VALIDITY OF THE NITRATE TEST STRIP TECHNIQUE FOR USE ON CEREALS

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ABSTRACT

The nitrate test strip technique for determination of sap nitrate levels was investigated for sources of variability and for its reliability as a predictive tool in estimating yield and grain quality of malting barley (*Hordeum vulgare*). The technique was performed on a malting barley crop, on barley plants at controlled temperatures and on standard solutions. A regression equation valid over a wide temperature range was derived to calibrate test results. Correlation of readings with grain yield and quality are discussed.

Readings from standard solutions measured by the nitrate test strip technique varied significantly with temperature, as did readings from barley plants measured at controlled temperatures. Diurnal fluctuations occurred in the sap nitrate test results of field-grown plants. The nitrate test strip technique may be useful for estimating grain yield and grain quality of malting barley, but care is needed in choice of testing conditions as temperature and diurnal fluctuation may influence results.

INTRODUCTION

Since Scaife (1979) reported that Merck nitrate test strips could be used as a safe, accurate and convenient measure of sap nitrate, the test has been applied in a number of ways as a crop monitoring tool.

Cornforth (1980) formulated relationships between early sap nitrate levels and yield in maize. Withers (1982) found a relationship between sap nitrate levels at intermediate growth stages and grain yield in wheat and barley. Papastylianou & Puckridge (1981) found an asymptomatic increase in stem nitrate with nitrogen treatments on cereals in dry seasons, but a close relationship between stem nitrate at early growth stages and final yield in seasons with good rainfall.

Withers (1986) suggested that late measurements of sap nitrate could be used to monitor grain nitrogen levels in wheat.

Commercial nitrogen fertiliser recommendations for wheat and barley have been published in New Zealand based on the use of the nitrate sap test as a monitoring tool.

Given the current use of the strips for monitoring sap nitrate levels in cereal crops, the experimental programme described in this paper was designed to:

1. Develop a reliable calibration equation for the test strips when used at concentrations above 500 ppm NO_3.
2. Determine the relevance of the technique for use in predicting and correcting yield quality and quantity of malting barley.
3. Test for sources of variability in the nitrate test strip technique.

MATERIALS AND METHODS

Calibration: Merck brand nitrate test strips are small plastic strips with filter paper attached to one end. The paper is impregnated with an aromatic amine and N-(1 naphthyl) ethylene diamine. These reagents turn purple on contact with aqueous nitrate. Up to 500 ppm NO_3, concentration of the test solution is determined by degree of colour change, to be compared against standards illustrated on the test strip container.

Nitrate test strips may be used to test solutions above 500 ppm NO_3 by measuring time taken to reach full colour change on the test strip (Scaife & Stevens, 1977; Scaife, 1979; Scaife (1979) and Prasad & Speirs (1984) have calibrated time taken to reach maximum colour on the test strips with concentration. However, all these calibrations vary, and are based on empirical curves.

Nitrate test strips were calibrated using standard solutions of K_2NO_3 at controlled temperatures. A total of 338 readings were made, recording seconds taken for the test strips to reach maximum colour after applying a drop of the test solution. Standard solutions ranging from 500 to 10,000 ppm NO_3 were tested at 9, 16, 23 and 26 °C. Test results were regressed onto actual concentrations of solutions tested.

Field experiment: A field experiment was conducted at Aranui Road, Kairanga, Manawatu in the 1988-89 spring season using nitrogen fertiliser treatments on a commercial malting barley (*Hordeum vulgare* cv. Corniche) crop.

The crop was sown on 17 November 1988 at a seeding rate of 120 kg/ha with drilled 15:10:10 NPK fertiliser equivalent to 18.5 kg/ha nitrogen. This fertiliser formed the lowest nitrogen treatment. Additional urea at two rates was applied to form treatments at 10 days after sowing (DAS) (Zadok's growth stage (Z10) and at 35 DAS (Z25) (Table 1).

Treatments were applied in a randomised complete block design with four replicates. Plant population in each plot was sampled at emergence, and analysed for differences among nitrogen treatments.

Rainfall was monitored on site throughout the experiment. Daily maximum and minimum temperatures were obtained from readings taken at the DSIR's Aorangi site, situated about 5 km from the trial site.

Nitrate sap tests were performed on the crop at ten stages between 15 and 56 DAS. The tests were conducted by collecting six plants at random from a single plot and crushing their white tiller bases in a garlic crusher. Expressed sap was applied to the nitrate test strip, and time taken to reach maximum colour change recorded. Two readings were taken from each plot at each time. Readings were transformed before analysis using the derived regression equation.

Zadok's growth stage measurements and soil moisture measurements (using an IRAMS brand Time Domain Reflectormeter) were taken at the same time as the nitrate sap tests. Zadok's growth stage was measured on two samples of six plants from each plot using 30 cm probes, and were transformed from meter readings to soil moisture percentages using the regression equation derived by Rahardjo & Clothier (pers.comm., 1989).

At maturity (17 February 1989, 92 DAS), two quadrats of approximately 0.45 m^2 were harvested from each plot. Plant population in each plot was sampled again, and tested for differences among nitrogen treatments.

Harvested quadrats were used to determine seed moisture, grain nitrogen (Kjeldahl method, adjusted to 14 % grain moisture), spikelets/ear (including lost and aborted grain), grains/ear, grain yield/m^2, vegetative tillers/m^2, reproductive tillers/m^2 and total dry weight/m^2. All these characters were tested by analysis of variance. Grain nitrogen, thousand seed weight and grain yield/m^2 were correlated with nitrate sap test results on a per plot basis.

Diurnal experiment: On 17 December 1988, sap nitrate tests were performed on two plots of the field experiment every two hours from 0800 until 1800 h. The two plots used were an S1 and an S3 treatment (Table 1). Temperature was recorded during each reading.

Readings were transformed using the derived regression equation and analysed by time and treatment for variation.

Standards: Standard solutions of 500, 1,000, 3,000, 5,000 and 10,000 ppm NO_3 were each divided into three portions and placed in growth cabinets at controlled temperatures of 14, 18 and 25 °C. After three hours for temperature equilibration, the standards were tested with nitrate test strips.

Readings were transformed using the derived regression equation and analysed by original concentration and temperature for variation.

Pot experiment: A pot experiment was conducted on barley plants (*Hordeum vulgare* cv. Fleet) grown in a growth cabinet at 18 °C. Three rates of nitrogen fertiliser (as urea) were mainplot treatments. Subplot treatments were created by removing pre-determined pots to growth cabinets at different temperatures for three hours (for temperature equilibration) before conducting sap nitrate tests (Table 2).

The treatments were grown in a single growth cabinet at 18 °C. The experimental design was a randomised complete block with three replicates. Nitrate sap tests were performed on the primary tillers of two plants from each treatment at 17 and 31 DAS after sowing. Pots were returned to the 18 °C cabinet after each test.

At 48 DAS the remaining plants were weighed, fresh weight and dry weight, and the total nitrogen (Kjeldahl method) of the mainstem tillers were measured.

The experiment was analysed as a double split block (over temperature and time).

Table 1: Field experiment treatments.

Treatment	N rate (kg/ha)	Time applied	Zadoks	Date
S1	18.5	sowing	0	17 November
S2	38.5	emergence	10	27 November
S3	98.5	emergence	10	27 November
T1	38.5	tillering	25	22 December
T2	98.5	tillering	25	22 December

Nitrate sap tests were transformed using the derived regression equation before analysis. Total nitrogen in mainstem tillers at 48 DAS was correlated with sap nitrate at 17 and 31 DAS on a treatment basis.

RESULTS

Calibration: A $\log_{10}$ transformation of both nitrate test strip readings (seconds taken to reach maximum colour) and concentration of standard solutions (ppm NO_3) was required to give a linear relationship between variables. The following equation was derived:

$\log_{10}$ concentration (ppm NO_3) e
$= -1.1468 \log_{10}$ seconds $+ 4.8830$
($r^2 = 0.829$; $P < 0.0001$)

The equation was valid for temperatures from 9 °C to 26 °C. Removing readings taken at either temperature extreme from the data set did not significantly alter the regression equation.

Table 2: Pot experiment treatments.

Treatment	Nitrogen rate (kg/ha)	Testing temperature (°C)
1	30	14
2	30	18
3	30	25
4	80	14
5	80	18
6	80	25
7	130	14
8	130	18
9	130	25

Field Experiment: The 1988-89 spring season at Kairanga was hotter and drier than the ten-year average during the December growth period (Table 3.)

Table 3: Rainfall and temperature at the field experiment site.

Month	Rainfall[1] (mm)		Mean temperature[1] (C°)	
October	77	(87)	13.8	(12.4)
November	80	(62)	15.1	(14.0)
December	26	(70)	17.3	(15.8)
January	103	(62)	19.3	(17.5)
February	77	(41)	17.4	(17.6)

[1] Figures in parentheses are 10 year average (1970 - 1980).

No significant differences ($P > 0.05$ throughout the experiment) were recorded among nitrogen treatments for Zadok's growth stage or soil moisture measurements (Table 4).

Significant differences in sap nitrate levels were found between times of measurements and in the time by nitrogen interaction (Table 4).

No significant differences in sap nitrate concentrations occurred until 22 DAS (Z18). All treatments showed a steady decline in sap nitrate concentrations from 15 DAS (Z12) until 35 DAS (Z25). A levelling-off in the decline of sap nitrate occurred in all treatments between 35 DAS and 49 DAS (Z46), after which sap nitrate concentrations of the treatments diverged. Sap nitrate concentrations in all treatments declined again between 53 DAS (Z53) and the final measurement at 56 DAS (Z57) (Figure 1).

Table 4: Zadok's growth stage, soil moisture and sap nitrate results, field experiment.

DAS	Z	Soil Moisture (%)	Sap Nitrate (log_{10} ppm)				
			S1	S2	S3	T1	T2
15	12a*	31.9e	3.6p	3.6nop	3.6op		
19	16b	26.8c	3.5m	3.5mn	3.5mno		
22	18c	28.8d	3.4lm	3.4kl	3.5lm		
26	20d	19.1a	3.3jk	3.4kl	3.3ij		
30	25e	23.6b	3.0g	3.2hi	3.1h		
33	25e		2.9defg	3.0g	3.0g		
35	25e	18.2a	2.8cde	2.9cdefg	2.9efg		
49	46f		2.9cdef	2.9cdef	2.9cdefg	2.9cdefg	2.9defg
53	53g	22.2a	2.8bcd	2.8cde	3.0c	2.8cde	2.9cdefg
56	57h		2.8abc	2.7ab	2.9cdefg	2.7a	2.8abc

* Values with one or more letters in common within each variable are not significantly different.

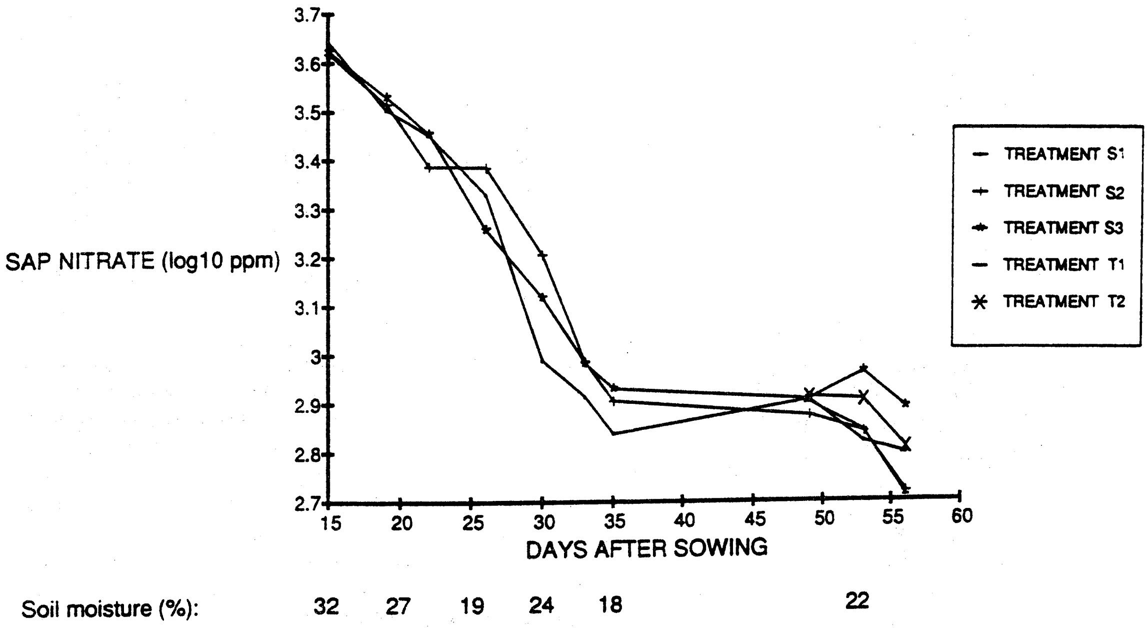

Figure 1: Sap nitrate levels over time in the field experiment

Treatments S1 and S2 had the lowest sap nitrate concentrations, or were not significantly different from the treatment with the lowest sap nitrate level, at most times. Treatment S3 had the highest sap nitrate level, or was not significantly different from the treatment with the highest sap nitrate level, at all times except 26 DAS After 35 DAS (Z25) treatment T1 had sap nitrate concentrations not significantly different from treatments S1 and S2. Similarly, sap nitrate concentrations in treatment T2 were not significantly different from those in treatment S3 at all times.

Grains/ear, grain yield/m², reproductive tillers/m², total tillers/m² and total dry weight/m² showed no significant differences among nitrogen treatments at harvest (92 DAS) (Table 5).

There were no significant differences in plant population/m² at emergence, but at 92 DAS treatments S1, S2 and S3 had a plant population/m² approximately 10 % lower than treatments T1 and T2 (Table 5). Seed moisture, grain nitrogen, thousand seed weight, spikelets/ear and vegetative tillers/m² were also significantly different among nitrogen treatments (Table 5). Seed moisture at 92 DAS was highest in treatment S3 (18.6 %), which was significantly different from the two treatments with the lowest seed moisture, T1 and T2 (15.2 % and 14.6 % respectively) (Table 5). Grain nitrogen was highest in treatments S3 and T2, followed by treatments T1, S2 and S1 in that order. All grain nitrogen levels (adjusted to 14 % grain moisture) were within the 2 % limit allowable for malting (Table 5). Treatment S3 had the lowest thousand seed weight, which was 6 % lower than the mean for treatments T2, S1 and T1 (Table 5). Treatment S1 had the most spikelets/ear, but differed significantly only from treatment S3 (Table 5). Treatment S1 had the highest number of vegetative tillers per m², with over twice the number of treatment T2 (Table 5).

Grain nitrogen was correlated with sap nitrate at 53 DAS (Z53) ($r = 0.65$, $P = 0.009$). Thousand seed weight displayed a negative correlation with sap nitrate readings at 35 DAS (Z25) ($r = -0.53$, $P = 0.04$) and at 56 DAS (Z57) ($r = -0.55$, $P = 0.035$). Grain yield was significantly correlated with sap nitrate at 15 DAS (Z12) ($r = 0.56$, $P = 0.03$).

Diurnal fluctuation experiment: There were no significant differences in sap nitrate levels among nitrogen treatments or in the nitrogen by time of day interaction during the day of measurements. However, sap nitrate levels did show significant differences by time of day (Table 6).

Sap nitrate readings taken at 1200 hours were nearly double those recorded at 1800 hours. Although temperatures at 1000 hours (20.3 °C) and at 1600 hours (20.2 °C) varied by only 0.1 °C, readings at these times were also significantly different.

Standards: Nitrate test strip readings on standard solutions differed significantly with both concentration and temperature (Table 7 & 8). The concentration by temperature interaction was not significant.

Readings taken at 25 °C were significantly higher (+30 %) than those taken at 14 or 18 °C (Table 7).

Cabinet experiment: Sap nitrate tests results from plants temporarily removed to different temperatures were significantly different by temperature. The nitrogen by date interaction was also significant.

The highest sap nitrate test results (+75 %) were obtained from plants tested at the growth temperature of 18 °C (Table 9).

At 17 DAS, there were no differences in sap nitrate levels among nitrogen treatments. Nevertheless, by 31 DAS, each nitrogen treatment could be differentiated by significantly different sap nitrate levels (Table 10).

The nitrogen level by temperature interaction was significant for the percentage nitrogen concentration of the primary tillers at 48 DAS (Table 11).

Plants for the two lower nitrogen treatments that remained at 18 °C during sap nitrate samplings had final tiller nitrogen levels higher than those moved to 14 °C or 25 °C environments (Table 11).

A correlation of $r = 0.90$ was obtained between sap nitrate levels recorded at 31 DAS, and percentage nitrogen content of primary tillers harvested at 48 DAS.

The fresh and dry weight of primary tillers were significantly greater at 80 and 130 kg N/ha than at 30 kg N/ha (Table 12). There was no significant effect of temperature on the fresh or dry weight of primary tillers.

Differences in fresh weight were greater than those in dry weight, indicating increased succulence as well as increased dry matter at the higher nitrogen rates.

DISCUSSION

The regression equation derived for calibration of nitrate test strip results is more convenient to use than a calibration curve, and allows simple and consistent transformations of data sets. Transformed data bears a close and linear relationship to the variable being measured (i.e. sap nitrate), allowing conventional analyses to be undertaken.

Dry weather in December 1988 reduced nitrogen responses in the field experiment from expected levels. Grain yield did not differ significantly with nitrogen treatment, but was significantly correlated with sap nitrate at 15 DAS. This is consistent with the findings of Papastylianou & Puckridge (1981, 1983) and

Table 5: Harvest data from field experiment.

	S1	S2	S3	T1	T2
Grains/ear	16.7a*	15.7a	16.1	15.7a	16.3a
Grain yield/m² (g DW)	501a	516a	523a	500a	553a
Reproductive tillers/m² (harvest)	750a	827a	851a	814a	852a
Total tillers/m²	845a	869a	909a	862a	886a
Total dry weight/m2 (g)	810a	840a	914a	871a	909a
Plants/m2 at emergence	232a	218a	205a	209a	213a
Plants/m2 at harvest	161a	177a	187ab	191b	196b
Seed moisture at harvest (%)	16.8abc	17.2ab	18.3a	15.2bc	14.6c
Grain nitrogen (%)	1.64a	1.70ab	1.90d	1.77bc	1.83cd
Thousand seed weight (g)	39.8b	39.7b	37.6a	39.2ab	40.4b
Spikelets/ear	19.2b	17.8ab	17.3a	17.8ab	18.6ab
Vegetative tillers/m² at harvest	94.8b	41.4ab	57.6ab	47.4ab	33.6a

* Within each variable values with one or more letters in common are not significantly different

Table 6: Sap nitrate measurements from the field experiment over a single day.

Time	Temperature (°C)	Sap nitrate (log_{10} ppm)
0800	17.5	2.94ab*
1000	20.3	3.13cd
1200	23.0	3.21d
1400	23.5	3.06bc
1600	20.2	2.97ab
1800	19.0	2.86a

* Values with one or more letters in common are not significantly different.

Table 7: Nitrate test strip results from standard solutions by temperature.

Temperature (°C)	Test Result (log_{10} ppm NO_3)
14	3.35a*
18	3.35a
25	3.47b

* Values with one or more letters in common are not significantly different.

Table 8: Nitrate test strip readings from standard solutions by concentration.

Solution concentration (ppm NO_3)	Test result (log_{10} ppm NO_3)
500	2.50a*
1,000	3.06b
3,000	3.60c
5,000	3.80d
8,000	3.98e

* Values with one or more letters in common are not significantly different.

Table 9: Sap nitrate concentration of pot-grown barley plants by temperature.

Temperature (°C)	Sap nitrate (log_{10})
14	3.19a*
18	3.41b
25	3.15a

* Values with one or more letters in common are not significantly different.

Table 10: **Sap nitrate concentrations (log_{10} of pot-grown barley plants.**

Nitrogen level (kg/ha)	DAS	
	17	31
30	3.40cd*	2.60a
80	3.45d	3.22b
130	3.52b	3.31bc

* Values with one or more letters in common are not significantly different.

Table 11: **Total nitrogen content of pot-grown barley plants.**

Nitrogen level (kg N/ha)	Temperature (°C)		
	14	18	25
30	0.85a*	1.01b	0.88a
80	1.20c	1.59de	1.13bc
130	1.23c	1.51d	1.71e

* Values with one or more letters in common are not significantly different.

Table 12: **Fresh and dry weight of primary tillers of pot-grown barley plants.**

Nitrogen level (kg/ha)	Primary tiller	
	Fresh weight (g)	Dry weight (g)
30	1.43a*	0.38a
80	1.97b	0.51b
130	2.05b	0.51b

* Values with one or more letters in common are not significantly different.

Cornforth (1980), who also observed close relationships between early sap nitrate levels and grain yield.

Grain nitrogen was significantly correlated with sap nitrate at 35 and 53 DAS, as was thousand seed weight at 56 DAS. Correlations of thousand seed weight and grain nitrogen with sap nitrate levels may be useful in determining whether a crop will meet malting specifications, but apply too late for corrective action to be viable. Level of nitrogen fertiliser applied at emergence was positively related to sap nitrate levels throughout the growth period. Nitrogen fertilizer applied at emergence was positively correlated with sap nitrate levels throughout the growth period. Nitrogen fertiliser applied at tillering gave sap nitrate levels which were not significantly different from the same rates applied at emergence.

Sap nitrate trends over time were similar to those observed by Withers & Palenski (1984) on wheat, although the early rise in sap nitrate levels observed by those authors was not reproduced. Sap nitrate levels decreased steadily from a peak of 3.6 log_{10} ppm NO_3 (3,981 ppm), having never reached the level of 5,000 ppm NO_3 (1,000 ppm NO_3) at Z25 (tillering), earlier than recommended by Withers & Palenski (1984) possibly because of the dry December. Although subsequent grain yields were only moderate (5.00 - 5.52 t/ha), grain nitrogen levels form all nitrogen treatments were within acceptable bounds for malting (1.64 - 1.90 % N).

Application of 98.5 kg N/ha at emergence reduced thousand seed weight from that in the lower nitrogen treatments. Nitrogen applied at tillering did not reduce thousand seed weight. Nitrogen at 98.5 kg N/ha, applied at emergence, significantly increased grain nitrogen, as did 38.5 and 98.5 kg/ha applied at tillering. Nitrogen fertiliser applied at sowing gave higher seed moisture levels at harvest than later nitrogen applications which perhaps indicated an induced delay in maturity.

Sap nitrate readings taken on two nitrogen treatments over the course of a day showed significant differences by time of reading, but not by nitrogen treatment. The differences observed were not directly related to ambient temperature, as readings taken at different times with similar temperatures were significantly different. Readings taken after the midday temperature peak were lower than those taken at similar temperatures in the morning, suggesting a lowered sap nitrate flow.

In pot-grown plants change in temperature directly before a sap nitrate test lowered test result. Differences in sap nitrate levels among temperature treatments were correlated (r = 0.90) with plant total nitrogen, suggesting that transient temperature changes caused permanent variation in plant nitrogen status.

High temperature significantly increased test strip results (log_{10} ppm NO_3) taken on standard solutions. A chemical effect on speed of test strip reaction is indicated, but appears to be overridden by stress effects such as temperature change when tests are conducted on plants.

CONCLUSIONS

1. A regression equation useful for transforming data sets of sap nitrate test strip readings was derived.
2. Significant correlations were obtained between early sap nitrate levels and final grain yield, and between late sap nitrate levels and grain nitrogen and thousand seed weight.
3. Increasing temperature increased speed of reaction in Merck nitrate test strips.
4. Changes in ambient temperature created a plant stress of unidentified mechanism which lowered sap nitrate levels. This effect overrode the chemical effect on the reaction time of the test strip, causing lowered sap nitrate levels after a temperature change, even at increased temperatures. Lowered sap nitrate levels after temperature fluctuations were reflected by lowered total nitrogen of the plants.
5. Nitrate sap test results were more affected by time of day than ambient temperature or nitrogen treatment.

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