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Symptoms of nutrient deficiencies in young olive trees and leaf nutrient concentration at which such symptoms appear



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ARTICLE INFO

Article history: Received 21 January 2016 Received in revised form 20 June 2016 Accepted 2 July 2016 Available online 16 July 2016

Keywords: Nitrogen deficiency Potassium deficiency Magnesium deficiency Calcium deficiency Iron deficiency Manganese deficiency Zinc deficiency Boron deficiency

1. Introduction

Plants affected by nutritional deficiencies or toxicity exhibit visual symptoms, particularly when the nutritional disorder is severe. These symptoms include unusual colors, necrosis, distortion of plant parts, and abnormal growth. Reduction of yield or growth is not characterized by visual symptoms. Each mineral nutrient induces a specific symptomatology, so that visual symptoms are useful for identifying nutritional disorders in plants. However, visual symptoms are not enough to diagnosis the nutritional status of a plant since other non-nutritional factors, biotic or abiotic, may induce symptoms similar to those produces by nutritional deficiencies or excess. The diagnosis by visual symptoms may be further complicated when more than one nutrient is deficient or when a deficiency of one nutrient is induced by the excess of another (Marschner, 2012). Successful diagnosis also requires plant analysis and information on cultural and climatic conditions (Shear and Faust, 1980). Nevertheless, knowledge of symptomatology is a complementary tool in the diagnosis.

The olive (*Olea europaea* L.) is an evergreen tree cultivated from ancient times and adapted to adverse growing conditions. Many

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http://dx.doi.org/10.1016/j.scienta.2016.07.002 0304-4238/© 2016 Elsevier B.V. All rights reserved.

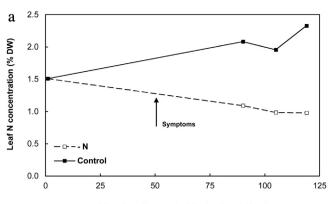
ABSTRACT

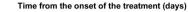
Mist-rooted 'Picual' olive cuttings growing in 1 L pots containing a mixture of washed sand and perlite, were used to induce symptoms of N, K, Mg, Ca, Fe, Mn, Zn and B deficiencies. Plants were growing in a shadehouse protected from the rain from June to December, and then placed in a growth chamber at 25/15 °C (day/night) with a 14 h photoperiod and 65% humidity until the end of the experiment. Plants were drip-irrigated with desioned water and once a week with 100 mL of a nutrient solution free in the element under treatment. Leaves were sampled periodically to determine leaf-nutrient concentration. When a symptom appeared, the symptomatic leaves were collected to be photographed. Deficiency symptoms appeared in the range of 50 days after the initiation of treatments in N and 34 weeks in K. Leaf-nutrient concentrations to which the symptoms appeared were determined in these plants.

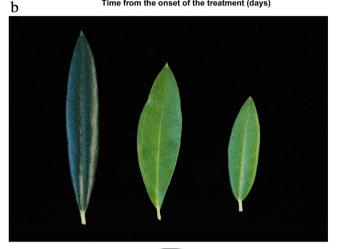
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plant species adapted to low-nutrient environments develop foliar symptoms of toxicity when fertilized with some nutrients, particularly phosphorus (Shane et al., 2004), but do not develop visual symptoms of deficiency since they adjust their growth to the most limiting nutrient (Chapin, 1983). This may explain that in the olive, nutrient deficiencies enough severe to produce symptoms in leaves are rare in mature trees growing under field conditions. The exceptions are K when growing in the drylands (Restrepo-Díaz et al., 2008,), Fe when growing on calcareous soils (Fernández-Escobar et al., 1993), and probably Ca on acid soils. Taking into account the low amounts of nutrients removal from olive orchards (Fernández-Escobar et al., 2015), it is easy to understand that most of the symptomatology associated to nutrient deficiencies are unknown in the olive.

Growth usually decreases before the appearance of visual symptoms, so the absence of symptoms does not necessarily indicate an optimum nutritional status (Shear and Faust, 1980). Leaf-nutrient analysis is the best method for diagnosing this status (Benton Jones, 1985). Their use as a tool for determining fertilization requirements must be based on standardized sampling methods, and that results must be compared only with standard values obtained by those procedures. But the standard values obtained for mature trees may not be the same than those for young trees, probably because the limited capacity to store nutrients in the tissues of young trees (Shane et al., 2004). Determining leaf-nutrient concentration to







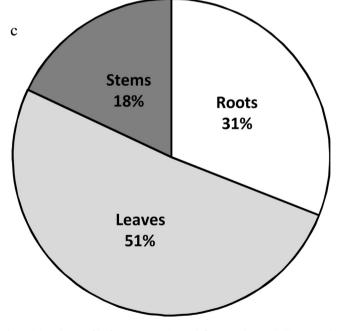


Fig. 1. (a) Evolution of leaf-N concentration in deficient and control olive trees. (b) N deficiency symptoms in leaves. Left, a control leaf. (c) N partitioning into leaves, roots and stems

which symptoms appear could be an interesting reference in diagnosing nutritional disorders, although plant growth usually has reduced before.

The objective of the present work was to induce symptoms of nutrient deficiencies in young olive trees, and to determine leafnutrient concentration to which such symptoms appear.

2. Materials and methods

2.1. Plant material and growth conditions

Mist-rooted 'Picual' olive cuttings were transferred to 1 L plastic pots containing a substrate of sand, which was previously washed to ensure the complete removal of nutrients, and perlite. Plants were placed in a shade-house located at Rabanales Experimental Farm (University of Córdoba, Spain) between mid-June and mid-December 2014 with a temperature range of 35–15 °C. During this period, plants were sheltered to avoid rain-fed. Thereafter they were moved into a controlled growth chamber with a day-night temperature of 25/15 °C, a photoperiod of 14 h of light and relative humidity around 65%. During the whole experiment, the plants were drip-irrigated with desioned water to control the supply of nutrients. Water was delivered through drippers $(2Lh^{-1} flow rate)$ individually installed in each pot. The irrigation dose was adjusted to growth conditions and intended to avoid nutrient accumulation in the substrate.

After 21 days of acclimation and before the initiation of treatments, the cuttings were pruned to a single shoot per plant, and the plants were divided into two groups: one to capture visual symptoms of nutrient deficiencies and the other to collect leaves to determine the leaf-nutrient concentration to which such symptoms appeared. Ninety uniform plants were selected per group and arranged in nine rows, with 10 plant replications, corresponding to each nutrient deficiency to study and the control plants.

2.2. Treatments

To induce leaf visual symptoms of N, K, Mg, Ca, Fe, Mn, Zn and B deficiency, plants were watered by hand once per week with 100 mL of a nutrient solution free in the element under treatment. Eight different element-free nutrient solutions, one per treatment, were individually prepared by omitting the element under study from a standard nutrient solution (SNS). This solution was Hoagland's nutrient solution (Hoagland and Arnon, 1950) with the following composition: 5 mM Ca(NO₃)₂, 5 mM KNO₃, 2 mM MgSO₄, 1 mM KH₂PO₄, 25 µM H₃BO₃; 2 µM MnSO₄; 2 µM ZnSO₄; 0.5 µM CuSO₄; 0.4 µM (NH₄)₆Mo₇O₂₄ and 20 µM Fe-ethylenediamine-dio-hydroxy-phenylacetic acid (Fe-EDDHA). N, K, Mg and Ca-free nutrient solutions were further supplemented with the following salts: N-free (5 mM CaCl₂ and 5 mM KCl); K-free (0.5 mM $Ca(H_2PO_4)_2$; Mg-free (2 mM Na₂SO4); Ca-free (5 mM NaNO₃). In this way, all the element-free nutrient solutions provided every element essential for plant growth except the one under treatment. Control plants were watered with the SNS. In all cases Ca(OH)₂ was used to adjust the pH of the nutrient solution to 5.5.

2.3. Data collection

Fully expanded leaves were sampled at the beginning of treatments application to determine the initial leaf-nutrients concentration of the plants. Thereafter, the collection of leaf samples was started ninety days after initiating the treatments and subsequently every fifteen days until the end of the experiment. At each time, 10 fully expanded leaves, which were developed during the experiment, were collected from each treatment to analyse the concentration of the corresponding element. All nutrients were analyzed in control plants. Once leaf visual symptoms of each nutrient deficiency appeared leaves were photographed. The experiment lasted between 125-275 days, depending on the treatment. At the end of the experiment plants were harvested and leaves, stems and roots were removed separately from each plant to determine dry matter and the concentration of every nutrient in each organ. Leaf samples and plants organs (leaves, stems and

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