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Sugar beet response to N fertilization as assessed by late season chlorophyll and leaf area index measurements in a semi-arid environment

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Abstract

In a 3-year experiment (2002-2004), five N rates (0, 60, 120, 180, 240 kg N ha^{-1}) were applied to sugar beets arranged in a Randomized Complete Block design with six replications. Experimentation took place under the semi-arid, irrigated conditions of central Greece. The aim of this work was to study the late season response of sugar beet crop to N fertilization as assessed by non-destructive measurements of chlorophyll (SPAD) and Leaf Area Index (LAI). Physiological measurements were conducted from early August to mid-September, every two weeks. Sugar yield response to N fertilization was evident only in 2004. The highest root and sugar yields were found in 2002 as a result of the unusually high, for Mediterranean conditions, rainfall during summer. The highest petiole NO_3-N concentrations and the lowest sucrose content in root fresh weight were also recorded in 2002. In 2003, sugar beets were water stressed at late August and thus LAI was minimum. Thus, the lowest root and sugar yields were found in 2003. In 2004, SPAD and LAI values were related with root and sugar yields. SPAD readings of ca 38.00 were the optimal for maximum yield. Optimal LAI values for root and sugar yields were 3.99 and 3.88, respectively. SPAD was, also, related with petiole NO_3-N concentration and α-amino N in roots. A healthy and green canopy at the late stages of the growing season can contribute to high sugar beet yield. Optimal N rate for maximum root and sugar yield was close to or higher than 200 kg ha^{-1} N. Chlorophyll (SPAD) and LAI determination by instruments could be a sensitive tool in revealing sugar beet response to N fertilization and non-destructively assessing sugar beet N nutrition status.

***Keywords*:** Drought; Photosynthesis; Plant nutrition; SPAD; Sugar yield

Introduction

Sugar beet productivity worldwide is heavily affected by the interaction of weather variability with nutrient availability (Freckleton et al., 1999; Märländer et al., 2003). Thus, optimizing nutrient and especially N input, which is the most limiting nutrient, is a provoking goal for sugar beet crop (de Koeijer et al., 2003). This effort is more challenging under Mediterranean conditions because supplemental irrigation complicates the interaction.

Nitrogen affects both root and sugar yields (Draycott and Christenson, 2003). Adequate N supply supports a Leaf Area Index (LAI) of 3 or more maximizing sun-light interception, sucrose synthesis in leaves and sugar accumulation in roots. Excessive N affects negatively the partitioning of photosynthetic product increasing the proportion of petiole and leaf mass (Scott and Jaggard, 1993; Draycott and Christenson, 2003). Except from a dense canopy, effective radiation turn over into sucrose requires N investment in photo-assimilatory machinery and thus in chlorophyll, the main compound participating in photosynthesis. Nitrogen availability affects the total amount of sucrose produced by sugar beets and it has detrimental effects on white sugar recovering, during industrial processing, *via* the increase of non-sugar molassigenic factors (K, Na, α-amino N) in roots (Harvey and Dutton, 1993).

Recently, there is a trend to substitute laborious and time-consuming physiological measurements with instrumental, non-destructive methods. SPAD-502 has been developed as a non-destructive leaf chlorophyll meter and close relationships between SPAD readings and destructively determined leaf chlorophyll concentration in many species were established (Yamamoto et al., 2002; Wang et al., 2004). Since chlorophyll is a nitrogenous compound, close relationships between SPAD readings and leaf N concentration were found (Duru, 2002; Wang et al., 2004) and thus SPAD is used as crop N indicator (Singh et al., 2002; Wiesler et al., 2002) or yield predictor (Ramesh et al., 2002; Singh et al., 2002). Non-destructive methods have, also, been evolved for assessing canopy characteristics such as the percentage of crop cover and LAI. In sugar beet, Röver and Koch (1995) found strong relationship between destructive and instrumental LAI determinations.

Although much work has been conducted to understand physiology at the early stages of sugar beet, crop responses at the later stages have not been studied in depth. Maintenance of a healthy and green canopy during autumn could contribute significantly to harvested yield (Ober et al., 2003). Thus, instrumental measurements of physiological traits like chlorophyll and LAI during the late stages could provide useful information about sugar beet physiology and yield.

The aim of this work was to study the effect of N fertilization on late season, non-destructively determined chlorophyll content and LAI and to relate these traits with sugar beet N nutrition and yield.

Materials and methods

Site and experimental set up

A 3-year (2002-2004) experiment was conducted in Nikaia, Thessaly Plain, central Greece (39º 33΄ N, 22º 27΄ E, 98m asl). Five N rates (N_0=control, N_{60}= 60, N_{120}= 120, N_{180}= 180 and N_{240}= 240 kg N ha^{-1}) were applied in a Randomized Complete Block design with six replications. Seeds of cv Rizor (SESVANDERHAVE NV/SA, Tienen, Belgium) were mechanically sown in eight rows (8 m long) per plot, at 50 cm apart and at 15 cm spacing in the row. The 2:3 of N were applied as basal (ammonium sulphate) and the rest as top-dressing (ammonium nitrate) given during the first half of May. Before sowing, 200 kg P_2O_5 ha^{-1} (hyper-phosphate) and 320 kg K_2O ha^{-1} (potassium sulphate) were incorporated into soil. Table 1 presents agronomic information and Table 2 some soil characteristics determined in soil samples taken from the center of control plots before fertilizer application. The climate of the region is typical Mediterranean with July and August being the hottest months (25ºC and 26.5ºC mean monthly temperature, respectively) and with negative water budget (precipitation + irrigation – evapotranspiration) during growing season. Water budget was more deficit in 2003 (-54 mm), followed by 2004 (-38 mm) and 2002 (-22.5 mm). Supplemental irrigation was provided according to the recommendations of Agronomic Service of Larissa factory and the availability of irrigation water. Full protection against cercospora, powdery mildew, insects and weeds were taken by sprayings. On early July, 12 petioles per plot were randomly collected, oven-dried (at 75^{o}C for 48 h) and analyzed for NO_3-N (Ulrich, 1950). Chlorophyll content and LAI were measured four times beginning at early August and every 15 days. Leaf chlorophyll content was assessed by SPAD-502 (Minolta Co Ltd, Osaka, Japan) on ten full-expanded, intact and full sun-lit leaves per plot. LAI was determined using SunScan Canopy Analysis System (Delta-T Devices Ltd, Cambridge, UK). Two measurements were taken between the 4th and 5th rows in each plot.

The three internal rows (3rd, 4th, 5th) were harvested by hand in a length of 7 m (10.5 m^2) per plot. Sugar beets were topped by hand, root number was counted and root and top weights were measured. A randomly selected root sample (25-30 roots), from each plot, was transferred to factory's tare house for qualitative determinations (% sucrose content in fresh root, K, Na, α-amino N content). Root quality was determined using Venema automatic beet laboratory system (Venema automation b.v., Groningen, Holland) connected with a BETALYSER® analyzing system (Dr Wolfgang Kernchen GmbH, Seelze, Germany). Moisture content of roots and tops was estimated after drying a sample of ~200 g at 75^{o}C till constant weight. Harvest Index was calculated as the percentage of root dry weight to the total dry mass (roots + tops). On harvest date, soil profile (0-30 cm and 30-60 cm) samples were taken from the center of each plot using a cylindrical probe and soil NO_3-N was determined by a colorimetric method (Bremner, 1965).

Data analysis

Soil data before experiment establishment were subjected to one factor (depths) Analysis of Variance (ANOVA) combined over years. Data of CEC were subjected to one factor (years) ANOVA. Productive traits (root number, root and sugar yields, Harvest Index, sucrose content in fresh or dry weight, α-amino N) and NO_3-N concentration in petioles were analyzed as one factor (N rates) Randomized Complete Block design combined over years. SPAD and LAI were analyzed as a two factor (N rates and dates) Randomized Complete Block design with split plot combined over years. NO_3-N in soil at harvest was analyzed as a two factor (N rates and depths) Randomized Complete Block design with split plot combined over years. Means were compared by LSD test using M-STAT statistical package (MSTAT-C, version 1.41, Crop and Soil Sciences Department, Michigan State University, USA).

Table 1. Agronomic information of the experiments.

Year	Previous crop	Sowing date	Harvest date*	Irrigation number	Irrigation Water (mm)
2002	Cotton	19 March	3 October (198)	5	214
2003	Wheat	18 March	1 October (196)	6	231
2004	Cotton	23 March	24 September (184)	6	260

In parenthesis is given Days After Sowing (DAS)

Table 2. Soil characteristics, at two depths (0-30 cm and 30-60 cm), of the experimental sites. In the same column, means labeled with the same letter did not differ significantly at P<0.05.

	Soil analysis									Exchangeable cations		
	Sand	Silt ($g\,kg^{-1}$)	Clay	pH (1:1)	$CaCO_3$	Org. matter ($g\,kg^{-1}$)	Total N	NO_3-N	P-Olsen ($mg\,kg^{-1}$)	K	Na ($mg\,kg^{-1}$)	CEC ($cmol\,kg^{-1}$)
Depth												
							2002					
0-30 cm	288 a	175 d	537 a	8.12 b	9.3 c	12.3 c	1.19 c	6.8 b	15.8 a	320 b	152 b	41.9 a
30-60 cm	267 a	197 c	537 a	8.07 c	14.3 c	11.9 c	1.13 c	6.8 b	14.8 ab	306b	156 b	
							2003					
0-30 cm	270 a	273 a	457 c	8.08 bc	56.2 b	13.8 ab	1.46 a	9.7 a	5.7 c	307 b	31 c	32.4 b
30-60 cm	283 a	255 a	462 c	8.17 a	101.5 a	8.6 d	1.00 d	6.7 b	1.9 d	253 c	39c	
							2004					
0-30 cm	268 a	217 b	515 b	8.20 a	12.0 c	14.6 a	1.33 b	8.3 ab	14.6 ab	365 a	272 a	40.9 a
30-60 cm	275 a	203 bc	522 b	8.20 a	14.5 c	13.0 bc	1.21 bc	8.0 ab	12.3 b	316 b	283 a	

Results

NO_3-N in petioles and soil at harvest

The highest NO_3-N concentration was measured in 2002 (8223 mg kg^{-1}) and the lowest in 2003 and 2004 (4309 and 2461 mg kg^{-1}, respectively). Higher N rates (N_{120}, N_{180}, N_{240}) increased significantly NO_3-N in petioles (Figure 1). Soil NO_3-N concentration at harvest was higher in the upper layer (0-30 cm). The highest concentration was found in 2003 when an increase of the residual NO_3-N in the soil with increasing N rate was evident. In 2002 and 2004, no significant differences were found between years or N treatments (Figure 1).

SPAD and LAI measurements

Years and dates significantly affected both SPAD and LAI. Nitrogen rates had no significant effect on SPAD but LAI was strongly affected but the simple interactions (years×N rates, years×dates, N rates×dates) were significant for both traits (Figure 2). LAI was highest in 2002 and lowest in 2003 but in both years, no significant effects of N rates were evident. In 2004, a positive reaction of LAI to N fertilization was found since the higher N treatments (N_{120}, N_{180}, N_{240}) had higher LAI values than the lower rates (N_0, N_{60}). In all years, LAI showed a decreasing trend with the progress of season. At early August, LAI did not differ significantly between years. In 2003, a collapse of plant canopy occurred between mid-August and early September when LAI was reduced to half (Figure 2).

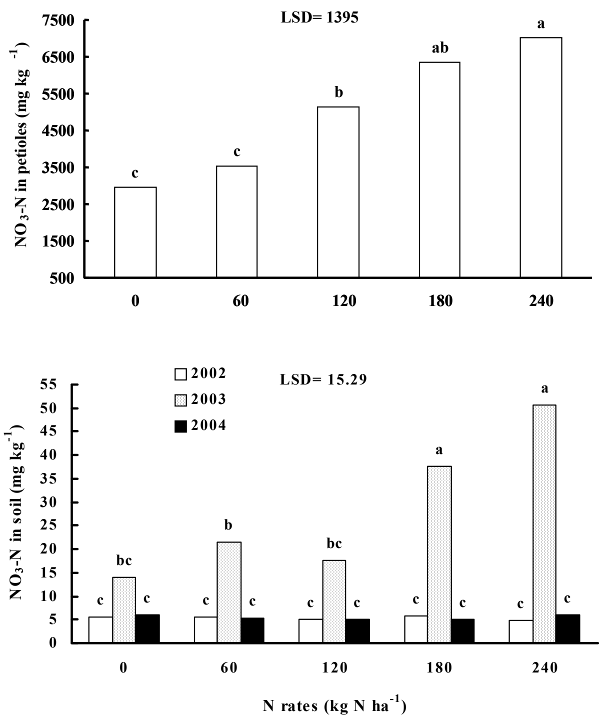

Figure 1. Concentration of NO_3-N in petioles and soil for the five N rates.

The highest SPAD values were found in 2003, the lowest in 2004 while SPAD values in 2002 were moderate. In 2002 and 2003, SPAD was unaffected by N rates but in 2004, the higher N treatments (N_{180}, N_{240}) showed higher SPAD readings. In 2004, SPAD gradually increased toward mid-September (Figure 2).

Productive traits

Fertilization affected significantly sugar yield, sucrose content in dry weight and α-amino N in roots. The years×N rates interaction was significant only for sugar yield. Root number was affected by year being higher in 2002 and 2003 (96 510 and 94 860 plants ha^{-1}, respectively) compared with 2004 (84 950 plants ha^{-1}). Root yield was highest in 2002 (118.4 t ha^{-1}), lowest in 2003 (73.7 t ha^{-1}) and moderate in 2004 (87.4 t ha^{-1}). Sucrose content in fresh weight was significantly affected by years, being highest in 2004 and 2003 (15.9 and 15.5%, respectively) and lowest in 2002 (12.4%). The highest sugar yield was found in 2002 and 2004 (14.7 t ha^{-1} and 13.8 t ha^{-1}, respectively) and the lowest in 2003 (11.4 t ha^{-1}). When data combined over years, higher N rates had a positive effect on sugar yield (N_{120}, N_{180}, N_{240}) compared with the lower ones (N_0, N_{60}). Also, significant effects of N rates on sugar yield were evident in 2004 (Table 3).

Sucrose content in dry weight did not differ between years although sucrose concentration in 2002 (68.1%) was 2% lower than that found in 2003 and 2004 (70.2 and 70.0%, respectively). Significantly higher sucrose content in dry weight was found in N_{60} and N_{120} (72.3% and 69.9%, respectively). Harvest Index was highest in 2003 (82.35 %), lowest in 2002 (79.56 %) and moderate in 2004 (80.63 %) but it was not affected by N rates. Harmful N in roots increased by the highest N doses (N_{180}, N_{240}). The lowest α-amino N was measured in 2004 (199.1 mg N 100 g^{-1} sugar) and the highest in 2002 and 2003 (408.7 and 373.2 mg N 100 g^{-1} sugar, respectively).

Relationships between yield and fertilization or physiological traits

Figure 3 presents the relationships between root and sugar yields with N rates for data combined over years and in 2004. From the quadratic functions best fitted to data, the optimum N doses were calculated. For both combined and 2004 data, root yield was maximum at higher N rates (217.25 and 227.88 kg N ha^{-1}, respectively) compared with sugar yield (171.25 and 218.57 kg N ha^{-1}, respectively).

We also plotted root and sugar yields against LAI and SPAD values in order to determine the optimum of physiological traits maximizing yields in 2004 (Figure 4). Optimum LAI for maximum root yield was calculated at 3.99 and for sugar yield at 3.88. Optimum SPAD were 38.30 and 38.26, respectively. Figure 5 presents the relationship between SPAD and NO_3-N concentration in petioles or α-amino N in roots during 2004. Quadratic functions revealed that NO_3-N in petioles was maximized at 38.56 SPAD reading and α-amino N at 39.21 SPAD. No significant relationship between SPAD or LAI values and yields, NO_3-N in petioles or α-amino N in roots was found in 2002 and 2003 data.

Table 3. Comparison of means for years, N rates and their interaction of the traits (root and sugar yield, sucrose content in fresh weight, sucrose content in dry weight, Harvest Index) determined. Means labeled with the same letter did not differ significantly at P<0.05.

N rate (kg ha^{-1})	Root yield (t ha^{-1})				Sugar yield (t ha^{-1})				Harvest Index (%)			
	2002	2003	2004		2002	2003	2004		2002	2003	2004	
0	113.7a	71.9c	75.5c	87.0b	14.2a	11.1bc	11.9 bc	12.4 b	80.3b-f	82.6ab	82.2 a-c	81.7 a
60	118.9a	75.7c	79.0c	91.2ab	14.8a	12.0bc	12.4 b	13.1 ab	79.0d-f	82.1a-c	81.3 a-e	80.8 a
120	117.8a	77.8c	92.3b	96.0a	14.9a	11.8bc	14.7 a	13.8 a	78.4f	83.5a	80.9 b-e	80.9 a
180	120.3a	68.5c	95.7b	94.8a	14.6a	10.6c	15.2 a	13.5 a	80.0c-f	82.1a-c	78.9 ef	80.3 a
240	121.6a	74.6c	94.7b	97.0a	14.8a	11.5bc	14.9 a	13.8. a	80.0c-f	81.4a-d	79.9 c-f	80.5 a
	118.4a	73.7c	87.4b		14.7a	11.4b	13.8 a		79.6b	82.4 a	80.6 ab	

N rate (kg ha^{-1})	Sucrose content in fresh weight (%)				Sucrose content in dry weight (%)			
	2002	2003	2004		2002	2003	2004	
0	12.5b	15.4a	15.8a	14.6a	66.5cd	69.6a-d	68.1 b-d	68.1 b
60	12.5b	15.8a	15.7a	14.7a	71.4ab	73.3a	72.1 ab	72.3 a
120	12.7b	15.2a	16.0a	14.6a	70.8a-d	68.4b-d	71.0 a-c	69.9 ab
180	12.2b	15.5a	16.0a	14.5a	66.2d	69.1a-d	69.8 a-d	68.4 b
240	12.3b	15.5a	15.8a	14.5a	66.1d	70.7a-c	69.1 a-d	68.6 b
	12.4b	15.5a	15.9a		68.1a	70.2a	70.0 a	

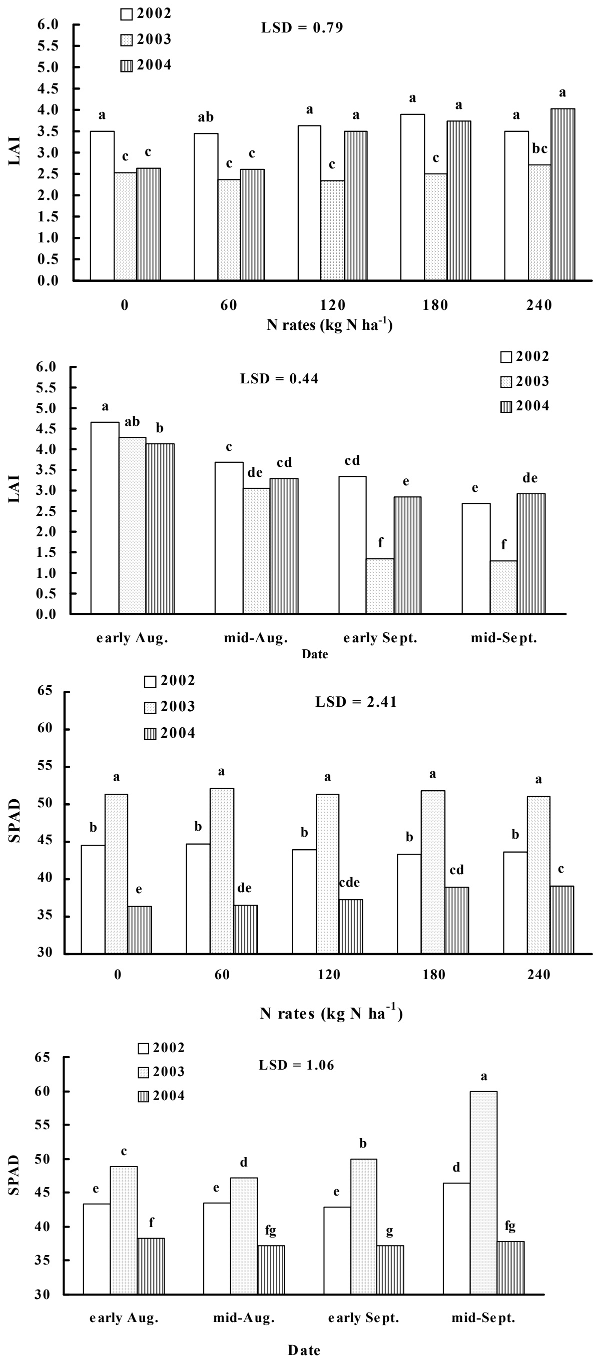

Figure 2. SPAD and LAI values of the N rates and dates.

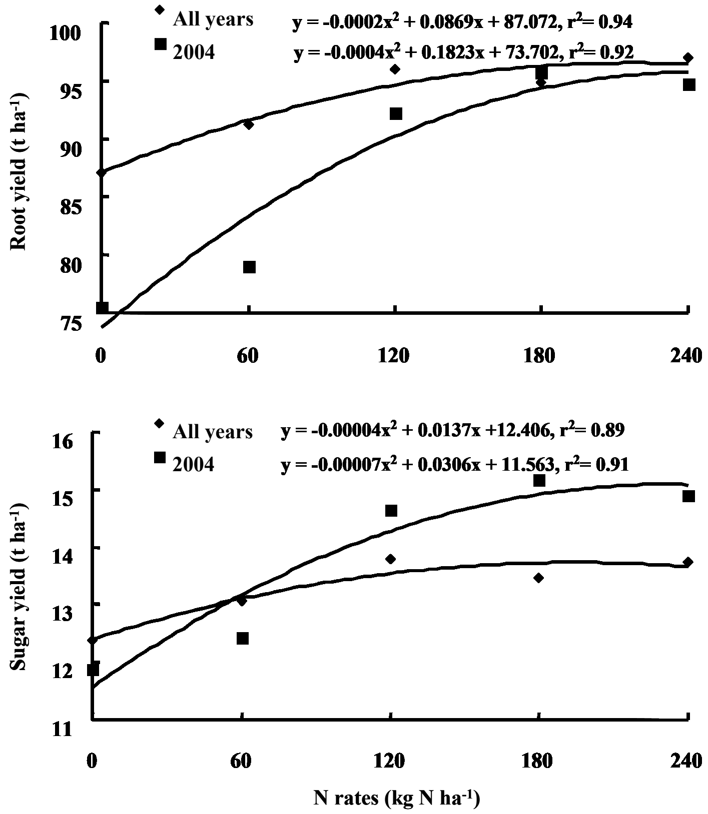

Figure 3. Quadratic functions between root or sugar yields and N rates.

Discussion

Experiments took place on inorganic, clayey soils, typical of Thessaly Plains, central Greece. In those soils, seed-bed aggregates reduced plant emergence in 2004 (Dürr and Aubertot, 2000) but N fertilization or crop sequence had no effect on plant population on contrary to other reports (Draycott and Christenson, 2003; Moyer et al., 2004). However, in all cases, plant number exceeded 75 000 plants ha^{-1}, the lower limit for maximum yield (Scott and Jaggard, 1993).

Since sugar beet response to N is highly site- and year-specific (de Koeijer et al., 2003; Märländer et al., 2003), a positive effect of N fertilization on yield was found only in 2004. Optimal N rate, for maximum root and sugar yields, was close to or exceeded 200 kg N ha^{-1}, in accordance with previous reports in Greece (Tsialtas and Maslaris, 2005).

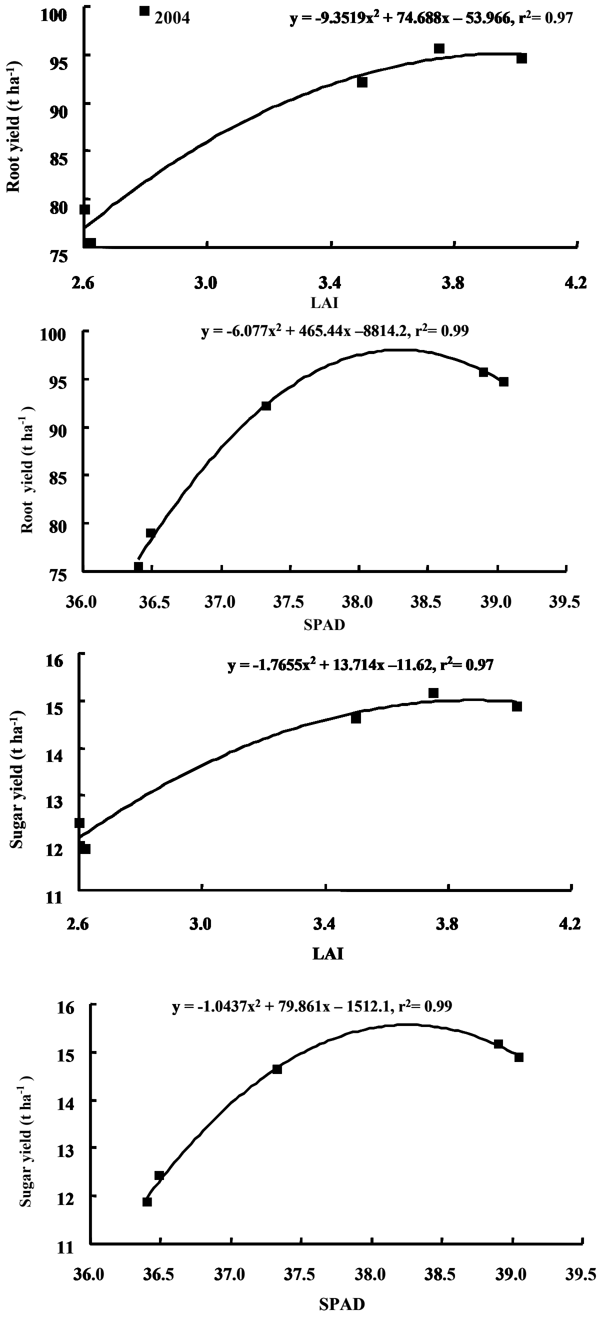

Figure 4. Quadratic functions between root or sugar yields and LAI or SPAD in 2004.

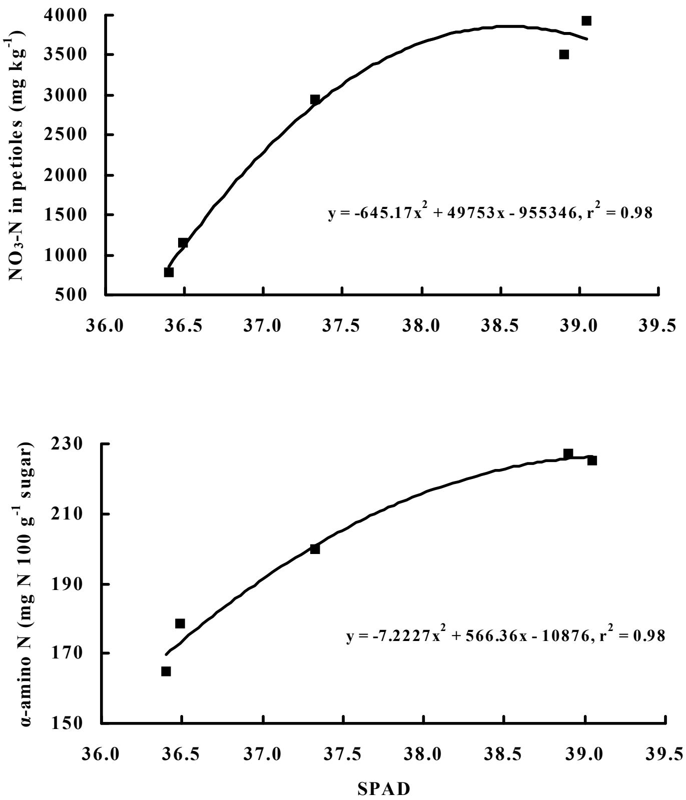

Figure 5. Relationships between LAI or SPAD and root or sugar yields in 2004.

Total N need of sugar beet is ca 250 kg N ha^{-1}, half of which is provided by residual and mineralized N (Märländer et al., 2003). Under Greek conditions, high optimal N rates are probably ascribed to the low levels of soil mineralizable N since the two traits are inversely related (Allison et al., 1996).

The highest root yield harvested in 2002 is the result of the unusual, for Mediterranean conditions, rainfall occurred from July onward. In semi-arid areas, soil N availability depends on water availability (Tsialtas et al., 2001) and thus, in 2002, sugar beets took up more N as it was also indicated by the high α-amino N in roots (Pocock et al., 1990). Nitrogen uptake was translated to higher NO_3-N concentration in petioles and higher foliage and total biomass production (Scott and Jaggard, 1993). In 2002, soil resources (water, nitrogen) availability favored partitioning of photo-assimilates to the aboveground biomass decreasing Harvest Index (Werker et al., 1999). Moreover, even not significant, sucrose content in dry weight was lowest in 2002; an indication of partitioning to aboveground parts. The lowest sucrose content in fresh weight found in 2002 is also ascribed to the high water availability, which increased root moisture content and diluted sucrose (Follett, 1991). In 2003, no response to N was accompanied with moderate NO_3-N concentration in petioles and high partitioning to roots (high HI). These findings could be ascribed to limited exploitation of soil NO_3-N by sugar beets probably due to soil water deficit, which resulted to increased residual NO_3-N. Low levels of residual NO_3-N and no significant differences between N rates were evident in 2002 and 2004, in accordance with Allison et al. (1996). In 2004, a positive response to N fertilization was found for rates higher than 120 kg N ha^{-1} with an optimum at ca 200 kg N ha^{-1}. NO_3-N in petioles estimated successfully the inadequacy of N nutrition since N_0 and N_{60} treatments had NO_3-N concentration lower or close to the critical level of 1000 mg NO_3 kg^{-1} (Ulrich and Hill, 1990). Crop showed a typical response to N by increasing top and root weights and consequently sugar yield without a negative effect of N on sucrose content in fresh weight. Harmful N in roots increased by N fertilization and in all cases, exceeded the acceptable limit (150 mg N 100 g^{-1} sugar) for inorganic soils (Palmer and Casburn, 1985; Tsialtas and Maslaris, 2005).

Recently, substitution of laborious, destructive physiological measurements with non-destructive methods gains interest, especially under field conditions. LAI is an important predictor of crop productivity and is used in growth models (Yin et al., 2000). Critical LAI for maximum light interception by sugar beets ranges between 3.5 and 4.0 (Scott and Jaggard, 1993) and uptake of 103-130 kg N ha^{-1} is necessary in order to be achieved (Malnou et al., 2006). Hoffmann and Blomberg (2004) found no relationship between LAI determined in June or September and yield. In our work, root and sugar yields in 2004, were maximized at LAI values (3.99 and 3.88, respectively) within the optimum range for maximum light absorption. After maximum LAI reached, LAI maintenance over 2.5 is necessary for yield maximization (Milford et al., 1985). This was evident in the high-yielding treatments (N_{120}, N_{180}, N_{240}) in 2004 while in 2003, LAI collapsed between mid-August and early September, a typical indication of crop subjection to water or nutrient stress.

SPAD is a non-destructive assessment of leaf chlorophyll and N content (Duru, 2002; Wiesler et al., 2002). As an indirect chlorophyll meter failed to distinguish high-yielding genotypes (Pulkrábek et al., 2001) but it is a useful indicator of in season sugar beet N demand (Tugnoli and Bettini, 2000; Wiesler et al., 2002). In 2004, root and sugar yields were maximized at SPAD values (38.30 and 38.26, respectively) slightly higher than the threshold value of 38.00 set as the lower limit of N efficiency in sugar beet (Tugnoli and Bettini, 2000). In Greece, sugar beets are left without irrigation for about a month in order sucrose concentration in fresh mass to be increased. In that case, leaf SPAD drops below 38.00 and thus yield is negatively affected. Consequently, higher N rates could maintain "green" foliage for a longer period during irrigation withholding and thus yield losses could be restricted. In 2003, high SPAD values could be ascribed to water stress conditions (García-Valenzuela et al., 2005) but the dark green foliage was not advantageous for CO_2 assimilation and sucrose accumulation in root (Pulkrábek et al., 2001; Draycott and Christenson, 2003). In our work, SPAD was linearly related to NO_3-N in petioles up to 3000 mg NO_3 kg^{-1} and SPAD value of 38.56 was the optimal for maximizing NO_3-N in petioles. Sexton and Carroll (2002) found linear relationship between the two traits up to

10000 mg NO_3 kg^{-1} in petioles and supported that NO_3-N in petioles is a more accurate predictor of sugar beet N nutrition. Finally, sugar beet N uptake, as assessed by α-amino N in roots (Pocock et al., 1990) was maximized at a SPAD reading of 39.21.

Conclusions

Yield response to N fertilization was found only in 2004. Optimal N rate for maximum root and sugar yields approached or exceeded 200 kg N ha^{-1} for 2004 or combined data. In 2004, root and sugar yields were related to SPAD and LAI. Optimal SPAD for maximum yields was ca 38.00 while optimal LAI ranged from 3.88 for sugar yield to 3.99 for root yield. Also, in 2004, significant quadratic functions between SPAD and NO_3-N in petioles or α-amino N in roots were evident.

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