The Uptake of Nitrate by *Lolium perenne* from Flowing Nutrient Solution

II. EFFECT OF LIGHT, DEFOLIATION, AND RELATIONSHIP TO CO₂ FLUX

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ABSTRACT

Experiments with simulated swards of perennial ryegrass (*Lolium perenne* L.) grown in flowing nutrient solution with NO₃⁻ held at 0-1 mg N l⁻¹ show that the rate of NO₃⁻ uptake was related to diurnal, day-to-day, and seasonal changes in radiation. In summer the diurnal variation in NO₃⁻ uptake ranged from 25 to 50 mg N m⁻² h⁻¹ and the day-to-day variation ranged from 500 to 1500 mg N m⁻² d⁻¹. Mean daily rates of uptake over 12 d periods in summer and in winter averaged 908 and 44 mg N m⁻², respectively. The pattern of NO₃⁻ uptake followed that of CO₂ flux with the maximum rate of the former occurring 5 or 6 h after the maximum CO₂ influx. After defoliation, NO₃⁻ uptake was severely curtailed for 2 d concomitant with a very small influx of CO₂. Analysis of the changes that occurred in the rate of NO₃⁻ uptake immediately after the switching on or off of artificial light suggests that two reversible processes may be involved in the relation between NO₃⁻ uptake and radiation, one with a longer and the other with a shorter time constant.

INTRODUCTION

It is widely recognized that nutrient uptake by the roots of plants is regulated by the activity of the shoots (Hatrick and Bowling, 1973). Detailed information on the effects of light on nutrient uptake has, however, mostly been derived from experiments with algae and other simple plants (Raven, 1971), and some of the work on the uptake of nutrients by higher plants has involved the use of roots excised from the shoots. The results from such experiments, although difficult to apply quantitatively to field situations, show that the effects of light and of the activity of the shoots may be large and of significance in relation to the uptake of NO₃⁻ by crops. There follows an account of three experiments with simulated swards of perennial ryegrass grown in a system of flowing solution culture by means of which it was possible to monitor the uptake of NO₃⁻ by the plants from solution with NO₃⁻ concentration and pH held nearly constant. These experiments comprised studies of the immediate and the longer term effects of variation in light.

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and of partial defoliation upon NO\textsuperscript{−} uptake; aspects of the relationship between NO\textsubscript{2} uptake and CO\textsubscript{2} flux were also examined.

MATERIALS AND METHODS

Three experiments were conducted with simulated swards of perennial ryegrass (Lolium perenne L. cv. S23) grown in a system of flowing solution culture as previously described (Clement, Hopper, and Jones, 1978). In experiment 1, seed was sown on 4 April and studies of NO\textsubscript{2} uptake in relation to radiation and of uptake in relation to CO\textsubscript{2} flux were conducted during June. In experiment 2, seed was sown on 26 April and similar studies were conducted during June and July. In experiment 3, seed was sown on 18 October and studies of NO\textsubscript{2} uptake in relation to radiation were conducted during November to January. The effects of defoliation were studied in experiments 2 and 3. The plant culture units, each comprising 24 culture vessels giving a simulated sward of 0.8 m\textsuperscript{2}, were situated in a south-facing glasshouse with air temperature controlled at approximately 25 °C day, 15 °C night. Experiments 1 and 2 were conducted in natural light only, whereas in experiment 3 artificial as well as natural light was used for certain studies. Radiation from natural light was measured using a Kipp solarimeter. Artificial light from a 2 kW mercury halide lamp (Philips HPI/T) mounted 2 m above the plants in a light tube lined with aluminized film (Silver polyester KS 80/15\textsuperscript{1}) gave approximately 6-25 MJ m\textsuperscript{−2} d\textsuperscript{−1} during a photoperiod of 9 h. In all experiments the concentration of NO\textsubscript{2} in the nutrient solution was recorded at intervals of 10 min and held near constant at 0.1 mg N l\textsuperscript{−1}, i.e. 7-14 \textmu M, by means of the ion monitoring and control assembly as previously described (Clement, Hopper, Canaway, and Jones, 1974).

In experiments 1 and 2, NO\textsubscript{2} uptake and CO\textsubscript{2} flux were measured concomitantly for periods of 3 d. For this purpose all plants were removed except those growing in nine adjoining vessels of a plant culture unit. The remaining sward (0.25 m\textsuperscript{2}) was covered by a 500 mm x 500 mm transparent plastic film enclosure (see Plate 1) and CO\textsubscript{2} flux measured using an apparatus described by Stiles and Leafe (1970) and Stiles (1977).

RESULTS

NO\textsubscript{2} uptake and CO\textsubscript{2} flux under natural light

The daily rates of NO\textsubscript{2} uptake and of radiation were closely related during periods of 12 d both in summer (experiment 2, 9–20 July) and in winter (experiment 3, 26 November–7 December) (Fig. 1). However, in summer the mean rates for the 12 d period were respectively 908 mg N m\textsuperscript{−2} (standard deviation for individual daily values = 285 mg N m\textsuperscript{−2}) and 11.8 MJ m\textsuperscript{−2} (s.d. = 4.6) whereas in winter the corresponding values were 44.1 mg N m\textsuperscript{−2} (s.d. = 33.4) and 1.06 MJ m\textsuperscript{−2} (s.d. = 0.83). Thus, in winter the mean rates of NO\textsubscript{2} uptake and of radiation were respectively about 5 and 10% of those in summer. Although variation in the daily rate of NO\textsubscript{3} uptake was proportionally greater in winter than in summer it was, nevertheless, significantly correlated \((r = 0.7)\) with daily variation in radiation in both seasons.

Figure 2 shows the hour-to-hour variation in the rate of NO\textsubscript{2} uptake during a period of 7 d in summer (experiment 1, 4–11 June). During this period radiation exceeded 20 MJ m\textsuperscript{−2} d\textsuperscript{−1} on 5 and 6 June only, and on each of these days the maximum rate of uptake exceeded 50 mg N m\textsuperscript{−2} h\textsuperscript{−1}. Subsequently the weather deteriorated and on 10 June radiation was less than 5 MJ m\textsuperscript{−2} d\textsuperscript{−1} and the rate of uptake fell below 15 mg N m\textsuperscript{−2} h\textsuperscript{−1}. On all days except 10 June there was evidence of a diurnal pattern of uptake, with the rate falling to a minimum during the morning and rising to a maximum in the evening. On the brighter days the

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Simulated swards of perennial ryegrass growing in two plant culture units with flowing nutrient solutions containing NO$_3$ at 0.1 mg N l$^{-1}$. Plants have been removed from the nearer unit leaving a sward of 0.25 m$^2$ which is covered by a transparent plastic film enclosure for concomitant measurement of CO$_2$ flux and NO$_3$ uptake.
FIG. 1. Daily rates of NO$_3^-$ uptake by simulated swards of perennial ryegrass and of radiation from natural light during 12 d in July (experiment 2) and in November–December (experiment 3). Plants were grown in flowing nutrient solutions containing NO$_3^-$ at 0-1 mg N l$^{-1}$.

FIG. 2. Hourly rate of NO$_3^-$ uptake by a simulated sward of perennial ryegrass during 7 d in June (experiment 1). Plants were grown in flowing nutrient solution containing NO$_3^-$ at 0-1 mg N l$^{-1}$. ▲, sunrise to sunset, midpoint 1200 GMT; ▼, sunset to sunrise, midpoint 2400 GMT.
maximum rate of uptake was apparently reached later in the evening, coinciding approximately with sunset (2000 GMT).

Concomitant hourly rates of NO$_3^-$ uptake and CO$_2$ flux during periods of 72 h in summer (experiments 1 and 2) are shown in Fig. 3. Positive values represent net influx of CO$_2$ from the atmosphere to the shoot when carbon assimilation exceeded respiration, and negative values the net efflux when respiration exceeded assimilation. Efflux of CO$_2$ from the roots as a result of root respiration is not included in these values. In both experiments the maximum rate of NO$_3^-$ uptake commonly occurred 5 or 6 h after the maximum CO$_2$ influx and often during the period when efflux was beginning to exceed influx. Conversely, relatively low rates of NO$_3^-$ uptake were often coincident with maximum rates of CO$_2$ influx. As is commonly observed (Leafe, 1972), there was a close and immediate relationship between CO$_2$ influx and level of radiation.

![Graph showing NO$_3^-$ uptake and CO$_2$ flux](image-url)
NO$_3^-$ uptake under artificial light

The effect of radiation on the hourly rate of NO$_3^-$ uptake was examined further using plants growing under artificial light which was switched on and off to give a 9 h photoperiod; these studies were conducted over a period of 7 d (experiment 3). The values in Fig. 4 are means for measurements made at the same time on each of the 7 d. During the first 2 h after the light was switched on the rate of NO$_3^-$ uptake increased from 30 to 40 mg N m$^{-2}$ h$^{-1}$, then remained constant for about 5 h, and then started to increase again. After the light was switched off, the rate of uptake fell during the first 3 h, then remained constant for 8 h, and then fell sharply until the light was switched on at the beginning of the next photoperiod.

![Graph showing NO$_3^-$ uptake under artificial light](image)

**Fig. 4.** Effect of radiation on the hourly rate of NO$_3^-$ uptake by a simulated sward of perennial ryegrass growing under artificial light with a 9 h photoperiod (experiment 3). Plants were grown in flowing nutrient solution containing NO$_3^-$ at 0.1 mg N l$^{-1}$. Mean values are shown for measurements at the same time on each of 7 d. Vertical bar is standard error for hourly mean.

Effect of defoliation on NO$_3^-$ uptake and CO$_2$ flux

The defoliation treatment consisted of cutting the plants at 5 cm above the base of the shoots. This treatment, which removed about 70% of the total dry weight of the shoot, is analogous to the extent of defoliation which occurs when grass is harvested in the field. Figure 5 shows the immediate effect of such defoliation on NO$_3^-$ uptake with plants growing under artificial light with a 9 h photoperiod (experiment 3).

Before the shoots were cut and removed the rate of NO$_3^-$ uptake was increasing in accordance with the diurnal pattern shown in Fig. 4. Immediately after cutting, however, the rate started to fall, and within the next 2 h it had fallen to less than 15 mg N m$^{-2}$ h$^{-1}$, i.e. to less than 40% of that at the time of cutting. Thereafter it continued to fall, but more slowly.

During the first hour after cutting an attempt was made to collect the sap which accumulated on the cut ends of the shoots. It was found to contain 470 mg NO$_3^-$ N l$^{-1}$ and the total amount of NO$_3^-$ collected was equivalent to about 3 mg N m$^{-2}$.
Fig. 5. Effect of defoliation on the hourly rate of NO$_3^-$ uptake by a simulated sward of perennial ryegrass growing under artificial light with a 9 h photoperiod (experiment 3). Plants were grown in flowing nutrient solution containing NO$_3^-$ at 0.1 mg N l$^{-1}$, and cut 3 h after the start of the photoperiod at 5 cm above the base of the shoots.

Fig. 6. Effects of defoliation on the daily rate of NO$_3^-$ uptake by simulated swards of perennial ryegrass growing in winter under both natural and artificial light (experiment 3). Plants were grown in flowing nutrient solutions containing NO$_3^-$ at 0.1 mg N l$^{-1}$, and cut at 5 cm above the base of the shoots.
The longer term effects of defoliation on NO$_3^-$ uptake were studied using plants growing under both natural and artificial light (experiment 3). Because this study was conducted in winter the recovery of uptake after cutting was much slower under natural than under artificial light (Fig. 6). At the low level of natural light there was no detectable uptake during the first 7 d and during the next 5 d it remained at less than 100 mg N m$^{-2}$ d$^{-1}$. In contrast, under artificial light there was a 'lag' period of only 2 d after which uptake increased rapidly to 300 mg N m$^{-2}$ d$^{-1}$ at 3 d and reached 900 mg N m$^{-2}$ d$^{-1}$ at 12 d after cutting.

![Graph of NO$_3^-$ uptake and CO$_2$ flux](image)

**Fig. 7.** Effects of defoliation on the hourly rates of NO$_3^-$ uptake and of CO$_2$ flux by simulated swards of perennial ryegrass growing in summer under natural light (experiment 2). Plants were grown in flowing nutrient solutions containing NO$_3^-$ at 0.1 mg N l$^{-1}$ and cut at 5 cm above the base of the shoots. ---, 1200 GMT.

The longer term effects of defoliation on concomitant NO$_3^-$ uptake and CO$_2$ flux were also studied using plants growing under natural light in summer (experiment 2). The hourly rates of these two processes are shown in Fig. 7. Although CO$_2$ flux fell immediately after cutting to a negative value as respiration exceeded assimilation, the rate of NO$_3^-$ uptake did not fall as rapidly as under artificial light in winter (cf. Fig. 5) and took about 12 h to reach a negligible value. For 2 d after cutting, CO$_2$ influx showed small peak values at midday but NO$_3^-$ uptake remained at a very low level (<4.0 mg N m$^{-2}$ h$^{-1}$). Subsequently, CO$_2$ flux showed a pattern with positive values each day and negative each night, but NO$_3^-$ uptake increased day by day in a stepwise fashion with a less pronounced diurnal pattern than shown by intact plants.

**DISCUSSION**

The rate of NO$_3^-$ uptake by perennial ryegrass was related to diurnal, day-to-day, and seasonal changes in radiation and was also severely curtailed by cutting the
foliage at 5 cm above the base of the shoots. Several processes appear to be involved in these relationships.

Firstly, the growth of the shoot creates a demand for N in two ways: (a) demand for reduced or metabolized N as a constituent of the protoplast and (b) possible demand for \( \text{NO}_3^- \) as an anionic component of the solutes required for vacuolar expansion (Mott and Steward, 1972). Edwards and Barber (1976) considered that, although demand by the shoot might not lead to an immediate increase in N uptake, the development of a nutrient stress within the shoot would eventually increase the capacity of the root to attain a higher rate of uptake. We did not determine \( \text{NO}_3^- \) concentration within the shoots, but Minotti and Stankey (1973) found with \textit{Beta vulgaris} L. that there was a peak in concentration at about 2000 GMT, or about 4 h after sunset, and another within an hour or so of sunrise. Thus, the delayed development of a \( \text{NO}_3^- \) stress within the shoot could be one reason for the observed lag of about 6 h between the maximum rate of \( \text{CO}_2 \) influx and maximum rate of \( \text{NO}_3^- \) uptake (Fig. 3) and likewise for the observed displacement of the diurnal maximum and minimum rates of uptake by about 6 h after noon and midnight respectively (Fig. 2).

Secondly, there is evidence that the rate of \( \text{NO}_3^- \) uptake depends on the metabolic activity of the root (Rao and Rains, 1976) which, in turn, would require a supply of assimilate originating from the photosynthetic activity of the shoot. Further, it has been found in \(^{14}\text{C} \) studies that the supply of assimilate to the root was severely reduced for 2 d after the removal of only half the photosynthetic surface of \textit{Hordeum distichon} L. plants (Ryle and Powell, 1975). These observations and the very low rates of \( \text{NO}_3^- \) uptake that we observed during the 2 d after defoliation (Figs 6 and 7) are consistent with the hypothesis that \( \text{NO}_3^- \) uptake depends on a supply of assimilate to the root. This dependence has been suggested by Brouwer and De Wit (1969) as a general process whereby the shoot regulates ion uptake and they specifically discussed its significance in relation to the uptake of \( \text{NO}_3^- \). In their opinion the experimental evidence supported the hypothesis that ‘leaves and roots are competing for carbohydrates and N and the organ which will be most successful in obtaining its requirement is that which is nearest to the source’. It follows that \( \text{NO}_3^- \) uptake may be influenced by the extent to which there is assimilate available that is surplus to the immediate need of the shoot. Furthermore, the size of a surplus would depend not only upon the total amount of assimilate in the shoot but also upon the amount of \( \text{NO}_3^- \) transported from the root and available for reduction and growth of the protoplast. This suggests a feedback process whereby a deficiency of \( \text{NO}_3^- \) in the shoot may stimulate its uptake by the root. Such a process would presumably result in an increase in root respiration coincident with periods of increased rates of uptake. In this context, it is notable that Huck, Hageman, and Hanson (1962) reported that maximum respiration by roots of intact plants was at the end of the photoperiod, coincident with the period when \( \text{NO}_3^- \) uptake was approaching a maximum (Figs 2 and 3). Likewise, Hansen (1977) reported that changes in the respiration of the roots of \textit{Lolium multiflorum} Lam. induced by day-to-day variation in artificial light were delayed in a manner parallel to the relationship between radiation and \( \text{NO}_3^- \) uptake (Fig. 4).
The results of Huck et al. (1962) and Hansen (1977) support our general conclusion that the major processes relating the level of radiation incident on the shoot and the rate of \( \text{NO}_3^- \) uptake by the root have a delayed action corresponding to a time constant of up to 6 h. There was however evidence of a more immediate effect of changes in radiation, which calls for further analysis. When plants were growing under artificial light with a 9 h photoperiod (experiment 3) there was a rapid change in the rate of \( \text{NO}_3^- \) uptake immediately after the light was switched on or off (Fig. 4). During the photoperiod, equation (1) relating the rate of \( \text{NO}_3^- \) uptake \( (U_N) \) and hours after switching the light on \( (t) \):

\[
U_N = 31.2 + 1.9 \times 10^{-6} e^{1.75t} - 8.2e^{-1.75t}
\]

accounted for the observed variance with a residual standard deviation of only 0.57 mg N m\(^{-2}\) h\(^{-1}\). A similar equation (2):

\[
U_N = 30.8 - 2.9 \times 10^{-4} e^{0.67t} + 7.0e^{-0.67t}
\]

accounted for the observed variance when the light was off with a residual standard deviation of 0.24 mg N m\(^{-2}\) h\(^{-1}\). The close fit to the observed values of these two double exponential equations, one being a mirror image of the other, with the general form as shown in equation (3):

\[
U_N = a \pm be^{xt} + ce^{-xt}
\]

suggests two reversible processes, one with a relatively long and the other with a relatively short time constant corresponding respectively to the second and third terms. The process with the shorter time constant would probably not be apparent under natural conditions with radiation changing relatively slowly and its underlying mechanism and physiological role remain matters for further study.

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LITERATURE CITED


