

Diurnal variations in nitrate content of red beets

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Abstract. The diurnal variations in nitrate content of red beet (*Beta vulgaris* L. var. *conditiva*) roots and leaves were observed in cultivations with either no nitrogen fertilizer or with Ca(NO₃)₂ addition. With less nitrate in the soil the diurnal reduction of nitrate content in the afternoon was greater; on the average 55 % in the leaves and 20 % in the roots of the maximum values in the middle of August. With nitrogen fertilizing (100 kg of nitrogen/ha) the corresponding values were about 15 to 20 %. The amplitude of alteration decreased toward the end of the growing season, as the nitrate content in the leaves in particular was reduced. In our experimental conditions nitrapyrin, a nitrification inhibitor, had no effect on the nitrate content in roots or leaves.

Introduction

In recent years, special interest has been directed to the cultivation conditions of nitrate accumulating plants. The incentive has been the renewed legislation and recommendations issued in many countries. The nitrate content of red beets can be affected with fertilizers, irrigation and nitrification inhibitors.

Nitrogen fertilizers increase the content of total nitrogen (SHANNON et al. 1967, CANTLIFFE 1973, PECK et al. 1974), amino acids (LEE 1973), protein (SPLITTSTOESSER and VANDERMARK 1974), and nitrate (LEE et al. 1971, CANTLIFFE 1972, PECK et al. 1974; KALLIO et al. 1980) in beets. Nitrate in the plant is in

dynamic anabolic circulation. Rapid changes in the nitrate balance in beets are controlled through nitrate reductase (E.C. 1.6.6.1.). Activity of the enzyme depends on the type and age of tissue; it is the highest in young leaves (PECK et al. 1974). Conversely, in root the low activity increases toward maturation (PECK et al. 1974). Increasing intensity of light activates the reduction of nitrate in beets (CANTLIFFE 1972, 1973, MINOTTI and STANKEY 1973).

Loss of nitrate-nitrogen in soil has been estimated to be usually very high, 20 to 80 % (GORING 1964, PRASAD et al. 1971), depending markedly, however, on the type and history of the soil (JAAKKOLA 1982, 1984). Con-

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version of different nitrogen sources to nitrate in soil and at the same time acceleration of the loss of nitrogen fertilizers has been retarded by using nitrification inhibitors. The most common and effective is nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) (GORING 1962 a, b). It inhibits nitrification of ammonium nitrogen and nitrification of fertilizers producing ammonium nitrogen in soil (GORING 1962 a, TURNER et al. 1962, BUNDY and BREMNER 1974).

Nitrapyrin has been seen to reduce nitrate content in radish (MILLS et al. 1976), spinach (BENGTSSON 1968, von KICK and MASSEN 1973, von SIEGEL and VOGT 1975, MILLS et al. 1976), and red beet roots (KALLIO et al. 1980). In the case of red beet the reduction has been observed while using urea as the sole nitrogen source (KALLIO et al. 1980).

This paper is a report on the diurnal variation in the nitrate content of red beet roots and leaves in field conditions. Beets fertilized with $\text{Ca}(\text{NO}_3)_2$ on the one hand and with no nitrogen fertilizer on the other hand were investigated in the middle of August and at the end of September. Effect of nitrapyrin is also reported.

Material and methods

The object of the study was the globe red beet, *Beta vulgaris* L. var. *conditiva* of a commercial variety Rubia Sv. The field experiment was carried out at the Sahalahti Experimental Farm of Saarioinen Ltd. (61°28'N, 24°20'E) during the 1978 growing season. As a basal dressing to the analysed medium heavy sandy silt (6–12 % OM), potassium chloride, superphosphate, ammonium molybdate, boron and manganese sulphate (330, 1000, 0.5, 10, and 10 kg ha⁻¹, respectively) were distributed and incorporated into the soil.

The number of treatments was four, with four replicates each. Area of a plot was 30 m² (randomized plot design). The trial units were: (1) = no nitrogen fertilizer nor nitrapyrin; (2) = no nitrogen fertilizer with

nitrapyrin (11 kg ha⁻¹) with limestone as carrier; (3) = nitrogen 100 kg ha⁻¹ in form of $\text{Ca}(\text{NO}_3)_2$ (14.1 % of $\text{NO}_3\text{-N}$, 1.4 % of $\text{NH}_4\text{-N}$) without nitrapyrin; and (4) = same as (3) supplemented with nitrapyrin (8 kg ha⁻¹). The fertilizers were incorporated into the soil at a depth of about 13 cm in rows 17.5 cm apart. The seeds were planted on June 6th in rows (63 seeds m⁻¹) in the middle of the fertilizer rows. Normal farming practice was carried out.

Beets in all the plots were studied four times, twice in the latter half of August and twice at the end of September or in the beginning of October. The replicates of each trial unit were always analyzed parallelly. Five medium-sized plants were taken for a sample from each plot every three hours from 0500 to 1700 hours.

To observe the diurnal variation in individual roots twenty red beets were marked in two of the replicates of all four trial units. A 3-mm diameter sample was bored through each marked beet, avoiding moving of the plant. All twenty wands of one replicate were pooled for the analysis. These analyses were carried out in September to October.

For the nitrate analyses the finely ground samples (5 g) were shaken for 15 min with boiling distilled water, and the nitrate in the extract was determined potentiometrically using a nitrate selective electrode (Orion Ltd, Helsinki, Finland). Each analysis was made in duplicate.

Results and discussion

The variation of nitrate content in beets in August is seen in Figure 1. Both the uptake and reduction of nitrate are induced by light (CANTLIFFE 1972, 1973, MINOTTI and STANKEY 1973). The induction of uptake was fastest and the net accumulation was the highest in the morning. This indicates that the nitrate permease system and the nitrate reductase can be separate enzymes. According to Figure 1 it is possible that the metabolizable nitrate may act as inductor. The delay between the

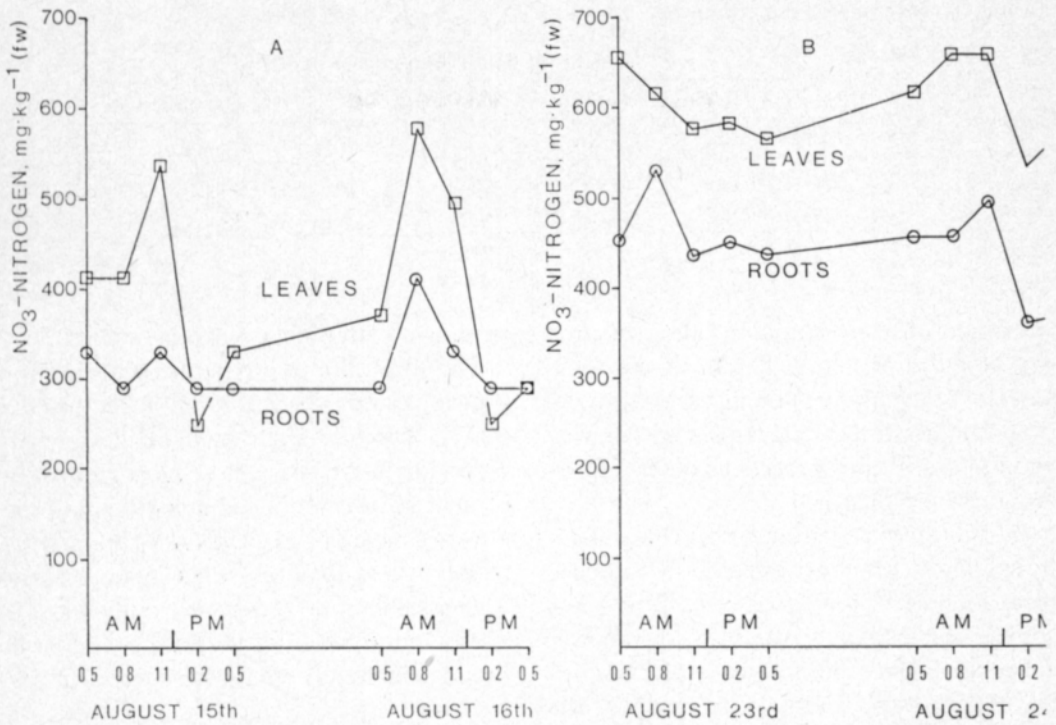


Fig. 1. Diurnal variations of nitrate content in red beet leaves and roots. (A) with no nitrogen fertilizers, (B) with Ca(NO₃)₂ fertilizer (100 kg of nitrogen/ha). Each curve is a mean of eight replicates.

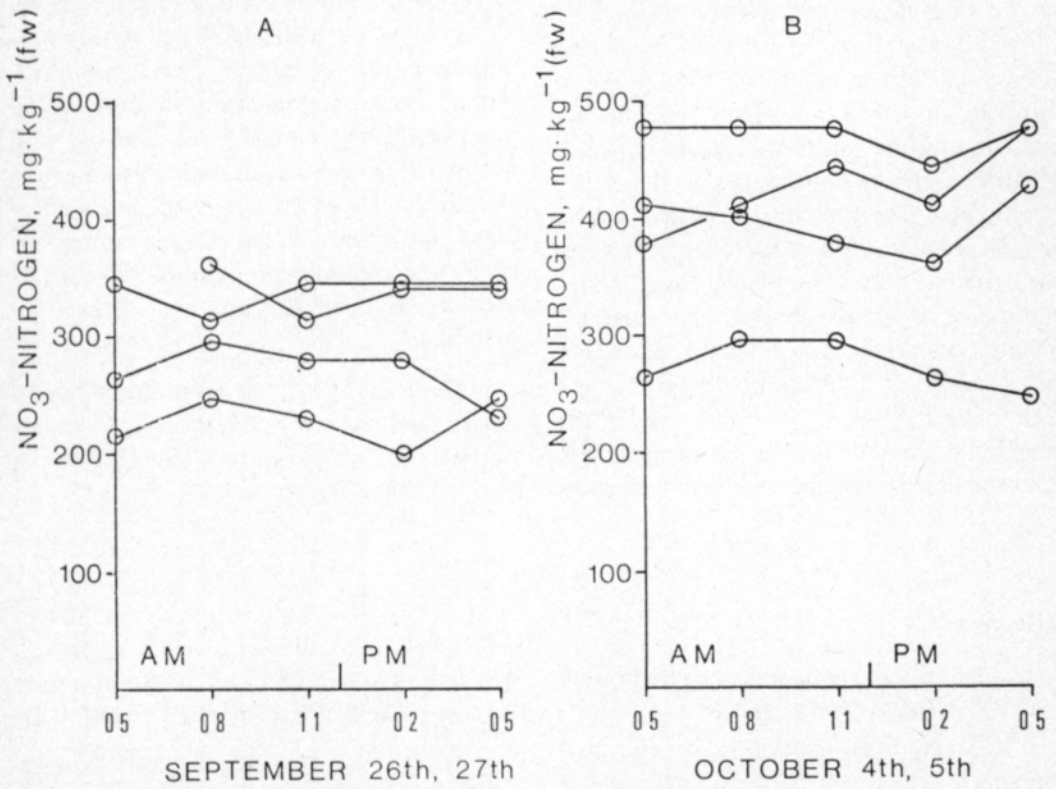


Fig. 2. Diurnal variation in nitrate content of red beet roots analyzed by biopsies in late fall. (A) with no nitrogen fertilizer, (B) with Ca(NO₃)₂ fertilizer (100 kg of nitrogen/ha). Each curve represents one replicate.

Table 1. Content of nitrate in red beets.

	Content of nitrate, NO ₃ -N mg · kg ⁻¹ , f.w. (n)			
	No nitrogen fertilizer		Ca(NO ₃) ₂ fertilizer	
	Aug 15 to 16	Sep 26 to 27	Aug 23 to 24	Oct 4 to 5
Leaves	400 ± 230 (40)	270 ± 140 (24)	610 ± 180 (40)	460 ± 160 (23)
Roots	320 ± 140 (40)	300 ± 80 (49)	440 ± 160 (40)	420 ± 120 (40)

activation of assimilation and the reduction was about three hours. The BUTZ and JACKSON (1977) membrane-bound tetrameric enzyme model would not explain the diurnal variation of nitrate in beets or other nitrate-accumulating plants.

In the leaves the diurnal reduction of nitrate content often exceeded 50 % in about three hours in the afternoon. The roots showed an analogous pattern, but with lower amplitude. Thus the nitrate, stored both in leaves and roots, is rapidly liberated for the protein and other syntheses in the afternoon. The effect was clearer when the beets were grown in deficiency of nitrogen (Fig. 1 A) rather than with ample Ca(NO₃)₂ fertilizing (Fig. 1 B). In the latter case the uptake of nitrogen from soil was easier, and the assimilated nitrate was more rapidly replaced. Hence the growth limiting factor, deficiency of available nitrogen, was avoided. Later in fall (September 26th to October 5th) when the beets had almost stopped growing the assimilation of nitrate slowed down and the nitrate peaks such as in Figure 1. could not be observed. Only a minor depression was seen in the afternoon. Non-uniformity of the trial units caused greater deviation in the fall than the diurnal changes in single beet roots

as shown with biopsy techniques (Fig. 2).

The total nitrate was reduced during the growing season, especially in the leaves (Table 1). The very high standard deviations, especially in August, of the nitrate nitrogen content are due to the unhomogeneity of the growing medium, plant density, the genetic variation and the cyclic photoperiodical nitrate assimilation. These also explain the difficulty in prediction of the nitrate content in commercial beets even in well controlled cultivations.

It was no surprise that nitrapyrin had no effect on the nitrate content of the red beet leaves or roots with Ca(NO₃)₂ fertilizer or with no nitrogen fertilizing. With urea fertilizer a clear reduction of nitrate both in soil and beets has previously been observed with nitrapyrin (KALLIO et al. 1980). Most effective (as much as 50 %) is the nitrapyrin-based reduction of the nitrate content in beets when ammonium nitrate nitrogen is used as fertilizer (KALLIO and TIKANMÄKI, unpubl.).

In this experiment, nitrapyrin could have a minor positive effect on the crop without nitrogen fertilizing (growth from 27 000 kg ha⁻¹ to 33 000 kg ha⁻¹).

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SELOSTUS

Punajuuren nitraattipitoisuuden vuorokausivaihtelu

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Työssä tutkittiin punajuuren (*Beta vulgaris* L. var. *conditiva*) lehtien ja juuren nitraattipitoisuuden muutoksia vuorokauden eri aikoina. Viljely suoritettiin tyypillisällä (100 kg salpitarina) tai ilman. Alhaisella tyypitasolla elokuun puolivälissä nitraatin vähenemä oli voimakkainta iltapäivällä; lehdissä keskimäärin 55 % ja

juuressa 20 %. Tyypilannoitetta käytettäessä vastaavat vähenemät olivat pienemmät. Muutosten vuorokautinen amplitudi aleni kasvukauden loppua kohti, jolloin kasvien kokonaisnitraattisisältökin aleni. Juurinäytteen otossa käytettiin biopsiatekniikkaa.