A model for irrigation of vineyards under limited water availability

Thesis submitted for the degree of

"Doctor of Philosophy"

By

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1. Abstract

Israel and other semi-arid regions suffer from a limited water supply and, in recent years, this problem has developed into a critical condition that will require better water-use management practices, particularly in agriculture. In this work, the different aspects of water usage in Israeli vineyards will be discussed.

From the lysimeters to the vineyard: development of an irrigation model for table grapes

Freshwater consumption of table-grape vines (*Vitis vinifera* cv. Superior Seedless) was measured during six growing seasons (1999, 2001-2005) using 12 drainage lysimeters. The lysimeters were installed as part of a one-hectare vineyard in the Lachish region in southern Israel. Maximum experimentally determined evapotranspiration (ET_c) values in different seasons ranged from 50.7 to 60 L day $^{-1}$ vine^{-1} and seasonal ET_c, from day of year (DOY) 91 through DOY 304, ranged from 1087 to 1348 mm over the six growing seasons. Reference evapotranspiration (ET_o) was calculated from local meteorological data using the modified Penman-Monteith equation. Seasonal curves for the crop coefficient (K_c) were calculated as $K_c = ET_c / ET_o$. Leaf area index (LAI) was measured monthly using the SunScan Canopy Analysis System (Delta-T Devices, Cambridge, UK). Maximum LAI ranged from 4.2 to 6.2 m^2 m^{-2} for the 2002-2005 seasons. The high ET_c and K_c values that were observed are explained by the wide canopy layout of the large open-gable trellis system.

According to our model, maximal water consumption of commercial vineyards can be calculated in the following ways:

1. DOY to K_c correlation can be used in Lachish region.
2. GDD to K_c correlation can be used in other table grape-growing regions (GDD - growing degree days).
3. Correlation of LAI to K_c is the most precise method, but it is also more labor intensive.

Using the lysimeter-based model, irrigation treatments were examined in a field trial in order to test the applicability of the model under semi-commercial conditions. Three

irrigation treatments, 80% (high), 60% (medium) and 40% (low) of the ET_c of the lysimeter-grown vines, were applied in the vineyard and vegetative and reproductive growth were observed. Increased irrigation (from 40% ET_c to 80% ET_c) increased the size of the vine canopy and yield. The positive effects of the increased irrigation on vegetative and reproductive growth are presented in a way that will help the vine-grower choose an irrigation coefficient that can be applied in his vineyard.

The effects of treated waste water (TWW) and irrigation volume in table grape vineyards

Over the past few years, the use of TWW (treated wastewater) has increased and now accounts for more than 50% of all water used in Israeli agriculture. The use of TWW raises many questions regarding irrigation and fertilization practices.

In the same vineyard that was mentioned previously, an additional experiment was conducted in the 2002-2007 growing seasons. The aim of this study was to examine the effects of irrigating with treated wastewater (1.88 dS m^{-1} ,230 mg l^{-1} Na^+), with and without fertilizer, as compared to freshwater treatments (1.30 dS m^{-1}, 117 mg l^{-1} Na^+). Each water quality treatment included three irrigation levels set according to the model that was developed for irrigation with freshwater.

In this study water, soil and leaves were periodically sampled and analyzed and the concentrations of a wide range of chemical elements were monitored. Na^+ stood out as the major "damage potential ion" under TWW-irrigation conditions. Soil Na^+ concentrations and SAR naturally fluctuated over the years, but fluctuations were significantly greater in the soil of TWW treatments, particularly in the high volume irrigation treatment. Adding fertilizer to TWW treatments appeared to have a positive effect on the SAR values, which may be due to changes in soil pH and the addition of cations that compete with Na^+ for adsorption onto the exchange complexes of clays. Na^+ concentrations in the leaf petioles rose significantly each year from the third year on, with maximum values exceeding 6500 mg Kg^{-1} Na^+ in TWW treatments, which is more than three times higher than the concentration observed in the control. The effects of increased soil Na^+ on leaves were established after five years of continuous irrigation.

In the present study, new methods were developed for collecting xylem sap in the spring and sampling bark and wood for mineral analysis. Significantly higher Na^+ concentrations were observed in the wood, bark and xylem sap of the TWW-treated vines.

It appears that in table-grape vineyards on heavy soils that receive relatively high levels of irrigation, Na^+ does is not leach well from the root zone and therefore poses a greater problem for the plant and soil. However, even under the trial's elevated Na^+ conditions, no significant yield effects were noted. This may be due to the effective stability of the cv. Paulsen rootstock under saline conditions and the tolerance of cv. Superior to increased Na^+.

A mature commercial vineyard of own-rooted cv. Red Globe grafted on its roots, which was located near the experimental site and maintained under similar irrigation conditions, collapsed after only three years. Wood samples taken from the trunks of those vines showed [Na^+] of 2271-2294 mg/kg. This may indicate that wood analyses could be used, year round, as a tool for identifying developing salt stress.

Leaf patch clamp pressure probe (LPCP) as a tool for irrigation management

An advanced and non-invasive LPCP was developed for online monitoring of the water balance in leaves of table grapes (cv. Superior Seedless in the Lachish region) and wine grapes (cv. French Colombard in the Gedera region). The probe measures the attenuated output patch clamp pressure of a clamped leaf in response to an external magnetic pressure. The measured pressure is inversely correlated with the leaf turgor pressure. Measurements showed that changes in transpiration rate and stomatal conductance induced by environmental parameters were reflected in the probe readings nearly immediately. The LPCP was successfully tested on leaves of irrigated and non-irrigated table-grape vines under field conditions. Data show that slight changes in the microclimate and/or water supply are reflected in the leaf turgor pressure. In wine grapes, the LAI to K_c model was examined along with LPCP measurements and the data were correlated with yield and yield components.

Contents

2. Introduction

Overview of *Vitis*

Vitis is a genus of deciduous perennial plants that grow grapes. Grape is a non-climacteric fruit, botanically defined as a true berry, which grows on compound raceme inflorescences that are often called panicles. The vines produce fruit that is eaten raw or made into wine, raisins, vinegar and other commercial products.

The most commonly grown species, *Vitis vinifera*, originated in the Mediterranean region and central Asia. Other vine species such as *Vitis riparia, Vitis labrusca* and *Vitis rotundifolia* are used for grape and wine production in North America. Cross-breeding among *Vitis berlandieri, Vitis riparia* and *Vitis rupestris* varieties has produced widely used rootstocks that determine many vine characteristics, particularly various stress tolerances.

Table grapes and wine grapes

Modern agronomic practices for wine and table grapes differ, even though the basic biology of these two crops is the same. Some of the differences are derived from historical selective breeding practices that were aimed at achieving different goals. Wine grapes have small berries with relatively thin skin, which contains most of the aroma and color components (mainly in red wine varieties). In contrast, in table grapes, seedless big berries with relatively thick skin are desired. Table grapes are usually harvested earlier than wine grapes, when their TSS (total soluble solids) reaches 15-16^{o} Brix. Wine grapes are usually harvested when their TSS reaches 23-25^{o} Brix.

Table grapes are usually grafted onto rootstocks that encourage plant vigor and trellis systems are constructed to support a wide canopy (open gable, Geneva double curtain, overhead pergola); whereas wine grapes are grafted onto less vigorous rootstocks and, therefore, their canopies and trellis systems are more compact (vertical shoot position, lyre, smart-Dyson). Two main pruning practices are used in grape crops: long-cane pruning, which is generally used in table grapes, and spur pruning, which is generally used for wine grapes. These practices affect the growth and dimensions of the crop canopy as well as yield potential.

Consequently, these differences affect the canopy size expressed as leaf area index (LAI). The LAI of table-grape crops is much greater than that of wine-grape crops (maximum LAI in the present field experiment was ~4.5 m^2 m^{-2} in cv. Superior Seedless and ~1.3 m^2 m^{-2} in cv. French Colombard). Increased LAI in table grapes results in a higher transpiration rate, necessitating higher amounts of irrigation than are needed for wine grapes. In wine grapes, imposing controlled water stress is a common practice used to encourage the production of smaller berries with a stronger aroma (higher phenolic content) and skin color (anthocyanin content) (Esteban et al., 2001; Roby et al., 2004). This method is often called regulated deficient irrigation (RDI) (Acevedo-Opazo et al., 2010; Cifre et al., 2005). This practice and a smaller canopy are the reasons why less water is used in the production of wine grapes.

Vine water consumption

In order to define plant water consumption and to distinguish it from irrigation requirements, the following terms were suggested (Doorenbos and Pruitt, 1977).

1. Plant water consumption: The amount of water a plant loses by transpiration as well as the water loss from the soil surface due to evaporation.
2. Irrigation requirements: The amount of water that is needed to meet the evapotranspiration demands (as effective precipitation and available groundwater is reduced), in order to produce an acceptable yield, rinse salts from the soil and support other agricultural practices that demand water (e.g., fertilization).

There are a few flaws in the aforementioned definitions. First, irrigation is applied in order to satisfy evapotranspiration demands but, at some specific phenological stages during the season, it is common to limit irrigation for a certain period without any subsequent yield loss. In addition, the irrigation definition ignores the timing of and intervals between irrigation applications. The term "acceptable yield" is subjective and differs among varieties, years and locations and even between various plots within a vineyard.

In the semi-arid conditions of the Lachish region, daily irrigation is the common practice in table-grape cultivation. In the present study, vine water use was calculated based on

measurements with 13 drainage lysimeters. Lysimeters are commonly used for ET (evapotranspiration) evaluations (Allen et al., 1998).

Lysimeters

A lysimeter is a device used to measure the actual plant transpiration and soil evaporation of woody or herbaceous plants planted in containers of soil. The amount of water lost by evapotranspiration can be calculated from the amount of irrigation (and precipitation) that an area receives and the amount of moisture that is lost by transpiration and directly from the soil. There are two types of lysimeters: weighing / floating lysimeters and drainage / overflow lysimeters. Weighing / floating lysimeters are usually large soil-containing tanks that are installed onto weight-load cells. The accuracy of these measurements is relatively high and hourly evapotranspiration can be measured. However, they are relatively expensive.. Therefore, usually, few of these measuring devices are used at a time. Drainage lysimeters are cheaper to build, but their operation may be more labor-intensive and the data is only obtained on a daily or periodic basis.

For many years, lysimeters have been used as a standard tool for measuring water consumption. Measurements have been made in woody species, such as peaches (Ayars et al., 2003), oranges (Yang et al., 2003), pears (Chalmers et al., 1992) and wine grapes (Evans et al., 1993). In experiments on table grapes, measurements have been obtained using both drainage lysimeters (Van Rooyen et al., 1980; Prior and Grieve, 1987; Evans et al., 1993; Shani and Ben-Gal, 2005) and weighing lysimeters (Williams, 1998; Williams et al., 2003a, b; Williams and Ayars, 2005). In addition to these woody species, lysimeter tests have also been performed on herbaceous species such as wheat and maize (Liu et al., 2002), cotton (Ayars and Hutmacher, 1994) and bermudagrass (Young et al., 1996).

Irrigation with treated wastewater

Wastewater is defined as water whose quality has been influenced by anthropogenic use; sewage water is a subset of wastewater.

The need to increase the use of treated wastewater (TWW) in agriculture is the result of increased demand for freshwater for domestic use and increased scarcity and fluctuations in yearly precipitation. From 1960 to 2004, the use of treated wastewater for irrigation in Israel increased to account for about 30% of all water used in agriculture (Aharoni and Cikurel, 2006; Fuchs, 2007) and this figure is expected to reach more than 50% in the next few years. The use of treated wastewater for irrigation started in cotton more then 20 years ago and was later introduced in orchards (Avnimelech, 1993). Large-scale use of TWW for irrigation of table grapes began six years ago. As a consequence of irrigation with TWW, increased visual symptoms and chlorosis started to appear on leaves and, in some cases, there was a total collapse of yield-bearing vines.

Public health risks associated with the use of TWW

TWW use may posses various health risks, including exposure to pathogenic bacteria, viruses, protozoa and parasitic worms (Gerardi and Zimmerman, 2005). Consumption or exposure to heavy metals, pharmaceutically active compounds and hormones can cause serious health problems. In 1989, the World Health Organization (WHO) developed guidelines for managing health risks that may arise from exposure to microbes present in TWW. Guidelines for treating sewage water require that biological oxygen demand (BOD) and suspended solids (SS) not exceed 20mg L^{-1} and 30 mg L^{-1}, respectively (WHO, 1989). The TWW that is released from the purification plant should not contain *E. coli* concentrations higher than 100 bacteria per 100 ml of water (WHO, 1989).

In order to avoid outbreaks of human diseases, specific guidelines were adapted for the use of TWW for agricultural purposes in Israel (Halperin, 1999). In order to be approved for TWW irrigation, several legally defined physical "barriers" must be installed in vineyards that use trellis systems. Regulations require two or more of the following safety practices: disinfection of water, maintaining a certain physical distance between the fruit and the drip line, the use of a plastic covering on the drip line that is resistant to sun damage and/or the use of an underground drip line.

In the current study, water was disinfected with chlorine, and a minimum of one meter was kept between the fruit clusters and the drip line, in addition to a 30 cm wide plastic sheet that covered the drip line. Microbial tests that were preformed at harvest during the present experiment confirmed that no coliform bacteria were present on the fruits.

Agricultural risks associated with the use of TWW

TWW usually contains a high organic matter load (Morgenroth et al., 2002) and may contain elements that are toxic to plants (Toze, 2006; Yermiyahu et al., 2007). Extended use of TWW for irrigation could potentially lead to the leaching of nitrate and, in some situations, phosphorus into groundwater (Bond, 1998).

High concentrations of organic matter components can affect soil properties by coating soil particles and eventually change the soil surface properties, rendering the soil hydrophobic, a condition that makes it harder for water to infiltrate the soil. Evidence of organic matter contamination is amplified when soil is dried (Wallis et al., 1991; Tarchitzky et al., 2007). In addition, bacterial films may clog drippers and drip irrigation lines.

High salinity content of TWW was found to be a central problem, which may affect crop performance and the chemical and physical properties of the soil. Salinization of water and soil is frequently identified with increasing concentrations of NaCl. Reduced growth, early leaf senescence and the appearance of chlorotic and necrotic spots on leaves are external symptoms of salt stress (Greenway and Munns, 1980;Tester and Davenport, 2003). It is generally accepted that salt stress in plants has two components: an osmotic component, in which growth is affected by reduced water uptake (Munns, 1993; Munns et al., 2000; Munns, 2002), and a slower, metabolic component, which is a result of specific ion toxicity.

The standard procedures for treating TWW at the purification plant do not reduce the concentration of most dissolved salts and the only way to reduce salt content is to control it at its source (Agassi et al., 2003). When crops are irrigated with water containing elevated levels of salts, sodium is present in the soil as an exchangeable form and may replace cations adsorbed on the exchange complex of clays, usually calcium and magnesium. This may cause infiltration problems due to the decreased aggregate

stability, clay dispersion and the swelling of expandable clays, which affect the soil's permeability. The sodium adsorption ratio (SAR) is the common parameter used to calculate the ratio of the relative concentration of sodium in relation to that of calcium and magnesium: $SAR = [Na]/([Ca+Mg]/2)^{1/2}$. In arid regions, decreased infiltration rates can result in increased surface runoff during precipitation events, which leads to the decreased availability of water for plants (Suarez et al., 2006). The ability to cope with increasing salinity and SAR in soils is a great challenge given the fact that of the current 230 million ha of irrigated land, 45 million ha (19.5%) are salt-affected (FAO, 2008). Aside from all of the abovementioned potential risks, the quality of TWW is affected by many constantly fluctuating factors. Therefore, the use of TWW for irrigation must be accompanied by intensive monitoring of water, soil and plant parameters, to ensure sustainable crop production with minimal damage to the environment.

Grapevines and salinity

All types of irrigation water (aside from distilled water) contain a certain amount of salts. A combination of many different dissolved salts may contribute to overall salinity levels that may induce plant toxicity and soil damage. Salinity is usually measured as electrical conductivity (EC) of irrigation water and/or the soil-water solution and is measured from a soil-paste extract (EC_e).

The most common method for studying the effect of salinity on grape vines is to sample and analyze leaf petioles growing opposite the fruit clusters at flowering or at harvest (Christensen, 1969). The measured values of the different salt components can be compared with previously published threshold levels for toxicity to grapevines (Reuter and Robinson, 1997).

Grapevines can be characterized either as moderately salt-sensitive (Mass and Hoffman, 1977; Mass, 1990; Francois and Mass, 1994) or salt-tolerant (Downton, 1977b; Garcia and Chabaji, 1993). The salt sensitivity or tolerance of vines is affected by the scion-root combination, irrigation system, irrigation amount and intervals, climate and soil properties. Scion-root combination also alters vine vigor (Yunusa et al., 1997), vine life duration and levels of tolerance and sensitivity to other types of biotic and abiotic stress. It is assumed that rootstocks with better vigor may be more salt tolerant. Mass and

Hoffman (1977) set the threshold value at 1.5 dS m^{-1} of soil saturation paste (EC_e) and each dS m^{-1} unit above that value was found to decrease yield by 9.6%. Zhang et al. (2002) argued that this value is too low, basing their argument on the results of three Australian field experiments that included a wide range of scion-related thresholds (1.8-4 dS m^{-1}), in which they observed 2.3-15% yield reduction per increase of 1 dS m^{-1}. The rootstock of the line 1103 Paulsen (which was also used in the present study) was the most salt tolerant, with no yield reduction until EC_e exceeded about 4 dS m^{-1} (Zhang et al., 2002).

The objectives of the present research were:

(1) To develop a novel irrigation model based on actual evapotranspiration measurements.
(2) To establish the connections between evapotranspiration and canopy and climate seasonal changes.
(3) To determine the application and impact of the irrigation model on grape production in semi-commercial conditions.
(4) To point out the potential risks of TWW application according to the model.
(5) To determine the applicabilty of a novel physiological tool (LPCP) for online monitoring of water balance in leaves of table and wine grapes.
(6) To determine the applicabilty of the LAI-based model and the LPCP on wine grapes.

3. Methodology

Experimental site Lachish – Table grapes cv. Superior Seedless: lysimeters, irrigation model and effluent irrigation.

The following studies were conducted between 1999 and 2009. The experimental area was a one-hectare vineyard of table grapes cv. Superior Seedless (also known as 'Sugraone') grafted onto the '1103 Paulsen' rootstock, located at the Lachish agricultural research and development station in southern Israel (lat. 31.4° N, long. 34.8° E). Vines were trained to a 2-m-high, Y-shaped, open-canopy gable system with six foliage wires on each side, as described in detail by Netzer et al. (2009). Vine spacing was 2 m between vines and 3.5 m between rows. Rows were organized in a north–south direction. During the winter (Jan.-Feb.), vines were pruned into eight fruiting canes with 14 buds per vine. The vineyard structure was similar in the lysimeter rows (cultivar, rootstock, row spacing, training, planting date and pest control).

A drip irrigation system with one line per row and pressure-compensated drippers spaced 0.5 m apart from each other (Netafim, Israel) was employed and was operated by an irrigation control unit (Talgil, Israel). The vines were planted in both the vineyard and the lysimeter tanks April 1997. The water consumption (ET_c) of table grapes was studied using 13 drainage lysimeters located on the borders of the vineyard. The ET_c data obtained from 12 lysimeters were used as the baseline for irrigation treatments in the vineyard. The remaining lysimeter (without a vine) was used for evaporation measurements; vines planted outside of the lysimeter's area shaded this lysimeter. Field- and lysimeter-grown vines were handled similarly over the duration of the experiment, aside from the amounts of water applied.

Lachish vineyard experimental design

The experimental field was divided into nine different irrigation treatment sections, with four replicates per treatment arranged in a randomized block design. Each irrigation treatment was applied to a total of 168 vines, with 10 measured vines per replicate and another 32 border vines. The effects of two factors were examined during 2002-2007 seasons: irrigation water type and amount. Three water types and three irrigation levels: high, medium, and low (nine treatments in all). The daily drip irrigation schedule was

determined on a five-day basis according to data obtained by the lysimeter. In cases of drastic changes in the weather, the irrigation schedule was determined daily. In treatments that included fertilizer, the fertilizer contained 1.29 mM NO_3^-, 0.64 mM NH_4^+, 0.87 mM K and 0.19 mM P and was applied daily through the irrigation system. Standard practices to control insect pests, fungi and weeds were employed each year.

Structure of the lysimeters

As mentioned before, water consumption of table grapevines was measured using 13 drainage lysimeters (Figs. 1-3). One vine (*Vitis vinifera* cv. Superior Seedless) was planted in each lysimeter (excluding the evaporation lysimeter) during March 1997 and full measurements commenced in April 1999. Each lysimeter tank had a volume of 1.3 m^3 and was 1.05 m in diameter and 1.5 m deep. Seven lysimeters were filled with local soil (clay loam soil composed of 30% sand, 28% silt and 42% clay) packed to the original bulk density and the remaining six were filled with tuff gravel (volcanic scoria, 0 to 4 mm diam.). The lysimeters were installed in the ground with their top edges flush with the soil surface. To ensure the drainage of water from the lysimeter into the receiver tank, the bottom of the tank was packed with a 10-cm-thick layer of gravel, 10 cm of rock wool and 10 cm of milled quartz (Netzer et al., 2005). A 3-m-long pipe was attached to the bottom of the each lysimeter. The drainage pipe led to a partially underground tunnel that was constructed parallel to the row containing the lysimeters (Fig. 4). Each lysimeter was irrigated separately from an 80-L water tank positioned 50 cm above the soil surface (Fig. 3), about 2 m away from the lysimeters. The drip line that was connected to the water tank was equipped with four (non-pressure-compensated) 2.4-L h^{-1} drip emitters spaced 30 cm apart. Drainage water was collected in receiver tanks located in a 2.5-m-deep tunnel.

Figure 1. Birdseye view of the lysimeter arrangement and the tunnel (white roof).

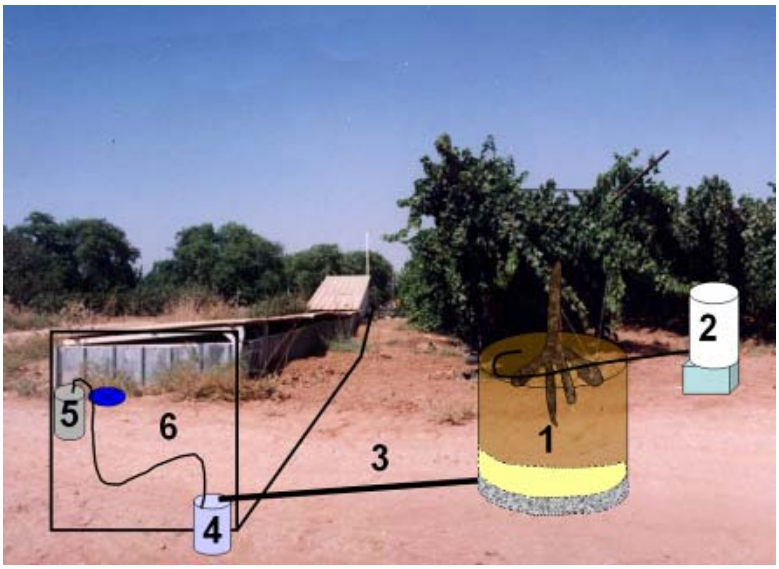
Figure 2. Arrangement of lysimeter tanks in the soil before they were covered.

Figure 3. 80-L irrigation tanks (right) with the irrigation lines of each lysimeter (left).

Figure 4. Inside the tunnel. The drainage pipes lead to collection tanks located under the floor.

Figure 5. Lysimeter scheme:

1. Lysimeter tank
2. Irrigation tank
3. Drainage pipe
4. Collection tank
5. Suction pump and volume-measuring tank
6. Underground tunnel



Lysimeter maintenance

Every morning, each lysimeter was irrigated separately from an 80-L water tank that was refilled manually with a known volume of water. The volume of water that drained through each lysimeter was measured every morning. To ensure that the growth of the vines was not limited by water availability, the volume of water supplied by irrigation exceeded the vines' estimated daily water consumption by 20-30%. Daily irrigation began at 7:00 am and continued for 4-8 hours depending on the amount of water needed at that point during the growing season.

Meteorological data, calculation of ET_o

An automated weather station located at the Lachish research station (about 100 m from the vineyard) monitored solar radiation (CM-11, Kipp & Zonen, Delft, Netherlands), wind speed and direction (type 05103; R. M. Young, Traverse, MI, USA), air temperature and relative humidity (type HMP 45C; Campbell Scientific, Inc., Logan, Utah, USA). Data were collected every 5 min. Reference evapotranspiration (ET_o) was calculated using the Penman-Monteith equation, as modified for the California Irrigation Management Information System (CIMIS; Snyder and Pruitt, 1985).

ET_c and K_c calculations

Daily water consumption, ET_c (liters), was calculated by subtracting the volume of water collected as drainage during a 24-h period from the amount that was supplied by irrigation during the same period. ET_c (mm) was calculated by multiplying the average daily consumption of water per vine, as measured by the lysimeters, by 0.143 (1430 vines per hectare). The daily crop coefficient (K_c) was calculated by dividing daily ET_c (mm day^{-1}) by daily ET_O (mm day^{-1}) (Doorenbos and Pruitt, 1977; Allen et al., 1998).

Leaf area index measurements

The leaf area index (LAI) is a dimensionless variable representing the leaf area per unit ground surface area (Jonckheere et al., 2004). In our case, LAI is the ratio of the total surface area of the leaves (one-side), shoots and fruit (when present) to the unit of land area allocated for each vine. The LAI values of the lysimeter-grown vines and the vineyard-grown vines were estimated several times during the 2001-2005 growing seasons using a non-destructive canopy analysis system (SunScan model SS1-R3-BF3; Delta-T Devices, Cambridge, UK). This canopy analysis system uses a line quantum sensor array that is sensitive to photosynthetically active radiation (PAR). This method of estimating LAI (gap fraction inversion) is based on measurements of light beneath the canopy collected parallel to measurements of above-canopy sun radiation (Grantz and Williams, 1993; Cohen et al., 1997; Wilhelm et al., 2000).

The instrument was operated using the standard protocol recommended by the manufacturer. Each sample consisted of equally spaced readings (20 cm apart) at ground level, starting from the center of the row to half the distance to the adjacent row, with the linear probe positioned parallel to the rows (Fig. 7). LAI values obtained by this non-destructive method were verified by a tissue-destructive method. For those measurements, leaves were harvested (Fig. 8) from two meter-long sections along the row, one meter from each side of the trunk. Leaf area was then measured using an area meter (model 3100; Li-Cor, Lincoln, NE, USA). The leaf areas of 18 sections, two meters each, were measured at different times during the growing seasons. Estimated and measured LAI values were found to be highly correlated ($y = 0.9821x - 0.0366$, $R^2 = 0.983$, $p < 0.0001$, $n = 18$)(Fig. 6). It should be mentioned that, in using this method, we

measured total green surface area, including leaves, shoots and fruits. The "LAI" contributed by the trunk and the canes (0.4-0.6 m^2 m^{-2}) was subtracted from the total LAI.

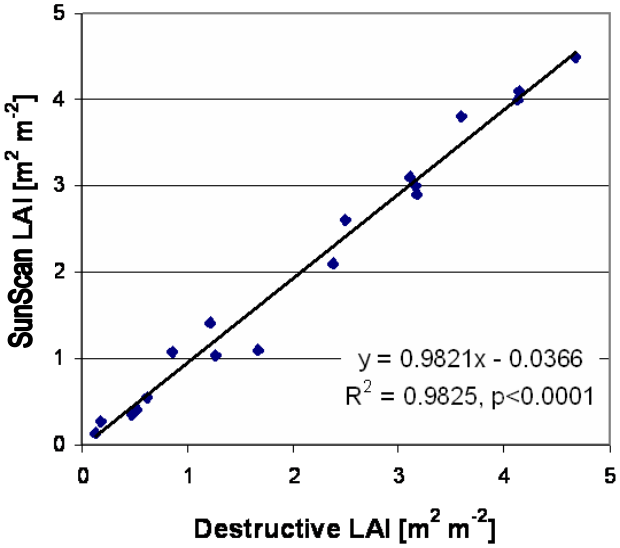


Figure 6. Correlation between estimated (SunScan) and measured LAI values (destructive leaf scan) (n = 18).

Figure 7. LAI measurement equipment. The beam fraction sensor (BFS) is located on top of the tripod (left). The line quantum sensor array and the data logger (Work about) is located beneath the vine canopy. A 20-cm graduated scale is located adjacent to the rows.

Figure 8. Leaf removal and LAI measurement to validate the accuracy of the non-destructive measurements.

Soil sampling and analysis

Soil was sampled annually (2002-2007) during the spring (March) before the beginning of irrigation and in autumn (October), after the end of the irrigation period, so that its chemical and physical characteristics could be examined. Soil samples were taken by soil auger from depths of 0-30, 30-60 and 60-100 cm in the middle row of each plot. Samples were taken from 40 cm perpendicular to the drip line and parallel to the dripper that was closest to the midpoint between two vines. Saturated paste extracts of soils oven-dried at 65°C were chemically analyzed.

Soil saturated paste and chemical analysis

Soil pH was determined by plugging a pH Orion combined electrode directly into the saturated paste (Rhoades, 1996). Soil electrical conductivity and concentrations of Ca, Mg, K, Cl, and NO_3^- were analyzed in the solution extracted from the saturated paste (Rhoades, 1996). The concentration of Ca^{2+} + Mg^{2+} was determined by EDTA titration and the concentration of NO_3^- was determined with Orion specific electrode. K concentration was determined using a 410 Sherwood flame photometer and electrical conductivity was determined using a platinum cell electrode (Standard Methods, 2005). Chloride was determined by potensiometric titration with a Ag-AgCl electrode (APHA, 2005). Soil ammonium was extracted with 1 M KCl according to the Nesler method (Greweling and Peech, 1960). Soil Zn, Fe, Cu, Mn were extracted with DTPA-TEA-$CaCl_2$ solution as described by Loeppert and Inskeep (1996) and were analyzed using a 220FS Varian atomic absorption spectrophotometer. Soil phosphate content was determined using the Olsen method, as described by Kuo (1996). Soil calcium carbonate content was determined based on the gravimetric loss of carbonates as carbon dioxide in the presence of excess hydrochloric acid (U.S. Salinity Laboratory staff, 1954). Soil organic matter was determined by wet oxidation with dichromate (Nelson and Sommers, 1982)

Leaf sampling and chemical analysis

From each replicate, 30 leaves (basal leaves growing opposite a bunch cluster) were sampled during harvest (mid-July to mid-August). Leaves were rinsed in tap water several times with a final rinse in double-distilled water. Petioles and blades were

separated and oven-dried at 70°C for 72 h. Samples were subsequently pulverized in an electric mill and 150 mg of dry matter was digested with 5 ml of concentrated reagent-grade nitric acid at 130°C. The digest was brought to a volume of 50 ml with double-distilled water and kept at 4°C in the dark until Na^+ concentrations could be analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Arcos, Spectro, Kleve, Germany).

For chloride and nitrate analyses, 100 mg of dry matter was shaken for 2 h with 5 ml of double-distilled water. The mixture was filtered using Whatman No. 1 filter papers. Chloride contents were determined by the silver ion-titration method with a Corning 926 automatic chloridometer (Corning Ltd., Halstead Essex, UK), according to the method described by Gilliam (1971). A hand-held reflectometer RQflex2 (Merck, Darmstadt, Germany) was used to measure nitrate concentration.

Trunk wood and xylem sap sampling and chemical analysis

Samples of trunk wood and xylem sap were taken during March 2007, two weeks before budbreak. Samples of trunk wood (Fig. 9) were obtained from four vines from each plot, 50 cm above ground level using a 7-mm-diameter tree corer (Mattson, Mechaniska, AB, Mora, Sweden). All samples were placed in 50-ml centrifuge vials and kept cool as they were transported to the laboratory (maximum 4 h). Each core was separated into wood and bark tissues, using a sharp blade, before drying for 72 h at 70°C. Dry material was ground into a fine dust using an electric mill and digested by the same method used for the leaves.

A xylem sap collection device (Fig. 11) was inserted into each pinhole left by the sample coring. The sap-collection device was composed of a 20-cm long, 8-mm diameter plastic tube and a 50-ml centrifuge vial covered with aluminum foil. One end of the plastic tube was attached to the centrifuge vial, while the other end was attached to the pinhole created by the corer. A small (10 cm long, 1 mm diam.) plastic tube was connected to the centrifuge tube cap to release air pressure. The device was attached to the vine for 24 h. The surrounding trunk area was wrapped in plastic wrap to prevent moisture loss and contamination.

Sufficient root pressure is established during the early spring season as result of root activity, which is accelerated by increased soil temperatures. When temperatures rise and

soil water content increases, xylem sap bleeding will be observed when canes are cut (Fig. 10). The xylem sap collection device (Fig. 11) should be installed during this period. Once budbreak has occurred and transpiration has resumed, the hydraulic pressure in the xylem will be negative and the xylem bleeding will stop. Installation during budbreak will not result in xylem sap collection.

Figure 9. (left) Wood sampling using a 7-mm-diam. tree corer.

Figure 10. (right) Xylem sap bleeding from a cane.

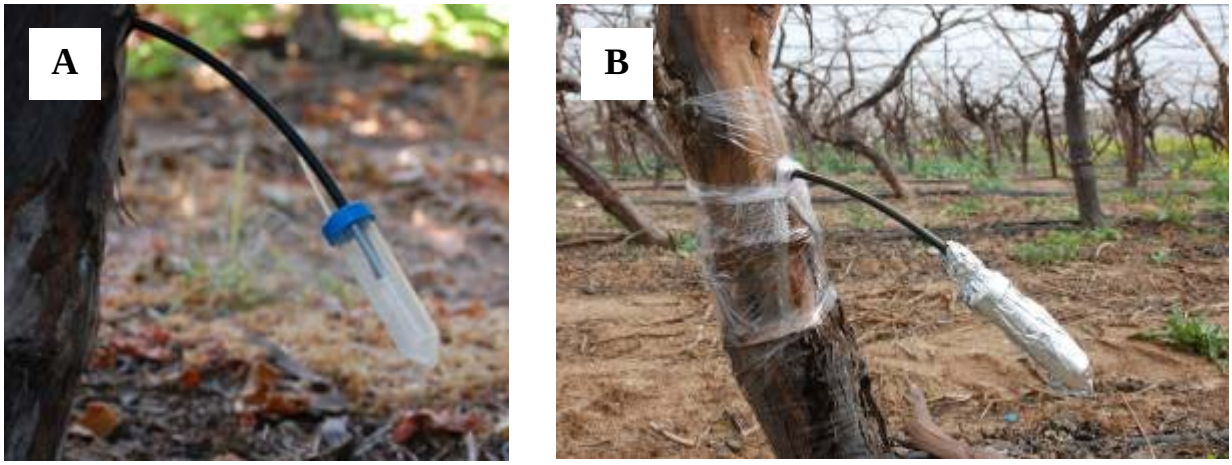


Figure 11. Xylem sap collection device. **A.** During installation. **B.** Tightly attached to the trunk and covered with aluminum foil after completion of installation.

Leaf patch clamp pressure probe –LPCP

The magnetic LPCP probe is composed of two planar circular metal pads integrated into two magnets (Fig. 12A). The lower pad contains a receptacle for integration of the pressure sensor chip. Turgescence is determined by measuring the pressure transfer function of the leaf patch. The probes were connected to small battery-powered telemetric transmitters (Teltow, Germany). The transmitters (Fig. 12B) read and amplified the analog signals of the probes. Every 5 min, the digitized data were wirelessly sent, together with the transmitter ID code, to an RF receiver unit via the ISM band of 433 MHz, over a distance of more than 100 m. The receiver was connected to a GPRS modem via an RS-232 interface. Using a mobile communication network, the data and the time stamps were transferred to an Internet server at the University Würzburg, Germany (NTBB Systemtechnik GmbH, Zeuthen, Germany), enabling remote control of the experiments. Solar photovoltaic panels (Fig. 12C) provided electricity for this system. For the device's measurements, a small leaf patch is used as a sensing element for turgor pressure changes in the entire leaf. The stomata in the patch must be closed and the patch must be in hydraulic contact with its surroundings when the magnetic pads are attached to the leaf.

Leaf turgescence is determined by measuring the pressure transfer function of the leaf patch, i.e., by measuring the output leaf patch pressure (P_p) upon application of an external pressure (P_{clamp}). P_{clamp} can be adjusted by changing the distance between the upper and lower magnet. Zimmermann et al. (2008) has shown that P_p is a power function of the turgor pressure (P_c):

$$P_p = \left(\frac{b}{aP_c + b} \right)^{\frac{1}{a}} \cdot F_a \cdot P_{clamp}$$

where a and b are constants. F_a is the leaf-specific attenuation factor, which takes into account the fact that only a constant fraction of P_{clamp} arrives at the cell level, as a result of losses due to the compressibility of the silicone in the sensor chip and of leaf-specific structural elements (cuticle and cell walls).

Control experiments performed in an accessible climate chamber showed that any effects

of temperature on the pressure reading of the probes could be excluded (<2 kPa between 10°C to 35°C). Furthermore, pressure probes mounted in a non-contact mode close to the leaf surface also showed only negligible pressure changes over the entire temperature range under field conditions. A detailed description and theoretical background is reported in the 4th article (pages 71-82 in this work).

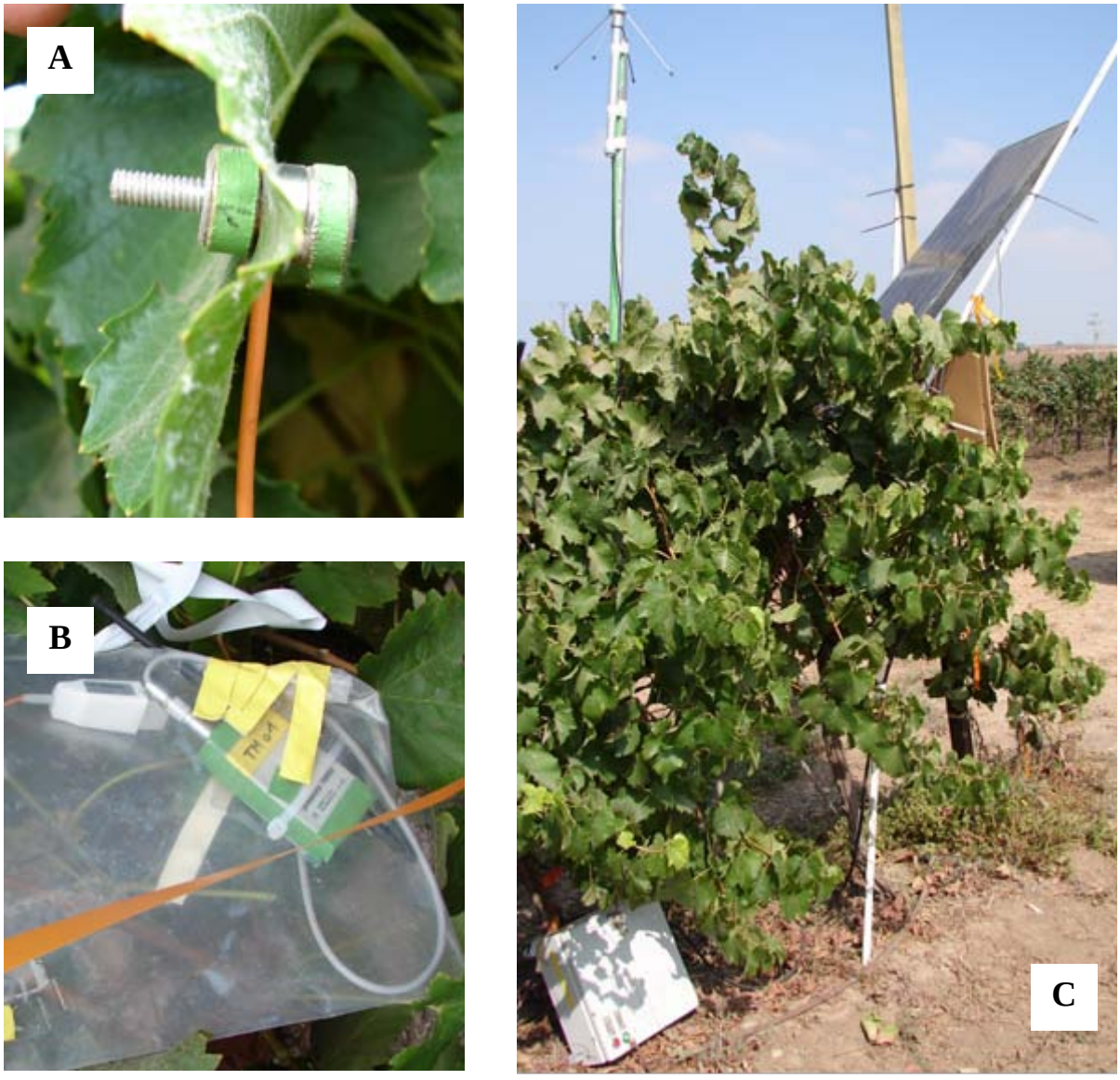


Figure 12. A. Leaf patch clamp pressure probe attached to vine leaf. **B.** Battery-powered transmitter unit protected against humidity in a plastic bag. **C.** Receiver unit (bottom left), mobile communication antenna (upper left), solar photovoltaic modules (upper right).

4. Results

4.1 First publication

Netzer Y, Yao C, Shenker M, Cohen S, Bravdo B and Schwartz A (2005) **Water consumption of 'Superior Seedless' grapevines grown in a semiarid region.** Acta Hort (ISHS) 689:399-406

Water Consumption of 'Superior' Grapevines Grown in a Semiarid Region

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Abstract

Water use of grapevines (*Vitis vinifera* 'Superior') trained to a gable system was measured in 12 drainage lysimeters for 4 growing seasons (1999, 2001-2003) in a semiarid region of southern Israel. The drainage lysimeters, with a volume of 1.3 m^3 each, were installed in a 1 ha vineyard. The vines were drip irrigated with 4 emitters at a rate of 2.4 L h^{-1}. The volume of water supplied each day exceeded the estimated vine water consumption by 20%. Maximum crop evapotranspiration (ET_c) ranged from 7.6 to 8.2 mm d^{-1} (based on an area of 7 m^2 per vine) across the 4-year study. Seasonal ET_c ranged from 988 to 1081 mm. The seasonal pattern of the crop coefficient (K_c) was similar from one year to the next. Monthly leaf area index (LAI) was measured the last two years of the study. The LAI measured with the SunScan Canopy Analysis System was highly correlated (R^2 = 0.99) with direct leaf area measurements. Maximum LAI was 4.2 and 4.9 in 2002 and 2003, respectively. The increase in the seasonal K_c was explained primarily by the increase in LAI (R^2 = 0.84). The high water consumption of the lysimeter-grown vines may be explained by higher LAI and stomatal conductance values due to the fact that those vines were supplied with unlimited amounts of water.

INTRODUCTION

Most of the water supplied to a crop returns to the atmosphere by evaporation from the soil and via transpiration from the canopy. The evaporative demand of the atmosphere along with canopy characteristics, such as stomatal conductance, and the irrigation method are the main factors affecting evapotranspiration (ET). The water use of grapevines, grown in different regions using different management practices, has been published (Behboudian et al., 2001; Bravdo and Hepner, 1987; Van Rooyen, 1980; Yunusa et al., 1997). The methods used to measure water use in grapevines have included models based on water and soil parameters (Erie et al., 1982; Van Rooyen et al., 1980), meteorological data (Oliver and Sene, 1992; Yunusa et al., 2000), sap flow sensors (Braun and Schmid, 1999; Ginester et al., 1998) and weighing (Williams, 1999; Williams et al., 2003) and drainage lysimeters (Evans et al., 1993; Prior and Grieve, 1986; Van Rooyen et al., 1980).

The relationship between ET_c and reference evapotranspiration (ET_o) is termed the crop coefficient ($K_c = ET_c/ET_o$) (Doorenbos and Pruitt, 1975). As vine water consumption changes during the growing season and due to cultural practices including trellis type and row spacing, it is expected that the seasonal K_c would also be affected.

One of the objectives of the present study was to determine seasonal crop water use of *Vitis vinifera* 'Superior' table grapevines grown under unlimited water supply in a semiarid region of southern Israel. Another objective was to establish seasonal K_c curves for these grapevines.

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Acta Hort. 689, ISHS 2005

MATERIALS AND METHODS

Water consumption of 'Superior' table grapes was determined with the use of 12 drainage lysimeters. The lysimeters were located in a 1 ha vineyard at the Lachish agricultural research station in Israel (31.4° N, 34.8° E). The vines, grafted onto '1103 Paulsen' rootstock, were planted both in the vineyard and in the lysimeter tanks in April 1997. The vineyard soil was deep and well drained, and was composed of 30% sand, 28% silt and 42% clay. Rows were oriented north to south. Vine spacing was 2 m within rows and 3.5 m between rows (1430 vines ha^{-1}). The vines were trained to a double canopy gable system with 8 fruiting canes per vine of 14 buds per cane. The volume of each lysimeter tank was 1.30 m^3 and its dimensions were 1.05 m in diameter and 1.5 m in depth. Six lysimeters were filled with undisturbed local soil and the remaining six were filled with tuff gravel (up to 4 mm in diameter). The lysimeters were installed in the ground with their surface equal to the soil surface of the vineyard. To assure the free flow of drainage water from the lysimeter to a receiver tank, the bottom three layers of the tanks were 10 cm of gravel, 10 cm of rock wool and 10 cm of milled quartz.

The vines in the lysimeters were irrigated above ground with 4 emitters at a discharge rate of 2.4 L h^{-1}. The water that percolated through the lysimeters was collected in the receiver tanks and the volume was measured each morning. To ensure that the drainage solution could be obtained daily, the volume of water supplied by irrigation exceeded the estimated daily water consumption by at least 20%. Daily irrigation began at 0700 h and continued for at least 6 h. The receiver tanks were located in a 2 m deep underground tunnel constructed along the row of lysimeters. Apart from irrigation, field and lysimeter-grown vines were treated similarly over the 5 year period.

Daily vine ET_c was calculated by subtracting the volume of water collected as drainage over a 24 h period from the amount that was supplied by irrigation for the same period. Vine ET_c was calculated multiplying the average daily water consumption per vine by 143 (143 vines per 0.1 hectare). Reference ET was calculated according to the Penman-Monteith equation as modified for the California Irrigation Management Information System (CIMIS) (Snyder et al., 1985). The meteorological data for calculation of ET_o were obtained from a weather station at the Lachish research station near the site of the experiment.

Leaf Area Index (LAI) is the ratio of total green surfaces, including leaves, shoots and fruit (when present) to unit of land area allocated for each vine. The LAI of the lysimeter grown vines and of field-grown vines was estimated several times during the 2002 and 2003 growing seasons, using a canopy analysis system (SunScan model SS1-R3-BF3; Delta-T Devices, Cambridge, UK). The canopy analysis system uses a line quantum sensor array sensitive to photosynthetically active radiation (PAR). The method of estimating LAI ('gap fraction inversion') is based on light measurements beneath the canopy (Cohen et al., 1997; Grantz and Williams, 1993; Wilhelm et al., 2003). The analyzer was operated using the standard protocol recommended by the manufacturer. Each sample consisted of equally spaced observations (20 cm apart), starting from the center of the row to half the distance between adjacent rows with the linear probe positioned parallel to the rows (Cohen et al., 1997). The LAI values obtained by this method were correlated with destructive harvesting of leaves along the row, 1 m on either side of the vine's trunk. Leaf area was then measured using an area meter (model 3100; Li-Cor, Lincoln, Nebraska). Leaf area of 14 sections was measured at different times during the growing season. A comparison between the two methods indicated they were highly correlated with one another ($y = 0.0133 + 0.983x$, $R^2 = 0.99$, $n = 14$).

RESULTS AND DISCUSSION

Budbreak for this cultivar occurred at the beginning of March between days of year (DOYs) 60 and 74 (Table 1). Harvest generally began the beginning of August at 15.5 °Brix. A LAI of 1.0, equivalent to a leaf area of ~ 7.0 m^2 per vine was obtained the middle of April. The LAI reached a maximum of 4.2 (29.3 m^2 $vine^{-1}$) and 4.9 (34.2 m^2 $vine^{-1}$) the beginning of August in 2002 and 2003, respectively (Fig.1). The LAI gradually

decreased until late fall.

The seasonal ET_o curves are typical for the hot and dry summers of the Mediterranean region. Maximum ET_o was measured during July (Fig. 2A). Seasonal ET_o and E_{pan} from April to October of the 1999 – 2003 seasons were 1142 and 1216 mm, respectively (Table 1). The average ET_o was 4.6 mm d^{-1} in early April and 7.1 mm d^{-1} during July each year. There was a general decrease in ET_o from September onwards each year.

Seasonal ET_c for 4 years of the study ranged from 6913 to 7559 L $vine^{-1}$; 988 and 1081 mm, respectively (Table 1). Average ET_c increased from 1.8 mm d^{-1} in April (DOY 105) to a maximum of 7.7 mm d^{-1} in August (DOY 217) when ET_o values were 5.2 and 6.6 mm d^{-1}, respectively (Fig. 2A). Crop ET exceeded ET_o at the end of June (DOY 176) and remained higher during July and August. A gradual decrease in ET_c was observed from the end of August to the end of the season.

The crop coefficient K_c is a dimensionless number obtained by dividing ET_c by ET_o (Doorenbos and Pruitt, 1975) and it can be used to estimate daily vine water consumption in vineyards using the same cultivar and cultural practices and grown under similar environmental conditions. The seasonal K_c curve for 'Superior' grown in the Lachish district was approximately 0.35 at the end of March, just after budbreak (Fig. 2B) and reached a value of 1.2 during September and October (DOY 250-304). The K_c was a linear function of LAI (Fig. 3). The maximum K_c of 1.2 reported here is higher than the published values for grapevines (Allen et al., 1998; Williams et al., 2003) but close to that calculated by Stevens and Harvey (1996).

The average seasonal water consumption of 1027 mm measured here is approximately 20% greater than that for 'Thompson Seedless' grown in the San Joaquin Valley of California (Williams et al., 2003) even though seasonal ET_o was similar at both locations. The difference in ET_c between this and the 'Thompson Seedless' studies may be explained due to different cultivars, differences in trellis system and greater leaf area of the 'Superior' vines.

Calculated ET_c of vines in the lysimeters from budbreak to until harvest (659 mm) is greater than the amount of water traditionally used by the growers in the area during the same period (400 mm). Similarly, ET_c from harvest through the end of the season measured in this study (476 mm) is about double the amount used by growers (250 mm). The higher ET_c measured in the present experiment is due to the fact that vines in the drainage lysimeters were irrigated with water amounts significantly higher than ET_c and therefore the vines did not experience water stress at any time. Additionally, growers tend to reduce irrigation substantially after harvest causing a gradual increase in water stress but still maintaining a viable canopy through the end of the season. The ET_c values obtained in the lysimeters served as a reference for an irrigation experiment conducted close by where values of 0.4, 0.6 and 0.8 of calculated ET_c were applied (not shown).

CONCLUSIONS

Vine ET_c at the beginning of the season was about 30% that of ET_o while ET_c exceeded ET_o beginning the end of June and remained higher through July and August. Average yearly ET_c and ET_o were 1028 mm and 1096 mm, respectively. The K_c was 0.35 at the end of March and increased to a maximum of 1.2 in August. The highest LAI was measured at the beginning of August, just before harvest. It was 4.2 and 4.9 m^2 m^{-2} in 2002 and 2003, respectively. The K_c was a linear function of LAI. The seasonal ET_c measured here using the lysimeters was almost double the amount of water (650 mm) used by the growers in this area. The high water consumption of the lysimeter-grown vines may be explained by higher stomatal conductance due to the fact that the vines in the lysimeters were supplied with an unlimited amount of water.

Literature Cited

Allen, R.A., Pereira, L.S., Raes, D. and Smith, M. 1998. Crop evapotranspiration: guidelines for computing crop water requirements. FAO irrigation and drainage paper

56, Rome, Italy.
Ayars, J.E. and Hutmacher, R.B. 1994. Crop coefficients for irrigating cotton in the presence of ground water. Irrig. Sci. 15:42-52.
Ayars, J.E., Johnson, R.S., Phene, C.J., Trout, T.J., Clark, D.A. and Mead, R.M. 2003. Water use by drip-irrigated late-season peaches. Irrig. Sci. 22:187-194.
Behboudian, M.H. and Singh, Z. 2001. Water relations and irrigation scheduling in grapevine. Hort. Rev. 27:189-225.
Braun, P. and Schmid, J. 1999. Sap flow measurements in grapevines (*Vitis vinifera* L.) - 1. Stem morphology and use of the heat balance method. Plant Soil 215:39-45.
Bravdo, B. and Hepner, Y. 1987. Water management and effect on fruit quality in grapevines. In: Lee T (ed) Proceedings of the 6th Australian Wine Industry Technical Conference, 14–17 July, Adelaide. Australian Industrial Publications, Adelaide. p. 150–158.
Chalmers, D.J., Andrews, P.K., Harris, K.M., Cameron, E.A. and Caspari, H.W. 1992. Performance of drainage lysimeters for evaluation of water-use by asian pears. Hortscience 27:263-265.
Cohen, S., Rao, R.S. and Cohen, Y. 1997. Canopy transmittance inversion using a line quantum probe for a row crop. Agric. For. Meteorol. 86:225-334.
Doorenbos, J. and Pruitt, W.O. 1975. Guidelines for predicting crop water requirements. FAO Irrigation and Drainage Paper 24, Rome, Italy.
Erie, L.J., French, O.F., Bucks, D.A. and Harris, K. 1982. Consumptive use of water by major crops in the southwestern United States. (Conservation research report no. 29) United States Department of Agriculture, Washington. D.C.
Evans, R.G., Spayd, S.E., Wample, R.L., Kroeger, M.W. and Mahan, M.O. 1993.Water use of *Vitis vinifera* grapes in Washington. Agric. Water Manag. 23:109-124.
Ginestar, C., Eastham, J., Gray, S. and Iland, P. 1998. Use of sap-flow sensors to schedule vineyard irrigation. I. Effects of post-veraison water deficits on water relations, vine growth, and yield of Shiraz grapevines. Amer. J. Enol. Vitic. 49:413-420.
Grantz, D.A. and Williams, L.E. 1993. An empirical protocol for indirect measurement of leaf area index in grape *Vitis vinifera* L. Hortscience 28:777-779.
Liu, C.M., Zhang, X.Y. and Zhang, Y.Q. 2002. Determination of daily evaporation and evapotranspiration of winter wheat and maize by large-scale weighing lysimeter and micro-lysimeter. Agric. For. Meteorol. 111:109-120.
Oliver, H.R. and Sene, K.J. 1992. Energy and water balances of developing vines. Agric. For. Meteorol. 62:167-185.
Prior, L.D. and Grieve, A.M. 1987. Water used and irrigation requirements of grapevines. In: Lee T (ed) Proceedings of the 6th Australian Wine Industry Technical Conference, 14–17 July, Adelaide. Australian Industrial Publications, Adelaide. p. 165-168.
Smart, R.E. and Coombe, B.J. 1983. Water relations of grapevines. In Kozlowski T.T. (ed) Water deficits and plant growth. VII:137-196.
Van Rooyen, F.C. 1980. The water requirements of table grapes. Decid. Fruit Grower, South Africa, March, p.100-106.
Van Rooyen, F.C., Weber, H.W. and Levin, I. 1980. The response of grapes to a manipulation of the soil–plant–atmosphere continuum.II. Plant–water relationships. Agrochemophysica. 12:69-74.
Wilhelm, W.W., Ruwe, K. and Schlemmer, M.R. 2000. Comparison of three leaf area index meters in a corn canopy. Crop Sci. 40:1179-1183.
Williams, L.E., Phene, C.J., Grimes, D.W. and Trout, T.J. 2003. Water use of mature Thompson Seedless grapevines in California. Irrig. Sci. 22:11-18.
Williams, L.E. 1998. Water use of Thompson Seedless grapevines measured with a weighing lysimeters during a late season dry down period. Proc. 1st ISHS Workshop on Water Realations of Grapevines. Acta Hort. 493:161-167.
Yang, S.L., Aydin, M., Yano, T. and Li, X. 2003. Evapotranspiration of orange trees in greenhouse lysimeters. Irrig. Sci. 21:145-149.
Yunusa, I.A.M., Walker, R.R., and Blackmore, D.H. 1997. Characterization of water use

by Sultana grapevines on their own roots or on Ramsy rootstock drip-irrigated with water of different salinities. Irrig. Sci. 17:77-86.
Yunusa, I.A.M., Walker, R.R., Loveys, B.R. and Blackmore, D.H. 2000. Determination of transpiration in irrigated grapevines: comparison of the heat-pulse technique with gravimetric and micrometeorological methods. Irrig. Sci. 20:1-8.

Tables

Table 1. Seasonal crop water use (ET_c) of 'Superior' grapevines and reference evapotranspiration (ET_o) measured from Day of Year 91 to 282. Vine ET_c was measured with 12 drainage lysimeters with a single vine in each lysimeter. Data for the calculation of ET_o was obtained from a weather station located at the research station, near the vineyard. Date of 50% budbreak and harvest for the lysimeter's vines are given.

Year	ET_c (L vine^{-1})	ET_c (mm)	ET_o (mm)	E_{pan} (mm)	Date of 50% Budbreak (DOY)	Date of harvest (DOY)
1999	7559	1081	1100	1225	12 March (71)	14 July (195)
2000*			1148	1288	15 March (74)	24 July (205)
2001	7259	1038	1151	1207	01 March (60)	13 August (225)
2002**	7020	1004	1011	1145	05 March (64)	8 August (220)
2003***	6913	988	1120		13 March (72)	5 August (217)

* ET_c data not given due to technical problems

** measured from Day of Year 121-282. The ET_c data were collected from 10 lysimeters.

*** measured from Day of Year 101-282. The ET_c data were collected from 8 lysimeters.

Figures

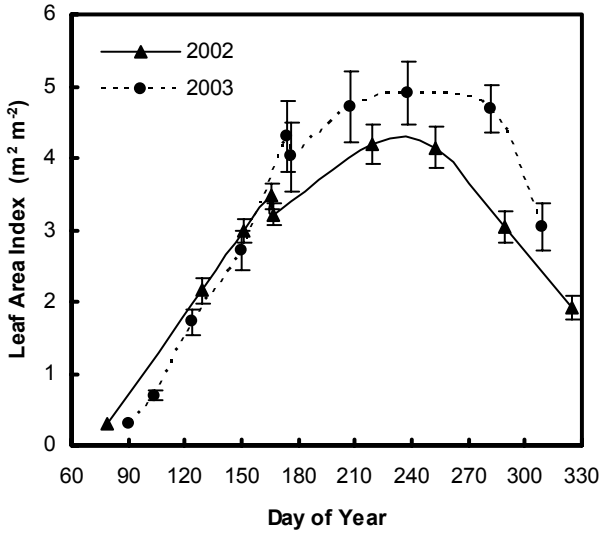

Fig. 1. The seasonal course of the Leaf Area Index (LAI) measured on 'Superior' grapevines grown in the lysimeters. LAI was estimated during the 2002 and 2003 growing seasons using a non-destructive method. Each data point represents an average of 10 vines in 2002 and 8 vines in 2003. Bars represent two times the S.E. The LAI curve for 2002 was best described by the equation: $y = -0.0002x^2 + 0.0831x - 5.2059$, $R^2 = 0.9708$ and that for 2003 by the equation $y = -0.0002x^2 + 0.0991x - 6.8899$, $R^2 = 0.9348$.

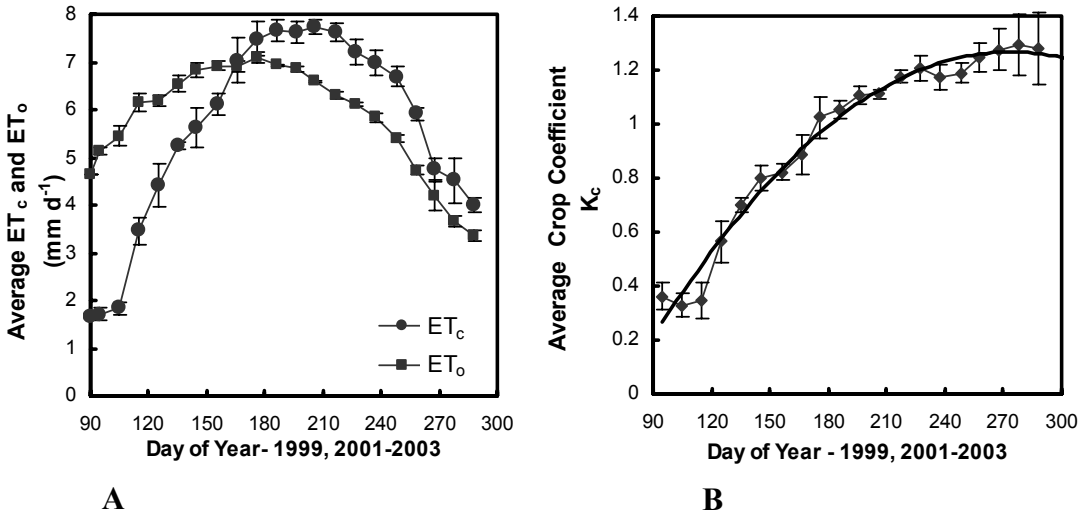

Fig. 2. (A) Seasonal curves of ET_c of 'Superior' grapevines measured with drainage lysimeters and ET_o. Each data point presents an average of 4 years, 1999 and 2001 to 2003. (B). Seasonal K_c curve averaged across 4 years. The seasonal K_c values were fitted to the following equation: $y = -0.00003x^2 + 0.0172x - 1.112$, $R^2 = 0.9783$. Error bars respresent two times the S.E. of the mean.

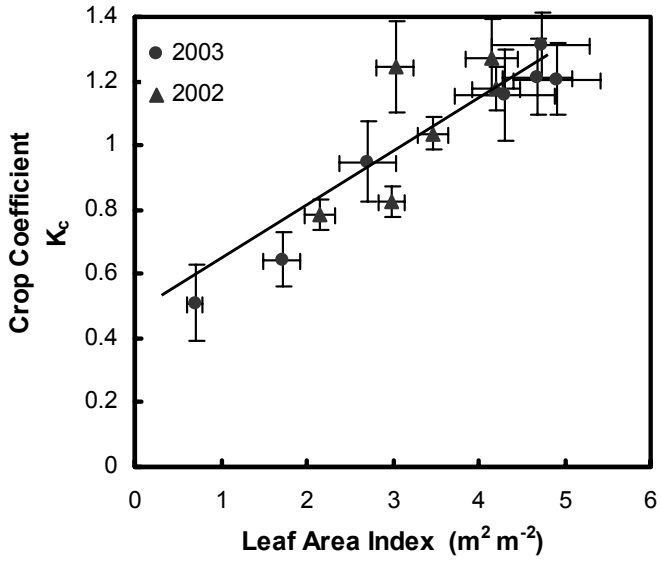

Fig. 3. The relationship between the Crop Coefficient (K_c) and Leaf Area Index (LAI) measured on 'Superior' grapevines. Each data point is the mean LAI value of 10 vines in 2002 and 8 vines in 2003 and an average (K_c) of 20 days. K_c values were fitted to the following equation: y = 0.1843x + 0.4051, R^2 = 0.8386. Error bars represent two times the S.E. of the mean.

4.2 Second publication

Netzer Y, Yao C, Shenker M, Cohen S, Bravdo B, Schwartz A (2009) **Water use and the development of seasonal crop coefficients for Superior Seedless grapevines trained to an open-gable trellis system.** Irrig Sci. 27: 109-120. doi: 10.1007/s00271-008-0124-1

Irrig Sci (2009) 27:109–120
DOI 10.1007/s00271-008-0124-1

ORIGINAL PAPER

Water use and the development of seasonal crop coefficients for Superior Seedless grapevines trained to an open-gable trellis system

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Abstract Water consumption of table grapevines (*Vitis vinifera* cv. Superior Seedless) trained to a large open-canopy gable system was measured during six growing seasons (1999, 2001–2005) using 12 drainage lysimeters. The lysimeters (1.3 m^3 each) were installed as part of a one-hectare vineyard in a semi-arid region in southern Israel. Water consumption of the lysimeter-grown vines (ET_c) was used as the basis for the calculation of irrigation applications in the vineyard. Three irrigation treatments, 80% (high), 60% (medium) and 40% (low) of ET_c of the lysimeter-grown vines, were applied in the vineyard. Reference evapotranspiration (ET_o) was calculated from regional meteorological data according to the Penman–Monteith equation. Seasonal curves for the crop coefficient (K_c) were calculated as $K_c = ET_c/ET_o$. Maximum ET_c values in different seasons ranged from 7.26 to 8.59 mm day^{-1} and seasonal ET_c (from DOY 91 through DOY 304) ranged from 1,087 to 1,348 mm over the six growing seasons. Leaf area index (LAI) was measured monthly using the SunScan Canopy Analysis System. Maximum LAI ranged from 4.2 to 6.2 $m^2\ m^{-2}$ for the 2002–2005 seasons. A second-order polynomial curve relating K_c to LAI ($R^2 = 0.907$, $P < 0.0001$) is proposed as the basis for efficient irrigation management. The effects of the irrigation treatments on canopy growth and yield are presented. The high ET_c and K_c values that were observed are explained by the wide canopy layout that characterize the large open-gable trellis system.

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Introduction

The need to optimize water use in table grapes has become more important given the decrease in the amount of water available for agricultural use and the increase in its cost. This situation underscores the need for appropriate and effective water management systems to improve fruit production efficiency (Fuchs 2007).

Most of the water supplied to a crop returns to the atmosphere via transpiration from leaves and evaporation from the soil. The term evapotranspiration (ET) refers to the total amount of water that is evaporated and water that is transpired. The evaporative demand of the atmosphere, canopy characteristics, stomatal conductance and irrigation practices are the main factors affecting ET. Any biotic or abiotic stress on the crop may also affect its water use patterns.

The region of Lachish, in southern Israel, is one of the largest table grape-growing areas in the country. Most of the vineyards in the area are planted with the table grape *Vitis vinifera* cv. Superior Seedless trained on large open-gable trellis systems. The climate of the region is representative of Mediterranean semi-arid areas, with an average annual precipitation of 385 mm between November and March. Summer irrigation is essential for the vines to reach their full yield potential. Vineyards in this area are drip-irrigated daily, based on the accumulated knowledge of the growers supported mainly by the use of tensiometers for measuring water tension in the soil.

The characterization of water use of grapevines grown in different regions according to different management practices has received the attention of many researchers (van Rooyen et al. 1980; Bravdo and Hepner 1987; Yunusa et al. 1997a, b; Behboudian and Singh 2001; Williams et al. 2003a, b). If water availability in the soil is insufficient to meet the ET demand, reduced yields can be expected. It has been well established that growth, as well as yield components like number of clusters and berry size, are sensitive to reductions in water availability (Smart and Coombe 1983; Matthews et al. 1987; Matthews and Anderson 1989; Hamman and Dami 2000; Salon et al. 2005; Shellie 2006). In contrast, excessive irrigation is costly and can cause vigorous vegetative growth that leads to reduced sugar accumulation in the berries and to the shading of clusters (Bravdo and Hepner 1987), as well as the percolation of water below the root zone, leaching nitrate and other chemicals into groundwater. Excessive canopy growth may increase the need for canopy management treatments and increase the severity to plant diseases (personal observation).

Doorenbos and Pruitt (1977) and Allen et al. (1998) described the crop coefficient of a particular crop (K_c) as the ratio of experimentally determined evapotranspiration (ET_c) to the reference evapotranspiration (ET_o) calculated from weather data ($K_c = ET_c/ET_o$).

Crop coefficient values for grapevine may vary with cultural practices and between modes of trellising (Williams and Ayars 2005b). K_c changes over the course of the growing season, along with the increase in leaf area index (LAI) and the crop's different phenological stages (Peacock et al. 1987; Jagtap and Jones 1989; Grimes and Williams 1990).

Lysimeters have been used for many years as the standard for measuring water consumption. Measurements have been made in woody species such as peaches (Ayars et al. 2003), oranges (Yang et al. 2003) and pears (Chalmers et al. 1992), and in herbaceous species such as wheat and maize (Liu et al. 2002) and cotton (Ayars and Hutmacher 1994). The technical methods employed to measure the water use of grapevine include models based on water and soil parameters (van Rooyen et al. 1980; Erie et al. 1982), models based on meteorological data (Oliver and Sene 1992; Yunusa et al. 2000) and monitoring the flow of xylem sap in the stem (Eastham and Gray 1998; Ginestar et al. 1998; Braun and Schmid 1999; Tarara and Ferguson 2001) and various other physiological parameters, as recently reviewed by Cifre et al. (2005). Measurements of ET_c in mature vines grown under different environmental conditions and using different training and agro-technical practices have been made using drainage lysimeters (van Rooyen et al. 1980; Prior and Grieve 1987; Evans et al. 1993; Shani and Ben-Gal 2005) and weighing lysimeters (Williams 1998; Williams et al. 2003a, b; Williams and Ayars 2005b). In the present study, we used drainage lysimeters to quantify ET_c of a table grape vineyard trained to a large trellis system in a semi-arid region with no summer rains, in which growth and yield are totally dependent on intensive irrigation. The measured ET_c and meteorological data were used to establish a seasonal K_c curve to be used as the basis for irrigation management. Since there may be some season-to-season variation in canopy size, the use of the relationships between K_c and LAI and between K_c and growing degree days (GDD) for the estimation of ET_c is also discussed.

The objectives of the present research were:

(1) To determine seasonal crop evapotranspiration and the crop coefficient of 'Superior Seedless' table grapevine trained to large trellis systems in a semi-arid region;
(2) To establish the relationships between the seasonal changes in LAI and changes in ET_c and K_c.
(3) To study the effect of irrigation at three ET_c levels on vegetative growth and grape yield.

Material and methods

A 7-year (1999–2005) study to determine ET_c of grapevines used for table grape production was conducted in a one-hectare vineyard of 'Superior Seedless' (also known as 'Sugarone') grafted onto 1103 'Paulsen' rootstock. The vines were planted in April 1997 at the Lachish agricultural research station in southern Israel (lat. 31.4°N, long. 34.8°E) in both the drainage lysimeters and the surrounding vineyard. Water consumption of single vines, ET_c, was measured using drainage lysimeters that were installed on the border row of the vineyard. To minimize the edge effect, a 2-m-high wind-breaking screen was erected parallel to the lysimeter's row 2 m from the middle of the row. Except for the difference in irrigation, the vineyard- and lysimeter-grown vines were established and treated similarly (row spacing, training, pruning, pest control and canopy management).

Twelve lysimeters were planted with one vine per lysimeter and one lysimeter was left unplanted, but was irrigated like the others (termed evaporation lysimeter). The soil surface of the lysimeter without a grapevine was shaded by the canopy of the adjacent vine to the same degree as the soil surface of the planted lysimeters. For technical reasons, the evaporation lysimeter was used only during the 2004 and 2005 growing seasons.

Lysimeters: structure and maintenance

Each lysimeter tank was 1.05 m in diameter and 1.5-m deep, for a total volume of 1.30 m^3. Seven lysimeters were filled with local soil (clay loam soil composed of 30% sand, 28% silt and 42% clay) packed to the original bulk density

and the remaining six were filled with tuff gravel (volcanic scoria, 0–4 mm in diameter). The lysimeters were installed in the ground with their top surfaces flush with the soil surface. To ensure drainage of water from the lysimeter into the receiver tank, the bottom of the tank was packed with a 10-cm-thick layer of gravel, 10 cm of rock wool and 10 cm of milled quartz (Netzer et al. 2005).

Each lysimeter was irrigated separately from an 80-L water tank that was refilled manually every morning with a known volume of water. The tanks were positioned 50 cm above the soil surface, about 2 m from the lysimeters. The drip line that was connected to the water tank was equipped with four 2.4-L h^{-1} drip emitters spaced about 30 cm apart. Drainage water was collected in receiver tanks located in a 2.5-m deep underground tunnel that was dug parallel to the row containing the lysimeters. The volume of water that drained through each lysimeter was measured every morning. To ensure that the vines were not limited by water availability the volume of water supplied by irrigation exceeded the vines estimated daily water consumption by 20–30%. Daily irrigation began at 7:00 am and continued for 4–8 h depending on the amount of water that was needed. The ET_c data from the 2000 growth season were omitted because, during that season all of the lysimeters were irrigated from a single drip line and, although the drippers were tested at the beginning of the season, doubts were raised concerning the uniformity of the water supply to the different lysimeters.

ET_c, ET_o and K_c calculation

The daily water consumption, ET_c (liters), was calculated by subtracting the volume of water collected as drainage during a 24-h period from the amount that was supplied by irrigation during the same period. ET_c (mm) was calculated by multiplying the average daily water consumption per vine, as measured in the lysimeters, by 0.143 (1,430 vines ha^{-1}). Reference evapotranspiration (ET_o) was calculated according to the Penman–Monteith equation, as modified for the California Irrigation Management Information System (CIMIS, Snyder and Pruitt 1985). The meteorological data used for calculating ET_o were obtained from a weather station located at the Lachish research station, about 100 m from the vineyard. The daily crop coefficient (K_c) was calculated by dividing daily ET_c (mm day^{-1}) by daily ET_o (mm day^{-1}) (Doorenbos and Pruitt 1977; Allen et al. 1998).

Vineyard structure and irrigation treatments

Vine spacing was 2 m within rows and 3.5 m between rows. Rows were oriented north–south and the vines were trained to a 2-m-high, Y-shaped, open-canopy gable system with six foliage wires on each side (Fig. 1). Each vine was pruned to eight fruiting canes of 14 buds each. The canes were tied to the second and third lower foliage wires supported by the Y-shaped cross-arms. Similar row spacing, training and trellis systems are used in commercial table grape production in the area. Shoots that protruded more than 20 cm above the upper foliage wire were usually hedged. Hedging took place between DOY 165 and 185. The clusters were sprayed with gibberellic acid (Tivag, Qianjiang Biochemistry Co. Ltd., China) at a rate of 1.5 g a.i. ha^{-1} when 50% of the berries had reached a diameter of 4 mm and a week later at a rate of 5 g a.i. ha^{-1}. Vines were not girdled. Pesticide application for diseases and pests were terminated at the end of August. The vineyard was kept weed-free each year of the study. The vineyard was drip-irrigated daily using a computer-controlled drip irrigation system with one line per row and 2.4-L h^{-1} in-line, pressure-compensated drippers spaced 0.5-m apart (Netafim, Israel).

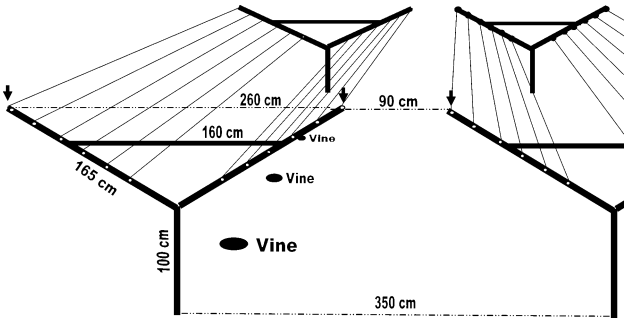

Fig. 1 The dimensions of the Y-shaped open-gable trellis system, with six foliage wires per cross-arm

Three irrigation treatments were imposed in the vineyard. Each treatment consisted of four replicates in a randomized block design with ten vines per replicate, and each replicate was surrounded by 32 border vines that received the same treatment. The irrigation control unit (Talgil, Israel) was manually set to satisfy 80% of ET_c before harvest and 60% of ET_c after harvest in the high-volume irrigation treatment, 60% of ET_c before harvest and 40% after harvest in the medium-volume irrigation treatment and 40% of ET_c before harvest and 20% after harvest in the low-volume irrigation treatment (Table 2). The daily drip irrigation schedule was determined on a 5-day basis according to data obtained by the lysimeter. In cases of large changes in weather conditions, the irrigation schedule was determined daily. Fertilizer concentrations of 1.29 mM NO_3^-, 0.64 mM NH_4^+, 0.87 mM K and 0.19 mM P were supplied daily through the irrigation water.

Leaf area index measurements

The leaf area index (LAI) represents the leaf area per unit of ground surface area (Jonckheere et al. 2004). In our case,

LAI is the ratio of the total one-side leaf surface area and shoot and fruit area per land area allocated for each vine. The LAI values of the lysimeter-grown vines and the vineyard-grown vines were estimated several times during the 2001–2005 growing seasons using a non-destructive canopy analysis system (SunScan model SS1-R3-BF3; Delta-T Devices, Cambridge, UK). The canopy analysis system uses a line quantum sensor array that is sensitive to photosyntheticlly active radiation (PAR). This method of estimating LAI (gap fraction inversion) is based on light measurements beneath the canopy (Grantz and Williams 1993; Cohen et al. 1997; Wilhelm et al. 2000). The analyzer was operated using the standard protocol recommended by the manufacturer. Each sample consisted of equally spaced readings (20 cm apart) at ground level, starting from the center of the row to half the distance to the adjacent row, with the linear probe positioned parallel to the rows.

Until midsummer, the measurements were conducted when the zenith angle of the sun was less than 30°. (For example, in April, measurements were taken between 10:10 and 13:20.) At the end of the season, the measurements were taken when the zenith angle was less than 45° (between 10:00 and 13:00 in October). LAI values obtained using this non-destructive method were verified by direct measurement of leaf area following leaf removal from 2-m-long sections along the row, 1 m from each side of the trunk (Netzer et al. 2005). Leaf area was then measured using an area meter (model 3100; Li-Cor, Lincoln, NE, USA). Leaf areas of 18 row sections of 2-m each were measured at different times during the growing seasons. Estimated and measured LAI values were highly correlated with one another ($y = 0.9821x - 0.0366$, $R^2 = 0.983$, $P < 0.0001$, $n = 18$). It should be mentioned that, using this method, we measured total green surface area, including leaves, shoots and fruits. The "LAI" contributed by the trunk and the canes (0.4–0.6 $m^2\ m^{-2}$) was subtracted from the total LAI. The trunk and cane data were obtained from the Sun Scan's measurements taken under the defoliated vine mentioned above.

Yield

Each replicate was harvested during July or August (Table 1), when the fruit total soluble solids (TSS) reached 15–15.5°Brix.

Statistical analysis

The treatments in the vineyard were arranged in a factorial randomized block design. Data were analyzed via analysis of variance and means were separated according to the least significant difference (LSD) at $P \leq 0.05$. The software program JMP IN 5.1 (SAS Institute, Inc., Cary, NC, USA) was used for all statistical procedures.

Results

Visual estimation indicated that budbreak occured in Lachish during the first half of March (Table 1). Measurements of the daily water consumption (ET_c) of the lysimeter-grown vines were initiated during the first week of April (DOY 91), at which point leaf area index (LAI) was still under 1 $m^2\ m^{-2}$ (1 $m^2\ m^{-2}$ is equivalent to a leaf area of 7.00 m^2 per vine; Fig. 2a). From the beginning of April to the middle of June (DOY 91 to 170), ET_o increased 1.46 times, from an average of 4.72 mm day^{-1} during the first 10 days of April to an average of 6.93 mm day^{-1} between the 11th and 20th of June. This period is characterized by intensive vegetative growth, which was expressed as a 7.5-fold increase in LAI, from 0.54 to 4.1 $m^2\ m^{-2}$ (Fig. 3a). Consequently, ET_c increased 3.82-fold, from 1.71 to 6.56 mm day^{-1} (Fig. 2a), leading to a concomitant increase in K_c, from ~ 0.4 to ~ 1 (Fig. 2b).

Table 1 Seasonal vine water use, ET_c (L $vine^{-1}$ $season^{-1}$ and mm $season^{-1}$), reference crop evapotranspiration, ET_o (mm $season^{-1}$), and soil evaporation, E (L $vine^{-1}$ $season^{-1}$), of *Vitis vinifera* cv. Superior Seedless from April (DOY 91) through October (DOY 304)

	ET_c [L $vine^{-1}$ $season^{-1}$]	ET_c [mm $season^{-1}$]	E [L $vine^{-1}$ $sseason^{-1}$]	ET_o [mm $season^{-1}$]	Date of 50% budbreak (DOY)	Date of harvest (DOY)
1999	8,585	1,228	No data	1,238	12 March (71)	14 July (195)
2000	No data	No data	No data	1,243	15 March (74)	24 July (205)
2001	8,104	1,159	No data	1,225	01 March (60)	13 August (225)
2002[a]	8,600	1,230	No data	1,257	05 March (64)	8 August (220)
2003[a]	7,599	1,087	No data	1,232	13 March (72)	5 August (217)
2004[a]	9,425	1,348	364	1,235	16 March (75)	19 July (200)
2005[a]	8,968	1,282	663	1,238	15 March (74)	29 July (210)

Dates of 50% budbreak and harvest of the lysimeter vines are presented. The missing data of ET_c in the 2002 and 2003 seasons were evaluated by extrapolating from the data for all seasons

[a] The ET_c data were collected from 10, 9, 6 and 4 lysimeters in 2002, 2003, 2004 and 2005, respectively

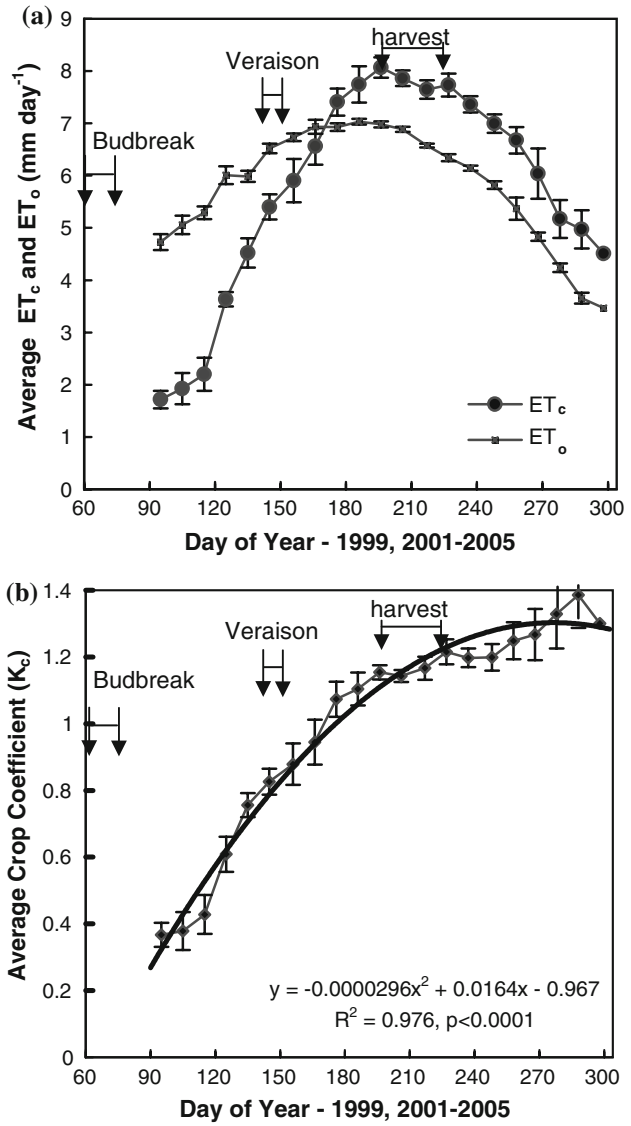

Fig. 2 **a** Seasonal curves of water use (ET_c) of *Vitis vinifera* cv. Superior Seedless, as measured using drainage lysimeters, and of reference evapotranspiration (ET_o) calculated using the Penman–Monteith equation as modified for CIMIS. Each data point represents an average of 10–11 days during six seasons, 1999 and 2001 through 2005. *Vertical error bars* represent the double S.E. of the mean. **b** Seasonal curves of crop coefficient (K_c) for the 1999 and 2001–2005 growing seasons. Each data point represents an average of 10–11 days. The seasonal K_c as a function of DOY was fitted to the quadratic equation: $y = -0.00000296x^2 + 0.0164x - 0.9673$; $R^2 = 0.976$

From the middle of June through the end of July (DOY 167–212), ET_o remained fairly stable, while ET_c and K_c continued to rise as a result of the further increase in canopy size (Figs. 1a, b, 2a). The crop was harvested when the total soluble solids (TSS) of the grape juice reached 15–15.5°Brix (Table 1), which was usually between the middle of July and the middle of August (DOY 195–225).

Between August and October (~ DOY 238–304), ET_o and LAI decreased gradually, leading to a decrease in ET_c (Figs. 1a, 2a). During this period, K_c remained high (more than 1.2) due to the parallel changes in ET_c and ET_o.

Seasonal ET_c values for the different seasons ranged from 1,087 to 1,348 mm (ET_c data of the 2000 growing season was omitted for technical problems; Table 1). The calculated seasonal ET_o values were between 1,225 and 1,257 mm (Table 1). Evaporation from the surface of the evaporation lysimeter (unplanted control lysimeter) in the 2004 and 2005 seasons accounted for 3.9 and 7.4% of the total seasonal evapotranspiration in the 2 years, respectively (Table 1).

A second-degree polynomial correlation exists between LAI and ET_c, as shown in Fig. 4a. A similar correlation curve was observed when ET_c was normalized by ET_o ($K_c = ET_c/ET_o$), and K_c is presented as a function of LAI (Fig. 4b). To reveal the dynamic relations between LAI, ET_c and K_c over the course of a season, we used DOY as a matching parameter. Figure 5a and b show the curves fitted between LAI and ET_c and LAI and K_c, respectively, and several time points are marked on the graph to indicate the corresponding DOY values. From DOY 91 to DOY 206, a linear relationship existed between ET_c and LAI (Fig. 5a). Between harvest and the end of the season (DOY 206 through DOY 304), ET_c decreased from about 8 to 2 mm day^{-1}, while LAI changed only moderately, from 4.8 to 3.5 m^2 m^{-2}. Likewise, K_c and LAI were linearly related until about DOY 206. After harvest (DOY 195–225), K_c continued to increase until DOY 250. After that point, it decreased slightly, by less than 10% (from the maximum K_c value), over the remainder of the season; as LAI declined from 4.71 to 3.41 m^2 m^{-2}.

Based on the average water consumption of the lysimeter-grown vines over six seasons, the average ET_c of the vineyard (1,430 vines ha^{-1}) was expected to be 1,220 mm; 660 mm from the beginning of the season until harvest and 560 mm from harvest until the end of season. The highest average daily water consumption, 60.06 L $vine^{-1}$ (8.59 mm day^{-1}), was measured during the last 10 days of July 2005 (Table 2), when the average ET_o value was 7.13 mm day^{-1}.

The pattern of canopy development, as demonstrated by the change in LAI over the course of the season, was similar in the lysimeter-grown vines and in the vineyard (Fig. 3). The seasonal pattern of the change in LAI in the vineyard demonstrates the differences between the irrigation treatments and, to a lesser extent, the differences in canopy development between growing seasons (Fig. 3). A small decline in LAI was often observed after harvest due to the removal of clusters and damage inflicted on foliage. An example of this is the observed decrease in LAI in the lysimeter-grown vines after the harvests of 2004 and 2005 (Fig. 3a).

A rapid increase in LAI is evident from budbreak through July or August, when the average maximum value of

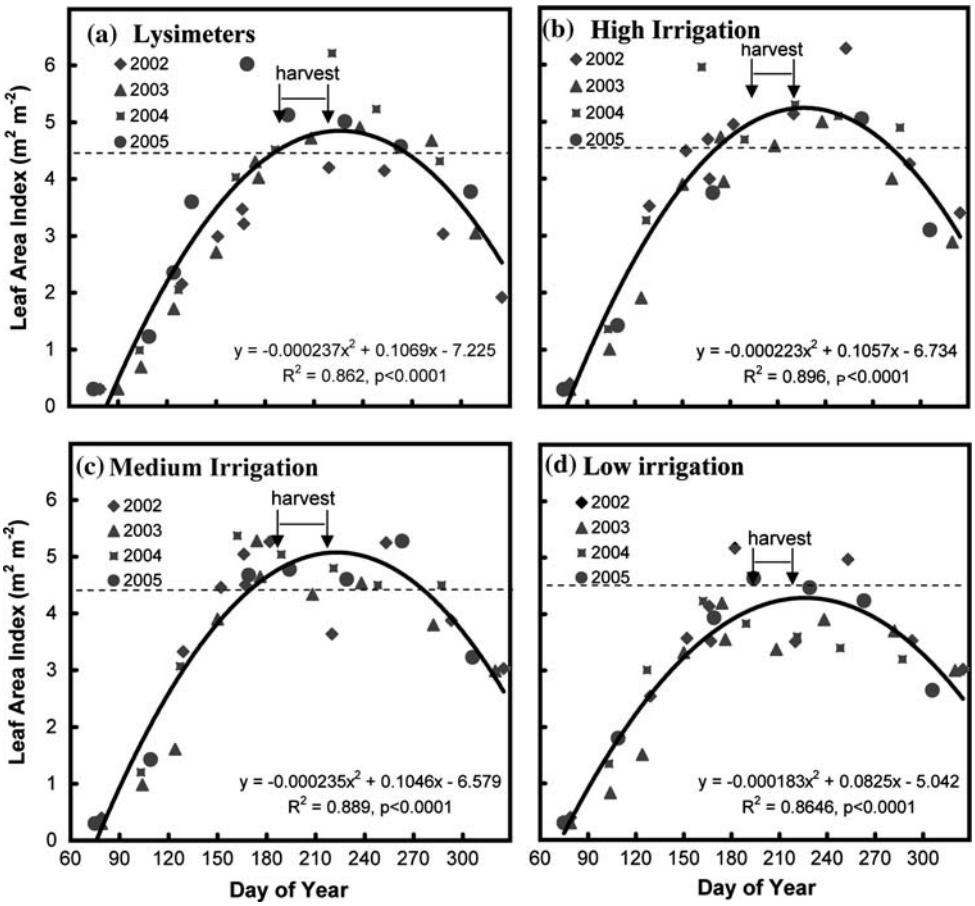

Fig. 3 The seasonal course of leaf area index (LAI) values of **a** lysimeter-grown *Vitis vinifera* cv. Superior Seedless and **b** vineyard-grown vines of the same cultivar irrigated with 80 and 60% of ET_c (before and after harvest, respectively), **c** 60 and 40% of ET_c; and **d** 40 and 20% of ET_c LAI was estimated during the 2002–2005 growth seasons using a non-destructive method. Each data point in **a** represents an average of data from all active lysimeters. For **b**–**d**), each data point represents an average of data from 16 vines (four vines per replicate). The curves were fitted to quadratic polynomial equations. *Arrows* indicate the harvest dates. The *dashed line* (at 4.5 m^2 m^{-2}) was inserted at the top of all of the charts to enable the easy visual comparison of the different charts

4.8 m^2 m^{-2} is generally reached. Higher LAI values were observed in the high- and medium-volume irrigation treatments, as compared to the low-volume treatment (Fig. 3b–d); and this effect was significant at harvest time for all growing seasons, with the exception of the 2005 season (Table 4).

The seven-season average yield of the lysimeter-grown vines was 34.9 tons ha (Table 3). The average yields of the three irrigation treatments in the vineyard were 9.1, 16.9 and 29.5% lower in the 80, 60 and 40% of ET_c respectively, as compared to the yields obtained from the lysimeter-grown vines (Table 3). The average yields harvested in the plots of the three irrigation treatments are linearly related to the volume of water that was applied $y = 0.0145x + 19.483$ ($R^2 = 0.982$). Although the difference between the high-volume and low-volume irrigation treatments was significant in only two out of the seven seasons (2001 and 2004), when the pooled yields (across all seasons) were analyzed, the low-volume irrigation treatment was associated with yields significantly lower than those of the high-volume treatment.

Discussion

Seasonal water consumption (ET_c) of an intensively managed table grape vineyard trained to a large, open-gable trellis system in a semi-arid region was measured using drainage lysimeters. No growth limitations due to water stress, disease, pests or lack of oxygen were noticed in the lysimeter-grown vines. While irrigation continued in the same manner until the end of the season, routine plant protection treatments like sprays against diseases and pests were discontinued at the end of August. The average yield obtained from the lysimeter-grown vines was equivalent to 34.9 t ha^{-1}, which is about 20% higher than the average yield in the area (about 25 ton ha^{-1}) and also higher than the average yield in the high-volume irrigation treatment in this experiment (Table 3).

Profitable management of table grape vineyards in semi-arid regions that lack summer rains and are characterized by high average temperatures and a small number of cloudy days is greatly dependent on irrigation. Decreasing the amount of water that was supplied by irrigation to 40% (low-volume irrigation treatment) of the amount used by the lysimeter-grown vines (100% ET_c) reduced the average seven-season yield by 29% (Table 3). Due to the fluctuating bearing habit of 'Superior Seedless', significant yield differences between the 80% of ET_c (high-volume) treatment and the 40% of ET_c (low-volume) treatment were observed only in seasons characterized by higher overall yields (2001 and 2004). Similarly, in the table grape cv. Crimson Seedless

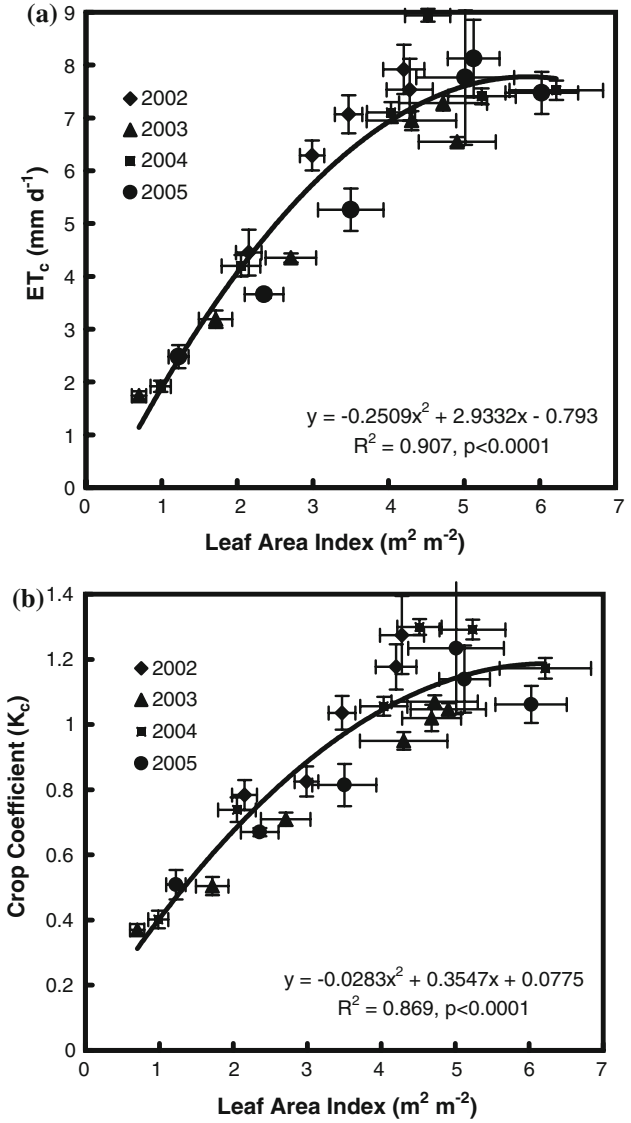

Fig. 4 **a** The relationship between leaf area index (LAI) and evapotranspiration (ET_c) and **b** the relationship between LAI and crop coefficient (K_c) for *Vitis vinifera* cv. Superior Seedless grown in lysimeters ($n = 23$). LAI was estimated using a non-destructive method during the 2002–2005 growing seasons. Each ET_c and K_c data point represents an average value over 20, 10 days before the LAI measurement and 10 days after the measurement. Each data point represents the average LAI value of 10, 9, 6 and 4 vines in the 2002, 2003, 2004 and 2005 growing seasons, respectively. *Vertical* and *horizontal error bars* represent the SE of the means. The *curves* were fitted to quadratic polynomial equations. Data from dates after DOY 259 (mid-September) were excluded. Including this data would change the equation in **a** to: $y = -0.1625x^2 + 2.2833x - 0.114$, $R^2 = 0.8023$, $n = 29$; and the equation in **b** to: $y = -0.0497x^2 + 0.5037x - 0.0697$, $R^2 = 0.7279$, $n = 29$

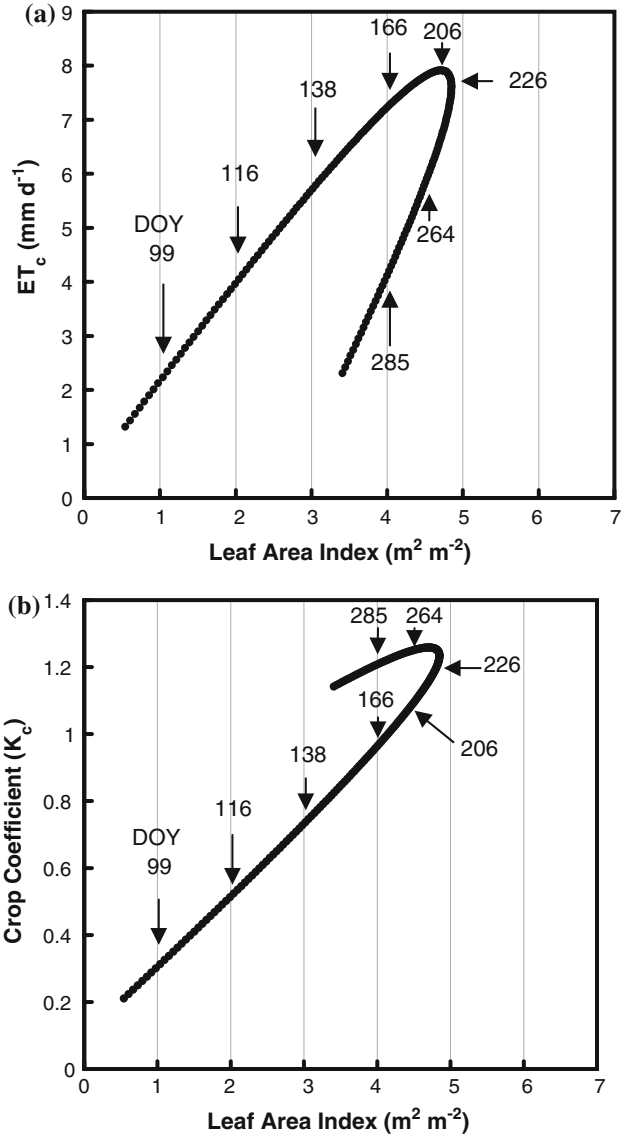

Fig. 5 **a** The relationship between the calculated seasonal changes of leaf area index (LAI) and evapotranspiration (ET_c). and **b** the relationship between the calculated seasonal changes of LAI and crop coefficient (K_c) values for *Vitis vinifera* cv. Superior Seedless grown in lysimeters, 2002–2005. The numbers repesent the corresponding days of the year (DOY). LAI data was calculated from the best fit second-degree polynomial equation (Fig. 3a): $y = -0.000237x^2 + 0.1069x - 7.2251$. ET_c data was calculated from the best fit second-degree polynomial equation for the 2002–2005 growing seasons: $y = -0.00053x^2 + 0.2167x - 13.9666$. K_c data was calculated from the best fit second-degree polynomial equation for the 2002–2005 growing seasons: $y = -0.000041x^2 + 0.02059x - 1.3230$

grown in a semi-arid region in Chile, an increase in irrigation from 39 to 96% of ET_c (average of 377 to 926 mm season^{-1}) increased the avarage yield by about 20% (Ferreyra et al. 2006). Although, as mentiond above, there is a large amount of variability in the yields of consecutive seasons, the 7-year average yields of the three irrigation treatments in the vineyard are linearly related to the irrigation levels applied.

Comparisons between the water consumption patterns and yields of different cultivars grown in different regions using different cultural practices may be complicated and can sometimes be misleading. Neverthless, to best of our

Table 2 Average daily vine water consumption [ET_c (mm day^{-1}) over 10–11 days] of *Vitis vinifera* cv. Superior Seedless in the lysimeters and in the irrigation treatments (mm day^{-1}) in the vineyard from DOY 91 to DOY 304 of the 2005 growing season

Date	Day of year	Lysimeters	High irrigation (80–60% of ET_c)	Medium irrigation (60–40% of ET_c)	Low irrigation (40–20% of ET_c)
April					
1–10	91–100	2.06	1.65	1.22	0.80
11–20	101–110	2.35	1.90	1.40	0.95
21–31	111–120	2.77	2.20	1.60	1.10
May					
1–10	121–130	4.05	3.30	2.40	1.60
11–20	131–140	4.31	3.40	2.60	1.70
21–31	141–151	5.66	4.55	3.40	2.25
June					
1–10	152–161	6.49	5.20	3.90	2.60
11–20	162–171	7.35	5.90	4.40	2.95
21–30	172–181	7.90	6.30	4.75	3.15
July					
1–10	182–191	7.69	6.15	4.60	3.10
11–20	192–201	8.24	6.60	4.95	3.30
21–31[a]	202–212	8.59	6.60	5.00	3.30
August					
1–10	213–222	7.77	4.65	3.10	1.55
11–20	223–232	8.03	4.80	3.20	1.60
21–31	233–243	7.35	4.40	2.95	1.45
September					
1–10	244–253	6.82	4.10	2.70	1.35
11–20	254–263	6.18	3.70	2.45	1.25
21–30	264–273	5.99	3.60	2.40	1.20
October					
1–10	274–283	5.77	3.45	2.30	1.15
11–20	284–293	5.39	3.20	2.15	1.10
21–31	294–304	4.85	2.90	1.95	1.00
Total (mm season^{-1})	101–304	1,282	903	647	392

Water use of the vines (L) in the lysimeters divided by 7 (m^2 of surface area per vine) is equivalent to mm of water. Irrigation treatments were 80 and 60% of ET_c before and after harvest, respectively, in the high-volume irrigation treatment, 60% and 40% of ET_c in the medium-volume irrigation treatment and 40 and 20% of ET_c in the low-volume irrigation treatment

[a] Harvested on July 29 (DOY 210)

Table 3 Yields (t ha^{-1}) of *Vitis vinifera* cv. Superior Seedless table grapes grown in lysimeters and in the three irrigation treatments in the vineyard in Lachish, 1999–2005

	Lysimeters	High irrigation (80–60% of ET_c)	Medium irrigation (60–40% of ET_c)	Low irrigation (40–20% of ET_c)
1999	49.8	33.9 a	33.6 a	25.8 a
2000	29.1	25.9 a	20.7 a	18.6 a
2001	23.8	47.0 a	40.0 ab	31.6 b
2002	39.6*	33.2 a	30.4 a	32.9 a
2003	33.4*	23.9 a	22.3 a	20.3 a
2004	50.0*	46.1 a	47.0 a	33.1 b
2005	18.9*	11.9 a	8.9 a	10.1 a
AVE	34.9	31.7 a	29.0 ab	24.6 b

Means within a row followed by different letters are significantly different at $\alpha = 0.05$

* The yield data were collected from 10, 9, 6 and 4 lysimeters in 2002, 2003, 2004 and 2005, respectively

knowledge, the avarage yield as calculated from the lysimeter-grown vines and the vines of the high-volume irrigation treatment in Lachish is similar or greater than the yields of intensively managed table grapes grown elsewhere. Average yields of vineyards in California (2000–2003) were 20.5 t ha^{-1} for table grape varieties (Lobel et al. 2006). Yields of up to 21.2 t ha^{-1} for cv. Red Globe in Mexico (Valenzuela-Ruiz et al. 2005), 25 t ha^{-1} for 'Crimson Seedless' in Chile (Ferreyra et al. 2006) and 28.5 t ha^{-1} for cv. Flame Seedless in California (Dokoozlian and Hirschfelt 1995) have also been reported. The average seasonal ET_c of 1,220 mm, as measured for the lysimeter-grown vines in

Table 4 Leaf area index (m^2 m^{-2}) of *Vitis vinifera* cv. Superior Seedless table grapevine grown in lysimeters and in the three irrigation treatments in the vineyard, as measured using a SunScan canopy analysis system close to harvest time at 2001–2005. Means within a row followed by different letters are significantly different at $\alpha = 0.05$. The data presented here are averages of LAI values for vines of the lysimeters and at 8 different locations (2 per replicate) for each of the irrigation treatments

	DOY	Lysimeters	High irrigation (80–60% of ET_c)	Medium irrigation (60–40% of ET_c)	Low irrigation (40–20% of ET_c)
2001	189	No data	5.17ab	5.62 a	4.47 b
2002	220	4.20*	5.14 a	3.64 ab	3.51 b
2003	217	4.72*	4.58 a	4.34 ab	3.37 b
2004	189	4.52*	4.69 ab	5.02 a	3.83 b
2005	229	5.13*	No data	4.63a	4.47a

* The data were collected from 10, 9, 6 and 4 lysimeters in 2002, 2003, 2004 and 2005, respectively

Lachish, is considerably higher than amounts reported for grapevines in other studies (van Rooyen et al. 1980; van Zyl and van Huyssteen 1980; Rollin et al. 1981; Evans et al. 1993; Trambouze et al. 1998). The relatively large ET_c can be attributed not only to the environmental conditions of the area, which are expressed in ET_o, but also to the high LAI and the type of trellising system used (Fig. 1). In the large open-gable trellis system, a large portion of the leaves are exposed to direct sunlight most of the day. It is estimated that from the beginning of June to September the canopy covers more than 80% of the ground. The fact that the type of trellising may have a significant effect on K_c was recently demonstrated in lysimeter-grown vines in California (Williams and Ayars 2005b). In that study, an increase in K_c from 0.9 to 1.3 was achieved by changing the amount of leaf area that was exposed to direct sunlight, thus demonstrating that water consumption is determined not only by LAI, but also by the type of trellising and the structure of the canopy.

The amount of water consumed by the lysimeter-grown vines (Table 1) was also substantially greater than that commonly applied by irrigation to commercial table grapes of the same cultivar in the area. Growers in Lachish apply about 350 mm of water in the period before harvest and 150 mm from harvest to autumn (using drip irrigation). The operation of drainage lysimeters requires the daily irrigation volumes to be greater than ET_c, so that a significant amount of drainage can be collected each day (20–30% higher than ET_c). The abundant supply of water resulted in vines that did not experience any significant water stress and the stomatal conductance of these plants was significantly higher than that of the field-grown vines (data not shown). In addition, after harvest, the growers usually reduce irrigation to about 1 mm day^{-1}, an amount that is assumed to be the minimal quantity necessary for maintaining a viable canopy until autumn.

Evaporation (E) from the soil surface, as measured in the unplanted lysimeters, was 4.2 and 7.4% of the annual ET_c of the planted lysimeters during the 2004 and 2005 seasons, respectively (Table 1). We estimate that, in the vineyard, the amount of E from the soil surface per vine is not more than double the amount of E from the soil surface of the lysimeters, although the area allocated to each vine in the vineyard is much larger (7.00 vs. 0.86 m^2). This estimation is based on the fact that the actual volume of wetted soil under the drip line next to a single vine in the field is not much larger than the 1.3 m^3 wetted soil in the lysimeter.

The presented 'Superior Seedless' seasonal ET_c values are about 40% higher than the ET_c values that were measured in cv. Thompson Seedless in California using a weighing lysimeter (Williams et al. 2003b). Average seasonal ET_o values in Lachish and in the San Joaquin Valley of California were rather similar, 1,238 and 1,172 mm, respectively. The difference in the ET_c values observed in the two locations can be related to the different cultivars, growth habits and trellising systems in the two locations, which led to a larger average leaf area in Lachish (32.2 vs. 27 m^2 $vine^{-1}$ in Lachish and San Joaquin, respectively).

K_c values of various crops range between 0.1 and 1.2. The highest K_c value observed in the present study (1.2) is close to the K_c of cv. Colombard grown in Australia (Stevens and Harvey 1996), as well as a K_c observed for 'Thompson Seedless' in California (Williams et al. 2003b). The relatively small season-to-season variation in K_c curves justifies their use as a basis for managing irrigation in the area.

Since temperature is the most dominant climatic factor affecting growth and development, annual (year-to-year) variation in K_c can be mostly avoided by plotting K_c as a function of growing degree days (GDD) rather than as a function of DOY, as suggested for corn and sorghum (Sammis et al. 1985; Slack et al. 1996). The variation between the K_c curves of 'Thompson Seedless' grapevines in different years, especially in the early part of the season, was smaller when K_c was plotted against GDD rather than against DOY (Williams et al. 2003b). It was therefore

suggested that the GDD–K_c curve could be a useful tool for scheduling irrigation of vineyards in areas with climatic conditions that differ from those of the original K_c-measuring site. Based on the meteorological data collected at the research site, the calculated R^2 of the GDD to K_c relationship (calculated from 50% of budbreak) differed from that of the DOY–K_c curves by only 0.0631. This can be attributed to the fact that the year-to-year variation in temperature in the area was rather small.

The average maximal LAI of the lysimeter-grown vines was 4.8. A polynomial relationship between LAI and ET_c exists when the entire season is considered. The two parameters were linearly related until harvest, i.e., until LAI reached about 4.5 $m^2\ m^{-2}$ (31.45 $m^2\ vine^{-1}$), after which point ET_c gradually leveled off. This gradual leveling-off is probably a result of the reduction in the amount of light penetrating the dense canopy and the increase in the thickness of the leaf boundary layer, both factors that can reduce transpiration rates (Fig. 4a). The relationship between K_c and LAI (Fig. 4b) shows a similar pattern. K_c and LAI were linearly related until LAI reached about 4.5 $m^2\ m^{-2}$ ($y = 0.218x + 0.202$, $R^2 = 0.893$, $n = 16$). However, at higher LAI values, only a small increase in K_c was observed. The relationship between LAI and K_c can serve as an additional tool for estimation of K_c in situations in which LAI can be measured directly or accurately estimated in vienyard with similar trellis system.

Detailed analysis of the relationships between LAI and ET_c and between LAI and K_c over the course of the season revealed that the decrease in ET_c toward the end of the season, from DOY 226 through DOY 304, can be attributed mainly to a decrease in atmospheric demand, since only a small change in LAI was observed during the same period (Fig. 5a). ET_c remained 10–20% higher than ET_o ($K_c = 1.1$–1.2) due to the continuous loss of water from the canopy through stomatal transpiration and probably also as a result of the damage inflicted on the leaves from gradual increase in downy mildew (*Plasmopara viticola*) and red thrips (*Retithrips syriacus*) infection, which may affect leaf conductance to water vapor. In a recent study, stomata of grapevine leaves that were infected by *Plasmopara viticola* remained open in the dark, even under water-stress conditions (Allègre et al. 2007). The fact that K_c values did not decrease toward the end of the season can also be attributed to the fact that, unlike the situation in the vineyard, the lysimeter-grown vines were irrigated at full ET_c until the end of the season. A similar K_c pattern was reported for lysimeter-grown Thompson Seedless table grape vines (Williams and Ayars 2005a). Those findings indicate that the reduction in K_c after the middle of the growing season, which was reported for grapevine by Allen et al. (1998), may relate to the fact that these plants were not sufficiently watered.

Conclusions

The high ET_c of the lysimeter-grown vines can be explained by the high LAI values of vines trained to a large open-gable trellis system fully irrigated during all growing season. The calculated K_c has been shown to be a useful tool for scheduling irrigation, as demonstrated in the irrigation trial in which the effects of three irrigation levels (amounts that were calculated as percentages of ET_c) were tested.

The linear relationship between yield and the applied irrigation level can assist growers to predict the expected yield, based on K_c values, even when the amount of the available water for irrigation is smaller than the ET_c obtained under favorable conditions where irrigation is an unlimited factor. Both K_c and ET_c were highly correlated to LAI. These correlations can be also used for precise irrigation management based on LAI measurements.

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References

Allègre M, Daire X, Héloir MC, Trouvelot S, Mercier L, Adrian M, Pugin A (2007) Stomatal deregulation in *Plasmopara viticola*-infected grapevine leaves. New Phytol 173:832–840

Allen RA, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration: guidelines for computing crop water requirements. (FAO Irrigation and Drainage Paper 56) FAO, Rome

Ayars JE, Hutmacher RB (1994) Crop coefficients for irrigating cotton in the presence of ground water. Irrig Sci 15:42–52. doi:10.1007/BF00187794

Ayars JE, Johnson RS, Phene CJ, Trout TJ, Clark D, Mead RM (2003) Water use by drip-irrigated late-season peaches. Irrig Sci 22:187–194. doi:10.1007/s00271-003-0084-4

Behboudian MH, Singh Z (2001) Water relations and irrigation scheduling in grapevine. Hortic Rev 27:189–225

Braun P, Schmid J (1999) Sap flow measurements in grapevines (*Vitis vinifera* L.): 1. Stem morphology and use of the heat balance method. Plant Soil 215:39–45. doi:10.1023/A:100475600 2983

Bravdo B, Hepner Y (1987) Water management and effect on fruit quality on grapevines. In: Lee T (ed) Proceedings of the 6th Australian wine industry technical conference, Adelaide, July 14–17, 1987. Australian Industrial Publications, Adelaide, pp 150–158

Chalmers DJ, Andrews PK, Harris KM, Cameron EA, Caspari HW (1992) Performance of drainage lysimeters for evaluation of water use by Asian pears. HortScience 27:263–265

Cifre J, Bota J, Escalona JM, Medrano H, Flexas J (2005) Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): an open gate to improve water-use efficiency? Ecosyst Environ 106:159–170

Cohen S, Rao RS, Cohen Y (1997) Canopy transmittance inversion using a line quantum probe for a row crop. Agric For Meteorol 86:225–334. doi:10.1016/S0168-1923(96)02426-4

Dokoozlian NK, Hirschfelt DJ (1995) The influence of cluster thinning at various stages of fruit development on 'Flame Seedless' table grapes. Am J Enol Vitic 46:429–436
Doorenbos J, Pruitt WO (1977) Crop water requirements. (FAO Irrigation and Drainage Paper 24) FAO, Rome
Eastham J, Gray SA (1998) A preliminary evaluation of the suitability of sap flow sensors for use in scheduling vineyard irrigation. Am J Enol Vitic 49:171–176
Erie LJ, French OF, Bucks DA, Harris K (1982) Consumptive use of water by major crops in the southwestern United States (Conservation Research Report no. 29). United States Department of Agriculture, Washington, D.C
Evans RG, Spayd SE, Wample RL, Kroeger MW, Mahan MO (1993) Water use of *Vitis vinifera* grapes in Washington. Agric Water Manage 23:109–124. doi:10.1016/0378-3774(93)90035-9
Ferreyra R, Selles G, Silva H, Ahumada R, Munoz I, Munoz V (2006) Effect of applied water on water relations and productivity of 'Crimson Seedless' table grapes. Pesqui Agropecu Bras 41:1109–1118
Fuchs M (2007) Impact of research on water use for irrigation in Israel. Irrig Sci 25:443–445. doi:10.1007/s00271-006-0050-z
Ginestar C, Eastham E, Gray S, Iland P (1998) Use of sap-flow sensors to schedule vineyard irrigation. I. Effects of post-veraison water deficits on water relations, vine growth, and yield of 'Shiraz' grapevines. Am J Enol Vitic 49:413–420
Grantz DA, Williams LE (1993) An empirical protocol for indirect measurement of leaf area index in grape *Vitis vinifera* L. Hortscience 28:777–779
Grimes DW, Williams LE (1990) Irrigation effects on plant water relations and productivity of 'Thompson Seedless' grapevines. Crop Sci 30:255–260
Hamman RA, Dami IE (2000) Effects of irrigation on winegrape growth and fruit quality. Horttechnology 10:162–168
Jagtap SS, Jones JW (1989) Stability of crop coefficients under different climate and irrigation management practices. Irrig Sci 10:231–244
Jonckheere I, Fleck S, Nackerts K, Muys B, Coppin P, Weiss M, Baret F (2004) Review of methods for in situ leaf area index determination. Part I: theories, sensors and hemispherical photography. Agric For Meteorol 121:19–35
Liu CM, Zhang XY, Zhang Y (2002) Determination of daily evaporation and evapotranspiration of winter wheat and maize by large-scale weighing lysimeter and micro-lysimeter. Agric For Meteorol 111:109–120. doi:10.1016/S0168-1923(02)00015-1
Lobel DB, Field CB, Cahill KN, Bonfils C (2006) Impacts of future climate change on California perennial crop yields: Model projections with climate and crop uncertainties. Agric For Meteorol 141:208–218. doi:10.1016/j.agrformet.2006.10.006
Matthews MA, Anderson MM (1989) Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficits. Am J Enol Vitic 40:52–60
Matthews MA, Anderson MM, Schultz HR (1987) Phenologic and growth responses to early and late season water deficitsin 'Cabernet Franc'. Vitis 26:147–160
Netzer Y, Yao C, Shenker M, Cohen S, Bravdo B, Schwartz A (2005) Water consumption of 'Superior Seedless' grapevines grown in a semiarid region. Acta Hortic (ISHS) 689:399–406
Oliver HR, Sene KJ (1992) Energy and water balances of developing vines. Agric For Meteorol 61:167–185
Peacock WL, Christensen LP, Andris HL (1987) Development of a drip irrigation schedule for average-canopy vineyards in the San Joaquin Valley. Am J Enol Vitic 38:113–119
Prior LD, Grieve AM (1987) Water used and irrigation requirements of grapevines. In: Lee T (ed) Proceedings of the 6th Australian wine industry technical conference, Adelaide, July 14–17, 1987. Australian Industrial Publications, Adelaide, pp 165–168
Rollin H, Meriaux S, Boubals D (1981) Sur l'irrigation de la vigne dans le midi de la France. Prog Agric Vitic 98:447–459
Salon JL, Chirivella C, Castel JR (2005) Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. Am J Enol Vitic 56:1–8
Sammis TW, Mapel CL, Lugg DG, Lansford RR, McGuckin JT (1985) Evapotranspiration crop coefficients predicted using growing degree days. Trans ASAE 28:773–780
Shani U, Ben-Gal A (2005) Long-term response of grapevines to salinity: osmotic effects and ion toxicity. Am J Enol Vitic 56(2):148–154
Shellie KC (2006) Vine and berry response of 'Merlot' (*Vitis vinifera* L.) to differential water stress. Am J Enol Vitic 57:514–518
Slack DC, Martin EC, Sheta AE, Fox FJ, Clark LJ, Ashley RO (1996) Crop coefficients normalized for climate variability with growing-degree days. In: Proceedings of the international conference on evapotranspiration and irrigation scheduling. American Society of Agricultural Engineers, pp 892–898
Smart RE, Coombe BJ (1983) Water relations of grapevines. In: Kozlowski TT (ed) Water deficits and plant growth. Academic Press, New York, pp 137–196
Snyder R, Pruitt W (1985) Estimating reference evapotranspiration with hourly data. In: Snyder R et al. (eds) California Irrigation Management Information System Final Report. Univ. of California-Davis, Ch. 7
Stevens RM, Harvey G (1996) Soil water depletion rates under large grapevines. Aust J Grape Wine Res 2:155–162
Tarara JM, Ferguson JC (2001) Device for simulating high rates of sap flow in grapevines. Am J Enol Vitic 52:260–265
Trambouze W, Bertuzzi P, Voltz M (1998) Comparison of methods for estimating actual evapotranspiration in a row-cropped vineyard. Agric For Meteorol 19:193–208
Valenzuela-Ruiz MJ, Contreras FR, Grijalva-Contreras RL, Duarte RM (2005) Effect of 'Harmony' and 'Freedom' rootstocks on yield and quality of `Red Globe' table grapes. HortScience 40:993–1147
van Rooyen FC, Weber HW, Levin I (1980) The response of grapes to a manipulation of the soil–plant–atmosphere continuum. II: plant–water relationships. Agrochemophysica 12:69–74
van Zyl JL, van Huyssteen L (1980) Comparative studies on wine grapes on different trellising systems: I. Consumptive water use. S Afr J Enol Vitic 1:7–14
Wilhelm WW, Ruwe K, Schlemmer MR (2000) Comparison of three leaf area index meters in a corn canopy. Crop Sci 40:1179–1183
Williams LE (1998) Water use of 'Thompson Seedless' grapevines measured with weighing lysimeters during a late season dry down period. Proceedings of the 1st ISHS workshop on water relations of grapevines. Acta Hortic 493:161–167
Williams LS, Ayars JE (2005a) Water use of Thompson Seedless grapevines as affected by the application of gibberellic acid (GA3) and trunk girdling—practices to increase berry size. Agric For Meteorol 129:85–94
Williams LS, Ayars JE (2005b) Grapevine water use and crop coefficient are linear functions of the shaded area measured beneath the canopy. Agric For Meteorol 132:201–211
Williams LE, Phene CJ, Grimes DW, Trout TJ (2003a) Water use of young 'Thompson Seedless' grapevines in California. Irrig Sci 22:1–9. doi:10.1007/s00271-003-0066-6
Williams LE, Phene CJ, Grimes DW, Trout TJ (2003b) Water use of mature 'Thompson Seedless' grapevines in California. Irrig Sci 22:11–18. doi:10.1007/s00271-003-0067-5
Yang SL, Aydin M, Yano T, Li X (2003) Evapotranspiration of orange trees in greenhouse lysimeters. Irrig Sci 21:145–149. doi:10.1007/s00271-002-0062-2
Yunusa IAM, Walker RR, Blackmore DH (1997a) Characterisation of water use by 'Sultana' grapevines (*Vitis vinifera* L.) on their own

roots or on 'Ramsey' rootstock drip-irrigated with water of different salinities. Irrig Sci 17:77–86. doi:10.1007/s00271-0050025

Yunusa IAM, Walker RR, Guy JR (1997b) Partitioning of seasonal evapotranspiration from a commercial furrow-irrigated 'Sultana' vineyard. Irrig Sci 18:45–54. doi:10.1007/s002710050043

Yunusa IAM, Walker RR, Loveys BR, Blackmore DH (2000) Determination of transpiration in irrigated grapevines: Comparison of the heat-pulse technique with gravimetric and micrometeorological methods. Irrig Sci 20:1–8. doi:10.1007/PL00006714

4.3 Third chapter

Netzer Y, Shenker M, Schwartz A (2010) **Effects of irrigation using treated wastewater on table grape vineyards: dynamics of sodium accumulation in soil and plant**. Not yet published in scientific literature.

Effects of irrigation using treated wastewater on table grape vineyards: dynamics of sodium accumulation in soil and plant.

Abstract

The effect of using treated wastewater (TWW) for irrigation of a table grape (*Vitis vinifera* cv. Superior Seedless) vineyard on sodium interactions with plant and soil was examined during six seasons. TWW (230 mg l^{-1} Na^{+}) with and without added fertilizer, and fresh water with fertilizer (FW+F: 117 mg l^{-1} Na^{+}), were each supplied at three irrigation levels. Soil solution Na^{+} concentration and sodium adsorption ratio (SAR) fluctuated over the years, but were significantly higher in the TWW-irrigated soils. This effect developed faster and was more pronounced as irrigation level increased. Adding fertilizer to TWW treatments alleviated the increase in SAR. Leaf petiole Na^{+} content in TWW-irrigated vines rose to maximum values of above 6500 mg kg^{-1}, more than threefold those of the FW+F-irrigated vines. Na^{+} concentrations in wood, bark and xylem sap from the stems of the TWW-irrigated vines were higher than those found in the FW+F-irrigated vines. We conclude that in clay soils under relatively high levels of irrigation, Na^{+} may pose a greater potential risk to plants and soil. On the other hand, significant effects on yield have not yet been recorded, perhaps due to the effective saline-resistance of the 'Paulsen' rootstock used in the experiment.

Keywords Wastewater, Effluent, Sodium, Superior Seedless, Table grape, *Vitis vinifera*

1. Introduction

Treated wastewater (TWW) is considered a valuable source of water for irrigation in many arid and semiarid regions throughout the world. The use of TWW is expected to rise with increasing water demand and the concomitant decrease in water availability. From 1960 to 2004, the use of TWW for irrigation rose in Israel to about 30% of total agricultural water used (Aharoni and Cikurel, 2006; Fuchs, 2007), and it is expected to reach more than 50% in the next few years. The need to increase the use of TWW results from the increasing demand for fresh water (FW) in domestic and industrial uses, due to a combination of population growth and increasing scarcity and fluctuations in annual precipitation.

Use of TWW for irrigation calls for intensive monitoring of water, soil, and plant parameters to ensure sustainable agriculture with minimal damage to the environment. The main concerns associated with TWW used for irrigation are its potential risk as a vector for pathogens (Gerardi and Zimmerman, 2005), its high organic matter load (Morgenroth et al., 2002), and its direct effects on crop production through the introduction of toxic elements to plants (Toze, 2006; Yermiyahu et al., 2007). TWW is also more sodic than FW and can therefore harm plants indirectly by degrading soil structure, aeration, and hydraulic conductivity (Agassi et al., 2003; Bhardwaj et al,. 2007; Tarchitzky et al., 1999). In 1989, the World Health Organization (WHO) developed guidelines for managing health risks that may arise from microbial exposure. Guidelines for treating raw sewage water require that the biochemical oxygen demand (BOD)

and suspended solids values in the TWW not exceed 20 mg l^{-1} and 30 mg l^{-1}, respectively (WHO, 1989). The TWW released from the purification plant should not contain over 100 *Escherichia coli* bacteria per 100 ml. In Israel, specific guidelines have been adopted for agricultural purposes (Halperin, 1999), calling for the use of "barriers", such as subsurface drip lines, mechanical covers over drip lines and water chlorination. These measures are designed to reduce the exposure of fruits that are harvested for consumption to the risks of TWW.

The high salinity and solute composition of TWW constitute a central problem, affecting crop performance and chemical and physical soil properties. TWW salinity results mainly from an increase in the concentrations of Na^+, Cl^-, and HCO_3^- and to a lesser extent, SO_4^{2-}, Ca^{2+} and Mg^{2+}. Extended use of TWW for irrigation can potentially cause leaching of NO_3^- and other soluble pollutants into the groundwater (Bond, 1998). In addition, TWW-originated organic matter may coat soil particles and eventually render the soil surface hydrophobic, reducing its water-infiltration capacity (Bhardwaj et al., 2007; Tarchitzky et al., 1999, 2007). The standard procedures for secondary and tertiary treatment of wastewater do not reduce the concentration of most dissolved salts. Therefore, the only efficient way to reduce salt content is to control it at the source (Agassi et al., 2003; Roland et al., 2000). When irrigating soils with water that has elevated Na^+ levels, the introduced Na^+ may replace other exchangeable cations in the soil's exchangeable complex. Exchanging Ca^{2+} and Mg^{2+} with Na^+ may cause clay swelling and dispersion, decreased aggregate stability, impaired soil aeration, reduced soil permeability and infiltration rates, and increased runoff and soil erosion (Agassi et al., 2003; Tarchitzky et al., 1999). The sodium adsorption ratio (SAR) is a common measure used to evaluate the exchangeable sodium ratio in the soil's exchangeable cation complex and is commonly calculated from the cation composition in the soil solution: $SAR = [Na]/[Ca + Mg]^{1/2}$ (square brackets indicate cation concentration in millimolar). Na^+ is introduced into the water by anthropogenic activities and therefore Na concentration and SAR in TWW are typically larger than those in FW. In Israel, the SAR value of FW is commonly about 2.5, but it ranges from about 5 to 8 by the time the water reaches the wastewater treatment facility (Feigin et al., 1991; Avnimelech, 1993). Thus, local regulations for unrestricted irrigation use were set to a maximum value of 5 (Inbar Committee, 2003). This SAR value, when combined with low salinity such as that encountered during the rainy season, may result in clay dispersion and decreased infiltration rates. In clay soils, even an increase in the SAR from 2 to 4 may result in increased surface runoff during rainstorms (Suarez et al., 2006).

Plant roots absorb essential nutritional elements such as N, P, and K, while to a certain extent excluding toxic elements such as Cl^- and Na^+; the efficiency of this exclusion in agricultural crops determines their salt tolerance (Munns, 1993). Reduced growth, early leaf senescence and the appearance of chlorotic and necrotic spots on leaves are external symptoms of salt stress (Greenway and Munns, 1980; Tester and Davenport, 2003). It is generally accepted that salt stress in plants has an osmotic component, in which growth is affected by reduced water uptake (Munns, 1993, 2002; Munns et al., 2000; Shani and Ben-Gal, 2005), and a slower, metabolic component, which is a result of specific ion toxicity stemming from, for example, the competition between Na^+ and K^+ for uptake pathways and binding sites in K^+-dependent

metabolic and biosynthetic processes (Carden et al., 2003; Flowers and Yeo, 1986; Munns, 1993, 2002; Tester and Davenport, 2003).

In Israel, large-scale use of TWW for irrigation has been applied in table grape vineyards for the past 5 years and as a consequence, an increase in visual symptoms of salinity have appeared on the leaves; in some cases, total collapse of yield-bearing vines has occurred.

Grapevines are defined as moderately salt-tolerant (Downton, 1977b; Francois and Mass, 1994; Garcia and Charbaji, 1993; Maas, 1990; Maas and Hoffman, 1977). Salt sensitivity/tolerance in vines is affected by the scion-root combination, which also influences vine vigor (Yunusa et al., 1997). For grapevine, Maas and Hoffman (1977) set a threshold electrical conductivity (EC) value of 1.5 dS m^{-1} for soil saturation paste (EC_e), with each 1 dS m^{-1} above that value decreasing yield by 9.6%. Following three field experiments conducted in Australia, Zhang et al. (2002) concluded that this value is too conservative. Their experiments exhibited a wide range of scion-related thresholds that ranged between 1.8 and 4 dS m^{-1}, with slopes ranging between 2.3 and 15% yield reduction per 1 dS m^{-1}. The rootstock 1103 'Paulsen' (used in the present study) was the most salt-tolerant, exhibiting no yield reduction until the EC_e exceeded about 4 dS m^{-1} (Zhang et al., 2002). The ability to cope with increasing salinity and SAR in soils is highly important given the fact that of the current 230 million ha of global irrigated land, 45 million ha (19.5%) are salt-affected soils (FAO, 2008).

The purpose of this research was to study the effects of irrigation with TWW on table grapes (*Vitis vinifera* cv. Superior Seedless) in a semiarid region. We tested the potential risk of Na-related damage from TWW irrigation, and whether TWW can be an appropriate substitute for FW in this crop. We examined the long-term (six seasons) dynamics of Na^+ accumulation in the soil, changes in soil SAR, changes in mineral content in the leaf and trunk, and effects on total fruit yield.

2. Materials and methods

2.1 Experimental site

A 6-year study (2002-2007) was conducted in a 1-ha vineyard of table grapes (*Vitis vinifera* cv. Superior Seedless, also called Sugraone grafted onto 1103 'Paulsen' rootstock, at the Lachish Agricultural Research and Development Station in the southern part of Israel (lat. 31.6° N, long. 34.8° E). The vines were planted in April 1997. The region of Lachish is one of the largest table grape-growing areas in the country. It is characterized by a semiarid Mediterranean climate with no summer rains. The soil is clay loam composed of 30% sand, 28% silt, and 42% clay, with a cation-exchange capacity of 27.3 meq 100 g^{-1}, $CaCO_3$ content of 18% and organic matter content of 0.6%. The average winter precipitation was 372 mm during the 6-year trial (Table 1). Average reference evapotranspiration from April to October (calculated by the Penman-Monteith equation) was 1272 mm per season.

Table 1 Total winter precipitation, reference crop evapotranspiration (ET_o), and amount of water applied seasonally for the three irrigation levels during the 2002-2007 growing seasons (mm)

	Precipitation[a] (mm)	**ET_o[b] (mm season^{-1})**	**High irrigation (mm)**	**Medium irrigation (mm)**	**Low irrigation (mm)**
2002	388	1257	923	615	394
2003	509	1232	815	544	348
2004	312	1235	944	674	431
2005	382	1238	903	647	392
2006	329	1231	976	729	437
2007	314	1219	996	712	398

[a]The rainy season each year begins on 1 Nov of the previous year and ends on 30 Mar. For example: the 2002 season is from 1 Nov 2001 to 30 Mar 2002.

[b]Calculated from meteorological data according to the Penman-Monteith equation as modified for the California irrigation management system from 1 Apr through 31 Oct.

2.2 Irrigation treatments and vineyard structure

The effects of two factors were examined: irrigation water type and amount. Three water types were used: (i) fresh water with fertilizer (FW+F); (ii) treated wastewater (TWW); (iii) TWW with fertilizer (TWW+F). Each water type was supplied at three irrigation levels: high, medium, and low (nine treatments in all). Each treatment consisted of four replicates (plots) arranged in a randomized block design. Each treatment plot consisted of three rows, 14 vines per row. To minimize the border effect, only the 10 central vines from the middle row were sampled. Vine spacing was 2 m within rows and 3.5 m between rows. Rows were oriented from north to south and the vines were trained to a 2-m-high Y-shaped open-canopy gable system with six foliage wires on each side. Each vine was pruned to eight fruiting canes of 14 buds each. The canes were tied to the second- and third-lowest foliage wires supported by the Y-shaped cross-arms. Vine and row spacing, and training and trellis systems, were according to standard practice for commercial table grape production in Israel. Standard horticultural practices to control insects, fungi and weeds were employed throughout the experiment. A drip irrigation system with one line per row and in-line pressure-compensated 2.4 l h^{-1} drippers was employed, with 0.5-m spacing between drippers (Netafim Ltd., Hatzerim, Israel).

The irrigation control unit (Talgil Computing & Control Ltd., Haifa, Israel) was set for the high-volume irrigation treatment to satisfy 80% of ET_c (vine evapotranspiration obtained by lysimeters) before harvest and 60% of ET_c after harvest. In the medium-volume treatment, it was set to 60 and 40% of ET_c before and after harvest, respectively, as per common agricultural practice in the region. In the low-volume treatment, it was set to 40 and 20% ET_c before and after harvest, respectively (Table 1). The daily irrigation amounts were determined on a 5-day basis according to the ET_c data obtained from vines grown in 12 lysimeters that were located next to the vineyard and irrigated daily at 10 to 20% excess over consumption. A detailed description of the lysimeter setup can be found in Netzer et al. (2009).

2.3 Treated wastewater

Secondary treated municipal wastewater was used from a reservoir in Kibbutz Gat, located 2 km away from the experimental site. Municipal wastewater was treated by an activated-sludge process and left standing in the reservoir for a maximum of 180 days in June, and a minimum of 30 days in August. Chemical properties, determined by standard methods (Franson, 1998), are presented in Tables 2 and 3. Water samples contained organic matter of 10 to 30 mg l^{-1} BOD and 22-95 mg l^{-1} chemical oxygen demand (COD). Fertilizer was supplied daily through the irrigation water to the six fertilized treatments at a concentration of (mM): 1.29 NO_3^-, 0.64 NH_4^+, 0.87 K and 0.19 P. At the end of each drip line, a 50-cm tube led off the main line to a low-flow dripper (1 l h^{-1}) that was encapsulated in a sealed vial, and used for irrigation water sample collection. Irrigation water was collected every day, stored at a cool temperature (4°C), and condensed into a sample that represented 30 days of water collection. These water samples were used to test electrical conductivity (EC), pH, and the concentrations of Na^+, Cl^-, K^+, Mg^+, Ca^+, and NH_4^+.

2.4 Soil sampling and analysis

Soil was sampled in March 2002, before the beginning of the experiment, for chemical and physical characteristics. No significant differences were observed between plots (data not shown). Soil was sampled annually at the end of October, immediately upon termination of the irrigation period. Soil samples were taken by auger from depths of 0-30, 30-60, and 60-100 cm in the middle row of each plot. Samples were taken from 40 cm, perpendicular to the drip line and parallel to the dripper that was closest to the midpoint between two vines. Saturated paste extracts of 65°C-oven-dried soils were analyzed for EC, pH, Na^+, Cl^-, K^+, Mg^{2+}, Ca^{2+}, and NH_4^+ according to Page et al. (1982).

2.5 Leaf sampling and chemical analysis

From each replicate, 30 leaves were sampled during harvest (mid-July to mid-August)—the basal leaves opposite a bunch cluster. Leaves were rinsed in tap water several times with a final rinse in double-distilled water. Petioles and blades were separated and oven-dried at 70°C for 72 h. Samples were subsequently pulverized in an electric mill, and 150 mg of dry matter was digested with 5 ml of concentrated reagent-grade nitric acid at 130°C. The digest was brought to a volume of 50 ml with double-distilled water and kept at 4°C in the dark until analysis for Na^+ concentration by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Arcos, Spectro, Kleve, Germany).

2.6 Trunk and xylem sap sampling and chemical analysis

Trunk and xylem sap were taken during March 2007, 2 weeks before budbreak. Samples of trunk wood were obtained from four vines from each plot, 50 cm above ground level using a 7-mm-diameter tree corer (Mattson, Mechaniska, AB, Mora, Sweden). All samples were placed in 50-ml centrifuge vials and kept cool during transport to the laboratory (maximum 4 h). Each core was separated into xylem and bark tissues, using a sharp blade, before drying for 72 h at 70^{o}C. Dry material was ground to a fine dust in an electric mill and digested by the same method as the leaves.

A xylem sap collection device (Fig. 1) was inserted into each pinhole left by the sample coring. The sap-collection device was composed of a 20-cm long, 8-mm diameter plastic tube and a 50-ml centrifuge vial covered with aluminum foil. One end of the plastic tube was attached to the centrifuge vial, while the other end was attached to the pinhole left by the corer. A small (10-cm long, 1-mm diam.) plastic tube was connected to the centrifuge tube cap to release air pressure. The device was attached to the vine for 24 h.

Fig 1 Xylem sap collection device

2.7 Yield data

Each replicate was harvested and weighed during July or August when the fruit total soluble solids (TSS) reached 15 to 15.5 ^{o}Brix.

2.8 Meteorological data

The meteorological data, including precipitation amount and data used for calculating reference crop evapotranspiration (ET_o, according to the Penman-Monteith equation) were obtained from an automatic weather station located at the Lachish research station, about 100 m from the experimental site. The station monitored solar radiation (CM-11, Kipp & Zonen, Delft, Netherlands), wind speed and direction (type 05103; R.M. Young, Traverse, MI), air temperature and relative humidity (type HMP 45C; Campbell Scientific, Inc., Logan, UT).

2.9 Statistical analysis

The treatments in the vineyard were arranged in a factorial randomized block design. Data were analyzed via analysis of variance, and means were separated according to the least significant difference (LSD) at $p \leq 0.05$. The software program JMP IN 5.1 (SAS Institute Inc., Cary, NC) was used for all statistical procedures.

3. Results

3.1 Water and precipitation

The amount of winter-season precipitation fluctuated among the years of the experiment (Table 1): in 2004, 2006 and 2007, rainfall was below the annual average of 385 mm $year^{-1}$, while in 2003, it exceeded the average by 32%. The average seasonal (spring to autumn) irrigation amounts during the 6 years of the experiment were 926, 653 and 400 mm for the high, medium and low irrigation levels, respectively (Table 1). No summer rainfall was recorded.

The average EC of the FW+F was 0.69 dS m^{-1}, lower than that of the TWW+F, with fertilizer adding on average 0.17 dS m^{-1} to the EC. The lowest EC value was obtained for FW+F in 2007, while the highest EC was recorded for TWW+F in 2004 (Table 2). Na^+ concentration in the TWW and TWW+F was on average 1.8 times higher than that found in the FW+F, resulting in a 2-unit increase in SAR to about 4.35 in the TWW and TWW+F (Table 2). The pH of TWW+F was slightly lower than that of the TWW (Table 3) as a consequence of the fertilizer solution acidity (pH about 3.5). Cl^- concentrations in the TWW and TWW+F were about 30% higher than those found in the FW+F (Table 3). Only small and inconsistent differences were observed in the concentrations of Mg^{2+} and Ca^{2+} between the different water-quality treatments. Addition of fertilizer to the FW and TWW increased the concentrations of K^+ and NH_4^+ by about 20 mg l^{-1} and 16 mg l^{-1}, respectively (Table 3).

Table 2 Annual water parameters: electric conductivity (EC), Na^+ concentration and sodium adsorption ratio (SAR) of the three irrigation water-quality treatments[a] (means ± standard error, n = 8)

	2002	2003	2004	2005	2006	2007	Average
				EC (dS m^{-1})			
TWW	2.04±0.06	1.78±0.09	2.25±0.05	2.00±0.13	1.88±0.08	1.38±0.10	1.83
TWW + F	2.10±0.05	1.96±0.05	2.40±0.06	2.19±0.10	1.90±0.07	1.45±0.07	2.00
FW + F	1.64±0.04	1.41±0.05	1.22±0.05	1.47±0.10	1.20±0.03	0.9±0.17	1.31
				Sodium (mg l^{-1})			
TWW	286±2.6	241±14	197±2.2	224±11	233±3.1	203±15	230
TWW + F	288±8.2	234±14	198±1.3	220±11	234±5.4	195±20	228
FW + F	143±3.2	123±1	128±0.5	121±3.8	84±5.6	102±3.3	116
				SAR			
TWW	4.87±0.17	4.44±0.25	3.37±0.08	4.08±0.20	4.36±0.06	4.87±0.66	4.33
TWW + F	4.95±0.18	4.22±0.26	3.46±0.07	4.02±0.18	4.47±0.14	5.04±0.72	4.36
FW + F	2.45±0.03	2.35±0.02	2.09±0.02	2.44±0.21	1.62±0.08	2.37±0.19	2.22

[a]TWW, treated wastewater; TWW+F, TWW with added fertilizer; FW+F, fresh water with added fertilizer

Table 3 pH levels and Cl^-, K^+, Ca^{2+}, Mg^{2+} and ammonium (NH_4-N) concentrations in the irrigation water (for abbrev., see footnote to Table 2), 2002-2007 (means ± standard errors, n = 50)

	pH	Cl^- (mg l^{-1})	Ca^{2+} (mg l^{-1})	Mg^{2+} (mg l^{-1})	K^+ (mg l^{-1})	NH_4-N (mg l^{-1})
TWW	7.7±0.1	304.1±19.9	63.4±1.3	30.7±0.7	62.4±1.9	32.7±5.4
TWW + F	7.4±0.1	301.2±17.6	59.9±1.0	29.4±0.7	82.3±2.7	49.1±4.0
FW + F	7.3±0.1	232.6±10.9	60.9±1.2	30.7±0.7	42.7±3.0	15.3±1.5

3.2 Soil characteristics

Soil analysis data of Na concentration and SAR values in the saturated paste extraction of the 0-30, 60-90, and 60-100 cm layers exhibited similar trends of change with time, water quality, and water amount. In the following sections, we address the 30-60 cm layer to describe these tendencies.

After the first irrigation season, under all irrigation treatments, no difference was yet established in Na^+ concentration in the soil extracts. From the following year, in the plots with high irrigation level treatment, Na^+ concentrations in the soil, as measured in the saturated soil paste extracts, were significantly higher in the TWW-irrigated plots than in the FW+F-irrigated plots (Table 4). A similar pattern of Na^+ accumulation, but with lower absolute values, was found in soils of the medium irrigation level, although statistically significant differences between TWW and FW+F were established only during the fifth year of the experiment. In the low-level irrigation treatments, these differences were even smaller and significance was established only during the last year of the experiment. Adding fertilizer to the TWW treatments tended to decrease soil Na^+ concentrations, especially at the high irrigation level (Table 4). Multivariate testing for effects of water quality, water level, soil depth and years indicated that the main effect of irrigation level was significant: soil Na^+ concentrations were elevated under high irrigation (9.42 meq l^{-1}) as compared to

the medium irrigation (8.02 meq l^{-1}), and the latter was higher than that of the low irrigation (5.50 meq l^{-1}); statistical analysis grouped deviations as A, B, and C ($p < 0.0001$; n = 648).

A similar pattern was found for the SAR values in the soil extracts. By multivariate test, the SAR of the soil under varying irrigation levels developed a distinct pattern with significant differences: FW+F < TWW+F < TWW. Development of this pattern was accelerated as irrigation level increased.

From the second year (2003) on, significantly elevated SAR values were recorded in the TWW plots compared to FW+F plots under the high irrigation level. As irrigation level decreased, those significant differences were established later: from 2006 at the medium irrigation level, and in the last year of the experiment at the low irrigation level (Table 5). The effect of irrigation levels on SAR values under each water-quality treatment was analyzed separately. For the TWW treatment, the SAR ranking was significant: high > medium > low levels of irrigation. In the FW+F treatments, the ranking was high = medium > low irrigation levels. In the TWW+F treatments, there was no statistical difference among the various irrigation levels.

Table 4 Annual soil Na^+ concentrations (meq l^{-1}) in saturated paste extracts of soils from a depth of 30-60 cm under different levels of irrigation with the different water-quality treatments (for abbrev., see footnote to Table 2). Annual values for each irrigation water level followed by different letters differ significantly ($p < 0.05$) (n = 4)

	2002	2003	2004	2005	2006	2007	Average
				High Irrigation			
TWW	16.7 A	13.3 A	14.5 A	18.9 A	11.9 A	8.1 A	13.9 A
TWW + F	9.5 A	2.9 B	8.8 AB	11.9 B	10.9 A	7.1 A	8.5 B
FW + F	12.5 A	5.6 B	6.1 B	9.6 B	6.7 B	4.7 B	7.5 B
				Medium Irrigation			
TWW	11.8 A	12.0 A	14.2 A	8.9 A	10.4 A	7.5 A	10.8 A
TWW + F	8.9 A	4.3 A	9.6 A	10.7 A	11.9 A	8.4 A	9.0 AB
FW + F	11 A	4.7 A	6.6 A	8.1 A	6.2 B	4.0 A	6.8 B
				Low Irrigation			
TWW	4.0 A	3.5 B	6.0 A	5.4 A	8.9 AB	7.7 A	5.9 AB
TWW + F	3.2 A	6.3 A	4.8 A	11.8 A	11.9 A	5.3 B	7.2 A
FW + F	3.7 A	3.3 B	4.2 A	4.6 A	6.8 B	3.1 C	4.3 B

Table 5 Annual soil adsorption ratio (SAR) at a depth of 30-60 cm at different levels of irrigation with the different water-quality types (for abbrev., see footnote to Table 2). Annual values for each irrigation water level followed by different letters differ significantly ($p < 0.05$) (n = 4)

	2002	2003	2004	2005	2006	2007	Average
				High Irrigation			
TWW	6.02 A	4.92 A	6.19 A	15.25 A	9.37 A	9.38 A	8.52 A
TWW + F	3.27 A	2.19 B	3.62 AB	6.97 B	5.57 B	5.48 B	4.52 B
FW + F	5.16 A	2.57 B	2.63 B	5.72 B	4.83 B	3.90 B	4.14 B
				Medium Irrigation			
TWW	4.78 A	5.65 A	6.26 A	5.78 A	7.92 A	7.67 A	6.34 A
TWW + F	3.40 A	3.13 A	4.40 A	5.03 A	6.22 AB	8.67A	5.14 B
FW + F	4.95 A	2.98 A	3.60 A	4.66 A	4.78 B	3.34 B	4.05 B
				Low Irrigation			
TWW	3.11 A	3.04 A	3.55 A	3.95 A	3.67 A	5.59 A	3.83 A
TWW + F	2.35 A	2.87 A	2.76 A	4.44 A	5.10 A	4.38 AB	3.65 AB
FW + F	2.72 A	2.68 A	2.53 A	3.11 A	3.78 A	2.6 B	2.90 B

3.3 Plant characteristics

Na^+ concentrations in the dry matter of leaf petioles, sampled during harvest, showed significant general trends during the experiment (Fig. 2). In the 2002 and 2003 growing seasons, Na^+ concentration was stable for all treatments. From 2004 on, all leaf petioles of plants irrigated with TWW showed significantly higher Na^+ concentrations than petioles from plants irrigated with FW+F. In the FW+F treatment, petiole Na^+ concentration was quite stable with a slight decrease from 2002 to 2006, followed by a slight increase in 2007. For each of the water-quality treatments, petiole Na^+ concentration at the high irrigation level was significantly higher than that found at the low irrigation level, while values from the medium irrigation level did not differ significantly from either (Table 6). Regression values between Na^+ concentrations in the petioles and in the soil extracts at depths of 0-30 cm and 30-60 cm became significant from 2005 and 2004, respectively, with the correlation significance becoming stronger from that point on (Table 7).

Analyses of dry woody tissue from the xylem and bark, drilled from the main stem of the vine in 2008, showed an overall accumulation of Na^+ in perennial tissues. Samples from vines that were irrigated with TWW contained 30 to 60% more Na^+ in these tissues than their counterparts from the FW+F-irrigated vines (Table 8).

Sap bleeding from the xylem in the spring, prior to budbreak, collected by the device shown in Fig. 1, results from the buildup of positive hydrostatic pressure in xylem vessels due to metabolic uptake of minerals followed by osmotic water flow into the root xylem (Marangoni et al., 1986; Sperry et al., 1987). Na^+ concentrations in xylem sap collected from vines that were irrigated with TWW were significantly higher than those from vines of the FW+F treatment (Table 8).

Despite the significant differences between Na^+ concentrations among some of the treatments in the soil, leaf, xylem, and bark, no differences between treatments were found in fruit yield during the experiment (Table 9).

Table 6 Six-year averages of Na^+ concentrations in leaf petioles (dry matter) for each irrigation level and water-quality treatment (for abbrev., see footnote to Table 2): means within a column followed by different letters are significantly different ($p < 0.05$). Samples were collected during harvest in 2002-2007 (n = 24)

	TWW	TWW + F	FW + F
	- - - - - - - - -	Na^+ (mg kg^{-1})	- - - - - - - -
High irrigation	5610 A	5107 A	2631 A
Medium irrigation	4932 AB	4811 AB	2231 AB
Low irrigation	3772 B	3556 B	1980 B

Table 7 Equation and statistical evaluation of linear regression between soil Na^+ concentrations (meq l^{-1}) (x) vs. Na^+ concentrations in leaf petioles (mg kg^{-1}) (y). Soil samples were collected in the autumn from depths of 0-30 cm and 30-60 cm and leaf petioles (dry matter) were collected during harvest (n = 36)

	Na^+ in petiole vs. Na^+ in soil (0-30 cm)			Na^+ in petiole vs. Na^+ in soil (30-60 cm)		
	equation	R^2	*p*	equation	R^2	*p*
2002	y=35.56x+2843	0.163	0.016	y=50.64x+2700	0.144	0.025
2003	y=-4.28x+2962	0.0001	0.949	y=-30.15x+3109.2	0.0062	0.679
2004	y=20.47x+2756	0.0049	0.782	y=88.54x+2122.1	0.182	0.026
2005	y=206.65x+2787	0.194	0.007	y=161.28+2416.8	0.181	0.0097
2006	y=370.82x+1347	0.457	<0.0001	y=659.62x-1707	0.502	<0.0001
2007	y=661.55x+1127	0.440	<0.0001	y=578.28+1685	0.335	0.0006

Table 8 Na^+ concentration in xylem sap and in xylem and bark tissue (dry matter) for each water-quality treatment (for abbrev., see footnote to Table 2). Samples were taken 2 weeks before budbreak in 2008. Means within each column followed by different letters are significantly different ($p < 0.05$). Xylem sap values are averages of 22 samples; xylem and bark values are averages of 36 samples

	Xylem sap	Wood	Bark
	Na^+ (mg l^{-1})	- - - - Na^+ (mg kg^{-1}) - - - -	
TWW	43.6 A	821 A	398 A
TWW + F	48.6 A	834 A	476 A
FW + F	21.7 B	538 B	298 B

Table 9 Yields (t ha^{-1}) of Superior Seedless grapes in the three water-quality treatments (for abbrev., see footnote to Table 2). Means within each column do not differ significantly ($p < 0.05$, n = 36 for each treatment in each year)

	2002	2003	2004	2005	2006	2007	Ave
	- - - - - - - - - - - - - - - -	Yield (t ha^{-1})	- - - - - - - - - - - - - - - -				
TWW	34.9	19.0	40.6	15.2	47.4	20.0	29.5
TWW + F	32.7	22.1	43.4	10.3	44.4	20.1	29.0
FW + F	37.1	20.1	40.4	13.5	51.8	20.7	30.6

4. Discussion

Irrigation with TWW poses a great challenge, but one that is worth taking on given today's shortage of water resources, particularly in arid and semiarid regions. Irrigation with low-quality water requires continuous monitoring of soil and plant parameters to ensure that the salt components in the TWW are not having a pronounced negative effect on soil properties or directly on plant growth (Prior et al., 1992).

We monitored a broad range of elements, including macronutrients, micronutrients and heavy metals, with the aim of determining their potential risks. Sodium ions (Na^+) stood out as the most dominant hazardous element, affecting both plant and soil properties. Use of TWW may further complicate the difficult nature of salinity measurements: daily and seasonal fluctuations require continuous monitoring and analysis of ionic concentrations in the water. The average Na^+ concentration in the TWW for the six seasons of the present experiment was double that in the FW (Table 2). Clay soils that are continually exposed to irrigation with TWW or saline water with high SAR values show a gradual replacement of adsorbed Ca^{2+} by Na^+, causing an increase in soil sodicity. The Na^+ cation, having a large hydrated radius, increases the diffuse double layer of clay minerals. Thus, high SAR, when combined with low ionic strength of the soil solution such as that encountered in the rainy season, may result in clay swelling and dispersion, damaging the soil's physical properties and decreasing infiltration rates (Brady, 1990).

Soil Na^+ concentration in autumn, at the end of the irrigation season, is likely to be at its highest values for the year, reflecting the balance between the amounts of salt added and leached by the irrigation water. Spring soil Na^+ concentration on the other hand, is affected by winter leaching by rains (Agassi et al., 2003). The autumn-to-autumn changes are effected by both components, namely input by irrigation water and output by irrigation and rain leaching. For example, in this study, Na^+ concentration in the soil was lower in seven out of nine irrigation treatments in the autumn of 2003 than in the autumn of 2002 (Table 4). This decrease was related to the overall reduction of about 40 and 20 mg l^{-1} of sodium in the TWW and FW, respectively, during the 2003 irrigation season (Table 2), in addition to a 30% increase in rainfall during the winter of 2002/2003 over the average yearly precipitation levels during the experiment (Table 1). In 2007, there was a ca. 30% reduction in soil Na^+ concentration for all TWW-irrigation treatments compared to 2006. This was explained by reduced sodium concentrations in the TWW (Tables 2 and 4).

Significantly higher Na^+ concentrations were found in the soil of the high-level TWW-irrigated plots from the second year of the experiment, while at the medium and low irrigation levels, a pronounced difference in soil Na^+ concentrations between the TWW and FW treatments was noted only in the last 2 years of the trial (Table 4). A multiseasonal analysis of the different water-quality treatments at different soil depths revealed that overall soil Na^+ concentration for the high irrigation treatments was significantly higher than that in the medium irrigation treatments, and the latter was significantly higher than that in the low irrigation treatments. It has previously been suggested that maintenance of high soil moisture and consequently, high water potential in the root zone may diminish the osmotic effects of saline irrigation water (Michelakis et al., 1993; Oron et al., 2002). Others have suggested increasing irrigation over

evapotranspiration when using saline water to ensure a leaching fraction that will prevent salt accumulation in soil, a practice that would maintain salinity under the threshold value for a given crop (Ayers and Westcot, 1985; Bresler, 1987; Dudley et al., 2008). This practice may help maintain reasonable Cl^- concentrations but, according to our results, elevated irrigation amounts were not only insufficient to support Na^+ leaching, they led to a further increase in soil Na^+ concentrations. We conclude that in clay soils, this practice may accelerate Na^+ accumulation and increase SAR in the soil. The effect of increased SAR in the soil on soil water permeability has been well documented (e.g., Quirk and Schofield, 1955; Richards, 1954). It should be noted that the amount of irrigation for table grape vineyards in the Lachish region ranges between 350 and 550 mm per season. The two highest irrigation levels in our experiment exceeded this commonly used range and resulted in different leaching fractions.

At depths of 30-60 cm, soil Na^+ concentration in the TWW treatment was noticeably higher than in the TWW+F treatment during the first 4 years of the experiment under high irrigation levels (only two of those years showed a significant difference). The differences between the treatments became less noticeable with time, as the soil Na^+ concentration in the TWW+F treatment rose to levels similar to those in the TWW treatment. At all irrigation levels, the overall average value of soil Na^+ concentration in the TWW (10.20 meq l^{-1}) treatments was significantly higher than in the TWW+F treatments (8.23 meq l^{-1}), and the latter was significantly higher than in the FW+F treatments (6.2 meq l^{-1}) (Table 4). Multiseasonal analysis at different irrigation levels for different soil depths showed that overall soil Na^+ concentration in the TWW treatment was significantly higher than in the TWW+F treatment. These results suggest that components within the added fertilizer affect Na^+ accumulation, at least during the first years of irrigation with TWW. Since soils in this area contain a large percentage of clay (42%), it is likely that cations contributed by the fertilizer compete with Na^+ for sites within the soil's exchangeable complex. Ca^{2+} and Mg^{2+} levels did not differ greatly among the water-quality treatments (Table 3), but K^+ and NH_3^+ concentrations were greater by 30 and 50%, respectively, in the TWW+F vs. TWW treatments (Table 3). An additional explanation for this phenomenon might be related to the fact that the TWW fertilizer supplement reduces the pH of the water from 7.7 to 7.4 (Table 3). The soil pH in the two treatments was similar according to the extracted paste pH. The pH buffering was probably related to Ca^{2+}-carbonate, and to a lesser extent Mg-carbonate dissolution, which released Ca^{2+} and possibly Mg^{2+} that in turn, competed with the Na^+ cations. Different studies have shown that compared to Na^+, not only divalent cations (e.g., Ca^{2+} and Mg^{2+}), but also the monovalent K^+ and NH_4^+ cations improve the soil's structural stability and water permeability (Brooks et al., 1956; Chen et al., 1983; Rao and Mathew, 1995).

Soil swelling and dispersion, which in turn decrease water percolation and soil aeration, are expected when SAR values exceed 8 (Brady, 1990; McBride, 1994). Others have noted that these effects can occur at lower SAR values when water ionic strength is low, e.g., during rainstorms (Mace and Amrhein, 2001). In the high irrigation treatments, there was a similarity between the dynamics of Na^+ concentration and SAR in the soil (Table 5): in four out of the six seasons, significantly higher SAR values were found in the soil of the TWW irrigation treatments than in those of the TWW+F treatments (TWW+F had values similar to

FW+F). According to the SAR values, there is a lower risk of soil degradation as the amount of irrigation is reduced.

While chemical composition of the soil represents its long-term history, sampling at a fixed location—40 cm perpendicular to the dripper in our case, may introduce a certain bias due to differences in the volume of the onion-shaped wetted soil. The dimensions of the onion-shaped wetting pattern change during the season, especially between irrigation treatments. On the other hand, the plant, which is exposed to the overall soil salinity gradients via its root system, may better represent the concurrent integrated salinity status. Accordingly, the plant can be regarded as a spatial and temporal integrator of the whole root zone. In vines, leaf samples for mineral analysis are usually taken opposite the cluster (Christensen, 1969), and petioles, rather than blades, are considered more highly representative due to their higher mineral and salt contents (Downton, 1977b; Fisarakis et al., 2001; Prior et al., 1992).

The dynamics of Na^+ concentration in the leaves among treatments and seasons was less complex than the dynamics of Na^+ concentration in the soil. As new leaves appear each spring, Na^+ concentration in the newly formed leaves is a measure of that in the soil solution during the growing season plus that stored in the roots, trunk and canes. Significant differences in leaf Na concentration between TWW and FW treatments were recorded from the third year of the experiment (Fig. 2). Petiole Na^+ concentration in FW+F-irrigated plants remained relatively low, whereas that in the TWW- and TWW+F-irrigated vines reached values of nearly 7,000 mg kg^{-1}. The concentration of Na measured in the petioles during flowering is considered toxic at levels in excess of 5,000 mg kg^{-1} (Nagarajah, 1992; Prior et al., 1992; Reuter and Robinson, 1997). Symptoms of salt toxicity appear as leaf chlorosis or burns on the leaf margins followed by early defoliation (Fisarakis et al., 2001; Paranychianakis and Angelakis, 2008; Prior et al., 1992). Less severe visual symptoms of leaf chlorosis in our experiment appeared only in the TWW and TWW+F treatments from the 2005 season. Those mild chlorosis symptoms appeared sporadically from 3 weeks after

budbreak, and gradually disappeared toward veraison.

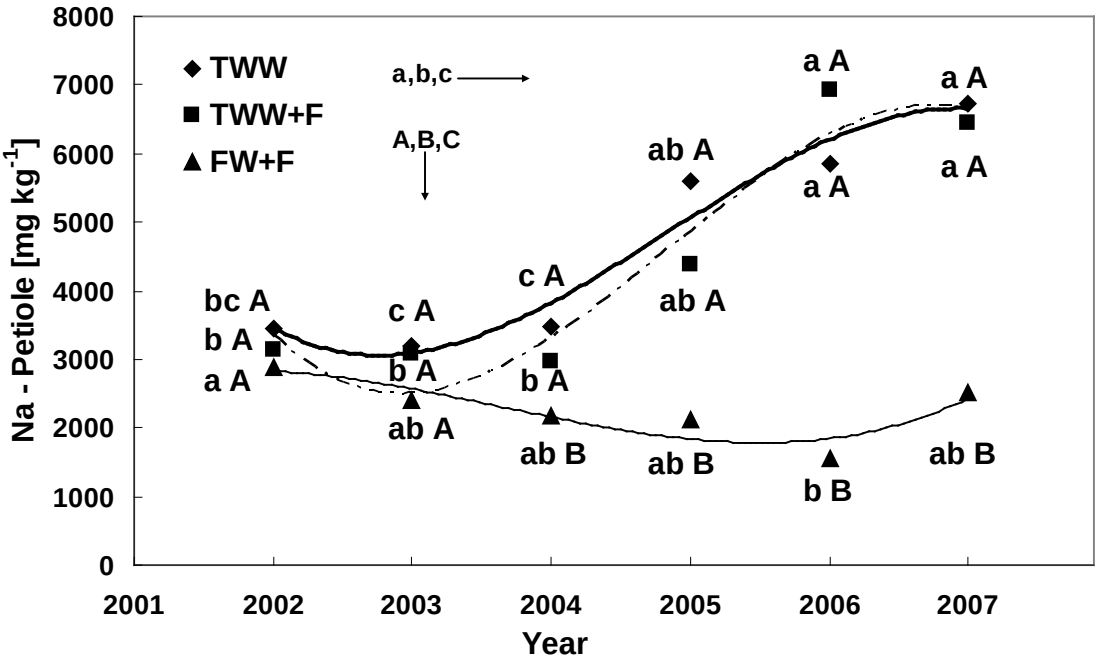

Fig 2 Na^+ concentrations in leaf petioles (dry matter) as a function of year for each water-quality treatment: each data point is an average of 12 plots (three irrigation level treatments x four replicates). Different capital letters indicate significant differences ($p < 0.05$) between water-quality treatments for a given year (read vertically). Different lowercase letters indicate significant differences between years for each water-quality treatment (read horizontally). Samples were collected during harvest. The curves are arbitrarily drawn according to the following equations (where y is petiole Na concentration and x is year):
TWW (thick line): $y = -99.7x^3+599725x^2-1*10^9x+8*10^{11}$; $R^2 = 0.95$
TWW+F (dashed line): $y = -148.7x^3+894216x^2-2*10^9x+10^{12}$; $R^2 = 0.92$
FW+F (thin line): $y = 40.2x^3+241493x^2+5*10^8x+3*10^{11}$; $R^2 = 0.81$
TWW, treated wastewater; TWW+F, TWW with added fertilizer; FW+F, fresh water with added fertilizer

Similar to the findings in the soil, Na^+ concentration in the leaves increased with increasing irrigation level (Table 6). Increasing irrigation by about 45% above the common regional FW irrigation practices (high irrigation level in the experiment) does not improve sodium leaching beyond the root zone; on the contrary, it increases Na^+ accumulation in the leaves (Table 6). Despite the different dynamics of Na^+ accumulation in soil and leaves, the correlations between the two strengthened during the final two years of the trial (Table 7).

At the end of the experiment, Na^+ concentration in the xylem and bark samples, isolated from the trunk of TWW- and TWW+F-irrigated vines, nearly doubled in comparison to FW+F-irrigated vines (Table 8). The perennial parts of the vines—roots, trunk and canes, probably provide an additional source of Na^+ that reaches the leaves with the transpiration stream during the growing season (Fisarakis et al., 2001).

Rootstock and scion varieties are important factors in vineyard success under salt-stress conditions (Downton, 1977a; Groot Obbink and Alexander, 1973). A mature commercial vineyard of own-rooted red globe grapes, which was located near the experimental site under similar irrigation conditions, collapsed after only 3 years of irrigation with similar quality TWW. Xylem samples taken from the vines' trunk showed a Na^+ concentration of 2271 to 2294 mg kg^{-1} (data not shown). Thus, xylem analyses can potentially be used for the identification of salt stress postmortem. The use of different rootstocks produces variability in Cl^- and Na^+ accumulation (Downton, 1977a; Fisarakis et al., 2001; Paranychianakis and Angelakis, 2008), but even rootstocks that are able to sequester Cl^- differ in their ability to sequester Na^+ (Sharma and Upadhyay, 2008). The 1103 'Paulsen' rootstock used in this experiment is categorized as one the most effective at tolerating salt (Fisarakis et al., 2001; Zhang et al., 2002).

Before budbreak in early spring, the xylem vessels fill as a result of positive hydraulic pressure, also known as root pressure, which builds up due to active ion uptake into the roots followed by diffusion of water into the root xylem. This process is accompanied by xylem bleeding from the cut surfaces of pruned branches (Sperry et al., 1987). In this study, we found Na^+ concentration in the xylem sap from the TWW and TWW+F treatments to be more than double that in the sap from the FW+F treatment (Table 8). Since xylem sap was sampled in spring, when soil Na^+ is at its lowest level, the difference among treatments in sap Na^+ concentration may reflect the differences in the amount of both Na^+ in the soil at that time and Na^+ that has accumulated in the root system during past seasons.

Despite the accumulation of Na^+ in the soil and vines, there was no significant impact of water-quality treatment on total grape yield. The pronounced differences observed between seasons were due to the alternate yield-bearing trait of cv. Superior Seedless.

Many studies on the effect of irrigation water salinity on grapevines have focused on Cl^- rather than Na^+ (Downton, 1977a; Fisarakis et al., 2001; Groot Obbink and Alexander, 1973; Woodham, 1956). High levels of Cl^- cause growth depression and typical damage symptoms on leaves (Downton, 1985). Cl^- concentration in petioles is considered toxic above 10,000 or 15,000 mg kg^{-1} (Nagarajah, 1992; Prior et al., 1992; Reuter and Robinson, 1997). In the present study, maximum values of Cl^- in the petioles did not reach the toxic threshold: in the TWW treatment, values were 9131 mg kg^{-1} and in the FW+F treatment, 6245 mg kg^{-1}. Cl^- can reach toxic levels in a number of ways: high concentrations of Cl^- in the source water, high evapotranspirative demand and poor management of soil moisture. Under conditions in which the evaporation fraction of evapotranspiration rises, such as when canopy shade is minimal (in case of wine grapes), there is a higher risk of soil salinity. If Cl^- does not reach toxic levels during a particular seasonal irrigation, most of the Cl^- will be effectively leached from the soil by winter precipitation. A comparison of soil salt concentrations in autumn and spring showed that the percentage of leaching during the winter ranges from 41-66% for Na^+ and 80-95% for Cl^- at 0-30 cm soil depth, and 15-54% for Na^+ and 73-93% for Cl^- at 30-60 cm soil depth (detailed data not presented). We assume that the moderate Cl^- concentrations in the TWW itself of ca. 300 mg l^{-1} (Table 3) and sufficient leaching of Cl^- during the winter prevent Cl^- accumulation to toxic levels in the vines.

5. Conclusions

Under the present experimental conditions, accumulation of sodium, rather than chloride, in the soil and in the plant emerged as the major problem related to TWW irrigation. Soil Na^+ concentrations and SAR values fluctuate in accordance with those in the source water and the amount of winter precipitation, and these soil parameters were significantly higher in the TWW-irrigated plots. Furthermore, adding fertilizer solution to the TWW moderated the soil Na^+ accumulation and SAR increase. In contrast to previous studies which called for increasing the leaching fraction to decrease solute accumulation in soils, in this study we found that increasing the leaching fraction by increasing irrigation amounts in a clay soil actually accelerates the buildup of Na^+ and the increase in SAR values.

Plant analyses indicated that vines exposed to continuous TWW irrigation, with or without fertilizer, show a clear pattern of elevated Na^+ levels in leaf petioles and in the xylem sap collected in spring, suggesting gradual Na^+ accumulation in the perennial parts of the vine. This effect of TWW was similar to that found in the soil, but differences in the plant were more significant and we could conclude that the plant, via its root system, may act as a spatial and temporal integrator of the entire root zone; thus plant data might be less sensitive to sampling protocols. Spring xylem sap and trunk wood are proposed as additional indicators of salinity development.

Unlike in the soil, addition of fertilizer to the TWW did not diminish Na^+ accumulation in the plant. This may support the assumed mechanism of cation competition underlying the fertilizer's dampening of the TWW effect on soil Na^+ accumulation: we suggest that the fertilizer-originated cations (K^+ and NH_4^+) and/or acid-dissolved soil-originated cations (Ca^{2+} and Mg^{2+}) compete with the TWW-originated Na^+ and restrict its accumulation as an exchangeable cation. Note that in this regard, plant Na^+ indicators may respond mainly to Na^+ concentration in the soil solution, and only to a lesser extent to Na^+ accumulation in the exchangeable soil complex. The latter may, however, affect the plant indirectly by degrading the soil's physical properties.

The yield of var. Superior table grapes grafted on 'Paulsen' rootstock was not significantly affected by the water-quality treatments during the 6 years of the experiment. Nevertheless, trends of Na^+ accumulation in the plant and soil may pose a potential risk in subsequent years under long-term use of TWW.

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References

Agassi, M., Tarchitzky, J., Kern, R., Chen, Y., Goldstein, D., Fizik, E., 2003. Effects of prolonged irrigation with treated municipal effluent on runoff rate. J. Environ. Qual. 32, 1053-1057.

Aharoni, A., Cikurel, H., 2006. Mekorot's research activity in technological improvements for the production of unrestricted irrigation quality effluents. Desalination 187, 347-360.

Avnimelech, Y., 1993. Irrigation with sewage effluents: the Israeli experience. Environ. Sci. Technol. 27, 1278-1281.

Ayers, R. S., Westcot, D. W., 1985. Water quality for agriculture. FAO Irrig. Drain. Pap. 29. F.A.O., Rome.

Bresler, E., 1987. Application of a conceptual model to irrigation water requirement and salt tolerance of crops. Soil Sci. Soc. Am. J. 51, 788-793.

Bhardwaj, A.K., Goldstein, D., Azenkot, A., Levy, G. J. 2007. Irrigation with treated wastewater under two different irrigation methods: effects on hydraulic conductivity of a clay soil. Geoderma 140, 199-206.

Bond, W. J., 1998. Effluent irrigation - an environmental challenge for soil science. Aust. J. Soil Res. 36, 543-555.

Brady, N. C., 1990. The nature and properties of soils. 10th edn. MacMillan Publishing Company. New York.

Brooks, R. H., Bower, G. A., Reeves, R. C., 1956. The effect of various exchangeable cations upon the physical condition of soils. Soil Sci. Soc. Am. Proc. 20, 325-327.

Carden, D. E., Walker, D. J., Flowers, T. J., Miller, A. J., 2003. Single-cell measurements of the contributions of cytosolic Na^+ and K^+ to salt tolerance. Plant Physiol. 131, 676-683.

Chen, Y., Banin, A., Borochovitch, A., 1983. Effect of potassium on soil structure in relation to hydraulic conductivity. Geoderma, 30, 135-147.

Christensen, P., 1969. Seasonal changes and distribution of nutritional elements in Thompson Seedless grapevines. Am. J. Enol. Vitic. 20, 176-190.

Downton, W. J. S., 1977a. Chloride accumulation in different species of grapevine. Sci. Hort. 7, 249-253.

Downton, W. J. S., 1977b. Influence of rootstocks on the accumulation of chloride, sodium and potassium in grapevines. Aust. J. Agric. Res. 28, 879-889.

Downton, W. J. S., 1985. Growth and mineral composition of the sultana grapevine as influenced by salinity and rootstock. Aust. J. Agric. Res. 36, 425-434.

Dudley, L. M., Ben-Gal, A., Shani, U., 2008. Influence of plant, soil, and water on the leaching fraction. Vadose Zone J. 7, 420-425.

F.A.O., 2008. F.A.O. Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/spush Last accessed 27/06/2010.

Feigin, A., Ravina, I., Shalhevet, J., 1991. Irrigation with sewage effluent. Advanced Series in Agricultural Science, vol. 17. Springer-Verlag, Berlin.

Fisarakis, I., Chartzoulakis, K., Stavrakas, D., 2001. Response of Sultana vines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. Agric. Water Manage. 51, 13-27.

Flowers, T. J., Yeo, A. R., 1986. Ion relations of plants under drought and salinity. Aust. J. Plant Physiol. 13, 75-91.

Francois, L. E., Mass, E. V., 1994. Crop response and management on salt-affected soils. In: Pessarakli, M. (ed) Handbook of plant and crop stress. Marcel Dekker, New York, pp. 149-181.

Franson, M. A. H., 1998. Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association,Washington, DC.

Fuchs, M., 2007. Impact of reaserch on water use for irrigation in Israel. Irrig. Sci. 25, 443-445. doi:10.1007/s00271-006-0050-z

Garcia, M., Charbaji, T. 1993. Effect of sodium chloride salinity on cation equilbria in grapevines. J. Plant Nutr. 16, 2225-2237.

Gerardi, M. H., Zimmerman, M. C., 2005. Wastewater pathogens. Wiley-Interscience, John Wiley & Sons, Inc., NJ, p. 179.

Greenway, H., Munns, R., 1980. Mechanisms of salt-tolerance in non-halophytes. Ann. Rev. Plant. Physiol. 31, 149-190.

Groot Obbink, J., Alexander, D. M., 1973. Response of six grapevine cultivars to a range of chloride concentrations. Am. J. Enol. Viticult. 24, 65-68.

Halperin, R., 1999. Regulations for water quality in Israel and internationally. Health in the Field 7, 3-6. [in Hebrew]

Inbar Committee., 2003. Effluent Standards Regulations, Ministry of Environmental Protection, Israel. [in Hebrew] http://www.sviva.gov.il/Enviroment/Static/Binaries/index_pirsumim/p0321_1.pdf Last accessed 27/06/2010

Maas, E.V., 1990. Crop salt tolerance. In: Tanji K.K. (ed) Agricultural salinity assessment and management. American Society of Civil Engineers, New York.

Maas, E.V., Hoffman, G.J., 1977. Crop salt tolerance - current assessment. J. Irrigation and Drainage Division of A.S.C.E., 103, 115-134.

Mace, J.E., Amrhein, C., 2001. Leaching and reclamation of a soil irrigated with moderate SAR waters. Soil Sci. Soc. Am. J. 65, 199-204.

Marangoni, B., Vitagliano, C., Peterlunger, E., 1986. The effect of defoliation on the composition of xylem sap from Cabernet franc grapevines. Am. J. Enol. Vitic. 37, 259-262.

McBride, M.B., 1994. Environmental chemistry of soils. Oxford University Press, New York/Oxford

Michelakis, N., Vougioucalou, E., Clapaki, G., 1993. Water use, wetted soil volume, root distribution and yield of avocado under drip irrigation. Agric. Water Manage. 24, 119-131.

Morgenroth, E., Kommedal, R., Harremoes, P., 2002. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment - a review. Water Sci. Technol. 45, 25-40.

Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16, 15-24.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239-250.

Munns, R., Guo, J., Passioura, J.B., Cramer, G.R., 2000. Leaf water status controls day-time but not daily rates of leaf expansion in salt-treated barley. Aust. J. Plant Physiol. 27, 949-957.

Nagarajah, S., 1992. Soil toxicity in Sunrasia vineyards. Aust. Grape Grower and Winemaker 334, 11-14.

Netzer, Y., Yao, C., Shenker, M., Bravdo, B., Schwartz, A., 2009. Water use and the development of seasonal crop coefficients for Superior Seedless grapevines trained to an open-gable trellis system. Irrig. Sci. 27, 109-120.

Oron, G., DeMalach, Y., Gillerman, L., David, I., Rao, V.P., 2002. Improved saline-water use under subsurface drip irrigation. Agric. Water Manage. 81, 237-247.

Page, A.L., Miller, R.H., Keeney, D.R., 1982. Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI , USA.

Paranychianakis, N.V., Angelakis, A.N., 2008. The effect of water stress and rootstock on the development of leaf injuries in grapevines irrigated with saline effluent. Agric Water Manage. 95, 375-382.

Prior, L.D., Grieve, A.M., Slavich, P.G., Cullis, B.R.,1992. Sodium chloride and soil texture interactions in irrigated field grown sultana grapevines. II. Plant mineral content, growth and physiology. Aust. J. Agric. Res. 43, 1067-1083.

Quirk, J.P., Schofield, R.K., 1955. The effect of electrolyte concentration on soil permeability. J. Soil Sci. 6, 163-178.

Rao, S.N., Mathew, P.K., 1995. Effects of exchangeable cations on hydraulic conductivity of a marine clay. Clays Clay Miner 43, 433-437.

Reuter, D.J., Robinson, J.B., 1997. Plant analysis- an interpretation manual, 2nd edn. CSIRO, Collingwood, Australia.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. United State Salinity Laboratory, Riverside, CA, USA.

Roland, B., Deletic, A., Crag, A., 2000. Techniques for water and wastewater management: a review of techniques and their integration in planning. Urban Water. 2, 197-221.

Shani, U., Ben-Gal, A., 2005. Long-term response of grapevines to salinity: osmotic effects and ion toxicity. Am. J. Enol. Vitic. 56, 148-154.

Sharma, J., Upadhyay, A.K., 2008. Rootstock effect on Tas-A-Ganesh (*Vitis vinifera* L.) for sodium and chloride uptake. Acta. Hort. 785, 113-116.

Sperry, J.S., Holbrook, N.M., Zimmermann, M.H., Tyree, M.T., 1987. Spring filling of xylem vessels in wild grapevine. Plant Physiol. 83, 414-417.

Suarez, D.L., Wood, D.J., Lesch, S.M., 2006. Effect of SAR on water infiltration under a sequential rain-irrigation management system. Agric. Water Manage. 86, 150-164.

Tarchitzky, J., Golobati, Y., Keren, R., Chen, Y., 1999. Wastewater effects on montmorillonite suspensions and hydraulic properties of sandy soils. Soil Sci. Soc. Am. J. 63, 554-560.

Tarchitzky, J., Lerner, O., Shani, U., Arye, G., Lowengart-Aycicegi, A., Brener, A., Chen, Y. 2007. Water distribution pattern in treated wastewater irrigated soils: hydrophobicity effect. Eur. J. Soil Sci. 58, 573-588.

Tester, M., Davenport, R.J., 2003. Na^+ transport and Na^+ tolerance in higher plants. Ann. Bot. (Lond) 91, 503-527.

Toze, S., 2006. Reuse of effluent water-benefits and risks. Agric. Water Manage. 80, 147-159.

W.H.O., 1989. Health guidelines for the use of wastewater in agriculture and aquaculture. Report of a W.H.O. scientific group, Technical Report Series No. 778. World Health Organization, Geneva.

Woodham, R.C., 1956. The chloride status of the irrigated Sultana vine and its relation to vine health. Aust. J. Agric. Res. 7, 414-427.

Yermiyahu, U., Ben-Gal, A., Sarig, P., Zipilevitch, E., 2007. Boron toxicity in grapevine (*Vitis vinifera* L.) in conjunction with salinity and rootstock effects. J. Hort. Sci. Biotechnol. 82, 547-554.

Yunusa, I.A.M., Walker, R.R., Blackmore, D.H., 1997. Characterization of water use by Sultana grapevines on their own roots or on Ramsy rootstock drip-irrigated with water of different salinities. Irrig. Sci. 17, 77-86.

Zhang, X., Walker, R.R., Stevens, R.M., Prior, L.D., 2002. Yield-salinity relationships of different grapevine (*Vitis vinifera* L.) scion-rootstock combinations. Aust. J. Grape Wine Res. 8,150-156.

4.4 Fourth publication

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RESEARCH PAPER

A non-invasive probe for online-monitoring of turgor pressure changes under field conditions

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ABSTRACT

An advanced non-invasive, field-suitable and inexpensive leaf patch clamp pressure probe for online-monitoring of the water relations of intact leaves is described. The probe measures the attenuated output patch clamp pressure, P_p, of a clamped leaf in response to an externally applied input pressure, P_{clamp}. P_{clamp} is generated magnetically. P_p is sensed by a pressure sensor integrated into the magnetic clamp. The magnitude of P_p depends on the transfer function, T_f, of the leaf cells. T_f consists of a turgor pressure-independent (related to the compression of the cuticle, cell walls and other structural elements) and a turgor pressure-dependent term. T_f is dimensionless and assumes values between 0 and 1. Theory shows that T_f is a power function of cell turgor pressure P_c. Concomitant P_p and P_c measurements on grapevines confirmed the relationship between T_f and P_c. P_p peaked if P_c approached zero and assumed low values if P_c reached maximum values. The novel probe was successfully tested on leaves of irrigated and non-irrigated grapevines under field conditions. Data show that slight changes in the microclimate and/or water supply (by irrigation or rain) are reflected very sensitively in P_p.

INTRODUCTION

Increasing worldwide shortages of fresh water, the continuous increase in water consumption by agriculture and the progressive salinisation of arable land provoked by irrigation are global problems (Läuchli & Lüttge 2002; Olesen & Bindi 2002; Jones *et al.* 2005; Fuchs 2007; Iglesias *et al.* 2007). Drip irrigation methods have saved water consumption in agricultural crops and have slowed down soil salinisation, but have highlighted the need for sensors to monitor sub-optimal water status of the crop. Water status can be determined by plant-based (*e.g.* xylem pressure/sap flow, turgor pressure, stomatal conductance, photosynthesis, transpiration, leaf temperature/thickness) and/or by soil-based (*e.g.* water content and water potential) indicators (Jones 2004). Plant-based sensing has many potential advantages over soil-based sensing, but a large number of practical difficulties have limited routine field application.

The water status of plants is usually determined by determination of the leaf water status using the pressure chamber (Scholander *et al.* 1965). The method is simple and thus very popular, but massively invasive, time-consuming and unsuitable for automation. A further drawback is that the number of leaves that can be measured is rather limited and, therefore, data can be misrepresentative of the overall *in situ* conditions (due to variability in height, sun exposure, microclimate conditions, canopy circumference *etc.*). Most importantly, and frequently ignored, the readings cannot always straightforwardly be

interpreted in terms of xylem pressure and/or turgor pressure (Zimmermann *et al.* 2004; Zimmermann *et al.* 2007). Both parameters are linked hydraulically to each other in turgescent cells and can quite accurately be measured by the probe techniques pioneered by Zimmermann and co-workers (Zimmermann *et al.* 1969; Balling & Zimmermann 1990). Even though these techniques are minimal-invasive and allow very accurate measurements on the single xylem vessel or cell level of intact plants, they are not suitable for long-term outdoor applications because of their susceptibility to gusting winds and heavy rain. There is a similar problem with ball tonometry, a non-destructive method by which cell turgor pressure is recorded by application of an external pressure (Lintilhac *et al.* 2000; Geitmann 2006). Online measurements of leaf thickness have been suggested as an indirect indicator for turgescence of leaves (*e.g.* Burquez 1987; McBurney 1992; Malone 1993). A number of instruments are commercially available for the routine monitoring of leaf thickness. However, leaf thickness sensors as well as other plant-based sensors have not found widespread applications in irrigation scheduling. The main reasons for this are the frequent insensitivity of leaf thickness to changes in the water status of the leaves and the anisotropy of leaf shrinkage (Jones 2004). Thus, development of a simple, inexpensive and field-suitable sensor for precise online monitoring of leaf turgor pressure or, generally speaking, of leaf water status of intact plants continues to be an important and indispensable aspect of any optimisation of irrigation management.

In this communication, an advanced online operating, non-invasive, field-suitable and inexpensive leaf patch clamp pressure probe is described. This probe measures the attenuated pressure of a leaf, P_p, in response to an external clamped pressure, P_{clamp}. The attenuation of P_{clamp} depends on the leaf transfer function. The magnitude of the leaf transfer function, and thus attenuation of external pressure signals, is determined by a plant-specific, turgor pressure-independent term (related to the compression of the cuticle, cell walls and other structural elements) and a turgor pressure-dependent term, T_f. The potential of the leaf patch clamp pressure probe for long-term measurements of leaf cell turgor pressure is demonstrated by measurements on various grapevine cultures under different climate and irrigation conditions. Concomitant pressure chamber and cell turgor pressure measurements (using the cell turgor pressure probe) demonstrated that this novel probe exceeds these techniques and other plant-based sensor technologies in all relevant performance criteria (see also Jones 2004).

MATERIALS AND METHODS

Measuring sites

Data collection on grapevine (used for the production of table grapes) was conducted during June and September 2007 at the Lachish Research and Development station near Quiryat-Gat, Israel (31°36′15.6″ N; 34°47′25.9″ E; elevation 146 m). The vineyard was irrigated by drip irrigation that could be switched off and on according to the experimental needs. The measuring site was characterised by hot and dry weather during the day (up to 38 °C) and by still relatively high temperatures during the night (about 18 °C). Diurnal changes in temperature (T) and relative humidity (RH) were practically identical over the entire week of experimentation. Drizzle occurred sometimes before predawn because of a relative humidity of 100%. During the day, RH dropped to about 40%. Field experiments on grapevines (used for the production of white wine grapes) were also conducted at Gedera, Israel (31°46′25.2″ N; 34°44′46.8″ E; elevation 50 m) in June/July 2008. Grapevines were irrigated according to an irrigation regime used by the owner of the vineyard in previous years. Maximum and minimum T and RH values were comparable to those recorded at Quiryat-Gat during the day and the night, but subject to more significant variations (see below). Measurements were also performed during August 2007 at the vineyards of the Schmachtenberger Farm close to Würzburg, Germany (49°46′6.7″ N; 9°58′12.0″ E; elevation 260 m). These vineyards were not irrigated. The rainfall was often extremely high during the experimental period (see below).

Plants and planting conditions

Vineyard Quiryat-Gat

Leaf patch clamp pressure, pressure bomb and cell turgor pressure measurements were performed on *Vitis vinifera* L. *cv. Superior Seedless*. The grapevines were about 2-m tall. Spacing between the rows was 3.5 m and between the plants, 2 m. The plant density was 1428 grapevines per ha. The first, outer row was equipped with drainage lysimeters. The plants in this row were irrigated daily between 06:00 h and 11:00 h (EET, Eastern European Time). For irrigation of the grapevine rows, fresh water was used, except for the second row, which was irrigated with effluent. Before and during the experimental period in June the irrigation amount per grapevine and day was 70 l. After harvest of the grapes in August, irrigation water was reduced to 50 l per plant and day. The leaf area index of the lysimeter-treated plants and of plants irrigated with effluent varied between 3.4 and 5.7.

Irrigation of the fifth row located in the centre of the vineyard and east of the 'lysimeter row' was stopped on June 18th and started again on June 24th at 11:25 h. The irrigation amount per grapevine and day was 19 l of fresh water and was reduced to about 10 l per day after harvest. The leaf area index of these plants varied between 3.9 (within the row) and 4.7 (at the end of the row).

An automatic weather station was installed at the vineyard and used to record solar radiation (CM-11, Kipp & Zonen, Delft, the Netherlands), wind speed and direction (type 05103; R. M. Young, Traverse, MI, USA), air temperature and RH (type HMP 45C; Campbell Scientific, Inc., Logan, UT, USA). During the experimental periods

in June and September, ambient T and RH were recorded close to the sites of the leaf patch clamp pressure probes using thermistors (Tinytag; RS Components GmbH, Mörfelden-Walldorf, Germany). Data were collected every 5 min.

Vineyard Gedera

Leaf patch clamp pressure and cell turgor pressure measurements were performed on *V. vinifera* L. *cv. French Colombard*. The grapevines were about 1.7-m tall. Spacing between the rows was 3.0 m and between the plants, 1.5 m. Each row contained 92 vines (2200 grapevines per ha). Plants were irrigated once a week from 10:00 h to 15:00 h. The irrigation amount per grapevine was about 50 l. The leaf area index of the plants was about 1.1. T and RH were recorded close to the sites of the leaf patch clamp pressure probes using Tinytag thermistors. Data were collected every 5 min.

Vineyard Würzburg

Leaf patch clamp pressure probe and pressure bomb measurements were performed on *V. vinifera* L. *cv. Bacchus*. The grapevines were about 1.8-m tall. Spacing between the rows was 1.6 m and between the plants, 1.35 m. The plant density was 4600 grapevines per ha. The leaf area index was about 1.4. T and RH were recorded close to the sites of the leaf patch clamp pressure probes using Tinytag thermistors. Data were collected every 5 min.

Leaf patch clamp pressure probe

For patch clamp pressure measurements, a leaf was positioned in the space between two planar circular pads, where one of the pads contained a receptacle (area: 4×2.5 mm^2, height: 0.8–1.2 mm) for integration of the pressure sensor chip. The principle of operation and a probe clamped to a leaf are shown schematically in Fig. 1a. The clamp pressure was generated magnetically. The pad containing the sensor chip was made of nickel and was fixed on a toric magnet (NdFeB, axial magnetised). The counter pad was made of aluminium (diameter: 8 mm). A further toric magnet with an inside thread (type M 4) could be moved along a threaded rod fixed on the back of the counter pad. The variation in the distance between the pad and the movable magnet allows adjustment of the magnetic force according to requirements (see Fig. 1b). The maximum force measured by a tensiometer was in the order of 400–600 g.

Two types of pressure sensors were used for recording the transfer function of the leaf. One was purchased from Raumedic AG (Helmbrechts, Germany) and the other from Keller AG (Druckmesstechnik, Winterthur, Switzerland). Both sensors are based on an electronic chip strain gauge coated with a thin silicone membrane. The sensors were calibrated by pressurisation in a pressure chamber equipped with an integrated manometer (LEO 1, Keller AG, Winterthur, Switzerland).

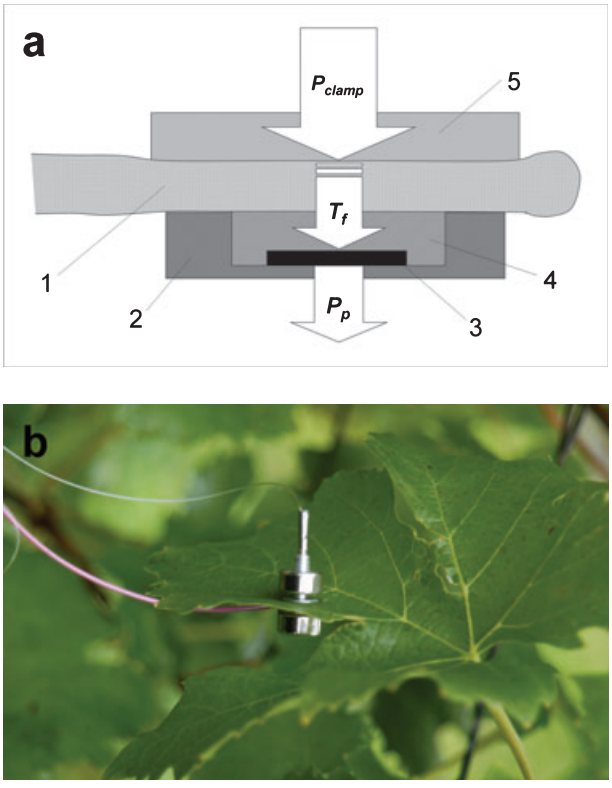

Fig. 1. Schematic diagram of the principle of operation of the leaf patch clamp pressure probe (a) and of a probe clamped on a grapevine leaf (b). (1) leaf patch, (2) pad containing the sensor chip, (3) sensor chip, (4) silicone membrane and (5) counter pad.

Data acquisition

The sensor signals were acquired by the telemetric system SENBIT (teleBITcom gmbh, Teltow, Germany). The transmitters read and amplified the analogue signals of the leaf patch clamp pressure probe. The digitised data were sent together with the transmitter ID code every 90 s or 5 min *via* the ISM band of 433 MHz to a RF receiver unit over a distance of up to 400 m. The receiver was connected *via* an RS-232 interface to a personal computer or to a GPRS modem linked to an Internet server (NTBB Systemtechnik GmbH, Zeuthen, Germany) *via* mobile phone network. The telemetric system allowed, in principle, simultaneous data processing of 32 sensors. Up to 17 sensor/transmitter units were installed in the vineyards for leaf patch clamp pressure recordings.

Pressure chamber

Diurnal changes in the balancing pressure (P_b) values of irrigated and non-irrigated grapevines were measured in parallel to online leaf patch clamp pressure measurements on 2 days in the vineyard at Quiryat-Gat and on 3 days in the vineyard close to Würzburg. The pressure bomb method is described in detail elsewhere (Scholander *et al.*

1965). For the measurements performed at Quiryat-Gat, a pressure bomb purchased from ARI (Kfar-haruv, Israel) was used. Measurements on the grapevines located at Würzburg were performed with a home-built device equipped with a high-resolution digitised manometer [LEO 1, Keller AG; for details, see Zimmermann *et al.* (2007)].

Balancing pressure values were determined on sun-exposed and shaded leaves. Leaves were sampled nearest to the site of the leaf patch clamp pressure probe.

Turgor pressure probe

Diurnal changes in cell turgor pressure were recorded concomitantly with online leaf patch clamp pressure measurements on 2 days in the vineyard at Quiryat-Gat and at Gedera using the cell turgor pressure probe (Zimmermann *et al.* 1969). The pressure probe technique is described in detail elsewhere (Zimmermann *et al.* 2004). The probe was inserted from the abaxial side of the leaves into the parenchymal cells close to the main vein. Leaf patch clamp pressure probes were clipped to nearby leaves and to leaves further away, and partly also to leaves of other branches. Turgor pressure measurements in the vineyard close to Würzburg failed because of tip clogging of the microcapillary of the pressure probe by abundant mucopolysaccharides.

Leaf area index

Leaf area of the vines was estimated using a non-destructive Sunscan canopy analysis system (model SS1-R3-BF3; Delta-T Devices, Cambridge, UK). The method ('gap fraction inversion') is based on PAR (photosynthetically active radiation) measurements under the canopy and parallel reference measurements above canopy (Cohen *et al.* 1997). Under each grapevine, 18 radiation measurements were taken (spaced every 20 cm) covering the soil surface completely under a given grapevine. Detailed information and instrument calibration is available in Netzer *et al.* (2005).

THEORETICAL CONSIDERATIONS

The input pressure seen by the cells of the leaf patch, P_{in}, is only equal to the external clamped pressure, P_{clamp}, if the pressure signal is transferred without loss to the cells. However, losses usually occur due to the compressibility and deformability of the silicone surrounding the sensor chip as well as the compressibility of the cuticle and other structural elements of the leaf. Therefore, theory shows that only a fraction of P_{clamp} is seen by the cells, *i.e.* the attenuation factor, $F_a = P_{in}/P_{clamp}$, is smaller than unity. F_a depends on the individual leaf properties. In the case of the rigid leaves of vines, F_a is *c.* 0.3 as evidenced by control experiments (data not shown). F_a can be assumed to be constant if the structural elements are completely pre-compressed by application of an appropriate P_{clamp} and if P_{clamp} is kept constant during the following experimental period. Thus the output patch clamp pressure, P_p, depends only on the cell transfer function, T_f (V), where V is the patch leaf volume. T_f (V) determines the fraction of P_{in} that is sensed by the probe (*i.e.* P_p). T_f is dimensionless and assumes values between zero and unity:

$$P_p = T_f(V) \cdot P_{in} \quad (1)$$

The function of T_f on leaf volume, V, is given at constant ambient temperature, T, by Equation (2):

$$T_f = -\left(\frac{\delta T_f}{\delta V}\right)_T \cdot V \quad (2)$$

The relative volume change $\delta V/V$ of the leaf patch is correlated to the turgor pressure change, δP_c, by the average volumetric elastic modulus, ε_p, of the tissue beneath the clamp (Philip 1958).

$$\left(\frac{\delta P_c}{\delta V}\right)_T = \frac{\varepsilon_p}{V} \quad (3)$$

ε_p is a very complex parameter and will depend *inter alia* on the magnitude of the turgor pressure. For a first approximation, we assume that ε_p increases linearly with P_c (support for this assumption is given by Zimmermann & Steudle 1978; Zimmermann & Hüsken 1980; Wendler *et al.* 1983):

$$\varepsilon_p = aP_c + b \quad (4)$$

where a and b are constants for an individual leaf. Because of the viscoelastic properties of the cell walls, the magnitude of the constants depends on the duration of pressure application (Zimmermann & Hüsken 1980). The constants are relatively large if rapid turgor pressure changes are induced (*e.g.* by using the cell turgor pressure probe), whereas slow turgor pressure changes (*e.g.* induced by transpiration) result in small values.

Combining Equations (2)–(4) leads to Equation (5):

$$\frac{dT_f}{T_f} = -\frac{dP_c}{aP_c + b} \quad (5)$$

Equation (5) can be integrated if we assume for a first approximation that at $P_c = 0$ $T_f = 1$ and that the internal osmotic pressure of the cells remains nearly constant in the range of cell turgescence. After appropriate re-arrangements Equation (6) is obtained:

$$T_f = \left(\frac{b}{aP_c + b}\right)^{\frac{1}{a}} \quad (6)$$

Introducing Equation (6) into Equation (1) yields a relationship between the parameters P_p and P_{in}:

$$P_p = \left(\frac{b}{aP_c + b}\right)^{\frac{1}{a}} P_{in} \quad (7)$$

Equation (7) can experimentally be proved. Inspection of the equation shows that the output patch clamp pressure, P_p, is a power function of the turgor pressure P_c. The exponent of the function is equal to or smaller than unity. If a = 1 and b << P_c, Equation (7) goes over into $P_p = b.P_{in}/P_c$, *i.e.* both parameters are directly reciprocally coupled with each other. Thus, T_f assumes low values if P_c is high and *vice versa*, a value close to unity if P_c is close to zero. Using appropriate values for a and b for a given leaf (see below) it can be shown that below P_c = 100 kPa, P_p must increase dramatically.

RESULTS

Evaluation of the leaf patch clamp pressure probe

One problem with semiconductor strain gauges is that they are frequently somewhat sensitive to temperature variations and tend to change resistance as they age, which, in turn, affects the attenuation factor $F_a = P_{in}/P_{clamp}$. Zero drifts can also occur. For measurements at constant temperature (*e.g.* under laboratory conditions or in clinical settings), this may not be a serious concern (Citerio *et al.* 2004), but under field conditions rapid temperature changes of 25 °C and more were quite common. According to the specifications of the manufacturer, the silicone-embedded sensors used here were temperature- and baseline drift-compensated. To prove this, the magnetic clamp probes were subjected to temperature regimes ranging from 10 °C to 35 °C. Measurements were performed in accessible climate chambers. Probes were only used that exhibited a systematic error smaller than 2 kPa. Changes of the leaf patch clamp pressure induced by temperature-dependent changes of the material properties of the clamp assemblies could also be excluded. This was verified by cooling, selectively and temporarily, components of the clamps (except the silicone sensor) using cooling spray. Pressure responses were only observed if the sensors exhibited relatively large temperature sensitivity.

Leaf movements induced by strong or gusting winds and/or rain did not affect the contact between the probe and the leaf surface. Finding of an optimum P_{clamp} that allowed monitoring over the range of leaf turgescence was performed empirically and depended on the compressibility and deformability properties of the individual leaves, which may vary considerably due to age, morphology, abiotic factors *etc*. Optimum P_{clamp} could easily be adjusted with the magnetic clamp probe. The P_{clamp} pressure was considered to be optimal if the output pressure, P_p, ranged between 10 and 25 kPa, after clamping in the early morning hours, *i.e.* at full turgescence of the leaf cells, or between 50 and 70 kPa after clamping around noon, when turgor pressure assumed minimum values.

Measurements showed that very reproducible results were obtained when the sensor-containing pad faced the abaxial side of the leaf. The reason for this was presumably because the abaxial side of the leaves was not covered by a dust layer or other dirt and its more elastic surface optimised the uniform contact between the leaf and the silicone membrane. However, it is important to note that adaxial clamping yielded similar results, excluding the possibility that the stomatal density plays a role. Viewing of the leaf patches under the microscope after removal of the probes showed that the area beneath the pads were practically as green as the surrounding leaf tissue. Only after 3 months of clamping (*i.e.* after harvest of the grapevines at the end of September) was the leaf patch a little lighter in colour (see Fig. 2), indicating that the chlorophyll concentration had somewhat decreased. Occasionally a very slight impression of the pads on the leaf

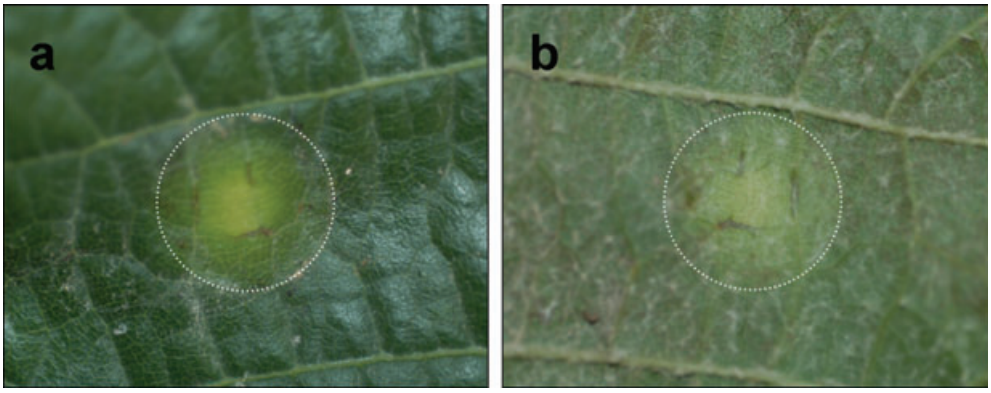

Fig. 2. Appearance of a leaf of a grapevine in the vineyard at Gedera, Israel, after removal of a probe clamped for an extremely long period (about 3 months). Leaves were taken after grape harvest at the end of September. Note that the area beneath the pads was somewhat lighter [on both the adaxial (a) and abaxial side (b)] than the surrounding leaf tissue, suggesting some decrease in chlorophyll concentration. It is evident that cells were still turgescent. Necroses or lesions were not observed, even after this long time of clamping. Note further that a decrease in chlorophyll concentration was not observed after about 2 months of clamping.

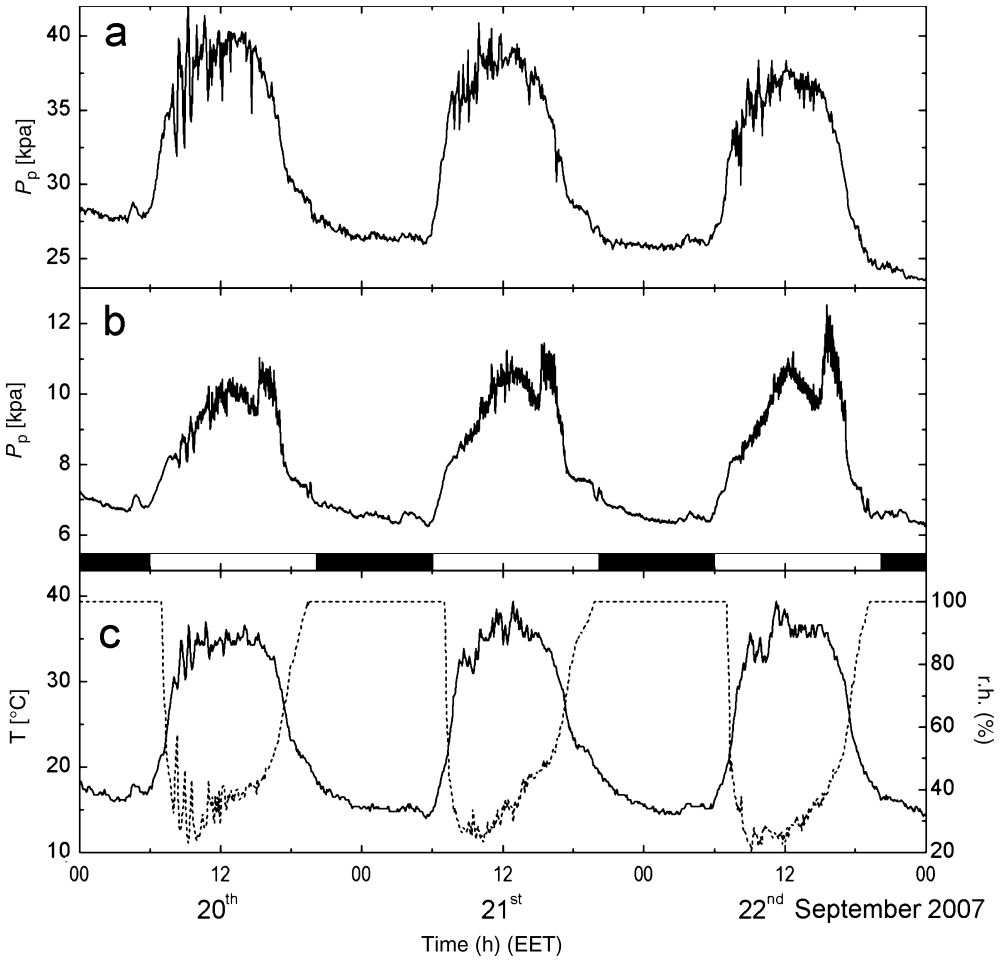

Fig. 3. Leaf patch clamp pressure (P_p) recordings on a sun-exposed leaf (area = 41 cm^2; a) and on a shaded leaf (area = 61 cm^2; b) of a grapevine in the 'lysimeter row' and irrigated daily (Quiryat-Gat, Israel). c: The corresponding diurnal changes in ambient temperature (T; black line) and relative humidity (RH; dotted line) measured in the neighbourhood of the sun-exposed leaf. Measurements were performed between September 20th and 22nd 2007. Note that the shaded leaf was exposed to sunlight between 15:15 h and 16:30 h, resulting in a second peak of the P_p values during afternoon. For further details, see text.

surface was found. Necroses or lesions on the leaves were never observed.

Clamping of several nearby leaves exposed to the same local environmental conditions demonstrated that the clamp pressure, P_{clamp}, has no effect on the diurnal profiles of the patch clamp pressure, P_p. The relative changes in the P_p values in response to changes in environmental conditions were always completely identical (data not shown). Similarly, using different-sized pads (from 20 mm^2 up to an area of 119 mm^2) resulted in comparable-shaped diurnal P_p curves. Problems only occurred if the diameter of the circular pads exceeded the spacing between the leaf veins. This could prevent a uniform contact between the leaf and the pads. Non-uniform contact resulted in a reduced pressure response of the probe or even, at high non-uniformity, in a reversal of P_p, *i.e.* P_p decreased with increasing transpirational water loss. Under these conditions, the probe is measuring changes in leaf thickness rather than turgor pressure-dependent pressure propagation through the leaf, as demonstrated by control experiments in which a force sensor with non-uniform pressure transfer was used (data not shown). Therefore, throughout the experiments, pads of 20 mm^2 area were used that allowed the placing between relatively small veins of the leaves.

Diurnal leaf patch clamp pressure profiles

Because of nearly constant climate conditions in June and September 2007, the diurnal changes in the P_p values of grapevines in the vineyard at Quiryat-Gat and irrigated regularly were very similar. At noon, peak temperatures of around 37 °C were usually measured. Minimum temperatures of 20 °C or slightly less were recorded at about 04:00 h (EET = Eastern European Time) in the morning. Conversely, RH dropped down to values of about 20% at

noon, whereas towards the early morning RH reached 100%, frequently leading to slight drizzle before predawn. Figure 3 represents P_p recordings performed on a grapevine in the 'lysimeter row' between September 20th and 22nd 2007. This grapevine was located towards the middle of the row. Figure 3 shows typical diurnal changes in the P_p values of a fully sun-exposed leaf (Fig. 3a; leaf area about 41 cm^2) and of a shaded leaf (Fig. 3b; leaf area about 61 cm^2). The shaded leaf was only exposed to direct sunlight between 15:15 h and 16:30 h. The corresponding diurnal changes in ambient T and RH measured in the neighbourhood of the sun-exposed leaf are given in Fig. 3c. The diurnal profiles of ambient T and RH in the neighbourhood of the shaded leaf were similar, except that T around noon was by about 2–3 °C lower than at the sun-exposed sites. Fig. 3a and b demonstrate that the diurnal changes in the P_p values were very reproducible over the experimental period. Consistent with the theory [see Equation (7) above], at noon, when the cell turgor pressures, P_c, usually assume minimum values, P_p values peaked, whereas during the night, when P_c assumed maximum values, the P_p values dropped to a minimum. A close examination of Fig. 3 further shows that the magnitude of the P_p values of the sun-exposed and shaded leaves was closely related to the diurnal changes in RH and T. Temporary changes in ambient RH and T were reflected in immediate changes in the P_p values. Changes in temperature and RH were more or less closely related to each other. Thus, it was not possible to unambiguously separate the effects of these parameters on the P_p values. The second peak of the P_p values of the shaded leaf, which occurred regularly in the afternoon, obviously resulted from the short-term exposure of the leaf and of the adjacent leaves to sunlight. Direct exposure to sunlight presumably changed the RH and T close to the leaf surfaces. Similar results (Fig. 3) were found for other leaves of regularly irrigated grapevines measured between June and September 2007.

Figure 4a shows a typical 1-week patch clamp pressure recording on a grapevine leaf in the vineyard at Quiryat-Gat after irrigation was stopped on June 19th 2007. The grapevine was in the middle of the fifth row. The probe was clamped on a leaf (area about 24 cm^2) at the top of the grapevine. The leaf was fully exposed to sunlight during the day. The concomitant online measurements of T and RH measured close to the site of the leaf patch clamp pressure probe are given in Fig. 4b. Inspection of the figure shows that during the night P_p assumed a low, nearly constant value over the entire period of non-irrigation (June 19th to 24th 2007). Peaking of the P_p values always occurred around noon. Interestingly, peaking of the P_p values (and thus turgor pressure loss) increased concomitantly with the proceeding non-irrigation, but dropped dramatically upon the onset of irrigation on June 24th. Irrigation also significantly lowered the P_p values during the night, suggesting improved water uptake and, in turn, turgescence of the cells. Data collection of recordings of P_p values on other leaves gave the same results, provided that sun-exposed leaves from the top of the grapevines were used (data not shown).

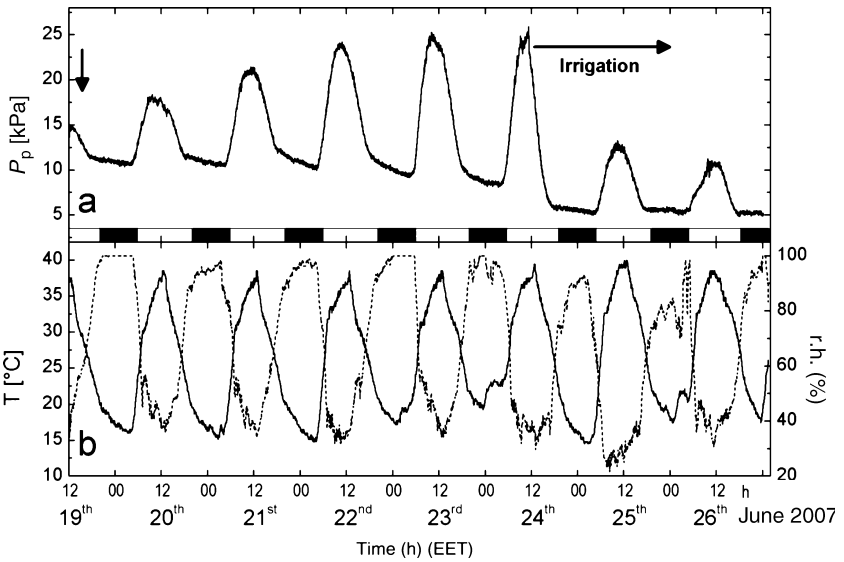

Fig. 4. Long-term leaf patch clamp pressure, P_p, recordings (a) together with the corresponding ambient T (solid line) and RH (dotted line) profiles (b) performed on a non-irrigated/irrigated grapevine in the vineyard close to Quiryat-Gat, Israel, during June 2007. Measurements were performed on a sun-exposed leaf (area = 24 cm^2). Irrigation was stopped at the beginning of the measurements (June 19th, downwardly directed arrow) and switched on again on June 24th (horizontally directed arrow). Note the effect of irrigation on the peak of the patch clamp pressure around noon and on the minimum value during the night. For further details, see text. Note that continuous irrigation leads to a permanent decrease of the peak P_p value.

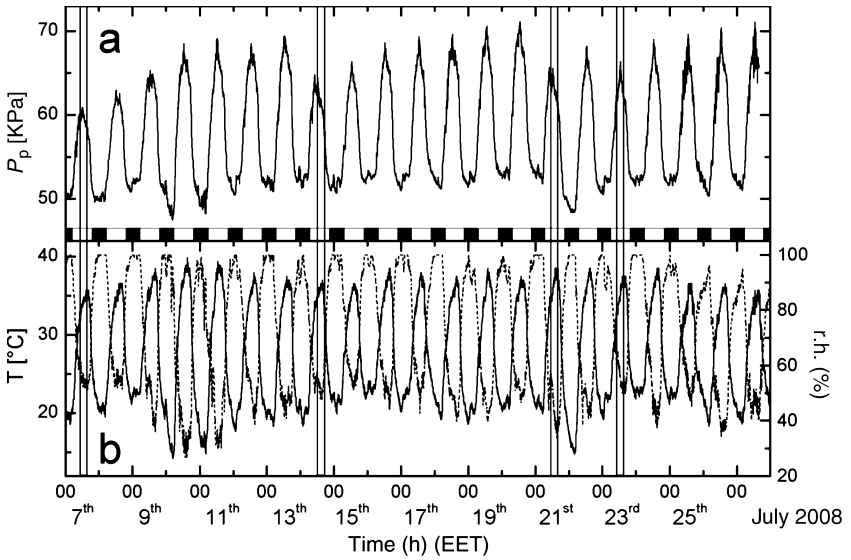

Fig. 5. Typical patch clamp pressure recordings taken from a 3-month long experiment (a) together with the corresponding ambient T (solid line) and RH (dotted line) profiles (b) recorded on a grapevine leaf (area = 113 cm^2) in the vineyard of Gedera between July 7th and July 27th 2008; the experiment ended at the end of September). Irrigation is indicated with open columns. Even though the diurnal changes in T and RH were more variable than during the experiments on grapevines in Quiryat-Gat (Fig. 4), the irrigation effects can clearly be distinguished from microclimate effects on the P_p values. Note that irrigation resulted in an immediate, temporary decrease of the peak P_p value at noon.

Figure 5a and b represent typical patch clamp pressure recordings together with the ambient T and RH profiles recorded on a grapevine leaf in the vineyard at Gedera between July 7th and July 27th 2008. A leaf of the plant at a height of 1.5 m was clamped. Days were also hot, but ambient changes in T and RH during the days and nights were more variable than during the experiments on grapevines in Quiryat-Gat. Despite this, it is obvious

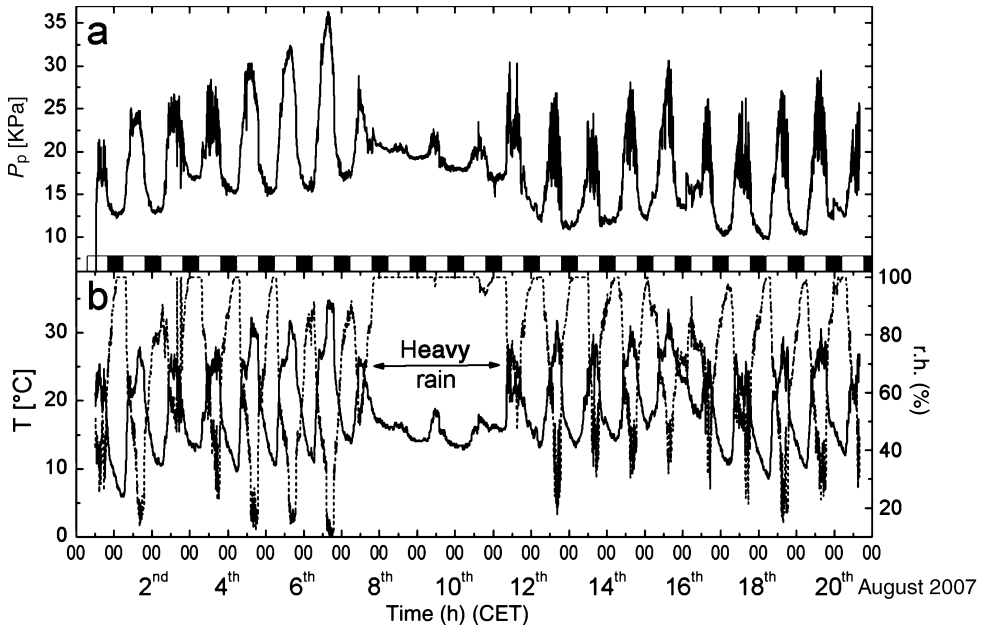

Fig. 6. Three-week leaf patch clamp pressure (P_p) recordings (a) together with the corresponding ambient T (solid line) and RH (dotted line) profiles (b) performed on a non-irrigated grapevine (leaf area = 121 cm^2) in the vineyard close to Würzburg, Germany, between August 1st and 20th 2007. Between August 7th and 9th it was very rainy (extremely heavy rainfall on August 8th: 38 l per m^2). Note that rainfall had a similar effect on P_p peaks at noon and on the P_p night values during the following days as the onset of irrigation in Figs 4 and 5.

from the figure that irrigation effects on the P_p values can clearly be distinguished from microclimate effects. Irrigation on July 7th, 14th, 21st and 23rd led to an immediate reduction of the peak P_p values at noon. In the following days after irrigation the peak values increased continuously, as observed under the irrigation and climate conditions of Quiryat-Gat (Fig. 4).

Fig. 6 represents typical measurements on grapevines in the non-irrigated vineyard close to Würzburg recorded between August 1st and 20th 2007 (CET = Central European Time), together with the corresponding T and RH profiles, as well as rainfall events. As indicated by the weather data, the days before August 7th were partly sunny, but were generally very cloudy, whereas the days between August 7th and August 9th were characterised by strong rainfall (38 l per m^2 on August 8th). The following days were partly cloudy, interrupted only occasionally by short duration rainfall. During the days of strong rainfall, the temperature did not exceed 20 °C, whereas the temperature usually reached 30 °C in the preceding and following days. Interestingly, in contrast to the environmental conditions at Quiryat-Gat and Gedera, RH dropped down to extremely low values (<10%) at noon, but usually reached 100% during the early morning.

Before the days of strong rainfall, the P_p peaks recorded at noon increased continuously from day to day, whereas during the night the P_p values assumed a nearly constant low value. During the rainy days, the P_p values remained at this low level, even during the day. The small changes in P_p values were in the range of accuracy of the sensor. The onset of significant diurnal changes in P_p values occurred immediately after rainfall stopped. Furthermore, on the following day the level of P_p values during the night dropped considerably further and remained nearly constant over the next few days. Similar findings were measured with other probes, indicating that shifts in 'night levels' of P_p values were not induced by baseline drift effects. Rather, the results are consistent with the findings on grapevines in vineyards at Quiryat-Gat and Gedera subjected to a non-irrigation/irrigation regime. Also, similar to the findings on grapevines in the vineyards at Quiryat-Gat and Gedera, magnification of the diurnal changes in P_p values demonstrated (not shown) that changes in T and particularly in RH were immediately reflected in changes in the magnitude of P_p values before and after the heavy rainfall.

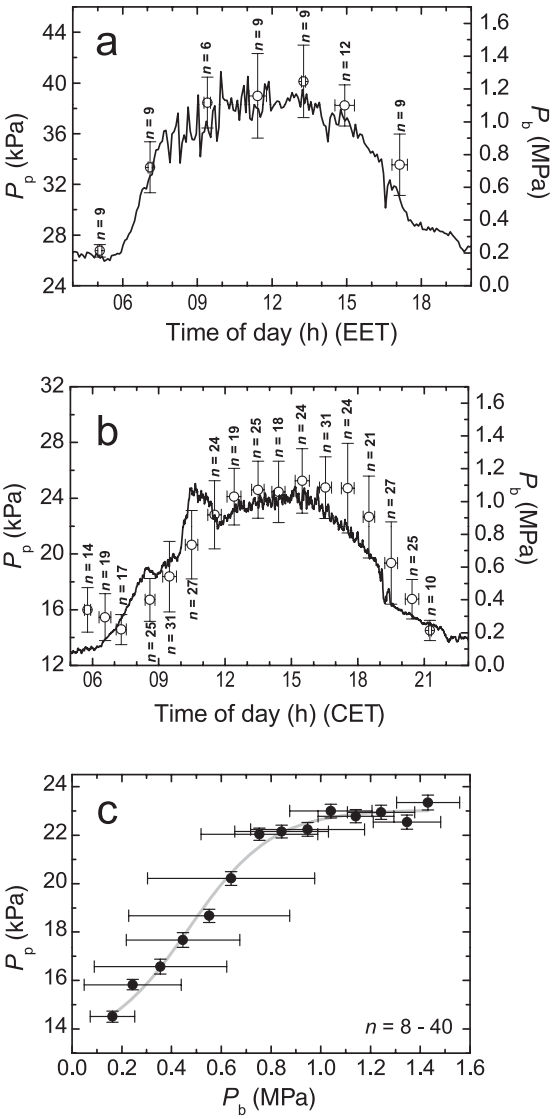

Fig. 7. Correlation between balancing pressure values (P_b; open circles) and leaf patch clamp pressures (P_p; solid lines). Measurements were performed on grapevines in the vineyard at Quiryat-Gat (a; probe was clamped on a leaf of area = 41 cm^2) and at Würzburg (b; probe was clamped on a leaf of area = 121 cm^2). c: Plot of the P_p values against the corresponding P_b values; data were taken from (b). Note that the sigmoid-shaped relationship between the P_p and P_b values can be approximated by a straight line up to a balancing pressure of about 600 kPa (least square method, r = 0.996). For further details, see text. [New figure added on 26 February 2009, after first online publication.]

Relationships between patch clamp pressure, balancing pressure and cell turgor pressure

Figure 7a and b represent diurnal changes in the leaf patch clamp pressure, P_p, and balancing pressure, P_b, values measured on leaves detached from vines close to those on which the leaf patch clamp pressure measurements were performed. The balancing pressure experiments in Quiryat-Gat were performed between June 19th and June 27th 2007 and at Würzburg between August 1st and August 7th 2007. Results are shown for grapevines at Quiryat-Gat (Fig. 7a) and Würzburg (Fig. 7b). It is obvious that diurnal changes in the P_b values coincided with diurnal changes in the P_p values if the limited accuracy of the spot measurements of the balancing pressure values is taken into account. The number of P_b data measured on grapevines at Würzburg was large enough to correlate the

P_b values with the P_p values. A cumulative plot of the P_p values against the corresponding P_b values yielded a sigmoid curve (Fig. 7c). Interestingly, up to a balancing pressure of 600 kPa, the relationship between P_p and P_b values was approximated a straight line.

The above theoretical considerations have shown that the transfer function of a defined leaf area depends only on turgor pressure if the structural elements do not contribute or constantly contribute to the pressure signal transfer of the external input pressure to the pressure sensor. In order to prove the theory, parallel measurements of leaf patch clamp pressure (P_p) and cell turgor pressure (P_c) were performed on grapevines of the second row in the vineyard at Quiryat-Gat that was irrigated daily (Fig. 8a). For technical reasons, the patch clamp pressure measurement was performed on the leaf that was closest to the leaf on which the cell turgor pressure was determined. Data recorded by the patch clamp pressure probe and the cell turgor pressure probe are given for September 19th 2007. During the morning hours the leaves were in the shade; they became sun-exposed around noon. As indicated in the figure, cell turgor pressure assumed values of about 500 kPa during the night and dropped to about 70 kPa around 14:00 h then increased continuously again during the afternoon (note the reverse scaling of the P_c ordinate in Fig. 8a). The diurnal changes in P_c correlated surprisingly well with the diurnal changes in P_p. Interestingly, during the morning hours the drop in turgor pressure lagged by about 20 min behind the corresponding increase in P_p. The delay in the response of P_p resulted most likely from the distance between the different measuring sites on the leaves. After the onset of transpiration, loss of turgor pressure of cells located at the periphery of the leaves (where P_p is measured) will immediately be compensated by water shifting from the xylem and the cells located close to the main vein (where P_c is recorded). Consistent with this explanation, towards the afternoon, when all cells throughout the leaves exhibit low turgor pressure, the increase in P_c and the decrease in P_p occurred nearly concomitantly. In Fig. 8b the P_p values are plotted against the corresponding P_c values measured during the morning hours (by neglecting the delayed response of P_p). As indicated in the figure, the data could be fitted quite well to Equation (7), particularly if the limited accuracy of the spot turgor pressure measurements at low values is taken into account. The relatively high temperatures measured at this time of day may also affect the elastic properties of the leaf and thus the constants a and b in Equation (4). Plots of the P_p values against the corresponding P_c data measured during the afternoon could also be fitted to Equation (7) (data not shown). A similar dependency of P_p on P_c was also obtained if the P_c values were plotted against P_p values recorded simultaneously on leaves located on the same branch or on a parallel branch up to 1.5 m away from the site of the turgor pressure measurements (Fig. 8b), indicating a hydraulic continuum between the measuring sites.

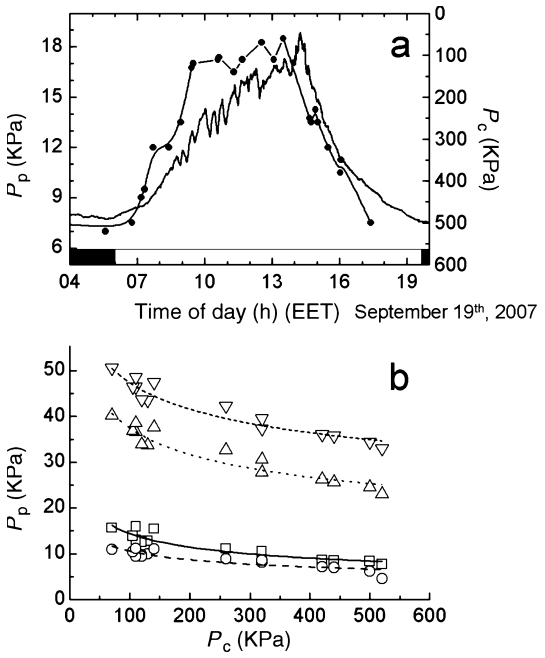

Fig. 8. Online recording of leaf patch clamp pressure, P_p, and average turgor pressure values from 3 to 8-min measurements, P_c, performed in parallel on a grapevine in the vineyard at Quiryat-Gat, Israel on September 19th 2007. Plants were irrigated with effluent. The turgor pressure probe was inserted into parenchymal cells located close to the main vein on the abaxial side of a mostly shaded leaf (area = 54 cm^2). The leaf patch clamp pressure probe was clipped to the nearest shaded leaf about 2 cm away from the leaf periphery (leaf area: 129 cm^2). a: Diurnal changes in T and RH were similar to those shown in Fig. 3 (maximum T and minimum RH between 13:00 h and 14:00 h: 34 °C and 45% respectively). Light irradiance measured at the leaf sites increased from about 13 to 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ between 05:30 h and 08:30 h; between 09:00 h and 11:40 h light irradiance was about 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (very cloudy) and increased to about 1600 $\mu E \cdot m^{-2} \cdot s^{-1}$ between 12:00 h and 14:00 h. At this time, the leaves were in shade (about 80 $\mu E \cdot m^{-2} \cdot s^{-1}$). Note that patch clamp pressure (P_p) and turgor pressure (P_c) show opposite, but very similar, diurnal changes. Note further that, during the morning hours, the increase in patch clamp pressure was delayed by about 20 min in comparison to the decrease in turgor pressure. b: Plot of P_p values measured by four leaf patch clamp pressure probes (denoted by squares, circles, triangles and inverse triangles) against the P_c values measured during the morning hours in (a). Even though the input pressures were different and the leaves clamped were located up to 1.5 m away on the same and on a parallel branch, respectively, the dependency of P_p on P_c could be fitted very well to Equation (7) (the following values for the input pressure, P_{in}, and the constants a and b defined by Equation (4) were used for fitting: squares: 70 kPa, 2.98 and 2.69 kPa; circles: 55 kPa, 3.51 and 2.69 kPa; triangles: 90 kPa, 4.06 and 11.84 kPa; inverse triangles: 95 kPa, 5.23 and 14.01 kPa).

Concomitant measurements of P_p and P_c on grapevines in the vineyard at Gedera, which were irrigated once a week, gave similar results (data not shown).

DISCUSSION

As shown here, the leaf patch clamp pressure probe provides very precise information about the supply of the leaves with water, independent of the size (*i.e.* age) of the leaves and knowledge of the transfer function of the leaves, which dictates the output patch clamp pressure, P_p. The patch clamp pressure also responded closely to the wetting and drying of the soil. This was demonstrated by modulation of the irrigation scheduling in the vineyards at Quiryat-Gat and Gedera, Israel (Figs 4 and 5) as well as by measurements on grapevines in the vineyard at Würzburg, Germany (Fig. 6), where the plants were exposed to very unsettled weather conditions.

For application of the leaf patch clamp pressure probes in the field, knowledge of the dependency of the transfer function on turgor pressure changes (and osmotic pressure changes which will be reflected in corresponding turgor pressure changes) is not required. It is only of relevance to know that the transfer function assumes small values at full turgescence and reaches nearly unity at low turgescence. These boundary values are easily found empirically under field conditions, particularly if the magnetic leaf patch clamp pressure probe is used. However, introduction of new methods requires calibration against currently used, physically sound methods, including the elucidation of their limits. Concomitant leaf patch clamp pressure and cell turgor pressure measurements have qualitatively demonstrated (Fig. 8) that the patch clamp pressure is inversely coupled with the turgor pressure, P_c, of the leaf cells. The theoretical analysis of the transfer function of the leaf cells yielded a relationship between clamp pressure, P_{clamp}, and the attenuated output pressure, P_p, which could explain quantitatively the experimental findings, as demonstrated by the fits of the curves $P_p = f(P_c)$ using Equation (7). The equation shows that the transfer function depends only on the turgor pressure, independent of the selected input pressure (and clamp pressure). Consistent with this, it was found that, despite different input pressures, concomitant patch clamp pressure measurements on various hydraulically connected leaves yielded relationships between P_p and P_c changes that could be fitted to Equation (7) (Fig. 8b).

Thus, theory and experiments show that P_p is a measure of turgor pressure. We can conclude that output patch clamp pressure values can be converted into turgor pressure values provided that the probe is calibrated with the cell turgor pressure probe under leaf clamp conditions. This opens up new avenues to study the effects of nutrients, pesticides and/or environmental factors on plant turgescence (and, in turn, on growth) as well as to unravel pathways of water and solute supply.

The online patch clamp pressure measurements have also provided insight into the parameters of the leaves that determine the magnitude of the balancing pressure, P_b, values measured by the Scholander pressure bomb technique. Provided that a large number of spot measurements of balancing pressure values are available, it can be shown (Fig. 7a and b) that the P_b values roughly reflect diurnal changes in the P_p values and, therefore, in turgor pressure. Plots of the two parameters against each other show (Fig. 7c) that a linear relationship between P_p and the P_b values exists up to a balancing pressure value of 600–700 kPa. Taking the dependency between patch clamp pressure and turgor pressure into account, we can conclude that the turgor pressure in the leaf cells must be close to zero at this external pressure value. Therefore, the further increase of the balancing pressure values, but not of the patch clamp pressure values, must be taken as evidence that excessive bomb pressure is required to shift the remaining water of the turgorless leaf cells to the cut end of the petiole. In the light of these findings, we can conclude that interpretation of balancing pressure values in terms of xylem pressure, as done by several authors (*e.g.* Koch *et al.* 2004), is apparently not correct, even in the range of turgescence because the probability of cavitation increases with increasing negative pressure, *i.e.* with decreasing turgor pressure (Zimmermann *et al.* 2004).

In summary, in the light of the results presented here, it is obvious that recordings of leaf patch clamp pressure can be used for early warning of the onset of water stress. The leaf patch clamp pressure probe paves the way to an irrigation on demand regime, both under field and greenhouse conditions. Patch clamp pressure recordings are also useful for basic research on water consumption and crop growth because they allow us to unravel fundamental processes and pathways of water and solutes in plants. Wireless transmission of data provides clear advantages in control, flexibility and cost.

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REFERENCES

Balling A., Zimmermann U. (1990) Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. *Planta*, **182**, 325–338.

Burquez A. (1987) Leaf thickness and water deficits in plants: a tool for field studies. *Journal of Experimental Botany*, **38**, 109–114.

Citerio G., Piper I., Cormio M., Galli D., Cazzaniga S., Enblad P., Nilsson P., Contant C., Chambers I. (2004) Bench test assessment of the new Raumedic Neurovent-P ICP sensor: a technical report by the BrainIT group. *Acta Neurochirurgica*, **146**, 1221–1226. (doi: 10.1007/s00701-004-0351-z).

Cohen S., Rao R.S., Cohen Y. (1997) Canopy transmittance inversion using a line quantum probe for a row crop. *Agricultural and Forest Meteorology*, **86**, 225–234. (doi: 10.1016/S0168-1923(96)02426-4).

Fuchs M. (2007) Impact of research on water use for irrigation in Israel. *Irrigation Science*, **25**, 443–445.

Geitmann A. (2006) Experimental approaches used to quantify physical parameters at cellular and subcellular levels. *American Journal of Botany*, **93**, 1380–1390.

Iglesias A., Garrote L., Flores F., Moneo M. (2007) Challenges to manage the risk of water scarcity and climate change in the Mediterranean. *Water Resource Management*, **21**, 775–788.

Jones H.G. (2004) Irrigation scheduling: advantages and pitfalls of plant-based methods. *Journal of Experimental Botany*, **55**, 2427–2436.

Jones G.V., White M.A., Cooper O.R., Storchmann K. (2005) Climate change and global wine quality. *Climatic Change*, **73**, 319–343.

Koch G.W., Sillett S.C., Jennings G.M., Davis S.D. (2004) The limits to tree height. *Nature*, **428**, 851–854.

Läuchli A., Lüttge U. (2002) *Salinity: Environment – Plants – Molecules*, 1st edition. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Lintilhac P.M., Wei C., Tanguay J.J., Outwater J.O. (2000) Ball tonometry: a rapid, non-destructive method for measuring cell turgor pressure in thin-walled plant cells. *Journal of Plant Growth Regulation*, **19**, 90–97.

Malone M. (1993) Rapid inhibition of leaf growth by root cooling in wheat: kinetics and mechanisms. *Journal of Experimental Botany*, **44**, 1663–1669.

McBurney T. (1992) The relationship between leaf thickness and plant water potential. *Journal of Experimental Botany*, **43**, 327–335.

Netzer Y., Yao C., Shenker M., Cohen S., Bravdo B., Schwartz A. (2005) Water consumption of 'Superior' grapevines grown in a semiarid region. *Acta Horticulturae (ISHS)*, **689**, 399–406.

Olesen J.E., Bindi M. (2002) Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy*, **16**, 239–262.

Philip J.R. (1958) The osmotic cell wall, solute diffusibility, and the plant water economy. *Plant Physiology*, **33**, 264–271.

Scholander P.F., Hammel H.T., Bradstreet E.D., Hemmingsen E.A. (1965) Sap pressure in vascular plants. *Science*, **148**, 339–346.

Wendler S., Zimmermann U., Bentrup F.-W. (1983) Relationship between cell turgor pressure, electrical membrane potential, and chloride efflux in *Acetabularia mediterranea*. *Journal of Membrane Biology*, **72**, 75–84.

Zimmermann U., Hüsken D. (1980) Turgor pressure and cell volume relaxation in *Halicystis parvula*. *Journal of Membrane Biology*, **56**, 55–64.

Zimmermann U., Steudle E. (1978) Physical aspects of water relations of plant cells. *Advances in Botanical Research*, **6**, 45–117.

Zimmermann U., Räde H., Steudle E. (1969) Kontinuierliche druckmessung in pflanzenzellen. *Naturwissenschaften*, **56**, 634.

Zimmermann U., Schneider H., Wegner L.H., Haase A. (2004) Water ascent in tall trees: does evolution of land plants rely on a highly metastable state? *New Phytologist (Tansley Review)*, **162**, 575–615.

Zimmermann D., Westhoff M., Zimmermann G., Geßner P., Gessner A., Wegner L.H., Rokitta M., Ache P., Schneider H., Vásquez J.A., Kruck W., Shirley St., Jakob P., Hedrich R., Bentrup F.-W., Bamberg E., Zimmermann U. (2007) Foliar water supply of tall trees: evidence for mucilage-facilitated moisture uptake from the atmosphere and the impact on pressure bomb measurements. *Protoplasma*, **232**, 11–34.

4.5 Fifth publication

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Remote monitoring of leaf turgor pressure of grapevines subjected to different irrigation treatments using the leaf patch clamp pressure probe

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Abstract

Background and Aims: Effects of four irrigation treatments on leaf turgor pressure of grapevines were studied using the novel leaf patch clamp pressure (LPCP) probe. Data were correlated with yield and yield components.

Methods and Results: The LPCP probe measures leaf water status by monitoring the attenuation of an external pressure applied magnetically to a leaf patch. The output pressure signals, P_p, are inversely correlated with cell turgor pressure. Measurements showed that changes in transpiration and stomatal conductance induced by environmental parameters were reflected nearly immediately in P_p. Ongoing non-irrigation resulted in a continuous increase of P_p, in the occurrence of stomatal oscillations and in an increased turgor pressure recovery phase during afternoon. Interestingly, analysis of the numerous diurnal P_p data sets showed that east-directed leaves responded more sensitively to water stress than west-directed leaves.

Conclusions: For the cultivar and conditions used in this study, the probe data as well as the yield data support irrigation on a 3-day basis with relatively small amounts of water.

Significance of the Study: The results show that the LPCP probe is a user-friendly, high precision instrument for online-monitoring of leaf turgor pressure in dependency on changes in microclimate and irrigation, thus helping growers to increase yield while simultaneously saving water.

Keywords: *grapevine, irrigation, microclimate, pressure probe, turgor pressure*

Introduction

Water shortage is an urgent problem in (semi-) arid zones and has focussed attention on the improvement of current irrigation treatments. How much water to irrigate and the optimal time of water application is a complex decision-making process. Severe under-irrigation stresses the plant and causes yield reduction (Smith and Griffiths 1993, Battilani and Mannini 2000). Over-irrigation wastes water, energy and labour (Brennan 2008). Furthermore, it leaches expensive nutrients below the root zone out of reach of plants, reduces soil aeration and thus crop yields. Crop quality also depends critically on water supply. Excessive water increases growth and yield of grapevines, but can have a negative impact on grape quality (Möller et al. 2007, Netzer et al. 2009). Monitoring of indicators that determine the irrigation need is therefore crucial. New irrigation programs were introduced in recent years in order to increase yield while maintaining high quality wines. Irrigation using the partial root drying method and regulated deficit irrigation method needs accurate and reliable indicators for monitoring plant water status (Bravdo and Naor 1996, Keller et al. 2008, Intrigliolo and Castel 2009). Measurements of stem water potential, stomatal conductance, daily variation in stem diameter, rate of sap flow in the stem and canopy temperature were reported as putative methods of controlling irrigation (as reviewed by Cifre et al. 2005; see also Turner and Long 1980). Plant-based sensing has several advantages, but a number of practical difficulties of implementation have prevented the development of

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commercially successful, user-friendly systems so far (Jones 1990a,b, 2004).

Recently, we have introduced a non-invasive, online-monitoring, low-cost, plant-based probe (termed leaf patch clamp pressure (LPCP) probe) for measuring the pressure transfer function of a clamped leaf patch (Zimmermann et al. 2008, Westhoff et al. 2009). The pressure transfer function is inversely correlated with the turgor pressure, i.e. at low turgor pressure the attenuation of an externally applied pressure by the leaf is less (=output pressure signals are high) than at high turgor pressure (=output pressure signals are low). Thus, the LPCP probe is an instrument that allows measurements of relative changes in leaf turgor pressure or – after calibration against the cell turgor pressure probe (Zimmermann et al. 2004) – absolute changes in turgor pressure. Recordings on tall lianas under greenhouse conditions and first measurements on grapevines have evidenced that changes in turgor pressure in response to environmental changes and/or watering were reflected in the output patch pressure of the LPCP probe.

In this communication, we have investigated in more detail the operating convenience, the functional efficiency and the precision of the probes to monitor relative changes in leaf turgescence on grapevines in dependency of microclimate and irrigation. The main objective was to identify useful, grapevine-specific, turgor pressure-related indicators for growers that allow very early detection of any divergence in leaf water status and that provide helpful information to a grower to tune or adjust irrigation according to desired plant water status and yield. To this end, probe measurements were performed on grapevines growing in a vineyard in Israel over the entire vegetation period. The grapevines were subjected to different irrigation treatments including different beginnings of irrigation and different amounts of water. After harvest yield, cluster and berry weight of the grapes was determined in dependency on the irrigation treatment. Microclimate and irrigation effects were sensed both on east- and west-oriented leaves by multiple probe readings in order to resolve possible spatial water stress effects. Probe data were transferred wirelessly via a mobile phone network to an Internet server, thus allowing remote real-time evaluation of the effect of irrigation regimes on leaf water status of the grapevines.

The results demonstrate that LPCP probe measurements yield the essential information for proper irrigation management and thus for profitable wine production.

Materials and methods

Plants and planting conditions

Measurements were performed on grapevines (for mass production of wine) between June and September, 2008 in a vineyard located close to Gedera, Israel (N 31°46′ 24.59″; E 34°44′ 47.22″). The vineyard was irrigated by drip irrigation. LPCP and turgor pressure measurements, P_c, were performed on *Vitis vinifera* L. *cv.* French Colombard grafted onto 1103 Paulsen rootstocks. Rows were aligned from north to south. Vines (about 1.7 m tall) were trained to a vertical shoot positioning trellis system. Spacing between the rows was 3 m and between the vines was 1.5 m (2200 grapevines/ha). *T* and *R.H.* were recorded close to the sites of the LPCP probes by using thermistors (Tinytag; RS Components GmbH, Mörfelden-Walldorf, Germany). Data were taken every 5 min.

Table 1. Irrigation amounts applied in the vineyard at Gedera, Israel, in litres per grapevine.

Date	*E-50*	*E-100*	*L-50*	*L-100*
14-04-2008	22	44	–	–
21-04-2008	26	52	–	–
28-04-2008	29	58	–	–
05-05-2008	32	64	–	–
12-05-2008	33	66	–	–
19-05-2008	37	74	–	–
26-05-2008	40	80	–	–
03-06-2008	41	82	41	82
09-06-2008	43	86	43	86
19-06-2008	46	92	46	92
24-06-2008	50	100	50	100
30-06-2008	53	106	53	106
07-07-2008	55	110	55	110
14-07-2008	56	112	56	112
21-07-2008	55	110	55	110
23-07-2008	13	26	13	26
28-07-2008	67	134	67	134
04-08-2008	67	134	67	134
11-08-2008	64	128	64	128
18-08-2008	60	120	60	120
25-08-2008	60	120	60	120
01-09-2008	60	120	60	120
06-09-2008			Harvest	
08-09-2008	34	68	34	68
22-09-2008	34	68	34	68
Total	1077	2154	858	1716

Bud break occurred at the end of March and irrigation usually started about 2 weeks later. In order to determine the effect of the irrigation amount on yield and to explore the possibility of saving water (at maintenance of high yield) by delaying the onset of first irrigation, four irrigation treatments were applied (see Table 1): (*Early*) Irrigation of part of the rows was started on 14 April when the length of the shoots was about 40 cm. Four replicates received the normal amount of water (*E-50*) while four other replicates received the double amount of water (*E-100*) by passing a second identical drip line along the row (see Table 1). (*Late*) Irrigation of plants was started on 3 June when first signs of drought stress (e.g. reduction of shoot elongation and leaf appearance) were visualised. Analogous to *E* irrigation four replicates received the normal (*L-50*) and four other ones the double (*L-100*) amount of water (see Table 1). Each treatment was designed in a randomised block distribution with three rows per replicate and 46 vines per row. Harvest was on

6 September when the fruit total soluble solids reached 20°Brix.

The above irrigation treatments were scheduled according to the following equation: $K_c = 0.2609$ *LAI + 0.3645 (LAI – lead area index). The equation was obtained from 7-year trails using drainage lysimeters (Netzer et al. 2009). LAI was determined monthly (see below) and K_c (crop coefficient) was calculated as following: $K_c = ET_c/ET_o$ (Allen et al. 1998), where ET_c is crop-determined evapotranspiration and ET_o is reference evapotranspiration calculated from regional weather data according to the American Society of Civil Engineers Standardized Penman–Monteith equation. The final amount applied to the treatments was 50% of ET_c at the *E-50* and *L-50* treatments, and 100% of ET_c at the *E-100* and *L-100* treatments.

LAI

Leaf area of the vines was determined using a non-destructive Sunscan canopy analysis system (model SS1-R3-BF3; Delta-T Devices, Cambridge, UK). The method ('gap fraction inversion') is based on photosynthetically active radiation measurements under the canopy and parallel reference measurements above canopy (Cohen et al. 1997). Under each grapevine, 18 radiation measurements were taken (spaced every 20 cm) covering the soil surface completely under a given grapevine (for details, see Netzer et al. 2005).

The leaf patch clamp pressure probe

The magnetic LPCP probe is shown in Figure 1a. The principle of the probe is described in detail elsewhere (Zimmermann et al. 2008). Briefly, the basic idea underlying the LPCP probe is that a relatively small patch of a leaf is used as a sensing element for turgor pressure changes in the entire leaf. To this end, the stomata in the patch must be closed; simultaneously, the patch must be in hydraulic contact with its surrounding. This is achieved by positioning an intact leaf between two planar circular metal pads integrated into two magnets. The lower pad contained a receptacle for integration of the pressure sensor chip. Leaf turgescence is determined by measuring the pressure transfer function of the leaf patch, i.e. by measuring the output leaf patch pressure, P_p, upon application of a constantly kept external clamp pressure, P_{clamp} (up to 250 kPa). P_{clamp} can be varied by changing the distance between the upper and lower magnet. A detailed analysis (Zimmermann et al. 2008) has shown that P_p is a power function of the turgor pressure P_c:

$$P_p = \left(\frac{b}{aP_c + b}\right)^{\frac{1}{a}} \cdot F_a \cdot P_{clamp} \qquad (1)$$

where a and b are constants. F_a is a leaf-specific attenuation factor which takes into account that only a constant fraction of P_{clamp} is arriving at the cell level because of losses due to the compressibility of the silicone of the sensor chip and of leaf-specific structural elements (e.g. cuticle and cell walls). In the case of the rigid leaves of the grapevines F_a was *c.* 0.3 as demonstrated by control experiments (data not shown). The inverse relationship between P_p and P_c was verified by concomitant leaf patch clamp pressure and P_c measurements on leaves under in situ conditions using the cell turgor pressure probe. In Figure 1b the P_p values measured by two probes are plotted against the corresponding P_c values. As indicated by the figure, P_{clamp} and, in turn, the P_p ranges of the probes were quite different. Despite this, by taking the different P_{clamp} values into account, the data points could be fitted quite well by Eqn 1 down to very low turgor pressure values (*c.* 50 kPa).

Control experiments performed in an accessible climate chamber showed that effects of T on the pressure reading of the probes could be excluded (<2 kPa between 10 to 35°C). Furthermore, pressure probes mounted in a non-contact mode close to the leaf surface also showed only negligible pressure changes over the entire temperature range under field conditions. Probes clamped to leaves which were excised after clamping, but kept in the same surrounding showed (after a transient P_p response) no significant response in the following 24 h. Under both conditions, the traces were very noisy.

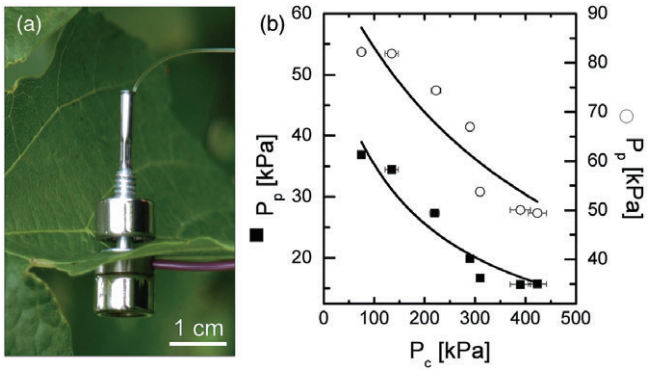

Figure 1. The magnetic leaf patch clamp pressure probe. (a) Photograph of a probe clamped on a grapevine leaf in the field and (b) calibration of two probes (open circles and filled squares) by short-term measurements of cell turgor pressure, P_c, using the cell turgor pressure probe (bars = standard deviation; $n = 23$). The different scaling of the ordinates shows that P_{clamp} and thus the range of the diurnal changes in P_p values of the two probes were quite different. Despite this, the data could be fitted by Eqn 1 by taking the different P_{clamp} values into account (circles: $P_{clamp} = 341$ kPa, $a = 1$, $b = 434$ kPa, $r^2 = 0.79$; squares: $P_{clamp} = 189$ kPa, $a = 1$, $b = 164$ kPa, $r^2 = 0.89$).

Data acquisition and telemetry

Up to 20 probes were connected to small telemetric transmitters developed by teleBITcom GmbH (Teltow, Germany). The battery-powered transmitters read and amplified the analogue signals of the LPCP probes. The digitised data were sent together with the transmitter ID code wirelessly every 5 min via the industrial, scientific and medical band of 433 MHz to a radio frequency receiver unit over a distance of more than 100 m. The receiver was connected via an RS-232 interface to a GPRS modem. Via a mobile communication network the data together with time stamps were transferred to an internet

server at the University Würzburg, Germany (NTBB Systemtechnik GmbH, Zeuthen, Germany) enabling remote control of the experiments. Electricity was provided by solar photovoltaic modules.

Turgor pressure probe
Construction and function of the pressure probe has been described in detail elsewhere (Zimmermann et al. 2004). The probe was inserted from the abaxial side of the leaves into the parenchymal cells close to the main vein.

Stomatal conductance measurements
Stomatal conductance was measured using the portable steady state porometer LI-1600 (Lincoln, Nebraska, USA). Each data point represents the average value of six measurements on different leaves.

Yield measurements
Total yield and number of clusters of each replicate was estimated on the basis of the yield of nine central vines located in the middle row of the replicate. Weight of the clusters and 100 berry weight was determined from 45 representative clusters taken from each replicate.

Statistical analysis
Average data of the output leaf patch pressure are given as mean ± standard error. Data were analysed by an unpaired Student's *t*-test using Origin 6.1 (Microcal Software Inc., Northampton, MA, USA). A value of $P < 0.05$ was considered to be statistically significant. The yield and its components were analysed by determination of the variance; means were compared with each other according to the least significant difference at $P < 0.05$. The software program JMP IN 5.1 (SAS Institute, Inc., Cary, NC, USA) was used for these statistical procedures.

Results

The magnitude of P_{clamp} had no effect on the relative P_p changes in response to changes of environmental factors. This was proven by clamping of several probes on nearby leaves exposed to the same microclimate. The relative diurnal P_p changes of all probes were identical (data not shown). Removal of the probes gave no indications of lesions. Sometimes very small impressions were seen. After *c.* 3.5 months clamping the adaxial and abaxial patches beneath the pads of the probes were somewhat less green than the surrounding arising from some degradation of chlorophyll. However, the patches were still turgescent and in hydraulic connection with the other cells outside the patch as shown by P_c measurements. This conclusion was also supported by the response of P_p to environmental factors and irrigation.

Diurnal P_p changes of weekly irrigated grapevines
The LAI of grapevines treated by the irrigation regime *E-100* and *L-100* was always slightly higher than the LAI of the grapevines which received the normal amount of water (*E-50* and *L-50*). The LAI values measured on 13 July were on average for *E-50* and *E-100* irrigation plants: 1.03 ± 0.07 and 1.23 ± 0.14, respectively, and for *L-50* and *L-100* irrigation plants 0.90 ± 0.07 and 1.03 ± 0.11,

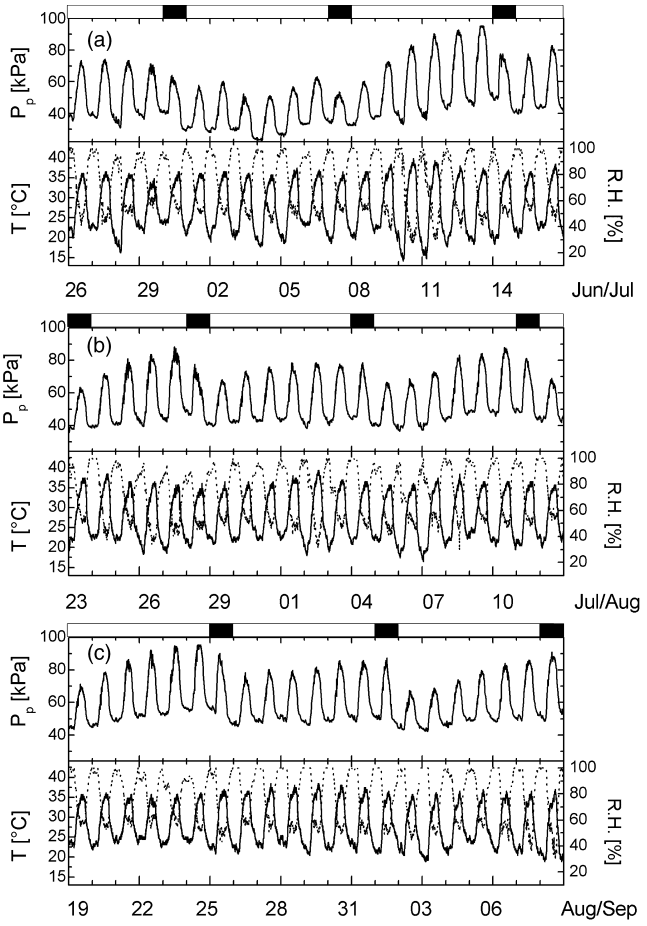

Figure 2. Parts of a typical 3-month P_p recording on a leaf of a weekly irrigated grapevine growing in the vineyard at Gedera. (a) 26 June–16 July (b) 23 July–12 August and (c) 19 August–8 September. The corresponding diurnal changes in ambient *T* (solid line) and *R.H.* (dotted line) are presented in each panel. Irrigation (filled rectangles) was started in April when the length of the shoot was *c.* 40 cm (*E-50* irrigation). The probe was clamped on a west-directed leaf at a height of 1.5 m. Note that non-irrigation resulted in a continuous increase of the minimum night P_p values and the peak P_p values at noon. This effect was reversible upon irrigation. For further details, see text.

respectively. P_c measurements on several leaves performed on irrigated plants yielded predawn and noon values which were comparable with those measured previously on grapevines in the vineyard at Kiryat Gat (Westhoff et al. 2009). The effect of weekly irrigation on the diurnal changes in P_p is shown in Figure 2. The figure shows parts of a typical 3-month recording on a grapevine together with the corresponding diurnal changes of *T* and *R.H.* The plant was subjected to *E-50* irrigation. The probe was clamped on a leaf in a western direction at a height of 1.5 m. The amplitude of night and noon P_p values increased continuously with progressing non-irrigation (Figure 2). *T* and *R.H.* effects on P_p are superimposed, but they are much lower than those of non-irrigation. After *c.* 4 days of non-irrigation P_p showed often strong oscillations between 08:00 h and 14:00 h (data not shown). Oscillations particularly occurred when *R.H.* had not reached 100% in the preceding night. Simultaneously, P_c values as low as 50 kPa were measured by using the cell

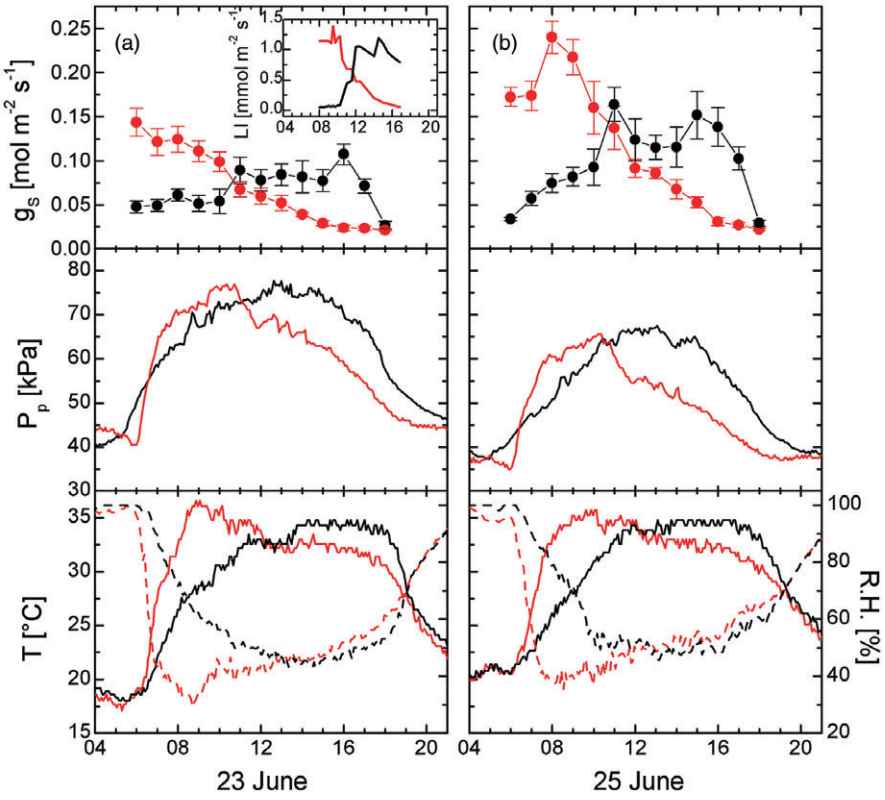

Figure 3. Concomitant measurements of diurnal changes in stomatal conductance (g_s) light irradiation (*LI*), P_p, *T*, and *R.H.* on an east-directed (red line) and west-directed (black line) leaf of two *L-50* irrigation plants. Data were recorded 1 day before (a) and 1 day after (b) irrigation on 24 June; upper panel: g_s; inset: *LI*; middle panel: P_p and lower panel: *T* (solid line) and *R.H.* (dotted line). For details, see text.

turgor pressure probe. Subsequent 5-h irrigation always resulted in a drop of the minimum value during the night and of the P_p peak value at noon of the following day in order to increase then again after 1–2 days. The irrigation/non-irrigation effects on P_p peaking at noon were very reproducible over the entire vegetation period, particularly from the beginning of July until grape harvest on 6 September. During this time period, the weather conditions were quite stable. When irrigation was switched off after harvest, a steady increase of the value occurred until leaf wilting (not shown).

The increase of the P_p peak value at noon upon non-irrigation and correspondingly the decrease of this value upon irrigation were independent of the microclimate in the neighbourhood of the clamped leaves. An example is given in Figure 3. P_p as well as *T*, *R.H* and light irradiation, (*LI*) together with stomatal conductance, g_S, were recorded on an east-directed (red lines) and a west-directed (black lines) leaf of two *L-50* irrigation plants 1 day before (Figure 3a) and 1 day after (Figure 3b) irrigation. As expected in the light of Figure 2, irrigation on 24 June resulted in a decrease of the P_p peak at noon both on the east and west side on the following day, even though the diurnal changes in *T*, *R.H* and *LI* at both probe sites were quite different. On both measuring days, the maximum and minimum, respectively, values of *T*, *R.H.*, g_S and *LI* (see inset in the upper panel of Figure 3a) were recorded at *c.* 08:00 h on the east side, but at *c.* 14:00 h on the west side. Correspondingly, P_p of the east-directed leaf increased faster after sunrise than the P_p value of the west-directed leaf (23 June: $\Delta P_p/\Delta t_{(east)} = 1.05\%$ min^{-1} vs $\Delta P_p/\Delta t_{(west)} = 0.33\%$ min^{-1}; 25 June: $\Delta P_p/\Delta t_{(east)} = 0.89\%$ min^{-1} vs $\Delta P_p/\Delta t_{(west)} = 0.27\%$ min^{-1}). P_p peaking at noon occurred earlier on the east side than on the west side (23 June: $t_{peak(east)} = 344$ min vs $t_{peak(west)} = 481$ min; 25 June: $t_{peak(east)} = 346$ min vs $t_{peak(west)} = 408$ min). Under non-irrigation conditions the relaxation time of the turgor pressure recovery phase in the afternoon, τ, of the east-directed leaf was considerably slower than that of the west-directed leaf (23 June: $\tau_{east} = 101$ min vs $\tau_{west} = 86$ min). Irrigation significantly decreased τ both on the east and west side. One day after irrigation turgor pressure recovery of the east-directed leaf was somewhat faster ($\tau_{east} = 64$ min) than that of the west-directed leaf ($\tau_{west} = 78$ min).

The above data indicate that irrigation significantly reduce τ and the P_p peak values at noon, but not $\Delta P_p/\Delta t$, and the time of P_p peaking after sunrise which were exclusively dictated by the microclimate parameters and, in turn, by stomatal conductance. Comparison of P_p data measured on east- and west-directed leaves of plants subjected to *E* and *L* irrigation treatments confirmed these conclusions (see Table 2): (i) On average, P_p increased on east- and west-directed leaves at the same time after sunrise. This was independent of the irrigation regime; (ii) There were indications (see Table 2) that P_p of *E* irrigation plants responded significantly slower after sunrise than plants subjected to *L* irrigation ($P < 0.05$). Furthermore, $\Delta P_p/\Delta t$ was generally much larger for leaves in eastern direction than in western direction (Table 2, $P < 0.05$). This was independent of the beginning of irrigation and of the amount of irrigation; (iii) On average, peaking at noon of east-oriented leaves occurred much earlier than of west-oriented leaves when the plants received the double amount of water ($P < 0.05$). This was found both for *E* and *L* irrigation plants. On irrigation days, *E-50* irrigation plants also showed large

Table 2. Parameters of the diurnal P_p changes measured on weekly irrigated grapevines in the vineyard at Gedera (average values ± standard error of eight probe recordings between 23 June and 18 August, 2008)†.

Parameters	Irrigation % of ETc	*E* (start of irrigation in April)		*L* (start of irrigation in June)	
		West (*n*)	East (*n*)	West (*n*)	East (*n*)
t_r (min)‡	50	34 ± 3 (52)	35 ± 3 (57)	46 ± 3 (57)	50 ± 4 (46)
	100	45 ± 6 (24)	47 ± 2 (53)	28 ± 3 (39)	43 ± 3 (51)
ΔP/Δt (%/min)§	50	0.29 ± 0.01 (56)	0.54 ± 0.03 (54)	0.34 ± 0.02 (57)	0.67 ± 0.05 (46)
	100	0.30 ± 0.03 (25)	0.38 ± 0.01 (53)	0.35 ± 0.01 (33)	0.52 ± 0.04 (53)
t_{peak} (min)¶	50	473 ± 5 (47)	479 ± 8 (47)	427 ± 5 (47)	353 ± 15 (38)
	100	571 ± 3 (22)	417 ± 11 (44)	529 ± 5 (33)	259 ± 11 (44)

†Non-irrigation did not significantly affect t_r and $\Delta P_p/\Delta t$. Therefore, the data were pooled. ‡t_r = rise time given in minutes after sunrise. §$\Delta P_p/\Delta t$ = percentage change of P_p per minute between sunrise and peaking at noon. ¶t_{peak} = time of P_p peaking around noon given in min after sunrise (without consideration of the irrigation days).

Table 3. The relaxation time, τ (min), of the turgor pressure recovery phase during the afternoon (average values ± standard error of eight probe recordings between 23 June and 18 August, 2008, on grapevines in the vineyard at Gedera).

Day after irrigation	*E* (start of irrigation in April)				*L* (start of irrigation in June)			
	50%		100%		50%		100%	
	West (*n*)	East (*n*)	West (*n*)	East (*n*)	West (*n*)	East (*n*)	West (*n*)	East (*n*)
0	78 ± 3 (9)	87 ± 6 (9)	59 ± 9 (4)	83 ± 7 (6)	58 ± 2 (9)	92 ± 10 (6)	72 ± 4 (5)	72 ± 8 (7)
1	90 ± 3 (8)	114 ± 5 (8)	71 ± 4 (4)	92 ± 4 (7)	66 ± 3 (8)	97 ± 7 (6)	79 ± 7 (5)	72 ± 7 (7)
2	84 ± 3 (9)	107 ± 3 (9)	61 ± 6 (4)	101 ± 5 (8)	76 ± 9 (9)	121 ± 12 (7)	74 ± 2 (7)	76 ± 6 (8)
3	95 ± 6 (8)	113 ± 8 (8)	74 ± 7 (4)	89 ± 4 (7)	81 ± 5 (8)	126 ± 9 (6)	90 ± 13 (6)	80 ± 5 (7)
4	81 ± 6 (8)	117 ± 8 (8)	79 ± 8 (4)	97 ± 7 (7)	81 ± 5 (8)	117 ± 12 (7)	86 ± 8 (6)	84 ± 9 (7)
5	95 ± 15 (7)	113 ± 7 (7)	73 ± 6 (3)	87 ± 5 (6)	84 ± 7 (7)	126 ± 7 (6)	91 ± 16 (5)	92 ± 9 (7)
6	92 ± 7 (6)	122 ± 14 (6)	74 ± 2 (3)	91 ± 3 (6)	96 ± 6 (6)	133 ± 8 (6)	71 ± 9 (5)	90 ± 6 (6)

Table 4. Yield and yield components†.

First irrigation	Irrigation % of ETc	Yield (t/ha)	No. clusters per vine	Cluster weight (g)	Berry weight (g)
E (April)	100	27.1^{a}	73.3^{a}	166.9^{a}	1.9^{a}
	50	24.4^{ab}	68.1^{a}	151.8^{ab}	1.7^{ab}
L (June)	100	20.4^{bc}	60.6^{ab}	142.9^{bc}	1.5^{bc}
	50	15.7^{c}	57.1^{b}	117.8^{c}	1.4^{c}

†Means within a column followed by different letters are significantly different at $P = 0.05$.

differences in the t_{peak} values between west-oriented and east-oriented leaves (408 ± 47 min vs 274 ± 37 min, $n = 10$); and (iv). Consistent with the finding in Figure 2 turgor pressure recovery during the afternoon was greatly affected by non-irrigation. As indicated in Table 3, τ increased on average with progressing non-irrigation. The magnitude of the increase in τ depended on the amount of irrigation and on the east/west orientation of the leaves.

Yield and yield components

Table 4 shows the effect of various irrigation treatments on the yield and its components. There was no significant difference in yield, cluster and berry weight between treatment *E-50* and *E-100*. A significant increase in yield, cluster and berry weight was also not observed when grapevines that were irrigated first on 3 June and received the double amount of water instead of the normal one (compare *L-50* with *L-100*). Significant higher values of yield, cluster and berry weight (but not so straightforward for the number of clusters per vine) were achieved when the grapevines were irrigated in early spring (*E-50* > *L-50*; *E-100* > *L-100*). Interestingly, starting irrigation in April and applying the normal amount of water was as effective as starting irrigation in June, when

applying the double amount of water. Regression analysis using the least squares method suggested that cluster weight dictates predominantly the yield and less the number of clusters per vine and the berry weight (r^2: 0.75, 0.66 and 0.33, respectively).

Discussion

To date cell turgor pressure could very accurately be determined by the cell turgor pressure probe (Tomos and Leigh 1999, Zimmermann et al. 2004, Wang et al. 2006; see in this context also Shabala 2006). However, the disadvantage of this technique is the high susceptibility to leaf movement. Therefore, measurements are restricted only to a few minutes under field conditions. As demonstrated here, relative changes in turgor pressure can be monitored with high sensitivity and robustness by determination of the pressure transfer function of the leaves. Theory and experiments performed here and previously (Zimmermann et al. 2008) have shown that the transfer function is inversely correlated with turgor pressure provided that a constant external pressure is applied and maintained during the measurements (Figure 1b). The pressure transfer function depends on the compressibility and thus the turgescence of the leaf tissue. An optimal value is easily found if the magnetic-based probe is used because these probes are user-friendly. The clamp pressure can be adjusted by simply changing the distance between the two magnets on the leaf. The finding of an optimal clamp pressure by using a spring (Zimmermann et al. 2008) is much more difficult and requires long-term experience because manipulations of the spring force after clamping frequently resulted in a displacement of the probe. A further disadvantage of spring-based probes have is that they can only be clamped at the leaf margins, which is unfavourable if the leaves are large. Problems with small-sized leaves are their relatively high weight (*c.* 9 g) which drags the leaf down into an interfered position. Moreover, when the leaves are exposed to strong wind, clamped leaves can break off. Probes made of (magnetic) metal dissipate heat much faster than probes made of plastic, thus temperature effects on the sensor or on the leaf patch are excluded (see further above). On the other hand, spring-based probes can be used under laboratory conditions and have the additional advantage that measurements can be combined with non-invasive nuclear magnetic resonance imaging methods simultaneously allowing determination of the dynamics of water shifting and flow in plants (Kuchenbrod et al. 1998, Köckenberger 2001).

The data reported here show that magnetic-based probes meet the demands for long-term measurements in the field. They are lightweight (*c.* 6 g). The leaf patches did not show any lesions, even after 3 months of clamping. The area of the pads of the probes was obviously kept small enough in order to avoid tissue damage. Furthermore, rapid leaf movements induced by wind and curling upon water stress did not lead to an interruption of P_p recordings over a measuring period of more than 3 months.

The data have evidenced that the probes sensed almost instantaneously changes in turgor pressure induced by changes of temperature, relative humidity and/or irradiation at high resolution while simultaneously not masking effects of water stress. Ongoing water stress was manifested in the continuous increase of the night P_p and particularly of the peak P_p values at noon and in an increase of the relaxation time of the turgor pressure recovery process in the afternoon. The effects could be reversed by irrigation and correlated well with stomatal conductance measurements.

The above data allow several conclusions. Water stress symptoms (such as rapid increase of P_p in the morning, increase in P_p peaking and slowdown of P_p relaxation in the afternoon with ongoing non-irrigation) became frequently manifested first in leaves on the east side. Thus, these leaves should be the preferential sites in future for monitoring water stress by using the LPCP probes. The data evidenced further that application of a double amount of water allayed the turgor pressure loss during the day and accelerated the turgor pressure recovery during the afternoon to some extent. However, watering with double the amount of water had, apparently, no significant effect on the yield and the yield's components (Table 4). Yield, cluster and berry weight were only significantly increased when irrigation was started in April (*E*) instead of June (*L*). The economic return of the improved productivity is, however, debatable if the total consumption of water per plant is considered.

A possible reason for the finding that excessive watering had no significant effect on the yield was the low turgor pressure at noon (particularly during the hot months of July and August 2008). The amplitude of the P_p peaking recorded after 6 days of non-irrigation showed that the turgor pressure was *c.* 50 kPa, i.e. close to zero turgor pressure. Grapevines responded to the severe water stress by the occurrence of oscillations in stomatal conductance and thus in P_p and P_c (data not shown). These were observed quite regularly after *c.* 4 days of non-irrigation (independent of the applied amount of water and of the beginning of irrigation). Oscillations of stomatal aperture reduce CO_2 assimilation and may be therefore compromising for growth and yield. This has to be proven in future. In any case, the data presented here suggest that for the cultivar and conditions used here, grapevines should be irrigated twice a week by applying the same normal amount of water to the plant as by weekly irrigation. This is corroborated by the dramatic increase of the noon P_p values (by *c.* 40%) and of the relaxation time of the turgor pressure recovery in the afternoon (by *c.* 25%) after 3–4 days. The setting of thresholds depends on the product quality and yield. As mentioned above, maintaining a certain level of stress (mainly in red vine cultivars) can be very beneficial in grapevine cultivation as it frequently stimulates optimal quality parameters without significantly compromising yield (Möller et al. 2007; see also in this context Stevens et al. 1995, 2008, Stevens and Walker 2002).

Conclusions

In the light of the results obtained here by online monitoring of turgor pressure of grapevines over the entire vegetation period using the magnetic clamp probe, it seems that the irrigation treatments used by the growers were not optimal. Given the special climate conditions in Israel, it is suggested that grapevines are irrigated twice a week by applying the same amount of water to the plant as by weekly irrigation. Furthermore, the results have shown that leaf water status should be measured on east-directed leaves because water stress is manifested in these leaves more clearly than in west-directed leaves. Indicators for unfavourable water supply are the steady increase of the peak value of the output pressure at noon and the increase of the time constant of the turgor pressure recovery phase in the afternoon, as well as the occurrence of oscillations after a few days of non-irrigation. In general, the measurements show that LPCP probe measurements allow the creation of a unique irrigation database for each vineyard, enabling the grower to reproduce, to a large extent, the desired yield and quality even under different climate conditions.

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References

Allen, R.A., Pereira, L.S., Raes, D. and Smith, M. (1998) Crop evapotranspiration: guidelines for computing crop water requirements. (FAO irrigation and drainage paper 56) FAO, Rome.

Battilani, A. and Mannini, P. (2000) Grapevine (*Vitis vinifera*) yield and quality response to irrigation. Acta Horticulturae **537**, 895–902.

Bravdo, B. and Naor, A. (1996) Effect of water regime on productivity and quality of fruit and wine. Acta Horticulturae **427**, 15–26.

Brennan, D. (2008) Factors affecting the economic benefits of sprinkler uniformity and their implications for irrigation water used. Irrigation Science **26**, 109–119.

Cifre, J., Bota, J., Escalona, J.M., Medrano, H. and Flexas, J. (2005) Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera L.*). An open gate to improve water-use efficiency. Agriculture, Ecosystems and Environment **106**, 159–170.

Cohen, S., Rao, R.S. and Cohen, Y. (1997) Canopy transmittance inversion using a line quantum probe for a row crop. Agricultural and Forest Meteorology **86**, 225–234.

Intrigliolo, D.S. and Castel, J.R. (2009) Response of *Vitis vinifera* cv. 'Tempranillo' to partial rootzone drying in the field: water relations, growth, yield and fruit and wine quality. Agricultural Water Management **96**, 282–292.

Jones, H.G. (1990a) Plant water relations and implications for irrigation scheduling. Acta Horticulturae **278**, 67–76.

Jones, H.G. (1990b) Physiological aspects of the control of water status in horticultural crops. HortScience **25**, 19–26.

Jones, H.G. (2004) Irrigation scheduling: advantages and pitfalls of plant-based methods. Journal of Experimental Botany **55**, 2427–2436.

Keller, M., Smithyman, R.P. and Mills, L.J. (2008) Interactive effects of deficit irrigation and crop load on cabernet sauvignon in an arid climate. American Journal for Enology and Viticulture **59**, 221–234.

Kuchenbrod, E., Kahler, E., Thürmer, F., Deichmann, R., Zimmermann, U. and Haase, A. (1998) Functional magnetic resonance imaging in intact plants – quantitative observation of flow in plant vessels. Magnetic Resonance Imaging **16**, 331–338.

Köckenberger, W. (2001) Functional imaging of plants by magnetic resonance experiments. Trends in Plant Science **6**, 286–292.

Möller, M., Alchanatis, V., Cohen, Y., Meron, M., Tsipris, J., Ostrovsky, V., Sprintsin, M. and Cohen, S. (2007) Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. Journal of Experimental Botany **58**, 827–838.

Netzer, Y., Yao, C., Shenker, M., Cohen, S., Bravdo, B. and Schwartz, A. (2005) Water consumption of 'Superior' grapevines grown in a semiarid region. Acta Horticulturae **689**, 399–406.

Netzer, Y., Yao, C., Shenker, M., Bravdo, B. and Schwartz, A. (2009) The use of crop coefficient for skilled irrigation of table grapes trained to a large trellis system. Irrigation Science **27**, 109–120.

Shabala, S. (2006) Transport from root to shoot. In: Plant solute transport. Eds. A.R. Yeo and T.J. Flowers (Blackwell Publishing: Oxford) pp. 214–234.

Smith, J.A.C. and Griffiths, H. (1993) Water deficits (Bios Scientific Publishers: Oxford).

Stevens, R.M. and Walker, R.R. (2002) Response of grapevines to irrigation induced saline-sodic soil conditions. Australian Journal of Experimental Agriculture **42**, 323–331.

Stevens, R.M., Harvey, G. and Aspinall, D. (1995) Grapevine growth of shoots and fruit linearly correlate with water stress indices based on root-weighted soil matric potential. Australian Journal of Grape and Wine Research **1**, 58–66.

Stevens, R.M., Pech, J.M., Gibberd, M.R., Walker, R.R., Jones, J.A., Taylor, J. and Nicholas, P.R. (2008) Effect of reduced irrigation on growth, yield, ripening rates and water relations of Chardonnay vines grafted to five rootstocks. Australian Journal of Grape and Wine Research **14**, 177–190.

Tomos, A.D. and Leigh, R.A. (1999) The pressure probe: a versatile tool in plant cell physiology. Annual Review of Plant Physiology and Plant Molecular Biology **50**, 447–472.

Turner, N.C. and Long, M.J. (1980) Errors arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. Australian Journal of Plant Physiology **7**, 527–537.

Wang, L., Hukin, D., Pritchard, J. and Thomas, C. (2006) Comparison of plant cell turgor pressure measurement by pressure probe and micromanipulation. Biotechnology Letters **28**, 1147–1150.

Westhoff, M., Reuss, R., Zimmermann, D., Netzer, Y., Gessner, A., Geßner, P., Zimmermann, G., Wegner, L.H., Bamberg, E., Schwartz, A. and Zimmermann, U. (2009) A non-invasive probe for online-monitoring of turgor pressure changes under field conditions. Plant Biology **11**, 701–712.

Zimmermann, D., Reuss, R., Westhoff, M., Geßner, P., Bauer, W., Bamberg, E., Bentrup, F.W. and Zimmermann, U. (2008) A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. Journal of Experimental Botany **59**, 3157–3167.

Zimmermann, U., Schneider, H., Wegner, L.H. and Haase, A. (2004) Water ascent in tall trees: does evolution of land plants rely on a highly metastable state. New Phytologist (Tansley Review) **162**, 575–615.

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5. Discussion and Conclusions

Vine irrigation in areas of limited water supply

Water scarcity is a major issue in the world, particularly in arid regions. Climate changes affect both the amount of precipitation and the pattern of its annual distribution. The impact of the water used to irrigate agricultural crops is far more wide reaching than simply its effects on plant vegetative and reproductive performance. Sustainable use of land must consider the ecological effects of irrigation on the chemical and physical properties of soils, while taking into account the long-term effects on the quality of groundwater and soil structure.

This research investigated the major aspects of irrigation of intensively cultivated vineyards under the semi-arid conditions that are typical of the Mediterranean region. Under these conditions, irrigation is necessary for profitable commercial grapevine production. The conclusions derived from this work may serve as useful and applicable tools for the development of irrigation programs for table-grape and wine-grape vineyards.

Scheduling irrigation based only on the percentage of estimated evapotranspiration without taking into account the different phenological stages of the vine at different points in the season can lead to the wasting of water during certain parts of the season and to a certain degree of drought stress at other times.

Many works that have examined the effects of irrigation on grapevines have been based on calculations of evapotranspiration obtained from meteorological measurements. The questions that arise from these measurements are: Is this method appropriate for irrigation of deciduous plants, such as vines? Does this method take into account phenological changes?

It is apparent that irrigation at constant rates based on meteorologically derived evapotranspiration data may be appropriate for a crop whose canopy dimensions remain relatively constant throughout the growing season. The use of such a method is more appropriate for wine grapes than table grapes, because the canopy of wine grapes is kept constant, by mechanical means, over the course of the growing season. In addition, during the early growth stages, which are characterized by rapid shoot elongation, irrigation has not yet started or is applied only for technical purposes such as the

application of fertilizers. The absence of irrigation during this period forces the plant to rely on water reserves from winter rains. This practice integrates well with the regulated deficient irrigation method.

On the other hand, irrigation practices for table grapes must take into consideration vegetative activity, i.e., the dynamic of canopy dimensions that greatly determine the plant's transpiration activity. This approach must take in account the fact that the canopy itself is influenced by water availability, nutrient levels and biotic and abiotic stresses. Over the past few years, there have been developments in techniques for measuring the size of the canopy. These new practices are particularly intriguing because they are not destructive (Cohen et al., 1997; Wilhelm et al., 2000) and some are operated via remote sensing (Gonsamo, 2010; Stamatiadis et al., 2010). Ben-Asher et al. (2006), Grantz and Williams (1993), Johnson et al. (2006) and others have reported on the performance of these measurement techniques in vineyard canopies. In the past, indirect canopy measurements were not available and, therefore, research methods relied on weighing the mass of pruned branches as an alternative method of estimating canopy size.

In the present study, vine water consumption was measured using drainage lysimeters. The choice of less costly lysimeters allowed for the use of a total of 13 measuring instruments. The following are concerns raised by the use of these lysimeters: How effectively do these lysimeters represent the water use needs of vines? In what ways do isolated lysimeter-grown vines represent plants growing in the vineyard? What are the effects of a restricted root zone on the plant? Are there problems with aeration of the growth medium in the lysimeters?

In the present study, it appears that the canopies of vines that were planted in the lysimeters were a maximum of 20% larger than vines in the nearby vineyard that received the smallest irrigation volumes. Gas exchange and water potential measurements showed that the vines in the lysimeters did not suffer from aeration stress, as indicated by their higher stomatal conductance and lower (less negative) leaf and stem water potential values, as compared to field-grown vines. Although the lysimeter vines had a larger canopy, the obtained lysimeter data provides us with a better understanding of the overall annual pattern of evapotranspiration.

The current study is unique because of the operational connection between the lysimeter- and field-grown vines. Use of the lysimeters together with meteorological data and canopy measurements allowed for the establishment of relationships between ET_c, ET_o, K_c and LAI. Similar work performed in California used one weighing lysimeter planted with cv. Thompson Seedless and the total water consumption reported in that study was almost identical to that reported in our own study (Williams et al., 2003b).

More recent publications have focused on table grape performance in the field, while irrigation amounts was based on lysimeter data (Williams et al., 2009; Williams et al., 2010). In these studies, yield increased linearly as a function of the amount of water supplied. In other studies, similar trends of a linear yield increase as function of amount of water supplied were reported (Van Rooyen et al., 1980; Salón et al., 2005; Marsal et al. 2008). Williams et al. (2010) reported a linear increase until 0.6 or 0.8 of ET_c. Beyond those values, yields level off and start to decrease due to excessive water in the soil and decreased soil aeration.

The innovation of the present work is its ability to quantify both the biotic (i.e., canopy) and abiotic (i.e., climate conditions) factors that together contribute to "maximal vine water consumption". The fact that we examined the long-term application of irrigation treatments and their effects on yield and yield quality make these findings particularly applicable for growers and researchers developing irrigation programs.

Use of treated waste water (TWW) in vineyards

In recent years, we have witnessed an increase in the use of TWW as a consequence of the need to disperse wastewater and conserve freshwater (Aharoni and Cikurel, 2006; Fuchs, 2007). The use of TWW requires a different approach to irrigation practices: new methods and devices, as well as changes in irrigation intervals, fertilization and water-soil-plant quality control. The quality of irrigation water has a direct influence on the quality of agricultural products. The quality of TWW is usually lower than that of freshwater. The source of the water used for irrigation influences the composition and concentration of dissolved salts in the crop and soil. Low or excessive salt contents can harm the quality and quantity of agricultural yields, in addition to affecting soil properties. When salts are dissolved in water, positively charged ions (cations) and

negatively charged ions (anions) are formed. The particular cations of interest are calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^{+}) and the anions of interest are chloride (Cl^{-}), sulfate (SO_4^{2-}) and bicarbonate (HCO_3^{-}). Additional ions that are found in lower concentrations include potassium (K^{+}), nitrate (NO_3^{-}) and, in some cases, boron (B) ions. In all types of soils, the soil solution contains salts, some of which are nutritional elements essential for proper plant growth. Negative effects on plants include the osmotic effects that inhibit plant water absorbance as well as specific ion toxicity, such as that presented by chloride, sodium and boron. Trees growing under saline conditions tend to accumulate salts and show visual symptoms such as leaf burn, chlorosis, early leaf senescence and defoliation. Continuous exposure of clay soils to irrigation with TWW or saline water with high SAR values leads to the gradual replacement of adsorbed calcium by sodium, causing an increase in soil sodicity, which may indirectly affect plant performance.

Potential risks of a large range of elements were monitored in this study. Even though salinity levels in the TWW we used were moderate (1.83 and 2 dS m^{-1} in TWW and TWW+F, respectively), the effects of salinity, particularly sodium ions, on both plant and soil properties were pronounced.

This study found that prolonged TWW irrigation leads to the following:

1. Sodium accumulation and elevated SAR values in the soil in relation to the amount of water applied and as factor of the amount of winter precipitation.
2. Elevated amounts of daily irrigation (80% of ET_c) can not be considered as leaching fractions. On the contrary, elevated amounts increase soil sodicity.
3. Sodium gradually accumulates in vine leaves and wood. Sodium concentrations were higher in the xylem sap of the TWW-irrigated treatments.
4. Sodium and chloride concentrations in xylem wood can serve as a good index of accumulated salinity in perennial tissues.

As discussed previously, grapevines are defined as moderately salt-tolerant (Downton, 1977a,b; Maas and Hoffman, 1977; Maas, 1990; Garcia and Charbaji, 1993; Francois and Mass, 1994) and the damage observed under saline conditions is primilarly caused by chloride ions (Ehling, 1960; Williams and Matthews, 1990; Walker, 1994). In a previous

studies, tissue salt content increased significantly with increasing salinity of irrigation water and chloride concentrations were always higher than sodium concentrations (Fisarakis et al., 2001). In leaf petioles, chloride concentrations above 10,000 or 15,000 mg kg^{-1}DW are considered toxic, while sodium concentrations of 5,000 mg kg^{-1} DW or more are considered toxic (Nagarajah, 1992; Prior et al., 1992; Reuter and Robinson, 1997). The findings of this study do not correspond with previously reported conclusions, in that we found that sodium (and not chloride) content was the most accumulated hazardous ion in vine tissues.

The soil in this trial was relatively high in clay (30% sand, 28% silt, and 42% clay) and most of the chloride (80-95% at 0-30 cm soil depth) was leached during winter precipitation (372 mm $season^{-1}$), while the sodium was leached to a lesser extent (41-66% at 0-30 cm soil depth). Soil moisture levels remained high because of the daily irrigation with relative large amounts of water (minimal irrigation at 40% of ET_c equal to 400mm $season^{-1}$). Continuous exposure to irrigation with moderately saline TWW with high SAR values led to the gradual replacement of absorbed calcium by sodium ions, causing an increase in soil sodicity. It is possible that the constant irrigation and low soil evaporation (more than 90% of soil surface was shaded by the canopy) did not allow for chloride concentrations to reach toxic levels. We assume that those conditions along with the moderate chloride concentrations in the TWW (~ 300 mg l^{-1}) were not sufficient to cause vine chloride toxicity and that any chloride remaining in the soil was efficiently leached during winter rains.

Development of physiological tools for optimal irrigation

The most common agricultural tool for supporting irrigation management decisions is the tensiometer. It is a rather cheap device whose use requires only basic operational skills. In order to evaluate the status of all wetting zones, a set of three devices at three depths is usually installed. Some of the concerns regarding the usage of these devices are: How effectively does one device represent the water availability of the entire vineyard? How many devices should be installed? How should we interpret contradicting information from different devices in the same field? And, how effectively does the soil-based information represents plant water status?

Tensiometers are widely used in table-grape vineyards, which are usually irrigated daily with relatively large amounts of water. In wine-grape vineyards, a deficient irrigation method is generally applied, with wider irrigation intervals and consequently much higher water tension values. These conditions rule out the use of tensiometers for most of the growing season of wine grapes.

Over the last decade, we have witnessed a strong trend of using diminished amounts of water in Israeli vineyards. This trend was initiated by the wine industry in order to improve wine quality. As a consequence of this trend, leaf drying and berry shrinkage has been observed, along with mature vines that have developed poor hydraulic systems. This phenomenon has challenged the deficient irrigation practice and questioned its sustainability.

Physiologically based tools for irrigation of wine grapes are used to maintain certain level of stress while attempting to avoid excessive or deficient irrigation. Cifre et al. (2005) reviewed several physiological tools for irrigation scheduling in grapevines, including sap flow sensor meters (Eastham and Gray, 1998; Ginster et al., 1998; Braun and Schmid, 1999; Fernandez et al., 2001), linear transducers of displacement (Fereres et al., 1999; Moriana et al., 2000), infrared thermometry, canopy reflectance indices and chlorophyll fluorescence indices.

It is known that low water availability decreases stomatal conductance (g_s) and worsens leaf water potential. The pressure chamber (Scholander et al., 1965) is a useful tool that provides a reliable method for determining the water status of vines (Choné et al., 2001; Olivo et al., 2009). Pressure chamber measurements can provide values of pre-dawn leaf water potential, daily leaf water potential and stem water potential. Daily stem water potential is influenced by whole plant transpiration and root/soil hydraulic conductivity. The disadvantages of the pressure chamber measurements are the fact that it is destructive as well as labor intensive and time consuming and the fact that information is sometimes collected after the plant has already been exposed to stress. In some cases, researchers have irrigated according to certain stem water potential values and decreased or increased irrigation based on data obtained by pressure chamber measurements.

In this study, we examined the leaf patch clamp pressure probe (LPCP), a novel physiological tool for monitoring of the water relations of table grape and wine grape

leaves. This probe was developed by a Prof. U. Zimmerman of the University of Wurtzburg in Germany. The LPCP was tested as a tool for scheduling irrigation in crops for the first time in the vineyard in Lachish, in collaboration with our laboratory. As a result of our input, the measuring system was improved substantially and a protocol for its use a tool for scheduling irrigation based on continous undistractive measurement of turgor pressure was developed. The probe measures the attenuated output patch clamp pressure of a clamped leaf in response to an external magnetic applied input pressure. The measured pressure is inversely correlated with leaf turgor pressure.

There are two components of plant water potential: turgor pressure (also called turgidity) and osmotic potential. Turgor pressure is defined as the force exerted outward on a plant cell wall by the water inside the cell vacuole. In water potential terms, turgor is expressed as the pressure component (Ψ_p) that gives the plant its rigidity, which keeps it erect. This is especially important in young tissues.

Our measurements showed that changes in transpiration and stomatal conductance were induced by environmental parameters and were reflected in probe readings nearly immediately. The advantages of the LPCP are its ability to track online water potential changes simultaneously at several probes attached to leaves that are dispersed throughout the field. Ongoing stress (as sometimes occurs during long intervals between irrigation applications) resulted in a pattern of oscillations that indicate a progression of stomatal patchiness. Further work has to be done in order to set irrigation thresholds and build the appropriate algorithm so that the LPCP can be used to automatically control irrigation and not only to measure current turgor status.

6. References

Acevedo-Opazo C, Ortega-Farias S, Fuentes S (2010) Effects of grapevine (Vitis vinifera L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. Agric Water Manage 97: 956–964

Agassi M, Tarchitzky J, Kern R, Chen Y, Goldstein D, Fizik E (2003) Effects of prolonged irrigation with treated municipal effluent on runoff rate. J Environ Qual 32:1053-1057

Aharoni A, Cikurel H (2006) Mekorot's research activity in technological improvements for the production of unrestricted irrigation quality effluents. Desalination 187: 347-360.

Allen RA, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration: Guidelines for computing crop water requirements. (FAO Irrigation and Drainage Paper 56) FAO, Rome

APHA (2005). Standard Methods for the Examination of Water and Wastewater, 21th Ed. American Public Health Association, Washington DC (2005) section 2340-C.

APHA (2005). Standard Methods for the Examination of Water and Wastewater, 21th Ed. American Public Health Association, Washington DC (2005) section 4500-Cl- D

Ayars JE, Johnson RS, Phene CJ, Trout TJ, Clark D, Mead RM (2003) Water use by drip-irrigated late-season peaches. Irrig. Sci: DOI 10.1007/s00271-003-0084-4

Ayars JE, Hutmacher RB (1994) Crop coefficients for irrigating cotton in the presence of ground water. Irrig Sci. DOI 10.1007/BF00187794

Avnimelech Y (1993) Irrigation with sewage effluents: the Israeli experience. Environ Sci Technol. 27: 1278–1281. DOI 10.1021/es00044a001

Ben-Asher J, Tsuyuki I, Bravdo B-A, Sagih M (2006) Irrigation of grapevines with saline water: I) leaf area index, stomatal conductance, transpiration and photosynthesis. Agric Water Manag 83:13–21

Bond WJ (1998) Effluent irrigation—an environmental challenge for soil science. Aust J Soil Res 36:543-555

Braun P, Schmid J (1999) Sap flow measurements in grapevines (*Vitis vinifera* L.) - 1. Stem morphology and use of the heat balance method. Plant Soil. DOI 10.1023/A:100475600 2983 215:39-45

Chalmers DJ, Andrews PK, Harris KM, Cameron EA, Caspari HW (1992) Performance of drainage lysimeters for evaluation of water-use by Asian pears. Hortscience 27: 263-265

Choné X, van Leeuwen C, Dubourdieu D, Gaudillère JP (2001) Stem water potential is a sensitive indicator of grapevine water status. Ann Bot 87:477–483

Christensen P (1969) Seasonal changes and distribution of nutritional elements in Thompson Seedless grapevines. Am J Enol Vitic 20:176-190

Cifre J, Bota J, Escalona JM, Medrano H, Flexas J (2005) Physiological tools for irrigation scheduling in grapevine (Vitis vinifera L.): An open gate to improve water-use efficiency? Agric Ecosyst Environ 106:159–170

Cohen S, Rao RS, Cohen Y (1997) Canopy transmittance inversion using a line quantum probe for a row crop. Agric For Meteorol. DOI 10.1016/S0168-1923(96)02426-4
Doorenbos J, Pruitt WO (1977) Crop water requirements. (FAO irrigation and drainage paper 24) FAO, Rome

Downton WJS (1977a) Chloride accumulation in different species of grapevine.
Sci Hort. 7:249-253

Downton WJS (1977b) Influence of rootstocks on the accumulation of chloride, sodium and potassium in grapevines. Aust J Agric Res 28:879-889

Ehling CF (1960) Effects of salinity on four varieties of table grapes grown in sand culture. Am. Soc Hort Sci. 76: 323–331

Erie LJ, French OF, Bucks DA, Harris K (1982) Consumptive use of water by major crops in the southwestern United States (Conservation Research Report no. 29). United States Department of Agriculture, Washington, D.C.

Esteban MA, Villanueva JM, Lissarrague JR (2001) Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (Vitis vinifera L) grape berries during ripening. J Sci Food Agric 81:409-420

Eastham J, Gray SA (1998) A preliminary evaluation of the suitability of sap flow sensors for use in scheduling vineyard irrigation. Am J Enol Vitic 49:171–176

FAO (2008) FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/spush

Fereres E, Goldhamer D, Cohen M, Girona J, Marta M (1999)Continuous trunk diameter recording is a sensitive indicator for irrigation of peach. Calc. Agric. 53: 21–25

Fernandez JE, Palomo MJ, Diaz-Espejo A, Clothier BE, Green SR, Giron IF, Moreno F (2001) Heat-pulse measurements of sap flow in olives for automating irrigation: tests, root flow and diagnostics of water stress. Agric Water Manage 51: 99–123

Fisarakis I, Chartzoulakis K, Stavrakas D (2001) Response of Sultana vines (V. vinifera L.) on six rootstocks to NaCl salinity exposure and recovery. Agric Water Manage 51, 13-27

Fuchs M (2007) Impact of reaserch on water use for irrigation in Israel. Irrig Sci 25:443-445. doi:10.1007/s00271-006-0050-z

Francois LE, Mass EV (1994) Crop response and management on salt-affected soils. In: Pessarakli M (ed) Handbook of plant and crop stress. Marcel Dekker, New York, pp 149-181

Garcia M, Charbaji T (1993) Effect of sodium chloride salinity on cation equilbria in grapevines. J Plant Nutr 16:2225-2237

Gonsamo A (2010) Leaf area index retrieval using gap fractions obtained from high resolution satellite data: Comparisons of approaches, scales and atmospheric effects. Int J Appl Earth Observ Geoinform 12:233-248

Gerardi MH, Zimmerman MC (2005) Wastewater pathogens. Wiley-Interscience, John Wiley & Sons, Inc., New Jersey, p 179

Gilliam JW (1971) Rapid measurement of chloride in plant material. Proceedings of American Soil Science Society 35:512–513

Ginestar C, Eastham E, Gray S, Iland P (1998) Use of sap-flow sensors to schedule vineyard irrigation. I. Effects of post-veraison water deficits on water relations, vine growth, and yield of ‘Shiraz’ grapevines. Am J Enol Vitic 49:413-420

Grantz DA, Williams LE (1993) An empirical protocol for indirect measurement of leaf area index in grape *Vitis vinifera* L. Hortscience 28:777-779

Greenway H, Munns R (1980) Mechanisms of salt-tolerance in non-halophytes. Ann Rev Plant Physiol 31:149-190

Greweling T, Peech M. (1960). Chemical soil tests. Cornell Exp Stn Bull 960.

Halperin R (1999) Regulations for water quality in Israel and internationally. Health in the Field 7:3-6 [in Hebrew]

Johnson LF, Roczen DE, Youkhana SK, Nemani RR, Bosch DF (2003) Mapping vineyard leaf area with multispectral satellite imagery. Computers and Electronics in Agriculture 38:33–44.

Kuo S (1996). Phospourus, pp 869-919 In: Methods of soil analysis, part 3 – chemicalmethods. In Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN,

Tabatabai MA, Johnson CT, Sumner ME, ed. Methods of Soil Analysis: Part 3-Chemical Methods, Vol. # 5 in the Soil Science Society of America Book Series. ASA and SSSA, Madison WI.

Liu CM, Zhang XY, Zhang Y (2002) Determination of daily evaporation and evapotranspiration of winter wheat and maize by large-scale weighing lysimeter and micro-lysimeter. Agri and Forest Meteo. DOI 10.1016/S0168-1923(02)00015-1

Loeppert RH., Inskeep WP (1996). Iron. In D. L. Sparks et al. (ed.) Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series 5:639-664

Maas EV (1990) Crop salt tolerance. In: Tanji KK (ed) Agricultural salinity assessment and management. American Society of Civil Engineers, New York

Maas EV, Hoffman GJ (1977) Crop salt tolerance—current assessment. J Irrigation and Drainage Division of ASCE 103:115-134

Marsal J, Mata M, del Campo J, Arbones A, Vallverdú X, Girona J, Olivo N (2008) Evaluation of partial root-zone drying for potential Field use as a deficit irrigation technique in commercial vineyards according to two different pipeline layouts. Irrig Sci 26:347–356

Morgenroth E, Kommedal R, Harremoes P (2002) Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment—a review. Water Sci Technol 45:25-40

Moriana A, Castro J, Humanes D, Orgaz F, Pastor M, Fereres E (2000) The relations between trunk diameter fluctuations and tree water status in olive trees (Olea europaea L.). Acta Hort 537:293–297.

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ 16:15-24

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239-250

Munns R, Guo J, Passioura JB, Cramer GR (2000) Leaf water status controls day-time but not daily rates of leaf expansion in salt-treated barley. Aust J Plant Physiol 27:949-957

Nelson DW, Sommers LE (1982). Total carbon, organic carbon and organic matter. pp. 539-579. *In:* Page et al. (ed.) Methods of soil analysis: Part 2. Chemical and microbiological properties. ASA Monograph Number 9

Oliver HR, Sene KJ (1992) Energy and water balances of developing vines. Agric For Meteorol 61:167–185

Olivo N, Girona J, Marsal J (2009) Seasonal sensitivity of stem water potential to vapour pressure deficit in grapevine. Irrig Sci 27:175–182 DOI 10.1007/s00271-008-0134-z

Prior LD, Grieve AM (1987) Water used and irrigation requirements of grapevines. In: Lee T (ed) Proceedings of the 6th Australian Wine Industry Technical Conference, 14–17 July, Adelaide .Australian Industrial Publications, Adelaide, 165–168

Reuter DJ, Robinson JB (1997) Plant analysis—an interpretation manual, 2nd edn. CSIRO, Collingwood, Australia

Rhoades JD (1996). Salinity: Electrical Conductivity and Total Dissolved Solids, p. 417-435, In Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnson CT, Sumner ME, ed. Methods of Soil Analysis: Part 3-Chemical Methods, Vol. # 5 in the Soil Science Society of America Book Series. ASA and SSSA, Madison, WI.

Roby G, Harbertson JF, Adams DO, Matthews MA (2004) Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. Aust J Grape and Wine Res 10:100-107.

Salón JL, Chirivella C, Castel JR (2005) Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield and wine quality. Am J Enol Vitic 56:1–8

Shani U, Ben-Gal A (2005) Long-term response of grapevines to salinity: osmotic effects and ion toxicity. Am J Enol Vitic 56:2:148-154

Scholander PF, Hammel HJ, Bradstreet A, Hemmingsen EA. (1965) Sap pressure in vascular plants. Science 148: 339-346

Stamatiadis S, Taskos D, Tsadila E, Christofides C, Tsadilas C, Schepers JS (2010) Precision 11:306–315 DOI 10.1007/s11119-009-9131-3

Suarez DL, Wood DJ, Lesch SM (2006) Effect of SAR on water infiltration under a sequential rain-irrigation management system. Agric Water Manage. doi:10.1016/j.agwat.2006.07.010

Tarchitzky J, Lerner O, Shani U, Arye G, Lowengart-Aycicegi A, Brener A, Chen Y (2007) Water distribution pattern in treated wastewater irrigated soils: hydrophobicity effect. Eur J Soil Sci 58:573-588

Tester M, Davenport RJ (2003) Na^+ transport and Na^+ tolerance in higher plants. Ann Bot (Lond) 91:503-527

Toze S (2006) Reuse of effluent water-benefits and risks. Agric Water Manage 80:147-159

U.S. Salinity Laboratory Staff. (1954). Alkaline-earth carbonates by gravimetric loss of carbon dioxide. p. 105. In: Richards LA (ed.) Diagnosis and improvement of saline and alkali soils. USDA Agric. Handb. 60. U.S. Government Printing Office, Washington, D.C.

Van Rooyen FC, Weber HW, Levin I (1980) The response of grapes to a manipulation of the soil–plant–atmosphere continuum.II. Plant–water relationships. Agrochemophysica 12:69–74

Wallis MG, Scotter DR, Horne DS (1991) An evaluation of the intrinsic Sorptivity Water Repellency Index on a range of New Zealand soils. Austrl J Soil Res 29:353-362

Walker RR (1994) Grapevine response to salinity. Bull. OIV 67: 635–660.

WHO (1989) Health guidelines for the use of wastewater in agriculture and aquaculture. Report of a WHO scientific group, Technical Report Series No. 778. World Health Organization, Geneva

Wilhelm WW, Ruwe K, Schlemmer MR (2000) Comparison of three leaf area index meters in a corn canopy. Crop Sci 40:1179-1183

Williams LE (1998) Water use of Thompson Seedless grapevines measured with a weighing lysimeters during a late season dry down period. Proc. 1st ISHS Workshop on Water Relations of Grapevines. Acta Hort 493:161-167

Williams LE, Ayars JE (2005) Grapevine water use and crop coefficient are linear functions of the shaded area measured beneath the canopy. Agric and Forest Meteo 132: 201-211

Williams LE, Matthews MA (1990) Grapevine. Irrigation of Agricultural Crops. Agr. Monog. No 30, ASA-CSSA-SSSA, Madison, WI 53711, USA, pp. 1019–1055 (Chapter 34).

Williams LE, Phene CJ, Grimes DW, Trout TJ (2003a) Water use of young Thompson Seedless grapevines in California. Irrig Sci. DOI 10.1007/s00271-003-0066-6

Williams LE, Phene CJ, Grimes DW, Trout TJ (2003b) Water use of mature Thompson Seedless grapevines in California. Irrig Sci. DOI 10.1007/s00271-003-0067-5

Williams LE, Grimes DW, Phene CJ (2009) The effects of applied water amounts at various fractions of measured evapotranspiration on water relations and vegetative growth of Thompson Seedless grapevines. Irrig Sci. doi:10.1007/s00271-009-0171-2

Williams LE, Grimes DW, Phene CJ (2010) The effects of applied water at various fractions of measured evapotranspiration on reproductive growth and water productivity of Thompson Seedless grapevines. Irrig Sci. 10.1007/s00271-009-0173-0

Yang SL, Aydin M, Yano T, Li X (2003) Evapotranspiration of orange trees in greenhouse lysimeters. Irrig Sci. DOI 10.1007/s00271-002-0062-

Yermiyahu U, Ben-Gal A, Sarig P, Zipilevitch E (2007) Boron toxicity in grapevine (Vitis vinifera L.) in conjunction with salinity and rootstock effects. J Hort Sci Biotechnol 82:547-554

Yunusa IAM, Walker RR, Blackmore DH (1997) Characterization of water use by Sultana grapevines on their own roots or on Ramsy rootstock drip-irrigated with water of different salinities. Irrig Sci 17:77-86

Yunusa IAM, Walker RR, Loveys BR, Blackmore DH (2000) Determination of transpiration in irrigated grapevines: Comparison of the heat-pulse technique with gravimetric and micrometeorological methods. Irrig Sci. DOI 10.1007/PL00006714

Young MH, Wierenga P.J, Mancino CF (1996) Large Weighing Lysimeters for Water Use and Deep Percolation Studies. Soil Science 161: 491-501

Zhang X, Walker RR, Stevens RM, Prior LD (2002) Yield-salinity relationships of different grapevine (*Vitis vinifera* L.) scion-rootstock combinations. Aust J Grape and Wine Res. doi: 10.1111/j.1755-0238.2002.tb00250.x

Zimmermann D, Reuss R, Westhoff M, Geßner P, Bauer W, Bamberg E, Bentrup FW, Zimmermann U (2008) A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. J Exp Bot 59: 3157-3167

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תקציר

במדינות בהן שוררים תנאים מדבריים וחצי-יובשניים המחסור במים היא עובדה קיימת. מצב זה מתפתח בשנים האחרונות למשבר מים ממשי. על רקע זה גדלה החשיבות של ייעול השימוש במים בכלל ובחקלאות בפרט. עבודה זו מקיפה היבטים מגוונים הקשורים לשימוש במים להשקיית כרמים בישראל.

מהליזמטר אל הכרם המסחרי: פיתוח מודל השקיה לכרמי ענבי מאכל.

במהלך שש עונות מדידה (1999, 2001-2005) נמדדה צריכת המים השפירים בענבי מאכל מהזן סופריור באמצעות 12 ליזמטרים של שטיפה. הליזמטרים הותקנו כמרכיב בחלקה בת 10 דונם במו"פ לכיש.

במהלך שש שנות המדידה ערכי המקסימום של האוופוטרנספירציה הנמדדת (ET_c) נעו בין 50.7 -60 ליטר לגפן ליום, וסך ההתאדות העונתית (1 באפריל עד 31 אוקטובר) נעה בין 1087-1348 מ"מ.

התאדות הייחוס (ET_o) חושבה עפ"י נוסחת פנמן-מונטית' המתוקנת על בסיס נתונים שהתקבלו מתחנה מטאורולוגית סמוכה. העקום העונתי של מקדם הגידול (K_c) חושב כיחס $K_c = ET_c / ET_o$.

הערכת שטח העלווה התבצעה ע"י מדידת אינדקס שטח העלווה אחת לחודש בעזרת מכשור בלתי הרסני (SunScan Canopy Analysis System). ערכי המקסימום העונתיים שנמדדו בגפני הליזמטרים עמדו על 4.2-6.2 מ"ר למ"ר בשנים 2002-2005. ערכי העלווה הגבוהים הנם נגזרת של שיטת ההדליה (וורנדה הכפולה) המקובלת בשימוש בארץ בגידול ענבי מאכל.

על בסיס המודל שפותח צריכת המים המקסימאלית (ET_c) של גפני ענבי מאכל ניתנת לחישוב בדרכים הבאות:

1. על בסיס מהלך עונתי של מקדם הגידול (K_c) . ניתן להשתמש בעקום זה באזור לכיש ובאזורים בעלי מאפייני אקלים דומים.
2. על בסיס קורלציית צבירת ימי מעלה (GDD)- מקדם הגידול. גרסה זו מתאימה לאזורי גידול בעל מאפיינים אקלימיים שונים מאזור לכיש.
3. על בסיס קורלציית שטח עלווה-מקדם הגידול. אפשרות זו היא המדויקת ביותר אך מצריכה מדידות עלווה שעדיין אינם זמינות דיין למגדלים.

בכל האפשריות נתוני התאדות הייחוס-פנמן (ET_o) השוררים באזור הגידול בנקודת זמן נתונה הנם מרכיב חיוני לחישוב צריכת המים המקסימאלית (ET_c).

בחינת יישום מודל הליזמטרים בתנאים חצי מסחריים התבצעה בכרם בן 10 דונמים הממוקם בצמוד לליזמטרים במו"פ לכיש. בכרם נבחנה השפעת שלושה טיפולי השקיה: השקיה גבוהה ($80\% ET_c$), השקיה בינונית ($60\% ET_c$) והשקיה נמוכה ($40\% ET_c$), על מדדי ווגטציה ורפרודוקציה. תוספת מנת המים המיושמת העלתה את גובה היבולים כמו גם את גודל הנוף. התוצאות מוצגות באופן שכורמים עשויים להיעזר במידע על מנת לקבוע את מקדם ההשקיה המתאים להם.

יישום המודל בבחינת השפעת השימוש בקולחים מול שפירים במנות מים שונות בכרמי ענבי מאכל.

השימוש במי קולחים בחקלאות ישראל מתרחב בצורה מהירה ועשוי להגיע בתקופה הקרובה לכדי 50% מכלל המים המשמשים לחקלאות. המעבר לשימוש במי קולחים מעלה שאלות רבות בנושא ממשק ההשקיה והדישון. בחלקת הכרם במו"פ לכיש הוקם (2002-2007) ניסוי הבוחן את השפעת ההשקיה בשלושה סוגי איכות מים, בשילוב שלוש רמות השקיה (סה"כ תשעה טיפולים במתכונת של בלוקים באקראי). טיפולי איכות המים כללו השקיה במי קולחים עם דשן וללא דשן ($1.88\ dS\ m^{-1}$, Na^+ $230\ mg\ l^{-1}$) מול מים שפירים ודשן ($1.30\ dS\ m^{-1}$, Na^+ $117\ mg\ l^{-1}$).

בעבודה זו נדגמו תקופתית מי ההשקיה, קרקע בשלושה עומקים, טרפי העלים ופטוטרותיהם, ובעונות המחקר האחרונות גם תכולת המינרלים במוהל העצה ובעצת הגזע. בוצעה סריקה רחבה של מגוון יסודות כימיים כדי לאתר את גורמי הסיכון לקרקע ולגידול. מתוך מגוון היסודות התבלט הנתרן כיסוד בעל פוטנציאל הנזק הגדול ביותר המצוי במי הקולחים. במהלך עונות המדידה ריכוזי הנתרן וכן ה-SAR בקרקע עלו וירדו בהשפעת הריכוז במי ההשקיה ושטיפת משקעי החורף, אולם הריכוזים הגבוהים ביותר נמדדו בטיפולי הקולחים בכלל ובאופן מיוחד בטיפולי ההשקיה הגבוהה. בשלוש השנים הראשונות ליישום הקולחים עם תוספת דשן אובחנה מגמה של דחייה בעליית ערכי SAR , ככל הנראה עקב החמצת הקרקע ותוספת של קטיונים חיוביים שהתחרו עם הנתרן על אתרים בקומפלקס החליף של חרסיות (הרכב מכני של הקרקע: 42% חרסית, 28% סילט, 30% חול). החל מהשנה השלישית החל להצטבר נתרן בפטוטרות, כאשר ערכי המקסימום עמדו על $6500\ mg\ l^{-1}$.

בטיפולי הקולחים ערכים אלה גבוהים במובהק עד פי שלושה מהערכים שהתקבלו בפטוטרות של הגפנים שהושקו במים שפירים. מגמות של הצטברות הנתרן בקרקע ניכרו לאחר חמש שנות השקיה מתמשכת בקולחים.

בעבודה זו נבחנה שיטה חדשה של איסוף מוהל קסילם באביב המוקדם, וכן דגימה ואנליזה כימית של עצת הגזע. ריכוזי נתרן גבוהים במובהק נמצאו בעצה ובסות הגזע וכן במוהל הקסילם של טיפולי הקולחים אל מול השפירים.

כפי הנראה בכרמי ענבי מאכל הנטועים בקרקעות חרסיתיות המושקים במנות מים גבוהות של מי קולחים, נתרן לא נשטף מהקרקע אלא להיפך: מצטבר בקרקע ובגפן על איבריה השונים. למרות המגמות הללו עדיין לא נצפתה פחיתת יבולים בטיפולי הקולחים בין היתר בגלל השימוש בכנה עמידה יחסית לנתרן (פולסן).

בחלקה סמוכה לאתר הניסוי הושקתה חלקה מסחרית במי קולחים באיכות דומה (רד גלוב על שורשיה), ונצפתה קריסה טוטאלית של כרם נושא יבול לאחר שלוש עונות השקיית קולחים. בחינת ריכוז הנתרן בגזע העלתה כי הריכוזים שהצטברו בו עומדים על ערכים הגבוהים פי שלושה מהריכוזים שהתקבלו בעצת הגזע בטיפול הקולחים של הניסוי. תוצאה זו יחד עם מגוון רחב של בדיקות בכרמים עם בעיות המלחה ברחבי הארץ מצביעות על היתכנות רבה של שימוש בבדיקות בגזע (גם לאחר תמותת גפנים) כאינדיקציה למצב המלחים שהצטברו בגפן.

בחינת המודל בענבי יין כמותיים בעזרת חיישני "לחץ טורגור" בעלים.

על בסיס קורלציית שטח עלווה-מקדם הגידול נבחן מודל ההשקיה (גירעונית) בענבי יין מהזן פרנץ' קולמבר בחוות נטע שע"י גדרה. הניסוי כלל שני מועדי התחלת השקיה ושתי מנות מים בארבע חזרות במתכונת של בלוקי באקראי. נבחנה השפעת הטיפולים על היבול ומרכיביו וכן על הצימוח העלוותי. בוצע מעקב רציף בעזרת מערכת חדשנית המודדת ערכים המצויים במתאם גבוה עם לחץ הטורגור של הרקמה. המדידות התבצעו במקביל למדדים פיסיולוגיים, כגון חילוף גזים ופוטנציאל מים. שילוב הגורמים אפשר בחינה טובה של השפעת יישום מקדמי השקיה שונים ומועדים שונים של תחילת השקיה על היבול, מרכיביו ואיכותו.

המערכת (LPCP) Leaf patch clamp pressure probe פותחה ע"י קבוצת מחקר גרמנית ומסוגלת למדוד ערכי לחץ (kPa) המצויים במתאם גבוה עם לחץ הטורגור של העלה באופן רציף בעלה השלם בזמן שהוא מחובר לגפן. המדידה מתבצעת באמצעות חיישן בקוטר 1 ס"מ המוצמד לעלה באמצעות מגנט זעיר. מערכת זו היא, ככל הידוע, היחידה המאפשרת לעקוב באופן רציף אחר "לחץ הטורגור" של העלים. המערכת נבחנה בהצלחה בענבי יין (וגם בענבי מאכל), ומהתוצאות עולה, כי גם שינויים קלים במיקרו אקלים נמדדו בעזרת מערכת LPCP.

תודות

"וְזָכַרְתָּ, אֶת ה' אֱ–לֹהֶיךָ כִּי הוּא הַנֹּתֵן לְךָ כֹּחַ, לַעֲשׂוֹת חָיִל" (דברים ח' י"ח)

תודה מיוחדת למדריכי, פרופ' אמנון שוורץ, שמלווה אותי שנים רבות ולימד אותי את רזי החשיבה והמחקר המדעיים. אמנון עשה זאת בדייקנות, במקצוענות, במאור פנים ובנפש חפצה, ועל כך אני מודה לו מעומק הלב.

תודה רבה לד"ר משה שנקר, שהדריך אותי בנפתולי המינרלים הקרקעיים בסבלנות רבה, ודלתו תמיד היתה פתוחה בפניי.

לצוות תחנת הניסיונות מו"פ לכיש: למדריך שירות השדה, עמרם חזן, שעמל הרבה על התפעול השוטף של הניסוי, לטכנאי המסור יוסי כהן על עבודת טכנאות מרובה בליזמטרים ולצוות המנהל של המו"פ: בני גמליאל ואברהם אליהו.

לכל הסטודנטים שהשתתפו הן בעבודת המעבדה והן בעבודת השדה: כרמית ליפשיץ, עידית דמבק, שירי נפתליהו, בת-אל ועקנין, אור בומץ', אוריאל אונר, מאור לוי, אביחי ברודץ', שראל מוניץ.

לכל החברים במעבדה: רון זליגמן, עידן בהט וענבר מאיר, שכיף גדול לעבוד בחברתם.

להוריי היקרים שבעמלם הרב הביאוני עד הלום.

לילדיי: יעלה, איתמר, שִׁילֹה, ערבה, אחָיָה ואורי שעוזרים, תומכים, שואלים, מתעניינים כל אחד בדרכו ובסגנונו ומקבלים את ה"גפן" כאחת מבנות המשפחה.

ואחרונה חביבה מוריה, "אשת חיל מי ימצא", שבלעדיה כלום לא היה קורה.

תודה.

תודה רבה.

בס״ד

מודל להשקיית כרמים בתנאי מגבלות מים

חיבור לשם קבלת תואר

״דוקטור לפילוסופיה״

מאת

ישי נצר

הוגש לסינט של האוניברסיטה העברית בירושלים

תשרי תשע״א, אוקטובר 2010