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introduction

Against the background of a future decrease in water availability, there is a need to use irrigation water with higher efficiency. To improve water management, it is crucial to clarify the role of irrigation water compared to soil water and additional water sources, including groundwater, which is often neglected by most water balance models.

An irrigation experiment applying ²H-enriched water was conducted in an apple orchard (*Malus domestica*, cv. Pinova, South Tyrol, Italy) to assess the uptake dynamics of irrigation water by apple trees.

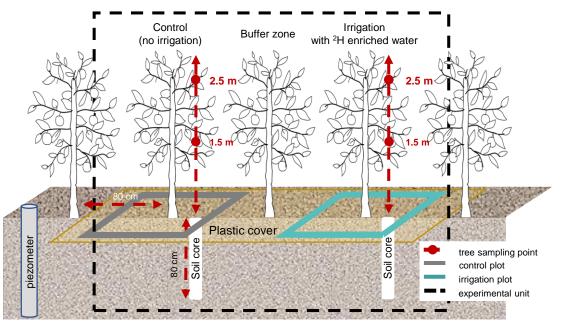
experimental design

In **Fig. 1**, a scheme of an experimental unit, consisting of three apple trees, is provided. The two lateral trees and their corresponding soil (1 m²) were chosen as **control** and **irrigated plot**, while the central tree was the buffer zone. In total, four experimental units were selected in consecutive rows and each was covered with plastic sheets, to prevent any additional water source to reach the soil.

The experiment started applying ²H-enriched water (40 L/m², δ^2 H = 1500‰) to the four irrigation plots at 9 AM (July 23 2019). The sampling was performed from 2 to 168 h after the irrigation cycle in both plots. Soil samples were collected to 0.8 m depth and divided into eight 0.1 m layers. Shoot axes and leaves were collected from the bottom/top of each canopy.

Soil water content (gravimetric) was determined for each sample. Daily evapotranspiration was estimated for the whole experimental period ($ET_0 = 3.25 \text{ mm/day}$). Through a piezometer in the proximity of one experimental unit, the groundwater level, rather shallow in this orchard (0.9 – 1 m depth), was monitored daily.

Total water was recovered from samples through cryogenic vacuum distillation. Isotopic analyses were performed at the IRIS (Isotope Ratio Infrared Spectroscopy) and at the IRMS (Isotope Ratio Mass Spectrometry) analyzer.



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Fig. 1. Scheme of a single experimental unit.



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results

→ soil and tree water analysis

Water content decreased from the surface to 0.4 - 0.5 m soil depth and then progressively increased again until 0.8 m depth in both irrigated and control soils (**Fig. 2**). Only the top soil significantly increased its moisture after the irrigation cycle. The relatively high soil moisture in the deeper layers might be related to a capillary rise (approximately 0.4 m from the groundwater table estimated by models for a silty loam soil).

The δ^2 H of subsequent soil layers evolved in time, showing the gradual infiltration of irrigation water and its mixing with pre-irrigation water. On average, the enrichment mainly affected the soil to 0.6 m depth (**Fig. 2**), where ca. 80% of the fine roots were concentrated. In this soil block, irrigation water represented ca. 20% of the total water.

In both shoots axes and leaves, a significant enrichment of the δ^2 H was measured starting from 8 h after irrigation (**Fig. 3**). From 24 h after irrigation, the δ^2 H settled to ca. 61‰ in the shoot axes collected from the bottom of the canopy, and to slightly lower values for top shoots. Leaves, as expected, had higher δ^2 H than shoot axes due to their transpiration. Transpiration rate also affected the degree of leaf enrichment. Specifically, leaves collected 72 h after the irrigation cycle were characterized by rather depleted δ^2 H (+7.1 ± 11.3‰ for bottom leaves) compared to the other sampling days (+45.9 ± 7.9‰), probably due to the low evapotranspiration registered that day (ET₀ = 1.8 mm/day).

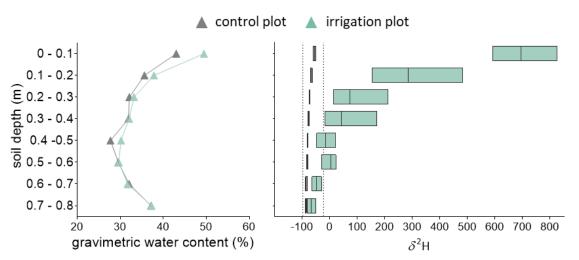


Fig. 2. Average gravimetric water content (left panel) and $\delta^2 H$ (right panel) in control and irrigated soil samples at each depth. Dot lines delimit the $\delta^2 H$ range in control soil, (± 4 sd).

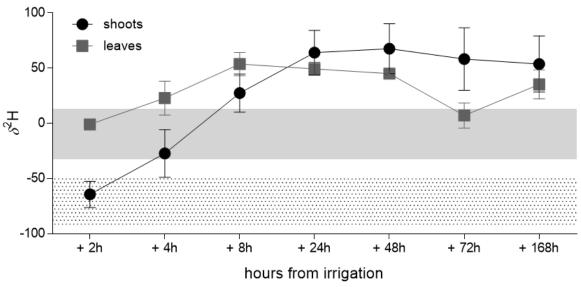


Fig. 3. Average $\delta^2 H$ in leaves and shoot axes of irrigated trees (bottom) at each sampling. Grey and dotted bands represent the $\delta^2 H$ range (± 4 sd) of control leaves and shoot axes, respectively.



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\rightarrow water sources for apple trees

Based on the obtained isotopic results, data were further elaborated to clarify the following points:

1. Irrigation water contribution to shoot water

The following two end-member mixing model was applied to calculate the percentage fraction of water present in the shoots and deriving from irrigation water ($f_{water (IRR, enr)}$):

$$f_{water(IRR,enr)} = \frac{\delta^2 H_{tree(IRR)} - \delta^2 H_{tree(CTRL)}}{\delta^2 H_{water(IRR,enr)} - \delta^2 H_{tree(CTRL)}}$$
(Eq.1)

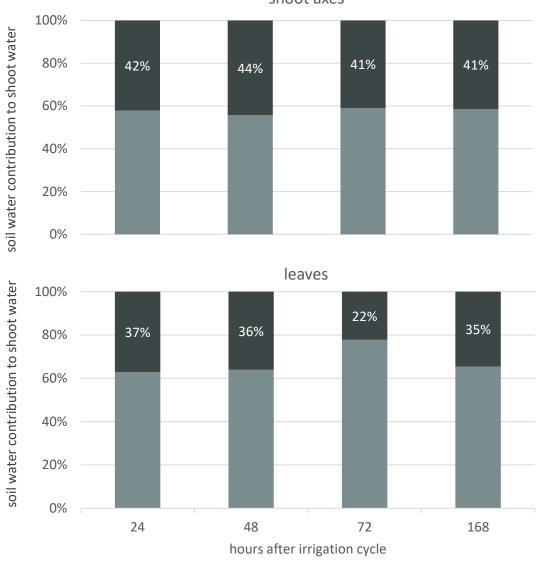
The irrigation water accounted for 8 and 3% of the shoot axes and leaf water, respectively (**Eq. 1**).

2. Soil water (0 – 0.6 m) contribution to shoot water

After calculating the contribution of pure irrigation water, the fraction of shoot water deriving from the water mixture in the upper soil layer (f_{soil} (0 - 0.6 m)), mostly affected by enriched irrigation, was calculated as follows:

$$f_{soil\,(0-0.6\,m)} = \frac{\delta^2 H_{tree\,(IRR)} - \delta^2 H_{soil\,(0.6-0.8\,m)}}{\delta^2 H_{soil\,(0-0.6\,m)} - \delta^2 H_{soil\,(0.6-0.8\,m)}}$$
(Eq. 2)

Assuming that tree roots do not discriminate between irrigation and soil water present before the irrigation, the estimated contribution of soil water from the first 0.6 m depth to tree water was ca. 35-45% (**Eq. 2, Fig. 4**).



shoot axes

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Fig. 4. Results of the two-end-member mixing model showing the fractional contribution of soil water from the upper (0 - 0.6 m) and the deeper (0.6 - 0.8 m) soil blocks to bottom tree samples (Eq. 2).



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3. Contribution of recently absorbed water to shoot water

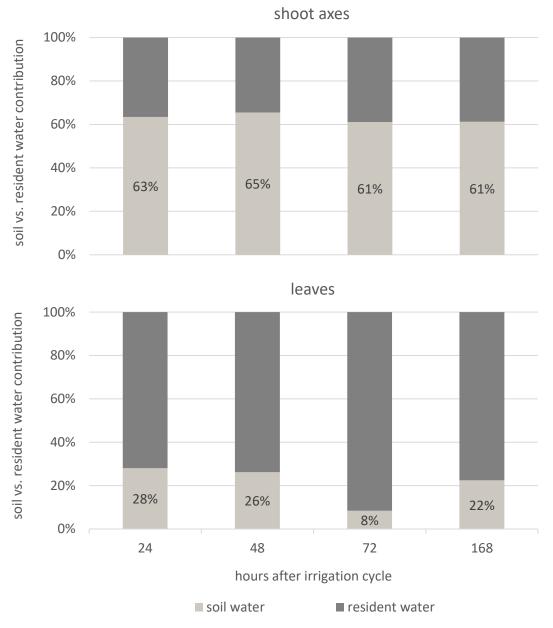
Comparing control and irrigated units, the ²H fraction (%) present in the shoots and recently absorbed from the soil was calculated applying the following equation:

$${}^{2}H_{from \,soil}\,(\%) = \frac{{}^{2}H_{irrigated \,tree} - {}^{2}H_{control \,tree}}{{}^{2}H_{irrigated \,soil} - {}^{2}H_{control \,soil}} \tag{Eq.3}$$

Around 60 % of the ²H in shoot axes derived from soil water, while in the leaves soil water represented only ca. 25 % of total water. (**Fig. 5**). The remaining water fraction present in shoot samples was ascribed to resident water, present in the shoots before irrigation.

conclusions

The present study highlighted that irrigation water accounted only for a relatively small amount of total shoot water. We hypothesize that in leaves, recently absorbed water rapidly transpire without mixing with resident water. These results highlight the complexity of soil-water-plant interactions and call for additional investigations to understand the competition between irrigation and pre-irrigation soil water and if there is a preferential absorption by roots. The next step of our research is to clarify the role of groundwater to the fulfilment of the water needs of our experimental apple orchard.



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Fig. 5. Estimation of the water fraction (%) coming from the soil in comparison with resident water (Eq. 3).