RAPID TISSUE TESTING FOR EVALUATING NITROGEN NUTRITIONAL STATUS OF (1) CORN AND (2) SORGHUM

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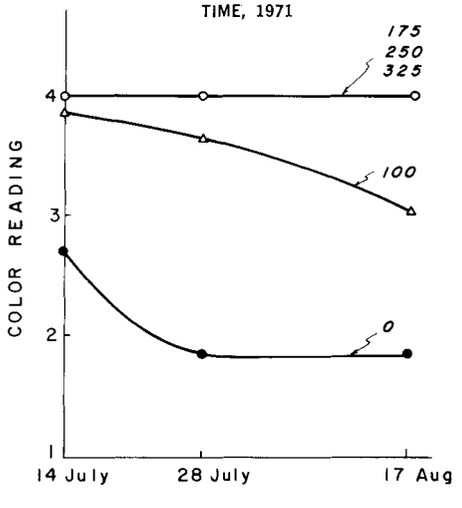
For several years, rapid tissue tests have been used in the field to evaluate plant nutritional status and to diagnose deficiencies. But they have been used only incidentally to make fertilizer recommendations, or to evaluate a fertilizer program, partly because they have lacked the accuracy of laboratory analysis.

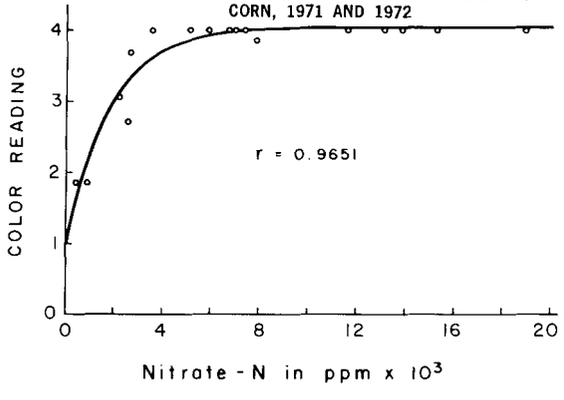
Research has revealed definite and consistent relationships between plant nutrient level and plant health. However, few crops are routinely tested to determine plant nutritional needs, because fertilizer has been plentiful and relatively low in cost, and because results from laboratory analyses are often delayed. Rapid tissue tests conducted in the field could overcome the problem of delay. By providing information about nutrients needed for a particular crop in any given field, the tests also enable growers to more fully utilize increasingly costly and limited fertilizer resources.

In the field investigations reported here, a rapid tissue testing procedure (developed by R. H. Bray in 1945 at the University of Illinois) was found useful as a guide for evaluation of the nitrogen nutritional status of corn and sorghum. Resulting guidelines are given here for recommending application rates for nitrogen fertilization and for evaluation at the end of the growing season.

GRAPH 1. CHANGE IN COLOR READING IN THE BASAL STEM OF CORN WITH RATE OF NITROGEN AND TIME, 1971



GRAPH 2. RELATIONSHIP BETWEEN THE MEAN COLOR READING AND MEAN NO_3-N IN THE BASAL STEM OF CORN, 1971 AND 1972



(1) RAPID TISSUE TESTING FOR EVALUATING THE NITROGEN NUTRITIONAL STATUS OF CORN

Two field trials were conducted in 1971 and 1972, to obtain data for calibrating the rapid tissue test in corn. The 1971 study was established using experiment station plots at Davis. The experimental design was a randomized complete block with five treatments and four replications. Plot size was 15 ft by 40 ft. Nitrogen treatments consisted of 0, 100, 175, 250, and 325 lbs per acre applied in a sidedress application as ammonium sulfate at the seedling stage of growth.

Commencing on July 14, 1971, tissue samples were obtained for laboratory analysis for nitrate-nitrogen (NO_3-N) and total nitrogen as determined by the Kjeldahl procedure (which was not modified to include nitrates). The first sampling date was approximately 35 days after emergence. Whole-leaf and mid-rib samples were selected from the first fully mature leaf of the plant. A basal three-inch section of the stem was obtained from plants sampled for leaves. The basal section of the stem was split lengthwise and one-half saved for laboratory analysis for NO_3-N and the remaining one-half was used for making a color reading in the field.

Reagent

The nitrate reagent powder used contained the following ingredients in the amounts listed: (1) 110 gm $BaSO_4$, (2) 10 gm $MnSO_4 \cdot H_2O$, (3) 2 gm finely powdered zinc, (4) 75 gm citric acid, (5) 4 gm sulfanilic acid, and (6) 2 gm alpha-naphthylamine. The coarse materials are ground to a fine powder and the ingredients thoroughly mixed. Store in black containers, since light affects the alpha-naphthylamine. Alpha-naphthylamine has been classified by the Occupational Safety and Health Act (OSHA) as a harmful substance, but in the mixture described above it is diluted to a concentration below the critical level established by OSHA. Nevertheless, prolonged skin contact with the reagent should be avoided.

Approximately 0.5 gm of the reagent powder is applied to the pith of the stem. A spatula or knife was used to make a paste with the cell sap. After three to five minutes, colors developed and were recorded, using the following code: 1 = no color, 2 = light pink, 3 = pink, and 4 = dark pink. Paint chips corresponding to the three shades of pink served as a guideline. Obtainable from Fuller Paint Co., the light pink chip was CC-46, pink was FF-47, and dark pink was FF-49. In each plot the color readings for several observations were averaged. Crop yields were calculated from weights obtained from the two center rows in each plot.

1972 tests

In 1972 two sets of plots were established, one at the experiment station, Davis, and the other at a grower's ranch in San Joaquin County. At Davis, six applications of 0, 50, 100, 150, 200, and 250 lbs of nitrogen per acre were replicated four times in a randomized complete block. Tissue samples for NO_3-N in the mid-rib and basal stem were taken at the first sampling date (June 28, 1972) and color readings were made on one-half the basal stem section, as in 1971. This first sampling date was approximately 35 days after emergence. Both silage and grain yields were obtained

from these plots. Silage yields were taken at the milk stage of growth.

Field study

At the Ripkin Ranch, San Joaquin County, a field study was initiated in cooperation with Einar Helgestad of Valley Nitrogen, Inc., to evalaute the efficacy of the color reading in predicting nitrogen fertilizer needs of the plants, and making a fertilizer recommendation. The whole field had received no nitrogen before planting. At about 40 days of age, the nitrogen nutritional status of the plants was evaluated by the rapid tissue test for nitrate. Based on the average of several observations, it was recommended that nitrogen be applied, and treatments consisted of 0, 100, and 200 lbs per acre applied as urea at the 40-day stage of growth. The nitrogen was sidedressed with shanks 8 to 10 inches from the row and 4 to 6 inches deep. Plot size was 24 ft by 490 ft. Four rows the length of the plot were harvested to obtain yields.

The data in the table (starting at the late vegetative stage on July 14, 1972), show that the nitrogen content of the plant tissue decreased over time. The most rapid drop in the NO_3-N concentration occurred in the mid-rib of the leaf. It appears that any of the corn tissues could be used in a laboratory analysis at the early stages of growth, but the mid-rib would be the least desirable at a later stage, since there is little difference in the NO_3-N content in this tissue over a wide range of nitrogen rates. The basal stem appears to be a suitable tissue for evaluation, and lends itself easily to the application of the reagent.

Graph 1 shows the relationship between the NO_3-N concentration in the basal stem section and the color readings. There was a high degree of correlation for these data, which represent the mean for four replications.

Graph 2 shows the change in color reading with time as a function of various nitrogen application rates. For the higher nitrogen application rates, the color readings were never below 4 (dark pink). All the plots receiving nitrogen had statistically significantly higher yields than the control and there was no difference between plots receiving various levels of applied nitrogen at the 95% confidence level (Duncan's multiple range test). Based on these data, color readings of 4 or nearly 4 indicate NO_3-N levels would be sufficiently high in the corn plant at the 35- to 40-day stage of growth to assure maximum yield—assuming no drastic loss in the supply of nitrogen to the plant, as through leaching or denitrification.

The 1971 data in graph 3 support the previous conclusion. When NO_3-N levels in the basal stem tissue reached between 4000 and 6000 ppm at approximately the 35-day stage of growth, the maximum yield was obtained. The very sharp break in the curve at that point is an artifact of fitting of the Mitscherlich curve to these data, which have relatively few points at lower NO_3-N levels in the plant tissue. The field was inherently high in nitrogen—an undesirable feature of a field study when trying to evaluate and calibrate a tissue test. Fortunately, the indigenous nitrogen level was not high enough to prevent a response to added nitrogen. The tendency for yield to drop at the higher NO_3-N concentrations (graph 3) resulted in a lower correlation when using the Mitscherlich equation than could be observed with a parabolic curve fitting. The relatively low maximum yield level was a function of the late planting.

Earlier planting

In 1972, because of earlier planting, the average maximum yield was approximately 1500 lbs per acre greater than in 1971. In a different field, but still one with high indigenous nitrogen, the 1972 relationships between color reading, NO_3-N in the basal stem, and yield, were the same as in 1971. A high degree of correlation ($r = 0.9518$) was found between applied nitrogen versus yield, as shown in graph 4, and also between applied nitrogen versus color reading ($r = 0.9998$) at the 35-day stage of growth for the 1972 field study at Davis.

There were no significant differences at the 95% confidence level between 50 and 250 lbs of applied nitrogen. All those plots receiving nitrogen had greater yields than the control. The same responses were observed for silage and grain yields. Also, the maximum yield was observed in those plots which showed a color reading of 4 in the basal stem at the 35-day stage of growth.

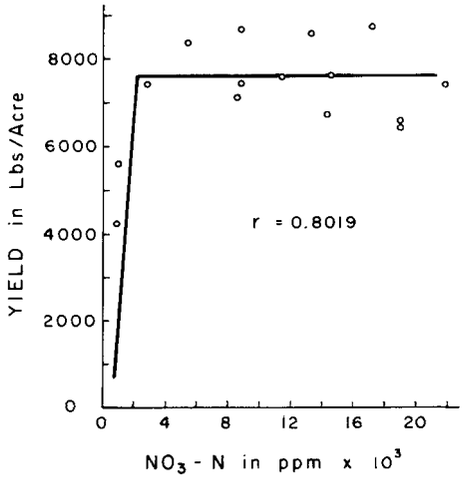
The results of the second field study conducted in 1972 showed no statistically significant differences between 100 and 200 lbs of nitrogen applied sidedress at the 40-day stage of growth. Both rates of applied nitrogen had greater yields than the control. Yields at 100 lbs of N per acre averaged 10,787 lbs per acre and at 200 lbs, 10,920 lbs per acre—as compared with the control yield, 8,138 lbs per acre. The rapid tissue test for nitrogen was used to evaluate nitrogen nutritional status of corn and the color reading obtained was the basis for making a fertilizer recommendation. Several basal stem sections were obtained and a color reading made for each one. The average reading obtained was 3. It was known from other experiments, a color reading of 4 was required to insure adequate nitrogen for maximum yield; therefore, the recommendation was made

NITROGEN CONTENT OF CORN PLANT TISSUES AS INFLUENCED BY APPLIED NITROGEN, U.C. DAVIS, 1971

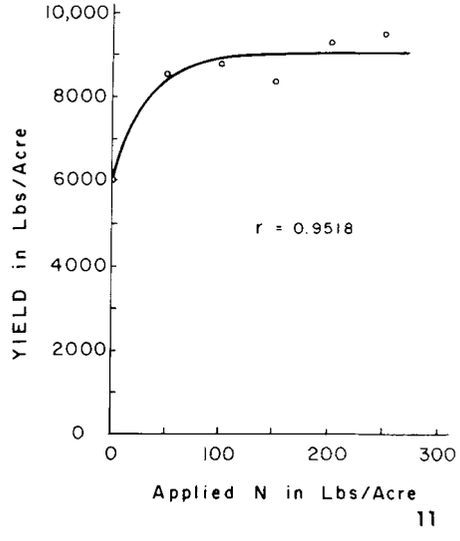
Sampling date	Pounds of Nitrogen Applied				
	0	100	175	250	3
	Whole leaf—% N*				
14 July	2.75	3.37	3.45	3.57	3.
28 July	1.91	2.55	2.75	2.91	2.
17 Aug.	2.23	2.55	2.59	2.66	2.
	Mid-rib—NO_3-N, PPM*				
14 July	1131	10140	22100	12090	144
28 July	351	520	1345	3555	48
17 Aug.	163	182	240	377	5
	Basal stem—NO_3-N, PPM*				
14 July	2568	7865	11635	13845	189
28 July	878	2659	6981	13143	152
17 Aug.	403	2216	3562	5122	58

* Values are means of 4 replications.

GRAPH 3. RELATIONSHIP BETWEEN YIELD OF CORN AND NO_3-N IN THE BASAL STEM, JULY 14, 1971



GRAPH 4. RELATIONSHIP BETWEEN YIELD OF CORN AND APPLIED NITROGEN, 1972



to apply nitrogen at the rates indicated previously. The yields obtained from the applied nitrogen were considered relatively high, indicating that no apparent yield loss resulted from fertilizing according to the rapid tissue test.

Conclusions

The rapid tissue test for nitrate-nitrogen in the basal stem is satisfactory for evaluating the nitrogen nutritional status of corn. When used at the 35- to 40-day stage of growth, the test color reading, obtained as an average of 10 to 20 basal stem sections, can be used as a basis for making nitrogen fertilizer recommendations. An average color reading of 4 indicates the presence of adequate nitrogen, while a color reading of less than 4 indicates the plant will become nitrogen deficient and yield will be reduced. Furthermore, the tissue test can evaluate the efficacy of the nitrogen fertilizer programs used during the season. An average color reading of 4 at the soft dough stage of development indicates that too much nitrogen was applied during the season, or that a late application was made, or both. Thus rapid tissue testing for nitrogen in the field can serve as a guide for diagnosing deficiencies, making fertilizer recommendations, and evaluating the fertilizer program for the season.

Rapid tissue testing for evaluating
(2) NITROGEN NUTRITIONAL STATUS OF SORGHUM

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THE STUDIES TO CALIBRATE the rapid tissue test with the nitrogen nutritional status of sorghum were conducted in a nitrogen deficient field on the Rosolia Ranch near Orland. In 1970, initial soil samples were taken and plots laid out in a randomized complete block design with six treatments and three replications. The plots were 15 ft wide and 50 ft long. The treatments consisted of 0, 50, 100, 150, 200, and 250 lbs of nitrogen applied per acre. The nitrogen (as ammonium sulfate) was applied pre-plant and disked into the soil. Sorghum, variety NK 222, was planted on July 4, 1970 by drilling on 12-inch row spacing.

Commencing on August 6, 1970 plant samples were taken from each plot. Midribs from the first fully matured leaf and the whole leaf of similar maturity from different plants were taken for laboratory analysis of NO_3-N and total nitrogen, respectively. A basal 3-inch section of the stem was also taken from the plants from which leaf samples were obtained. The stem section was split lengthwise and one-half was used for the laboratory determination of the NO_3-N content; the other half was used for evaluating the nitrate content using the nitrate reagent powder.

Tissue samples were collected and color readings were made four times during the growing season. Sampling dates roughly corresponded to different stages of growth: vegetative, floral initiation, boot and heading.

The nitrate reagent, color coding, and paint chips used in these tests with sorghum were identical with those used in the corn tests described in the companion article.

In 1971 another field trial was established on the Rosolia Ranch for further evaluation of the rapid tissue testing technique. Experimental design, plot size and cultural practices were the same as in 1970; however, another variety, NK 265, was planted. Treatments were changed to vary timing of application, as well as rate of nitrogen applied. Total nitrogen applied per acre was 0, 50, 100, and 150 lbs. In four treatments the total nitrogen used was applied pre-plant; in four other treatments either 50 or 100 lbs of nitrogen was applied pre-plant and an additional 50 lbs at the six-leaf and boot stage. The final plot was a control. Tissue samples were collected and color readings made at the vegetative, floral initiation and boot stages of growth.

Guidelines

A final field study was conducted in 1972 to determine whether the guidelines developed in the previous studies could be used to make nitrogen fertilizer recommendations. Three treatments were used: a control, 200 lbs of nitrogen applied preplant, and a third treatment to be fertilized in accordance with the results obtained by using the rapid tissue test at the appropriate stage of growth.

As part of the first study, the changing NO_3-N concentration in various tissues was measured over time. Table 1 shows that regardless of tissue sampled and nitrogen treatment, the nitrogen content decreased with time. In the basal stem, the rate of decrease was more gradual than in the mid-rib, making it more suitable for field use.

It appears that either the NO_3-N content of the basal stem, or the total nitrogen content of the first fully matured leaf, could be used to evaluate plant nitrogen nutritional status, since both reflect the applied nitrogen treatments throughout the growth period sampled. Between the floral initiation stage (Aug. 20), and boot stage (Sept. 3), total nitrogen content tended to level off in the leaf tissue. This period is also one of rapid nutrient uptake, indicating that the plant is growing rapidly and storing nitrogen for use during maturation of the head. Further evidence for this is the drop in total nitrogen content that occurs between the boot and heading stages of growth. At heading, the growth of the plant is practically complete and further gains in dry matter production are the result of carbohydrate accumulation rather than cell development.

In comparing the color readings obtained at the four sampling times (see graph 1), it became apparent that the values from the rapid tissue test follow the same general trend as observed in the NO_3-N concentration in the stem tissue. Data from the 1971 study show a greater range of NO_3-N concentrations in the basal stem, which corresponded well with the color readings taken (see graph 2).

Fitting the data to the Mitscherlich equation, a correlation coefficient of $r = 0.965$ was obtained between the color reading and the NO_3-N content in the basal stem. At low NO_3-N concentrations

GRAPH 1. CHANGE IN COLOR READING IN BASAL STEM OF SORGHUM WITH RATE OF NITROGEN AND TIME, 1970