Leaf nutrient variation in mature carob *Ceratonia siliqua*) trees in response to irrigation and fertilization

P. J. CORREIA\textsuperscript{1} and M. A. MARTINS-LOUÇÃO\textsuperscript{2}

\textsuperscript{1} AIDA, Apartado 302, 8100 Loulé, Portugal
\textsuperscript{2} Departamento de Biologia Vegetal, Faculdade de Ciências de Lisboa, Campo Grande, C2 Piso 4, 1700 Lisboa, Portugal

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**Summary**  Seasonal variations in leaf nitrogen, phosphorus and potassium concentrations were studied in a mature carob (*Ceratonia siliqua* L. cv “Mulata”) orchard subjected to a 4-year irrigation and fertilization experiment. Three irrigation regimes (0, 50 and 100%), based on the evaporation values obtained from a class A pan, were tested in combination with two nitrogen (N) supply regimes in which 21 kg ha\(^{-1}\) year\(^{-1}\) (low-N) and 63 kg ha\(^{-1}\) year\(^{-1}\) (high-N) were supplied as ammonium nitrate. Leaf nitrogen concentration increased throughout the experiment, independently of treatments. There were no significant differences in leaf N concentration between trees in the high-N and low-N treatments. Irrigation regimes had no effect on leaf mineral concentration but influenced the amount of leaves shed and slightly modified the pattern of leaf shedding that occurred during the summer drought period. Nutritional balances between N and P and N and K were both closely and significantly correlated. Potassium was translocated from leaves to fruits during spring, independently of treatments. Severe water stress periods occurring during spring or autumn induced shedding of leaves leading to nutrient mobilization. Nutrient retranslocation during these drought periods may represent an adaptive mechanism. Nitrogen retranslocation was higher for trees in the high-N treatments than for trees in the low-N treatments, whereas phosphorus retranslocation was independent of the irrigation and fertilization treatments.

**Keywords:** absolute shoot growth, leaf fall, nitrogen, N/K ratio, N/P ratio, phosphorus, potassium, retranslocation.

**Introduction**

Among the factors determining plant distribution are soil nutrient availability (Chapin and Kedrowski 1983) and soil water content (e.g., Pitman 1981, Borchert 1994). Chapin and Kedrowski (1983) identified several mechanisms that enable plants to adapt to nutrient stress including: (1) low growth rate; (2) high nutrient absorption capacity; (3) reduced nutrient loss; and (4) alterations in the biochemistry of nutrient use. In contrast, lowered tree water status may substantially enhance nutrient stress as a result of decreased phloem transport of ions (Pitman 1981). Furthermore, a sharp decrease in leaf water potential may lead to premature leaf abscission that, in turn, may result in nutrient stress because leaves are the most important nutrient storage organs in many evergreen species (Chapin and Kedrowski 1983), including carob (Diamantoglou and Kull 1988).

Several studies indicate that plants growing in nutrient-poor soils may increase their nutrient use efficiency (Pugnaire and Chapin 1992, 1993) by increasing retranslocation from senescing tissues. However, Pugnaire and Chapin (1992) also reported several examples showing that resorption efficiency changes with water availability but not with nutrient availability. Other results suggest that the pattern of nutrient response varies among nutrients (Boerner 1985). Nutrient retranslocation may also be influenced by source–sink effects, particularly when soil nutrient availability is low (Helmisaari 1992). It is generally believed that the reproductive structures represent a strong sink, and constitute a substantial energetic cost to overall plant production. The resources available for reproduction are linked to the supply of nutrients, which, in turn, is influenced by soil water availability (Bloom et al. 1985).

Nitrogen is an important factor limiting site productivity in the Mediterranean region (Correia and Martins-Loução 1993). It is known that the evergreen Mediterranean species Ceratonia siliqua L. cv “Mulata” (carob) is completely dependent on soil inorganic N because it lacks symbiotic nitrogen fixation, even though it belongs to the Leguminosae family (Martins-Loução 1985). Several short-term studies indicate that nitrogen fertilization enhances carob growth (Correia and Martins-Loução 1995) and fruit production (Lloveras and Tous 1992, Correia and Martins-Loução 1995). It has also been observed that, under drought conditions, the translocation of nitrogen from leaves to flowers influences carob productivity (Cabrita and Martins-Loução 1990, Correia and Martins-Loução 1993). Although El Gazzar et al. (1981) and Cabrita and Martins-Loução (1990) studied seasonal variations in some leaf nutrients of carob trees, there is no information on the long-term effects of nitrogen fertilization on leaf mineral content or nutrient retranslocation in mature carob trees.

We undertook a study of the effects of nitrogen fertilization on leaf mineral concentrations of carob trees to test four hypotheses. First, leaf mineral nutrition (N, P, K) is enhanced in response to long-term nitrogen application and irrigation.
Second, there is a close relationship between leaf nutrients and vegetative and reproductive growth. Third, nitrogen application and irrigation influence the balance between nitrogen and the other nutrients, inducing nutrient imbalances. Fourth, long-term nitrogen application and irrigation influence nutrient retranslocation and ameliorate leaf shedding during severe droughts that occur outside the normal summer drought season.

Materials and methods

Experimental site

The experimental orchard is located in southern Portugal (37°13′N, 7°28′W). Trees (cv “Mulata”) are 20–30 years old, and were established on a sandy loam soil at a 12 × 12 m spacing (69 trees ha⁻¹). Soil analysis is presented in Table 1 and refers only to the B horizon at 30–40 cm depth. It was not possible to obtain soil cores of deeper layers because of the presence of a schist bedrock. The climate is typically Mediterranean (Mitrakos 1981). Annual rainfall (450–500 mm) is mainly distributed in winter and early spring, and summer drought (approximately 5 months in duration) is associated with maximum air temperatures.

The field experiment consisted of three irrigation regimes (0, 50 and 100%) based on water loss by evaporation (Epan) from a class A pan, according to the formula

\[ I = E_{pan} \times CF \times K_p \]

(Marangoni et al. 1987, Nightingale et al. 1989), where CF is a conversion factor and Kp is the pan coefficient. The conversion factor (CF) (8.4 m²) was calculated as the average projected canopy area of all trees and the pan coefficient (Kp) was assumed to be 0.70 (Doorenbos and Pruitt 1977). The three irrigation regimes were combined with two nitrogen supply regimes (21 (low-N) and 63 kg ha⁻¹ year⁻¹ (high-N)) (Marti and Caravaca 1990, Pulizzi et al. 1993) to provide 0.3 (low-N) and 0.9 kg tree⁻¹ (high-N) of N, corresponding to 1.5 kg tree⁻¹ and 4.5 kg tree⁻¹ of total fertilizer. The fertilizer contained 15% calcium and 20.5% nitrogen with equal amounts of nitrate and ammonium, and was applied once per year. For trees receiving the 0% irrigation regime, fertilizer was applied 2 months before the other treatments to ensure dilution by rainfall. For trees in the 50 and 100% irrigation regimes, fertilizer was added at the beginning of the irrigation period (June).

Table 1. Chemical analysis of a composite soil sample obtained at a depth of 30–40 cm between tree rows at the beginning of the experiment (April 1991).

<table>
<thead>
<tr>
<th>Chemical characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Nitrogen (%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Phosphate (ppm)</td>
<td>0.44</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>92.7</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.6</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>0.9</td>
</tr>
<tr>
<td>C/N</td>
<td>8.4</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Fertilizer was spread over the area under the canopy and was mixed with top soil to avoid ammonia volatilization. Because there was a sharp decrease in leaf K concentration in the winter of 1992, each tree was supplied with 2.8 kg of potassium sulfate (1.4 kg tree⁻¹ year⁻¹) in the second year of the experiment (Cabrita and Martins-Loução 1990). Despite low concentrations of soil phosphorus (Table 1) no phosphorus fertilizer was added, because leaf phosphorus concentration at the beginning of the experiment (May 1991) was similar to previously reported values (Cabrita and Martins-Loução 1990).

Water was applied daily during the morning, between June and August 1991, 1992, 1993 and 1994. Trees were irrigated by means of a micro-sprinkler system, one per tree placed close to the tree trunk, delivering 40 l h⁻¹ with a range of 360°.

Each of the six treatments consisted of three replicates of four trees for a total of twelve trees per treatment distributed in a randomized design.

Phenological analysis

For phenological analysis, nine trees per treatment were selected, and eight branches on each tree were marked on the outside of the canopy. The terminal 100 cm of each branch was labeled and subsequent shoot length increment was followed monthly. Leaf shed and leaf number were also recorded for the same branches during the same period. Shoot growth and the number of developing fruits were recorded on the same branches during April and May of each year.

Leaf mineral concentration

In each treatment, two to three trees were selected for leaf nutrient analysis. Leaf samples, homogeneous in terms of age and canopy position, were randomly collected from among the selected trees in each treatment. A composite sample consisting of 30–40 mature leaves per treatment was prepared every 2 months from May 1991 until December 1994. Leaf nitrogen concentration was determined by the Kjeldahl method using a mixture of potassium sulfate and mercuric oxide as catalyst. Phosphorus was analyzed spectrophotometrically as the phosphorus molibdenovanadate complex and potassium was assayed by atomic absorption spectroscopy (Pye Unicam, Cambridge, U.K.). Element concentrations were expressed on a leaf area basis.

Retranslocation of N and P

Calculations of nutrient retranslocation were based on leaf shedding. Leaves that shed because of their age are sclerophyllous, green with some necrotic zones, and located in the innermost part of the branch. In contrast, leaves that shed in response to water stress are yellow and present no special location in the branch. The appearance of these yellow leaves was associated with the periods of exceptionally low precipitation that occurred in April 1992 and October 1991 and 1993 (Correia and Martins-Loução 1995).

Nutrient retranslocation was studied in leaves shed in response to water stress. Yellow leaves were collected before shedding, in October 1991 and 1993 and in April 1993. Retranslocation (R, %) was calculated according to the formula:

\[ R = \left( \frac{C_{\text{shed}}^*}{C_{\text{new}}^* \times 100} \right) \]

where Cshed is the concentration of the nutrient in the shed leaves and Cnew is the concentration of the nutrient in the new leaves. The asterisk symbolizes the treatment conditions.
where \([\text{Nutrient}]_{\text{max}}\) and \([\text{Nutrient}]_{\text{min}}\) represent, respectively, the maximum (green leaves) and minimum (yellow leaves) leaf N or P concentration (calculated on a leaf area basis) determined for each year.

**Statistical analysis**

Relationships between leaf nutrient concentrations and phenological parameters were examined by linear regression analysis. Differences in nitrogen concentration and retranslocation of N and P between the low-N and high-N treatments were compared by one-way ANOVA. All data analyses were made with the software program Statgraphics Version 5.0 STSC (Jandel Scientific Corp., San Rafael, CA).

**Results**

Leaf nitrogen concentration increased throughout the experimental period independently of the treatments. Irrigation did not influence seasonal variation in leaf nitrogen concentration (Figure 1A). Although leaves in the high-N treatments had slightly higher nitrogen concentrations than leaves in the low-N treatments, the differences were not statistically significant \((P > 0.05)\) (Figure 1A).

Seasonal patterns of leaf nitrogen concentration differed between years, particularly during the spring–summer period. In all years, leaf nitrogen concentration increased during late autumn–winter (October–March). In spring 1992 and 1994, there was a decrease in leaf nitrogen concentration followed by an increase in summer. The reverse situation occurred in 1993, with an accumulation in spring and a decrease in summer.

Leaf phosphorus concentration (Figure 1B) decreased slightly during the experimental period and the pattern was similar for all treatments. Leaf potassium concentrations were less than 2 g m\(^{-2}\) until the last year of the experiment in all of the treatments (Figure 1C). In 1994, there was a general increase in leaf potassium concentration, which was independent of the treatments, corresponding to the spring 1994 application of K fertilizer. In all years, leaf potassium concentration decreased during winter and increased from March–April until September–October.

At the time of vegetative flushing, in April 1992, 1993 and 1994, there was a negative and statistically significant \((P < 0.05)\) relationship between leaf nitrogen concentration and absolute shoot growth increment (Figure 2), indicating that the highest nitrogen concentration in mature leaves was associated with the period of lowest shoot growth increment.

Leaf N/P ratios increased during the experiment (Figure 3) as a result of the simultaneous effects of increasing nitrogen and decreasing P concentrations (Figures 1A and 1C). At the beginning of the experiment, N/P values ranged between 5 and 10 (Figure 3) but reached 30 in leaves in the high-N treatment by the end of the experiment (1994), indicating a change in nutrient balance during the study. Throughout the study, there were clear relationships between the concentrations of nitrogen and phosphorus as well as between the concentrations of nitrogen and potassium. The correlation coefficients were high and statistically significant \((P < 0.01)\), with only a few exceptions (Table 2).

During the April–May period when the fruits attained their maximum weight (Haselberg 1988), the number of fruits was inversely correlated with leaf potassium concentration \((P < 0.05)\) in both the high-N and low-N treatments (Figure 4).

The patterns of age-related leaf shedding (green leaves) are presented in Figures 5A and 5B. Age-related leaf shedding...
occurred mainly during the summer drought period about 14–17 months after leaf emergence, although some leaves were retained until winter (Figure 5). Nitrogen fertilization did not affect the pattern of age-related leaf shedding (Figure 5A), whereas irrigation delayed the onset of age-related leaf shedding and reduced the total number of shed leaves (Figure 5B). Thus, age-related leaf shedding occurred independently of nitrogen availability, but was closely associated with the summer drought.

Table 2: Effects of the irrigation and fertilization treatments on the correlation coefficients ($r$) between nitrogen and phosphorus (N–P) and nitrogen and potassium (N–K) leaf concentrations during the experimental period from June 1991 till December 1994 (** = significant at $P < 0.01$; * = significant at $P < 0.05$; and ns = not significant).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N–P</th>
<th>N–K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-N/0</td>
<td>0.42 ns</td>
<td>0.67**</td>
</tr>
<tr>
<td>Low-N/50</td>
<td>0.52 ns</td>
<td>0.66**</td>
</tr>
<tr>
<td>Low-N/100</td>
<td>0.77**</td>
<td>0.76**</td>
</tr>
<tr>
<td>High-N/0</td>
<td>0.85**</td>
<td>0.59*</td>
</tr>
<tr>
<td>High-N/50</td>
<td>0.87**</td>
<td>0.75**</td>
</tr>
<tr>
<td>High-N/100</td>
<td>0.32 ns</td>
<td>0.30 ns</td>
</tr>
</tbody>
</table>

Discussion

Leaf nitrogen concentration increased throughout the experiment, independently of the fertilization treatment. This finding

Retranslocation percentages for nitrogen and phosphorus from leaves shed in response to water stress occurring outside the summer drought period are shown in Figures 6A and 6B. Because the irrigation treatments had no significant effect on retranslocation patterns ($P > 0.05$), the trees were assigned to either the low-N or high-N treatment. One-way ANOVA was used to evaluate differences between the two N treatments, independently of date. The percentage of nitrogen retranslocated from leaves was significantly ($P < 0.05$) higher for trees in the high-N treatment (mean 59%) than for trees in the low-N treatment (mean 52%). On the other hand, phosphorus retranslocation was similar for both groups of trees (54–55%).
contrasts with results for other species that exhibit an increase in leaf nitrogen concentration in response to fertilizer application (e.g., Pugnaire and Chapin 1992, Carranca et al. 1993, Nilsson and Wiklund 1994). Leaf nitrogen concentrations of our carob trees were similar and sometimes higher (e.g., in the high-N + 100% irrigation) than those reported by Cabrita and Martins-Loução (1990) for mature unfertilized carob trees. The irrigation treatments did not affect leaf N concentration. Similar findings have been reported for *Picea abies* (L.) Karst. (Nilsson and Wiklund 1994) and young kiwi fruit trees (Ara et al. 1993).

The absence of a fertilizer effect on leaf nitrogen concentration may have been associated with high variability among the leaf samples. Several factors that are known to regulate nitrogen distribution, including leaf age (Diamantoglou and Kull 1988), duration and intensity of irradiance (Meletiou-Christou et al. 1994), and location within the canopy (Chen et al. 1993), were accounted for by the leaf sampling method employed. However, other factors including biomass allocation and photosynthetic surface area were not considered in the experimental design. Large increases in shoot increment (Correia and Martins-Loução 1995), leaf growth and inflorescence number (Correia and Martins-Loução 1993) occur in response to fertilization and may have masked the treatment effects on leaf nitrogen concentration. Correia and Martins-Loução (1995) observed that irrigation and fertilization effects on leaf water potential are masked as a result of large treatment-induced increments in shoot and leaf biomass and, consequently, of canopy area, causing an altered distribution of water within the plant that triggers a decrease in leaf water potential.

As observed by Cabrita and Martins-Loução (1990) for carob and by Carranca et al. (1993) for *Citrus*, leaf nitrogen concentration varies widely within and between years. Nitrogen accumulated in carob leaves during winter, presumably in proteins as has been observed in other species (Chapin and Kedrowski 1983). It is known that Rubisco serves as the main N storage in many plant species (Millard 1988), being also responsible for photosynthetic capacity. The maximum photosynthetic activity registered during winter and early spring in natural carob trees (Nunes et al. 1992) paralleled the observed increase in leaf nitrogen concentration. The decrease in leaf nitrogen concentration in spring was associated with a period of intense vegetative growth (Cabrita and Martins-Loução 1990). It is not known if the decrease was a consequence of increased growth causing dilution effects or a consequence of the translocation of nitrogen to active meristems (i.e., source–sink effects); however, close relationships between both nitrogen concentration and absolute shoot growth increment and nitrogen concentration and inflorescences number have been observed (Correia and Martins-Loução 1993). Adequate foliage nitrogen concentration at the time of both vegetative and

![Figure 5](http://www.heronpublishing.com)

![Figure 6](http://www.heronpublishing.com)
Nitrogen fertilization had no significant effects on either leaf phosphorus or leaf potassium concentration. Leaf phosphorus concentration was low compared with that of other species (Benton Jones et al. 1991) and decreased during the experimental period, especially in the last year of the experiment (1994). Nevertheless, it was within the range observed for carob (Cabrita and Martins-Loução 1990) and cork oak (Oliveira et al. 1995). Although the phosphorus concentration of the soil solution was similar to that in other carob orchards, these orchards have different soil characteristics (Cabrita and Martins-Loução 1990, Correia and Martins-Loução, unpublished results) and a higher soil pH (Cabrita and Martins-Loução 1990), which may facilitate phosphorus uptake (Mengel and Kirkby 1987). We did not account for the phosphate buffer capacity of the soil or soil phosphorus concentration when determining phosphorus fertilizer requirements.

The increase in N/P ratio in response to nitrogen fertilization highlights the close coupling between nitrogen and phosphorus metabolism (Chapin and Kedrowski 1983, Canadell and Vilà 1992). Similarly, Fahlsson (1992) observed that nitrogen fertilization increased both N/P and N/K ratios in *Fagus sylvatica* L. However, the correlations between N and P and between N and K were not significant in *Fagus sylvatica* leaves, whereas significant correlations were found for N and P and N and K in carob leaves.

Leaf potassium concentrations were similar to those obtained by Cabrita and Martins-Loução (1990). Nitrogen fertilization did not markedly affect leaf potassium concentrations as has been observed for *Citrus* spp. (Carranca et al. 1993). This difference between the species may be associated with source–sink effects on potassium mobilization, because carob pods are effective potassium sinks (Cabrita and Martins-Loução 1990). Normally, from March until the end of May, there is a significant increase in fruit potassium concentration that is accompanied by a decrease in leaf potassium concentration (Cabrita and Martins-Loução 1990), which may indicate that potassium is mobilized from leaves to fruits. Other evidence in support of this suggestion is the finding of a significant negative correlation between leaf potassium concentration and fruit number, indicating that the number of developing carob fruits strongly enhances potassium movement. Thus, it appears that, in carob trees, potassium is mainly translocated from leaves to fruits (cf. Mengel and Kirkby 1987).

### Nutrient retranslocation

In addition to summer drought periods, the Mediterranean region is subject to droughts during the spring and autumn, at which times there occurs vegetative and reproductive growth, respectively. To avoid water stress during these critical growth periods, carob trees shed leaves. Because neither the nitrogen fertilization nor the irrigation treatment prevented leaf shed during the water-stress periods that occurred during the spring and autumn (Correia and Martins-Loução 1993, 1995), we conclude that leaf shedding during these periods represents a physiological adaptation to these special conditions. By shedding less efficient leaves, water loss from transpiring surfaces is minimized, and by mobilizing nutrients from these leaves to the most demanding sinks, nutrient use efficiency is maximized.

Nitrogen retranslocation was slightly higher in the carob trees than in evergreen *Quercus* spp. (between 31% and 45%) (Escudero et al. 1992), probably because retranslocation in carob was associated with both age-related leaf shedding and the leaf shedding that occurred during the spring and fall periods of water stress, whereas it was associated only with age-related leaf shedding in the evergreen species. During age-related leaf shedding, phosphorus retranslocation is normally higher than nitrogen retranslocation (Escudero et al. 1992); however, similar amounts of phosphorus and nitrogen were retranslocated during the leaf shedding that occurred in response to the spring and fall periods of water stress.

The higher percentage of nitrogen retranslocation from leaves in the high-N treatment compared with the low-N treatment is probably associated with a higher concentration of soluble-N compounds in leaves in the high-N treatment. Previously we have shown that high-N treatment increases growth (Correia and Martins-Loução 1995) and fruit yield (Correia and Martins-Loução 1993). Several studies (e.g., Chapin and Kedrowski 1983, Helmisarai 1992) have shown that increasing soil nutrient availability leads to increased retranslocation. On the other hand, results obtained by Boerner (1985) and Pugnaire and Chapin (1993) showed that both nitrogen and phosphorus retranslocation were higher in plants growing on nutrient-poor soils than in plants growing on nutrient-rich soils, resulting in a positive correlation between the fraction of soluble nitrogen and retranslocation. However, under conditions of low fertility, the soluble N forms increase, probably as a result of the synthesis of stress compounds such as proline (Pugnaire and Chapin 1993, Lansac et al. 1994).

In Mediterranean regions, severe water stress occasionally occurs in the spring and fall corresponding to periods of vegetative growth and independently of summer drought and irrigation regimes, representing a critical physiological period that leads to premature leaf abscission. Under these conditions, nutrient retranslocation may represent an adaptive mechanism to sustain the nutritional demands of vegetative and reproductive sinks. During these particular periods, fertilization effects are more pronounced than irrigation effects.

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### References


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LEAF NUTRIENT VARIATION IN MATURE CAROB TREES

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