Determination of a Critical Nitrogen Dilution Curve for Winter Wheat Crops

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A set of N-fertilization field experiments was used to determine the 'critical nitrogen concentration', i.e. the minimal concentration of total N in shoots that produced the maximum aerial dry matter, at a given time and field situation. A unique 'critical nitrogen dilution curve' was obtained by plotting these concentrations N_{ct} (% DM) *vs.* accumulated shoot biomass DM (t ha^{-1}). It could be described by the equation:

$$N_{ct} = 5.35DM^{-0.442}$$

when shoot biomass was between 1.55 and 12 t ha^{-1}. An excellent fit was obtained between model and data ($r^2 = 0.98$, 15 d.f.). A very close relationship was found using reduced N instead of total N, because the nitrate concentrations in shoots corresponding to critical points were small. The critical curve was rather close to those reported by Greenwood *et al.* (1990) for C_3 plants. However, this equation did not apply when shoot biomass was less than 1.55 t ha^{-1}. In this case, the critical N concentration was independent of shoot biomass: the constant critical value $N_{cr} = 4.4\%$ is suggested for reduced-N.

The model was validated in all the experimental situations, in spite of large differences in growth rate, cultivar, soil and climatic conditions; shoot biomass varying from 0.2 to 14 t ha^{-1}.

Plant N concentration was found to vary by a factor of four at a given shoot biomass level. In the heavily fertilized treatments, shoot N concentration could be 60% higher than the critical concentration. Most (on average 80%) of the extra N accumulated was in the form of reduced N. The proportion of nitrate to total N in shoot mainly depended on the crop stage of development. It was independent of the nitrogen nutrition level.

Key words: Winter wheat, *Triticum aestivum*, arable crops, plant N concentration, aerial biomass, critical nitrogen, dilution curve, fertilization, reduced N, nitrate.

INTRODUCTION

Various studies have indicated that N concentration of graminaceous grasslands decreases during the growth cycle (Salette and Lemaire, 1981; Salette, 1982; Lemaire and Salette, 1984). This is also true for other cultivated plants (Greenwood *et al.*, 1990; Lemaire *et al.*, 1990), including legumes which also derive their N from atmospheric nitrogen (Lemaire *et al.*, 1985). Plant N concentration in field crops has been related to the aerial biomass according to the general equation:

$$N_t = aDM^{-b} \qquad (1)$$

where DM is the amount of dry matter accumulated in the shoots expressed in t ha^{-1}, and N_t is the total N concentration in shoots expressed in % DM.

Salette and Lemaire (1981) have considered that the decrease in N concentration was tied to plant growth as part of a 'dilution' phenomenon of plant nitrogen by carbon assimilates. The 'dilution law' defined by eqn (1) corresponds to the existence of an allometric relation between accumulated nitrogen N_a(kg N ha^{-1}) and accumulated dry matter in shoots, accounting for the following equations:

$$N_t = \frac{N_a}{10DM} \qquad (2)$$

$$N_a = 10aDM^{1-b} \qquad (3)$$

The coefficient $10a$ represents the amount of N present in the shoots when the aerial dry matter produced is 1 t ha^{-1}; $(1-b)$ is the allometric ratio between N accumulation and dry matter accumulation in shoots, since the derivation of eqn (3) yields:

$$RNR = (1-b)RGR \qquad (4)$$

where RGR is the relative growth rate and RNR the relative accumulation rate of nitrogen in the shoots; RNR being defined similarly to RGR:

$$RNR = \frac{1}{N_a}\frac{dN_a}{dt} \qquad (5)$$

Caloin and Yu (1986) and Greenwood *et al.* (1991) have proposed to use another dilution equation:

$$N_t = N_{\omega} + \frac{a}{b+DM} \qquad (6)$$

This hyperbolic formulation allows to account for a minimal N concentration in shoots (N_{ω}) which would be obtained

for high aerial biomasses. On a theoretical point of view, it can be derived from the assumption that plant N is composed of two pools: a 'metabolic' pool, associated with photosynthesis and growth processes, and a 'structural' pool corresponding to N storage, structure and other metabolic functions (Greenwood *et al.*, 1991).

N dilution is due to two processes: self-shading of leaves (Lemaire *et al.*, 1991; Lemaire and Chartier, 1992) and change in the leaf:shoot ratio during crop development (Lemaire *et al.*, 1985, 1992). The decrease in N mean concentration in shoots is also observed for isolated plants (Caloin and Yu, 1986; Lemaire and Chartier, 1992). In fact, shaded lower leaves contain less N than upper leaves, the latter being richer in enzymatic proteins. Furthermore, the proportion of cell wall constituents (cellulose, lignin) increases in stem tissues during aging and the protein concentration simultaneously decreases when compared to that of leaf blades (Lemaire *et al.*, 1992). Finally, senescence of older leaves due to intra- and inter-plant competition for light and mineral nutrients is accompanied by N remobilization towards growing organs. Therefore, during active growth, a plant needs less and less N to build each new unit of dry matter (Salette and Lemaire, 1981).

However, the dilution law defined by eqn (1) can be applied only for plant populations having a regular N uptake. This condition is usually satisfied when the plants do not receive high levels of N fertilization during the growing season because then, the mineral N uptake from the soil occurs smoothly. Nevertheless, even for temporary or permanent grasslands fertilized once in spring before bud break and where N availability is rather progressive, it has been observed that the N quantity absorbed by the plants does not strictly follow dilution eqn (3) since the soil supply and the N uptake can fluctuate (Lemaire and Salette, 1984). The dilution curves can even be more irregular for annual crops such as wheat receiving high levels of N fertilization (Machet, 1989). Lemaire and Salette (1984) have tried to establish a 'limit dilution curve', also called 'reference curve' for grasslands, defined by the fact that 'below the curve, N availability would become a limiting factor of dry matter growth'; they suggested the following equation:

$$N_r = 5.31DM^{-0.477} \qquad (7)$$

This curve could be applied when aerial dry matter varies between 1 and 12 t ha^{-1}. For biomass values less than 1 t ha^{-1}, dilution would not be significant. In this case, since plant canopy is not closed, leaves self-shading and competition for light among plants are low; there would not be a significant decrease in N concentration of aerial parts.

The preceding authors have also suggested another 'reference' curve equation when dry matter ranges from 1 to 6 t ha^{-1}:

$$N_r = 4.79DM^{-0.324} \qquad (8)$$

Lemaire and Denoix (1987) confirmed the validity of this curve for spring growth of tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*), in this range of shoot DM. Lemaire, Gastal and Salette (1989) have then suggested that the curve could be applied to all pure grass swards.

Greenwood *et al.* (1990) have tried to generalize the N dilution curves to various crop species. They have selected

situations whereby N was not a growth limiting factor. Two 'critical' curves have been suggested: one for C$_3$ species (tall fescue, lucerne, potato, wheat, rape, beans, cabbage), and one for C$_4$ plants whose N concentration is lower (sorghum, maize, setaria):

C$_3$ plants: $N_c = 5.7DM^{-0.5}$ (9)

C$_4$ plants: $N_c = 4.1DM^{-0.5}$ (10)

In fact, each of these critical curves was defined as the average fitting curve of all non limiting N treatments. However, if all the experimental points are really in non limiting N situations (which is, according to the authors, not certain), the critical N dilution curve should correspond to the lower curve and not to the average fitting curve. A more precise definition of the critical curve seems to be necessary.

It is also necessary to test whether only one critical N dilution curve can be applied in different growth conditions. Thus, Lemaire *et al.* (1985) have noticed that the leaf:stem ratio in lucerne was not the same for spring and summer growth. This might result in the existence of several critical dilution curves. The objective of this study was to evaluate the validity of a critical N dilution curve for winter wheat at stages of development ranging from tillering to anthesis, for different cultivars and growth conditions.

MATERIALS AND METHODS

Field experiments

Several N fertilization experiments in various regions of Northern France were used in this study including some described by Meynard (1985). The experiments that we have retained are rather heterogeneous since they were not all designed to determine a critical N nutrition curve for wheat (Table 1). The cultivars used were Appolo, Centurion, Fidel, Lutin, Talent, Thésée and Soissons. Seeds were sown in autumn except for expt 11 (carried out in Aulnois in 1990) sown in Jan.

The rates of fertilizer-N applied varied between experiments; each experiment consisted of one or two rates of N assumed to be non limiting and several rates supposed to be limiting growth, at least before anthesis. Samples were taken (three or four replicates) between stage Feekes 3 (tillering) and Feekes 10.5.1 (onset of flowering), according to Large's scale (1954). Measurements were made during either part or whole growth cycle. At each sampling time, aerial dry matter and N concentration (Kjeldahl or Dumas method) were measured. Nitrate concentration in shoots was measured on all samples except for expts 1 and 2 (carried out at Mons in 1984 and Aulnois in 1985, respectively).

Analytical methods

Total N concentration in shoots was determined using either the Kjeldahl 'classical' method or the Dumas method. The Kjeldahl method consisted of a wet digestion in concentrated sulphuric acid for 4 h at 400 °C, without specific reduction for nitrate. The catalyst used was a mixture of selenium oxide and potassium sulphate. This method was applied to expts 4–7 and 19–22 (Table 1), except for treatments used to determine the critical curve (Table 2).

TABLE 1. *Characteristics of field experiments*

	Description of field experiments				Nitrogen treatments (Applied N in kg N ha^{-1})	Sampling stages (Feekes Scale)
No.	Location	Year	Soil texture	Cultivar		
1	Mons (80)	84	Clay loam	Fidel	0–120–170–220	5–7–9–10.5.2
1				Talent	0–120–170–220	5–7–9–10.5.1
2	Aulnois (02)	85	Calcareous loam	Fidel	0–80–123–166–210	5–6–7–8–9–10.3
3	Aulnois (02)	89	Calcareous loam	Appolo	0–150–180–410	3–4–5–6–7–8–10.5.1
4	Le Robillard (14)	89	Clay sandy loam	Centurion	0–50–100–150	4–5–6–7–8–9–10
5	Le Robillard (14)	90	Clay sandy loam	Centurion	0–50–100–150	5–6–7–8–10.2
6	Le Robillard (14)	91	Clay sandy loam	Thésée	0–50–100–150	4–5–6–7–8–9–10–10.3
7	Auxerre (89)	91	Loam	Soissons	120–180–240	5–6–7–10
8	Pouilly (02)	91	Sandy clay loam	Soissons	60–110–160–210–360	3–4–5–6–7–8–9–10–10.3
9	Pouilly (02)	90	Sandy clay loam	Thésée	10–40–80–130–170–340	3–4–5–6–7–8–10.1–10.4
10	Samoussy (02)	90	Sandy loam	Thésée	20–130–260	4–5–6–9–10.1–10.4
11	Aulnois (02)	90	Calcareous loam	Thésée	20–80–140–310	4–5–6–7–8–9–10.3–10.5
12	Grignon (78)	91	Clay loam	Thésée	15–65–100–130–180	4–5–6–7–8–9–10.1
13	Vraux (51)	92	Calcarious loam	Soissons	0–100–200–300	6–7–10
14	Bignan (56)	92	Clay sandy loam	Soissons	0–30–70–110–150–190–230	6–7–10.1
14				Thésée	0–30–70–110–150–190–230	6–7–10.1
15	Vraignes (80)	93	Clay sandy loam	Soissons	0–90–130–170–210	6–9–10.5.3
16	Binges (21)	93	Loamy clay	Soissons	0–50–90–130–170–210–250	6–7–10.1
17	Bourgogne (51)	93	Calcareous loam	Soissons	0–50–90–130–170	6–8–10–10.5.1
18	Bignan (56)	93	Clay sandy loam	Soissons	0–30–70–110–150–190–230	6–7–9–10.5
18				Thésée	0–30–70–110–150–190–230	6–7–9–10.5
19	Champagne* (51)	77	Chalk	Talent	0–20–60–100	4–5
20	Champagne* (51)	78	Chalk	Talent	0–20–60–100	4–5
21	Noyon* (60)	81	Calcareous loam	Fidel	0–40–90	4–5
21				Lutin	0–40–90	4–5
22	Noyon* (60)	83	Clay loam	Fidel	0–40–90	4–5
22				Lutin	0–40–90	4–5

* Experiments from Meynard (1985).

TABLE 2. *Experimental treatments selected from statistical analysis (see text) and calculated critical points for total-N* (N_{ct}) *and reduced-N* (N_{cr})

	Experiment			Calculated critical point		
No.	Location	Cultivar	Feekes stage	DM (t ha^{-1})	N_{ct} (% DM)	N_{cr} (% DM)
3	Aulnois 89	Appolo	4	0·80	5·23	4·98
7	Auxerre 91	Soissons	5	0·85	5·05	4·79
4	Le Robillard 89	Centurion	5	0·90	4·41	4·31
4	Le Robillard 89	Centurion	5	0·93	4·55	4·43
4	Le Robillard 89	Centurion	6	1·65	4·19	4·05
7	Auxerre 91	Soissons	6	2·01	3·99	3·95
5	Le Robillard 90	Centurion	6	2·06	3·95	3·89
2	Aulnois 85	Fidel	6	2·46	3·42	—
5	Le Robillard 90	Centurion	7	2·73	3·63	3·57
8	Pouilly 91	Soissons	7	3·30	3·16	3·10
4	Le Robillard 89	Centurion	7	3·37	2·96	2·92
6	Le Robillard 91	Thésée	8	4·72	2·86	2·77
1	Mons 84	Talent	8	5·60	2·49	—
2	Aulnois 85	Fidel	9	6·58	2·39	—
12	Grignon 91	Thésée	8	6·77	2·33	2·30
5	Le Robillard 90	Centurion	10.1	7·13	2·39	2·34
9	Pouilly 90	Thésée	9	7·53	2·16	2·15
7	Auxerre 91	Soissons	9	7·84	2·04	2·01
12	Grignon 91	Thésée	10	9·26	2·02	2·01
1	Mons 84	Talent	10.5.1	11·42	1·73	—
18	Bignan 93	Thésée	10.5	11·89	1·69	1·67

— Shoot nitrate concentration not determined.

The Dumas method was used for all other experiments. It involved combustion of plant powder at about 1800 °C (flash combustion), reduction of N oxides by reduced Cu at 600 °C and analysis or N_2 by catharometry (Carlo Erba NA1500 analyser). This method takes into account the total N contained in the plant, including nitrate.

Nitrate was extracted from dried and finely ground plant tissues with a 1 M KCl solution. The plant powder was stirred for 2 min in the solution; the mixture was then left for 16 h at 4 °C and filtered. The nitrite formed after the nitrate reduction on a cadmium column was measured using the Griess method. The spectrometric reading was done at 540 nm with a continuous flow equipment (Skalar).

DATA PROCESSING

Definition of the critical dilution curve

The N concentration of each point of the critical dilution curve is the minimum N concentration observed at a given date among all N treatments that produced the maximum amount of dry matter up to that date. It corresponds to a N concentration level at which the aerial dry matter does not significantly increase when the N fertilization increases despite an increase in crop N uptake and shoot N accumulation.

Statistical analysis

At each measurement date and for each experiment, the amounts of aerial dry matter produced and the N concentrations were compared in the different N treatments. The Student's two tail t test was used to test the hypothesis of means equality at 10% level of probability. The means were said to be equal when the calculated t was lower than the theoretical t. The 10% significance level was preferred over the 5% significance level classically used in order to reduce the occurrence of Type II error (Dagnélie, 1975).

The experimental treatments retained in order to compute the critical total N concentration are composed of three, four or five points (DM_i, N_i) corresponding to different N

levels. Two cases can be distinguished depending on the position of these n points (Fig. 1 A and B). Each set of points must satisfy the following inequalities (statistical meaning):

First case (Fig. 1 A):

$$DM_1 < DM_2 = \ldots = DM_n \qquad (n = 3, 4 \text{ or } 5)$$

$$N_1 < N_2 \leqslant \ldots < N_n$$

Second case (Fig. 1 B):

$$DM_1 < DM_2 < DM_3 = \ldots = DM_n \qquad (n = 4 \text{ or } 5)$$

$$N_1 < N_2 < N_3 \leqslant \ldots < N_n$$

For each measurement date, the variation of total N concentration *vs.* shoot dry matter was assimilated to a bilinear relation composed of: (*a*) an oblique line of joint increase in dry matter and N concentration: in the first case (Fig. 1 A), this line passes through points D1 and D2; in the second case (Fig. 1 B), it is the regression line between points D1, D2, and D3, minimizing the sum of square errors; (*b*) a vertical line corresponding to an increase in N concentration without variation in the aerial dry matter: its abscissa is equal to the mean abscissa of points D2 to Dn (Fig. 1 A) or D3 to Dn (Fig. 1 B).

In both cases, the critical N concentration corresponds to the ordinate of the intersection point of the oblique and vertical lines.

RESULTS

Experimental dilution curves

Figure 2 gives an example of experimental N dilution curves obtained from the four treatments of expt 2 (at Aulnois in 1985). It indicates that the N dilution phenomenon is not systematic: the fertilizer-N supplied on 18 Apr. when the plants were at a '1 cm ear' stage of development resulted in an increase in the N concentration of the shoots at the onset
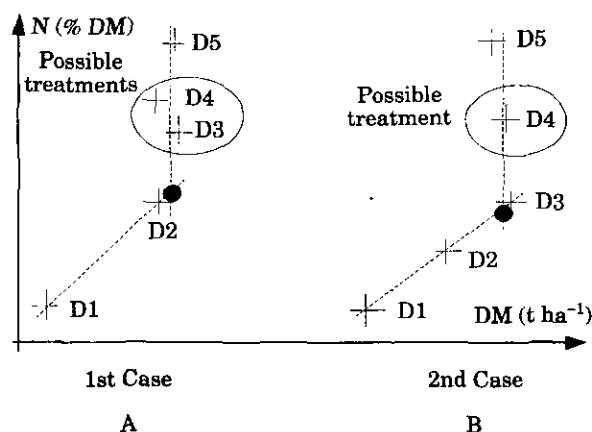

FIG. 1. Diagram representing N concentration versus accumulated dry matter in shoots. (+) Values measured in the different nitrogen fertilization treatments (D1–D5) at a given date; (●) calculated 'critical' point, at this date.
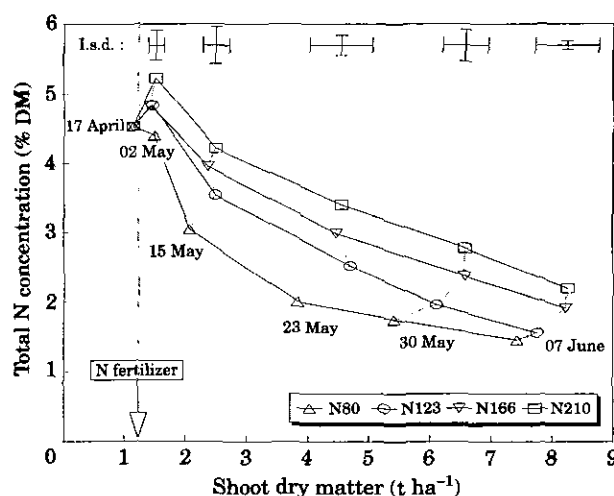

FIG. 2. Experimental 'dilution' curves observed in the four N fertilization treatments of trial 2 (Aulnois 1985). The four treatments (N80, N123, N166 and N210) received 80 kg N ha^{-1} at tillering (25 Feb. 1985) and 0, 43, 86, 130 kg N ha^{-1} at the stage 'ear at 1 cm' (18 Apr. 1985), respectively. l.s.d., little significant difference (Newman and Keuls).

of elongation (2 May). This was particularly obvious for treatment N_{210} which had received the highest level of N. A more regular dilution kinetics was observed after this date because the plants did not receive any other N supply. In this experiment and on a more general standpoint, statistical differences in aerial biomass between experimental treatments appeared less rapidly than differences in total N concentration or in amounts of N accumulated in shoots. This is due to less variability among repetitions of total N measurements than among repetitions of shoot dry matter measurements, and also to the fact that N uptake rate varies more between treatments than growth rate. It is therefore difficult to estimate, based only on one experiment, the critical N concentration for a given amount of dry matter. This example also illustrates the chronology of a nitrogen deficiency: first, the nitrogen concentration of the aerial shoots decreases while growth remains the same; then, growth rate decreases and dry matter accumulation is reduced.

Critical dilution curve

Among the set of experiments studied (Table 1), only 21 sampling dates out of 120 fulfilled the previously defined statistical criteria. Each of these situations allowed us to compute, using the computation method described above (Table 2), a critical point of N concentration (total and reduced) for a given level of biomass.

Four situations corresponded to a low level of aerial biomass (less than $1\,t\,ha^{-1}$). The critical total N concentration (N_{ct}) found for these four points varied between 4·41 and 5·23%. In fact, the estimate of the critical concentration is less precise in those situations because the slope of the oblique line used in calculations is very steep (Fig. 3). This is particularly true for points 1 and 2 for which the slope (expressed in $kg\,N\,ha\,t^{-2}\,DM$) is 67 and 62, respectively (whereas it is 30 and 21 for points 3 and 4). The method of calculation probably tends to overestimate the true critical concentration of the first two points.

The 17 other critical points, having a biomass higher than or equal to $1·65\,t\,ha^{-1}$, are precisely estimated since the slope of the oblique line was always lower than 20 (Fig. 3). We have fitted these data to different mathematical functions by the non linear regression (Huet, Jolivet and Méssean, 1992). The power [eqn (1)] and hyperbolic [eqn (6)] functions fit closely to the experimental data, according to the following equations:

$$N_{ct} = 5·35DM^{-0·442} \qquad (11)$$

$$N_{ct} = 0·77 + \frac{14·12}{2·48 + DM} \qquad (12)$$

The models account for 98% of the variance: $r^2 = 0·982$ (15 d.f.) and $r^2 = 0·981$ (14 d.f.), respectively. In both cases, residuals are randomly distributed: the models are unbiased. The hyperbolic model involves three parameters which are very strongly correlated with each other, whereas the power function contains only two parameters much less correlated. Therefore, we prefer to use the power function [eqn (11)] shown in Fig. 4.
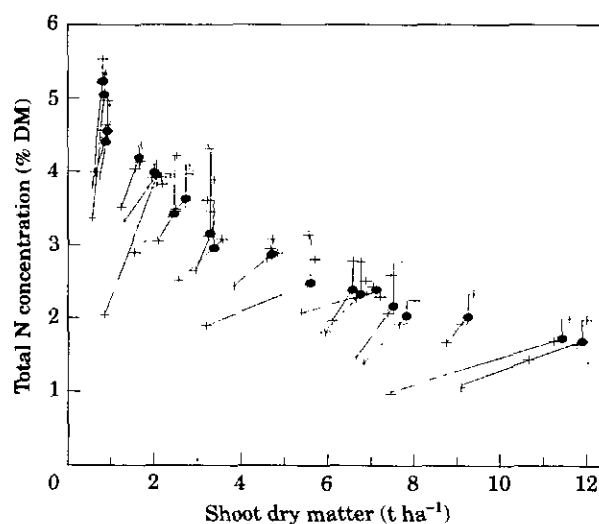

Fig. 3. Diagram representing N concentration versus accumulated dry matter in shoots, for the selected data satisfying the statistical criteria defined in text. (+) Values measured in the different nitrogen fertilization treatments (D1–D5) at a given date; (●) calculated 'critical' points (N_{ct}), at each date (method shown in Fig. 1).
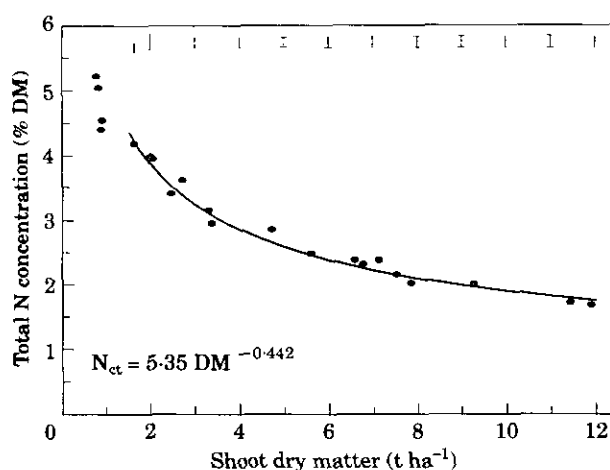

Fig. 4. Critical points (N_{ct}), and critical N dilution curve obtained by non linear fitting applicable to the range $1·55$–$12\,t\,DM\,ha^{-1}$ (development stages varying between Feekes 5 and 10.5). Curve equation: $N_{ct} = 5·35DM^{-0·442}$. Vertical bars indicate confidence intervals on fitted curve $(P > 0·95)$.

The confidence interval ΔN_{ct} (at $P > 0·95$) varies between $\pm 0·07\%$ for a shoot biomass of $5\,t\,ha^{-1}$ and $\pm 0·14\%$ for a biomass of $1·55\,t\,ha^{-1}$.

The power function characterizes well the critical dilution curve of winter wheat: it can be applied when shoot dry matter varies between $1·6$ and $12\,t\,ha^{-1}$ and for stages of development ranging from '1 cm ear' (Feekes 5) to the onset of flowering (Feekes 10.5.1) (Table 2).

Measurements of $N\text{-}NO_3^-$ in shoots have been made in 13 out of 17 treatments used to build this curve. The critical concentrations of reduced nitrogen (N_{cr}) were then calculated in these 13 situations (Table 2) and a critical dilution curve was determined for reduced-N:
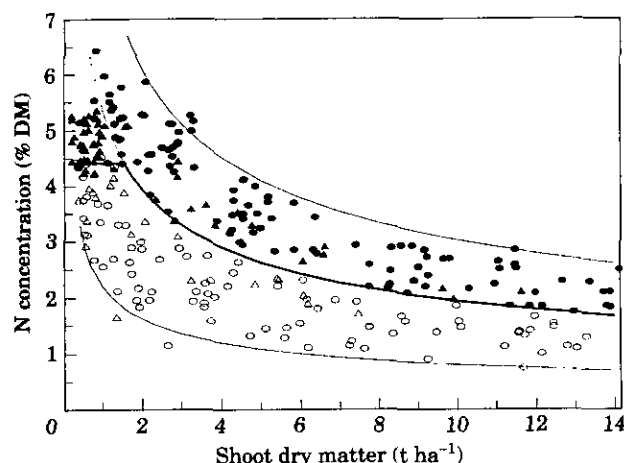
$$N_{cr} = 5·29DM^{-0·443} \qquad (13)$$

FIG. 5. Diagram representing N concentration versus accumulated dry matter in shoots, for all experimental treatments either N limiting or non-limiting growth. ($\bigcirc$), ($\triangle$) Treatments where N was statistically limiting factor of shoot growth. ($\bullet$), ($\blacktriangle$) Treatments where N was not statistically limiting factor of shoot growth. ($\bigcirc$), ($\bullet$) Analysis by Dumas' method. ($\triangle$), ($\blacktriangle$) Analysis by Kjeldahl's method. (—) Critical dilution curve [eqn (11)]; (———) 'envelope'-curves N_{min} and N_{max} [eqns (16) and (17)].

The fitting is good due to its low dispersion ($r^2 = 0.981$; 11 d.f.) and the absence of bias in residuals. In fact, the critical curves of total-N [eqn (11)] and reduced-N [eqn (13)] are very close since the experimental points in the vicinity of the critical curve correspond to plants containing low levels of $N-NO_3^-$: on average, 1 % of the total nitrogen of these plants was in the form of nitrate. Gastal, Belanger and Lemaire (1992) had also noticed that fescue plants that received an optimum fertilization (near the critical curve) contained low levels of $N-NO_3^-$ (0.1 %).

Validation of the critical curve

The situations not retained by the statistical criteria defined earlier were used to test the validity of the critical curve. A new selection was operated on these data in order to retain the significantly either 'N limiting or non-limiting treatments. A treatment was considered to be N limiting when its shoot dry matter was significantly smaller ($P > 0.90$) than the shoot DM of the treatment having received the rate of fertilizer-N immediately higher. Several treatments were considered N non limiting when their shoot DM were statistically equal ($P > 0.90$), although they had received different N rates. This selection provided 245 data points, 104 being N limiting and 141 being N non-limiting.

Figure 5 shows that the critical curve was validated for all these situations retained within the biomass range used to establish it (1.65–12 t ha^{-1}). It was also validated for biomass varying from 12 to 14 t ha^{-1} for flowering populations (Feekes 10.5.1 to 10.5.4). This result indicates that growth rate, density (at least according to our experimental conditions, that is, between 150 and 400 plants m^{-2}), cultivar and pedoclimatic conditions did not really affect the critical nitrogen concentration.

In contrast, the equation of the critical curve cannot be applied to low biomasses (close to 1 t ha^{-1}): the values

corresponding to dry matter of 1.0, 0.75, and 0.5 t ha^{-1} would be 5.35, 6.08 and 7.27 %, respectively; these values are well above those observed in the non limiting treatments (Fig. 5).

In the range 0.2–1.65 t ha^{-1}, the critical concentration should be between 4.20 % (minimum concentration of non limiting points) and 4.55 % (maximum concentration of limiting points). Our results suggest that the critical concentration could be constant over this range of shoot biomass. The mean value, 4.4 %, can be retained since it is close to the critical concentrations of points 3 and 4 which were 4.41 and 4.55 % (Table 2). The curve N_{ct} [eqn (11)] and the horizontal line of ordinate 4.4 % meet at point of abscissa DM = 1.55 t ha^{-1}.

This critical dilution curve should allow an accurate diagnosis of wheat N nutrition. Lemaire *et al.* (1989) have proposed to characterize the nitrogen nutrition level of a population by using the 'N nutrition index' (NNI) defined as:

$$NNI = \frac{N_t}{N_{ct}} \quad (14)$$

where N_t is the total nitrogen concentration measured and N_{ct} is the critical nitrogen concentration corresponding to the amount of shoot dry matter produced. The nitrogen nutrition is considered 'optimal' when the NNI is 1, limiting when it is lower than 1, and in excess ('luxury consumption') when it is higher than 1. The NNI can be used to quantify the N stress intensity after the onset of a nitrogen deficiency (Ågren, 1985a; Meynard and David, 1992; Justes, 1993).

Proportion of nitrate and reduced nitrogen

When growth is maximum, nitrogen accumulation in shoots can be higher than the 'optimum' value defined by the N critical curve. The excess accumulation is often described as a 'luxury consumption' (Ågren and Ingestad, 1987; Hirose, Freijsen and Lambers, 1988), and the extra N often supposed to be non assimilated-N (nitrate). We have tested this hypothesis.

Figure 6 shows the ratio of nitrate nitrogen (N_n) to total nitrogen (N_t) *vs.* the nitrogen nutrition index (NNI), in all situations where shoot nitrate was determined. The fraction of nitrate in shoots (N_n/N_t) increases markedly when the NNI increases.However, a large variability is encountered at a given level of N nutrition. The amount of $N-NO_3^-$ varies from 0.1 to 4 % when the NNI is lower than 0.85 (significant N deficiency; Justes, 1993), from 0.1 to 7 % when the NNI is close to 1 ('optimal' N nutrition) and from 2 to 18 % when the NNI is higher than 1.15 (significant excess of N).

It is also interesting to determine the ratio of nitrate nitrogen to the extra nitrogen accumulated in the shoots beyond the critical concentration, when N is not a limiting factor for aerial growth (NNI statistically greater than 1). This fraction was calculated as:

$$f = \frac{N_n}{N_t - N_{ct}} \quad (15)$$

where N_{ct} is the critical total nitrogen concentration corresponding to the amount of dry matter observed.
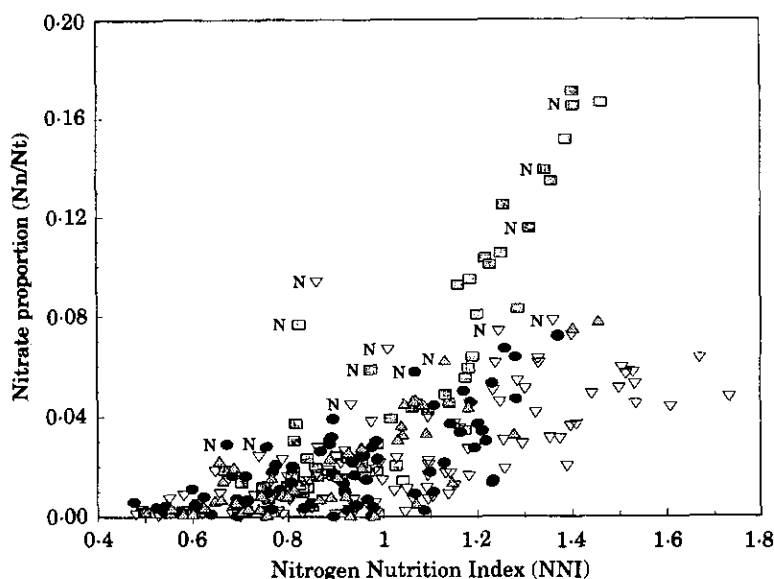
FIG. 6. Proportion of nitrate-N to total N found in shoots (N_n/N_t) vs. nitrogen nutrition index (N_t/N_{ct}) in all field experiments where shoot nitrate was measured. Development stages: (▦), Feekes 3–5 (tillering); (▽), Feekes 6–7 (stem elongation); (●) Feekes 8–10 (booting) and (△), Feekes 10.1–10.5 (earing-anthesis). Number of points = 291. N indicates that fertilizer-N had been applied during the week preceding the measurement. The surrounded points correspond to low densities (< 150 plants m^{-2}).
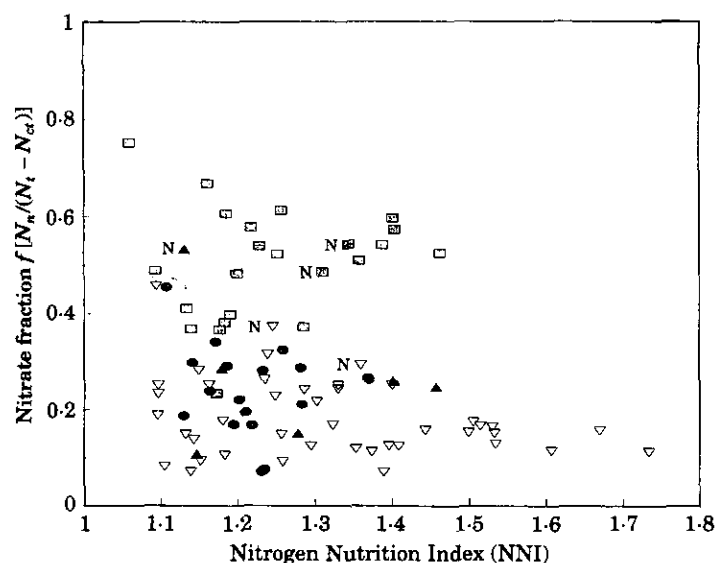

FIG. 7. Proportion of nitrate-N to extra N (supplement of N to critical curve) found in shoots $[f = N_n/(N_t - N_{ct})]$ vs. nitrogen nutrition index (N_t/N_{ct}) in all treatments where N was not a limiting factor of shoot growth (NNI $\geqslant 1$ + confidence interval). Number of points = 89. Symbols as in Fig. 6.

We found that this fraction depended mainly on the stage of development. For a given stage, it did not depend on the former N nutrition level defined by the NNI (Fig. 7): there was no correlation between f and NNI. The proportion of nitrate to extra N (f) was more important in the treatments that had recently been fertilized (during the week preceding the measurement), although this effect was highly dependent on the chemical composition of the fertilizer (Justes, 1993).

The highest proportion of nitrate nitrogen was observed during tillering (Feekes 3 to 5): f varied between 37 and 77% (mean value: 50%) for young stages and was not dependent on the level of N nutrition. Our results are in complete agreement with those reported by Zhen and Leigh (1990) in controlled environmental conditions. These authors have shown that nitrate accumulation in plants occurs in significant quantity only when the N needs to reach maximum growth were fulfilled. They have also found that the proportion of nitrate accumulated beyond the critical concentration (f) was about 50% at similar young stages, remained constant for different levels of N nutrition

$(1 \leqslant NNI \leqslant 1\cdot45)$ and did not vary between plant organs (leaf, stem, root).

The proportion of nitrate (f) was smaller during and after stem elongation: no difference was observed between stages Feekes 6–7, Feekes 8–10 and Feekes $\geqslant 10\cdot1$. Depending on the situations, the amount of nitrate (f) varied from 7 to 52%, no matter what the former level of nutrition. If low densities $(< 150\ \text{plants m}^{-2})$ and situations whereby the plants had been fertilized the previous week are not taken into account, this fraction f was equal to 20% and was little variable $(\pm3\%)$: therefore, most (80%) of the nitrogen accumulated beyond the critical curve was reduced nitrogen.

DISCUSSION

Critical nitrogen concentration for low shoot DM

For stages of development varying from Feekes 3 to Feekes 5, it was estimated that a constant critical concentration of 4·4% could be applied. The range of N concentrations reported by different authors for 'optimal' conditions of N nutrition is large: 4·8–5·5% (Jonard and Odent, 1967), 3·5–4·5% (Karlen and Whitney, 1980), 3·6–4·7% (Masle, 1980). The concentration obtained in this study is close to the 4·5% 'optimal' concentration suggested by Meynard (1985) for wheat at tillering stage. Ingestad (1979) found a 4·29% value for leaves of seedlings of birchtree (*Betula pendula*), grown in controlled environmental conditions. Our estimate is equal to the 4·41% value measured by Jarvis (1987) for perennial ryegrass.

The values obtained by Devienne (1993) are higher: the critical total-N concentrations (N_{ct}) of spring wheat varied from 5·27 to 5·53% at stages of development between Feekes 3 and 5 (Table 3). However, the critical reduced-N concentrations (N_{cr}) were lower, from 4·43 to 4·54%. These results were obtained in a growth chamber giving 250 μmol m^{-2} s^{-1} of light which is much lower than what is usually encountered in field conditions. It is known that the nitrate concentration in shoots increases when light intensity decreases (Gastal and Saugier, 1986a). The results of Table 3 indicate that the wheat shoots did contain a large proportion of N-NO$_3^-$, between 16 and 20% of the total-N; this is markedly higher than the 2–5% observed in our

TABLE 3. *Total-N* (N_{ct}) *and reduced-N* (N_{cr}) *concentrations observed in wheat shoots during tillering in 'optimal' conditions for hydroponic cultures. 'Optimal' conditions correspond to the minimal N concentration in solution which yielded the maximum shoot growth. Values in brackets are standard errors. Results are from Devienne (1993)*

Days after sowing	35	42	49
Development stage (Feekes scale)	3	4	5
'Optimal' nitrate concentration in solution (moles m^{-3})	1·5	0·8	0·8
Total-N concentration N_{ct} (% DM)	5·53 (0·09)	5·40 (0·07)	5·27 (0·04)
Reduced-N concentration N_{cr} (% DM)	4·43 (0·03)	4·54 (0·06)	4·43 (0·06)

experiments near the critical curve (points 1–4; Table 2). Considering that nitrate concentration in shoots is higher and more variable at early stages of development than during stem elongation and ear emergence (Fig. 6), it is probably better to define the critical concentration with respect to reduced N in the case of low biomasses or early stages. The mean of Devienne's results and our results (points 3 and 4, better estimated than points 1 and 2) is:

$$N_{cr} = 4\cdot42 \pm 0\cdot07\%.$$

In our model, the decrease in critical N concentration in shoots is effective only when DM exceeds 1·55 t ha^{-1}, that is when the leaf area index is around 2·5. According to Puckridge and Donald (1967) and Meynard (1985), competition for light among plants has already started at this level of LAI or biomass. The self-shading of leaves is such that N dilution becomes unavoidable. Ågren (1985b) indicates that the critical N concentration is constant only when growth is exponential, i.e. when competition for light is negligible. N dilution would then be effective before the shoot DM reaches 1·55 t ha^{-1}. However, our results indicate that dilution, if it exists, remains low at this biomass level.

Comparison with available 'reference' curves

Figure 8 shows that our critical N curve defined for winter wheat is clearly below the reference curve established by Lemaire *et al.* (1989) for grassland [eqn (8)]. It is closer to the first curve proposed by Lemaire and Salette (1984) for tall fescue or to the critical curve proposed by Greenwood *et al.* (1990) for C$_3$ plants. However, the differences in critical N concentrations between our curve [eqn (11)] and that established by Greenwood *et al.* [eqn (9)] are not negligible. The relative differences between the two models are 8% for a biomass of 12 t ha^{-1}, corresponding to a difference of 17 kg N ha^{-1} of accumulated N in shoots. The differences are more important for low biomasses since we suggested the use of a unique critical concentration for DM lower than 1·55 t ha^{-1} whereas, according to Greenwood *et al.* (1990), the power function could still be applied (at least between 1 and 1·5 t ha^{-1}).

Greenwood *et al.* (1991) have calculated two critical curves for winter wheat using one experiment made in Belgium (curve B) and one experiment conducted in Sweden (curve S). Curve B is in good agreement with our results, except when shoot DM is lower than 2 t ha^{-1} where discrepancies occur (Fig. 8). The curve S is on the contrary very far from our critical curve on a large range of DM. It cannot account for many experimental situations shown at Fig. 5. It is unsatisfactory at least in our environmental conditions.

Variability of total N concentration in shoots

We observed an important variability in the shoot N concentrations for a given level of shoot DM in our experimental conditions although more variable environmental conditions can be found in wheat crops in France. Two envelop-curves have been defined: a curve of minimum N concentration (N_{min}) valid from 0·5 to 1·4 t ha^{-1}, and a
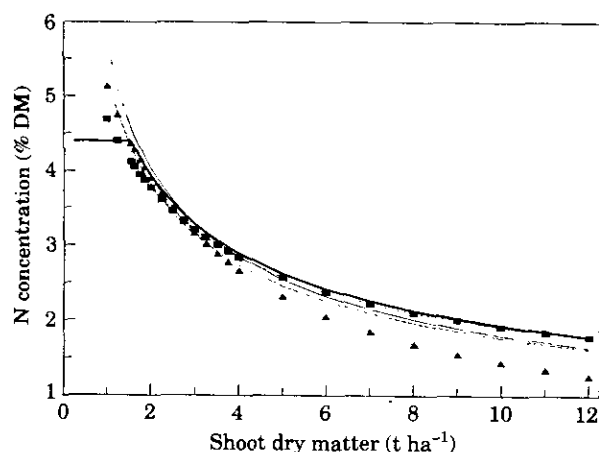
Fig. 8. Comparison of different critical dilution curves. (--) Lemaire and Salette (1984): (.....) Lemaire *et al.* (1989): (——) Greenwood *et al.* (1990); (---) this paper; (..—..) Greenwood *et al.* (1991) with: (▲) Wheat Sweden (S) and (■) Wheat Belgium (B).

curve of maximum N concentration (N_{max}) valid from 1·5 to 14 t ha^{-1} (Fig. 5). They correspond to the following equations:

$$N_{min} = 2·2DM^{-0·44} \tag{16}$$

$$N_{max} = 8·3DM^{-0·44} \tag{17}$$

For a given level of aerial DM, the N concentrations observed can vary by a factor of 3·8. Total N concentrations can vary markedly on both sides of the critical dilution curve: by a factor of 1·6 above and 2·4 below.

The N_{max} curve [eqn (17)] can be considered as the first estimate of a 'maximum N dilution curve', that is a curve corresponding to the maximum N uptake (or more precisely, to its maximum accumulation capacity in the aerials). This curve is very different from the critical curve as suggested by Angus and Moncur (1985). It could be obtained in treatments where the growth and N accumulation rates are maximum. In these conditions, the N absorption rate would be regulated by mechanisms directly associated with growth (Clement, Hopper and Jones, 1978; Deane-Drummond, 1984; Gastal and Saugier, 1986*b*; Macduff and Wild, 1988; Oscarson, Ingemarson and Larsson, 1989; Rufty, Mac-Kown and Volk, 1989; Raper, Vessey and Henry, 1991) or indirectly associated to it *via* N metabolism (Lee and Ratcliffe, 1986; Cooper and Clarkson, 1989; Touraine, Muller and Grignon, 1992). From eqns (2), (3) and (17), we get:

$$\left(\frac{dN_a}{dt}\right)_{max} = k\left(\frac{dDM}{dt}\right)_{max}$$

with $\qquad\qquad k = 46·5DM^{-0·44}$

The maximum absorption rate is directly related to the maximum growth rate, but the proportionality coefficient *k* depends on the accumulated shoot biomass.

Below the maximum curve, N absorption would be determined by the N availability in the root medium. In the area included between the critical curve and the maximum curve, N absorption would be regulated independently from growth rate since this one is maximum. Finally, in the area

below the critical dilution curve, N absorption would be limited by mineral N availability in the soil which would determine the growth rate. The minimum curve, N_{min}, is probably an overestimate of the minimum limit because N availability could have been lower in other experimental conditions. According to Penning de Vries (1982), N_{min} can be defined as the inferior limit at which the metabolism would cease to function.

Analysis of total N or reduced-N?

The reduced-N concentration would certainly be a better criterion for the diagnosis of N nutrition than the total-N concentration since nitrate is the major non-assimilated form of nitrogen. However, this measurement is more difficult to obtain than that of total-N using Kjeldahl or Dumas' method.The problem encountered with 'the' Kjeldahl method is that the analysis can include a variable fraction of nitrate nitrogen that is generally not known, unless specific reducing agents are used. The nitrate reduction rate with this method highly depends on the (varied) digestion procedure: possible reducing reagents, type of catalysts, heating procedure,... (Guiraud and Fardeau, 1977). On the other hand, the recovery of nitrate-N is 100% with the Dumas method.

We found that the critical curves for total-N (N_{ct}) and reduced-N (N_{cr}) were very close in the case when shoot DM > 1·55 t ha^{-1}. The diagnosis of N nutrition will not be dependent upon the amount of nitrate taken into account by the method of analysis: the method used (Kjeldahl or Dumas) can change the calculation of NNI when the latter is higher than 1, but will result in the same diagnosis.

For low biomasses, it has been observed that the shoots can contain a variable amount of N (0·1–18%) in the form of nitrate-N. The method used to define the critical concentration as well as the method of analysis used could influence the nutrition diagnosis. The safer recommendation seems to be the analysis of reduced-N and the use of the constant critical concentration $N_{cr} = 4·4\%$. It is then necessary to analyse both total-N (Dumas' method, by preference) and nitrate-N in shoots.

The results indicate the validity of a critical N dilution curve, for different conditions of growth and development, cultivar and population density of wheat crops. However, the growth rates and development stages remained rather highly correlated in these experiments; each biomass level corresponded to a relatively restricted range of stages of development. The critical curve might not be applicable to situations of very low productivity where similar stages of development would be obtained with low growth. For example, the critical concentration could be different for plants having a biomass of 5 t ha^{-1} at ear emergence (Feekes 10) in comparison with conventional situations (at this stage in our experiments, the aerial biomasses varied between 7·5 and 9·5 t ha^{-1}) since the leaf:stem ratio could be different.

The critical dilution curve can be applied for wheat from tillering to flowering. It allows the establishment of a diagnosis of N nutrition at any time during the growth cycle up to flowering. However, post-flowering growth can be important, as important as pre-anthesis growth (Machet,

1989).The 'optimal' growth of a wheat population up to flowering (i.e. along the critical curve) does not necessarily lead to a maximum growth between flowering and maturity nor does it necessarily lead to maximum yield.

ACKNOWLEDGEMENTS

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