Diagnosis Using Stem Base Extract: JUBIL Method

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Contents

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10.1 Introduction

In this chapter, the principle and validation of the JUBIL method are presented. The value and limits of this method for winter wheat and maize crops are then analyzed. This research was carried out in collaboration with ITCF[5] and AGPM[6].

10.2 Methods of N Fertilization Recommendations

10.2.1 Use of a Soil Indicator: the Balance-Sheet Method

In order to reduce losses of nitrogen (N) from agricultural areas to atmosphere and water, N fertilization of arable crops must be monitored as precisely as possible. In France, N fertilization recommendations for the major crops are mainly based on the balance-sheet method (Hébert 1969; Rémy and Hébert 1977; Rémy 1981). The balance considers variations in the soil mineral N in the potential rooting zone between midwinter and crop harvest. A predictive balance is made to determine the amount of fertilizer N needed to supplement the soil N supply to meet the crop N requirements of nonlegume crops. The most complete balance of the mineral N pool can be written (in kg N ha^{-1}):

$$R_f - R_i = (M_n + F + A) - (N_f - N_i + G + L),$$

where R_f = residual inorganic N in the soil at harvest[7]; R_i = residual inorganic N in the soil at mid-February[8]; M_n = net mineralization in soil; F = fertilizer N; A = atmospheric N (dry and wet) deposition and nonsymbiotic fixation; N_f = crop N accumulation at harvest; N_i = crop N accumulation at mid-February[8]; G = gaseous losses (ammonia volatilization and denitrification); L = leaching losses[9].

The simplification which is usually made is that the atmospheric inputs are compensated for by the gaseous losses (A ≅ 1 G, assessed at about 15–25 kg N ha^{-1} in arable cropping systems; Rémy 1981). In the case of cereals, the N crop uptake can be considered as proportional to the yield:

$$N_f = bY,$$

where bY = crop N requirements, b = amount needed per unit of grain (30 kg N t^{-1} for winter wheat; Coïc 1956) and Y = grain yield target (t ha^{-1}). Net mineralization (M_n) is also described as the sum of three contributions:

[5] ITCF: Institut Technique des Céréales et des Fourrages (France)
[6] AGPM: Association Générale des Producteurs de Maïs (France)
[7] A depth z greater or equal to the rooting depth
[8] This may be large for early sown winter crops (rape, barley)
[9] Occurring below depth z between mid February (R_i) and harvest (R_f)

$$M_n = M_h + M_r + M_a,$$

where M_h = net mineralization from humus, M_r = net mineralization from residues of previous crop, and M_a = net mineralization from organic (fresh and old) amendments.

The simplified balance-sheet model (Machet et al. 1990) can therefore be written:

$$bY + R_f = (R_i - L) + (M_h + M_r + M_a) + F.$$

The different components constituting the soil N supply are either measured (R_i), or estimated from models (M_h and L) or references (b, M_r, and M_a). The N fertilizer requirements of various arable crops (wheat, barley, maize, sugarbeet, potato, rapeseed, vegetables, etc.) can be evaluated for each individual field using the updated computer program AZOBIL (Machet et al. 1990).

This method is based on the time course of inorganic soil N, and a soil indicator is used to determine the initial N content. The soil indicator corresponds to the measurement (over the rooting depth) of inorganic N (NO_3^- and NH_4^+) present in the soil at mid-February (R_i). The balance-sheet method is well adapted to loamy and deep soils for various crops (Machet et al. 1990), in situations where little water deficit occurs, and has been used successfully in chalky soils for winter wheat (Meynard et al. 1981). It has been shown more recently that the use of the balance-sheet method minimizes the risks of nitrate pollution without penalizing yield (J.M. Machet, pers. comm.).

However, since this method gives a prediction over a long period of time (from mid-February to harvest), it may not be reliable enough if the soil N supply and root interception are not correctly estimated. The risk of poor prediction is greater in situations where the soil N supply is large: this is particularly the case for summer crops, after legumes or grasses, and after a fallow or applications of organic wastes rich in N. Under these conditions, the fertilizer N recommendations are small or zero, and farmers often apply larger rates to ensure that no N deficiency will occur. Consequently, the nitrate pollution risks are increased. These situations highlight the interest of using another indicator that allows more precise adjustment of the N fertilizer rate to meet plant requirements.

10.2.2
Use of a Plant Indicator: the Nitrate Concentration in Stem Base Extract (SBE)

It has been shown previously (Chaps. 1 and 2, this Vol.) that the nitrogen nutrition index (NNI) allows a reliable crop N diagnosis. Unfortunately, the determination of NNI requires a measurement of both the aerial biomass and its N concentration, which cannot be routinely done by farmers. It is therefore necessary to propose more practical plant tests.

Various authors (e.g., Papastylianou et al. 1982; Wehrmann et al. 1982, Scaife and Stevens 1983; Darby et al. 1986; Gonzalez-Montaner 1987; Gonzalez-Montaner

et al. 1987) have pointed out the interest of measuring plant nitrate concentration to evaluate the N nutrition status of vegetables or cereals (nitrate test). The nitrate concentration in the stem base extract has been particularly studied for the winter wheat crop because of its sensitivity and suitability for making rapid tests (semi-quantitative measurement of nitrate or the snappy sap test). The nitrate concentration of the stem base extract has been shown to be of interest for diagnosis of the N nutrition status of wheat (Papastylianou et al. 1982; Gonzalez-Montaner 1987). However, this nitrate test cannot be used alone to predict the development of the N crop status on a long-term basis (for example, measurements at tillering are inappropriate to predict N status up to flowering).

Gardner and Jackson (1976) and Papastylianou et al. (1984) proposed the use of measurements made on the stem dry matter for long-term predictions, but other authors (Beringer and Hess 1979; Pettygrove et al. 1984) concluded that it was impossible to establish a useful nitrate concentration threshold to make a long-term diagnosis. More recently, it has been shown that the long-term predictive critical concentration of maize crops can vary according to growth stage (Geyer and Marschner 1990), climatic conditions (Iversen et al. 1985), soil type (MacClenahan and Killorn 1988; Binford et al. 1990), and light intensity (Fox et al. 1989). This is expected, considering that net N mineralization, at least, fluctuates with climatic conditions and soil type.

On the other hand, it appears that the nitrate test can be used to predict the development of N crop status in the short term, from tillering to the onset of stem elongation (Darby et al. 1986; Gonzalez-Montaner 1987) or from the booting stage to ear emergence (Wehrmann et al. 1982; Knowles et al. 1991); but the nitrate test does not allow determination of the amount of fertilizer N required to remove any limitation of growth by N availability from the soil. It is necessary to build a new N fertilizer strategy that integrates a plant indicator with the previously N fertilizer application scheme.

10.2.3
Use of a Combination of a Plant and Soil Indicator: the JUBIL Method

We have suggested the combination of the balance-sheet method (using a soil indicator) with the nitrate test method (using a plant indicator): both indicators are used to form decision-making rules which define the JUBIL method (Justes 1993; Justes et al. 1994a).

In situations where the balance-sheet method gives an unsatisfactory prediction of fertilizer-N amount, it is likely that it will overestimate the amount to be applied. We propose a reduction in the balance-sheet N fertilizer dose (corresponding to a higher soil N supply), to avoid nitrogen excess (a cause of nitrate pollution). Then, we need to monitor the N crop status during growth to detect situations where it is necessary to add N fertilizer to compensate for insufficient soil N supply. The nitrate test is used during stem elongation (Feekes 6 to 9; Feekes scale from Large 1954) to determine whether a supplementary N dressing is

needed. Its use corresponds to a short-term predictive N diagnosis.

At the present time, the JUBIL method has been developed for winter wheat cereals. It involves four successive steps:

1. Calculate the overall N fertilizer requirements using the balance-sheet method: this defines the total rate, X (kg ha^{-1});
2. Apply a reduced amount of fertilizer N (X minus 40 kg ha^{-1}), considering that favorable conditions of crop N uptake may happen. One part (40–60 kg ha^{-1}) is applied at tillering (Feekes 3) and the remainder at stage ear 1 cm (stage Feekes 5);
3. Measure the NO_3^- concentration in the main stem base extract during stem elongation to detect N deficiency and to predict the short-term N status of the crop: one to three measurements are needed, depending on the situation. The first measurement is made at stage Feekes 6 (first node). The second measurement takes place at Feekes 7 (second node), and the third at Feekes 8–9 (flag leaf emergence).
4. Apply or omit the last N dressing (40, 60 or 80 kg N ha^{-1}), according to the NO_3^- concentration of the stem base and the growth stage. An N application is required to maintain the target yield if NO_3^- concentration falls below a threshold value.

10.3 Measurement, Interpretation, and Calibration of the Nitrate Test

10.3.1 Measurement Protocol

The nitrate test is carried out on fresh plant material. A 2-cm-long segment is cut at the base of the maiu stem. This is mainly tissue without chlorophyll. The extraction is made with a small manual press (Routchencko 1967; Gonzalez-Montaner 1987). Around 60 main stem bases are collected together to represent a farmer's field. Results are expressed in mg $NO_3 l^{-1}$ of stem base extract. The sampliug is carried out iu the field, between sunrise and 2 h after. During transport, the stems must be protected against light and excessive temperature (preferentially in an ice box). On arrival in the laboratory (or at the farm), the roots are cut at the tillering plate level (i.e., at the crown); the dead leaves, leaf sheaths, aud diseased stems are removed; the stems are washed and wiped; the stem bases are cut and then pressed; and the extract is diluted with deionized water (1:10) to avoid coloration interference when using rapid test strips. Under laboratory conditious, the nitrate concentration is measured precisely using the Griess method. Under farm couditions, the nitrate concentration is measured with rapid test strips (e.g., Merckoquant or Reflectoquant nitrate), which can be read with a hand reflectometer (e.g., Nitrachek or RQflex). The reflectometer reading yields the nitrate concentration with less than 10% error, which is good enough for making the N diagnosis.

The extract is sometimes erroneously called sap; we prefer to call it stem base extract (SBE), since it is a mixture of different solutions: apoplasmic water, xylem sap, phloem sap, cytosollic and vacuolar water, the vacuolar liquid being the major contributor of SBE. The nitrate concentration of the SBE will be referred to in the following as NSBE.

10.3.2 Interest and Physiological Significance of NSBE

First of all, the nitrate test (NSBE) can be used if the absorption form of N is primarily nitrate and not ammonium. In that case (usual in France and under temperate climate), the NSBE must fulfill several conditions in order to be useful in the diagnosis of plant N status:

1. Its variation must be small during the day/night period.
2. Its change must be large when the crop becomes deficient in N.
3. The activity of nitrate reduction in the roots must be small, so that the major N component transported into the xylem sap is nitrate (and not amino acids).
4. The indicator must be a short-term integrator of the N absorption flux (i.e., over a few days). It must be neither an instantaneous signal such as the xylem nitrate flux nor a signal that is an integral over several weeks, such as the nitrogen index (NNI).
5. The relationship between NSBE and NNI must be significant; so that it is possible to determine the N fertilizer decision-makiug threshold value of NSBE.

These five conditions are discussed in turn below.

10.3.2.1 Time Course of NSBE During the Day

The variation of NSBE during the day/night period has been studied in winter wheat crops by Justes (1993). An example of NSBE evolution for three N treatments is shown in Fig. 10.1; the measurements were made at Feekes 7 stage. In this example, the NNI indicated that treatment N_1 was deficient, treatment N_2 was starting to lack N, and N_3 was N-deficient, although it had received an N dressing 3 days before the measurements. The NSBE tended to decline during the day in treatments N_1 and N_2, whereas the trend was for an increase in N_3. The latter could result from a dominant effect of the recent N dressing over the daily effect, this daily effect being a slight decline during the day and an increase during the night. However, in such cases, the spatial variability of the nitrate concentration between experimental blocks is often equal to or greater than the daily variation, as is the case in Fig. 10.1.

In order to avoid this possible daily variation, we have recommended that the SBE samplings should be made at the same period of the day, the 2-h period following sunrise being preferred (Justes 1993). However, this is a precaution rather than a uecessity.

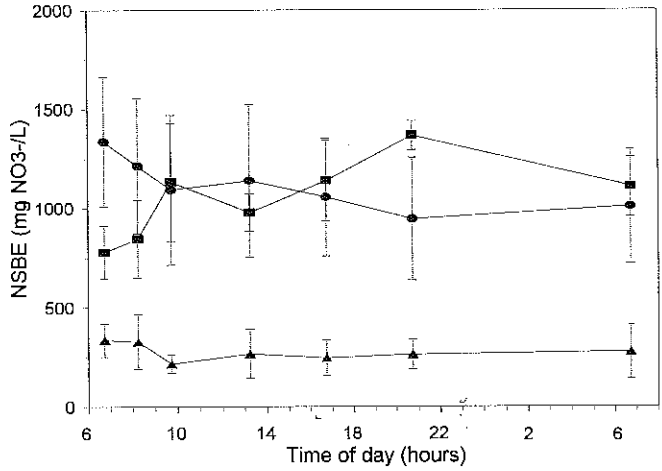

Fig. 10.1. NSBE versus time of day for winter wheat crops at Feekes 7 stage (experiment at Grignon, France, 1992). ▲ N1 treatment; ● N2 treatment; ■ N3 treatment (see text); *vertical bars* standard error

10.3.2.2
Time Course of NSBE During the Crop Growth Cycle

Figure 10.2a shows an example of the time course of the NSBE during crop growth in one experiment including five N treatments (15, 65, 100, 130, and 180 kg N ha^{-1}), taken from Justes (1993). In Fig. 10.2b, which shows the plant N% curves for the five treatments and the critical plant N% curve (Justes et al. 1994b), N180 treatment is N-nonlimiting during all the period monitored (tillering to anthesis), but the other treatments are N-limiting at different growth stages. The NSBE quickly decreases when N deficiency approaches and becomes very low (near zero) when N deficiency is evident; this is the case for the four low N treatments. The NSBE also decreased in the nonlimiting N treatment (N180), but the rate of decline was much slower than in the N-limiting treatments. This is generally the case in experiments carried out in France, except in situations where the soil N supplies are very large (cropping systems with large applications of manure), while the SBE nitrate concentration can remain high (or slightly decrease) during stem elongation, and then decreases after appearance of the ears.

Comparison of the time course of NSBE and NNI in two out of the five treatments previously studied (Fig. 10.2c) shows that these two indicators reacted at different speeds. The NSBE decreased after the appearance of N deficiency and did not increase later when N deficiency was evident. The NNI did not change, or decreased slightly but not significantly. Later, NNI decreased regularly even if the NSBE did not alter.

These two indicators are complementary tools for diagnosis: the NBSE is the indicator for earliest detection of the appearance of N deficiency (Gonzalez-Montaner 1987; Justes 1993), while NNI allows us to quantify it (Justes 1992).

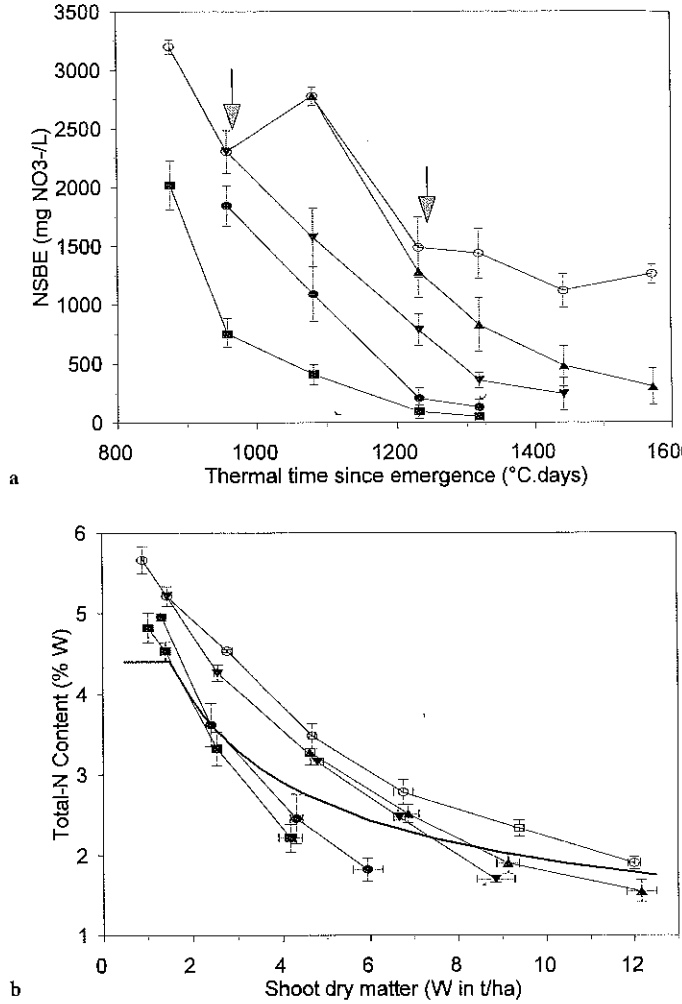

Fig. 10.2. a NSBE versus thermal time from emergence for winter wheat crops for five N treatments. **b** Plant N% curves observed for the five N treatments and critical N dilution curve ($N_{ci} = 5.35W^{-0.442}$). ■ N15 treatment (which received 15 kg N ha^{-1}); ● N65 treatment; ▼ N100 treatment; ▲ N130 treatment; ○ N180 treatment (N nonlimiting). **c** NSBE (primary y-axis) and NNI (secondary y-axis) versus thermal time from emergence for two N treatments: N180 (N nonlimiting) and N15 (N limiting since Feekes 6); □ NSBE and ■ NNI for N15 treatment; ○ NSBE and ● NNI for N180 treatment; *vertical* and *horizontal bars* represent standard error; *arrows* indicate fertilizer N application for N180 treatment (experiment at Grignon, France, spring 1991, from Justes 1993)

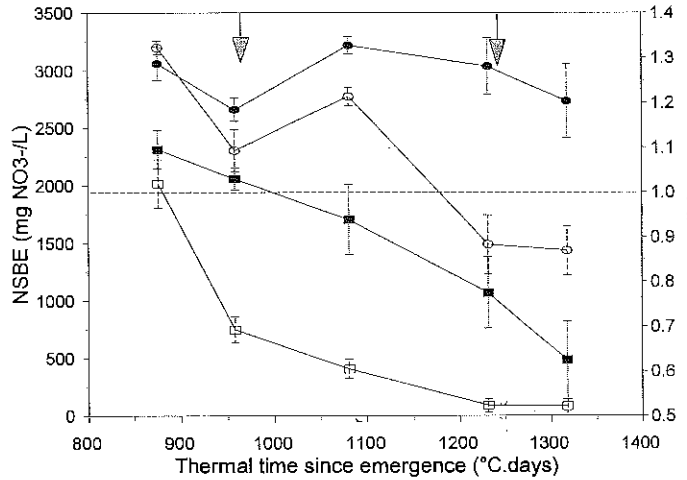

Fig. 10.2c

10.3.2.3
Time Course of NBSE After Applicatian of Various N Fertilizers

Nitrate is known to be the major form of N absorption of arable crops (except rice) in temperate climatic conditions (e.g., Beevers and Hageman 1980), because nitrification is a rapid phenomenon in normal soil conditions (e.g., Recous et al. 1988). After the application of an N fertilizer containing ammonia, plants can take up a large proportion of their N as ammonia over a short period; but across the growing period as a whole, arable crops mainly take up nitrate. However, Justes (1993) showed that after an N application during stem elongation (Féekes 6 to 10), the nitrogen index, NNI, always increased, but the NSBE reacted differently according to the chemical form of the fertilizer and the soil moisture. Under these experimeutal conditions, the NSBE increased when the fertilizer form was NH_4NO_3 or Ca $(NO_3)_2$ (Fig. 10.3a), but did not iucrease when urea was used (Fig. 10.3b). N fertilizer can be takeu up by the foliar route; in this case, nitrate is immediately reduced to amiuo acids by the leaves, and so the nitrate cannot accumulate iu the stem. Thus, N diagnosis based on the NSBE indicator can be wrong (Justes 1993).

Therefore, no NBSE measurement should be made to evaluate the N status of a winter wheat crop when the deficiency has become large and after a third N application during stem elongation.

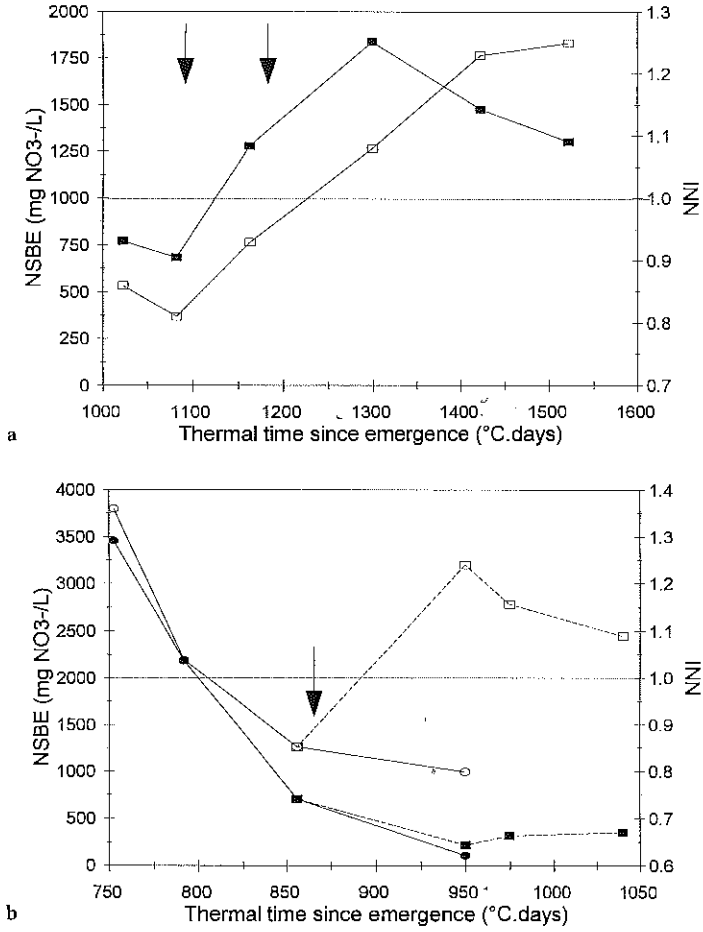

Fig. 10.3a,b. Curves of NSBE and NNI versus thermal time from emergence for winter wheat crops after fertilizer N application. (Justes 1993). **a** Fertilizer N corresponding to NO_3NH_4 application on two occasions with 50 kg N ha^{-1} (Laon 1990): ■ NSBE; □ NNI. **b** Fertilizer N corresponding to urea application with 150 kg N ha^{-1} (Grignon 1991). ● NSBE; ○ NNI without fertilizer N application; ■ NSBE; □ NNI after fertilizer N application

10.3.2.4
The Contribution of Roots to Nitrate Reduction in Winter Wheat Crops

Nitrate reduction, which is the major source for N nutrition of higher plants, is believed to occur principally in green leaves (Beevers and Hageman 1980). Although numerous results in the literature support this hypothesis, some authors have proposed that roots may have a significant role in reduction, particularly during early growth (Talouzie et al. 1984; Gojon et al. 1986). However, most of the studies were carried out with young plants in the greenhouse or growth chamber.

Furthermore, the methodology of estimating root nitrate reduction using either NRA (nitrate reductase activity) measurements or the ratio of amino N to total N (reduced N plus nitrate N) in the xylem sap has been contested, because of the rapid translocation of amino acids between shoots to roots via the phloem and their return to roots via the xylem (Cooper et al. 1986a). The best methodology relies on the determination of the ratio of amino-^{15}N to total-^{15}N over short intervals after $^{15}NO_3^-$ application. This methodology has been applied to field experiments at different growth stages by Cooper et al. (1986b) and Justes (1993). They both show that, for winter wheat crops during stem elongation, less than 30% of the nitrate is reduced in the roots, whatever the growth conditions. Furthermore, Justes (1993) reported a close relationship between the contribution of roots to nitrate reduction and the N absorption rate measured daily: the root contribution increased when N absorption increased, a result opposite to that of Beevers and Hageman (1980) for young plants of barley. Thus, during tillering and stem elongation (Feekes 3 to 10.5), with an N absorption rate of about 2.5 kg N ha^{-1} in northern Frauce, the contribution of the root is on average around 10–15% of the total nitrate reduction. Consequently, the major part of nitrate absorbed by the roots during the growth cycle is reduced in the shoot.

Hence, we can conclude that an aerial tissue such as the stem base extract could be used to reflect N absorption of the whole winter wheat.

10.3.2.5
Relationship Between Nitrate Concentrations in SBE and Xylem Sap

Justes (1993) showed that the SBE nitrate concentration was 1.5 to 7 times higher than that of the xylem sap, according to growth stage and the time of day that measurements were made. He carried out a field experiment with $^{15}NO_3^-$ (labeled at 50%) to assess the turnover time of nitrate in the stem base and in the xylem sap. The ^{15}N atom% excess of nitrate in xylem sap and SBE was measured between 3 and 72 h following the $^{15}NO_3^-$ application, for various developmental stages. Figure 10.4 shows the results obtained at Feekes 7 stage. The $^{15}NO_3^-$ applied was rapidly absorbed and translocated to shoots via the xylem sap. The isotopic excess of the xylem sap increased up to 48 h after application and then decreased. The same pattern was observed for the ^{15}N-NO_3^- atom% excess of the SBE, the latter being much less labeled. The ratio of the two isotopic excesses (xylem sap:SBE) decreased from 24 to 5. This indicates that the nitrate in the SBE became progressively equilibrated with the nitrate in the xylem sap. This equilibration is, however, not complete 3 days after ^{15}N application. Equilibrium was obtained after 2 days at stage Feekes 7 (ratio = 1). These differences between stages may be explained by the volume differences in xylem sap and SBE according to growth stages.

The nitrate test does not reflect the nitrate concentration of the xylem sap. Thus, daily variations in transpiration do not significantly influence directly the NSBE, which is why the daytime variation of the latter is relatively limited.

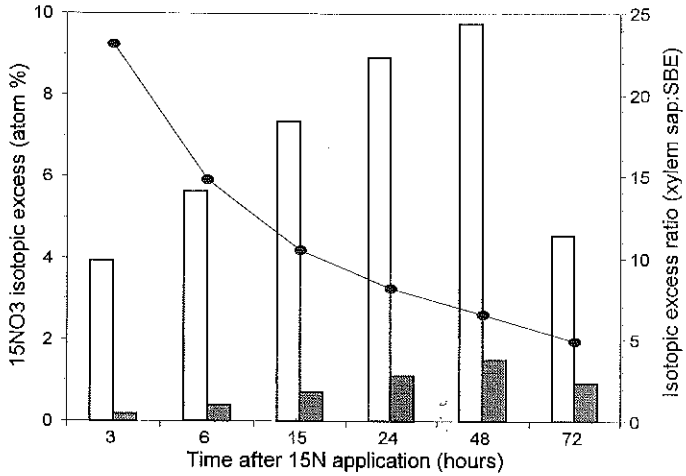

Fig. 10.4. Nitrate isotopic excess ($^{15}N\%$) in the xylem sap (□) and in SBE (■) versus hours after ^{15}N application (primary y-axis) for winter wheat crop. Isotopic ^{15}N excess ratio between xylem sap and SBE (-●-) versus hours after ^{15}N application (secondary y-axis) (experiment at Grignon, France, April 1992, at Feekes 7 stage, from Justes 1993)

10.3.2.6
Conclusion: the Physiological Significance of NSBE

From the above survey, it appears that the nitrate concentration of the SBE (NSBE) represents an integrated value of the NO_3^- exportation flux from the roots to the shoots, and an integrated value of the absorption flux during the days preceding the measurement.

10.3.3
Determination of a Critical Value of Nitrate Concentration in SBE

Figure 10.5 shows the relationship obtained in different field experiments between the NSBE and the index of nitrogen nutrition (NNI + 50), calculated for 50 degree-days (base 0) later (Justes 1993). We have indicated that the NSBE indicator responds earlier than NNI to a nitrogen deficiency: the delay in the latter has been estimated at 50 degree-days from results obtained by Gonzalez-Montaner (1987) and Justes (1993). Therefore, the NNI + 50 was estimated at day d plus 50 degree-days by linear interpolation, in relation to thermal time, between NNI measured at day d and NNI measured at the following sampling.

Justes (1993) obtained a significant correlation between NSBE and NNI + 50 for the different situations (various plant population densities, developmental stages, cultivars, soil types, countries, and growth conditions), ($r^2 = 0.754$; 175 degree of freedom). The covariance analysis (SAS Institute Inc 1987) indicated that

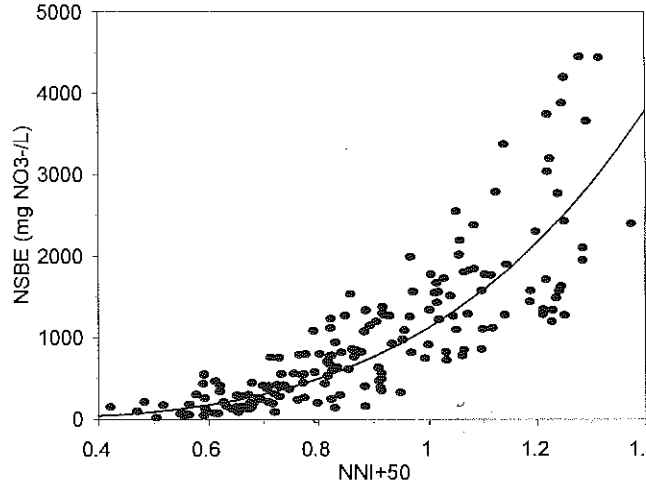

Fig. 10.5. Relationship between NSBE (measured at day d) and NNI + 50 which is NNI at day d plus 50 degree-days (+50°C days) for winter wheat crops (Justes 1993). Each *symbol* represents one point of measurement (mean of three replicates) and *curve* shows the global model (see text)

two factors have significant effects on this relationship: the plant density and growth stage. If we separate these factors into classes (two classes for plant density: normal and low, four classes for Feekes stage: 3 to 5, 6 to 7, 8 to 9 and 10 to 10.5), the complete model accounts for a greater proportion part of the variance (r^2 = 0.862; 176 degrees of freedom). The NSBE is significantly lower for the same NNI level when the plant density and the growth stage increase (Justes 1993).

Therefore, the NSBE threshold below which N is limiting growth (and the N fertilizer decision-making threshold) depends on the plant density and the developmental stage. In order to use the NSBE as an indicator of fertilizer management, we need to know the link between the wheat yield (or the number of grains m^{-2}) and the NNI.

We have shown previously (Chap. 2, this Vol. Cereal crops) that the grain number was not significantly different from the maximum grain number if NNI at anthesis was equal to or greater than 0.9. The threshold for N fertilizer decision-making has been defined as the nitrate concentration of SBE which existed for around days at 100°C (5 to 10 days in April and May in northern France) before the N deficiency could be detrimental, to allow for the time necessary for the N fertilizer action.

Complementary experiments allowed different threshold values to be established according to the pedoclimatic situation (three classes: north and middle of France, south and west of France and chalky soils of Champagne crayeuse) and cultivar (four classes according to earing precocity) (Justes et al. 1994a; Laurent et al. 1996).

For example, the NSBE threshold for fertilizer decision-making is:

- 1500 mg $NO_3^- l^{-1}$ for a standard density with the cultivar Soissons in the north of France at Feekes 6 stage, and 1000 mg $NO_3^- l^{-1}$ at Feekes 8–9 stage.

- 2300 mg $NO_3^-l^{-1}$ for a standard density with the cultivar Soissons in the chalky soils of the Champagne crayeuse at Feekes 6 stage, and 1800 mg $NO_3^-l^{-1}$ at Feekes 8–9 stage.
- 2000 mg $NO_3^-l^{-1}$ for a low density with the cultivar Soissons in the west of France at Feekes 6 stage, and 1500 mg $NO_3^-l^{-1}$ at Feekes 8/9 stage.

The method (balance-sheet + NSBE), called JUBIL, has now been commercialized in France by the company Challenge Agriculture. Farmers can use an interpretation grid, corresponding to crop density class and pedoclimatic situation, to interpret their value immediately. Consequently, a decision is proposed to omit or apply N fertilizer between the first node stage (Feekes 6) and booting stage (Feekes 8/9). The small case provided for the JUBIL method contains the equipment needed to make the nitrate test (NSBE indicator) under farm conditions: handle press, cutter, rapid test strips, reflectometer, interpretation grids, and the guidelines for couverting the nitrate analysis into fertilizer recommendations.

10.3.4
Qualities and Limits of Utilization of the NSBE Indicator

The nitrate test is a good indicator for decision making because of its qualities (see Meynard et al., Chap. 9, this Vol.), particularly when an N fertilizer application is needed.

Due to the close relationship between NSBE and NNI + 50, aud the lesser importance of root contribution to nitrate reduction, NSBE is closely related to the plant N status and thus its specificity is quite satisfactory. It is very sensitive in detecting the appearance of N deficiency. It may be used in all situations where soil inorganic N is primarily in nitrate form (which is the case in temperate climates).

The use of the NBSE indicator is easy for agronomists and also for farmers. The diagnosis of N nutrition is not influenced by the time of day that plants are sampled, or by large variations in crop growth rate, temperature, or solar radiation. Nevertheless, to use the NSBE correctly and give a robust diagnosis, it is necessary to take into account the plant density, the growth stage, the soil type, and the cultivar. Under these conditions, the nitrate test may be successfully applied across the large diversity of situations in France.

10.4
Validation of the JUBIL Method for Winter Wheat Crops

The JUBIL method was tested in France in 40 farmers' fields during the years 1991 to 1993. Various soil types, climatic conditions, winter wheat cultivars, and preceding crops were represented (Justes et al. 1994a).

In each case, four fertilizer N rates were applied (in two applications, at tillering and the beginning of stem elongation): X (balance-sheet rate), X-40

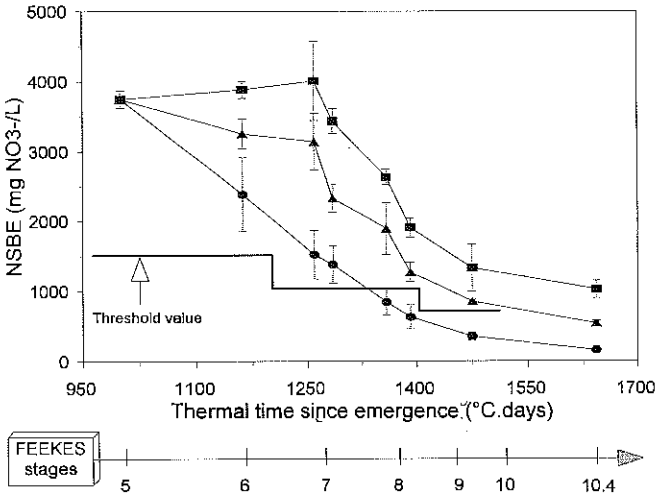

Fig. 10.6. NSBE versus thermal time from emergence for three N treatments. ■ X treatment; ● X-40 treatment; ▲ X-80 treatment (see text); *vertical bars* represent standard error (experiment at Eure et Loir, France in spring 1991; Justes 1993)

(JUBIL rate), X + 40 (safety rate) and 0 (no fertilizer). Sometimes, a rate of X-80 was also tested.

During stem elongation, the NSBE was measured in treatment X-40 (and sometimes X-80), and a third N dressing was applied on part of the experimental plot if NSBE was lower than the threshold value previously established, in order to maintain the yield objective.

Wheat yield components were analyzed in each treatment, including those which received an N dressing during stem elongation.

The mineral N remaining in soil at harvest was measured in six experiments in 1991 to evaluate the risk of nitrate leaching during the following winter (Justes 1993).

Figure 10.6 shows an example of the time course of NSBE on one site (Eure et Loir Veillard, 1991). The nitrate concentration decreased regularly during stem elongation and was clearly greater in the crops receiving more N. The NSBE measurements indicated that treatment X-80 became N deficient (lower than the threshold value) after the second node stage, and that N fertilizer was required to maintain the yield objective. On the other hand, the treatments X-40 and X did not require additional N, since NBSE always remained higher than the threshold value.

10.4.1
Evaluation of the JUBIL Method on Yield and Protein Content

We found that the NSBE in the X-40 treatment remained greater than the threshold value in 20 out of the 40 farmers' fields. In these fields, the JUBIL method recom-

Table 10.1. Average grain yields and protein contents observed in farm wheat fields (1991–1993)

NO_3^- concentration	> Threshold		< Threshold	
No. of sites	20		20	
N fertilizer rate	X-40	X	X-40 + 40	X
Yield ($t\,ha^{-1}$)	8.75a	8.84a	9.18a	9.05a
Protein content (%)	11.94b	12.58a	11.93a	11.67b

Letters a and b represent Newman-Keuls groups.

mended that extra N should not be applied and allowed a saving of $40\,kg\,N\,ha^{-1}$ compared to the balance-sheet rate X. In these X-40 treatments, the grain yield was not significantly different than in the conventional X treatments. The grain protein content was slightly reduced, on average by 0.6%, but stayed satisfactory. Thus, rate X-40 was the optimum amount of N fertilizer in terms of yield (Table 10.1).

In the 20 other fields, NSBE became lower than the threshold value during the measurement period, and a third dressing of $40\,kg\,N\,ha^{-1}$ was therefore applied. The total amount applied was the same in the X conventional treatment but three applications were made iustead of two. Splitting N had no negative effect on yield; it even increased yield significantly in many cases, and slightly increased the protein content of grains.

10.4.2
Value of the JUBIL Method for Reducing the Risks of Nitrate Pollution

The amount of soil nitrate N at harvest was significantly reduced with the JUBIL method compared to the nominal X rate, in plots where the last dressing was not applied (X-40 rate) and even when the third $40\,kg\,N\,ha^{-1}$ dressing was applied (X-40[+40] rate) (Table 10.2).

The attraction of the JUBIL method to limit soil NO_3^- at harvest is more evident when one considers the "safety" rates (X + 40) frequently used by farmers. The nitrate in soil at harvest time is the form of N most susceptible to being leached during the next wiuter. By considering a mean of drainage of 150 mm (about the

Table 10.2. Nitrate-N in soil (depth: 0–90 cm) at harvest and nitrate concentration of water drainage calculated by the model of Burns (1976) for an average 150 mm drainage (mean in northern France)

Method	JUBIL	Balance sheet	Safety
N fertilizer rate	X-40 or X-40 + 40	X	X + 40
$N\text{-}NO_3^-$ ($kg\,ha^{-1}$)	26	36	50
NO_3^- in water ($mg\,l^{-1}$)	38	53	74

average of the last 20 years in the north of France) and using the model of Burns (1976), we can calculate that the mean concentration of nitrate in drained water would be 38, 53, and 74 mg $NO_3^-l^{-1}$ in treatments JUBIL, X, and X + 40, respectively. This shows the value of minimizing the residual nitrate in soil at harvest with this method.

These results have been confirmed by Laurent et al. (1996) for tests carried out in 1994 and 1995.

10.5 Use of Nitrate Concentration Analyses for Maize Crops

Following the work of Routchenko (1967) and Geyer and Marschner (1990), Plénet et al. (1994) developed a diagnostic tool for maize crops based on nitrate concentration in stem base using the concepts previously demonstrated in wheat (JUBIL method).

10.5.1 Measurement Protocol of Nitrate Test for Maize

The operative protocol of the NSBE in maize is as follows (Plénet et al. 1994):

- Ten plants on four to six plots are sampled in early morning. The plants are cut just above the soil (first visible internode) and at the third internode level of stem, so as to keep only the second internode without the leaf sheaths. The samples are preserved in an ice box during transport to the laboratory or the farm. Before the 12-13-leaf stage, the plant tissues selected correspond to "*pseudo*-stem".
- Disks of 1–2 cm thickness are sampled at the middle of the second stem internode and the extraction is carried out with a manual press.
- The nitrate concentration is measured using a colorimetric method with an autoanalyzer or with rapid test strips and a reflectometer.

10.5.2 Time Course of the NSBE in the Maize Crops: Elements of Physiological Significance

An example of the time course of SBE nitrate concentration during maize crop growth is shown in Fig. 10.7, and this can be compared to NNI shown in Fig. 10.8. In these experiments fertilizer N is applied twice, with 30 kg N ha^{-1} at planting and the remainder at the 8-9-leaf stage. The second application produces a large increase in the NSBE, except with the 30N treatment. This can be interpreted as the result of root nitrate reduction capacity being exceeded when the nitrate supply is very large (Gojon et al. 1986). In this case, the majority of nitrate absorbed by the roots is translocated to the shoots, where the reduction capacity and supply of carbohydrates are not limiting under favorable conditions of solar radiation.

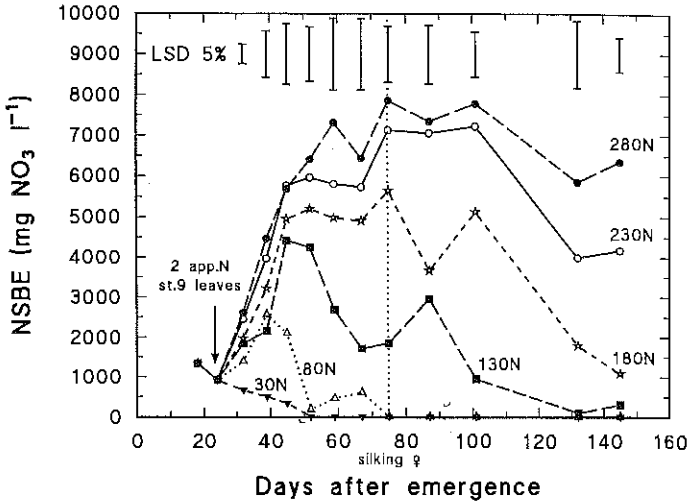

Fig. 10.7. Effects on N application rates (six N rates from 30 to 280 kg N ha^{-1}) on the time course of NSBE (mg NO_3^{-1} l^{-1}) during the growing season at Onard 1992 (France). Data are the mean of four replicates

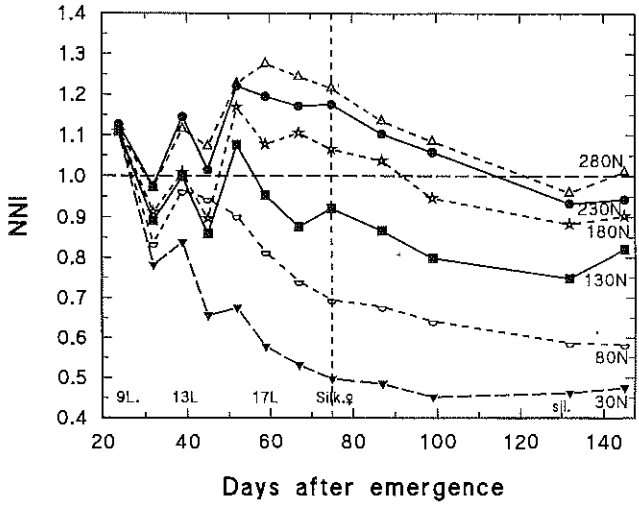

Fig. 10.8. Time course of NNI (NNI = $\%N_{act}/\%N_c$ with $\%N_c = 3.40\ W^{-0.37}$) as a function of N rates (six N rates from 30 to 280 kg N ha^{-1}) at Onard 1992 (France). Data are the mean of four replicates

When the N supply is limiting, which produces a decrease in NNI (Fig. 10.8), the NSBE declines quickly and approaches zero. Even if the absence of nitrate in the basal stem does not signify the cessation of nitrogen absorption by the roots, it does indicate that the flux of reduced N is inadequate for N growth demand, at least for the 10-leaf stage and flowering. During this period, NSBE is a very precise indicator for detecting the appearance of N deficiency, showing a response 6 days

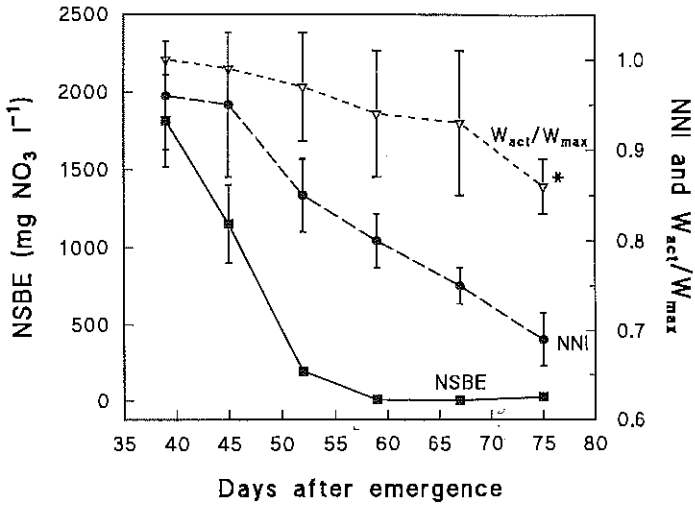

Fig. 10.9. Comparison of the time course of NSBE (mg NO_3^- l^{-1}), the NNI (NNI = $\%N_{act}/\%N_c$ with $\%N_c = 3.40\,W^{-0.37}$) and the relative shoot dry matter (W_{act}/W_{max}) on the treatment 80N (80 kg N ha^{-1}) during the period from 39 days after emergence (13-leaf stage) to 75 days after emergence (silking stage). *Error bars* show standard error of the mean of three replicates and * shoot dry matter is significantly different at $P < 0.05$

(65°C-days) before NNI decreased, and 28 days (350°C-days) before a significant reduction in shoot growth (Fig. 10.9).

After flowering plus 20 days, the stem base is primarily a storage organ for excess nitrate. This storage can reach up to 35 kg N ha^{-1} (8 t DM ha^{-1} of stems at 20% DM with a gradient of nitrate concentration between 0 to 10 000 mg NO_3^- l^{-1} from the base of the stem to its top), which represents a considerable amount compared to the 7 to 8 kg N ha^{-1} which are necessary to make 1 t DM ha^{-1} during this period. However, this storage pool does not always seem to be used well during the grain-filling period. This difficulty of the plants in using stem nitrate at the end of the grain accumulation period was reported by Below et al. (1981). It seems to be related to the absence of nitrate reductase activity in the stem at 30 days after anthesis (Ta 1991). On the other hand, high nitrate concentrations in SBE at the silage and maturity stages give a good indication of excessive N supply, as has been shown by Binford et al. (1992) on extracts carried out on dried stems.

Use of nitrate test on maize seems possible from the 10-leaf stage to the 20 days postflowering stage, at the latest. However, in areas where N is applied twice, it is necessary to carry out several measurements of the nitrate concentration in the young plants, so as to have a precise diagnosis of plant nutrition before the 14-leaf stage. The agronomic value of the nitrate test at the juvenile stage (influence of absorption of ammonium in cold soils) and after flowering (decrease of nitrate reductase activities and increase of proteolytic activities) must still be demonstrated before the test is used in these cases.

10.5.3
Relation Between the Nitrate Test and Nitrogen Nutrition Index: Determination of Nitrate Concentration Threshold for Maize Crops

The nitrate test is a partial indicator of plant N status, as it is carried out on a small part of the plant and takes into account only nitrates, which represent a small part of the total N of the plant. Therefore we have compared the nitrate test with the NNI in maize crops, which is an indicator of the overall N status of the crop. Figure 10.10 shows the NSBE in relation to the NNI at different stages of maize crop on several locations in France from 1992 to 1994 (Plénet 1995, and unpubl. data).

The NSBE is low when NNI is lower than 1. This shows that nitrate reduction must occur mainly in the roots when the N supply is less than the demand. The N is transported from root to shoot in the form of amino acids and there is no nitrate accumulation in the stem base. On the other hand, the NSBE tends to increase strongly when NNI > 1; but there is no linear relation between the two indicators: the nitrate concentration may range from 1000 to 8000 $mg\,l^{-1}$ for values of NNI between 1 and 1.6. In this case, nitrate storage in the stem base indicates that the supply is greater than the demand. The amount of nitrate in SBE depends on the availability of carbohydrates and of reduced energy carriers produced by the light

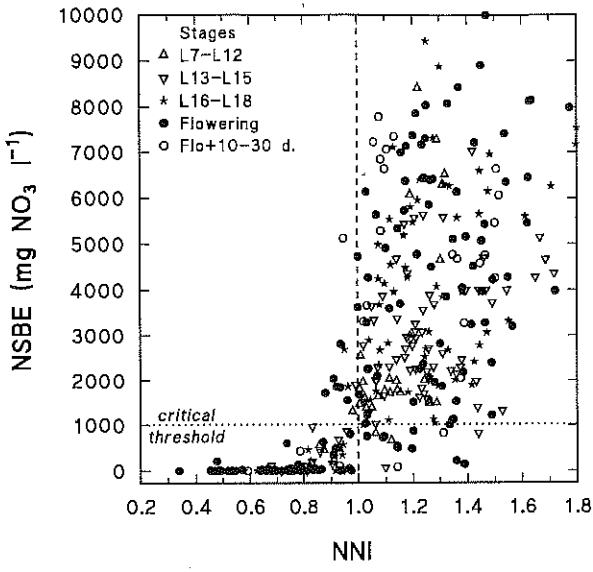

Fig. 10.10. Relationship between NSBE ($mg\,NO_3^{-1}\,l^{-1}$) and NNI (NNI = $\%N_{act}/\%N_c$ with $\%N_c = 3.40\,W^{-0.37}$). The two indicators of N status are measured at the same date during the period from 7-leaf stage to 20 days after flowering. Data of 20 experiments realized in France from 1992 to 1994. The critical threshold of 1000 $mg\,NO_3^{-1}\,l^{-1}$ was determined in using the procedure presented in the text and Table 10.3 ($n = 352$)

reactions, which relate to environmental conditions (Hageman 1986; Touraine et al. 1994).

To determine a nitrate concentration threshold, we have used NNI = 1, because it corresponds to the minimal level of N nutrition that allows the maximal growth at a given date. Furthermore we have previously shown (Chap. 5, this Vol.) that the number of grains per unit area will not be significantly different from the maximum when NNI is equal to or greater than 1 at flowering (Plénet 1995). The relatiouship between NNI and the critical nitrate concentration ($NO_3^-{}_c$) in SBE must be described by: NNI < 1, $NO_3^- < NO_3^-{}_c$ and NNI > 1, $NO_3^- > NO_3^-{}_c$. The experimental variability introduces two sorts of errors: error A when NNI < 1, but $NO_3^- > NO_3^-{}_c$ and error B when NNI > 1, but $NO_3^- < NO_3^-{}_c$. For every stage, we have calculated the probability of realization of the two types of errors for different nitrate concentrations (Table 10.3). The nitrate concentration threshold which maintain error A at less than 5% probability is between 750 and 1250 mg NO_3^{-1} l^{-1}, according to the growth stage. The critical threshold of 1000 mg NO_3^{-1} l^{-1} has been selected because it minimizes the two errors. To confirm this threshold, the same analysis was made when using the relative shoot biomass (DM/DM_{max}) measured 14 days after the nitrate test (short-term prediction). The purpose of this analysis was to check whether dry matter accumulation was significantly limited by N, and when the NSBE was higher than 1000 mg NO_3^{-1} l^{-1}. Except for one case, when the nitrate test result was greater than the threshold value, the shoot biomass was not limited (data not presented).

To use the nitrate test as a tool in N fertilization management of maize crops, a short-term prognosis must be carried out integrating the time necessary for farmers to carry out the N fertilizer application and for the N fertilizer to be available for the plant (at least 7–15 days). The critical concentratiou that is applied corresponds to an intervention threshold. Based on 20 experiments, we adopted an intervention threshold of 1500 mg NO_3^{-1} l^{-1}, which corresponds to the

Table 10.3. Probability of two diagnostic error types as a function of different nitrate concentrations in stem base extract at different stages in maize (L: number of visible leaves)

Stage	Number data	Nitrate concentration in SBE (mg NO_3^- l^{-1})											
		500		750		1000		1250		1500		2000	
		A*	B	A	B	A	B	A	B	A	B	A	B
		(%)											
7L-12L	32	6.3	0.0	3.1	3.1	3.1	6.3	3.1	6.3	0.0	21.9	0.0	56.3
13L-15L	77	5.2	1.3	3.9	1.3	1.3	2.6	1.3	3.9	1.3	7.8	0.0	15.6
16L-18L	82	3.7	0.0	2.4	0.0	2.4	0.0	2.4	4.9	2.4	4.9	1.2	12.2
Flowering	122	8.2	2.5	5.7	4.1	4.9	6.6	4.9	10.7	4.9	10.7	1.6	14.8
Flowering + 10 to 30 days	39	2.6	2.6	2.6	5.1	2.6	7.7	2.6	10.3	2.6	10.3	2.6	12.8
Total	352	5.7	1.4	4.0	2.6	3.1	4.3	3.1	7.4	2.8	9.7	1.1	17.9

*A Error: NNI < 1, but $NO_3^- > NO_3^-{}_c$ and B Error: NNI > 1, but $NO_3^- < NO_3^-{}_c$.

critical threshold of 1000 mg NO_3^{-1} l^{-1}, to which we have added 500 mg NO_3^{-1} l^{-1} to allow for the time necessary for the N fertilizer action (about 7 days). We advise that the nitrate test be used during the period from the 12-leaf stage to flowering. The nitrate indicator permits only a short-term prognosis, as the threshold of intervention corresponds to the nitrate concentration above which the N nutrition will not penalize growth in the 15 days following the date of the nitrate test. This threshold of intervention is being tested for maize crops at the national level in France in 1996.

10.5.4
Use and Perspectives for Maize Crops

The nitrate test proves to be a rapid and reliable diagnostič tool for maize crops, which can be used to evaluate a strategy of fertilization, as for the JUBIL method proposed for winter wheat. The management of fertilizer application in maize is based on four steps:

1. Determination by the balance sheet method of the total N fertilizer requirements: rate X (kg N ha^{-1}).
2. Application of the amount equal to X minns 50 kg N ha^{-1} at the start of cropping (planting plus 8-leaf stage).
3. Monitoring of crop N status with the plant nitrate test from the 12-leaf stage to flowering stage (three to four measuring dates).
4. If the nitrate test is lower than 1500 mg NO_3^{-1} l^{-1} during one of the measurement dates, a supplementary dose of 50 kg N ha^{-1} is applied in the following week.

Nevertheless, in view of the height of the maize crop, such management of N fertilizer applications by monitoring crop N status necessarily implies the use of specific techniques to spread the supplementary fertilizer on the crop after the 12-leaf stage: N applied with irrigation water or a high-clearance vehicle equipped with soil injectors. The large areas of irrigated maize cropping high leaching potential and/or low capacity for water retention (systems which are vulnerable to nitrate pollution), are an incentive to perfect the nitrate test so that the N supply is well adjusted to the growth needs.

In those areas where it is not possible to carry out the supplementary N application, the validation of a rapid diagnostic tool proves to be essential for a posteriori analysis of the experimental results, so as to increase the precision of reference data for each pedoclimatic situation. The risks to the environment will thus be progressively reduced.

10.6
Conclusion

This chapter has shown the interest of linking a simple predictive model of N fertilization (balance-sheet method) and a plant iudicator (NBSE) to optimize N

fertilizer for a given target yield and to reduce nitrate pollution as much as possible. The NBSE has shown its value for detecting the appearance of N deficiency and for deciding on the need for an extra N dressing during stem elongation in wheat and maize. However, it cannot be used to verify crop N status after an N fertilizer application carried out during stem elongation of winter wheat.

The use of a plant indicator in the decision-making rules for N fertilization may allow farmers to change the management of risks. To avoid N deficiency, farmers usually apply more fertilizer N than the amount calculated by the balance-sheet method (i.e., overestimation of the target yield). Thus, farmers prefer to obtain a slight excess of nitrogen supply which is less detrimental to yield (e.g., lodging, plant diseases, etc.) than N deficiency. In these conditions, there is no a priori risk of N deficiency to ensure the variable margin that is aimed for. This attitude is potentially bad for the environment. Models to predict N fertilization (e.g., the balance sheet method) do not allow a complete change in this attitude. On the other hand, the nitrate test gives information to apply a last N dressing; it is possible then to apply a priori a reduced amount of fertilizer N without any consequence for yield. Hence, risks of overapplications of fertilizer are strongly reduced without any risk of yield decrease due to N deficiency.

The JUBIL method has been implemented for winter wheat in 1994 by extension services (ITCF) with farmers' groups, to test the method's feasibility on a large scale in France. It received a promising reception because farmers have easily adopted the technique and used it on a large scale (200 000 ha of winter wheat were managed in 1995 using the JUBIL method). Its use showed be made a priority on the fields where N fertilizer rate is more difficult to adjust (after fallow, organic application, legume crops, etc.). It could be, in a second phase, extended to a larger number of cereal fields.

Its adaptation to other crop species (rapeseed, potatoes, sugarbeet, etc.) should be studied, particularly for summer crops because the net mineralization component of the N balance is more important and variable according to climatic conditions than is the case for winter crops. However, practical problems such as those evoked for maize could be a brake to extending the use of the JUBIL method. Indeed, N dressings applied in summer are not easily available for root absorption: in this case irrigation is necessary.

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