Scientia Horticulturae 123 (2010) 336–342

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'

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ARTICLE INFO

Article history:
Received 9 February 2009
Received in revised form 23 September 2009
Accepted 29 September 2009

Keywords:
Carbohydrate
Nitrogen deficiency
Olive tree
Photosynthesis
Pigments
Photosynthetic nitrogen use efficiency

ABSTRACT

The effects of nitrogen deficiency on CO_2 assimilation, carbohydrate content and biomass were studied in two olive (*Olea europaea* L.) cultivars ('Meski' and 'Koroneiki'). One-year-old plants were grown in pots and subjected to four nitrogen levels for 58 days.

Nitrogen-deficient plants had significant lower leaf nitrogen and chlorophyll *a* contents. They also showed a significant reduction in their photosynthetic capacity. A tolerance difference between cultivars was observed: 'Meski' proved to be more efficient in maintaining CO_2 assimilation rates than 'Koroneiki' under nitrogen deficiency, which was reflected by increased photosynthetic nitrogen use efficiency. Accumulation of carbohydrates, especially starch, mannitol, sucrose and glucose, was observed in nitrogen-deficient leaves. This indicates that both the high carbohydrate and the low nitrogen content inhibit photosynthesis in nitrogen-deprived olive plants. Total biomass was strongly reduced (mainly caused by a decrease in leaf dry weight) under nitrogen deficiency for both cultivars, but root:shoot ratio was hardly affected. Elongation of fine roots was enhanced in 'Koroneiki' under severe nitrogen-deprivation.

1. Introduction

Modern farming requires increasingly fine management of nitrogen (N) fertilisation due to economical and environmental constraints. Moreover, excess fertiliser application is expensive and leads to N losses by leaching with negative impacts on the environment. Fertilisation must, however, be sufficient to provide an optimal final yield and the desired product quality. Thus, the required level of accuracy for fertilisation practices is within 10% (Gastal and Lemaire, 2002).

Olive tree (*Olea europaea* L.) is one of the major crops in the Mediterranean basin with a cultivated area of about 8.2 Mha. Traditionally, fertilisers as well as other crop inputs are applied to olive orchards without considering spatial variability in field characteristics. Such agricultural management might be inefficient due to under-application or over-application of field inputs in specific orchard areas. Under-treated zones will not reach optimum yield levels whereas in over-treated ones there might be a higher risk of environmental pollution and reduced cost efficiency (Bouma, 1997).

N shortage results in a marked decrease in plant photosynthesis in many crops. This is to be expected, because more than half of the total leaf N is allocated to the photosynthetic apparatus (Makino and Osmond, 1991). Photosynthetic capacity and total amount of leaf N per unit leaf area are usually correlated (Field and Mooney, 1986; Sage and Pearcy, 1987; Walcroft et al., 1997).

There is nowadays clear evidence that N deficiency induces sink limitation within the whole plant due to decreased growth (Paul and Foyer, 2001). This leads, in turn, to feedback down-regulation of photosynthesis. N deficiency results in accumulation of carbohydrates (sugars and starch) in the leaves, higher levels of carbon allocated to the roots and an increase in root-to-shoot biomass ratio (Hirai et al., 2004; Scheible et al., 2004; Remans et al., 2006). N deficiency therefore affects, to various extents, primary photosynthesis, sugar metabolism and/or carbohydrate partitioning between source and sink tissues (Paul and Driscoll, 1997; de Groot et al., 2003; Scheible et al., 2004). Although N content in leaves did not correlate with shoot growth, it was negatively correlated with the proportion of carbon allocated to the roots (de Groot et al., 2003; Scheible et al., 2004). Plants indeed constantly sense the changes in their environment and, when mineral elements are scarce, they often allocate a higher proportion of their biomass to the root system (Lawlor et al., 2001). This response is a consequence of metabolic changes

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doi:10.1016/j.scienta.2009.09.023

in the shoot and an adjustment of carbohydrate transport to the roots.

In Tunisia, N deficiency is frequently observed in olive tree orchards, and is responsible for considerable losses of productivity (Braham and Mhiri, 1997). N deficiency is also found to be associated with pistil abortion in olive trees (Morettini, 1950). Nitrogen excess does not increase yield or vegetative growth (Fernández-Escobar et al., 2004), but it negatively affects fruit quality or that of derived products, such as olive oil (Fernández-Escobar et al., 2006). However, in current horticultural practice N is often applied in higher amounts than those needed to ensure a good production (Sánchez et al., 1995).

N fertiliser recommendations for olive trees are traditionally based on soil N status and to a lesser extent on foliar analysis (Boussadia et al., 2006). A rationalization of fertilisation is a prerequisite in the light of the factors up on which productivity depends, namely photosynthesis, translocation of assimilates, growth and production.

To gain a better insight in the optimal use of N fertilisers, one must correctly evaluate the plant's responses to possible deficiencies. This paper therefore aims at evaluating nutritive N deficiency in two olive tree cultivars 'Meski' and 'Koroneiki'. To our knowledge, there is hardly any information available on this functional approach linking olive tree growth with the N status of the leaves. To achieve this goal, repeated measurements were performed in a greenhouse experiment in order to better understand the mechanisms adopted by young olive trees subjected to four levels of N. The effects of N deficiency on chlorophyll concentration, leaf photosynthesis, carbohydrate pools, crop growth and biomass partitioning were quantified in order to assess the differences in response in both cultivars.

2. Materials and methods

2.1. Growth conditions and nitrogen stress treatment

One-year-old olive trees (*Olea* L. 'Meski' and 'Koroneiki') were grown hydroponically in vermiculite (2 l containers) under greenhouse conditions from 22 January till 21 April 2008. Air temperature fluctuated between 20 and 32 °C and the relative humidity of the air ranged between 60 and 70%. Plants were fertigated with a full-strength modified Hoagland's solution (EC = 2.5, pH = 6.5) (Table 1) in a hydroponic system (Hartmann and Brown, 1956). After 34 days of culture, the plants were randomly allocated to four groups and each group consisted of 16 plants (8 plants for each cultivar). Four N levels were provided for 58 days:

100N received a full-strength nutrient solution throughout the experiment (NO_3^- = 23.96 meq l^{-1})
40N reduced N to 40% N (mild nitrogen stress with NO_3^- = 9.58 meq l^{-1})
20N reduced N to 20% N (moderate nitrogen stress with NO_3^- = 4.79 meq l^{-1})
0N reduced N to 0% N (severe nitrogen stress with NO_3^- = 0 meq l^{-1})

Table 1
Modified full-strength Hoagland's nutrient solution (100N) and its adjustments supplied for the N-deprivation treatments (40N, 20N and 0N).

Macronutrients (g/100l)	100N	40N	20N	0N
$Ca(NO_3)_2$	109.6	43.84	21.9	0
KNO_3	27.4	10.96	5.48	0
$MgSO_4$	27.4	27.4	27.4	27.4
NH_4SO_4	13.7	5.48	2.74	0
KH_2PO_4 (MKP)	13.7	13.7	13.7	4.1
EDTA Na Fe	4.1	4.1	4.1	4.1
$CaCl_2{\cdot}2H_2O$	0	48.24	64.34	80.4
KCl	0	11.91	15.88	19.85

All other nutrients were provided as specified by the modified Hoagland's solutions reported in Table 1.

2.2. Gas exchange measurements

Leaf gas exchange measurements were performed weekly on the 8th fully expanded leaf counted from the top of the olive tree using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA), along a period of 58 days (from 24 February till 21 April 2008). Measurements took place between 9 and 17 h and were done in 3 replications. The replications were measured, respectively, at 9, 12 and 15 h for each treatment and each cultivar in order to cover as such the daily variability. The photosynthetic active radiation (PAR) was supplied with a 6400-02B LED light source and photosynthetic light response curves were determined by decreasing light intensity from 2000 to 0 μmol m^{-2} s^{-1} in 9 steps. The temperature inside the leaf cuvette was set to 25 °C, and the CO_2 concentration was set to 450 μmol mol^{-1}.

Photosynthetic parameters were obtained by fitting the light response curve to the experimental data of each individual plant according to Drake and Read (1981):

$$A = \frac{A_{max}(I - I_c)\alpha_c}{A_{max} + (I - I_c)\alpha_c}$$

where A and A_{max} are respectively the net assimilation and the maximum net assimilation rate (μmol CO_2 m^{-2} s^{-1}), α_c the quantum efficiency (μmol CO_2 (μmol PAR)$^{-1}$), I the light intensity and I_c the light compensation point (μmol PAR m^{-2} s^{-1}).

The dark respiration rate R_d (μmol CO_2 m^{-2} s^{-1}) was calculated from the relationship describing the light-limited part of the photosynthesis light response curve, characterized by its linear response to increasing PAR intensities:

$$A = \alpha_c I - R_d$$

For $I = I_c$, A equals zero and consequently R_d equals $\alpha_c I_c$ from which α_c can be derived.

After deriving individual plant parameters, mean photosynthetic parameter values for each N treatment level and each cultivar were calculated and used in the analysis.

2.3. Chlorophyll a concentration

Chlorophyll *a* (Chl *a*) concentration was measured at weekly intervals according to Moran (1982). Leaf discs (7 mm × 19.6 mm/plant) of randomly chosen fully expanded leaves for each treatment and cultivar were extracted with N,N-dimethylformamide (DMF) and absorbance was measured at 664 and 647 nm (UV-VIS, Biotek Uvikon XL). Each measurement was done using seven plants selected from each treatment and per cultivar.

2.4. Leaf nitrogen content

Young fully expanded leaves (one leaf per plant) were randomly harvested from eight plants in each treatment and combined into a composite sample, weighed and analyzed. Leaf tissue nitrogen (N) was determined by the Kjeldahl method (Martin-Prével et al., 1984). Photosynthetic nitrogen use efficiency (PNUE) was calculated as A_{max} per unit of foliar N content.