ESTABLISHMENT OF CONTINUOUS CRITICAL LEVELS FOR INDICES OF PLANT AND PRE-SIDEDRESS SOIL NITROGEN STATUS IN THE POTATO CROP

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ABSTRACT

Critical levels for six plant and pre-sidedress soil nitrogen indices were established for the growing season and their relative accuracy to diagnose the need for supplemental sidedress N was also compared. Field trials were conducted from 1996 to 1998 with irrigated potato in North-eastern Portugal. Fertilizer treatments included several pre-plant and sidedress N rates. Petiole nitrate concentrations (determined by a standard laboratory method and with the portable RQflex reflectometer), leaf N content, leaf greenness (SPAD-502 chlorophyll meter), pre-sidedress soil NO_3-N and pre-sidedress soil inorganic-N (NO_3-N + NH_4-N) were selected as N indicators. The Cate-Nelson graphical method and an analytical procedure using Mitscherlich type curves were used to determine critical levels. In both cases, a yield reduction of 10 % was accepted. The accuracy of the diagnostics was estimated from the Cate-Nelson graphical method, quantifying the point percentage that appears in negative quadrants (the error rate). The graphical method yielded lower critical levels, appearing as the most conservative sidedress N recommendation basis. The critical levels decreased linearly between 15 to 45 days after emergence (DAE). The linear equations achieved provide continuous critical levels for the growing season and are shown below:

Petiole NO_3-N (g kg^{-1}, dry wt basis) = - 0.737 DAE + 36.879 (r^2 = 0.92);

Petiole NO_3 (g kg^{-1}, from fresh tissue) = - 0.182 DAE + 9.417 ($r^2 = 0.69$);

Leaf N (g kg^{-1}, dry wt basis) = - 0.453 DAE + 61.028 ($r^2 = 0.91$);

Chlorophyll-SPAD (SPAD units) = - 0.463 DAE + 64.400 ($r^2 = 0.93$);

Soil NO_3-N (mg kg^{-1}) = - 1.096 DAE + 49.279 ($r^2 = 0.92$); and

Soil inorganic-N (mg kg^{-1}) = - 1.245 DAE + 56.599 ($r^2 = 0.92$).

The N indicators with lower error rate were the pre-sidedress soil NO_3-N and pre-sidedress soil inorganic-N (both showing an error rate of 8.3 %), followed by petiole nitrate concentration (determined in laboratory, 12.0 %, and with RQflex reflectometer,12.5 %), leaf N content (13.0 %) and leaf greenness (14.6 %). Error rates were similar throughout the growing season, meaning that is possible to get information about the need for supplementary N in the very early growth stages.

INTRODUCTION

Fertilizer-N recommendation systems are constantly under discussion all around the world, due to the ecological significance of N. The major unsolved problem with N management is the difficulty in making fertilizer recommendations for this element before the start of the growing seasons. Notwithstanding the effort of soil scientists in the last decades, and consequently the abundant literature on the subject (1-4), there are no methods yet, based on pre-plant soil analysis, providing accurate information about the potential availability of N in soils that could be used on a global scale. Thus, a successful strategy regarding N management of annual crops, like potato, could be the application of a small fraction of the eventually needed N with in-season adjustments on N rate, based on plant N nutritional status indices and/or pre-sidedress soil N tests.

The N nutritional index widely used in potato is petiole nitrate concentration (5-8). Leaf N content is used, for instance, in multi-element determinations for DRIS (Diagnosis and Recommendation Integrated System) interpretation (9, 10). More recently, leaf greenness, estimated by portable chlorophyll meters, is also frequently used (11-13). The pre-sidedress soil nitrate test acquired great importance on corn (14-17) and could be used in other crops, such as potatoes.

Results of plant and soil N indicators are usually interpreted from the establishment of critical or threshold values. The concept of critical level presupposes the existence of a simple point on the curve that relates the level of the N indicator with the yield. Below and above that point, there is a high probability that the crop responds or not to the application of more nutrient. Although the critical level is theoretically a largely consensual concept there are many ways to estimate it. Based on the relationships between N indicators and yield, different researchers use values corresponding to a yield reduction of either 5 % (18) or 10 % (19) as the critical level or even the point where the tangent drawn on the response curve has a unit slope ($\delta y/\delta x = 1$) (20). Researchers who use the linear plateau model consider the point of interception between the straight line segments as critical level (21). The Cate-Nelson graphical method (22) is also largely used either in its original form (23) or modified by the fixation of the horizontal line at more or less arbitrary values of relative yield (16, 24). All these computational forms can provide diverse critical levels. However, this fact usually is not taken into account when comparing different researchers' results.

Due to the variation of nutritional indices during the growing season, critical levels are usually defined for specific crop growth stages. This limits farmers or field consultants who intend to use these N management strategies. Thus, we lay out in this paper continuous critical levels for the first half of the growing season for petiole nitrate concentrations, leaf N concentrations, leaf greenness, pre-sidedress soil NO_3-N and pre-sidedress soil inorganic-N.

Furthermore, the results of two different methods of critical levels' determination as well as the relative accuracy of the N tests to diagnose the need for supplemental N were also compared.

MATERIAL AND METHODS

Field Experiments

Local and general crop growth conditions - The studies were conducted between 1996 and 1998 in Bragança, NE Portugal. Local climate is of Mediterranean type, hot and dry during the summer growing season. The soil is a eutric Cambisol, loamy textured, with pH(H_2O) of 6.5 and 15 g kg^{-1} of organic matter. The potato crop was grown in a triennial rotation of silage maize / triticale – potato / triticale – silage sorghum / triticale. The triticale crop was included as winter inter-cropped silage, and mowed by the end of April, at the 60^{th} growth stage of Zadoks (25), which corresponds to anthesis.

Experimental design - The experimental setup was a split-plot field design. As main plots the pre-plant N treatments were included. The main plots were sub-divided to include the sidedress fertilizer-N treatments as subplots. The pre-plant treatments were 0, 50, 100, 200 and 300 kg urea-N/ha and Poultry Manure (PM), Cow Manure (CM) and Municipal Solid Waste (MSW). The organic amendments were applied in variable rates in order to equal 100 kg of total N per ha. Five sidedress urea-N rates 0, 25, 50, 100 and 200 kg/ha were arranged as subplots. Three replications were used for all fertilizer treatments. Individual plots consisted of seven rows 5 m long.

Crop management – Phosphorus (as superphosphate, 18 % P_2O_5) and potassium (as potassium chloride, 60 % K_2O) rates were applied according to pre-plant soil analysis. In the three consecutive years of experiments (1996, 1997 and 1998), 26, 52 and 26 kg P/ha and 75, 125 and 66 kg K/ha were respectively applied. Pre-plant fertilizers and amendments were broadcast and incorporated with pre-planting tillage. Whole pre-sprouting tubers (cv. Désirée) with sizes of 28-45mm (1996) and 45-60mm (1997 and 1998) were machine-planted on 28 May 1996, 28 May 1997 and 21 May 1998. Seed rate was 44 000 tubers/ha, arranged at 70 cm apart with in-row spacing of 32 cm. Crop emergence (50 % of visible shoots) occurred 14, 13 and 15 days after planting in 1996, 1997 and 1998, respectively. The urea of the sidedress N treatments applied on 17 July 1996, 9 July 1997 and 24 June 1998 was incorporated with irrigation water. Solid set sprinkler system irrigated the crop during the growing season. Depth and interval irrigation application were based on FAO methods (26). Crop protection included weeds, late blight (*Phytophthora infestans*) and beetle (*Leptinotarsa decemlineata*) control. Just before potato shoots emerge Metribuzin was applied on soil surface for weed control. Cymaxonil, metalaxyl, folpet, propineb and mancozeb, used alone or in formulated mixtures, were foliarly applied as required for blight control. Carbaryl, chlorpyrifos and lambda-cyhalothrin were alternately used during the three years of study for insect control. The harvests occurred every year in the last week of September after the full senescence of the plants. From samples of eighteen potato plants the fresh weight of marketable tubers (> 35 mm) was recorded.

Laboratory Analysis and in Situ Measurements

Soil inorganic N content – This analysis used similar methodology to the pre-sidedress soil nitrate test (PSNT) proposed for corn (14). Soil cores were taken from the first 30 cm of the soil profile, field-moist sieved (6 mm) and stored frozen until analysis. Thereafter, the samples were thawed, extracted with 2 *M* KCl (1:2) and the extracts analysed for NO_3-N and

NH_4-N separately. Nitrate-N concentrations were determined by the sulfanalamine method and NH_4-N by the Berthelot method (27). Both ions were analysed in a segmented flow analyser.

Leaf N content – Leaf samples consisted of twenty to thirty of the most recently mature leaves. The leaves were dried in a forced air oven at 65 ºC and ground to pass through a 1 mm^2 sieve. Leaf N content was determined by the Kjeldahl method, consisting of steam distillation and acid titration in a Kjeltec Auto 1030 Analyzer after the digestion of plant tissues in sulphuric acid.

Petiole nitrate content - Samples had 30 to 40 petioles of the most recently mature leaves. A random sub-sampling of 5 g of these petioles was used for quick testing. Fresh tissues were macerated and boiled for 15 minutes with ≈100 ml of distilled water. After cooling, final volumes of 300 ml were lined up and nitrate concentrations in extracts determined by Reflectoquant test strips (MERCK), read with the portable RQflex reflectometer (MERCK). Petioles for laboratorial nitrate analysis were dried and ground. The extracts were prepared by adding 50 ml of distilled water to 1.0 g of the sample. The mixtures shaken for 1 hour were filtered. Nitrate-N concentrations were determined as described for soil NO_3-N.

Leaf greenness – The greenness of the leaves was determined by the Minolta model SPAD-502 chlorophyll meter. The SPAD meter provides adimensional estimates of chlorophyll content of leaves and the results expressed in SPAD units. Readings were made on the distal leaflet of the most recently fully matured leaves (11). The SPAD value of each plot corresponds to the average of 30 individual readings.

Soil and plant N tests were made throughout the growing season. Table 1 shows plant and soil sampling dates.

Critical Levels and Error Rates

Critical levels and associated error rates were estimated for each sampling dates for the following plant and soil N indicators: (i) petiole NO_3-N concentrations (dry weight basis), determined by laboratory analysis; (ii) petiole NO_3 (fresh weight basis), obtained with the portable RQflex reflectometer; (iii) leaf N content; (iv) leaf greenness, expressed in SPAD units; (v) leaf greenness, expressed in relative terms, as N sufficiency index, using the equation:

N sufficiency index (%) = Average bulk reading/Average reference strip reading x 100,

where the reference strip is a well fertilized plot (28); (vi) pre-sidedress soil NO_3-N content; and (vii) pre-sidedress soil inorganic-N (NO_3-N + NH_4-N).

To standardize the relationships between tuber yield and N indicators among the years, tuber yields were expressed as relative yields. The critical levels were estimated by the Cate-Nelson graphical method and with a mathematical procedure. The Cate-Nelson graphical method was modified by declaring the horizontal line to be a fixed value (16, 24). The horizontal line was fixed at 90 % of relative yield and the vertical line was displaced as to keep in the positive quadrants the greatest amount of the points (figure 1 a). With the analytical method, critical levels were determined by fitting Mitscherlich type curves to the relationship between relative tuber yield and N indicators and solving the equations for 90 % of relative yield (figure 1 b).

The modified Cate-Nelson graphical method helped to determine the error rates associated with the critical levels. The error rate represents the point proportion that remains in the negative quadrants and corresponds to a failure in the diagnosis, i.e., situations where N would be recommended when unnecessary and vice-versa.

The effect of the treatments on tuber yield was evaluated by analyses of variance, using the SYSTAT 5.02 program. Fisher's LSD test separates the means with significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Tuber Yield

The results of tuber yield as a function of pre-plant N treatments and years are shown in table 2. Nitrogen treatments had a significant effect ($P < 0.05$) on tuber yield in 1997 and 1998. During these years, in the control plots and in the plots of low urea-N rates and organic amendments maximum tuber yield was not reached. Crop response was also significantly influenced by the year, when comparing between the control plots or between the plots with low N rates and organic amendments. Reversely, the results of the treatments with higher N doses were quite similar for all the years, always reaching values of tuber yield of about 50 Mg/ha. The effect of the year on crop production can be explained by differences in native soils N availability. This aspect was confirmed by the results of an *in situ* incubation technique performed throughout the three growing seasons (29). Marketable tuber yields of 50 Mg/ha are usual in the region (30, 31) and, for the purposes of this work, such value represents the yield potential of the cropping system.

Critical Levels

Critical levels determined by both graphical and analytical methods are shown in table 3. The response pattern of N indicators was similar, despite some variability among the years: critical levels for petiole nitrate, leaf N, leaf greenness and soil inorganic N, decreased along the growing season. The decrease in some of these N indicators throughout the growing season is well documented (5, 6, 11, 12, 18). Soil N dynamics, particularly crop N uptake, justifies the decrease in soil inorganic N, while the decrease in plant N nutritional indices can be justified by the depletion of soil N availability and by the dilution factor associated with plants ageing.

Significant linear equations were established between critical levels and sampling dates for all N indicators. The result was exemplified in the figure 2 with leaf N. These equations represent continuous critical levels for the growth period considered. The establishment of critical levels from the linear equations, comparatively to the critical values obtained for each individual sampling date, has as advantage the removal of the variability among sampling dates and years. The equations achieved for all the N indicators from both methods of critical levels determination are shown in table 4. Solving the equations for a random value of DAE reaches the critical level for this DAE. In order to enable an easier result interpretation, table 4 also include critical values obtained by solving the equations for regular intervals of the growth season (15, 25, 35 and 45 DAE).

The analytical method provided critical levels higher than the graphical one. The observation is valid for all the indices and for the entire growth period considered (table 4), with differences increasing over time. This discrepancy between the results of the two methods may be attributed to the greater sensitivity of the mathematical method to dispersed points as well as to the difficulty of the curvilinear models in fitting the data in the transition zone (where the index changes from the deficient to the adequate range). Thus, the results seem to support the opinion of Black (32) who considered, when comparing critical levels the form in which they are determined must always be taken into account.

Critical concentrations of petiole NO_3-N (lab. analysis), determined by Cate-Nelson graphical method, varied from 25.7 to 3.4 g kg^{-1} between 15 and 45 DAE. The results of the analytical method decreased from 27.3 to 8.2 g kg^{-1} in the same period (table 4). These results are not very different from those that were registered by other researchers (5, 7, 19, 21, 33). This is more so, if it is taken into account that many factors could influence petiole NO_3-N concentrations, as for instance the effect of cultivar (19, 34, 35) and the general crop growth conditions of each experiment. In addition, other factors could limit comparative results of different researchers as the use of different calendar/physiological scales of crop growth and the methods by which the critical levels are determined.

Critical NO_3 concentrations provided by the portable RQflex reflectometer are very similar to those obtained by the laboratory method, considering that the dry matter content of petioles was approx. 6 %. Previous calibration of RQflex results using standard solutions of $Ca(NO_3)_2$ yielded a significant linear regression with a unit slope and r^2 = 0.99 (29). The linear regression established with RQflex and laboratory results was also significant, with slope = 1 and r^2 = 0.92 (29). Being unaware of other published data obtained with this device, it seems these results have very satisfactory analytical quality.

Critical leaf N concentrations decreased over the growth season. At 15 DAE critical values ranged between 54.2 and 54.8 g kg^{-1} and at 45 DAE between 40.6 and 43.5 g kg^{-1} (table 4). Evanylo (23) published critical leaf N levels with cv. Superior of 59 g kg^{-1} for 14 DAE and 51.6 g kg^{-1} for 30-35 DAE, values that are slightly higher than ours. However, the difficulties in comparing different authors' results with leaf N are similar to those referred for petiole nitrate concentrations. In general, the critical values reported in table 4 are within the range of the norms published for the crop (36, 37).

Critical SPAD values determined by Cate-Nelson and analytical methods were quite similar at 15 DAE (57.4 and 57.9, respectively). However, as the growth season progressed the results of Cate-Nelson graphical method tended to be much lower, 43.4 against 47.9 at 45 DAE (table 4). Results published by Minotti et al. (12) with cv. Katahdin, Superior, Allegheny and Castile are not much different from our results. They report that, early in the season, between 29 to 37 days after planting, SPAD readings associated to the lowest N rates that give maximum yields, ranged between 49 to 56 SPAD units, depending on year, variety and location and potato variety significantly affected SPAD values in 8 of 12 situations. Minotti et al. (12) also recorded a decrease in SPAD units as the plants aged, similar to table 4 values. Contrarily, results published by Vos and Bom (11) with cv. Vebeca seem to be much lower than our results during the whole season. During the growth season, critical relative SPAD values had a proportional decrease to critical SPAD values. A decrease in critical relative SPAD values calls into question the possible use of a critical sufficiency index of 95 %, regardless of the crop growth phase, as suggested by Peterson et al. (38). On corn, Piekielek et al. (24) already reported relative SPAD values different from 95 %, decreasing in the course of the growth season.

Critical levels of pre-sidedress soil NO_3-N test varied from 33.9 and 32.8 to 4.7 and 0.1 mg kg^{-1} between 15 and 45 DAE, when determined by the analytical or graphical methods, respectively. Notwithstanding the abundant information for other crops, such as corn (14-17), there are no published reports with this N indicator for potato allowing for a comparison.

Error Rates

The error rate, as previously defined, was used to compare the relative accuracy of N indicators to diagnose the need for supplemental N. Table 5 shows those results. The quality of the diagnoses did not improve during the cultural cycle, except for the leaf greenness index (table 5). Thus, we can conclude that it is possible to make acceptable diagnoses early in the season, enabling to proceed with fertilizer adjustments while crop N use efficiency is high. In agreement, Singh (19) has verified that it is possible to detect a shortage of N with petiole nitrate index at 25 days after planting. When sampling dates are delayed there is no improvement in diagnostic accuracy.

The best diagnostics were made with soil NO_3-N and soil inorganic-N tests, both with an error rate of 8.3 %. The pre-sidedress soil nitrate test has also given very good results in several studies on corn (14, 16, 17). The inclusion of NH_4-N did not improve index quality. The increment in critical levels of the inorganic N relative to NO_3-N was proportional to the contribution of NH_4-N to the soil inorganic N as described above. Sims et al. (16) recorded similar results on corn. In contrast, Meisinger et al. (15) found advantages in NH_4–N inclusion. Meisinger et al. (15) considered that N would probably not completely nitrify at PSNT sampling and, thus, the inclusion of NH_4-N could more accurately represent the total availability of organic-N to the crop.

The error rate of critical levels of petiole nitrate (lab. analysis) was 12 %. The result was slightly better than those of petiole nitrate determined with the RQflex reflectometer (12.5 %). Petiole nitrate content is the N nutritional index widely used in potato crop. However, in this study, the accuracy of its diagnosis was only slightly better than the other plant N indicators. Leaf N index showed a satisfactory diagnostic quality, with an error rate of 13 %. Leaf greenness index provided the worst result, with 14.6 % of error rate. The sampling of 10 DAE contributed greatly to this poor result. Without the result of 10 DAE, the error rate improved to 12.5 %, a value similar to those of other plant N nutritional indices. In other studies, the use of leaf greenness in the very early growth stages has often given inconsistent results. It does not seem possible to detect significant differences among N fertilizer treatments (11) or to establish satisfactory relationships between leaf greenness and yield (39).

The conversion of SPAD values into relative ones did not improve the accuracy of the diagnosis, in contrast with the observations reported by Minotti et al. (12). The concept of relative SPAD was developed to solve problems of experimental variability, related to differences in ecological conditions and among cultivars (28). Since these variables were not part of this study, it was not possible to benefit from that form of result interpretation.

CONCLUSIONS

The critical levels estimated by Cate-Nelson graphical method were always lower than those estimated by the analytical method. Taking into consideration the need for reducing the use of excessive N, the graphical method seems to be a more conservative sidedress N recommendation. On the other hand, the range of differences confirms the need to take this into consideration when comparing results of different researchers. The linear equations, presented below, provide continuous critical levels for the six N indicators estimated from the Cate-Nelson graphical method.

Petiole NO_3-N (g kg^{-1}, dry wt basis)	= - 0.737 DAE + 36.879	(r^2 = 0.92);
Petiole NO_3 (g kg^{-1}, from fresh tissue)	= - 0.182 DAE + 9.417	(r^2 = 0.69);
Leaf N (g kg^{-1}, dry wt basis)	= - 0.453 DAE + 61.028	(r^2 = 0.91);
Chlorophyll-SPAD (SPAD units)	= - 0.463 DAE + 64.400	(r^2 = 0.93);
Soil NO_3-N (mg kg^{-1})	= - 1.096 DAE + 49.279	(r^2 = 0.92); and
Soil inorganic-N (mg kg^{-1})	= - 1.245 DAE + 56.599	(r^2 = 0.92).

The best N indicators of the need of supplemental sidedress N were pre-sidedress soil NO_3-N and pre-sidedress soil inorganic-N, both with an error rate of 8.3 %. Chlorophyll-SPAD readings had the worst error rate (14.6 %). However, we should take also into account the use of SPAD-502 chlorophyll meter since it is user-friendly and provides accurate diagnoses after the second week after emergence. Diagnoses' quality provided by the N indicators did not improve during the growing season. Satisfactory diagnoses of N deficiency from the early growth stages were obtained, allowing for fertilizer adjustments while crop N use efficiency is high. Critical relative SPAD values decreased during the cultural cycle. This result calls into question the possibility of using the constant value of 95 % for the entire growing season as a critical sufficiency index.

ACKNOWLEDGEMENTS

The author thank Rita Diz and Ana Pinto for laboratory assistance. Financial support was provided by the Ministry of Agriculture, Rural Development and Fisheries through the Project PAMAF 6107.