 uptake by the leaf was only partly correlated with the stomatal conductance. The results suggest a large 'additional' uptake of SO_2 by the leaves. Similar results have also been reported by others for different SO_2 concentrations and plant species (Taylor and Tingey 1983; Johansson 1983; Olszyk and Tingey 1985). An explanation for this additional uptake might be adsorption on the leaf surface. However, the uptake of SO_2 was determined in this study after a steady state situation in the leaf chamber was reached. A previous study showed a negligible SO_2 adsorption in this situation. The same was found for SO_2 transport through the cuticle (Van Hove et al. 1989). Therefore, these pathways can presumably be excluded. Consequently, the observed effect must be related to the stomatal pathway. A plausible explanation may be the assumption made by Taylor and Tingey (1983).

They postulated that SO_2 molecules into the leaf have a shorter pathway than effluxing H_2O molecules due to a reaction of SO_2 molecules with the stomatal tube or with cells in the substomatal cavity immediately adjacent to the stomatal pore. Thus, as compared to SO_2 molecules the effluxing H_2O molecules encounter an 'extra' resistance. In the analysis of figure 6.3a no correction for this resistance was made. It can be derived from this figure that the stomatal resistance for SO_2 (r_s) has to be lowered with a value of 40 to get a linear relationship between $(r_s)^{-1}$ and $(r_t-r_b)^{-1}$, with slope unity (see also equation 6.2). Taking into account the differences in diffusion coefficients between SO_2 and H_2O molecules, this would imply that the 'extra' resistance encountered by the H_2O molecules is about 20 $s.m^{-1}$.

The microscopic observations of Black and Black (1979a,b) seem to support this assumption. For *Vicia faba L.* they found that SO_2 affects the epidermal cells immediately adjacent to the stomata, which may result into a passive stomatal opening.

Black and Black (1979a,b) observed also injury of epidermal cells for SO_2 concentrations comparable to those applied in our study. However, these effects were not observed in the present study. In addition, no significant influence of these gases on the linear relationship between the CO_2 assimilation rate and stomatal conductance could be detected suggesting that there were also no invisible effects on the stomata, e.g. an effect of these gases on the turgor balance of the cells of the stomatal complex. Clearly, no satisfactory explanation can be given for this discrepancy between our results and those of Black and Black (1979b). Genetic factors and differences in environmental variables during the exposure period might be responsible for the difference in sensitivity of poplar and *Vicia faba* leaves for SO_2.

Numerous reports have established the activity of SO_2 as an inhibitor of net photosynthesis (Mudd, 1975; Hälgren, 1978, 1984; Heath, 1980; Black, 1982). However, controversy exists about the question whether this inhibition is reversible or irreversible (Black and Unsworth 1979; Gezelius and Hällgren 1980; Hällgren and Gezelius 1982; Carlson 1983; Parry and Gutteridge 1984; Kropff 1987). Part of this controversy may be explained by the difference in the applied SO_2 and SO_3^{2-} concentrations. The reversible inhibition has often been observed in experiments where relatively high SO_2 and SO_3^{2-} concentrations have been applied.

In *vitro* studies have shown that in this case the inhibition of photosynthetis may be due to competition between CO_2 and SO_3^{2-} and HSO_3^- for active binding sites on RUBISCO (Ziegler 1975; Khan and Malhotra 1982).

In our study no indication for a competitive inhibition was found. Similar results have been reported by Saxe (1983) for *Phaseolus vulgaris L.*. This would imply that the reduction in P_{max} caused by a prolonged exposure to a low SO_2 concentration is of a more structural nature. Several mechanisms can be proposed to account for this result such as; an SO_2 induced increase in H_2O_2 (Alscher 1984), an effect on translocation of assimilates (Gorissen and Van Veen 1988), a gradual acidification of the chloroplast stroma (Pfanz and Heber 1986; Pfanz et al. 1987a,b), premature senescence (Saxe 1983) or a direct effect on photosynthesis electron transport and photophosphorylation (Shimazaki and Sugahara 1980).

The fluorescence measurements showed no effect on the minimal fluorescence (F_o), nor an effect on the maximum photochemical efficiency (F_v/F_{max}). This precludes the possibility of a reduced activity of PSII, as proposed by Shimazaki and Sugahara (1980). Remarkable was that leaves exposed to 112 $\mu g.m^{-3}$ SO_2 had a reduced P_{max}, but a higher quantum yield. This implies that these leaves had a higher efficiency of photosynthesis in normal air when the light intensity is the limiting factor. In addition, the fluorescence measurements indicated that the Calvin cycle of these leaves was more rapidly activated after transition from dark to light (fig. 6.5). This suggests that a moderate SO_2 concentration may have a direct or an indirect influence on electron transport rate through the thylakoid membrane, e.g. by an effect on reactions involved in the light-activition, as has been proposed by Alscher (1984). However, this assumption is still very speculative and more research is required to elucidate the underlying mechanisms of the effects of low SO_2 concentrations on leaves.

SO_2 concentrations lower than 25 $\mu g.m^{-3}$ are usually measured in the field (Vermetten, 1988). The results of the present study indicate that under rather optimal environmental conditions a long term exposure to these concentrations may have no or only a minor effect on photosynthesis of leaves. The same is probably true for a mixture of this gas with a moderate concentration of NH_3 in the air.

Only SO_2 concentrations exceeding 100 $\mu g.m^{-3}$ may induce a significant reduction (>10%) of CO_2 assimilation under these conditions.

Also for other plant species the first notable effects on photosynthesis have been observed at about the same SO_2 concentration in the air (Black and Unsworth 1979; Saxe 1983). This suggest that the capacity of the leaf to cope with the SO_2 flux, e.g by reduction of the produced sulfite and subsequent metabolic conversion into S-containing amino acids or by compartimentalization, becomes limiting at this SO_2 concentration. The fact that leaves exposed to 112 $\mu g.m^{-3}$ SO_2 showed an increased dark respiration, provides evidence for this assumption. From the results of Pierre and Queiroz (1981) it can be derived that this may be related to an SO_2 induced acceleration of the enzymatic and metabolic operation of the cell. Another indication might be the results of Brunold et al. (1983) showing an increase in sulfate content in leaf cells at this SO_2 concentration in the air.

An impression of the load that a SO_2 concentration of 112 $\mu g.m^{-3}$ may give on leaf metabolism, can be obtained by comparing the acquired S by the leaves at this concentration with the total 'S-need' on a leaf area basis. The total 'S-need' of a leaf can be estimated from the measured net CO_2 assimilation, assuming that a constant percentage of the acquired C is used for leaf growth and that a constant C:S ratio in the leaf is maintained. The comparison is made for the light intensity in the fumigation chambers which was, expressed in PFD, about 200 $\mu mol.m^{-2}.s^{-1}$. Figure 6.1 shows that the (net) CO_2 assimilation at this light intensity was about 8 $\mu mol.m^{-2}.s^{-1}$, which corresponds to a C-supply of 96 $\mu g.m^{-2}.s^{-1}$. Results of growth experiments with poplar shoots revealed that about 60% of the acquired C is used for leaf growth (Pieters, unpublished results). The C- and S-content of a leaf are usually about 40% and 0.2% of leaf dry matter respectively (Anderson 1978). This would imply that a S-flux of 0.46 $\mu g.m^{-2}.s^{-1}$ is required to maintain the C:S ratio in the leaf. From figure 6.1b and 6.3a it can be derived that the stomatal resistance for SO_2 transfer at this light intensity will be about 125 $s.m^{-1}$. The boundary layer resistance for SO_2 transfer towards the leaf surface was found to be 30 $s.m^{-1}$ at a wind velocity of 1 $m.s^{-1}$. Using these resistances the SO_2 flux can be calculated according to: $F= C_a x(r_b+r_s)^{-1}$ (C_a=SO_2 concentration). At a SO_2 concentration of 112 $\mu g.m^{-3}$ the calculated flux is 0.7 $\mu g.m^{-2}.s^{-1}$ or 11.3 $nmol.m^{-2}.s^{-1}$. This corresponds to a S-supply of 0.36 $\mu g.m^{-2}.s^{-1}$ or 80% of the total 'S-need' of the leaves. Although only a rough estimation could be made here, this calculation clearly demonstrates that a low SO_2 concentration in the air may contribute considerably to the relatively small S-need of the leaf.

This would imply that the acquired S by the leaves may be beneficial for the plant when S-supply from the roots is insufficient. This has been observed indeed by Faller (1972). However, such a situation is unlikely to occur for plants in the field and so it can be concluded, that at a relatively low SO_2 concentration in the air the leaf may reach its maximum capacity to incorporate this gas. It can be postu-lated that as a result the leaf is more sensitive to other stress factors. For instance, it can be calculated for the leaves of the present study, which were 200 μm thick, that a SO_2 concentration of 112 $\mu g.m^{-3}$ would give an [SIV] concentration of 3.3 mM after just one day (i.e. 16 h) of exposure assuming that no detoxification occurs. This concentration is high enough to affect cellular processes in the plant (Heath 1980; Sasaki and Kondo 1985; Malhotra and Khan 1984). Similarly, at the concentrations used here the contribution of the NH_3 influx to the total N-need is less than 10%. From this it can be concluded that the capacity of leaves to detoxify NH_3 is far larger as compared to the capacity to detoxify SO_2. As a consequence leaves should be less sensitive to NH_3 than to SO_2. However, calculations show that in case of an inhibition of the detoxification process, e.g. as result of a low temperature, the NH_3/NH_4^+ concentration in 200 μm thick leaf may reach a toxic level of 6 mM after one day of exposure. So, both exposure to a low SO_2 concentration and a moderate NH_3 concentration in the air may enhance the sensitivity of plants to other environmental 'stress' factors, which is confirmed by field observations (Van der Eerden 1982; De Temmerman et al. 1988; Mansfield et al. 1988) and preliminary studies conducted at our department. Therefore, the interaction of pollutant gases with other 'stress' factors will require more attention.

CHAPTER 7

The effect of wind velocity, air temperature and humidity on NH_3 and SO_2 transfer into bean leaves (*Phaseolus vulgaris L.*)

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(submitted for publication)

Abstract

The influence of wind velocity, air temperature and vapour pressure deficit of the air (VPD) on NH_3 and SO_2 transfer into bean leaves (*Phaseolus vulgaris L.*) was examined using a leaf chamber. The measurements suggested a transition in the properties of the leaf boundary layer at a wind velocity of 0.3-0.4 $m.s^{-1}$ which corresponds to a Re_{crit} value of about 2000. At higher windvelocities the leaf boundary layer resistance (r_b) was 1.5 to 2 times lower than can be calculated from the theory. Nevertheless, the assessed relationships between r_b and wind velocity appeared to be similar to the theoretical derived relationship for r_b. The NH_3 flux and in particular the SO_2 flux into the leaf strongly increased at a VPD decline. The increase of the NH_3 flux could be attributed to an increase of the stomatal conductance (g_s). However, the increase of the SO_2 flux could only partly be explained by an increase of g_s. An apparent additional uptake was also observed for the NH_3 uptake at a low temperature and VPD. The SO_2 flux was also influenced by air temperature which could be explained by a temperature effect on g_s. The results suggest that calculation of the NH_3 and SO_2 flux using data of g_s gives a serious underestimation of the real flux of these gases into leaves at a low temperature and VPD.

Introduction

It has been shown that environmental variables such as light intensity, temperature and relative humidity may have an important influence on responses of plants to air pollutants (Taylor and Selvidge 1985; Black and Unsworth 1980; Barton et al. 1980; McLaughlin and Taylor 1981; Norby and Kozlowski 1982; Jensen and Roberts 1986). The environmental conditions may indirectly modify the influence of a pollutant gas on physiological processes in the plant such as on photosynthesis, either by altering the flux of a pollutant gas into the plant via changes in stomatal conductance, or by affecting the detoxification rate. Alternatively, environmental variables themselves may directly affect the pollutant sensitivity of the physiological processes in the plant.

Little is known about the mechanisms with which these environmental variables influence plant responses to air pollutants. A better understanding can be obtained, if more is known about the influence of environmental variables on the uptake of pollutant gases into leaves. Once the different relations between environmental variables and the uptake of pollutant gases into leaves have been assessed and integrated into a mechanistic model, a better understanding of the influence of environmental variables on physiological and biochemical responses to air pollutants can be obtained.

In the present study the influence of wind velocity, air temperature and humidity on the transfer of NH_3 and SO_2 into bean leaves (*Phaseolus vulgaris L.*) was studied.

Transfer of gases into leaves is mainly dependent on the resistance of the boundary layer (r_b) between the leaf surface and the atmosphere and the resistance of the stomata (r_s) of the leaf. The magnitude of r_b strongly depends on the wind velocity across the leaf surface, while also leaf morphology and leaf surface structure are of influence. The functions that are used to calculate r_b from the wind velocity, are based on empirical relationships derived from studies with leaf replicas (Monteith 1973; Goudriaan 1977; Grace 1981). However, leaf roughness and microtopography cannot be represented in model leaves. In a real leaf much of the resistance to transfer may reside in the sublayer trapped between hairs, grooves or papillae. Therefore, it can be questioned whether these functions can also be used for leaves. In the present study the validity of these functions was examined. The relation between wind velocity and r_b was derived from measurements of leaf transpiration, NH_3 and SO_2 uptake into the leaves at different wind velocities.

Most canopy deposition models make use of data for r_s obtained from leaf transpiration measurements to estimate the flux of a pollutant gas into leaves (Meyers 1987; Hicks et al. 1987). In addition, the flux of a pollutant gas may be derived from the net CO_2 assimilation of leaves. For many plant species a linear relation between the stomatal conductance ($=r_s^{-1}$) and CO_2 assimilation rate has been found (Goudriaan and Van Laar 1978; Louwerse 1986; Bell 1982; Farquhar and Sharkey 1982). It is postulated that the leaf regulates its stomata to such an extent that a constant ratio between the internal and external CO_2 concentration is maintained (about 0.7 for C_3 plants). This C_i/C_a-ratio is used in the crop ecological models to calculate stomatal conductance from net CO_2 assimilation using the resistance model for CO_2 diffusion through stomata. Therefore, these models can, in principle, also be applied to calculate the flux of a pollutant gas into leaves. In this connection the influence of air humidity and temperature on the relationships between photosynthesis, stomatal conductance and transfer of NH_3 and SO_2 into leaves was examined in the present study.

Material and methods

Plant material

The experiments were performed with three weeks old bean plants (*Phaseolus vulgaris L.* cv. 'witte zonder draad'). The plants were grown in 12 cm pots containing a mixture of sterilized peat and sand (3:1), to which lime was added. The following conditions were maintained: day and night temperature 20 °C, relative humidity 60-70% and a 14 h photoperiod at a light intensity of 70 $W.m^{-2}$ (PAR, Philips fluorescent tubes TMLF 140 W/33 RS).

Gas exchange measurements

The gas exchange measurements were conducted with a leaf chamber in which the top leaf of the first trifoliate leaf attached to the plant was enclosed. The leaf chamber is part of an open system (Jarvis and Catsky 1971), where uptake of a pollutant gas, transpiration and CO_2 assimilation of a leaf is determined by measuring the difference between inlet and outlet concentration of the leaf chamber. The gas exchange fumigation system and leaf chamber have been described in detail elsewhere (Van Hove et al., 1987, 1988). The wind velocity across both leaf surfaces is homogeneous in the leaf chamber and can be varied up to a maximum of about 3 $m.s^{-1}$.

Different air humidities were obtained by passing the airstream through waterbaths at a constant temperature before entering the leaf chamber. NH_3 and SO_2 concentrations at the inlet of the leaf chamber were 100 $\mu g.m^{-3}$. The leaves were exposed to these concentrations for a maximum period of three days. The NH_3 concentrations were measured with a NO_x analyzer (Mon Labs 8840), equipped with a catalytic converter to oxidize NH_3 to NO (accuracy ± 2 ppb, converter efficiency > 85%) (Aneja et al.,1978). The SO_2 measurements were conducted with an UV pulsed fluorescent analyzer (Mon Labs 8850). The analyser has been modified to be able to measure SO_2 concentrations lower than 100 $\mu g.m^{-3}$. The CO_2 and H_2O concentrations were measured with infrared analysers (ADC 225 Mk 3). The analysers were calibrated for the different air humidities and temperatures applied in the experiments.

Experimental procedure and data analysis

Wind velocity

The following relation between the leaf boundary layer resistance for H_2O transfer ($r_{b,v}$ in $s.m^{-1}$) and wind velocity (u in $m.s^{-1}$) can be derived (Monteith 1973; Goudriaan 1977; Grace 1981):

$$r_{b,v} = \alpha(w/u)^{0.5} \qquad (7.1)$$

, with α = 168 and where w is the length of the leaf parallel to the wind direction. The constant α in equation (7.1) was obtained for the roughness factor of a leaf surface of 1.1. α is inversely proportional to this roughness factor. Equation (7.1) is valid for hypostomatous leaves such as the bean leaves used in this study. In case of an amphistomatous leaf a multiplication factor (<1.0) has to be introduced, which accounts for the boundary layer resistances of the upper and lower side being connected in parallel. The leaf boundary layer resistances for a gas ($r_{b,g}$) is related to that for H_2O transfer according to;

$$r_{b,g} = (D_v/D_g)^{0.66} x r_{b,v} \qquad (7.2)$$

The coefficient 0.66 accounts for the fact that mass transfer through the boundary layer partly takes place by turbulence (Monteith 1973; Goudriaan 1977; Grace 1981). D_v and D_g are diffusion coefficients of water vapour and gas, respectively.

It was examined in the present study whether the equations (7.1) and (7.2) can be used to approximate r_b for leaf transpiration or NH_3 and SO_2 transport towards the leaf surface. However, r_b is in series with the resistance of the stomata (r_s), which is parallel with a cuticular resistance, and an internal resistance (r_i). Consequently, the influence of wind velocity on the total resistance (r_t) for H_2O, NH_3 or SO_2 transfer has to be considered. It is assumed that H_2O transport out of the leaf is not hindered by an internal resistance (r_i=0) and that transport through the cuticle is negligible. There are strong indications that the same assumptions can be made for NH_3 or SO_2 transport into the leaf. So, apart from r_b this transport mainly depends on r_s. A relation between wind velocity and r_b can only be assessed if r_s is kept constant. Therefore, the measurements were carried out at a saturating light intensity (485 μmol.m^{-2}.s^{-1} PFD), a constant air temperature (21 °C) and humidity (60-70% R.H.), and a constant CO_2 concentration (330±10 μl.l^{-1}).

The total resistance for H_2O transfer was determined by measuring the transpiration rate of the leaves. The resistance was directly calculated from the water vapour deficit between substomatal cavity and the leaf chamber air (Jarvis, 1971). The saturated water vapour concentration in the substomatal cavity was calculated from the leaf temperature which was measured with three thermocouples (copper-constantan) pressed against the abaxial side of the leaf. The thermocouple wires (1 mm thick) were situated parallel to the wind direction in order to reduce disturbances in the wind profile at the leaf surface.

On the assumption that the NH_3 or SO_2 concentration at the point of removal in the leaf is zero, the total resistance for transfer of these gases into the leaf can be determined according to (Van Hove et al. 1987):

$$r_t = (C_a - 0)/F \qquad (7.3)$$

, where C_a is the gas concentration in the leaf chamber. Just like the transpiration rate the uptake (F) of these gases by a leaf is calculated from the difference in concentration between the inlet and outlet of the leaf chamber (ΔC in g.m^{-3}), air flow through the leaf chamber (f in m^3.s^{-1}) and projected leaf area (A in m^2):

$$F = (f \times \Delta C)/A \qquad (7.4)$$

Values for r_t were plotted against reciprocal values of the wind velocity (u^{-1}) which gives a linear relationship. By extrapolating to $u^{-1}=0$ a value representing r_s and the other resistances in series with r_b could be determined from the intercept at the Y-axis. This value was substracted from r_t to obtain r_b. A fitting program (Eureka, Borland) was applied to get an equation for r_b as a function of the wind velocity.

Air humidity and temperature

In a first series of experiments NH_3 or SO_2 uptake, stomatal conductance for H_2O and CO_2 assimilation rate were measured for each leaf at a series of increasing or decreasing vapour pressure deficits (VPD) of the air. The measurements were done at an air temperature in the leaf chamber of 20, 25 and 15 °C respectively. The other conditions were: a maximum light intensity of 485 $\mu mol.m^{-2}.s^{-1}$ PFD, a wind velocity of 1 $m.s^{-1}$ and a CO_2 concentration of 330 ±10 $\mu l.l^{-1}$.

The measurements were partly repeated for the empty leaf chamber in order to correct for possible reactions of the gases at internal surfaces of the leaf chamber. In these measurements the humidity was adjusted to that during the experiments with the leaves.

In the second series of experiments the effect of air humidity and temperature on the relation between assimilation rate and stomatal conductance was examined. In addition, transfer of NH_3 and SO_2 into the leaf was further analysed. For each leaf a light response curve was assessed either at different air temperatures and similar VPD-ranges or at different VPDs and a constant air temperature (20 °C). The light intensity was varied in a range from 0 to 485 $\mu mol.m^{-2}.s^{-1}$ (PFD).

Net CO_2 assimilation rate (P_n) was calculated in a similar way as the NH_3 or SO_2 flux into the leaf (equation 4). Only a minor correction (<0.8%) for the influence of water vapour efflux on CO_2 transfer had to be made (Von Caemmerer and Farquhar 1981). The relation between P_n and stomatal conductance was assessed by plotting values for P_n against those for the leaf conductance for CO_2 ($g_{t,c}$). This gives a linear relationship described by:

$$P_n = g_{t,c} x (C_a - C_i) \qquad (7.5)$$

$g_{t,c}$ was calculated from the stomatal and boundary layer resistance for H_2O taking into account the differences in diffusion coefficients between CO_2 and

H_2O. From the slope of the line ($=(C_a-C_i)$) and the CO_2 concentration in the leaf chamber air (C_a) the internal CO_2 concentration (C_i) was calculated (Goudriaan and Van Laar 1980).

Transfer of NH_3 or SO_2 into the leaf was analysed by comparing $(r_t-r_b)^{-1}$ calculated from the determined NH_3 or SO_2 flux into the leaf with r_s for these gases calculated from the transpiration rate (Van Hove et al. 1987):

$$(r_t-r_b)^{-1} = (r_s+r_i)^{-1} + (r_c+r_{ic})^{-1} \qquad (7.6)$$

r_s for NH_3 or SO_2 is calculated from r_s of H_2O ($r_{s,v}$) according to:
$r_s = r_{s,v} \times (D_v/D_g)$. D_v and D_g are diffusion coefficients of water vapour and gas, respectively. r_i is the internal resistance encountered by the gas in the leaf, whereas $(r_c+r_{ic})^{-1}$ represents the pathway for gas transfer into the leaf via the cuticle. If values of $(r_t-r_b)^{-1}$ determined at different light intensities are plotted against those for $(r_s+r_i)^{-1}$, a straight line with slope unity and intercept $(r_c+r_{ic})^{-1}$ should be obtained, according to equation (7.6).

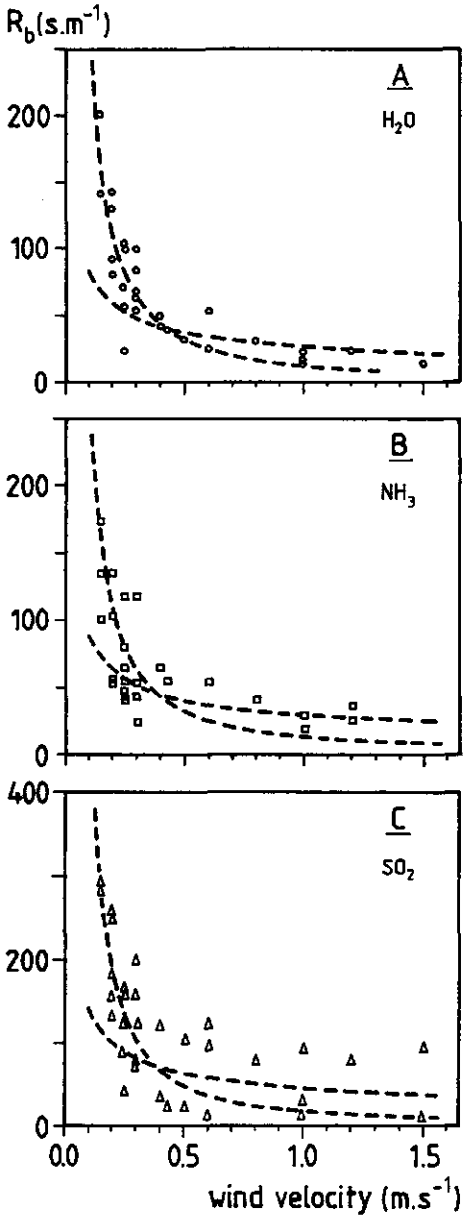

Figure 7.1. Boundary layer resistance (r_b in $s.m^{-1}$) for transpiration (A), NH_3 transfer (B) and SO_2-transfer (C) into the leaf as a function of wind velocity (in $m.s^{-1}$). The experiments were carried out with bean leaves (Phaseolus vulgaris L.) with a length parallel to the wind direction of 8 cm. Dashed lines: fitted curves (see text).

Results

Wind velocity

Figure 7.1 shows the leaf boundary layer resistances (r_b) for transpiration and for NH_3 and SO_2 transfer into the leaf as a function of wind velocity. The resistance strongly declined when wind velocity in the leaf chamber increased from 0.1 to 0.4 $m.s^{-1}$. The fast decline in this range of wind velocities could be fitted with an empirical equation in which r_b changes roughly with $u^{-1.5}$ and not with $u^{-0.5}$ (see equation 7.1). After this fast decline only a small decline of r_b was observed at a further increase of the wind velocity (>0.4 $m.s^{-1}$). This decline can nicely be fitted with equation (7.1), except for the value of α which was found to be much lower (α = 94). Thus, the plotted values in figure 7.1 could only be fitted by two curves: one curve for the fast decline of r_b and the other one for the relatively small decline at wind velocities >0.4 $m.s^{-1}$. Almost identical equations for H_2O, NH_3 or SO_2 were obtained taking into account the corrections for the differences in diffusion coefficients (see equation 7.2).

Microscopic examination showed that the smaller value for α was not due to the presence of stomata on both sides of the leaves. Consequently, the smaller value must be a result of a larger surface roughness of our plant material.

Air humidity and temperature

Figure 7.2 shows the influence of vapour pressure deficit of the air (VPD) and air temperature on the conductance for NH_3 uptake into the leaf calculated from the measured flux ($[r_t-r_b]^{-1}$), and on net CO_2 assimilation (P_{max}) at light saturation. An increase of $[r_t-r_b]^{-1}$ was observed when VPD declined at 20 or 25 °C. Similar values and patterns were found for the stomatal conductance for NH_3 ($g_{s,n}$) calculated from the transpiration rate under the same conditions. This indicates that at these temperatures the increase of NH_3 uptake by the leaves at a lower VPD was a result of the increase of the stomatal conductance. This is also shown in figure 7.3 in which values for $(r_t-r_b)^{-1}$ at 20 °C determined at different light intensities and VPDs were plotted against those for $g_{s,n}$ (= r_s^{-1}). All points could be well fitted by one regression line with slope unity (0.95) indicating that there is no internal resistance (r_i) against NH_3 transport into the leaf. In addition, the small value of the intercept indicates that transport through the cuticle was negligible as compared to transport via the stomata.

So, apart from the boundary layer resistance NH_3 uptake into leaves only depends on the stomatal resistance, irrespective of the VPD of the air.

However, the results obtained at 15 °C were different from those obtained at 20 or 25 °C. Figure 7.2a shows that $(r_t-r_b)^{-1}$ strongly increased at a low VPD in contrast to $g_{s,n}$. This implies that the measured NH_3 flux at 15 °C was larger than the flux that can be calculated using $g_{s,n}$. This discrepancy was also observed for smaller values of $(r_t-r_b)^{-1}$ and $g_{s,n}$ assessed at lower light intensities.

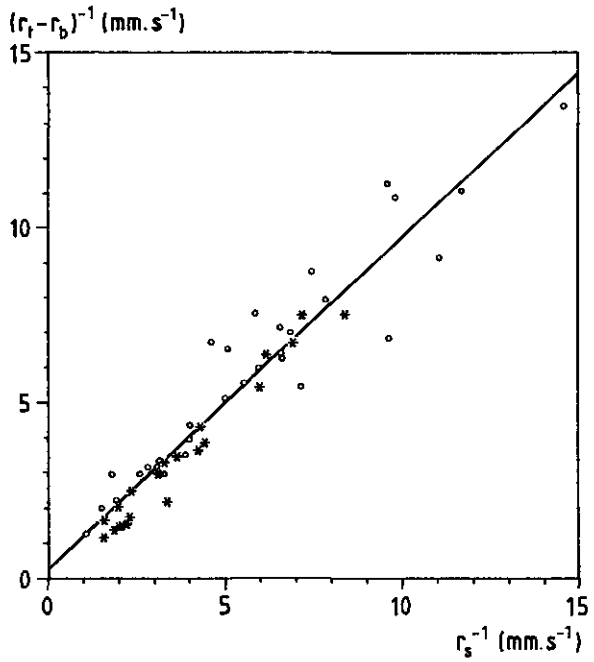

Figure 7.3. Analysis of NH_3 transfer into bean leaves (*Phaseolus vulgaris L.*) at different vapour pressure deficits of the air (VPD in kPa). Values for the NH_3 flux into the leaf were compared with those obtained for leaf transpiration according to equation (7.6). The internal resistance (r_i) against NH_3 transport in the leaf was assumed to be zero in this analysis. The resistances determining transport of NH_3 through the leaf cuticle ($[r_c+r_{ic}]^{-1}$) can be derived from the intercept of the lines. Symbols: NH_3; o,x: VPD of 0.3 and 0.8 respectively. Air temperature was 20 °C.

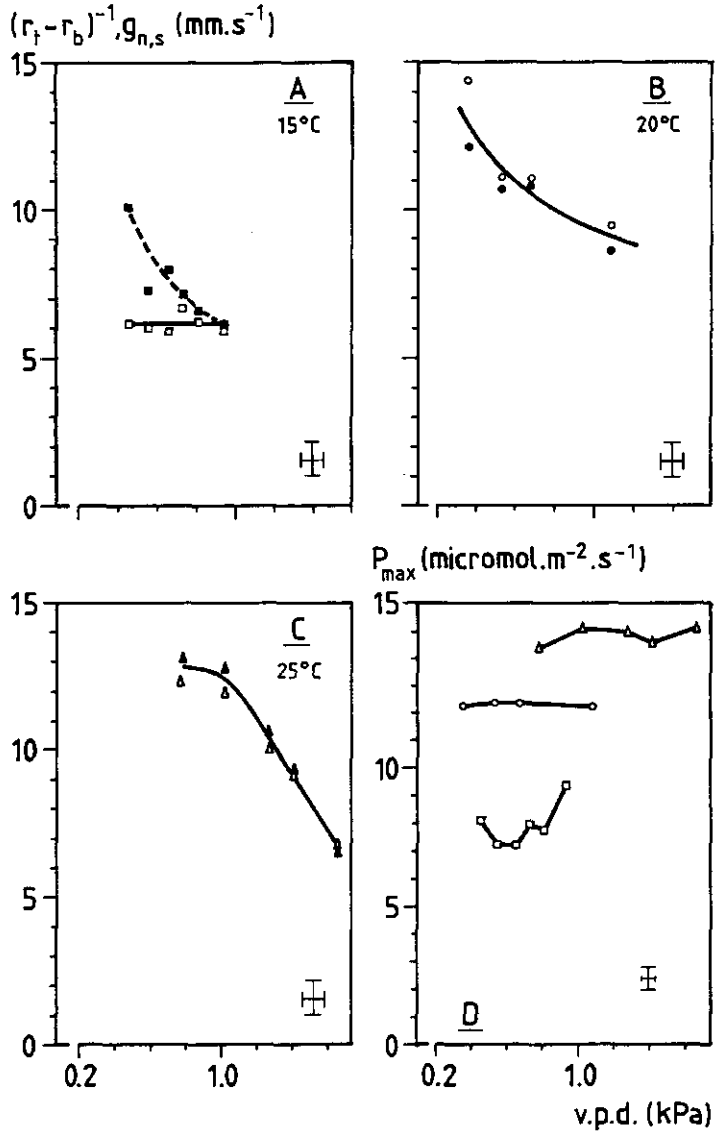

Figure 7.2. Conductance for NH_3 transfer into the leaf ($[r_t-r_b]^{-1}$) calculated from the measured flux (closed symbols), stomatal conductance for NH_3 ($g_{s,n}$ in mm.s^{-1}) calculated from leaf transpiration (open symbols) (a-c) and net CO_2 assimilation (d) at light saturation (P_{max}) as a function of vapour pressure deficit of the air (VPD in kPa) at different air temperatures. The experiments were conducted with bean leaves (*Phaseolus vulgaris L.*). Light intensity was 485 μmol.m^{-2}.s^{-1} (PFD). Each point represents the mean value of 6 measurements. ⊢⊣ standard error of the mean (S.E.).

The results of a similar study with SO_2 are shown in figure 7.4 and 7.5. Values for $(r_t-r_b)^{-1}$ calculated from the measured SO_2 flux at light saturation appeared to be larger than those of the stomatal conductance for SO_2 ($g_{s,s}$) calculated from the transpiration rate (fig. 7.4). Thus, the real flux into leaves was larger than can be calculated using $g_{s,s}$.

At each temperature $(r_t-r_b)^{-1}$ increased strongly at a low VPD, while only a small increase in $g_{s,s}$ was observed. In figure 7.5 values for $(r_t-r_b)^{-1}$ assessed at different light intensities were plotted against those for $g_{s,s}$ ($=r_s^{-1}$). The measurements were carried out at a low and high VPD (0.7 and 1.3 kPa respectively) and a constant air temperature (20 °C). Also from this figure it can be derived that at a low VPD the real SO_2 flux into leaves was larger than can be calculated from $g_{s,s}$. However, on the other hand no 'additional' uptake can be derived from the results obtained at the high VPD. The regression line has a slope of 0.9 indicating that SO_2 uptake into the leaf under this condition was almost solely dependent on stomatal conductance. Both lines show a low intercept value at the Y-axis. This indicates that the strong increase of $(r_t-r_b)^{-1}$ at a low VPD was not due to a large increase of cuticular transport.

The uptake of SO_2 into leaves was also examined at different air temperatures and a constant VPD (0.8 kPa ±0.1). An increase of the uptake was observed at a higher temperature. However, no significant effect on the relationship between $(r_t-r_b)^{-1}$ and $g_{s,s}$ could be detected. This implies that the effect of temperature on the SO_2 flux can solely be attributed to an effect on the stomatal conductance.

The net CO_2 assimilation at light saturation was hardly influenced by a VPD decline (fig. 7.2d and 7.4d) in contrast to g_s. This suggests that a low VPD induces a change in the relationship between stomatal conductance and net CO_2 assimilation. As a result also the ratio between intercellular and external CO_2 concentration (C_i/C_a ratio) changes. The dependency of the C_i/C_a ratio on VPD is shown in figure 7.6. The ratios were estimated from the light response curves for CO_2 assimilation and stomatal conductance assessed at the different VPDs (see equation 7.5). It appeared that the C_i/C_a ratio increased at VPDs lower than 0.7 kPa.

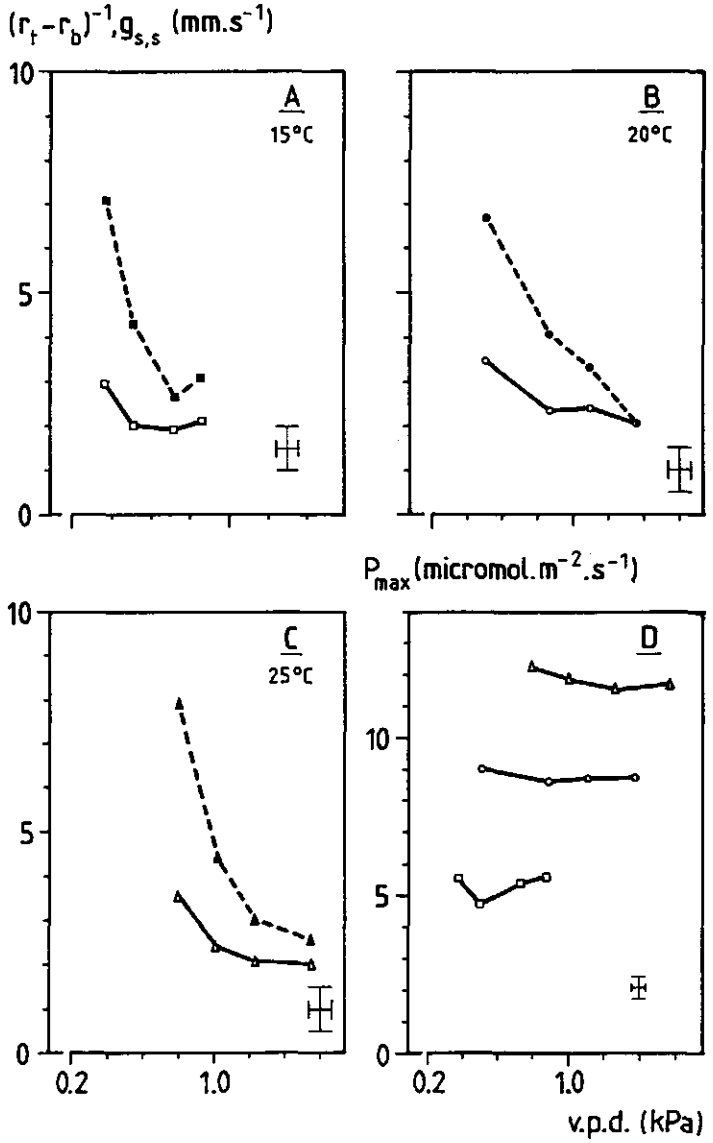

Figure 7.4. Conductance for SO_2 transfer into the leaf ($[r_t-r_b]^{-1}$) calculated from the measured flux (closed symbols), stomatal conductance for SO_2 ($g_{s,s}$ in mm.s^{-1}) calculated from leaf transpiration (open symbols) (a-c) and net CO_2 assimilation (d) at light saturation (P_{max}) as a function of vapour pressure deficit of the air (VPD in kPa) at different air temperatures. The experiments were conducted with bean leaves (*Phaseolus vulgaris L.*). Light intensity was 485 μmol.m^{-2}.s^{-1} (PFD). Each point represents the mean value of 6 measurements. ⊢⊣ standard error of the mean (S.E.).

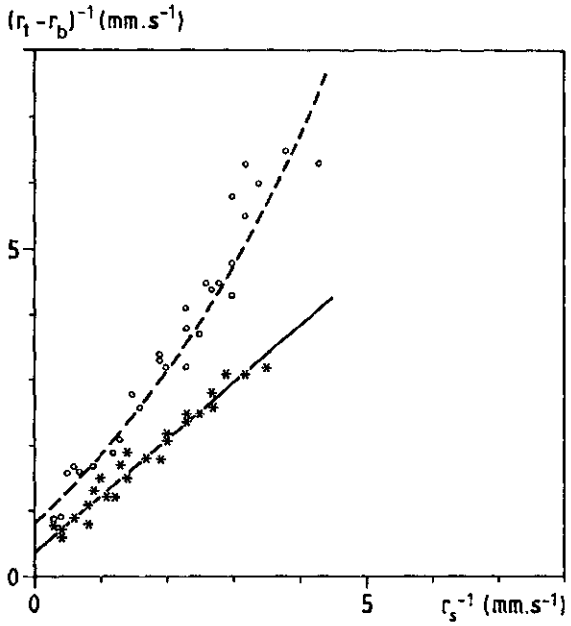

Figure 7.5. Analysis of SO_2 transfer into bean leaves (*Phaseolus vulgaris L.*) at different vapour pressure deficits of the air (VPD in kPa). Values for the SO_2 flux into the leaf were compared with those obtained for leaf transpiration according to equation (7.6). The internal resistance (r_l) against SO_2 transport in the leaf was assumed to be zero in this analysis. The resistances determining transport of SO_2 through the leaf cuticle ($[r_c+r_{lc}]^{-1}$) can be derived from the intercept of the lines. Symbols: SO_2; o,x: VPD of 0.7 and 1.3 respectively. Air temperature was 20 °C.

Discussion

The results of the present study suggest a transition in the properties of the leaf boundary layer at a wind velocity between 0.3 and 0.4 m.s^{-1}. Similar observations have been reported by Grace and Wilson (1976) for poplar leaves (*Populus euramericana L.*). Using a hot-wire anemometer they measured a strong increase in turbulence of the air flow along the leaf surface at a critical value of the Reynolds number (Re_{crit}) of 3.4×10^3. This is much lower than Re_{crit} values of (1 to 10)$\times 10^5$) reported for smooth flat plates in a laminar air flow (Grace 1982). Even lower Re_{crit} values can be calculated from the results of the present study ((1.6 to 2.1)$\times 10^3$).

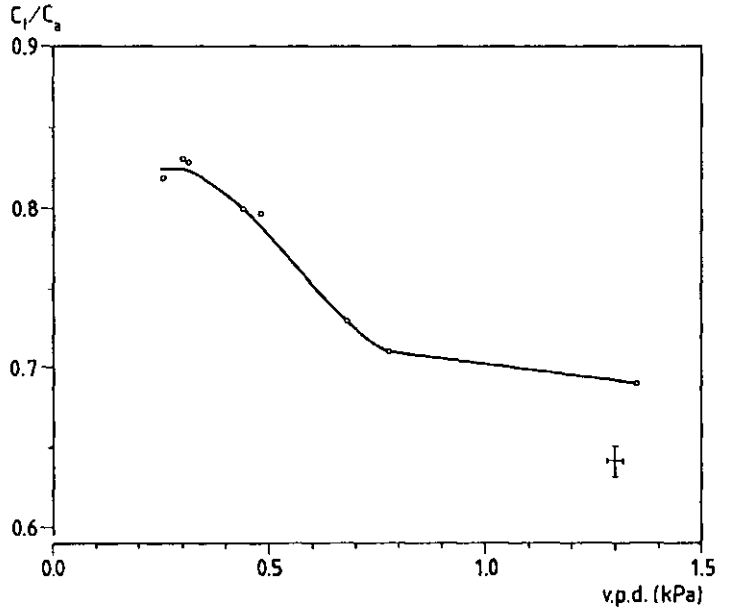

Figure 7.6. The ratio between internal and external CO_2 concentration (C_i/C_a) of bean leaves (Phaseolus vulgaris L.) as a function of vapour pressure deficit of the air (VPD in kPa). The experiments were carried out at 20 °C and a CO_2 concentration of 330 $\mu l.l^{-1}$ (±10). Each point represents the average value of 5 measurements. ⊣⊢ standard error of the mean (S.E.).

This may be due to actual turbulence in the air flow along the leaf and a complex topography and roughness of the leaf surface, which promote turbulence in the leaf boundary layer. The small Re_{crit} values observed in this study are consistent with the view that turbulence in the leaf boundary layer prevails, except at very low wind velocities (Grace 1982). This might also explain that the leaf boundary layer resistance assessed at wind velocities >0.4 $m.s^{-1}$ was a factor 1.5 to 2 smaller than can be calculated from the equations for r_b derived from model leaves. Nevertheless, a similar relation between r_b and wind velocity was found ($r_b = \alpha(w/u)^{0.5}$). From this it can be concluded that the equations derived from model leaves may give a good approximation of the leaf boundary layer resistance provided that a larger correction for roughness of the leaf surface is made and the wind velocities are >0.4 $m.s^{-1}$.

Our results indicate that a low VPD may induce a change in the relationship between stomatal conductance (g_s) and net CO_2 assimilation (P_n) as a result of which the internal CO_2 concentration increases. This has also been found for other plant species (Ward and Bunce 1986; Morison 1987; Woledge et al. 1989). Sofar no mechanism for this effect of VPD has been identified. The effect of temperature on this relationship is little known (Morison 1987). In pre-liminary measurements we found no significant effect of temperature on this relationship. However, the measurements were carried out at the same VPD range (0.8±0.1 kPa). A different situation may occur at other combinations of temperature and VPD. In view of the consequences for modelling gas exchange of leaves, it is important that more attention is paid to the influence of temperature and VPD on the relationship between g_s and P_n.

It appeared that at 20 or 25 °C the increase of the NH_3 flux at a low VPD could be attributed to the increase of g_s. However, no proportionality between NH_3 flux and g_s was found at a low temperature and VPD. The results suggest that under this condition a calculation of the NH_3 flux using experimental data obtained for r_b and g_s will give an underestimation of the real flux into leaves.

A rather strong discrepancy between measured and calculated flux was observed for SO_2. The strong increase of the SO_2 flux at a low VPD could only partly be explained by the increase of g_s, suggesting a large additional uptake of this gas by the leaf. Figure 7.3 shows that at a low VPD (<0.5 kPa) the measured SO_2 flux was about a factor 1.5 to 2 larger than the flux that can be calculated from data on r_b and g_s. The results are consistent with earlier experimental results obtained for poplar leaves (*Populus euramericana L.*) (Van Hove et al. In prep.). Similar observations have also been reported by others for different SO_2 concentrations and plant species (Hällgren et al. 1982; Taylor and Tingey 1983; Johansson 1983; Olszyk and Tingey 1985). The extent with which the measured flux differed from the calculated flux, was found to be strongly dependent on the VPD of the air. This might be an indication that the 'additional' uptake of SO_2 is a result of adsorption of the leaf surface. A previous study showed that a VPD decline may cause a strong increase of the SO_2 adsorption on the leaf surface (Van Hove et al. 1989). However, it should be noted that the uptake of SO_2 in the present study was determined after a steady state situation in the leaf chamber was reached.

It has been shown that SO_2 adsorption on the leaf surface in this situation already has taken place. A similar result was obtained for NH_3 adsorption on the leaf surface. Furthermore, no clear influence of a low VPD on transport of these gases through the cuticle could be detected (Van Hove et al. 1989). This can also be derived from the results of the present study. Therefore, these pathways can presumably be excluded. Consequently, the observed effect must have its origin in the stomatal pathway.

A possible explanation might be the assumption made by Taylor and Tingey (1983). They postulated that SO_2 molecules moving into the leaf follow a shorter pathway than effluxing H_2O molecules due to a reaction of SO_2 molecules inside the stomatal pore or with cells in the substomatal cavity immediately adjacent to the stomatal pore. Thus, as compared to SO_2 molecules moving inside the effluxing H_2O molecules encounter an 'extra' resistance. According to equation (7.6) (see material and methods) it can be calculated that the stomatal resistance for SO_2 (r_s) in figure 7.5 has to be lowered with a value of 80 to obtain a linear relationship between $(r_s)^{-1}$ and $(r_t-r_b)^{-1}$, with slope unity. Taking into account the difference in diffusion coefficients between SO_2 and H_2O, this would imply that the 'extra' resistance encountered by H_2O molecules is 40 $s.m^{-1}$. It can be postulated that the reaction of SO_2 inside or with cells close to the stomatal pore is promoted at a low VPD by an effect on the formation of a waterfilm covering the walls of the stomatal pore. This might also explain the apparently shorter pathway for NH_3 at low temperatures, since the solubility of this gas in water strongly increases at a temperature decline.

The microscopic observations of Black and Black (1979a,b) seem to sustain this assumption. For *Vicia faba L.* they found that SO_2 preferentially affects the epidermal cells immediately adjacent to the stomata. However, more research is required to obtain conclusive evidence for this assumption.

The results of this study indicate that canopy deposition models or crop ecological models may give an underestimation of NH_3 or SO_2 fluxes into leaves. Particularly at a low temperature and VPD a large error may be made. This can be illustrated by the following example in which the effect of a concurrent temperature and VPD decline on maximum NH_3 and SO_2 flux into the leaf was similated using the relations assessed in this study. The initial conditions are: an air temperature of 20 °C and a VPD of 0.9 kPa (=60% R.H.). Figure 7.2 shows that P_{max} decreases with 40% at a temperature decline to 15 °C.

If we assume that the moisture content of the air is not changed, the VPD will decrease to 0.3 kPa due to this temperature decline. As a result the C_i/C_a ratio increases from 0.7 to 0.8 (fig. 7.6) assuming that temperature alone has no effect on this ratio. It can be calculated that due to this increase of the C_i/C_a ratio g_s declines with only 10% in stead of 40%. Furthermore, the NH_3 transfer into the leaf at 15 °C was found to be larger than can be calculated from g_s. So, in stead of an expected decrease of the NH_3 flux the opposite may occur at a temperature decline. From figure 7.4 it can be derived that SO_2 transfer into the leaf will be even larger due to the strong increase of the 'additional' uptake by the leaf at a decreasing VPD of the air.

The relations assessed in this study can be used to construct a simple descriptive model for NH_3 and SO_2 transfer into leaves as a function of wind velocity, light intensity, air temperature and humidity. The model can be used in conjunction with e.g. crop ecological models to provide realistic simulations of the influence of environmental variables on whole leaf responses to air pollutants. However, more work is needed to determine plant responses to a wider range of conditions. Also more data about the influence of these climatic factors on NH_3 and SO_2 transfer into leaves of other plant species are required to obtain a more general model.

CHAPTER 8

GENERAL DISCUSSION

In crop ecological research models have been developed to simulate canopy exchanges of CO_2 and water vapour as a function of environmental factors, plant structure and developmental stage (Goudriaan 1977, 1979; Penning de Vries and Van Laar 1982; Jarvis et al. 1985). In principle these models can also be applied for the prediction of (dry) deposition and effects of pollutant gases on vegetation (O'Dell et al. 1977; Murphy et al. 1977; Unsworth 1981; Kercher et al. 1982; Murphy and Lorenz 1985; Hicks et al. 1987; Meyers 1987; Wesely 1989; Kropff 1989).

However, the application of these models requires that more is known about the uptake of pollutant gases by leaves. In the present research the uptake of NH_3 and SO_2 by individual leaves has been examined. A major question was whether the current leaf resistance models developed for analyzing the exchange of CO_2 and water vapour between a leaf and the surrounding atmosphere, are equally useful for the estimation of NH_3 and SO_2 uptake by leaves. Also it was examined whether a long term exposure to low concentrations of these gases causes direct physiological effects on leaves. This may be of importance for the uptake of these gases by leaves as well. The experiments were performed with bean (*Phaseolus vulgaris L.*) and poplar (*Populus euramericana L.*). The uptake was analyzed according to a resistance analogue presented in *chapter 3*.

Boundary layer effects

As gases approach the leaf surface, they have to pass through the boundary layer of the leaf where transfer no longer takes place by turbulence, but partly by molecular diffusion. The results of the present research indicate that at wind velocities below 0.4 $m.s^{-1}$ the leaf boundary layer constitutes a large resistance against transfer of gases. The resistances for H_2O, NH_3 and SO_2 were found to be larger than can be calculated according to the functions derived from measurements with flat leaf replicas. These low wind velocities may occur within a close canopy (Vermetten, pers. comm.). Consequently, in model calculations an overestimation of the transfer of gases towards leaves in general will be made in this situation. However, the error made will largely depend on the value of the stomatal resistance.

The boundary layer resistance strongly declined at an increase of the wind velocity. This decline could be described by an empirical relation in which the resistance changes roughly with $u^{-1.5}$ (u=wind velocity). The results suggest a transition in the properties of the leaf boundary layer at a wind velocity between 0.3 and 0.4 $m.s^{-1}$. Similar results have been reported by Grace and Wilson (1976). Using a hot-wire anemometer they measured a strong increase in turbulence of the air flow along the leaf surface in this range. The result of the present research indicate that at wind velocities larger than about 0.4 $m.s^{-1}$ the theoretical functions derived for flat leaves may give a good approximation of this resistance for a gas, provided that a larger correction for roughness of the leaf surface is made.

Adsorption on the leaf surface

Relatively little is known about the adsorption of air pollutants on leaf surfaces. Moreover, most information refers to the adsorption of SO_2 and fluoride, whereas hardly any information about other gases is available. The reported amounts for e.g. SO_2 adsorption have usually been obtained indirectly (Garsed and Read 1977a, 1977b; Fowler and Unsworth 1979; Black and Unsworth 1979; Elkiey and Ormrod 1981; Taylor and Tingey 1983). For instance, they were derived from the difference between uptake of a canopy surface during day time and night time when the stomata are closed.

Only a few workers have attempted to determine the adsorption directly. However, the accuracy of the applied techniques can be questioned. For instance, it is possible that the washing procedure employed by some authors to determine this adsorption removes more pollutant than is actually sorbed on the leaf surface.

In the present research a more direct method for the determination of NH_3 and SO_2 adsorption was applied. The adsorbed quantities of these gases were found to be relatively small as compared to the quantities taken up via the stomata (*chapter 4*). Only at a low vapour pressure deficit of the air (VPD) and with both gases present in the atmosphere, a significant adsorption of these gases will occur. It can be estimated (see *chapter 3*) that under these conditions the adsorbed quantity of NH_3 at a concentration of 100 $\mu g.m^{-3}$ is 12% of the quantity transported into the leaves after a 12h photoperiod. A similar estimation for SO_2 adsorption shows that the largest quantity adsorbed will be 32% of that transported inside. However, these estimations are only valid for leaves with a relatively large stomatal conductance.

For other plant species with a much lower stomatal conductance the relative contribution of adsorption to the total uptake will be much higher. An impression of the relative importance of adsorption in this situation can be obtained by making a similar estimation for such a plant species with the assumption that there are no significant differences in adsorption per unit leaf area between plant species. For instance, the relative contribution of NH_3 and SO_2 adsorption to the total uptake by needles of the Douglas fir (*Pseudotsuga menziesii*) can be estimated. The Douglas fir constitutes a large part of the total forest area in the Netherlands and therefore much research carried out in the framework of the Dutch Acidification Program is focussed on this species. In experiments with three years old trees we assessed an average maximum leaf conductance[1] of about 2.0 $mm.s^{-1}$ for current and one-year-old needles (Van Hove and Mensink, unpublished results). Taking into account the difference in leaf area between flat and round leaves it can be estimated that the maximum adsorbed NH_3 quantity, i.e. at a low VPD and in the presence of SO_2, will be 60% of that transported inside after a 12h photoperiod. A similar estimation for SO_2 shows that the quantity adsorbed will be about 1.5 times larger than the quantity transported into the needles under this condition. Although only rough estimations could be made here, they clearly demonstrate that a relative large NH_3 and SO_2 adsorption on needles may occur.

No significant desorption of NH_3 and SO_2 from the external leaf surface could be detected. In addition our results indicate that only a minor transport of these gases through the cuticle occurs. Hence, it can be assumed that the bulk of the adsorbed NH_3 and SO_2 remains associated with the cuticle and can only be removed by (rain) water or dew, after which a renewed adsorption may occur. This wash off by rain water followed by a renewed adsorption might explain the large quantities found in throughfall and stemflow (Van Breemen et al. 1982; Heil et al. 1987, 1988; Roelofs et al. 1985).

The strong increase in NH_3 and SO_2 adsorption at a low VPD suggests an important role for 'water' in the adsorption of these gases on the leaf surface. According to Schönherr (1982) the cutin matrix can be compared with a porous membrane: the cutin matrix contains water filled pores which develop only as a result of hydration of the polar functional groups in the cutin matrix. With increasing vapour pressure the water mobility and water content of the cuticle increases.

[1] calculated for projected needle area.

This hypothesis can also be applied to explain the strong increase of NH_3 and SO_2 adsorption at a low VPD. Moreover, the observed similarities between the reaction of NH_3 and SO2 with the leaf surface and their reaction with water suggest that the cuticle-water-system might behave like a free water layer. This would offer interesting possibilities for modelling adsorption. The amount of adsorption of a gas on the leaf surface could then be estimated using calculating models for the dry deposition of these gases on water films (Adema et al. 1986; Heeres and Adema 1989).

However, more research is required to provide evidence for this assumption. For instance, an unknown variable is the pH at the leaf surface which is probably of crucial importance for the dissolving of NH_3 and SO_2 in cuticle-water-system. In this connection also more data about the influence of CO_2 and of substances excreted by the leaves such as metabolites, cations and anions, are required (Evans 1984). Also uncertainties exist with respect to the dissolving of NH_3 and SO_2 in the lipid phase of the cuticle. Results of Lendzian (1984) for SO_2 suggest that this may be of importance too. More refined techniques such as the application of isotopes and ion-sensitive micro-electrodes, are required to examine the influence of these variables.

Cuticular transport

Transport of NH_3 and SO_2 through the cuticle was found to be very small, even at high air humidities. The results of our study indicate that the cuticular resistance for NH_3 and SO_2 is probably in the same range as that for water vapour (i.e. (2 to 40)x10^3 s.m^{-1}). Similar results have been obtained for other pollutant gases such as NO_2 and O_3 (Grennfelt et al. 1983; Rowland-Bamford and Drew 1988; Aben 1988).

Light and electron scanning microscopy showed that a prolonged exposure to moderate concentrations of NH_3, SO_2 or a mixture of both gases did not damage the leaf cuticle under the experimental conditions of the present study. Neither an increased cuticular transpiration was measured (*chapter 5* and *6*), indicating that the permeability of the cuticle was not influenced.

However, the maximum exposure time in the present research was relatively short as compared to common field situations. Field examinations showed that a long term exposure to air pollutants may cause erosion of epicuticular wax structures around the stomata of needles (Huttunen and Soikkeli 1982). Damage of the leaf cuticle may also be enhanced in case of a wet leaf surface. For instance, it was shown that exposure to a combination of NH_3 and artificial

rain containing $(NH_4)_2SO_4$ enhances the erosion of the epicuticular wax layer of Douglas fir (Van der Eerden et al. 1989). Similar observations for combinations of fog and low concentrations of NO_x and SO_2 have been reported (Freer-Smith 1988). This damage may increase cuticular transpiration as well as transfer of pollutant gases through the cuticle into leaves. Also this damage may lead to an increase of ion leaching such as K^+ and Mg^{2+} from leaves.

NH_3 transfer into leaves

The results of the present research indicate that NH_3 transport into leaves only depends on the boundary layer and stomatal resistance of the leaf. No internal resistance against NH_3 transfer in the leaf could ever be detected suggesting that leaves are an almost infinite sink for NH_3. Also other studies indicate that NH_3 transport into the leaf encounters no internal resistance and only depends on the boundary layer and stomatal resistance (Porter et al. 1973; Meyer 1973; Rogers and Aneja 1980). Furthermore, no clear interaction with SO_2 was observed (*chapter 6*). Apparently, the main reaction between these two gases takes place on the leaf surface.

It can be concluded that NH_3 molecules moving into the leaf follow a similar pathway as effluxing H_2O. This implies that leaf data assessed for the boundary layer and stomatal resistance for H_2O can be used to estimate NH_3 transport into leaves. However, it should be noted that this conclusion is only valid for optimal environmental conditions. Our results indicate that a different situation exists under less favourable conditions, e.g. at a low VPD and at a temperature below 15 °C. Under those conditions NH_3 molecules moving into the leaf encountered less resistance than effluxing H_2O molecules suggesting a difference in pathway between both molecules. This would imply that in model calculations a significant underestimation of the NH_3 flux into leaves is made under those conditions.

Ammonia compensation point

Besides uptake into leaves also emission of NH_3 from leaves has been reported for a number of plant species (Porter et al. 1972; Meyer 1973; Denmead et al. 1978; Stutte and Weiland 1978; Stutte et al. 1979; Farquhar et al. 1979, 1980, 1983; Lemon and Houtte 1980; Harper et al. 1983).

This would imply that the internal NH_3 concentration remains above zero and that plants have a compensation point for NH_3 uptake at which no net NH_3 uptake occurs.

Rather high compensation points ranging from 10 to 20 $\mu g.m^{-3}$ were assessed by Meyer (1973) in growth chambers. Similar measurements made by Farquhar et al. (1980) yielded much lower compensation points (1.5-5 $\mu g.m^{-3}$ NH_3). Also senescent maize leaves showed a compensation point in about the same range (Farquhar et al. 1979). Emission of NH_3 has also been observed occasionally in the field using micrometeorological techniques but, in such cases, the soil may have been the source (Denmead et al. 1978; Harper et al. 1983).

In the present research we measured no significant uptake of NH_3 into leaves at concentrations lower than about 5 $\mu g.m^{-3}$ (*chapter 3*). However, neither a significant emission of NH_3 could be detected when clean air was passed over the leaf. Similar results were obtained in a repetition of the experiments of chapter 3 at different temperatures (15, 20 and 25 °C). (Van Hove, unpublished results). The main difficulty is that no clear distinction between emission from the leaf and the release of NH_3 from the internal surfaces of the leaf chamber can be made at these low concentrations. This release might have been from a water film covering the internal surfaces of the leaf chamber. However, it appeared that also a small release of NH_3 from teflon in the leaf chamber may occur (see chapter 2). This 'memory-effect' has also been observed for other materials. So far we have found no construction material which is inert for NH_3 (Sauren et al. 1989). Consequently, the accuracy of the reported results for NH_3 emission of most studies can be questioned. It seems likely that the emission rates have been overestimated in many cases. Also it can be questioned whether large quantities of NH_3 actually are able to escape from leaves. Results of studies in which the dry deposition of NH_3 on wet surfaces has been examined, show that NH_3 may react with CO_2 as a result of which ammonium carbonate is formed (Heeres and Adema 1989). A similar reaction is likely to occur at the leaf surface or inside the leaf. The results of Van Breemen et al. (1982) suggest that this reaction takes place indeed.

Our experimental results indicate that the compensation point for NH_3 uptake, if present at all, is very small and probably in the same range as assessed by Farquhar et al.(1979, 1980). Thus, an almost zero internal NH_3 concentration can be considered at model calculations.

The compensation point is in the same range as the low background concentrations measured in the atmosphere, i.e. 0.5-4 $\mu g.m^{-3}$ NH_3 (Asman 1987). Therefore, hardly any nitrogen loss as NH_3 directly from plant leaves will occur under common field situations.

SO_2 transfer into leaves

Although our results indicate that stomata play an important role in determining SO_2 uptake into leaves, a less clear relation between its uptake and the stomatal conductance was found. The measured flux is larger than can be calculated from the boundary layer and stomatal resistance for H_2O suggesting a large additional uptake of SO_2. Similar results have been reported by others for different SO_2 concentrations and plant species (Hällgren et al. 1982; Taylor and Tingey 1983; Johansson 1983; Olszyk and Tingey 1985; Kropff pers. comm.). Also for ozone (O_3) a similar effect has been observed (Johansson 1983; Aben 1988). On the other hand NO_2 uptake into leaves shows a close correlation with stomatal conductance (Grennfelt et al. 1983; Rowland-Bamford and Drew 1988). Furthermore our results show that the extent with which the measured SO_2 flux differs from the calculated flux, strongly depends on the VPD of the air (*chapter 7*). For example, no significant difference between measured and estimated flux at a high VPD was measured.

Since SO_2 strongly reacts with water, this suggests that the observed effect was caused by a surface reaction, rather than by an effect on the diffusion process itself. Also the fact that no discrepancy for NO_2 uptake is observed, may indirectly provide evidence for this assumption, because this gas shows only a minor reaction with water (Adema et al. 1986; Heeres and Adema 1989). The discrepancy might be a result of adsorption on the external leaf surface. However, the uptake was measured in these studies at a steady state situation. Earlier experimental results show that the adsorption has already taken place in this situation (*chapter 4*). Also no strong increase of transport through the cuticle was observed. Thus, these pathways can probably be excluded. Consequently the observed effect must have its origin in the stomatal pathway. It is proposed that SO_2 molecules moving into the leaf follow a shorter pathway than effluxing H_2O molecules due to a reaction inside the stomatal pore or with cells in the substomatal cavity immediately adjacent to the stomatal pore. Thus, as compared to the SO_2 molecules the effluxing H_2O molecules encounter an 'extra' resistance.

It can be derived from our results that the extra resistance would be approximately 20-40 $s.m^{-1}$ (for H_2O). This resistance is large in comparison with the maximum stomatal resistance for H_2O which was about 100 $s.m^{-1}$.

The largest part of the 'extra' resistance must reside in the stomatal pore, since the substomatal cavity constitutes only a minor resistance against diffusing H_2O or gas molecules (Monteith 1973).

This would imply that SO_2 molecules penetrate into the stomatal pore only over a shorter distance, i.e.: they mainly react with the walls of the guard cells. It can be postulated that a low VPD promotes this reaction by an effect on the formation of a water film covering the walls of the stomatal pore. This could also explain the discrepancy between measured and calculated NH_3 flux observed at a combination of a low temperature and VPD
(*chapter 7*), since the solubility of NH_3 in water strongly increases at a low temperature.

These assumptions are in accordance with results of Black and Black (1979a,b) and of Black and Unsworth (1980) showing that SO_2 preferentially affects cells of the stomatal complex.

The observed discrepancy for the ozone (O_3) flux can be explained by a similar reaction because O_3 is extremely reactive to a variety of substances found within living systems. Also O_3 decomposes spontaneously in aqueous solutions to form molecular oxygen and in water soluble products.

In conclusion it should be emphasized that these assumptions are still speculative. More research is required to get a better understanding of the pathways followed by SO_2 into the leaf and the influence of VPD and structure of the stomatal complex on this transport.

Physiological effects

Under the experimental conditions of the present research the physiological effects of a prolonged exposure to NH_3 or SO_2 became notable at concentrations of about 100 $\mu g.m^{-3}$.

The results indicate that a long term NH_3 exposure to this concentration may have a positive effect on photosynthesis and stomatal conductance. Moreover, the results indicate that this exposure may also have a positive effect on NH_3 uptake into leaves. The effect looks like an autokatalytic reaction: as a result of NH_3 uptake into leaves photosynthesis is stimulated, which subsequently leads to a larger stomatal conductance and NH_3 uptake into leaves.

A positive correlation between the nitrogen content of leaves and photosynthetic capacity is usually found (Evans 1983; Wong, Cowan and Farquhar, 1985; Hirose and Werger, 1987). This suggests that the NH_3 flux into leaves at a concentration of 100 $\mu g.m^{-3}$ may substantially contribute to the nitrogen content of leaves.

It can be estimated from the results of our study that exposure to 100 $\mu g.m^{-3}$ NH_3 may give a contribution of more than 10% to the total nitrogen need of leaves (*chapter 5*). Results of Faller (1972) and Lockyer and Whitehead (1986) provide evidence for this conclusion. For Italian ryegrass Lockyer and Whitehead (1986) found that a NH_3-concentration of 118 $\mu g.m^{-3}$ supplied about 20% of total plant N when the plants were grown at a low dosage of N in the root-medium, and about 10% of total plant N when plants were grown at a higher dosage. Also needles of conifers in areas with a high NH_3 deposition show an enhanced N-content (Van Dijk and Roelofs 1988), which seems likely to be the result of uptake of atmospheric NH_3 as well.

In addition these needles show an accumulation of amino acids such as arginine, glutamine and asparagine, indicating that leaves detoxify NH_3 by the formation of amino acids via the GS/GOGAT-cycle. A close correlation between N-content, RUBISCO activity and photosynthesis is observed (Von Caemmerer and Farquhar 1981; Evans 1983; Huffaker 1982; Makino et al. 1988). Hence, it can be postulated that the extra N-input into leaves inhibits the decline in RUBISCO activity, causing a delay in the rate of ageing of these leaves. This might also explain that leaves exposed to a moderate NH_3 concentration showed a smaller rate in decline of maximum CO_2 assimilation as compared to leaves exposed to filtered air.

Only few papers report on the physiological responses of plants exposed during a long time to low SO_2 concentrations. Moreover, conflicting results have been obtained. For instance, a reversible as well as an irreversible effect on photosynthesis has been reported for concentrations in the same range (Black and Unsworth 1979; Hällgren and Gezelius 1982; Saxe 1982).
A similar controversy exist with respect to the effect on stomatal conductance (Black and Unsworth 1979; Black and Black 1979a,1979b; Saxe 1983).
The results of the present research indicate that a SO_2 concentration of about 100 $\mu g.m^{-3}$ may cause a small reduction (about 15%) in maximum CO_2 assimilation and stomatal conductance. However, no clear effect on SO_2 uptake could be detected. Estimations suggest that the uptake into leaves at this SO_2 concentration may contribute almost completely to the total amount of sulphur needed by the leaves (*chapter 6*). It can be postulated that the leaf reaches its maximum capacity to cope with the influx of SO_2 at this concentration. This might also explain that the first inhibiting effects on photosynthesis can be observed at this concentration.

Also results of other experiments suggest that a decline in the capability of the leaf to cope with the SO_2 influx at concentrations in this range. For instance, experiments with bean leaves in which the relation between SO_2 uptake and concentration was examined, showed that at a concentration of 200 $\mu g.m^{-3}$ the relationship became less linear (Van Hove and Adema 1988).

The inhibiting effect on maximum photosynthesis was proven to be irreversible indicating that the effect was related to a structural change rather than to a short term reversible effect as result of e.g. a competitive inhibition between sulfite and CO_2 for RUBISCO. This is in accordance with results of Kropff (1987) obtained for *Vicia faba L.* showing that only SO_2 concentrations above 600 $\mu g.m^{-3}$ induce an acute, reversible reduction in maximum CO_2 assimilation and stomatal conductance. The same was observed for *Phaseolus vulgaris L.* (Van Hove, unpublished results).

The long term exposure to 100 $\mu g.m^{-3}$ SO_2 was also found to cause a higher dark respiration, which is in accordance with results obtained for *Vicia faba L.* by Kropff (1989) in field fumigation experiments with this species. Remarkably, the quantum yield for CO_2 fixation of these leaves was higher. This suggests that these leaves had a higher efficiency of photosynthesis under light limiting conditions. In addition the fluorescence measurements indicated that the Calvin cycle was more rapidly activated after a transition of the leaves from dark to light. From this it can be derived that this SO_2 exposure may have a direct or indirect influence on electron transport rate through the thylakoid membrane. For instance, Alscher (1984) proposed that SO_2 may influence the electron transport by an effect on reactions involved in the light-activation. However, this assumption is still very speculative. Probably there is no single mechanism to explain the action of SO_2 on photosynthesis. Many processes which will influence photosynthesis, both directly or indirectly, are likely to be affected by SO_2 to varying degrees. The effect on these processes will, among other things, depend on the concentration, duration and frequency of the exposure.

Usually average NH_3 and SO_2 concentrations below 100 $\mu g.m^{-3}$ are measured in the Netherlands (see *chapter 1*). This would imply that the NH_3 and SO_2 concentrations in the Netherlands hardly cause any direct physiological effects on plants. From this, one might also conclude that the observed effects on vegetation are mainly a result of an indirect effect of NH_3 and SO_2 via the soil, e.g. by contributing to soil acidification (Van Breemen et al, 1982).

However, it should be noted that the plants in the present study were grown under rather favourable conditions. Assuming that no detoxification occurs, it can be estimated for the leaves used in our study (chapter 6) that toxic concentrations of sulphite and NH_4^+ in leaf cells may be reached after about one day of exposure to a NH_3 or SO_2 concentration of 100 $\mu g.m^{-3}$. Thus, it can be assumed that due to the large uptake of these gases into leaves plants are more sensitive to other stress factors. This is confirmed by field observations showing a higher sensitivity of plants for NH_3 and SO_2 at low temperatures or at drought (Van der Eerden 1982; De Temmerman et al. 1988; Mansfield et al. 1988; Kropff 1989). Furthermore, it can be postulated that the uptake of these gases alters leaf metabolism in such a way that leaves have an increased sensitivity to other stress factors. For instance, it has been shown that NH_3 may act as an important regulatory agent in the leaf. It stimulates the activity of glutamine synthetase, as well as the activity of enzymes involved in the Krebs cycle. The higher activity of the Krebs cycle induces a rise in the concentration of α-ketoglutarate in cells that subsequently is used in the GS-GOGAT cycle to detoxify NH_3. So, the detoxification of NH_3 takes place at the expense of the synthesis of carbohydrates. Moreover, the amino acids formed by the GS-GOGAT might be used for the formation of RUBISCO resulting in a prolonged activity of the leaves. These both effects of NH_3 in leaves might explain that plants exposed during a long time to this gas show a higher frost sensitivity (Van der Eerden 1982; De Temmerman et al. 1988). The extra input of N may also cause relative shortages of other mineral nutrients such as potassium, phosphorus and magnesium (De Temmerman et al. 1988; Van Dijk and Roelofs 1988). In addition the acquisition of N and S by shoots may have implications for the acid-base regulation in plants (Raven 1988; Kropff 1989). These effects might affect the development and growth of plants on the long term.

Concluding remarks

The present results may have important consequences for the application of current leaf resistance models for the estimation of NH_3 and SO_2 uptake into leaves. Particularly at a low temperature and VPD these models will give a large underestimation of the real flux of these gases into leaves. In addition our results indicate that even a larger error is made when CO_2 assimilation data are used to calculate the uptake of these gases.

This is a result of a change in the relation between photosynthesis and stomatal conductance at a low VPD (*chapter 7*).

An improved model for NH_3 and SO_2 transfer into leaves can, in principle, be obtained with the relations assessed in the present research. The model can be used in conjunction with e.g. crop ecological models to provide more realistic simulations of the influence of environmental variables on whole leaf responses to air pollutants. However, verification of our results for a wider range of conditions and plant species is required to obtain a more general model. In addition field measurements are necessary for verification.

Also it can be concluded that the adsorption on leaf surfaces cannot be ignored. Otherwise, a significant underestimation of the total uptake of NH_3 and SO_2 by leaves is made. Consequently, it is important that a (sub)model describing this process is developed, with which the adsorbed quantities of NH_3, SO_2 and other pollutant gases can be estimated.

With respect to the physiological effects the conclusion can be drawn that the NH_3 and SO_2 flux into leaves at the concentrations measured in the Netherlands hardly cause any direct effect on photosynthesis or on stomatal conductance. However, a different situation may occur when plants grow under less favourable conditions. Therefore, the interaction of pollutant gases with other 'stress' factors will require more attention.

SUMMARY

In addition to deposition of sulphur dioxide (SO_2) and nitrogen oxides (NO_x) also a large deposition of ammonia (NH_3) occurs in the Netherlands. The NH_3 deposition originates almost entirely from emission sources in the Netherlands itself, in contrast to SO_2 and NO_x. The dominant emission of NH_3 (about 90%) arises from agricultural sources, mainly livestock wastes. It has been shown that the high NH_3 emission in areas where intensive livestock breeding is concentrated, is one of the major causes for the dieback and decline in number of plant species observed in these areas. However, also SO_2 originating from fossil fuels is suspected to play an important role. Both gases mutually stimulate their deposition by a reaction on the leaf surface, as a result of which ammonium sulphate is formed. The observed effects are mainly attributed to effects on the soil: Ammonium sulphate that reaches the soil after leaching by rainwater, may cause acidification and disturbances in the ionic balance in soils with a limited buffering capacity. Furthermore, the high inputs of acids and nitrogen compounds may lead to a relative shortage of other nutrient elements in leaves.

In the present research the uptake of NH_3 and SO_2 by leaves and the relation between uptake, photosynthesis and stomatal conductance has been examined. The method of analysis was similar to that commonly used in photosynthetic research where the gas exchange of leaves is analyzed by using electrical resistance analogues, with the total transfer resistance being partitioned into gas-phase and liquid-phase components. The experiments were performed with bean (*Phaseolus vulgaris L.*) and poplar (*Populus euramericana L.*). The uptake of NH_3 and SO_2 by leaves was experimentally determined using a leaf chamber. This leaf chamber was specially developed for the application of low concentrations of pollutant gases. The leaf chamber is part of an open system. The uptake is determined from the difference between inlet and outlet concentration of the leaf chamber. Simultaneously, the transpiration and net CO_2 assimilation of the leaves were measured. In this way the uptake of NH_3 and SO_2 could be directly related to stomatal behavior and photosynthesis of leaves.

Chapter 2 describes the properties and performance of the leaf chamber. The internal surfaces of the chamber are of glass and teflon. Both construction materials have a low adsorptive capacity for pollutant gases. Nevertheless, adsorption of SO_2 and NH_3 on the internal surfaces of the chamber occurs. Also a small permanent loss of ozone (O_3) is observed.

In contrast, the internal surfaces of the chamber show no reaction with nitrogen oxides (NO and NO_2).

The adsorption of NH_3 and SO_2 is directly related to the humidity of the air inside the leaf chamber and is not caused by the technique with which a specific air humidity in the air is generated. This suggests an important role for water in the adsorption of these gases. It is assumed that this water is adsorbed by any surface, resulting in the formation of a thin water layer or water film at higher relative air humidities. The water film phenomenon might set a limit to the performance of leaf chambers and other devices to be developed for measuring and handling these and other soluble pollutant gases. However, adsorption of NH_3 on the internal surfaces of the leaf chamber is also observed in dry air. Presumably this is caused by either a specific surface adsorption or a relatively high permeability of teflon for NH_3.

A wide range of air temperatures and humidities can be applied in the leaf chamber. The wind velocity across both leaf surfaces is homogeneous and can be varied up to a maximum of about 3 $m.s^{-1}$. Consequently, the relation between wind velocity and boundary layer resistance for a gas can be studied properly. Measurements with an infrared thermovision camera showed that the control of leaf temperature distribution at the leaf surface is improved as compared to the leaf chambers used so far. This enables a higher accuracy in the determination of the stomatal resistance.

The research first concentrated on the uptake of NH_3 into leaves. The results presented in *chapter 3* show that the flux into leaves increased linearly with the NH_3 concentration in the leaf chamber, even at concentrations of up to 400 $\mu g.m^{-3}$. From this it can be derived that leaves have a high capacity to assimilate NH_3 in the light. A possible assimilation mechanism in the leaves is proposed in chapter 5. The NH_3 taken up by the leaves was found to have no influence on photosynthesis. No significant uptake into leaves was measured at concentrations below 5 $\mu g.m^{-3}$, suggesting the presence of a compensation point for NH_3 uptake. However, no clear evidence for this compensation point could be obtained in the present research.

Analysis of the transport resistances revealed that NH_3 transfer from the adjacent air into leaves depends on the boundary layer and stomatal resistance; transport through the cuticle was found to be negligible under the experimental conditions of the study. The transfer of NH_3 in the leaf encounters no internal resistance.

Chapter 4 describes a study in which the adsorption of NH_3 and SO_2 on the external leaf surface has been examined. The adsorbed quantities of both gases on the 'dry' leaf surface increased strongly at a low vapor pressure deficit of the air (VPD). In contrast no clear effect of temperature in the range from 15 to 26 °C was found. The influence of temperature comes mainly, if not exclusively, into expression via an effect on the VPD of the air. Furthermore, the adsorbed quantities of NH_3 and to a less extent those of SO_2, appeared to be proportional with their concentration in the air. These results suggest an important role of water in the adsorption of these gases on the leaf surface. The presence of a water film has been assumed. However, there are indications that the adsorption process of these gases on a leaf surface is more complex. A theoretical model describing the adsorption process is presented in this chapter. The affinity of SO_2 for the leaf surface was found to be approximately twice that of NH_3. With both gases present in the atmosphere the adsorption of NH_3 and SO_2 increased with a factor 4 and 2 respectively. Under this condition the adsorption of NH_3, and in particular, that of SO_2 may contribute considerably to the daily uptake of these gases by leaves. Neither a significant desorption, nor a significant transport of these gases through the cuticle could be detected. This suggests that the bulk of the adsorbed NH_3 or SO_2 remains associated with the cuticle and can only removed by (rain-)water.

The effects of a long term exposure to low NH_3 concentrations (50 and 100 $\mu g.m^{-3}$) on photosynthesis, stomatal conductance and NH_3 uptake into leaves have also been examined (*chapter 5*). The experiments were carried out with poplar shoots which were exposed to NH_3 for 6 or 8 weeks. A significant effect was observed for leaves exposed to a NH_3 concentration of 100 $\mu g.m^{-3}$. These leaves appeared to have a higher photosynthetic activity than leaves which had been exposed to 50 $\mu g.m^{-3}$ NH_3 or charcoal filtered air.
In particular the maximum CO_2 assimilation was influenced, while no influence on the quantum yield was observed. Fluorescence measurements demonstrated that this can be attributed to a higher activity of the Calvin cycle. In addition, these leaves showed a larger stomatal conductance and uptake rate of NH_3.

In a following study (*chapter 6*) poplar shoots were exposed for 7 weeks to SO_2 (45 and 112 $\mu g.m^{-3}$), NH_3 (65 $\mu g.m^{-3}$) and a mixture of SO_2 and NH_3 (45 and 65 $\mu g.m^{-3}$, respectively). Exposure to 112 $\mu g.m^{-3}$ SO_2 caused a small irreversible inhibition of maximum CO_2 assimilation rate and stomatal conductance.
Leaves from this treatment showed also a slightly higher quantum yield and dark respiration.

Furthermore, the fluorescence measurements indicated that the Calvin cycle of these leaves was more rapidly activated after transition from dark to light. No satisfactory explanation for these effects can be given so far. The positive effect of the NH_3 exposure on CO_2 assimilation, stomatal conductance and NH_3 uptake into the leaves was counteracted by the low SO_2 concentration. No influence on the NH_3 transport into leaves was observed. This implies that in almost any case the NH_3 flux into leaves can be estimated from data on the boundary layer and stomatal resistance for H_2O transfer and NH_3-concentration at the leaf surface. In contrast, a less clear correlation between SO_2 uptake into leaves and stomatal conductance was found. The measured flux appeared to be larger than can be calculated from the boundary layer and stomatal resistance for H_2O, suggesting a lower diffusion resistance of this gas. An explanation might be that SO_2 molecules moving into the leaf follow a shorter pathway than effluxing H_2O molecules due to a reaction of SO_2 inside the stomatal pore or with cells in the substomatal cavity, close to the stomatal pore.

In *chapter 7* the influence of wind velocity, air temperature and vapor pressure deficit of the air on NH_3 and SO_2 transfer is described. At wind velocities below 0.3 $m.s^{-1}$ the boundary layer resistances for H_2O, NH_3 and SO_2 were found to be larger than can be calculated from theory. The results suggested a transition in the properties of the leaf boundary layer at a wind velocity of 0.3-0.4 $m.s^{-1}$.

It appeared that at higher wind velocities the theoretical functions may give a good approximation of this resistance provided that a larger correction for the roughness of the leaf surface is made.

The NH_3 flux and in particular the SO_2 flux into leaves strongly increased at a VPD decline. The increase of the NH_3 flux could be attributed to an increase of the stomatal conductance. The increase of the stomatal conductance was found to be independent of net CO_2 assimilation, as a result of which the internal CO_2 concentration increased. However, a different result for NH_3 uptake was obtained at a low VPD and temperatures below 15 °C. Under those conditions the measured NH_3 flux appeared to be larger than can be calculated from the stomatal conductance.

The results of this study also show a less clear correlation between the SO_2 flux and stomatal conductance. In addition the results show that the difference between measured and calculated flux strongly increased at a low VPD.

It is postulated that a low VPD promotes the reaction of SO_2 inside the stomatal pore by an effect on the formation of a water film covering the walls of the stomatal pore. This might also explain the discrepancy observed for NH_3 uptake at a low VPD and temperature, since the solubility of NH_3 strongly increases at a low temperature. However, these assumptions are still very speculative and more research is required to obtain conclusive evidence.

A general discussion is given in *chapter 8*. The application of current leaf resistance models for the estimation of NH_3 and SO_2 uptake by leaves is evaluated. Also the physiological effects caused by a long term exposure to low concentrations of NH_3 and SO_2 are discussed.

SAMENVATTING

De depositie van verzurende stoffen in Nederland wijkt in belangrijke mate af van die in andere landen van West en Noord Europa. Behalve een belangrijke depositie van zwaveldioxide (SO_2) en stikstofoxiden (NO en NO_2) vindt er ook een belangrijke depositie van ammoniak (NH_3) plaats, welke voor ongeveer éénderde bijdraagt tot de totale depositie van verzurende stoffen in Nederland. Deze depositie is bijna geheel afkomstig uit Nederland zelf, in tegenstelling tot de SO_2 en NO_x depositie. Het merendeel (ca. 90%) is afkomstig van agrarische bedrijven. In gebieden met veel intensieve veehouderij bedrijven is de totale depositie van stikstof vanuit de atmosfeer 10 tot 20 keer hoger dan de stikstof depositie van 5-10 $kg.ha^{-1}.jaar^{-1}$ die bij natuurlijke achtergrondsconcentraties van NH_3 plaatsvindt.

In deze gebieden wordt tevens een sterke vermindering van de vitaliteit van de bossen waargenomen. Een ander duidelijk waarneembaar effekt is de sterk toegenomen vergrassing van de heidevelden. Onderzoekingen hebben inmiddels een duidelijk causaal verband aangetoond tussen de hoge NH_3 emissie in deze gebieden en de waargenomen effekten op de vegetatie. Veel van de Nederlandse naaldbossen bevinden zich op voormalige zand- en heidegronden. Deze gronden zijn zwakzuur en nutriënten arm en daardoor gevoelig voor verstoringen van buitenaf. Vandaar dat de effekten voornamelijk worden toegeschreven aan een invloed van NH_3 depositie op de bodem. Echter, aangetoond is dat ook zwaveldioxide (SO_2) een belangrijke rol speelt. Beide gassen stimuleren hun depositie door een reactie op het bladoppervlak, ten gevolge waarvan ammoniumsulfaat wordt gevormd. In nitrificerende gronden wordt het ammonium-sulfaat dat via afspoeling in de bodem terecht komt, snel geoxideerd tot salpeter- en zwavelzuur, hetgeen kan leiden tot ernstige bodemverzuring en het vrijkomen van het toxische aluminium van het bodemcomplex. De hoge stikstof-depositie kan eveneens leiden tot een verstoring van de nutriëntenbalans in bladeren. Door een verhoogd stikstofgehalte in bladeren ontstaat er een relatief tekort aan andere nutrinten, zoals magnesium, kalium en fosfor.

De "ammoniakproblematiek" vormde de aanleiding tot het onderzoek van dit proefschrift. Onderzoek werd gedaan naar de opname van ammoniak (NH_3) en zwaveldioxide (SO_2) door bladeren en naar de relatie tussen opname, foto-synthese en geleiding van de stomata (huidmondjes) van het blad.

Het onderzoek vond onder laboratorium omstandigheden plaats. De analy-semethode was voor een belangrijk deel ontleend aan methoden die in het

fotosynthese-onderzoek gebruikelijk zijn. Het transportproces van NH_3 of SO_2 vanuit de atmosfeer grenzend aan het bladoppervlak tot in het blad werd geanalyseerd aan de hand van een weerstandsschema. Dit schema wordt beschreven in *hoofdstuk 3*. De proeven werden uitgevoerd met planten van de boon (*Phaseolus vulgaris L.*) en populieren stekken (*Populus euramericana L.*). De opname van afzonderlijke bladeren werd bepaald. Hiervoor werd een bladkamer gebruikt waarin een blad aan een plant wordt ingesloten. Een nieuwe bladkamer werd speciaal voor dit onderzoek ontwikkeld, aangezien de bestaande bladkamers niet geschikt bleken te zijn voor een nauwkeurige analyse van de NH_3- en SO_2-opname door een blad. De bladkamer is onderdeel van een zogenaamd open begassingssysteem: Door de kamer wordt continu geconditioneerde lucht met een bepaalde NH_3 of SO_2 concentratie geleid. Uit het verschil in concentratie tussen de in- en uitgaande lucht van de bladkamer kan de opname worden berekend. Op eenzelfde manier wordt, tegelijkertijd met de NH_3- of SO_2-opname, de transpiratie en fotosynthese van het blad gemeten. De opname kan zodoende direkt worden gerelateerd aan het gedrag van de stomata en fotosynthese van de bladeren.

Hoofdstuk 2 geeft een beschrijving van de bouw en eigenschappen van de bladkamer. De wanden en andere oppervlakken in de bladkamer zijn geheel van teflon en glas uitgevoerd. Beide materialen worden geacht inert te zijn m.b.t. een groot aantal luchtverontreinigende gassen. Desondanks wordt er adsorptie van NH_3 en SO_2 waargenomen. Ook treedt er een klein permanent verlies van O_3 aan de wanden van de bladkamer op. Daarentegen vindt er geen reactie met NO en NO_2 plaats. De adsorptie van NH_3 en SO_2 is sterk afhankelijk van de luchtvochtigheid in de kamer, hetgeen wijst op een belangrijke rol van water in het adsorptieproces.
Verondersteld wordt dat dit water door elk oppervlak wordt geadsorbeerd, hetgeen resulteert in de vorming van een dunne waterlaag of waterfilm bij een hoge luchtvochtigheid. De aanwezigheid van een waterfilm zou een beperking kunnen inhouden voor de verdere ontwikkeling van bladkamers en andere apparatuur voor het toepassen en meten van lage concentraties van NH_3, SO_2 en andere oplosbare gassen. Adsorptie van NH_3 wordt echter ook in droge lucht waargenomen. Dit zou een gevolg kunnen zijn van een specifieke adsorptie aan glas of teflon, of van een relatief hoge permeabiliteit van teflon voor NH_3.

Met de bladkamer kunnen temperaturen en luchtvochtigheden in een relatief groot bereik worden onderzocht. Tevens kan een windsnelheid van 0 tot 3 $m.s^{-1}$ over het bladoppervlak worden ingesteld, zodat de relatie tussen windsnelheid en grenslaagweerstand van een blad nauwkeurig kan worden onderzocht.

Bovendien hebben infraroodmetingen aangetoond dat in vergelijking met de tot nu toe gebruikte bladkamers een betere controle van de temperatuurverdeling over het blad mogelijk is. Dit is van belang voor een nauwkeurige bepaling van de stomataire weerstand van het blad.

Uit de resultaten van *hoofdstuk 3* kan worden afgeleid dat bladeren een grote opnamecapaciteit voor NH_3 bezitten. Zelfs bij hoge NH_3 concentraties (400 $\mu g.m^{-3}$) wordt een lineair verband tussen concentratie en NH_3 opname in het blad gevonden. Een schematische voorstelling van het verwerkingsmechanisme van NH_3 in het blad wordt in hoofdstuk 5 gegeven. De opgenomen NH_3 veroorzaakt evenwel geen meetbaar effekt op de fotosynthese van de bladeren. Beneden een concentratie van 5 $\mu g.m^{-3}$ vindt er geen duidelijke NH_3 opname plaats, hetgeen wijst op de mogelijke aanwezigheid van een compensatiepunt d.i.: een concentratie waarbij geen netto NH_3 opname in het blad plaatsvindt. Echter, een duidelijk bewijs hiervoor ontbreekt nog. De analyse van de transportweerstanden toont aan, dat het NH_3 transport vanuit de atmosfeer grenzend aan het bladoppervlak tot in het blad afhankelijk is van de grenslaagweerstand en de weerstand van de stomata van het blad. Het transport door de cuticula van het blad is te verwaarlozen. Verder wordt het NH_3 transport in het blad niet gehinderd door een interne weerstand.

Het blijkt dat de adsorptie van NH_3 en SO_2 aan het bladoppervlak sterk toeneemt met de luchtvochtigheid (*hoofdstuk 4*).
Bovendien wordt gevonden dat de geadsorbeerde hoeveelheden van NH_3 en in mindere mate, die van SO_2, evenredig zijn met de concentratie in de lucht. Daarentegen is er geen duidelijke invloed van de temperatuur merkbaar; de temperatuur in het bestudeerde gebied van 15 tot 26 °C blijkt alleen via een effekt op het dampdruktekort de adsorptie aan het bladoppervlak te beinvloeden. Uit deze resultaten kan men afleiden dat 'water' een grote rol speelt in het adsorptieproces.

Ook nu zou men de aanwezigheid van een waterfilm kunnen veronderstellen. Echter, er zijn aanwijzingen dat het adsorptieproces aan het bladoppervlak volgens een ingewikkelder mechanisme verloopt. Een alternatieve hypothese wordt in dit hoofdstuk gegeven. De adsorptie van SO_2 is, uitgedrukt per concentratie eenheid in de lucht, ongeveer tweemaal zo groot als de NH_3 adsorptie. De adsorptie van NH_3 en SO_2 neemt achtereenvolgens met een factor 4 en 2 toe, wanneer beide gassen in de lucht aanwezig zijn. In dit geval kan de adsorptie van NH_3, en vooral die van SO_2, een naar verhouding belangrijk deel uitmaken van totale opname van deze gassen door de bladeren.

Noch een duidelijke desorptie, noch een duidelijk transport van NH_3 of SO_2 via de cuticula in het blad wordt waargenomen. Hieruit kan worden afgeleid dat de geadsorbeerde hoeveelheden voornamelijk met de cuticula van het blad verbonden blijven en blijkbaar alleen door afspoeling kunnen worden verwijderd.

Ook is onderzoek gedaan naar de fysiologische effekten veroorzaakt door een langdurige begassing met lage NH_3 concentraties (50 en 100 $\mu g.m^{-3}$) (*hoofdstuk 5*). Het onderzoek is uitgevoerd met populieren stekken die gedurende 6 of 8 weken werden begast. De resultaten van hoofdstuk 5 laten zien, dat een langdurige begassing met 100 $\mu g.m^{-3}$ NH_3 een positief effekt kan hebben op de fotosynthese van bladeren. Met name de CO_2 assimilatie bij lichtverzadiging wordt bevorderd. Volgens de fluorescentie metingen is dit een gevolg van een verhoogde activiteit van de donkerreactie. Daarentegen is er geen duidelijke invloed op de 'quantum yield' voor CO_2-fixatie waarneembaar. Behalve een grotere fotosynthese-activiteit hebben deze bladeren een grotere stomataire geleiding en NH_3 opname dan bladeren die zijn begast met 50 $\mu g.m^{-3}$ NH_3 of met koolstof gefilterde buitenlucht.

In een daaropvolgend onderzoek (*hoofdstuk 6*) werden populieren stekken gedurende 7 weken blootgesteld aan SO_2 (45 en 112 $\mu g.m^{-3}$), NH_3 (65 $\mu g.m^{-3}$) en een combinatie van SO_2 en NH_3 (45 en 65 $\mu g.m^{-3}$, resp.). Het blijkt dat blootstelling aan 112 $\mu g.m^{-3}$ SO_2 een kleine, irreversibele remming van de maximum CO_2 assimilatie en stomataire geleiding veroorzaakt. Ook wordt bij deze bladeren een iets hogere 'quantum yield' en donkerrespiratie waargenomen. Verder kan uit de fluorescentie metingen worden afgeleid, dat de donkerreactie van deze bladeren sneller wordt geactiveerd na een overgang van donker naar licht. Echter, geen bevredigende verklaring voor deze effecten kan worden gegeven. Het positief effekt van de NH_3 expositie op de fotosynthese, stomataire geleiding en NH_3 opname van bladeren wordt geneutralizeerd door de lage SO_2 concentratie.

De verschillende begassingen veroorzaken geen merkbare verandering in de relatie tussen stomataire geleiding en CO_2 assimilatie. Dit betekent dat in principe deze relatie kan worden gebruikt om uit gegevens over de netto CO_2 assimilatie van bladeren de stomataire geleiding en daarmee ook de opname van NH_3 en SO_2 in het blad te berekenen. Ook hebben de begassingen geen merkbare invloed op het transportproces van NH_3 in het blad. Dus, de NH_3-opname in het blad kan worden berekend uit gegevens over de grenslaagweerstand en stomataire weerstand van bladeren en de NH_3-concentratie aan het bladoppervlak, ongeacht

of de bladeren worden begast met NH_3 of met een combinatie van NH_3 èn SO_2. Daarentegen wordt een minder duidelijke relatie tussen de SO_2-opname en stomataire geleiding gevonden. De gemeten flux in het blad blijkt groter te zijn dan kan worden berekend uit gegevens over de grenslaag en stomataire weerstand van het blad. Dit zou betekenen dat er een lagere weerstand voor SO_2 diffusie is. Een verklaring zou kunnen zijn dat SO_2 moleculen die via de stomataire opening in het blad diffunderen, een kortere weg afleggen dan uit het blad diffunderende H_2O moleculen. Deze kortere weg zou het gevolg kunnen zijn van een reactie van SO_2 met de wanden in de stomataire opening of hieraan grenzende cellen in de substomataire holte.

In *hoofdstuk 7* wordt de invloed van windsnelheid, luchtvochtigheid en temperatuur op het transport van NH_3 en SO_2 in het blad beschreven. Bij lage windsnelheden (<0.3 $m.s^{-1}$) is de grenslaagweerstand voor H_2O, NH_3 of SO_2 transport groter dan kan worden berekend volgens formules afgeleid uit metingen met bladreplica's. Een overgang in de eigenschappen van de grenslaagweerstand van een blad wordt waargenomen bij een windsnelheid tussen 0.3 en 0.4 $m.s^{-1}$. Het blijkt dat bij hogere windsnelheden de bestaande formules een goede schatting geven van de grenslaagweerstand van een blad, mits een grotere correctie voor de ruwheid van het bladoppervlak wordt gemaakt.

Een sterke toename van de NH_3 flux en vooral van de SO_2 flux in het blad wordt gevonden bij een stijging van de luchtvochtigheid. De toename van de NH_3 flux kan volledig worden toegeschreven aan een toename van de stomataire geleiding van het blad bij een hogere luchtvochtigheid. De toename van de stomataire geleiding is onafhankelijk van de CO_2 assimilatie, hetgeen een toename van de interne CO_2 concentratie tot gevolg heeft. Echter, een afwijkend resultaat voor de NH_3 opname wordt verkregen bij een laag dampdruktekort en temperaturen lager dan 15 °C. In dit geval is de gemeten NH_3 flux groter dan kan worden berekend uit de stomataire geleiding.

Ook de resultaten van dit onderzoek laten een minder duidelijke correlatie tussen de SO_2 flux en stomataire geleiding zien. Bovendien wordt waargenomen dat het verschil tussen de gemeten en berekende SO_2 flux sterk toeneemt met een daling van het dampdruktekort. Verondersteld wordt dat een klein dampdruktekort de reactie van SO_2 in de stomataire opening bevordert door een effekt op de vorming van een 'waterfilm' in de stomataire opening. De aanwezigheid van een waterfilm zou ook het verschil in de gemeten en berekende NH_3 flux bij een lage temperatuur kunnen verklaren. Immers, de oplosbaarheid van NH_3 in water neemt sterk toe bij een lage temperatuur.

Echter, voorlopig hebben deze veronderstellingen nog een sterk speculatief karakter. Verder onderzoek is nodig om meer concrete bewijzen in handen te krijgen.

In het laatste hoofdstuk (*hoofdstuk 8*) wordt de toepassing van bestaande weerstandsmodellen voor de berekening van de NH_3 en SO_2 opname door bladeren geëvalueerd. Ook wordt nader ingegaan op de fysiologische effekten veroorzaakt door een langdurige blootstelling aan lage NH_3 en SO_2 concentraties.

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Curriculum vitae

Lambertus Willem Adriaan van Hove werd op 28 april 1956 te Hillegom geboren. In 1974 behaalde hij het Atheneum-B diploma aan het Chr. Atheneum "*Adriaen Pauw*" te Heemstede. In hetzelfde jaar begon hij met de studie aan de Landbouwhogeschool te Wageningen waaraan hij in 1982 (*met lof*) afstudeerde in de studierichting: tuinbouwplantenteelt. Het doctoraal vakkenpakket bestond uit de hoofdvakken, tuinbouwplantenteelt en plantenfysiologie, en het bijvak fytopathologie. Tevens behaalde hij een onderwijsaantekening voor biologie. Na het vervullen van de militaire dienstplicht kwam hij in 1983 als wetenschappelijk assistent in tijdelijke dienst bij de vakgroep Luchthygiëne en -verontreiniging van de Landbouwuniversiteit. Vanaf augustus 1988 is hij werkzaam op een vervolgprojekt (projekt 110) van het Nationaal Programma Verzuringsonderzoek. Hierin wordt onderzoek gedaan naar de opname van ammoniak, zwaveldioxide en stikstofdioxide door naalden van de Douglas spar (*Pseudotsuga menziesii*) en de fysiologische effekten die hiervan het gevolg zijn.