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NITRATE ASSIMILATION IN POTATO: IMPLICATIONS FOR PREDICTING NITROGEN STATUS IN THE FIELD

B. Searle, M. Andrews, P. Jarvis, R.J. Lucas

Department of Plant Science
Lincoln College
Canterbury

ABSTRACT

A preliminary study was carried out to survey techniques for diagnosing nitrogen deficiency in potatoes, with a view towards more precise determination of requirements for nitrogen fertilizer. Potato plants, cv. Ilam Hardy were grown in the field under a wide range of nitrogen regimes. Nitrate reductase activity (NRA) was used to assess the nitrogen status of plants. The NRA, assayed in the presence ($NRA+NO_3$) and absence ($NRA-NO_3$) of added nitrate was measured in the petiole and lamina of the seventh leaf of young plants and in the root and stem. Similar measurements were carried out on the petiole and lamina of mature plants. In addition, nitrate concentrations in the lamina and petiole of the seventh leaf were measured using nitrate test strips and chemical analysis.

Data for young plants indicate that when plants received the range of nitrate concentrations likely to occur in agricultural soils, a substantial proportion of nitrate assimilation occurs in the shoot. The $NRA+NO_3$ was highest in the lamina regardless of the nitrogen supply and age of the plant, and did not change significantly when nitrogen fertilizer was applied. In the absence of nitrogen fertilizer, the amount of $NRA-NO_3$ was very low in all tissues. However in young plants, application of nitrogen fertilizer increased the activity in the lamina until it was similar to that of $NRA+NO_3$.

In mature plants, $NRA-NO_3$ was always greater in the lamina than in the petiole, and in both tissues, increased when nitrogen fertilizer was applied. In contrast, the concentration of nitrate was always higher in the petiole than in the lamina and concentrations in the petiole were increased by application of nitrogen. It is concluded that measurements of $NRA-NO_3$, in the lamina the ratio between $NRA-NO_3$:$NRA+NO_3$ in the lamina and the concentration of nitrate in the petiole would be useful in determining the nitrogen status of potato crops. These tests are relatively quick and simple to perform.

Additional Key Words: nitrate reductase activity (NRA), nitrate test strip

INTRODUCTION

Potato yields of 80-90 tonnes/ha have been obtained in the United Kingdom and New Zealand (Evans and Nield, 1981; Mountier and Lucas, 1981). However, the average commercial yield in New Zealand is much lower, and in order to remain profitable, growers need to improve yield and quality.

Agronomic factors affect both the yield and quality of potato crops. One of the main limitations to potato growth is lack of nitrogen. Higher potato yields resulting from application of nitrogen can be attributed to increases in leaf area and a lengthening of the duration of canopy cover thus extending the period of light interception (Dyson and Watson, 1971; Allen and Scott, 1980). However, excessive use of nitrogen fertilizer can delay tuber initiation and promote vegetative growth at the expense of tuber growth, thus reducing yield and quality (Allen and Scott, 1980). A reliable, simple method for predicting the nitrogen requirements of the crop is clearly needed.

Attempts to determine the nitrogen status of potato crops have been based primarily on determining critical concentrations of nitrate in the petiole usually using nitrate test strips but results vary considerably between site, season and cultivar (Carolus and Woltz, 1944; Tyler, *et al.*, 1961; Geraldson *et al.*, 1973).

It has been suggested that an indirect measure of the nitrogen status of the plant may be made by determining the amount of nitrate reductase activity (NRA) in the plant (Kapoor and Li, 1982). This can be easily measured by an *in vivo* assay (Andrews, 1986) and two *in vivo* assays for $NRA+NO_3$ and $NRA-NO_3$, are commonly used. The $NRA+NO_3$ assay, is believed to indicate the capacity of tissue to reduce nitrate when nitrate is not limiting and nitrate is added to the assay buffer (Andrews, 1986). In the $NRA-NO_3$ assay, nitrate is not added to the assay buffer and the nitrate reductase activity relies solely on nitrate stored in plant tissue. In some cases, the ratio of $NRA-NO_3$ to $NRA+NO_3$ may be a good indicator of the nitrogen status of a plant (Davison *et al.*, 1984).

Preliminary studies with the objectives of determining if the amount of $NRA+NO_3$, the amount of $NRA-NO_3$, the ratio between $NRA-NO_3$:$NRA+NO_3$ or the concentration of tissue nitrate can be used to determine the nitrogen status, and hence nitrogen requirement of a potato crop are reported here.

MATERIALS AND METHODS

Field Trial 1.

Seed of Ilam Hardy was planted on 30 October 1986 into a Wakanui silt loam at Lincoln College using a potato planter. Plots consisted of four rows, 30 m long and 0.8 m apart. The two inner rows of each plot were used for sampling. Superphosphate (40 kg P/ha) and potassium chloride (100 kg K/ha) were applied as basal fertilizer at planting with 2.5 kg a.i. per ha of disulfuton for aphid control. Weed control was obtained by applying metribuzin (800 g/ha) after mounding. A microjet irrigation system was used to apply 30 mm of water whenever a soil moisture deficit of 40 mm was reached. At planting, three rates of N fertilizer (0, 100 and 300 kg N/ha) were applied as calcium ammonium nitrate. There were three replicates.

On 23 December, just prior to flowering, two plants from each plot were dug. The petiole and lamina of the seventh leaf and the stem and root were separated from each plant and assayed for NRA. Nitrate test strips (Merckoquant) were used to determine the nitrate content in the sap of the leaf lamina and petiole of the youngest mature leaf (the leaf seventh from the shoot apex).

Field Trial 2.

Seed of Ilam Hardy was planted by hand on 21 October 1986 into a Templeton silt loam, at Crop Research Division, DSIR, Lincoln. Plots were 3 m x 5 m and consisted of four rows, each 0.75 m apart. Again, only the two inside rows of each plot were used for sampling. Applications of basal fertilizer and disulfuton were the same as for Field Trial 1. Despite the application of metribuzin (600 g/ha), there were some problems with wireweed (*Polygonum aviculare*). The rate of irrigation was the same as in Field Trial 1, but was applied with a spray boom.

Urea was applied at five rates; 0, 100, 200 kg N/ha and 100 or 200 kg N/ha each with a side dressing of 50 kg N/ha made in early January. Initial applications were by hand at planting. There were four replicates. On 11 February 1987, one stem was taken from each of two plants in each plot. The leaf seventh from the apex was separated from the stem, and the lamina and petiole tested for NRA as well as nitrate concentration (test strip and chemical analysis).

Chemical Analysis

All samples were taken between 1200 and 1400 hours and placed on ice. An *in vivo* assay, as described by Andrews *et al.* (1984), was used to measure NRA+NO_3 and NRA−NO_3. Briefly, 0.4 − 0.5 g of fresh tissue was vaccuum infiltrated in a flask containing phosphate buffer (100 mol m^{-3}) and propan-l-ol (3%). For NRA+NO_3 estimates, 50 mol m^{-3} NO_3 as KNO_3 was added to the buffer; KNO_3 was omitted for the estimate of NRA−NO_3. After vacuum infiltration for 10 minutes, a sample was taken at zero time. The flasks were shaken in the dark for 20 minutes at 30°C, and then a second sample taken and nitrite was determined in both samples. NRA is expressed in micromoles of nitrite produced per unit fresh weight of tissue per hour (μmol NO_2/g fwt/hr).

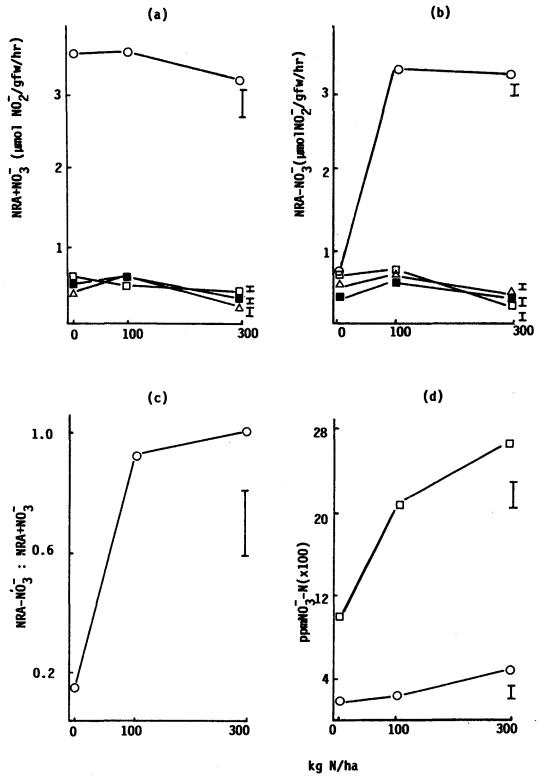

Figure 1 **Response of the lamina ( ) and petiole ( ) of the seventh leaf from the apex, stem ( ) and root ( ) of young plants to different rates of nitrogen fertilizer. a) NRA+NO_3, b) NRA−NO_3, c) NRA−NO_3: NRA+NO_3 ratio, d) nitrate concentration measured using test strips. Bars indicate S.E. of the mean.**

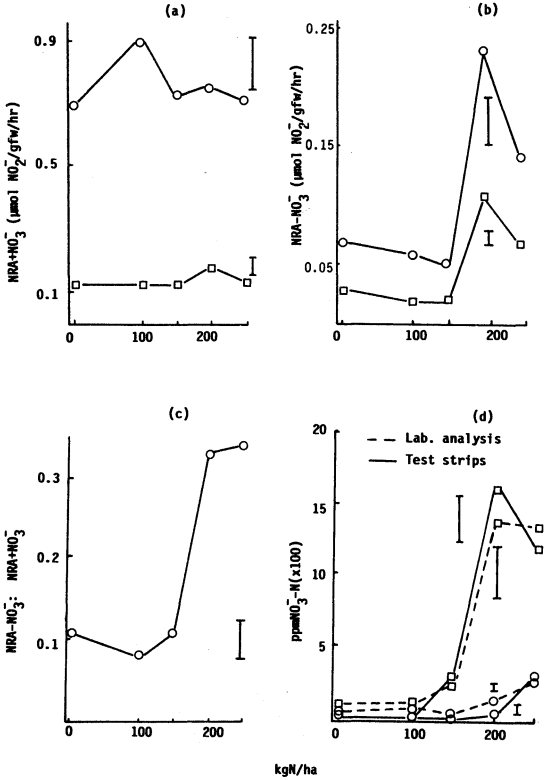

Figure 2 **Response of the lamina ( ) and petiole ( ) of the seventh leaf from the apex of mature plants to different rates of nitrogen fertilizer. a) NRA+NO_3, b) NRA−NO_3, c) NRA−NO_3: NRA+NO_3 ratio, d) nitrate concentration. Bars indicate S.E. of the mean.**

The concentration of tissue nitrate was determined on dried and ground samples using the method described by Mackereth *et al.* (1978). Samples were shaken overnight in distilled water and cadmium used to reduce nitrate to nitrite, which was then determined colourimetrically.

To test for nitrate using test strips, fresh samples were squashed between glass plates and the exuded sap squeezed onto Merckoquant test strips. The time taken to reach a standard colour was determined by comparison with a calibration colour chart.

RESULTS

Trial 1, Young Plants

In all tissues from young plants, NRA+NO_3 did not change significantly when different rates of nitrogen fertilizer were applied (Figure 1a). Activity in the lamina of the youngest mature leaf (seventh leaf) was approximately seven times greater than that in the petiole, the stem or the root at any given rate of nitrogen fertilizer. When no additional nitrogen fertilizer was applied, NRA−NO_3 values (Figure 1b) did not differ significantly between tissues. The amount of NRA−NO_3 in the petiole, stem and root did not increase when nitrogen was applied and values were similar to those for NRA+NO_3. However, NRA−NO_3 of the lamina of the seventh leaf increased significantly ($p < 0.05$) from 0.55 to 3.36 µmol NO_2/g fwt/hr as the rate of nitrogen applied increased from 0 to 100 kg N/ha. Further increases in nitrogen application did not significantly increase enzyme activity in the lamina. Thus, the ratio of NRA−NO_3:NRA+NO_3 in the lamina increased significantly from 0.12 to 0.94 when the nitrogen fertilizer was increased from 0 to 100 kg N/ha (Figure 1c). There was little additional change in the ratio as the rate of nitrogen application was increased from 100 to 300 kg N/ha.

Nitrate concentrations in the lamina of the seventh leaf determined by test strips, increased from 120 ppm to 530 ppm NO_3−N as the rate of applied nitrogen increased from 0 to 300 kg N/ha. Nitrate concentrations in the petiole were higher, and increased from 1000 ppm NO_3−N at 0 kg N/ha to 2700 ppm NO_3−N at 300 kg N/ha (Figure 1d).

Trial 2, Mature plants

As in young plants (Figure 1a), the amount of NRA+NO_3 in the lamina and petiole of the seventh leaf from mature plants was not affected by application of nitrogen (Figure 2a). Again, as in young plants, activity in the lamina was substantially greater than in the petiole. However, regardless of the nitrogen applied, the level of NRA+NO_3 was lower in mature plants than in young plants. The amount of NRA−NO_3 in the lamina and petiole of the seventh leaf (Figure 2b) did not change when 0 to 150 kg of nitrogen was applied per hectare. However, activity in the petiole increased significantly from 0.02 to 0.12 µmol NO_2/g fwt/hr, when fertilizer nitrogen was increased from 150 to 200 kg N/ha. Activity with rates of 250 kg N/ha was also higher than at 0 to 150 kg N/ha. The amount of NRA−NO_3 in the lamina of the seventh leaf increased significantly from 0.05 to 0.23 µmol NO_2 / g fwt/hr with an increase in applied nitrogen from 150 to 200 kg N/ha (Figure 2b) but did not change significantly as the amount of nitrogen applied increased from 200 to 250 kgNha. The ratio between NRA−NO_3:NRA+NO_3 in the leaf lamina was not significantly affected by rates of nitrogen less than 150 kg N/ha. However, the ratio did increase significantly from 0.11 to 0.33 as applied nitrogen was increased from 150 to 200 kg N/ha. Further increases to 250 kg N/ha did not cause any futher change in the ratio (Figure 2c).

Nitrate concentration in the leaf lamina, as determined by nitrate test strips did not change significantly with rates of applied nitrogen (Figure 2d). However, nitrate concentration of the petioles did increase significantly from 2.0 ppm NO_3−N as applied nitrogen increased from 0 to 200 kg N/ha. No further change in nitrate concentrations of the petiole occurred as the rate of nitrogen applied was increased to 250 kg N/ha. Nitrate levels in the tissues, measured by chemical analysis in the laboratory, were similar to those obtained using nitrate test strips (Figure 2d).

DISCUSSION

In young plants, NRA+NO_3 was higher in the lamina of the seventh leaf than in the petiole of that leaf, the stem or the root. In mature plants, NRA+NO_3 was also higher in the leaf lamina than the petiole, indicating that the leaf lamina is capable of reducing more nitrate than other tissues. Similar results for a range of potato cultivars (Kapoor and Li, 1982) suggest that this is a general feature of potato plants.

Since the amount of NRA+NO_3 and NRA−NO_3 in root, stem and petiole tissue is so low, and NRA−NO_3 of the leaf lamina showed the greatest response to nitrogen application irrespective of age, the leaf lamina appears to be the only tissue worth considering useful for NRA determination of plant nitrogen status. In the present study, the amount of NRA+NO_3 of all tisues measured in young and mature plants was not affected by applied nitrogen (Figures 1a, 2a). Davies *et al.* (1987) however, found that the amount of NRA+NO_3 in the youngest fully expanded leaf was greater in plants receiving 240 kg N/ha than in plants receiving no nitrogen fertilizer. However, even in their study there was little change in NRA+NO_3 when nitrogen was applied during the growing season so the usefulness of NRA+NO_3 as an indicator of plant nitrogen status appears limited.

In contrast to NRA+NO_3 the amount of NRA−NO_3 of the lamina of the youngest mature leaf did not reflect changes in the amount of nitrogen supplied to the plant. As NRA−NO_3 (Figures 1b, 2b) increased with nitrogen application while NRA+NO_3 (Figures 1a, 2a) did not change, then the ratio of NRA−NO_3:NRA+NO_3 (Figures 1c, 2c) also increased with higher rates of applied nitrogen. It appears that both NRA+NO_3 and the ratio between NRA−NO_3:NRA+NO_3 of the youngest mature leaf could be used as indicators of the nitrogen status of the potato plant.

Nitrate concentration in the petiole was also affected by the level of applied nitrogen (Figures 1d, 2d) and was always higher in the petiole than in the leaf lamina. This is in contrast to NRA+NO_3 at all rates of nitrogen and NRA−NO_3 at high rates of applied nitrogen. This suggests that nitrate is assimilated by the leaf, but stored in the petioles and may explain why many workers (Carolus and Woltz, 1944; Tyler *et al.*, 1961; Geraldson *et al.*, 1973, and Kleinkopf *et al.*, 1984) have found nitrate concentration in the petiole to be a good indicator of plant nitrogen status and to correlate well with yield.

In New Zealand, recommendations for petiole nitrate concentrations of plants 8-10 weeks old, determined using nitrate test strips, are 1200 - 1600 ppm NO_3−N (Clarke *et al.*, 1985). Similar concentrations were found in young plants given 100 kg N/ha. The nitrate concentration in mature plants measured by chemical analysis were similar to those measured by nitrate test strip and were about 1700 ppm NO_3−N (Figure 2d). These values are approximately ten times lower than the value of 15,000 ppm NO_3−N recommended as a minimum concentration for Russet Burbank potatoes grown in the U.S.A. (Kleinkop *et al.*, 1984). However, this value is related to dry weight and if the plant contained ten percent dry matter recommendations in NZ and the

USA would be similar.

The data suggest that $NRA-NO_3$, the ratio between $NRA-NO_3$:$NRA+NO_3$ and concentration of nitrate in the petiole can be used as indicators of the nitrogen status of potatoes. However correlations between the various methods of measuring nitrogen status and growth of the crop are needed before final conclusions can be drawn.

CONCLUSIONS

The amount of $NRA+NO_3$ in the plant is not affected by application of fertilizer nitrogen and appears to be of little value in determining the nitrogen status of a plant. The nitrate concentration of the petiole is measured quickly and is responsive to changes induced by nitrogen fertilizer. The amount of $NRA-NO_3$ and the ratio between $NRA-NO_3$:$NRA+NO_3$ of the youngest mature leaf also alter when nitrogen is applied to the plant and it is possible that one or all of these measurements could be used to predict the nitrogen status of the plant. To confirm this, correlations with growth must be made.

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