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Field diagnostic test for nitrate in tomato petiole sap

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Abstract

Four rapid tests for nitrate were assessed for use on tomato sap. The performance characteristics of each were examined. Merckoquant strips were favoured as there was acceptable differentiation and repeatability of response over a suitable working range.

Procedures are described for the collection of tomato petiole sap and for the estimation of nitrate in that sap using the rapid test. Sap was diluted 1:20 with water using plastic ware for the test.

A linear relationship was established between nitrate measured by the strip test and by an auto-analyser method. The linear model was used to successfully predict the nitrate content of further sap collected at Bundaberg, using the strip test.

There was no significant difference between operators of the strips nor between batches of strips.

INTRODUCTION

Drip irrigation systems can be designed to supply necessary plant nutrients and moisture throughout the growth cycle of crops. Moreover, the nutritional programme can also be readily altered as the demands of the plant for various nutrients change. To do this successfully there is a need for a method to monitor plant nutrient status.

Laboratories remote from the site are able to perform all necessary analyses but time taken to transport samples and perform analyses can be excessive. The adoption of a regular monitoring approach to assess crop nutrient status calls for a plant analytical procedure that can be performed cheaply, easily and quickly under field conditions.

Researchers have shown that the nitrate content of sap from the petiole of the youngest fully expanded tomato leaf can be a sensitive indicator of the nitrogen status of the plant (Gomez-Lepe and Ulrich 1974; Prasad and Spiers 1985; Scaife and Stevens 1983). In addition, the nitrogen concentration of sap correlated well with nitrogen concentration in dried plant material (Sonneveld and de Bes 1983).

Prasad and Spiers (1985) used Merckoquant nitrate test strips in trials with tomatoes to establish a desirable sap nitrate–nitrogen range of 880 to 1180 mg/L at the six week growth stage. In that work, undiluted sap from individual petioles was squeezed onto the nitrate sensitive strips and sap concentrations often exceeded the colour of the darkest (113 mg/L NO_3–N) standard. In practice it was difficult to read accurately above 113 mg/L NO_3–N (500 mg/L NO_3) because of the steepness of the strip calibration curve at high concentrations (Scaife and Stevens 1977). The same workers considered that quantitative dilution of sap was not practical on the farm (Scaife and Stevens 1983).

Our objective was to survey available tests for nitrate and, if necessary, develop a rapid field test for sap from tomato petioles. This paper describes the development of

such a test. For grower use, the test should require no special skills yet be reasonably accurate and precise. Hazardous chemicals are unsuitable, wet reagents undesirable and ingredients should have a long shelf life. Rather than sample individual petioles, it was our intention to use the test to make replicated measurements of nitrate from one bulk supply of sap thereby collecting sufficient petioles, at least ten (Scaife and Turner 1983), to give a good representative sample.

In preliminary experiments at Bundaberg (25°S, 152°30′E), the concentration of nitrate in sap at flowering ranged from 220 to 1355 mg/L NO_3–N and this was the target range for our field test. As the colour standards of commercially available tests are shown as mg/L NO_3, NO_3 rather than NO_3–N units are used in this paper.

MATERIALS AND METHODS

Collection of sap

Petiole sap was collected from plants in multi-rate nitrogen trials at Bundaberg. At least 5 mL of sap was collected for each sample. This entailed sampling at least ten petioles from youngest fully expanded leaves, chopping the petioles into small pieces (5–10 mm long) and expressing the sap using a garlic press.

Assessment of available tests for nitrate

Four rapid tests for nitrate in solution were assessed for accuracy and applicability to tomato petiole sap. These tests were:

'Nitratesmo' nitrate test strips. Strips were available from Mackerey-Nagel and Co. (MN), Duren, West Germany. The test strips are impregnated with an organic amine compound and in the presence of nitrate are based on the formation of a red colour when dipped in concentrated sulphuric acid.

Diphenylamine test. As reported in the Morgan Soil Testing System (Morgan 1935). Nitrate reacts with diphenylamine in sulphuric acid to give a blue colour, the intensity of which varies with the nitrate concentration.

Bray's test. Bray's test (Bray 1945) for nitrate is based on the formation of a pink dye through the interaction of nitrous acid with 1-naphthylamine and sulphanilic acid. We attempted to optimise reagent concentrations to give the best separation of response over the range 0 to 6000 mg/L NO_3. Eighteen combinations of reagents were tested. The volume of sap was held constant at 1 mL and the amounts of acetic acid diluent, barium sulphate mixture and colour developing solution varied from 5, 7 and 10 mL; 0.5 and 1 mL; 0.5, 1 and 2 mL respectively.

'Merckoquant' nitrate test strips. Strips were developed by C. Merck, Darmstadt, F. R. Germany and are available commercially. The test strips consist of a length of thin plastic to an end of which are glued two paper squares. Both of these are reactive to nitrite but one of them contains a reducing agent and reacts to nitrate, producing a violet colour. The reaction involves diazotisation and coupling, to produce an azo dye.

Analytical grade potassium nitrate was used to prepare nitrate standards to cover the expected range of nitrate in undiluted and diluted sap (range 0–9000 mg/L NO_3).

For tests on diluted sap, 1 mL of sap was pipetted (plastic pipette) into a plastic container calibrated to 20 mL and then made to volume with water.

Comparison of proposed field test and a quantitative method for nitrate

Eleven sap samples were selected for the comparison test. Samples were selected to cover the range 500 to 6000 mg/L NO_3. Sap was snap frozen then thawed before aliquots were taken for the field test and an auto-analyser finish as described by Spann and Lyons (1985). Results were compared using linear regression. Sap was quantitatively diluted 1:200 for the auto-analyser finish.

Use of the proposed field test for prediction of nitrate

A further ten samples of sap were collected and the field test was used to estimate the nitrate concentration using the regression co-efficients from the linear model.

As rapid strip tests involve subjective matching of colours, an experiment was performed to assess the effect of three different operators each using strips from three different batches of strips.

RESULTS AND DISCUSSION

Assessment of a suitable field test

'Nitratesmo' test strips were eliminated as a suitable field test. Concentrated sulphuric acid which was required to develop the red colour was considered potentially dangerous for a field test. There was an increasing response from 30 mg/L NO_3, which was about the limit of detection, to 250 mg/L NO_3. Quantification over that range however, was almost impossible; the strips were only suitable for the detection of nitrate.

The Diphenylamine test is very sensitive and hence has a fairly narrow practical working range. There was good differentiation of response over the range 2 to 60 mg/L NO_3, but 60 mg/L NO_3 was the definite upper limit of quantification. It was not practical to dilute sap to that extent. In addition, concentrated sulphuric acid which is used in the test would cause clothing and flesh burns. For these reasons, the Diphenylamine test was not considered suitable for grower use.

Bray's test uses reagents that do have an acceptable bench life and could be easily handled in the field. Indeed, some commercially available tests for nitrate in soil solution use a modification of the Bray's test. We tested eighteen combinations of reagents used in the Bray's reaction and found two combinations 7, 1, 1 and 10, 1, 0.5 that gave the best separation of response. That is 7 mL diluent+1 mL powder+1 mL colour developing solution and 10 mL diluent+1 mL powder+0.5 mL colour developing solution.

The first combination gave fair separation between 1000 and 6000 mg/L NO_3 while the latter gave good separation between 2000 and 4000 mg/L NO_3 but similar responses between 4000 and 6000 mg/L NO_3. Repeatability of the test proved to be very dependent on the accurate addition of reagents, especially the powder and colour developing solution. Because the test involved the addition of four reagents and was dependent on the accurate addition of reagents, it was considered too messy for grower use.

Merckoquant test strips proved to be best suited for grower use. There was good matching of each prepared standard with the illustrated colour on the label and the differentiation of colour was acceptable for a field test. It is claimed by the manufacturer of the strips that concentrations above 500 mg/L NO_3 can be determined by recording the time taken to reach the darkest colour (500 mg/L NO_3). We believe there is too much subjective judgement of colour and time in using the strips in this way, (see Table 1).

We believe a better approach is to dilute the sap, using plastic ware, to the working range of the strips. Our desired target range was 0 to 250 mg/L NO_3, as there are five

colour standards on the label over that range. There is only one colour standard outside that range that is, the 500 mg/L NO_3 standard. In addition, we found the pink colour started to fade after 120 seconds and that the colour should be taken as the maximum colour developed between 60 and 90 seconds.

Table 1. Times takes for high nitrate concentration to reach the colour of that illustrated for 500 mg/L NO_3 using Merckoquant test strips

Nitrate concentration (mg/L NO_3)	Time (s)				
	1	2	3	4	5
1000	55	50	38	50	35
2000	30	30	23	20	18
3000	20	20	15	12	11
4000	14	15	11	8	7
7000	9	9	9	5	5
9000	*	7	7	4	4

1 Obtained from literature accompanying Merckoquant strips.

2 Taken from data of Scaife and Stevens 1983.

3 Approximate times taken from data of Prasad and Spiers 1984.

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* Not available.

Comparison of methods, field versus laboratory

Table 2 shows nitrate concentrations in eleven sap samples determined by both the proposed Merckoquant strip test (after 1:20 dilution) and an auto-analyser method. The mean of three determinations using the field test was compared with the quantitative result, using classical linear regression.

Table 2. Relationship between nitrate content of tomato sap determined by auto-analyser (X) and by the proposed field test (Y)

Sample no.	Nitrate concentration (mg/L)				
	Auto-analyser (X)	Field test (Y)			Merck. strips on undiluted sap.*
		Rep. 1	Rep. 2	Rep. 3	
1	460	500	500	500	300
2	1020	1000	1000	1000	1000
3	1460	1600	1600	1600	1300
4	1740	2000	1600	2000	2900
5	2330	2000	2500	3000	3800
6	2920	4000	3500	3000	7000
7	3600	5000	4000	4000	4900
8	4070	5000	4000	6000	4900
9	4960	7000	5000	6000	9600
10	5530	6000	5000	6000	11000
11	6340	8000	8000	7000	11000

* Using calibration curve [NO_3] versus time constructed from data of Operator 4, Table 1. One estimate only.

Merckoquant strips tend to over-estimate nitrate, especially at higher concentrations; this is most likely due to the spread of the higher colour standards (100, 250, 500 mg/L NO_3). Similarly other workers (Prasad and Spiers 1984) report higher results for the strip method as nitrate concentration increases. From our observations, the relationship between field and laboratory results was reproducible and was of the form, $Y=b_1X$ where $b_1=1.159\pm0.056$ ($P=0.05$), Y=field test value and $X=AA$ value. The coefficient of determination (R^2) was 0.995.

An analysis of variance of data from an experiment involving different operators and batches of strips showed that most of the error was individual operator reading error. There was no significant difference between operators nor batches of test strips. Precision of the strip method was estimated at±15% relative based on the mean of three determinations.

Prediction of nitrate

The relationship between field and laboratory methods was tested on a further ten samples collected at Bundaberg. Table 3 shows predicted and quantitatively determined nitrate concentrations. The range of predicted concentrations was calculated from the 95% confidence interval of the slope (b_1) estimate.

Table 3. Prediction of nitrate concentration in sap using the proposed field test

Sample	Nitrate concentration (mg/L)		
	Field test* result	Predicted† value	Quantitative‡ result
A	300	260±20	205
B	1300	1120±50	955
C	3700	3190±150	2510
D	2600	2240±100	2005
E	4000	3450±180	3420
F	5500	4740±240	5190
G	6700	5780±300	5665
H	5000	4310±220	4425
I	4400	3800±190	3900
J	3300	2850±140	2750

* Mean of three replicates.

†Calculated from the linear regression model $Y=b_1X$, where $b_1=1.1595\pm0.056$. Predicted value±95% confidence interval.

‡Using the auto-analyser finish described by Spann and Lyons 1985.

There was good agreement between the predicted nitrate concentration and the quantitative result, especially for medium to high concentrations. It is in this range that we expect the critical concentration (that concentration which is just deficient for eventual maximum marketable yield) to fall.

We believe the use of Merckoquant strips on 1:20 diluted sap will prove a useful means of monitoring nitrogen status of tomatoes and of re-adjusting fertiliser schedules. The strip test has acceptable accuracy and precision. It is simple for grower or field officer use and requires plastic ware and water as diluent. Tap water could be used as diluent provided the concentration of nitrate did not exceed a maximum of 5 mg/L NO_3.

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