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Experiments to examine the significance of ammonia evolution from barley seedlings infected with the powdery mildew fungus, *Erysiphe graminis* f.sp. *hordei*

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SUMMARY

Experiments using intact seedlings and detached leaves failed to confirm previous reports that ammonia gas is evolved from barley leaves during the establishment of infection by the powdery mildew fungus *Erysiphe graminis* f.sp. *hordei*.

In the experiments using intact seedlings infection did, however, lead to greater concentrations of ammonium nitrogen in the senescing leaves and, in one experiment, the subsequent evolution of ammonia gas from these seedlings. Losses of nitrogen as ammonia gas from crops are probably small, but it is possible that under some circumstances they may represent a significant proportion of the otherwise unexplained nitrogen losses and hence be important in experiments which aim to study the nitrogen balance of crop–soil systems.

INTRODUCTION

Amounts of nitrogen applied to cereal crops in the United Kingdom have increased considerably over the last 30 years or so (Church & Lewis, 1977). In 1980 the average amount of nitrogen applied to spring barley, for example, was estimated to be 87 kg/ha and a proportion of crops received much larger amounts (Church, 1981). At 1982 prices the cost for each kilogram of nitrogen was *ca.* £0.33 (excluding the costs of applying it) so that the total expenditure on nitrogen applied to cereal crops is substantial. Maximizing the efficiency with which the applied nitrogen is used is, therefore, important in determining how profitable these crops are to the farmer.

Infection by leaf pathogens is one factor which can influence the efficiency with which nitrogen is used, and cereal crops protected from leaf diseases by fungicides typically respond much better to applied nitrogen than do unprotected crops (Dilz & Schepers, 1972; Ellen & Spiertz, 1975; Jenkyn, Finney & Dyke, 1983). However, relatively little is known about the underlying mechanisms although they are potentially complex (Jenkyn, 1977). For example, barley plants affected by powdery mildew (*Erysiphe graminis* f.sp. *hordei*) are likely to produce less dry matter than healthy plants and may, therefore, have a decreased capacity to utilize nitrogen, but it may be necessary to apply relatively more nitrogen to a diseased crop than to a healthy one to compensate for smaller, and less efficient, root systems (Last, 1962). Infection by *E. graminis* can also, apparently, decrease the recycling of organic nitrogen compounds within the plant, thus decreasing the efficiency with which nitrogen is used after it has been assimilated (Finney, 1979).

Effects of the powdery mildew fungus on the assimilation and metabolism of nitrogen in barley have also been reported by Sadler & Scott (1974), who showed that infection causes accumulation of ammonium (but not nitrate or nitrite) ions in the leaves. Furthermore, in experiments using detached leaves, they also demonstrated the evolution of ammonia gas from these leaves. Their paper suggests that the amounts evolved exceeded 30 μg ammonia/g fresh weight/h, during the fourth day after inoculation, which would imply that total losses from cereal crops could be very large. Correspondence with the authors, after we had completed the experiments described here, revealed, however, that the values in their fig. 2 represent amounts of ammonia evolved per 24 h, not per hour. Assuming their data relate to first seedling leaves, and further assuming a plant population density in a spring barley crop of 250 plants/m^2, we estimate that

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their value for the fourth day after inoculation corresponds to a nitrogen loss of *ca.* 6 g/ha/day. Such amounts are trivial in field terms, but if all attached infected leaves behave similarly, and if they evolve ammonia at similar rates for some days, then total amounts of nitrogen lost in this way might still represent a significant component in the total nitrogen balance of cereal crops infected with *E. graminis*.

The first two experiments described in this paper sought to confirm ammonia evolution from intact barley seedlings infected with the powdery mildew fungus, *E. graminis* f.sp. *hordei*. Ammonia gas was not, however, detected in significant amounts until leaves began to senesce, so the later experiments we describe attempted to repeat the original observations of Sadler & Scott (1974) using detached leaves.

MATERIALS AND METHODS

Experiments with intact seedlings

To measure ammonia evolution from intact barley seedlings we adapted the apparatus described by Jenkyn, Hirst & King (1973) which provides filtered air, under positive pressure, to discrete units each consisting of a plant pot and transparent plastic cover. This apparatus had been designed to allow the simultaneous culture of healthy and infected seedlings without cross-contamination occurring, but it allowed air to be sampled from a relatively restricted aerial environment around growing seedlings. A suction pump was connected to a manifold (mounted on the central manifold of the original apparatus) and used to draw air from a total of eight units (four on each side of the manifold) using a combination of glass and polythene tubing. In Expt 1, air was drawn through tubes inserted in the tops of the plastic covers and delivered to sample tubes (*ca.* 28 mm diameter boiling tubes), where it passed through disposable plastic pipette tips immersed in boric acid solutions. The plastic pipette tips were replaced each time the boric acid solution was changed. During this experiment condensation sometimes developed in the polythene tubing. In Expt 2, therefore, we extended the transparent plastic covers (using cylinders of Melinex sheeting), and the polythene tubing which delivered the air to the sample collectors was inserted at such an angle through the sides of the covers as to ensure that any condensate which did develop would flow into the sample collectors rather than into the isolation units in which the seedlings were growing. Total air flow through the system was measured through a flow meter and adjusted with a side-arm bleed to 15 l/min, giving a flow through each unit of nearly 2 l/min.

Each sample tube contained 20 ml of 1% boric acid solution to absorb the ammonia from the sampled air. After changing the boric acid solutions, evaporation losses were made good by the addition of distilled water and the pH adjusted by the addition of 5 ml of 1·0 N-NaOH to each sample. The concentration of ammonium ions was then measured using an Orion ammonium-sensitive electrode and a Corning Model 12 pH meter. Amounts of ammonium nitrogen in the leaves were measured by macerating samples (*ca.* 1 g fresh weight) in a ball mill with 10 ml of 1% boric acid solution for 10 min. After centrifuging, the supernatant was made up to 20 ml with 1% boric acid solution, and the concentration of ammonium ions measured as above.

The apparatus was situated in a glasshouse. For both experiments, pots were fitted with plastic flue pipes and Terylene wicks as described by Jenkyn *et al.* (1973) but to increase circulation of air around the seedlings a baffle (4 × 4 cm) was fitted just above the air inlet. Pots (13 cm diameter) were filled with a peat–sand compost ('Eff' compost, available from Eff Products Ltd, Glaziers Lane, Normandy, Guildford, Surrey) and sown with pregerminated barley seeds (cv. Zephyr) at five (Expt 1) or ten (Expt 2) per pot. Sampling began 18 and 12 days, respectively, after sowing, and 24 h before seedlings were inoculated by allowing spores of *E. graminis* f.sp. *hordei* to sediment on to them. The surface of the compost in each pot was covered with a layer of Cornish grit to decrease evaporation. The junctions between the pots and their covers were sealed with adhesive tape to ensure, as far as possible, that all air entering the units had been filtered and all air leaving the units had to pass through the boric acid solutions. Mildew became moderate to severe on inoculated seedlings but its development was not monitored in detail because this would have required excessive disturbance to the sealed system.

In Expt 1, the eight units were used to compare four treatments, each replicated twice: healthy (uninoculated) seedlings, mildew-infected seedlings, healthy seedlings with all first seedling leaves covered with aluminium foil to induce early senescence, and all seedlings cut at soil level and removed at the start of the test to measure ammonia evolution from soil and senescing roots. After 8 days, there was no evidence that significant quantities of ammonia had been released from the soil and measurements on these units were discontinued.

In Expt 2, the eight units were used to compare only two treatments: healthy (uninoculated) and mildew-infected seedlings.

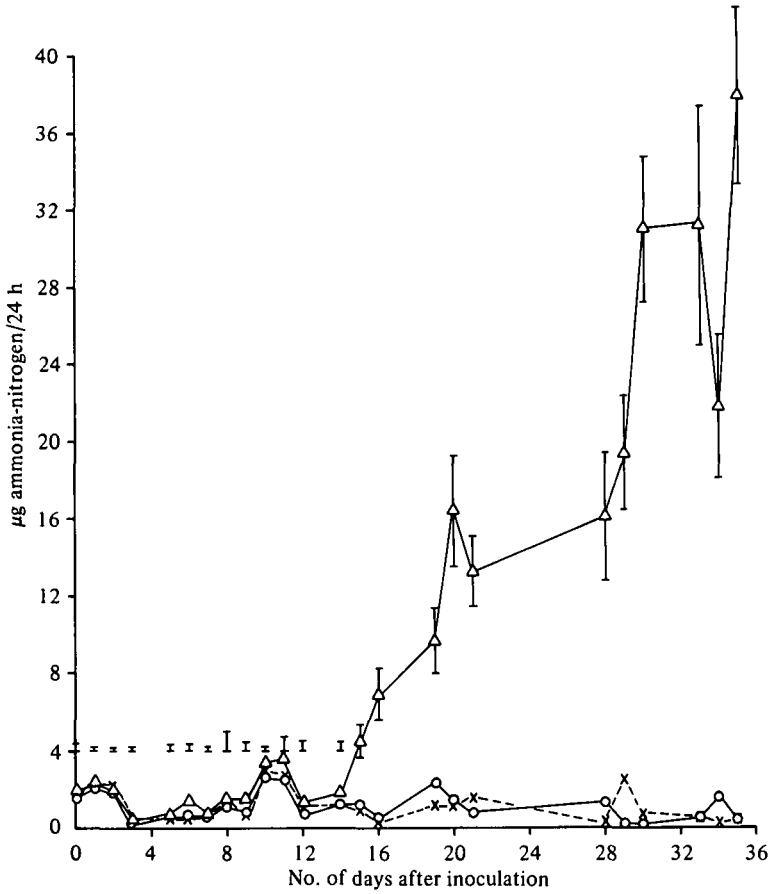

Fig. 1. Ammonia evolution from intact barley seedlings in Expt 1. Seedlings were either uninoculated (○—○), inoculated with *Erysiphe graminis* (△—△) or uninoculated and their first seedling leaves enclosed in aluminium foil (× -- ×); I, S.E. (3 D.F.)

Experiments with detached leaves

These experiments used Thunberg tubes and aimed to measure ammonia evolution from detached leaves. The methods used were similar to those described by Sadler & Scott (1974). Harvested leaves (1 g fresh weight) had their basal ends removed under water and were then placed in the main compartment of a Thunberg tube containing 1 ml distilled water. Boric acid solution (3 ml of a 5% aqueous solution) was placed in the side-arm, and the tubes then sealed with high-vacuum grease and incubated under constant illumination. After incubation (usually for periods of 24 h) the contents of the side-arm were made up to 20 ml with distilled water. The pH was adjusted using 5 ml of 1·0 N-NaOH and the concentration of ammonium ions measured, as above, using an ammonium-sensitive electrode. All seedlings were grown in a soil-less compost in pots on the propagator.

In Expt 3 we again used spring barley cv. Zephyr and compared all combinations of three treatments, namely uninoculated *v.* inoculated leaves; leaves with their bases immersed in the water in the Thunberg tubes or supported above it on plastic gauze; and two sampling regimes. In the first of the sampling regimes the leaves and the boric acid were changed daily for the 7 days after inoculation, the leaves always being replaced by others from seedlings which had been identically treated and similarly maintained on the propagator. In the second, the boric acid was changed daily but the leaves were not changed until after day 4. After a second 4-day period, during which the boric acid was again changed daily, the leaves then in the Thunberg tubes were left *in situ* for a further 10

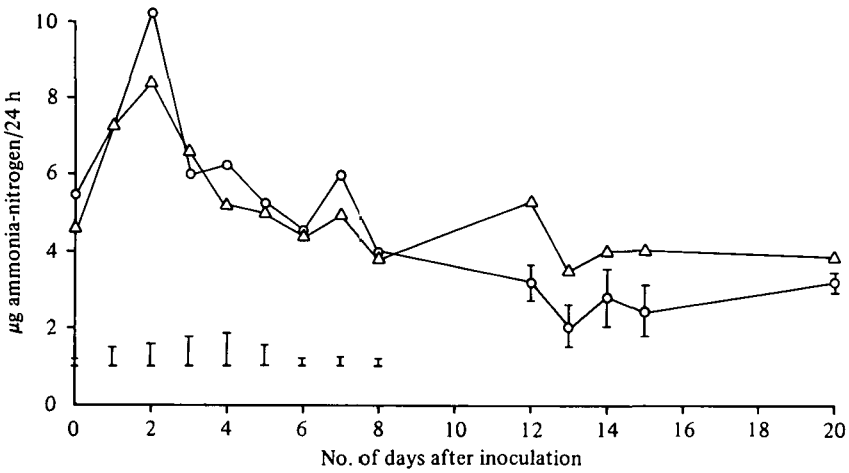

Fig. 2. Ammonia evolution from intact barley seedlings in Expt 2. Seedlings were either uninoculated (○—○) or inoculated with *Erysiphe graminis* (△—△); I , S.E. (3 D.F.)

days, to allow them to senesce, during which time the boric acid remained unchanged.

Experiment 4 compared only uninoculated and inoculated leaves (four replicates of each) of the spring barley cultivar Prior (as used by Sadler & Scott). Equal numbers of first and second seedling leaves were placed in every Thunberg tube, with their basal ends immersed in the water contained therein. The boric acid and the leaves were changed at 24 h intervals during the first 4 days after inoculation.

RESULTS

Experiments with intact seedlings

Experiment 1

Mildew became severe in this experiment. Although not assessed, its severity is illustrated by the differences in dry weights of healthy and inoculated seedlings which had been treated identically to those used in the experiment and which had been grown in units ventilated in the same way but not connected to the sampling system. Thus 8, 21 and 28 days after inoculation the dry weights of inoculated seedlings were 2, 12 and 49% less, respectively, than those of healthy seedlings.

Amounts of ammonia detected in air sampled from around healthy seedlings and those on which the first seedling leaves had been enclosed in aluminium foil were similar, but small, throughout the 35-day course of the experiment (Fig. 1). Pots containing inoculated seedlings produced similarly small amounts of ammonia during the first 14 days after inoculation but thereafter produced substantial and increasing quantities as infection progressed and as the older seedling leaves became progressively more senescent. Both of the pots containing inoculated seedlings produced large quantities of ammonia but they did behave differently, and this is the reason why these data have such large standard errors. In one replicate, ammonia evolution increased rapidly from 15 days after inoculation. In the second replicate there were small increases in ammonia from 19 days after inoculation but large amounts were not detected until 29 days after inoculation. Average amounts of ammonia detected in the subsequent samples taken from this replicate were, however, 67% greater than the amounts detected in the corresponding samples taken from the first replicate. Pots from which the top growth of seedlings had been removed (and, therefore, contained only soil and senescing roots) also evolved little ammonia during the first 8 days of the experiment, after which no further measurement was made.

Experiment 2

Initially, the amounts of ammonia detected in this experiment were somewhat larger than had been detected in Expt 1, and they did seem to increase in the first 2 days after inoculation (Fig. 2). However, there was no evidence that inoculated seedlings produced any more ammonia than the uninoculated. Between 3 and 8 days after inoculation, during which time mildew symptoms became apparent, ammonia evolution tended to decline, but remained similar for healthy and infected seedlings. Senescence of the lower leaves of infected

seedlings was pronounced by 12 days after inoculation and from that date slightly, but not significantly, more ammonia was detected in air from around infected seedlings than in that from around healthy seedlings. However, amounts were still smaller than had been detected at the start of the experiment and there was no evidence for the marked increase in ammonia evolution which coincided with leaf senescence in Expt 1. Analyses done at the end of the experiment (20 days after inoculation) did, however, show that the average concentrations of ammonium nitrogen in the old leaves of infected seedlings were much greater than in those of healthy seedlings; concentrations in the first seedling leaves averaged 215·0 and 5·5 and those in the third seedling leaves 30·5 and 4·1 μg NH_3-N/g fresh weight, respectively.

Experiments with detached leaves

Experiment 3

Mildew symptoms developed on inoculated seedlings after 5 days, but analyses of the boric acid solutions provided no evidence for ammonia evolution either during the incubation period or in the 3 days immediately after symptoms first appeared. Healthy and infected leaves which were allowed to remain undisturbed in the Thunberg tubes for a further 10 days both evolved small quantities of ammonia (equivalent to 6·2 and 14·2 μg NH_3-N/g fresh weight, respectively) but much larger amounts were found in the distilled water (whether or not the basal ends of the leaves were submerged in it), averaging the equivalent of 444 and 396 μg NH_3-N/g fresh weight, respectively.

Experiment 4

This experiment also provided no evidence for ammonia evolution from healthy or inoculated seedlings, during the course of symptom development.

DISCUSSION

In cereals, yield response to increasing amounts of applied nitrogen is certainly decreased by leaf pathogens (Dilz & Schepers, 1972; Ellen & Spiertz, 1975; Jenkyn *et al.* 1983) but the extent to which the different processes of uptake, assimilation and utilization are affected is only poorly understood. The experiments described in this paper were prompted by the work of Sadler & Scott (1974), who demonstrated the evolution of ammonia gas from mildew-infected leaves and in quantities which were apparently sufficient to be agronomically important. They deliberately limited their observations to the first 4 days after inoculation to avoid the complications of necrosis. However, they also used detached leaves, and it seemed to us possible that some compounds which they found to accumulate in response to infection might normally be translocated away from the infected tissue so that amounts of nitrogen lost as ammonia gas from intact plants could be agronomically less significant than their results might suggest. The results of our first experiment seem to support that view, because we detected little ammonia in the first 2 weeks after inoculation. The subsequent detection of large quantities of ammonia as the leaves senesced is further discussed below, but does indicate that had ammonia been evolved during the establishment of infection we would have detected it. Although in Expt 2 we initially detected more ammonia than in Expt 1, there was similarly no effect of the pathogen during the first 2 weeks after inoculation. Surprisingly, there was also no effect of the pathogen in the subsequent experiments using detached leaves and even in that which used cv. Prior and which was, as far as we could achieve, a repeat of Sadler's & Scott's experiment. We can offer no explanation for the contradictory results, but we have certainly obtained no evidence to confirm that large amounts of ammonia are evolved as a direct result of infection by *E. graminis*, or that the nitrogen lost in this way is likely to represent a significant component in the total nitrogen economy of cereal crops.

The results of our Expt 1 did, however, show that, at later stages, mildew-infected seedlings can evolve large amounts of ammonia. By this time, secondary infections were developing on the younger, later-formed leaves but it nevertheless seems probable that this ammonia was not derived from these leaves but rather from the older leaves which were then senescing. The evolution of ammonia gas from mildew-infected seedlings was not, however, confirmed in Expt 2. For this second experiment we extended the transparent covers and, therefore, increased the volumes of air around the seedlings. This might have decreased ammonia concentrations, but we also increased the number of seedlings from five to ten per pot and this should have increased ammonia concentrations. However, the contrast between the two experiments may not have been as great as at first appears because the mildew-infected leaves in Expt 2 were shown to contain large concentrations of ammonium nitrogen. Thus concentrations in first and third seedling leaves from infected plants were *ca.* 39 and 7 times greater, respectively, than those in corresponding leaves from healthy plants, a substantial difference even allowing for the fact that these concentrations are based on fresh weights and probably, therefore, tend to exaggerate the effect. It may be, therefore, that ammonia gas was not detected in Expt 2 simply because conditions did not favour its release from the leaves. Wetselaar & Farquhar (1980) argued that the evolution of ammonia gas from leaves is probably favoured by high temperatures, high light

intensities, ample moisture and high levels of nutrition. Leaf pH is probably important also and this may be affected by the senescence of leaves, and the dehydration which accompanies it, and by the growth of micro-organisms in the senescing tissue. Whatever the mechanisms involved, it seems reasonable to suggest that conditions in Expt 2 might subsequently have become favourable for ammonia evolution, and that ammonia gas would have been detected, if we had continued to sample beyond 20 days after inoculation. Results from our first experiment perhaps support this argument, because although amounts of ammonia began to increase from *ca.* 15 days after inoculation this was in only one replicate. In the second replicate there were small increases in amounts of ammonia from 19 days after inoculation, but large amounts were not detected until 29 days after inoculation, which was 9 days beyond the stage at which Expt 2 was terminated. We can offer no explanation for the marked difference between the two replicates in Expt 1. In retrospect, our decision to terminate Expt 2 as early as we did was unfortunate, but we did so because our principal interest then was in the direct effects of the pathogen during infection.

To what extent the greater accumulation of ammonium nitrogen in senescing, mildew-infected leaves than in senescing, uninfected leaves (whether or not it is subsequently evolved as ammonia gas) is a primary consequence of infection by *E. graminis* is uncertain. However, it seems that the disease does cause the retention of nitrogen compounds in the infected leaves (whether in the leaf tissue itself or in the fungal mycelium) (Finney, 1979) so that the recycling of nitrogen from old to young leaves and eventually to the developing grain is decreased. It seems likely that much of the ammonium nitrogen which accumulates in senescing, mildew-infected leaves may be derived from the autolysis of other nitrogen compounds in these leaves.

In reviewing the evidence for nitrogen losses from the tops of plants, Wetselaar & Farquhar (1980) concluded that direct gaseous losses could well be important. However, the available evidence suggested to them that ammonia volatilization probably accounted for only a small part of the total losses they were considering. Our evidence for ammonia evolution was obtained under very artificial conditions and we cannot be sure that it is relevant to field-grown crops. However, if it is assumed that crops do behave in a similar way then the average amounts detected from five seedlings in Expt 1 would correspond to *ca.* 0·2 kg N/ha on a purely proportional basis assuming 250 plants/m^2. As this quantity was evolved over a period of only 20 days, and from plants bearing relatively little senescent tissue, it is conceivable that total losses during a season could be equivalent to some kilograms per hectare. They are thus likely to be small in relation to the total amounts of nitrogen required by modern cultivars, and applied to them as fertilizer. Nevertheless, identifying losses of this magnitude may be important in work which aims to study the total nitrogen balance of crop–soil systems in different seasons and on different soils. Commonly, measurements of the amounts of nitrogen in winter wheat and remaining in the soil at the end of a growing season can account for 80% or more of the applied nitrogen (Powlson *et al.* 1983). The remainder, which is unaccounted for, represents a relatively small proportion of the total but it can vary considerably, e.g. from 10 to 30% (Powlson *et al.* 1983). The nitrogen which is not recovered is usually assumed to have been lost by leaching or by denitrification in the soil. However, our results suggest that, under some circumstances, losses of ammonia from diseased leaves might also be a significant contributory factor. Losses in spores may also be important but, as far as we are aware, have not been measured.

REFERENCES

CHURCH, B. M. (1981). Use of fertilisers in England and Wales, 1980. *Report of the Rothamsted Experimental Station for* 1980, *Part* 2, pp. 115–122.

CHURCH, B. M. & LEWIS, D. A. (1977). Information from the survey of fertilizer practice, 1942–1976. *Outlook on Agriculture* **9**, 186–193.

DILZ, K. & SCHEPERS, J. H. (1972). Stikstofbemesting van granen. 24. Effekt van ziektebestrijding op de stikstofreaktie van winter- en zomertarwe met en zonder toepassing van chloormequat. *Stikstof* **71**, 452–458.

ELLEN, J. & SPIERTZ, J. H. J. (1975). The influence of nitrogen and benlate on leaf-area duration, grain growth and pattern of N-, P- and K-uptake of winter wheat (*Triticum aestivum*). *Zeitschrift für Acker- und Pflanzenbau* **141**, 231–239.

FINNEY, M. E. (1979). The influence of infection by *Erysiphe graminis* DC. on the senescence of the first leaf of barley. *Physiological Plant Pathology* **14**, 31–36.

JENKYN, J. F. (1977). Nitrogen and leaf diseases of spring barley. *Fertilizer Use and Plant Health, Proceedings of the 12th Colloquium of the International Potash Institute, Izmir, Turkey*, pp. 119–128.

JENKYN, J. F., FINNEY, M. E. & DYKE, G. V. (1983). Effects of nitrogen fertilizer and tridemorph on mildew, growth and yield of spring barley 1975–7. *Journal of Agricultural Science, Cambridge* **101**, 517–546.

JENKYN, J. F., HIRST, J. M. & KING, G. (1973). An apparatus for the isolated propagation of foliar pathogens and their hosts. *Annals of Applied Biology* **73**, 9–13.

LAST, F. T. (1962). Analysis of the effects of *Erysiphe graminis* DC. on the growth of barley. *Annals of Botany*, N.S. **26**, 279–289.

POWLSON, D. S., JENKINSON, D. S., PRUDEN, G. & JOHNSTON, A. E. (1983). *Report of the Rothamsted Experimental Station for* 1982, *Part* 1, p. 263.

SADLER, R. & SCOTT, K. J. (1974). Nitrogen assimilation and metabolism in barley leaves infected with the powdery mildew fungus. *Physiological Plant Pathology* **4**, 235–247.

WETSELAAR, R. & FARQUHAR, G. D. (1980). Nitrogen losses from tops of plants. *Advances in Agronomy* **33**, 263–302.