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**Title**

Effects of Fe-chlorosis on the stomatal behaviour and water relations of field-grown peach leaves

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## Introduction

Iron (Fe) deficiency chlorosis is a common abiotic stress affecting plants in many areas of the world. This physiological disorder is mainly found in crops grown in calcareous and/or alkaline soils and occurs as a result of several causes acting simultaneously (Rombolà and Tagliavini 2006). Fe deficiency chlorosis has been shown to markedly reduce the photosynthetic rate of several plant species and cause decreases in stomatal opening, transpiration rates and water use efficiency (Larbi et al. 2006).

Recently, Fe chlorosis has been shown to affect the structure of peach leaves grown under field conditions (Fernández et al., 2008). Iron-sufficient leaves have been found to have more epicuticular waxes as compared to Fe-chlorotic leaves. Iron chlorosis also decreased the length of stomatal pores as compared to that of Fe-sufficient leaves (Fernández et al., 2008). The main focus of the present investigation was to assess the effect of Fe-chlorosis on leaf water relations and the functionality of stomata.

## Materials and Methods

The study was conducted in August and September 2008 with green and chlorotic leaves of 15 year-old peach (*Prunus persica* (L.) Batsch, cv. Miraflores) trees grown in a commercial orchard located in the Jalón River Valley, in the Zaragoza province, Spain. Iron-chlorotic trees did not receive any exogenous Fe input for 3 years and developed Fe deficiency symptoms in springtime. Green trees were treated with Fe(III)-EDDHA (40 g per tree applied in May) and remained fully green throughout the experimental period.

Gas exchange of green and chlorotic leaves of recently flood-irrigated trees was measured with a portable steady-state porometer (CIRAS-2 Portable Photosynthesis System). Measurements were performed with leaves attached to the trees (i) under normal ambient conditions, (ii) after withdrawal of external CO<sub>2</sub> or exposure of previously darkened leaves to high light intensities, and (iii) with leaves detached from the trees during the measurements. Water potentials in leaves were measured with a portable Scholander-type pressure chamber. Hydraulic conductivity of the xylem system supplying the leaves was estimated by wrapping heavily transpiring intact leaves tightly in aluminium foil and measuring the time course of recovery of leaf water potentials.

## Results and Discussion

Iron deficiency affected plant water relations and stomatal behaviour. Net photosynthesis, transpiration rates and water use efficiency were significantly lower in chlorotic leaves than in green leaves (Table 1). In green leaves the water potential decreased to values of about -2.0 MPa during the course of the day, while in chlorotic leaves minimum water potentials were about -1.0 MPa (Fig. 1a). The hydraulic conductivity of the xylem system was  $74 \pm 3 \mu\text{mol s}^{-1} \text{MPa}^{-1}$  in green leaves but only  $32 \pm 8 \mu\text{mol s}^{-1} \text{MPa}^{-1}$  in chlorotic leaves.

Withdrawal of external CO<sub>2</sub> or exposure of previously darkened leaves to high light intensities induced a rapid opening of stomata in green leaves, while stomata of chlorotic leaves did hardly respond (Fig. 1b). A different response of stomata was also observed between green and chlorotic leaves immediately after detaching the leaves from the tree. Once detached, transpiration rates of Fe-sufficient leaves decreased over time, regardless of prevailing irradiation conditions. In contrast, the transpiration rate of detached chlorotic leaves decreased slightly shortly after but increased thereafter to reach values similar to the ones measured prior to leaf detachment.

These results show that Fe deficiency not only causes a reduction in photosynthetic performance but also a fundamental disturbance of leaf functionality, also regarding the action of stomata, and of leaf water relations. While it has been frequently reported that soil or leaf application of Fe can recover the photosynthetic performance of leaves, it remains to be studied if re-supply with Fe can also restore the barrier properties of leaves and the functionality of stomata and of the xylem.

## References

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Table 1: Net photosynthesis ( $A$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), internal  $\text{CO}_2$  concentrations ( $C_i$ ) and water use efficiency (WUE) of green and chlorotic peach leaves. Means  $\pm$  standard error are shown (\*\*\*:  $p < 0.001$ , t-test  $n = 5$ )

Leaf type	$A$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	$g_s$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	$C_i$ ( $\mu\text{mol mol}^{-1}$ )	WUE ( $\mu\text{mol mmol}^{-1}$ )
green	$15.9 \pm 1.4$	$6.2 \pm 0.5$	$529 \pm 124$	$335 \pm 3$	$2.6 \pm 0.1$
chlorotic	$4.0 \pm 0.7$ ***	$4.4 \pm 0.3$ ***	$243 \pm 26$ ***	$365 \pm 5$ ***	$0.9 \pm 0.1$ ***

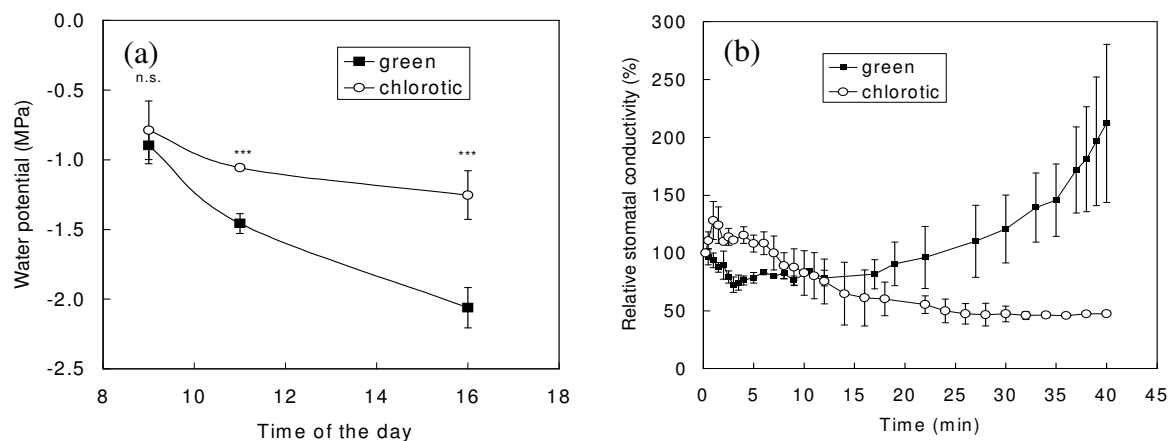


Fig. 1: (a) Diurnal course of leaf water potentials (\*\*\*:  $p < 0.001$ , t-test,  $n = 5$ ). (b) Relative stomatal conductivity after exposure of previously darkened leaves to high irradiation (photosynthetic active radiation:  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $n = 2$ ).