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Leaf-Atmosphere NH_3 Exchange in Barley Mutants with Reduced Activities of Glutamine Synthetase[1]

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Mutants of barley (*Hordeum vulgare* L. cv Maris Mink) with 47 or 66% of the glutamine synthetase (GS) activity of the wild type were used for studies of NH_3 exchange with the atmosphere. Under normal light and temperature conditions, tissue NH_4^+ concentrations were higher in the two mutants compared with wild-type plants, and this was accompanied by higher NH_3 emission from the leaves. The emission of NH_3 increased with increasing leaf temperatures in both wild-type and mutant plants, but the increase was much more pronounced in the mutants. Similar results were found when the light intensity (photosynthetic photon flux density) was increased. Compensation points for NH_3 were estimated by exposing intact shoots to 10 nmol NH_3 mol^{-1} air under conditions with increasing temperatures until the plants started to emit NH_3. Referenced to 25°C, the compensation points were 5.0 nmol mol^{-1} for wild-type plants, 8.3 nmol mol^{-1} for 47% GS mutants, and 11.8 nmol mol^{-1} for 66% GS mutants. Compensation points for NH_3 in single, nonsenescent leaves were estimated on the basis of apoplastic pH and NH_4^+ concentrations. These values were 0.75, 3.46, and 7.72 nmol mol^{-1} for wild type, 47% GS mutants, and 66% GS mutants, respectively. The 66% GS mutant always showed higher tissue NH_4^+ concentrations, NH_3 emission rates, and NH_3 compensation points compared with the 47% GS mutant, indicating that NH_4^+ release was curtailed by some kind of compensatory mechanism in plants with only 47% GS activity.

There are several reactions in plant tissues that release NH_4^+ from organic compounds. The most important are photorespiration and the utilization of N transport compounds (Joy, 1988). This NH_4^+ must be reassimilated if the plant is not to be depleted of N, since photorespiratory NH_4^+ release may occur at rates up to 10 times higher than primary NH_4^+ assimilation (Lea et al., 1992; Leegood et al., 1995). The major pathway for reassimilating NH_4^+ is through the enzyme GS, which is present in two isoforms in leaves. GS_1 is localized in the cytoplasm, but is present mainly in the phloem companion cells, and GS_2 is located in the chloroplasts (Woodall et al., 1996). Refixation of photorespiratory NH_4^+ is mediated by GS_2. Despite this efficient system for reassimilating NH_4^+, plants with normal activities of GS can, under certain conditions, lose NH_3 to the atmosphere. Barley (*Hordeum vulgare*) plants grown with NH_4^+ in the root medium were shown to accumulate NH_4^+ in their tissues and to emit NH_3 (Mattsson and Schjoerring, 1996; Mattsson et al., 1997). Inhibition of GS with Met sulfoximine resulted in a dramatic increase in NH_3 emission from barley (Mattsson and Schjoerring, 1996).

Plants have a compensation point for NH_3 (Farquhar et al., 1980), reflecting the fact that they can both absorb and emit NH_3. If the atmospheric mole fraction of NH_3 is below the compensation point, emission occurs, whereas at concentrations above the compensation point, NH_3 absorption takes place. In barley NH_3 compensation points between 0.9 and 8 nmol mol^{-1} have been measured (Husted et al., 1996). Since the NH_3 compensation point is highly dependent on the apoplastic pH and NH_4^+ concentration, it is important to measure these parameters to understand the regulation of NH_3 exchange. Husted and Schjoerring (1996) have also shown that the NH_3 compensation point can be predicted on the basis of the pH and NH_4^+ concentration in the leaf apoplast.

Mutant lines of barley have been isolated that lack chloroplastic GS_2 (Blackwell et al., 1987; Wallsgrove et al., 1987). Häusler et al. (1994a) have employed heterozygous plants with leaf GS activities in a range between 47 and 97% of the wild-type activity to study the control of CO_2 assimilation. A decrease in GS activity resulted in increases in leaf NH_4^+ and in decreases in leaf amino acids and protein, consistent with a decrease in the capacity of GS to reassimilate NH_4^+ in heterozygous plants. There was also evidence that a compensatory mechanism might be induced that restricts leaf NH_4^+ accumulation when the GS activity falls below about two-thirds that of the wild type (Häusler et al., 1994a, 1994b).

In the present study we have measured NH_3 exchange with the atmosphere in two GS mutants with 66 or 47% of

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Abbreviation: GS, Gln synthetase.

normal leaf GS activity. These were compared with wild-type plants to investigate how the level of GS activity influences NH_3 exchange, compensation points for NH_3, apoplastic pH, and NH_4^+ concentrations.

MATERIALS AND METHODS

Two lines of GS mutants of spring barley (*Hordeum vulgare* L. cv Maris Mink) with only 66 or 47% of wild-type GS activity (Häusler et al., 1994a) were grown simultaneously with wild-type plants in nutrient-rich soil (nutrients added: 180 g m^{-3} N as NO_3^-, 100 g m^{-3} P, and 210 g m^{-3} K) in 0.5-L pots in a climate chamber at 17°C with 16 h of light (350 μmol m^{-2} s^{-1}) and 8 h of darkness. Every 6 to 8 weeks the plants were repotted and propagated vegetatively by dividing the tillers. The plants were watered with deionized water three times a week. At the time of monitoring NH_3 exchange, the plants were in a growth stage just prior to anthesis.

NH_3-Exchange Measurements

NH_3 exchange was monitored in a cuvette system for continuous measurement (Mattsson and Schjoerring, 1996). The pots were put in polyethylene bags to avoid NH_3 uptake or emission from the soil surface. Control measurements showed insignificant NH_3 exchange between the air and pots where the shoots had been cut off. A cuvette made of polycarbonate and coated with Margard (General Electric) having low water- and NH_3-adsorption properties was used in the experiments. The plant cuvette was installed in a growth chamber in which temperature, light, and RH were controlled.

The air entering the cuvette (0.040 m^3 min^{-1}) was filtered to reduce the NH_3 concentration to a very low level. Experiments with NH_3-free air and with NH_3 mixed into the air at a known concentration were carried out. Flow controllers (model 1100, KDG Mobrey Ltd., Crawley, UK) were used to ensure that the flow rates were kept constant through the cuvette and NH_3 monitor. The NH_3 was collected in a rotating denuder and detected by conductometry (Wyers et al., 1993). CO_2 and H_2O concentrations were monitored simultaneously in a combined IR gas analyzer (CIRAS-1, PP Systems, Herts, UK). When light intensity (PPFD) or temperature was changed, the system was allowed to adjust for 45 min between measurements.

Plant Analysis

After gas-exchange measurements the shoots were cut off and the total leaf area was measured. Two to three green, nonsenescent leaves (approximately 0.6 g) were used for isolation of an apoplastic solution. The leaves were rinsed in deionized water, cut in 20- to 30-mm segments, and infiltrated with a 350 milliosmole isotonic sorbitol solution (280 mM) in a 50-mL syringe. Four syringes were mounted on a hydraulic infiltrator (Knapp Micro Fluid, Neutraubling, Germany) programmed to expose the leaves to 4 atm of pressure under a vacuum for 10 s. This procedure was repeated 10 times to ensure that the leaves were fully infiltrated. After centrifugation at 2000*g* for 15 min, apoplastic extracts were collected in microtubes. The concentration of NH_4^+ in the apoplastic extracts was determined with a flow-injection system (Tecator FIA Star 5020, Höganäs, Sweden). The pH values of the extracts were determined directly with a microelectrode (9802 BN, Orion, Boston, MA). To correct for the dilution of the apoplast during the infiltration procedure, the apoplastic air and water volumes were determined and a dilution factor of 1.72 was calculated according to the method of Husted and Schjoerring (1995).

The rest of the leaf material was freeze-dried for 24 h and ground to a fine powder, which was used for extraction of NH_4^+ with 0.025 M H_2SO_4 for 1 h. Tissue-extract NH_4^+ concentrations were determined by the same flow-injection system described above.

NH_3 Compensation Points

Since the distribution between gaseous and aqueous NH_3 in the plant leaf is very sensitive to the leaf temperature, it is possible to estimate the compensation point for NH_3 (χ_{NH3}) by changing the leaf temperature while keeping the surrounding NH_3 concentration constant. The plants were exposed to 10 nmol NH_3 mol^{-1} air and the leaf temperature was increased from 18 to 33°C. Each temperature was maintained for 45 to 60 min to obtain stable readings. Absorption of NH_3 decreased with increasing temperatures and at a certain temperature the plants started to emit NH_3, indicating a compensation point of 10 nmol NH_3 mol^{-1} air at this temperature. If it is assumed that the equilibrium between NH_3 in the intercellular air space and aqueous NH_3/NH_4^+ in the apoplast determines the χ_{NH3}, it is possible to recalculate the χ_{NH3} to any other leaf temperature using the thermodynamic equilibria:

$$NH_4^+ \leftrightarrow NH_{3(aq)} + H^+ \quad \Delta H^0_{dis} = 52.21 \text{ kJ mol}^{-1}; \; K_d^{25°C} = 10^{-9.25}$$

$$NH_{3(aq)} \leftrightarrow NH_{3(g)} \quad \Delta H^0_{vap} = 34.18 \text{ kJ mol}^{-1}; \; K_H^{25°C} = 10^{-1.76}$$

The enthalpy (ΔH) of these reactions is essentially independent of temperatures below 40°C, and the variation in ΔH with ionic strength is insignificant below 100 mM (Stumm and Morgan, 1981). Using a slightly modified Clausius-Clapeyron equation, the compensation point (χ_{NH3}) at 25°C could therefore be calculated according to Husted and Schjoerring (1996) by:

$$\ln \frac{\chi_2}{\chi_1} = \frac{\Delta H^0_{dis} + \Delta H^0_{vap}}{R} \times \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

where χ_1 is the actual NH_3 compensation point at temperature T_1 and χ_2 is the compensation point at a new temperature T_2. R denotes the gas constant (8.31 J K^{-1} mol^{-1}).

Another method for estimating the NH_3 compensation points employed the measured pH and NH_4^+ concentration of the apoplastic solution. Using the following equation the compensation point for NH_3 (χ_{NH3}) at 25°C was calculated:

$$\chi_{NH3} = K_H \times \frac{K_d \times C_{tot}}{K_d + (H^+)}$$

where C_{tot} is the NH_4^+ and NH_3 concentration of the apoplast and (H^+) is the H^+ activity of the apoplast.

RESULTS

Wild-type barley plants and GS mutants were similar in size, although the plants with 47% GS activity had slightly smaller leaves (and thus a lower leaf area) than the plants with 66% GS activity and wild-type plants (Table I). Leaf-tissue NH_4^+ concentrations increased from 1.9 μmol g^{-1} fresh weight for wild-type plants to 3.7 and 2.9 μmol g^{-1} fresh weight for 66 and 47% GS mutants, respectively (Table I). Emission of NH_3 from the leaves of wild-type plants was very low at 24°C, but increased in the mutants to an average of 0.58 and 0.41 nmol m^{-2} s^{-1} for 66 and 47% GS mutants, respectively. Emissions were stable during the morning hours, but often showed an increased rate during the late afternoon/evening hours (Fig. 1). Emission of NH_3 decreased during the night to about one-half of the daytime value in all three groups (Fig. 1).

Increasing leaf temperatures from 20 to 32°C resulted in increased NH_3 emission for all plants, but the increase was much more pronounced in the mutants (Fig. 2A). The NH_3 emission from the 66% GS mutant was higher compared with the 47% GS mutant at all temperatures. Increasing temperatures caused a decrease in photosynthesis, and the mutants showed marginally lower rates of photosynthetic CO_2 assimilation compared with the wild type (Fig. 2B). Transpiration increased at the same time, but did not show any differences between wild-type and mutant plants (Fig. 2C). Increasing the light intensity (PPFD) had a similar effect on NH_3 emission, as did increasing the temperature (Fig. 3). Emission of NH_3 increased more in the GS mutants compared with the wild-type plants as the light intensity was increased (Fig. 3).

Absorption and emission of NH_3 were measured by increasing the temperature from 18 to 33°C at a constant atmospheric NH_3 concentration of 10 nmol mol^{-1} air. At 18°C, the wild-type plants absorbed approximately 1 nmol m^{-2} leaf area s^{-1} NH_3 from the air coming into the cuvette, whereas the mutants absorbed slightly less NH_3 (Fig. 4). As the temperature was increased, NH_3 absorption decreased until a certain temperature was reached, at which time the plants started to emit NH_3. At this temperature the compensation point was 10 nmol mol^{-1} air (Fig. 4). Referenced to 25°C (see "Materials and Methods"), the NH_3 compensation points were 5.0, 11.8, and 8.3 nmol NH_3 mol^{-1} for wild-type and 66 and 47% GS plants, respectively (Table I).

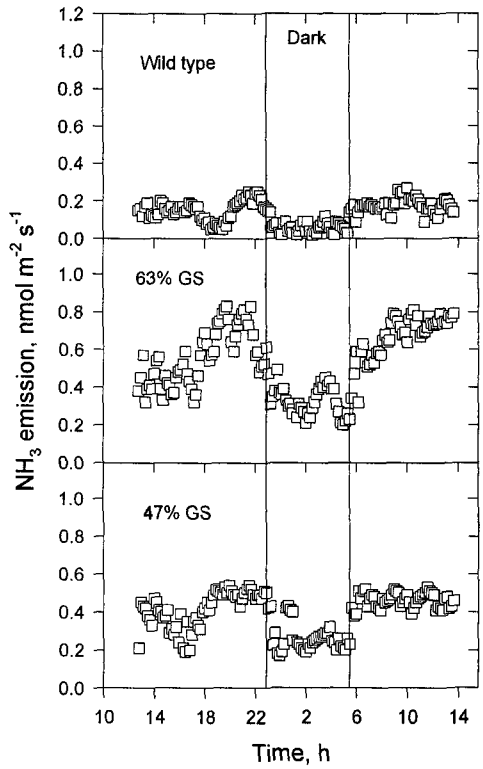

Figure 1. Typical curves of NH_3 emission at 24°C during the light and dark period of wild-type barley plants and mutants with 66 or 47% GS activity.

Both apoplastic NH_4^+ concentrations and pH values in nonsenescent leaves were higher in the GS mutants than in wild-type plants (Table II). The 66% GS mutant also showed higher NH_4^+ concentration in the apoplastic solution than the 47% GS mutant, whereas the pH values of the apoplast were not significantly different.

DISCUSSION

The exchange of NH_3 with the atmosphere was clearly affected by a decrease in GS activity in the barley mutants. This was unlikely to be due to any pleiotropic effects, since

Table I. *Intact shoot parameters: leaf area, leaf tissue NH_4^+ concentrations, NH_3 emission at 24°C, and NH_3 compensation points at 25°C for wild-type barley plants and two mutants with 66 and 47% GS*

NH_3 compensation points were determined on the basis of data for shoot-atmosphere NH_3 fluxes measured at different air temperatures and at a constant atmospheric NH_3 concentration of 10 nmol mol^{-1} air. Values are means ± SE of three replicates.

Plant	Leaf Area	Leaf Tissue NH_4^+	NH_3 Emission	NH_3 Compensation Point
	cm^2 pot^{-1}	*μmol g^{-1} fresh wt*	*nmol m^{-2} s^{-1}*	*nmol mol^{-1}*
Wild type	295 ± 32	1.95 ± 0.38	0.10 ± 0.02	5.02 ± 0.79
66% GS	307 ± 27	3.70 ± 0.73	0.58 ± 0.14	11.78 ± 0.41
47% GS	263 ± 23	2.87 ± 0.51	0.41 ± 0.05	8.32 ± 0.60

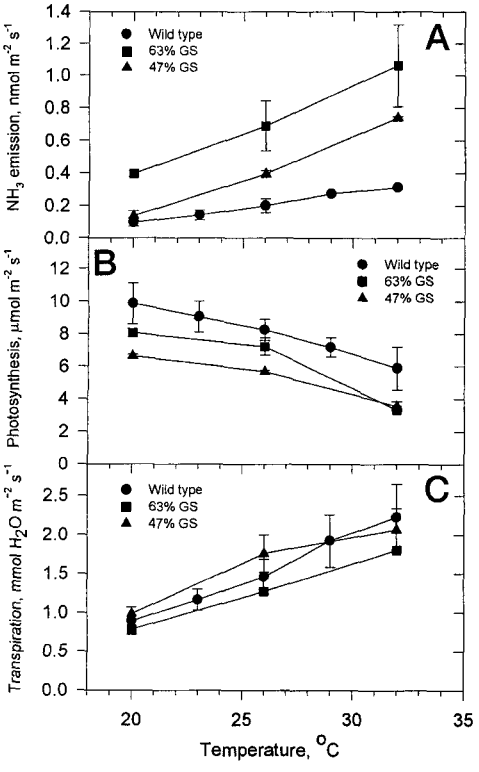

Figure 2. Effect of increasing leaf temperature from 20 to 32°C on NH_3 emission (A), CO_2 assimilation (B), and transpiration (C) in wild-type barley plants and mutants with 66 or 47% GS activity. Values are means ± SE.

parameters such as leaf protein, chlorophyll content, fresh weight, or a range of other enzyme activities were not affected in mutants compared with wild-type plants (Häusler et al., 1994a).

The emission of NH_3 was very low in wild-type barley plants (Fig. 1), in agreement with earlier observations of plants grown in nutrient solutions with only nitrate as the N source (Mattsson and Schjoerring, 1996). However, decreased GS activity resulted in NH_4^+ accumulation in leaf tissues and increased NH_3 emission to the atmosphere (Table I).

Temperature influences plant-atmosphere NH_3 exchange, both by affecting the concentration of atmospheric NH_3 in equilibrium with NH_3 in the apoplastic solution and by affecting physiological processes such as photorespiration, which generates NH_4^+ in leaf tissue (Schjoerring et al., 1997). Temperature effects on plant-atmosphere NH_3 exchange have previously been observed both over a natural forest (Langford and Fehsenfeld, 1992) and in chamber experiments (Farquhar et al., 1980; Stutte and da Silva, 1981). As the temperature was increased from 18 to 32°C, NH_3 emission increased and photosynthesis decreased, which clearly showed that the increased emission was not due to increased photosynthesis (Fig. 2). The temperature-dependent increase in NH_3 emission was much steeper in the mutants compared with the wild-type plants. This higher sensitivity of the mutants was probably a result of increased flux through the photorespiratory pathway, since

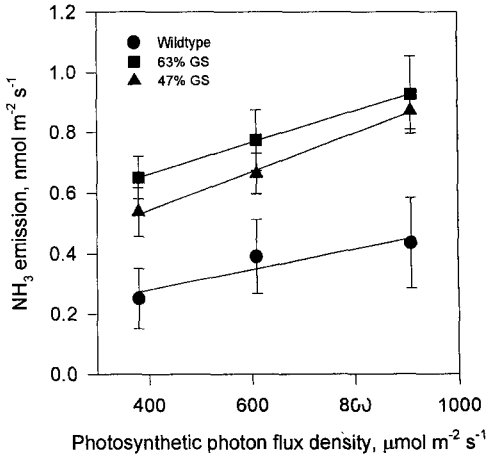

Figure 3. Effect of increasing light intensity (PPFD) on NH_3 emission at 24°C in wild-type barley plants and mutants with 66 or 47% GS activity. Values are means ± SE.

increasing temperatures favor the oxygenation of ribulose-1,5-bisphosphate (Leegood et al., 1995).

Photosynthesis was also slightly affected by the decreased GS activities of the mutants (Fig. 2B), which correlates to the decrease in total protein and therefore Rubisco observed by Häusler et al. (1994a). In the 47% GS mutant NH_3 emission was lower at all temperatures compared to the 66% GS mutant. Increasing the light intensity (PPFD) had a similar effect on NH_3 emission as increasing the temperature, except that the difference between the two mutants was smaller than with exposure to elevated temperatures. With increasing light intensities the photorespiratory rate increases as a result of increased rate of photosynthesis rather than as an increased proportion of total photosynthesis (Leegood, 1993).

In earlier studies barley mutants with decreased activities of chloroplastic GS were used to study the regulation and control of photorespiration (Häusler et al., 1994a,

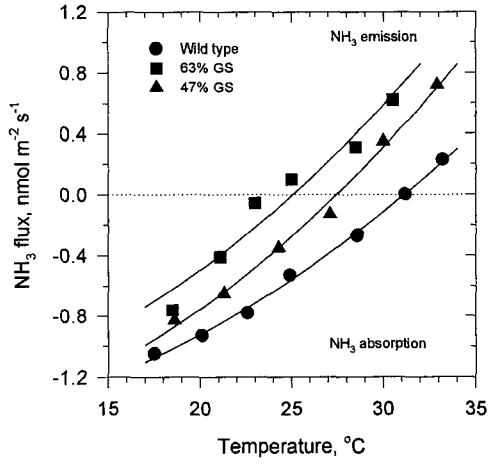

Figure 4. Effect of increasing leaf temperature from 18 to 33°C on NH_3 exchange in wild-type barley plants and mutants with 66 or 47% GS activity exposed to 10 nmol NH_3 mol^{-1} air. Curves represent a typical example from three replicates.

Table II. *Single-leaf parameters: apoplastic NH_4^+ concentration, apoplastic pH, and NH_3 compensation points at 25°C estimated on the basis of apoplastic NH_4^+ and pH for wild-type barley and two mutants with 66 and 47% GS activity*

Values are means ± SE of three replicate plants with two parallel samples taken from each.

Plant	Apoplastic NH_4^+	Apoplastic pH	NH_3 Compensation Point
	mM		nmol mol^{-1}
Wild type	0.36 ± 0.08	5.32 ± 0.06	0.75 ± 0.2
66% GS	1.08 ± 0.26	5.86 ± 0.14	7.72 ± 2.4
47% GS	0.69 ± 0.12	5.71 ± 0.09	3.46 ± 1.8

1994b; Leegood et al., 1995). Under conditions that favor photorespiration, contents of amino acids directly involved in the photorespiratory pathway, as well as NH_4^+ contents and the activation state of Rubisco, exhibit a biphasic response to a decrease in GS activity. When GS activity falls below 60 to 70% of the wild type, NH_4^+ accumulation is curtailed (Häusler et al., 1994a, 1996). Under similar conditions, photorespiratory CO_2 and NH_3 release is decreased (Häusler et al., 1994b). The data presented here are fully consistent with the notion that a decrease in GS results in an increase in NH_3 release down to 66% of the wild-type activity, but that once GS activity is further reduced (to 47% of wild-type activity), some further mechanism restricts NH_3 loss. The nature of this compensating mechanism is still unclear. Häusler et al. (1996) have suggested that it may involve the engagement of alternative pathways of glyoxylate metabolism.

The importance of leaf temperature for plant-atmosphere NH_3 exchange is clear from the data shown in Figure 4 for wild-type and mutant plants exposed to 10 nmol NH_3 mol^{-1} air at temperatures from 18 to 33°C. At 18°C both wild-type and mutant plants absorbed NH_3 from the air, but as the temperature was increased they switched from being sinks for NH_3 to being NH_3 sources (Fig. 4). At the temperature at which no net NH_3 exchange occurred the compensation point was 10 nmol mol^{-1}. At 25°C the estimated NH_3 compensation points (Table II) agreed well with compensation points measured using the same technique in oilseed rape (*Brassica napus*; 7.7 nmol NH_3 mol^{-1} at 25°C; Husted and Schjoerring, 1996). Previous measurements of NH_3 compensation points in agricultural crops range from 1 to 7 nmol NH_3 mol^{-1} air (Farquhar et al., 1980; Dabney and Bauldin, 1990; Schjoerring et al., 1993; Sutton et al., 1995; Husted et al., 1996). Differences in NH_3 compensation points between cultivars and developmental stages have also been shown for barley (Husted et al., 1996). The higher compensation points for the GS mutants agree with the observed tissue NH_4^+ and NH_3 emission data (Table I).

Leaf apoplastic pH values varied from 5.3 in wild-type plants to 5.9 for the 66% GS mutants. In most plant species apoplastic pH values range between 5.0 and 6.5 (Grignon and Sentenac, 1991; Husted and Schjoerring, 1995; Mattsson et al., 1997). Apoplastic pH values have been shown to increase with increasing NO_3^- concentrations in the root medium (Hoffmann et al., 1992; Kosegarten and Englisch, 1994; Dannel et al., 1995), probably as a result of a proton-nitrate cotransport (Dannel et al., 1995). Increasing NH_4^+ concentrations in the root medium also resulted in decreasing apoplastic pH values in the leaves (Mattsson et al., 1997). In the present experiment leaf apoplastic pH values increased in the mutants compared with wild-type plants (Table II), indicating a consumption of protons as NH_3/NH_4^+ was excreted from the leaf cells into the apoplast. Increasing apoplastic pH and NH_4^+ concentrations were also seen in oilseed rape plants after inhibition of GS with Met sulfoximine (Husted and Schjoerring, 1995). The apoplastic NH_4^+ concentration was 1.1 and 0.7 mM in the 66 and 47% GS mutants, respectively (Table II). These values are similar to those in NH_4^+-grown barley plants (Mattsson et al., 1997) and oilseed rape plants grown with a high N supply (Husted and Schjoerring, 1996).

Compensation points for NH_3 estimated on the basis of apoplastic NH_4^+ and H^+ in single, nonsenescent leaves were lower (Table II) than those calculated on the basis of NH_3 fluxes between whole shoots and the atmosphere under conditions of increasing temperatures (Table II). This difference is not very surprising if we take into account the fact that the latter measurements included plants with tillers of different developmental stages, having both old, senescent leaves and young leaves. Apoplastic solution was, on the other hand, collected only from young leaves, and was therefore not representative of the whole plant. In oilseed rape plants apoplastic NH_4^+ concentration was shown to increase with leaf age, and the best agreement between estimated and measured NH_3 compensation points was found in the early, vegetative growth stage (Husted and Schjoerring, 1996).

Another factor contributing to differences in NH_3 compensation points obtained by the two methods could be uncertainties in using the Clausius-Clapyron equation for referencing NH_3 compensation points to 25°C in the temperature-ramping measurements. Such uncertainties originate from the assumption that temperature is the only parameter affecting the compensation point, whereas the apoplastic NH_4^+ and H^+ concentrations should be constant. The latter may not have been the case, as shown by a steeper temperature response for NH_3 emission from the mutants than from the wild-type plants (Fig. 2A), indicating that the NH_4^+ concentration of the apoplast increased in the mutants as the temperature was increased, probably because there was not sufficient GS activity for assimilating the higher amounts of NH_3 released in photorespiration.

Although the apoplastic data resulted in lower NH_3 compensation points than the temperature-ramping measurements, in particular for wild-type plants, the values obtained by the two methods were in the same range (Tables I and II) and clearly show that the NH_3 compensation point is higher in mutants with reduced GS activity than in wild-type plants. The results presented in the present work thus clearly demonstrate the significance of GS in controlling the flux of NH_3 between plants and the atmosphere. It is therefore important to obtain more knowledge regarding variations in GS activity in plants growing under natural conditions, particularly during senescence, and whether certain stress factors or particular environmental conditions may reduce GS activity to an extent that leads to increased NH_3 emission.

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LITERATURE CITED

Blackwell RD, Murray AJS, Lea PJ (1987) Inhibition of photosynthesis in barley with decreased levels of chloroplastic glutamine synthetase activity. J Exp Bot **38:** 1799–1809

Dabney SM, Bouldin DR (1990) Apparent deposition velocity and compensation points of ammonia inferred from gradient measurements above and through alfalfa. Atmos Environ **A24:** 2655–2666

Dannel F, Pfeffer H, Marschner H (1995) Isolation of apoplasmic fluid from sunflower leaves and its use for studies on influence of nitrogen supply on apoplasmic pH. J Plant Physiol **146:** 273–278

Farquhar GD, Firth PM, Wetselaar R, Weir B (1980) On the gaseous exchange of ammonia between leaves and the environment. Determination of the ammonia compensation point. Plant Physiol **66:** 710–714

Grignon C, Sentenac H (1991) pH and ionic conditions in the apoplast. Annu Rev Plant Physiol Plant Mol Biol **42:** 103–128

Häusler RE, Bailey KJ, Lea PJ, Leegood RC (1996) Control of photosynthesis in barley mutants with reduced activities of glutamine synthetase and glutamate synthase. III. Aspects of glyoxylate metabolism and effects of glyoxylate on the activation state of ribulose-1,5-bisphosphate carboxylase-oxygenase. Planta **200:** 388–396

Häusler RE, Blackwell RD, Lea PJ, Leegood RC (1994a) Control of photosynthesis in barley leaves with reduced activities of glutamine synthetase or glutamate synthase. I. Plant characteristics and changes in nitrate, ammonium and amino acids. Planta **194:** 406–417

Häusler RE, Lea PJ, Leegood RC (1994b) Control of photosynthesis in barley leaves with reduced activities of glutamine synthetase or glutamate synthase. II. Control of electron transport and CO_2 assimilation. Planta **194:** 418–435

Hoffmann B, Planker R, Mengel K (1992) Measurements of pH in the apoplast of sunflower leaves by means of fluorescence. Physiol Plant **84:** 146–153

Husted S, Mattsson M, Schjoerring JK (1996) Determination of NH_3 compensation points in two cultivars of *Hordeum vulgare* L.: influence of plant developmental stage. Plant Cell Environ **19:** 1299–1306

Husted S, Schjoerring JK (1995) Apoplastic pH and ammonium concentration in leaves of *Brassica napus* L. Plant Physiol **109:** 1453–1460

Husted S, Schjoerring JK (1996) Ammonia flux between oilseed rape plants and the atmosphere in response to changes in leaf temperature, light intensity and air humidity. Plant Physiol **112:** 67–74

Joy KW (1988) Ammonia, glutamine and asparagine: a carbon-nitrogen interface. Can J Bot **66:** 2103–2109

Kosegarten H, Englisch G (1994) Effects of various nitrogen forms on the pH in leaf apoplast and on iron chlorosis of *Glycine max* L. Z Pflanzenernaehr Bodenk **157:** 401–405

Langford AO, Fehsenfeld FC (1992) Natural vegetation as a source or sink for atmospheric ammonia: a case study. Science **255:** 581–583

Lea PJ, Blackwell RD, Joy KW (1992) Ammonia assimilation in higher plants. *In* K Mengel, DJ Pilbeam, eds, Nitrogen Metabolism of Plants. Oxford Scientific Publishers, New York, pp 153–186

Leegood RC (1993) The Calvin cycle and photorespiration. *In* PJ Lea, RC Leegood, eds, Plant Biochemistry and Molecular Biology. John Wiley & Sons, New York, pp 27–45

Leegood RC, Lea PJ, Adcock MD, Häusler RE (1995) The regulation and control of photorespiration. J Exp Bot **46:** 1397–1414

Mattsson M, Husted S, Schjoerring JK (1997) Influence of nitrogen nutrition on ammonia volatilization from plant leaves. Nutrient Cycling in Agroecosystems (in press)

Mattsson M, Schjoerring JK (1996) Ammonia emission from young barley plants: influence of N source, light/dark cycles and inhibition of glutamine synthetase. J Exp Bot **47:** 477–484

Schjoerring JK, Husted S, Mattsson M (1997) Physiological parameters controlling plant-atmosphere ammonia exchange. Atmos Environ (in press)

Schjoerring JK, Kyllingsbaeck A, Mortensen JV, Byskov-Nielsen S (1993) Field investigations of ammonia exchange between barley plants and the atmosphere. I. Concentration profiles and flux densities of ammonia. Plant Cell Environ **16:** 161–167

Stumm W, Morgan JJ (1981) Aquatic Chemistry, Ed 2. Wiley Interscience, New York, pp 68–78

Stutte CA, da Silva PRF (1981) Nitrogen volatilization from rice leaves. I. Effects of genotype and air temperature. Crop Sci **21:** 569–600

Sutton MA, Schjoerring JK, Wyers GP (1995) Plant-atmosphere exchange of ammonia. Philos Trans R Soc Lond A **351:** 261–278

Wallsgrove RM, Turner JC, Hall NP, Kendall AC, Bright SWJ (1987) Barley mutants lacking chloroplast glutamine synthetase: biochemical and genetic analysis. Plant Physiol **83:** 155–158

Woodall J, Boxall JG, Ford BG, Pearson J (1996) Changing perspectives in plant nitrogen metabolism: the central role of glutamine synthetase. Sci Prog **79:** 1–26

Wyers GP, Otjes RP, Slanina J (1993) A continuous-flow denuder for the measurement of ambient concentrations and surface-exchange fluxes of ammonia. Atmos Environ **27A:** 2085–2090