

Ammonia Flux between Oilseed Rape Plants and the Atmosphere in Response to Changes in Leaf Temperature, Light Intensity, and Air Humidity¹

Interactions with Leaf Conductance and Apoplastic NH₄⁺ and H⁺ Concentrations

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NH₃ exchange between oilseed rape (*Brassica napus*) plants and the atmosphere was examined at realistic ambient NH₃ levels under controlled environmental conditions. Different leaf conductances to NH₃ diffusion were obtained by changing leaf temperature (10 to 40°C), light intensity (0 to 600 μmol m⁻² s⁻¹), and air humidity (20 to 80%), respectively. NH₃ adsorption to the cuticle with subsequent NH₃ transport through the epidermis had no significant effect on the uptake of atmospheric NH₃, even at 80% relative air humidity. NH₃ fluxes increased linearly with leaf conductance when light intensities were increased from 0 to 600 μmol m⁻² s⁻¹. Increasing leaf temperatures from 10 to 35°C caused an exponential increase in NH₃ emission from plants exposed to low ambient NH₃ concentrations, indicating that leaf conductance was not the only factor responding to the temperature increase. The exponential relationship between NH₃ emission and temperature was closely matched by the temperature dependence of the mole fraction of gaseous NH₃ above the leaf apoplast (NH₃ compensation point), as calculated on the basis of NH₄⁺ and H⁺ concentrations in the leaf apoplast at the different leaf temperatures. NH₃ fumigation experiments showed that an increase in leaf temperature may cause a plant to switch from being a strong sink for atmospheric NH₃ to being a significant NH₃ source. In addition to leaf temperature, the size of the NH₃ compensation point depended on plant N status and was related to plant ontogeny.

NH₃ is increasingly becoming recognized as an important atmospheric pollutant with impacts on atmospheric chemistry, on acidification of ecosystems, and on the stability and biodiversity of terrestrial oligotrophic biotopes (Bobbink et al., 1992; Sutton et al., 1992).

NH₃ emission from natural land is probably the most uncertain factor in the compilation of a global NH₃ budget (Dentener and Crutzen, 1994). Vegetation can act as both source and sink for atmospheric NH₃. NH₃ emission occurs if the mole fraction of NH₃ in the atmosphere is lower than the mole fraction of gaseous NH₃ above the mesophyll cell walls in the substomatal cavity (the NH₃ compensation point), whereas in the opposite case, NH₃ absorption takes place (Husted and Schjoerring, 1995a, 1995b). Plants growing in low N input ecosystems such as moorlands and forests generally seem to have NH₃ compensation points close to 0 (Langford and Fehsenfeld, 1992; Sutton et al., 1992; Kesselmeier et al., 1993), whereas agricultural crop species have NH₃ compensation points ranging from 1 to 6 nmol NH₃ mol⁻¹ air under field conditions (Dabney and Bouldin, 1990; Schjoerring et al., 1993; Sutton et al., 1995; Yamulki et al., 1996) as well as in controlled environments (Farquhar et al., 1980; Husted and Schjoerring, 1995a, 1995b). Exceptionally high NH₃ compensation points, exceeding 40 nmol NH₃ mol⁻¹ air during senescence, were reported for *Triticum aestivum* by Morgan and Parton (1989). However, their measurements were biased because of very high NH₃ background concentrations.

The NH₃ compensation point changes with plant ontogeny and differs between cultivars of barley (Husted et al., 1996). Plants having access to NH₄⁺ in the root medium seem to have a higher NH₃ compensation point than plants absorbing NO₃⁻ (Mattsson and Schjoerring, 1996). Very little information is available concerning the influence of various climatic parameters on the NH₃ compensation point and plant-atmosphere fluxes.

The objective of this work was to investigate the effect of leaf temperature, PPFD, and air humidity on the NH₃ compensation point and the NH₃ exchange between oilseed rape (*Brassica napus* L.) plants and the atmosphere. Experiments were carried out with plants growing under controlled environmental conditions, including atmospheric NH₃ concentrations, which were measured continuously by the use of on-line NH₃ monitors. The measurements of plant-atmosphere NH₃ exchange fluxes were combined with a sampling of leaf apoplastic solution, and used for determination of apoplastic H⁺ and NH₄⁺ concentrations. Calculated and experimentally determined NH₃ compensation points were compared and found to be in good agreement.

Abbreviations: NH_{3(aq)}, aqueous ammonia; RH, relative air humidity; χ_{NH₃}, NH₃ compensation point.

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MATERIALS AND METHODS

Seeds of oilseed rape (*Brassica napus* L. cv Global) were germinated in the dark for 4 d before planting in 0.0025-m³ self-watering pots (three plants per pot) filled with a growth medium containing 0.15 (2N), 0.30 (4N), or 0.50 (7N) mol of N and supplemented with additional nutrients as described by Husted and Schjoerring (1995b). Plants were grown in a greenhouse at 25 ± 5°C (70 ± 5% RH) under a 16-h photoperiod with PPFD > 400 μmol m⁻² s⁻¹.

Effect of Environmental Parameters on NH₃ Exchange

The effect of leaf temperature and RH on the NH₃ exchange was examined in 0.075-m³ cuvettes, which restricted the plant sizes to less than 80 cm in height, i.e. plants in vegetative growth stages. The plant cuvettes were made of polycarbonate coated with Margard (General Electric), ensuring a low water adsorption and an unchanged spectral composition of the photosynthetically active light (Husted and Schjoerring, 1995a). The plant cuvette was mounted in a growth chamber with 400-W HQI lamps (Power star, Osram, Stuttgart, Germany), which could be turned on and off individually, allowing stepwise control of PPFD in the range 0 to 600 μmol m⁻² s⁻¹ without changing the spectral properties of the light.

The air temperature in the chamber was regulated between 10 and 40°C. The incoming air was adjusted to the desired air humidity (20–80% RH) by passing through brine solutions and was subsequently cleaned with expanded aluminum hydroxide (Purafil, Atlanta, GA) and acid-coated charcoal (type WS425520; Chemviron Carbon, Neu Isenberg, Germany), ensuring <0.01 nmol NH₃ mol⁻¹ air. The incoming air (0.040 m³ min⁻¹) was mixed with NH₃ to preset values on flow controllers (model 1100, KDG Mobrey, Crawley, UK) enabling investigation of the plant response to NH₃ enrichment at realistic ambient levels (0–25 nmol NH₃ mol⁻¹).

Pneumatic valves (Mikrolab, Aarhus, Denmark) distributed air from the cuvette inlet and outlet to an NH₃ continuous flow denuder (Amanda, model 1263B, ECN, Petten, The Netherlands; Wyers et al., 1993), which was connected to a computer for data logging. The detection limit of the system (denuder plus cuvette) was 0.25 nmol NH₃ mol⁻¹ air, and values below this level were designated as NH₃-free air. Simultaneously, CO₂ and water vapor were measured by IR absorption (Ciras-1, PP-Systems, Hertshire, UK). When temperature, PPFD, RH, or NH₃ concentrations were changed, the system was allowed to adjust for 45 min before a new reading was recorded. Leaf temperatures were measured by small thermocouples mounted on the abaxial leaf surface (Testo 925, Testo, Lenzkirch, Germany). To correct for any possible effect of NH₃ exchange with the soil, control experiments were performed with soil-filled pots. However, the interference was usually negligible.

Determination of NH₃ Compensation Points

NH₃ compensation points were determined at three growth stages (early vegetative growth, anthesis, and middle senescence) by exposing five groups of plants to atmo-

spheric NH₃ mole fractions of 0, 5, 10, 15, and 25 nmol mol⁻¹. The NH₃ fumigation took place in five large cuvettes (0.45 m³) placed in growth chambers with an air temperature of 25 ± 1°C, an RH of 65 ± 5%, and a PPFD of 550 ± 50 μmol m⁻² s⁻¹ in a photoperiod of 16 h d⁻¹ (Husted and Schjoerring, 1995a). The use of large cuvettes allowed experiments to be performed for a full growth cycle with plants up to a height of 180 cm. NH₃ concentrations in the incoming and outgoing air were, after conversion to NO, measured by chemiluminescence in a modified NH₃ monitor (Thermo Environmental Instruments, Franklin, MA) with a detection limit of 0.9 nmol NH₃ mol⁻¹ air (cuvette plus NH₃ monitor). The measurements were carried out over 5 to 7 d, and two compensation points per d were determined in the light period according to principles described in detail by Husted and Schjoerring (1995a). The relationship between NH₃ flux and NH₃ fumigation level was linear, allowing calculation by linear regression of the NH₃ mole fraction at which the net NH₃ flux equaled 0. *SE*s of the estimated NH₃ compensation points were calculated according to statistical procedures described by Husted et al. (1996).

Determination of Apoplastic NH₄⁺ and H⁺ Concentrations

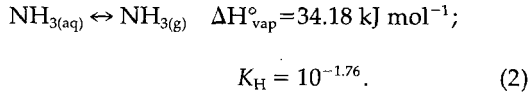
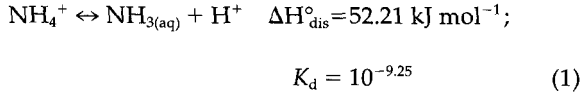
Leaves were sampled at four random positions at approximately one-half plant height. Leaf segments of 25 × 50 mm were infiltrated with a 350-mosmol isotonic sorbitol solution (280 mM) in a 50-mL syringe mounted on a hydraulic infiltrator (Knapp Micro Fluid, Neutraubling, Germany). The infiltrator was programmed to expose the leaf discs to 4 atm of pressure and vacuum for 10 s and to repeat the procedure eight times, ensuring full infiltration. The leaf segments were then centrifuged at 2000g for 15 min at 10°C, and the apoplastic extracts (approximately 75 μL) were collected in microtubes. The total sum of NH_{3(aq)} and NH₄⁺ was determined on a gas membrane coupled to a flow injection system with a detection limit of 1 μM (FIA Star 5020, Tecator, Helsingborg, Sweden). The pH values were determined directly in the tubes by a microelectrode (type 60236, Metrohm, Herisau, Switzerland). To correct for the dilution of NH₄⁺ and H⁺ by infiltration the apoplastic air and water volumes were determined by infiltration with high viscosity polydimethylsiloxane (Dow Corning, Poole, UK) and 50 μM indigo carmine, respectively, according to principles described in detail by Husted and Schjoerring (1995b).

Bulk tissue NH₄⁺ was determined by drying plant material at 80°C for 12 h, followed by extraction of NH₄⁺ with 0.025 M H₂SO₄ for 2 h. NH₄⁺ in the extracts was analyzed by the flow injection system described above. Comparison of extractable NH₄⁺ in oven-dried leaves (80°C for 12 h) with that in lyophilized leaves showed no significant difference between the methods.

Calculation of NH₃ Compensation Points Based on Apoplastic NH₄⁺ and H⁺ Concentrations

The NH₃ compensation point (χ_{NH_3}) is equal to the mole fraction of gaseous NH₃ above the water film in the meso-

phyllic cell walls (apoplast) and may be calculated on the basis of apoplastic NH₄⁺ and H⁺ concentrations using the following equilibria:



The thermodynamic constants given above are valid only at 25°C (298.15 K) and at an ionic strength equal to 0 (Atkins, 1990). Since the physiological ionic strength in the apoplast normally ranges between 14 and 28 mM (Cosgrove and Cleland, 1983; Speer and Kaiser, 1991) K_d was adjusted to an ionic strength of approximately 20 mM by the extended Debye-Hückel equation (Atkins, 1990), giving $K_d' = 10^{-9.32}$.

The total concentration of NH_{3(aq)} and NH₄⁺ in the liquid phase is given by $C_{\text{tot}} = [\text{NH}_4^+] + [\text{NH}_3]_{(\text{aq})}$. Using the mass balance equations for equilibria 1 and 2 gives:

$$[\text{NH}_3]_{\text{aq}} = C_{\text{tot}} \times \frac{K_d}{K_d + (\text{H}^+)} \quad (3)$$

$$\chi_{\text{NH}_3} = [\text{NH}_3]_{\text{aq}} \times K_H \quad (4)$$

After these two equations are combined, the NH₃ compensation point at 25°C (298.15 K) can be calculated:

$$\chi_{\text{NH}_3} = K_H \times \frac{K_d \times C_{\text{tot}}}{K_d + (\text{H}^+)} \quad (5)$$

The enthalpy (ΔH) for the above-mentioned reactions (Eqs. 1 and 2) is essentially independent of temperature below 40°C, and the variation in ΔH is insignificant at ionic strengths below 100 mM (Stumm and Morgan, 1981). K_d and K_H may therefore be corrected for temperature effects using a slightly modified Clausius-Clapeyron equation (Atkins, 1990):

$$\ln \frac{\chi_2}{\chi_1} = \frac{\Delta H_{\text{dis}}^\circ + \Delta H_{\text{vap}}^\circ}{R} \times \left(\frac{1}{T_1} - \frac{1}{T_2} \right), \quad (6)$$

where χ_1 is the actual NH₃ compensation point at temperature T_1 and χ_2 is the requested NH₃ compensation point at a new temperature T_2 . R designates the gas constant = 8.31 J K⁻¹ mol⁻¹, and $\Delta H_{\text{dis}}^\circ$ and $\Delta H_{\text{vap}}^\circ$ are the enthalpy of NH₄⁺ dissociation and NH₃ vaporization, respectively.

Calculation of Leaf Conductances for H₂O and NH₃

Leaf conductances for water vapor were calculated on the basis of transpiration rates at preset mole fractions of

water vapor in plant cuvettes using the following equation:

$$g = \frac{F}{X_{\text{in}} - X_{\text{atm}}}, \quad (7)$$

where g is the conductivity (mol m⁻² s⁻¹), F is the flux (mol m⁻² s⁻¹), and $X_{\text{in}} - X_{\text{atm}}$ is the gradient in mole fractions (mol mol⁻¹) of water vapor between intercellular air and the surrounding atmosphere. The intercellular mole fraction of water vapor was calculated at the actual leaf temperature from tabulated values, assuming that the air space was nearly saturated (99%). Leaf conductances for NH₃ were calculated analogously to those of H₂O (Eq. 7), using experimentally determined NH₃ fluxes in plants exposed to an NH₃-enriched atmosphere with a preset mole fraction and with the mole fraction of NH₃ in the intercellular air space (NH₃ compensation point) being calculated on the basis of apoplastic H⁺ and NH₄⁺ concentrations.

Prediction of NH₃ Fluxes on the Basis of NH₃ Compensation Points and Leaf Conductance

NH₃ fluxes were predicted on the basis of transpiration data and NH₃ compensation points using the following equation:

$$F_{\text{NH}_3} = g_{\text{H}_2\text{O}} \times 0.92 \times (\chi_{\text{NH}_3} - X_{\text{atm}}), \quad (8)$$

where the factor 0.92 corrects for the difference in diffusion coefficients of NH₃ and H₂O (Farquhar et al., 1983). It is assumed from this equation that the NH₃ exchange is independent of interactions with other gases diffusing in and out of the leaf and that the path length for gas diffusion from the intercellular air space to the leaf surface is equal for NH₃ and H₂O.

RESULTS

Effect of Light Intensity and Air Humidity on NH₃ Exchange

NH₃ absorption in *B. napus* plants exposed to 17 nmol NH₃ mol⁻¹ air increased with increasing PPFD in the range 0 to 600 μmol m⁻² s⁻¹ (Fig. 1). The response to PPFD was dependent on the RH of the air surrounding the plants. At 20% RH, NH₃ absorption responded nonlinearly to increasing PPFD, reaching maximum NH₃ uptake at a PPFD of approximately 350 μmol m⁻² s⁻¹. No saturation effect was observed at higher air humidities, causing NH₃ absorption to increase linearly ($P < 0.001$) over the entire range of PPFD values. At both 60 and 80% RH, the intercept of the linear regression line for NH₃ absorption versus PPFD did not differ from 0 ($P > 0.05$), showing no absorption of NH₃ through the leaf surface or through stomata in darkness.

Leaf conductances for NH₃ absorption as calculated from NH₃ flux data at different levels of PPFD and RH were linearly correlated with the leaf conductances for water vapor calculated on the basis of transpiration (Fig. 2). There was no significant difference between flux data obtained at 20, 60, and 80% RH, and regression analysis was consequently performed on the full data set (data not shown). The slope of the linear regression line between g_{NH_3} and

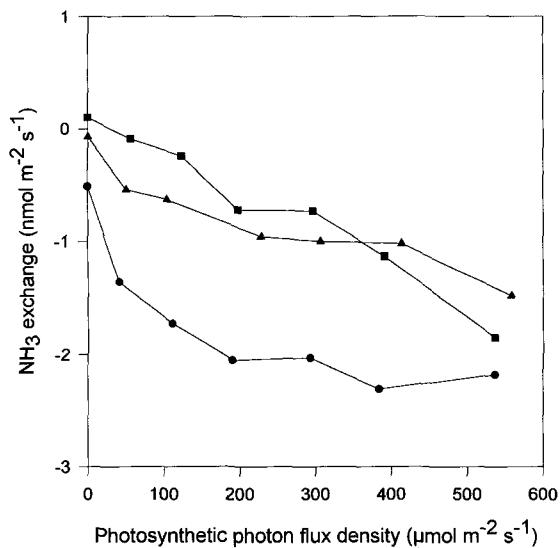


Figure 1. Effect of PPFD and RH on NH_3 exchange in vegetative growth-stage *B. napus* plants growing at a low level of N supply in the root medium (2N) and exposed to $17 \text{ nmol NH}_3 \text{ mol}^{-1}$ air at an air temperature of $25 \pm 1^\circ\text{C}$. The experiments were done in duplicate over a period of 6 d with relative ses $< 10\%$. ●, 20% RH; ■, 60% RH; ▲, 80% RH.

$g_{\text{H}_2\text{O}}$ was 0.95 ± 0.08 , which is very close to the theoretical ratio of 0.92 between diffusion coefficients for gaseous NH_3 and water vapor.

Effect of Leaf Temperature on NH_3 Exchange

Increasing leaf temperatures caused NH_3 emission to increase exponentially in relation to transpiration when

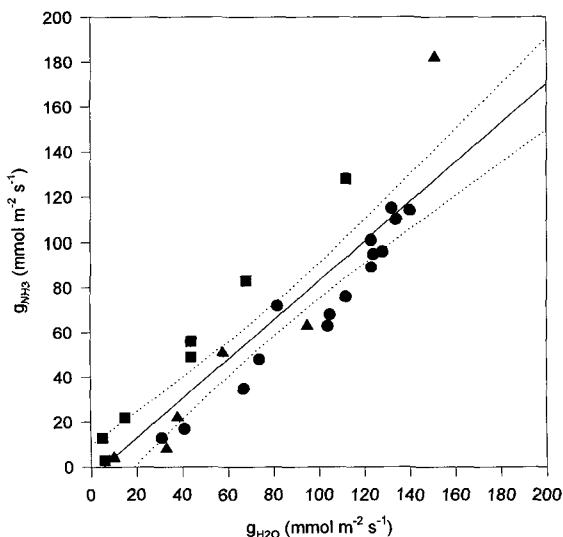


Figure 2. Leaf conductances for water vapor and NH_3 in vegetative growth-stage *B. napus* plants exposed to $15 \text{ nmol NH}_3 \text{ mol}^{-1}$ air at varying PPFD and air humidities at $25 \pm 1^\circ\text{C}$. Plants were grown at a low level of N supply (2N) in the root medium. The experiments were done in duplicate over a period of 6 d with relative ses $< 10\%$. ●, 20% RH; ■, 60% RH; ▲, 80% RH; area between dotted lines, 95% confidence limits.

plants were exposed to NH_3 -free air (Fig. 3). Effects of leaf temperature therefore deviated from effects of RH and PPFD, the two latter parameters causing a linear relationship between changes in NH_3 and H_2O transport.

Elevation of the leaf temperature was followed by a substantial exponential increase in NH_3 emission from low-, medium-, and high-N plants when exposed to NH_3 -free air (Fig. 4). The most pronounced effect occurred in high-N plants, in which NH_3 emission increased from $0.1 \text{ nmol NH}_3 \text{ m}^{-2} \text{ s}^{-1}$ at 15°C to $27 \text{ nmol NH}_3 \text{ m}^{-2} \text{ s}^{-1}$ at 32°C . NH_3 compensation points calculated on the basis of apoplastic H^+ and NH_4^+ were 0.91, 2.70, and $6.31 \text{ nmol NH}_3 \text{ mol}^{-1}$ air, for low-, medium-, and high-N plants, respectively, at 25°C . These NH_3 compensation points were corrected for the temperature effect using Equation 6, and strong correlation coefficients ($r^2 = 0.87\text{--}0.99$) were observed between NH_3 compensation points and NH_3 emissions.

NH_3 emissions predicted on the basis of NH_3 compensation points and leaf conductance for water vapor, adjusted for the difference in diffusivity between NH_3 and H_2O (Eq. 8), were strongly correlated with experimentally determined NH_3 fluxes in plants exposed to NH_3 -free air (Fig. 5). However, it was only in medium-N (4N) plants that the slope between predicted and measured NH_3 fluxes was close to the theoretical value of 1; in low- and high-N plants, the slope was 2.3 and 0.23, respectively, showing that NH_3 emission was overestimated in low-N plants and underestimated in high-N plants.

NH_3 absorption in high-N plants exposed to $15 \text{ nmol NH}_3 \text{ mol}^{-1}$ air just before anthesis decreased rapidly with increasing leaf temperature (Fig. 6). At $31 \pm 1^\circ\text{C}$, the plants started to emit NH_3 , thus indicating an NH_3 compensation point of $15 \text{ nmol NH}_3 \text{ mol}^{-1}$ air at this temperature. The

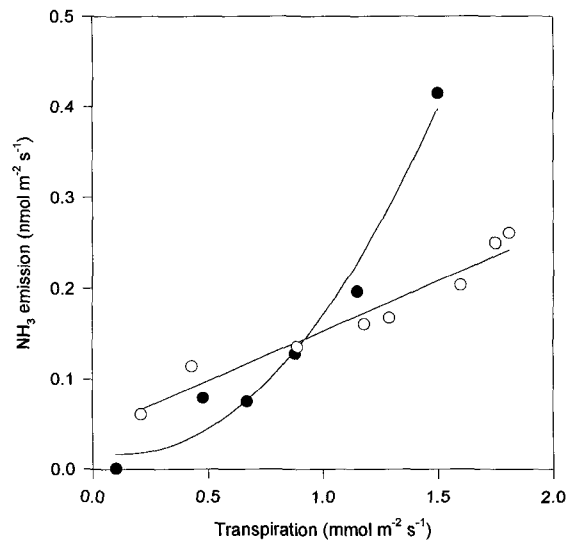


Figure 3. NH_3 emission from vegetative growth-stage *B. napus* plants exposed to NH_3 -free air at different transpiration rates obtained by varying the PPFD or the leaf temperature. Plants were grown at a high level of N supply (7N) in the root medium. The experiments were done in duplicate over a period of 6 d with relative ses $< 15\%$. ○, Light intensity; ●, leaf temperature.

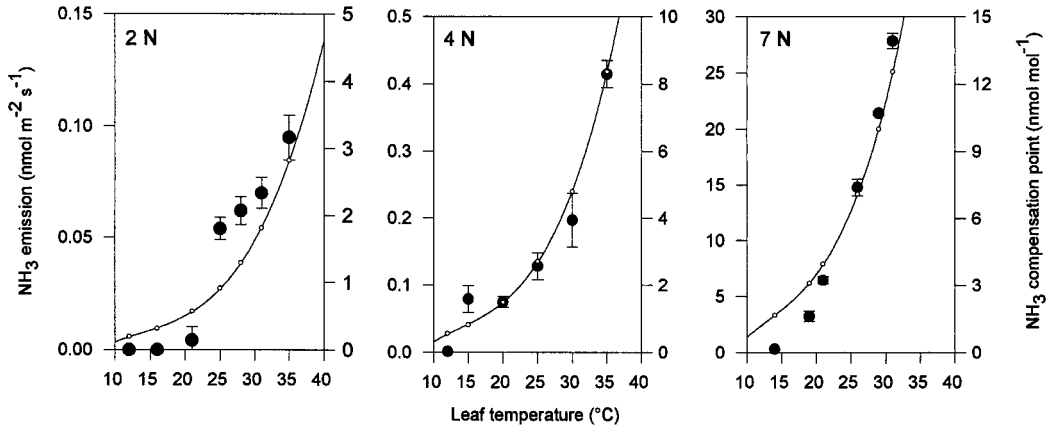


Figure 4. Effect of leaf temperature on NH₃ emission (●) and NH₃ compensation points (○) in vegetative growth-stage *B. napus* plants exposed to NH₃-free air growing at low- (2N), medium- (4N), and high- (7N) N supply. PPFD was 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and RH was 20% at 25°C. Apoplastic NH₄⁺ and pH values were measured at 25°C and used in combination with a modified Claussius-Clapeyron equation (see "Materials and Methods") for estimating NH₃ compensation points at the different leaf temperatures. The experiments were done in duplicate over 2 consecutive d with relative ses <15%.

corresponding NH₃ compensation point at 25°C was $7.7 \pm 0.8 \text{ nmol NH}_3 \text{ mol}^{-1} \text{ air}$, after correcting for temperature effects (Eq. 6). The apoplastic NH₄⁺ concentration and pH were $0.88 \pm 0.05 \text{ mM}$ and 5.90 ± 0.10 , respectively. The NH₃ compensation point estimated on the basis of these values was $5.80 \pm 1.24 \text{ nmol NH}_3 \text{ mol}^{-1} \text{ air}$ at 25°C.

Effects of Plant N Status and Age on NH₃ Exchange

Apoplastic NH₄⁺ concentrations increased at all growth stages with plant N status, and apoplastic pH was fairly constant at 5.70 ± 0.1 (Table I). Increasing plant N status also resulted in an increase in bulk leaf tissue NH₄⁺ concentrations (Table I). Bulk tissue NH₄⁺ concentrations increased much more with plant age than did apoplastic NH₄⁺ concentrations, causing their ratio to increase from

about 2 in vegetative growth-stage plants to about 15 during flowering and senescence.

NH₃ compensation points estimated on the basis of apoplastic pH and NH₄⁺ concentrations at the different growth stages increased markedly with plant age and plant N status (Table I). In the vegetative growth stage, there was a close agreement between predicted and experimentally determined NH₃ compensation points at all N levels. In later growth stages, predicted and measured NH₃ compensation points deviated in low-N plants, whereas a good agreement still was obtained in medium-N plants (Table I).

DISCUSSION

NH₃ exchange between *B. napus* plants and the atmosphere was highly dependent on the intensity of PAR in the

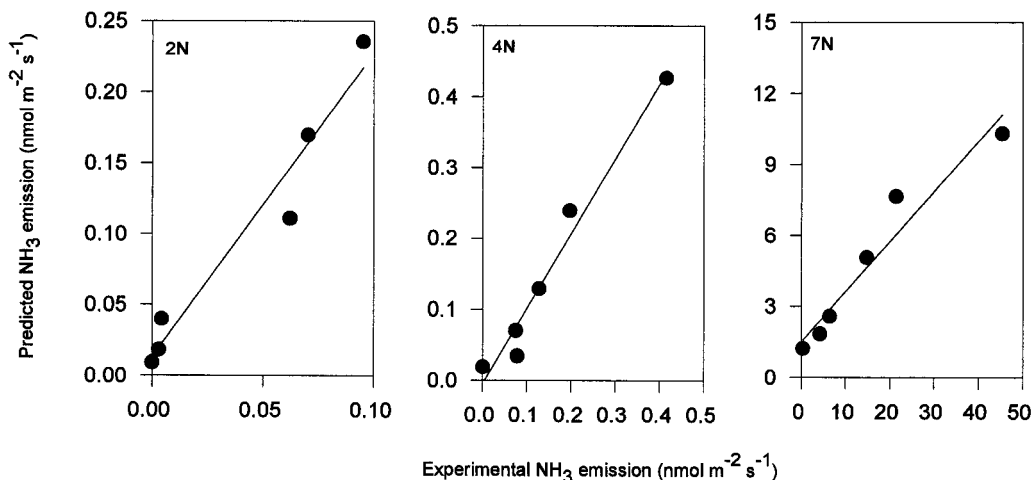


Figure 5. Predicted versus experimentally determined NH₃ emissions from low-, medium-, and high-N *B. napus* plants exposed to NH₃-free air in the vegetative growth stage. NH₃ emissions were predicted on the basis of apoplastic NH₄⁺ and H⁺ concentrations, and leaf conductances were calculated from transpiration data. The PPFD was 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and RH was 20% at 25°C. The experiments were done in duplicate over 2 consecutive d with relative ses <15%.

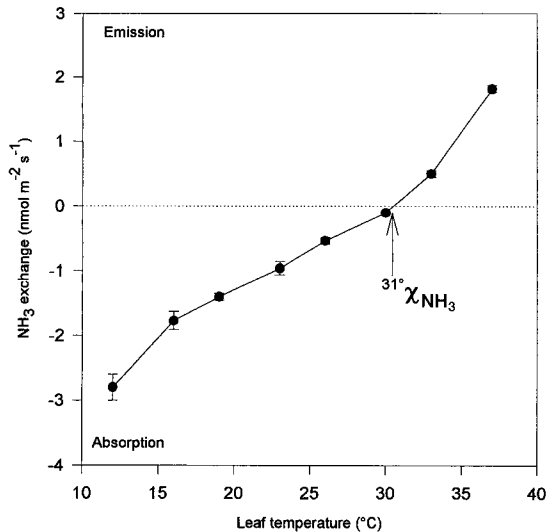


Figure 6. Effect of increasing leaf temperatures on the NH_3 exchange in *B. napus* plants exposed to $15 \text{ nmol NH}_3 \text{ mol}^{-1}$ air. Plants were growing with a high N level (7N) and measured in the vegetative growth stage. The PPFD was $550 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and the RH was 20% at 25°C. The experiments were done in duplicate over 2 consecutive d.

range 0 to $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and there was no difference in the light response at 60 and 80% RH (25°C). This, together with the fact that no NH_3 adsorption occurred in darkness (Fig. 1), indicates that a possible NH_3 adsorption to the leaf surface was not followed by a detectable NH_3 transport across the leaf cuticle into the leaf interior (Figs. 1 and 2). Several measurements have shown a large NH_3 adsorption to wet leaf surfaces because of the high solubility of NH_3 in water (Erismann and Wyers, 1993; Burkhardt and Eiden, 1994; Yamulki et al., 1996). Under field conditions, dew formation may result in a marked atmospheric NH_3 deposition to vegetation, but this phenomenon is only temporal because most of the adsorbed NH_3 will be emitted when the dew evaporates. Thus, it seems unlikely that

a significant amount of NH_3 adsorbed on the leaf surface will be assimilated and contribute to the N balance of plant canopies.

NH_3 absorption at low RH (20%) reached a maximum at a light intensity of approximately $350 \mu\text{mol m}^{-2} \text{ s}^{-1}$, whereas at higher air humidities NH_3 uptake did not saturate below $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 1). This reflects that stomata in a dry atmosphere reached full aperture at low PPFD as compared with a humid atmosphere, in which full stomatal aperture in *B. napus* normally would be obtained at 800 to $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Thus, under humid conditions the NH_3 exchange would be favored by high light intensities, whereas relatively large NH_3 exchange rates may be found under dry atmospheric conditions, even when light intensities are low (Fig. 1).

Under conditions with varied light intensity and air humidity leaf conductances for NH_3 and H_2O changed proportionally (Fig. 2), whereas at increasing temperatures NH_3 emission increased exponentially relative to water vapor transport (Fig. 3). Thus, increasing leaf conductivity at elevated temperatures could not solely be responsible for the increase in NH_3 emission. The influence that temperature has on plant-atmosphere NH_3 exchange has previously been recognized in other cases (Farquhar et al., 1983). Also, measurements over a mixed-pine, spruce-fir, and aspen forest show that atmospheric NH_3 concentrations were temperature-dependent when the air had passed the forest, although independent of temperature when the air was sampled before the forest (Langford and Fehsenfeld, 1992). Stutte and da Silva (1981) postulated that the process of volatile N loss from rice plants was a defense mechanism against ammonium toxicity under stress temperatures. However, when simple thermodynamic equations are used in combination with NH_3 compensation points derived from measurements of apoplastic NH_4^+ and H^+ concentrations, increasing NH_3 emission with increasing temperature could be explained in the present study entirely in

Table I. Apoplastic H^+ , NH_4^+ , bulk tissue NH_4^+ concentrations, and χ_{NH_3} s in leaves of *B. napus*

For all chemical analyses, $n = 3-4$ and experimentally determined compensation points were analyzed with $n = 5-7$.

Stage	Apoplastic NH_4^+ mM	Bulk Tissue NH_4^+ mM	Apoplastic pH	Calculated χ_{NH_3} at 25°C nmol mol ⁻¹	Measured χ_{NH_3} at 25°C nmol mol ⁻¹
Vegetative					
2N	0.29 ± 0.03	0.89 ± 0.01	5.28 ± 0.20	0.46 ± 0.17	0.44 ± 0.30
4N	0.64 ± 0.05	1.65 ± 0.07	5.55 ± 0.12	1.88 ± 0.47	1.72 ± 0.87
7N	2.03 ± 0.30	3.45 ± 0.04	5.52 ± 0.10	5.58 ± 1.45	4.10 ± 0.20
Anthesis					
2N	0.47 ± 0.04	7.54 ± 0.64	5.84 ± 0.06	2.70 ± 0.43	5.30 ± 0.80
4N	0.50 ± 0.05	7.84 ± 1.49	5.87 ± 0.01	3.07 ± 0.31	3.32 ± 0.90
7N	1.05 ± 0.16	12.21 ± 1.16	5.68 ± 0.12	4.17 ± 1.17	ND ^a
Senescence					
2N	0.71 ± 0.11	5.80 ± 0.84	5.73 ± 0.09	3.16 ± 0.76	<0.90
4N	0.76 ± 0.04	10.02 ± 0.47	5.68 ± 0.07	3.02 ± 0.48	2.60 ± 0.50
7N	1.36 ± 0.10	11.98 ± 1.13	6.10 ± 0.14	14.20 ± 4.12	ND

^a Not determined.

terms of effects on the equilibrium between gas phase and soluble NH₃ and NH₄⁺ in the apoplast (Fig. 4).

The marked effect of leaf temperature on plant-atmosphere NH₃ exchange is also shown by the fact that increasing leaf temperatures caused high-N plants exposed to 15 nmol mol⁻¹ to switch from being an NH₃ sink to becoming an NH₃ source at 31°C (Fig. 6). This clearly demonstrates the existence of an NH₃ compensation point, which for the investigated plants corresponded to 7.7 ± 0.8 nmol mol⁻¹ at 25°C. Calculation of the NH₃ compensation point on the basis of the apoplastic concentration of NH₄⁺ and pH in the same plants gave a value of 5.8 ± 1.2 nmol mol⁻¹, i.e. not significantly different from the measured NH₃ compensation point. However, determination of NH₃ compensation points by exposing plants to a well-defined atmospheric NH₃ concentration at increasing temperatures may be erroneous if concentrations of aqueous NH₃ and NH₄⁺ in the apoplast are not constant in the applied range of temperatures. NH₃ emissions in the present experiment were stable over a period of 3 h at a leaf temperature of 35°C (data not shown).

The high sensitivity of plant-atmosphere NH₃ exchange to leaf temperature makes information about the temperature very important when data on NH₃ compensation points are reported. Even small errors in determination of the leaf temperature may result in significant errors. As an example, the NH₃ compensation point in high-N *B. napus*, which was 7.7 nmol NH₃ mol⁻¹ air at 25°C, an absolute temperature deviation of only ±1.0°C would result in an error of 21%. Thus, determination of NH₃ compensation points under field conditions may be problematic because of large diurnal temperature variations (Sutton et al., 1995; Husted et al., 1996).

Several investigations have shown a disagreement between the theoretical and experimentally determined ratio of stomatal conductance to NH₃ and H₂O transport, and the reason for this remains to be resolved (van Hove, 1989; Husted and Schjoerring, 1995a; Husted et al., 1996). The new information obtained from the present study is that the ratio between leaf conductances for NH₃ and H₂O depends on the N status of the plants, with a relatively higher H₂O conductance in low-N plants, a similar conductance in medium-N plants, and a much higher relative NH₃ conductance in high-N plants (Fig. 5). The varying ratio between H₂O and NH₃ conductances may be due to differences in the diffusion path length of the two gases, with NH₃ absorption or emission occurring in different depths of the stomatal cavity, depending on the N status of the plant.

Apoplastic pH values were independent of the plant N status and plant ontogeny. The mean pH was 5.70 ± 0.10, which is consistent with the apoplastic pH values varying between 5.0 and 6.5 in most plant species (Grignon and Sentenac, 1991). Several experiments have shown that apoplastic pH is dependent on the N form and N concentration supplied to the roots. Nitrate nutrition leads to higher apoplastic pH compared with NH₄⁺, and in contrast to the present study, several experiments have shown increasing

pH values with increasing NO₃⁻ concentration in the root medium (Hoffmann et al., 1992; Kosegarten and Englisch, 1994; Dannel et al., 1995).

Apoplastic NH₄⁺ concentrations in *B. napus* ranged from 0.3 to 2.0 mM and were dependent on the N concentration in the growth medium and on the growth stage. Except for a previous study by Husted and Schjoerring (1995b), only one experiment has been reported in which apoplastic NH₄⁺ was measured in leaves. Mühling and Sattelmacher (1995) measured apoplastic NH₄⁺ in intact leaves of *Vicia faba* and found no detectable amounts of NH₄⁺ in the leaf apoplast, presumably because of a very efficient NH₄⁺ assimilation in this species.

In vegetative growth stages a good agreement was observed between measured and predicted NH₃ compensation points, whereas during anthesis and senescence, several cases of discrepancy were observed (Table I). NH₃ compensation points were determined as mean values for the whole plant, and obviously the spatial variation among leaves increased considerably during plant ontogeny, making it difficult to sample representative leaves when many had started senescence. In a previous study we demonstrated that the natural variation in apoplastic NH₄⁺ concentration between leaves and the resulting NH₃ compensation point may be considerable even during vegetative growth (Husted and Schjoerring, 1995b).

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