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Application of the OECT-Based In Vivo Biosensor Bioristor in Fruit Tree Monitoring to Improve Agricultural Sustainability

Filippo Vurro [1,†], Edoardo Marchetti [1,†], Manuele Bettelli [1], Luigi Manfrini [2], Adele Finco [3], Carlo Sportolaro [4], Nicola Coppedè [1], Nadia Palermo [1], Maria Grazia Tommasini [5], Andrea Zappettini [1,*] and Michela Janni [1]

1 Istituto dei Materiali per L'Elettronica e il Magnetismo (IMEM-CNR), Parco Area delle Scienze, 37/A, 43121 Parma, Italy; filippo.vurro@imem.cnr.it (F.V.); edoardo.marchetti@imem.cnr.it (E.M.); manuele.bettelli@imem.cnr.it (M.B.); nicola.coppede@imem.cnr.it (N.C.); nadia.palermo@imem.cnr.it (N.P.); michela.janni@imem.cnr.it (M.J.)
2 Department of Agricultural and Food Sciences, University of Bologna, V.le Fanin 44, 40127 Bologna, Italy; luigi.manfrini@unibo.it
3 Department of Agricultural, Food and Environmental Sciences (D3A), Polytechnic University of the Marche (UNIVPM), Via Brecce Bianche, 60131 Ancona, Italy; a.finco@staff.univpm.it
4 Gruppo Filippetti, Via Guglielmo Marconi, 60015 Ancona, Italy; csportol@gmail.com
5 Ri.NOVA Società Cooperativa, Via dell'Arrigoni, 120, 47522 Cesena (FC), Italy; mgtommasini@rinova.eu
* Correspondence: andrea.zappettini@imem.cnr.it
† These authors contributed equally to this work.

Abstract: Water scarcity is a major concern in agriculture worldwide. Fruit trees are severely affected by water deprivation in terms of growth, fruit yield, and quality. Plant monitoring combined with efficient irrigation is pivotal to achieve good quality standards and improve agricultural sustainability. This study reports the use of in vivo sensing technology to monitor fruit tree species continuously, in real time and in vivo, through an Organic Electrochemical Transistor (OECT)-based biosensor called Bioristor. The sensor was applied to grapevines, apples, and kiwis, revealing its capability to trace the plant water status for the whole productive cycle. A correlation between the sensor response index (R) and environmental parameters such as air humidity and temperature were recorded for fruit species. The day/night oscillation of the ionic content in the transpiration stream varies during plant growth and fruit maturation and during severe drought stress. Bioristor promptly detected the occurrence of drought stress. The gate current (Igs) trend supports the reduction in the saturation of the system due to the lower water availability. The use of Bioristor-acquired indices can be used to improve precision irrigation techniques according to the real plant needs.

Keywords: apple; kiwi; grape; Organic Electrochemical Transistor (OECT); bioelectronics; precision agriculture; water stress; field phenotyping; circadian clock; precision fruit growing

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1. Introduction

Global warming, a decrease in precipitation, and population growth have resulted in a 60% increase in food demand and pose increasingly serious challenges to world temperate fruit production in the upcoming years [1].

In the Mediterranean region, the rising temperatures are severely affecting yields and crop production mainly due to prolonged drought events [2,3].

Most fruit crops need an irrigation supply to produce a profitable yield when rain does not satisfy the crops' water requirements [4,5]. Deficit irrigation is a sustainable approach becoming more common in fruit management, limiting water overuse and improving water productivity [6–9].

Indeed, improving water use efficiency and increasing the resilience of agricultural systems to climate change (Objective 2, Target 2.4) are mandatory and are included in the

17 sustainable development goals defined by the United Nations Agenda 2030 [8]. This goal can be achieved either by developing and selecting novel varieties with improved tolerance to water deprivation or by introducing novel smart plant monitoring tools and precision agriculture approaches into farm management.

Currently, sophisticated biochemical molecular and genetic methods relying on invasive and destructive tissue sampling are used to trace the mechanisms underlying plant tolerance to abiotic and biotic stresses [10].

Recently, several devices have been applied for the continuous monitoring of fruit trees to measure sap flow [11] and water potential [12]. In addition, the image-based acquisition of vegetation indices through UAVs are of common use in precision agriculture (PA) and field phenotyping [8,13–19]. However, recently, bioelectronic technologies have been applied to complement the above-mentioned approaches and offer new possibilities for the real-time monitoring and dynamic modulation of plant physiology. Bioelectronic sensors can translate complex biological inputs to electronic readout signals, while bioelectronic actuators can modulate biological networks via electronic addressing [10,20].

Among the bioelectronic devices, the in vivo Organic Electrochemical Transistor (OECT)-based sensor named “Bioristor” was developed and implemented in plants [21]. The sensor can detect the changes in the composition of the plant sap in growing plants, in vivo and in real time, without interfering with plant functions [21–24].

The Bioristor system is based on two textile functionalized electrodes introduced into the plant stem. One electrode is connected at both ends and is used as a transistor channel, and the other electrode is the gate (Figure 1A,B). The electrodes are bridged by the plant sap. Upon application of a positive potential at the gate, the cations of the system are forced into the polymeric channel and, as a result, the conductivity of the channel is reduced. The Bioristor measures the currents flowing from the gate (Igs) and the drain (Ids) to the source electrode. Both Igs and Ids depend on the physical and chemical characteristics of the system, mainly on the concentration of ions in the plant sap [25] and on the saturation of the system.

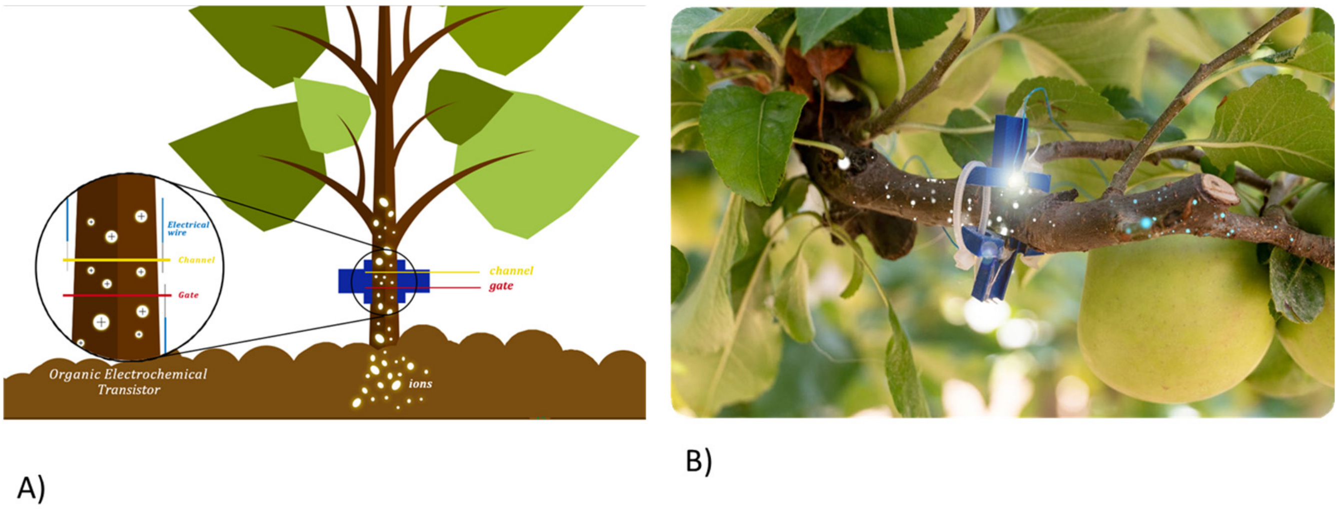

Figure 1. Bioristor device. (**A**) Working principle and configuration, (**B**) cartoon of ion flow and monitoring in apple.

Bioristor can measure ion concentrations and movements in the vascular tissues of the plant with a focus on xylem vessels being responsible for the water and mineral nutrients (xylem sap) transport from roots to leaves through the transpiration stream [22,25]. Bioristor enables the in vivo detection and monitoring of the plant’s physiological mechanisms related to ion movements and compartmentalization, under normal as well as stress conditions such as drought [22] and salt stress [23], or under environmental variations such as vapor pressure deficit (VPD) and increases in relative air relative humidity (RH, [24,26]).

The study reports the implementation of Bioristor in fruit trees in three main Italian fruit crops: grape, apple, and kiwi fruit. Plant monitoring through Bioristor for the entire growing season and the acquired indices are discussed in terms of sensor operability, opening up new perspectives of use to improve irrigation efficiency and sustainability.

2. Materials and Methods

2.1. OECT Sensor Device: Bioristor Preparation and Insertion in Plant Trees

Bioristor was fabricated, installed, and operated following the methods previously reported [22,26]. In brief, each textile thread was cleaned with a plasma oxygen cleaner (Femto, Diener electronic, Ebhausen/Germany) to improve the thread's wettability and facilitate the adhesion of the aqueous conductive polymer solution. This step enhances the performance of the sensors by removing any impurities or contaminants on the fibers and increasing the surface area for the polymer adhesion. An aqueous solution containing PEDOT:PSS (Clevios PH1000, Starck GmbH, Munich, Germany) and dodecyl benzene sulfonic acid (2% v/v) was prepared and stirred for five minutes to ensure homogeneity.

The solution obtained was deposited onto polypropylene fibers using a drop-casting technique in an amount of 50 μL/cm. This resulted in the formation of a thread with a cross-sectional area of 1.42 mm × 0.25 mm. The thickness and shape of the sensor can be controlled precisely, polypropylene fibers can be used as a substrate that offers biocompatibility and mechanical stability, and the fibers can be infused with the conductive polymer, which is essential for the sensors to function effectively.

The entire process was repeated three times to complete the preparation of the sensors. Next, the fibers were treated with highly concentrated sulfuric acid (95%) for 20 min. This improves the crystallinity, electrical properties, and durability of the polymer by replacing the use of ethylene glycol (10% v/v) treatments to improve the electrical conductivity and long-term stability of the polymer. Finally, the fibers were washed with water and subjected to annealing for 1 h at 130 °C [21,27].

The channel of the OECT was inserted into the plant stem by means of a 0.8 mm drill, and connected at both ends to a metal wire to form the source and the drain electrodes. The connections were secured through silver paste. Then, a gate electrode completed the design of the sensor device.

Bioristor signals were amplified by custom read-out electronics and connected to an IoT control unit based on the Arduino DUE system powered by a 12 V 12 Ah lead battery charged by a photovoltaic panel. The sampling rate was 1 Hz, and each control unit was able to read up to four sensors. The control unit has a 12-bit ADC (5 V full scale); the maximum current full scale is 7 mA, and the current resolution is about 1.5 μA.

A micro-weather unit was also incorporated into the control unit (DHT11 module, Seed Technology Inc., Shenzhen, China) to monitor the air temperature (°C) and relative humidity (RH).

The Bioristor data were locally saved on a micro-SD memory card and transferred to the cloud via a 4G connection. This overall setup allowed for the maximization of the signal-to-noise ratio using customized electronic circuits to amplify the Bioristor signals, as well as the local analysis of the raw data.

Bioristor was operated by applying a constant voltage (Vds = −0.1 V) across the transistor channel, along with a positive voltage at the gate (Vg = 0.5 V); the resulting transistor current (Ids) and gate current (Igs) were monitored continuously for the entire duration of the experiments.

2.2. Measuring the Electric Activity of the Plants Using OECT

The operating principle of an OECT is thoroughly described in Coppedè et al. (2017) [25,28]. Common nutrients absorbed through roots and circulating in the plant sap (NaCl, KCl, MgCl, ZnCl, and other salts) dissociate as cations (M+) and anions (A−). In the OECT device, the channel is the active part and it is made of PEDOT:PSS, a p-type conductive polymer. Upon the application of a positive voltage at the gate, cations are forced towards the transistor channel de-doping the PEDOT:PSS polymer. De-doping results in the removal of the charge carriers from the conducting polymer. The smaller number of holes available for conduction in the channel is a consequence of the incorporation of cations in the PEDOT:PSS. Cations entered into the PEDOT+:PSS− cause a reduction in the oxidized PEDOT+ and induce a decrease in conductivity upon reduction to PEDOT. This de-doping process causes a reduction in the current from drain to source (Ids). The Ids current is proportional to the cation concentration in the fluid.

The entire process is reversible. A voltage (Vds = −0.1 V) was applied across the source and drain terminals of the channel, resulting in a continuous flow of current. In addition, a positive voltage was applied to the gate (Vg = 0.5 V) for 15 min, causing a decrease in the conductivity of the channel due to the migration of cations from the electrolyte into the channel. When the gate voltage is switched off again for 15 min, the cations tend to return to solution through diffusion—this is the de-doping phase [29]. Vg varies in the time following a typical 50% duty cycle square wave, periodically oscillating between 0 and 0.5 V with a frequency of two oscillations per hour.

In this configuration, the gate and the drain are the cathodes and the source is the anode; thus, positive charges move from the gate to the source, and within the channel from the drain to the source.

The sensor response (*R*) is calculated as

$$R = \frac{|I_{ds} - I_{ds0}|}{I_{ds0}} \quad (1)$$

which is proportional to the positively charged ion concentration.

Ids0 represents the current flowing across the channel when Vgs = 0.

At the same time, the gate current Igs was also recorded in these trials and represents an overall estimation of the sap conductance. Igs was found to be a fundamental parameter to establish the device saturation, i.e., the device wet fraction [30] that was, thus, correlated with the transpiration flux [25]. In some sense, while R is correlated with the sap ion concentration, the Igs is related to the amount of sap that is wetting the device, thus, the amount of sap that is circulating in the plant.

2.3. Bioristor Setup in Tree Crops

In *apple*, the experiment was conducted in an apple orchard cv pink lady grafted on M9 rootstock for the whole month of July 2021, located in Cesena (Italy). Four plants were equipped with two sensors for each plant, for a total of eight Bioristors, on three-year branches. Irrigation was only performed for the entire month of July.

In *grapes*, the experiment was conducted in 2021 as described in Finco et al. (2022) [31] in the Marche Region (central Italy) near the municipality of Serra De' Conti. One Bioristor was implanted in each grape plant cv Verdicchio on two-year branches on a total of eight plants and eight sensors. The vineyard was conducted under a non-irrigation regime.

In *kiwi fruit cv G3*, the experiment was conducted in the Azienda Severi (Cesena, Italy) in 2019. One sensor was inserted in two branches on each plant for a total of sixteen sensors. Two plots were monitored. Irrigation was kept constant for both plots till July 5th, and then the two plots were subjected to different irrigation regimes: 100% and 120%, respectively.

2.4. Bioristor Data Analysis

R and Igs data were analyzed with MATLAB (https://uk.mathworks.com/) (MathWorks, Natick, MA, USA) and Microsoft Excel 2016 to smooth out variations related to the circadian cycle [21] by calculating the rolling mean. The R data were then subjected to ANOVA using MATLAB (https://uk.mathworks.com/) (MathWorks, Natick, MA, USA).

3. Results

Bioristor was successfully implemented in fruit trees (Figure 2). The plants were monitored continuously for 132 days in apples (Figure 3A), for 123 days in kiwi (Figure 3B), and for 167 days in grapes (Figure 3C).

Figure 2. Bioristor implementation in fruit trees. (**A**) Apple tree, (**B**) kiwi, (**C**) vineyard.

In all crops, as also observed in tomato (Vurro et al., under revision) the Bioristor traces the effects of rain on the plants. The trend of the sensor response under rain rapidly increased as a consequence of the increases in the air relative humidity (RH%), leading to decreased VPD [26].

The analysis of the sensor response allowed the dynamic and continuous tracing of the ionomic fluctuations in the plant following the initiation of the plant defense response [22].

In apple, a stable sensor response was observed until the end of the scheduled irrigation (26 July).

From 29 July, a strong decrease in the R slope was observed and the R signal became stable at low values, indicating the overall low water status of the plant (Figure 3A). This finding was also supported by the observed decreases in the slope of Igs, and thus of the system saturation (Figure 4A), due to the reduced water availability observed during July–August (30/07–30/08).

In kiwi, Bioristor correctly traced the dynamic changes in plant sap ion concentrations for 120 days in total (Figure 3B). In the first month of monitoring (5/6–5/7), no significant difference in the sensor response (R) was observed at the same water regime applied for the entire month of June up to early July (05/06–07/07). The application of different water regimes led to changes in the R index trend that is consistent with two irrigation regimes. In August, the sensor reported a strong reduction in R in the 120% water regime. When the Igs was analyzed, no differences were observed in the slope between the two water regimes (Figure 4B), suggesting that the alteration in the water availability affects the ion concentration more than the saturation. The saturation starts to decrease when the sensor response detects the differences between the two water regimes (06/08).

In grapes, Bioristor was operative for a total of 180 days continuously in the absence of irrigation [31]. A drop in the R trend was observed during summer (June–August, Figure 3C). In addition, a drop in the Igs was observed in correspondence to the reduction of the R index (Figure 4C).

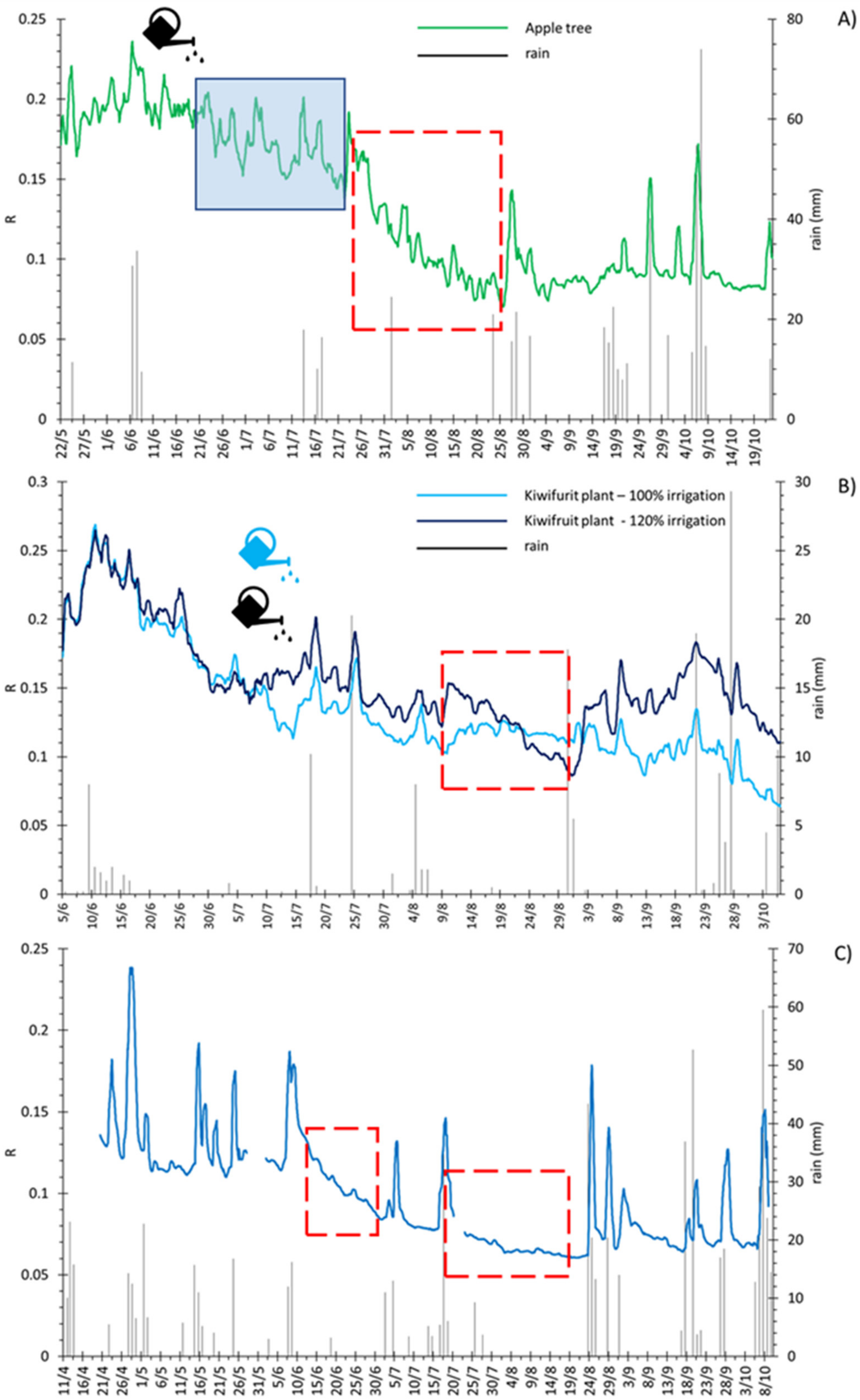

Figure 3. Plot of the Bioristor sensor response (R) in fruit tree species. (**A**) Apple, one watering can indicates the period of irrigation; (**B**) kiwi, one watering can indicates the beginning of the irrigation period and two watering cans indicate the different irrigation volumes (100%, dark blue and 120% light blue); (**C**) grapes, no irrigation is applied. Red boxes indicate the time window identified as a period of drought stress. Black lines indicate the occurrence of rainy events.

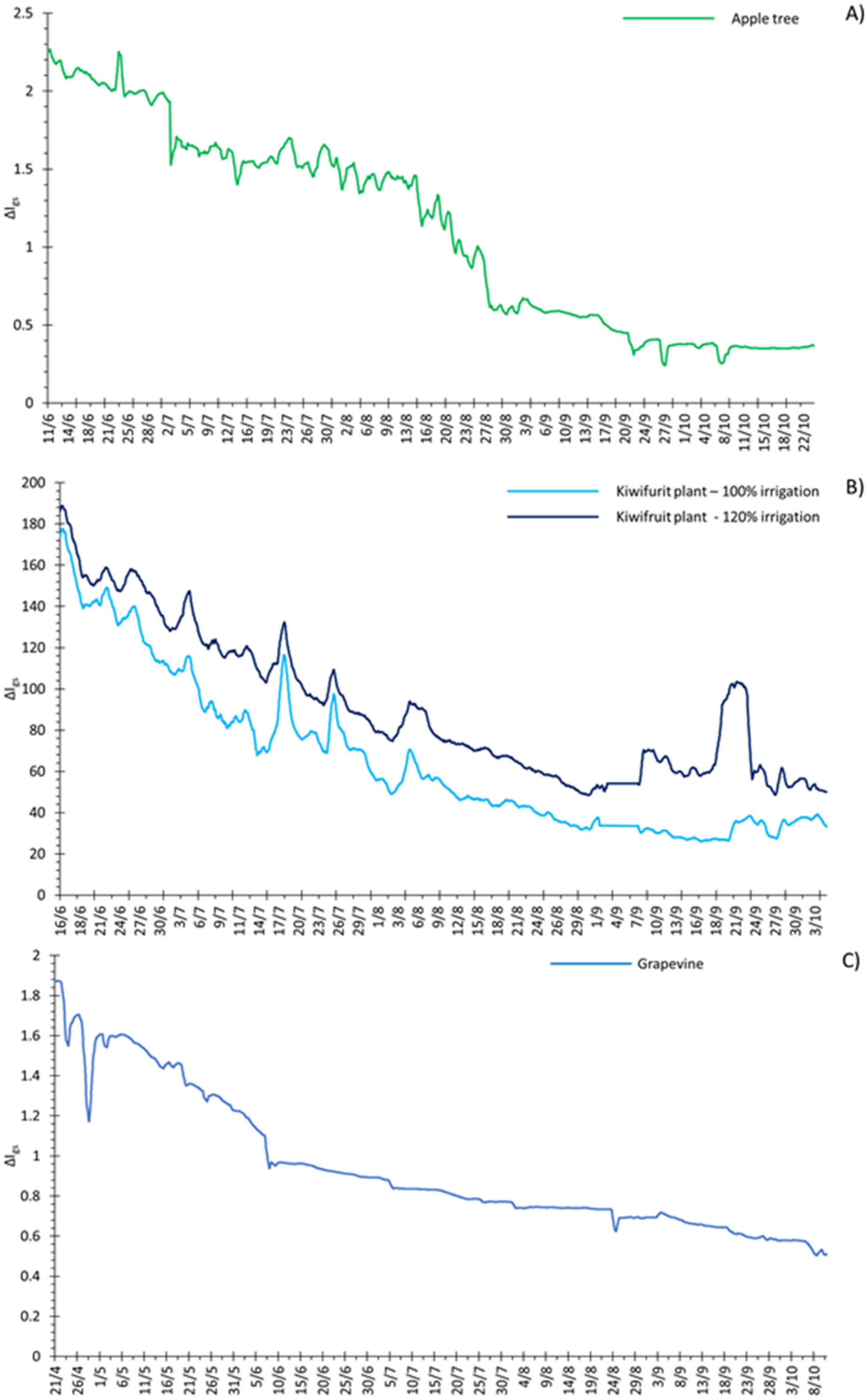

Figure 4. Plot of the Igs in fruit tree species. (**A**) Apple, (**B**) kiwi, (**C**) grape.

Day/Night Modulation Transpiration and Ion Content

The previously observed trend in day/night Bioristor response with an increase during the night and a decrease during the day [21] was also confirmed in fruit trees (Figure 5A–F).

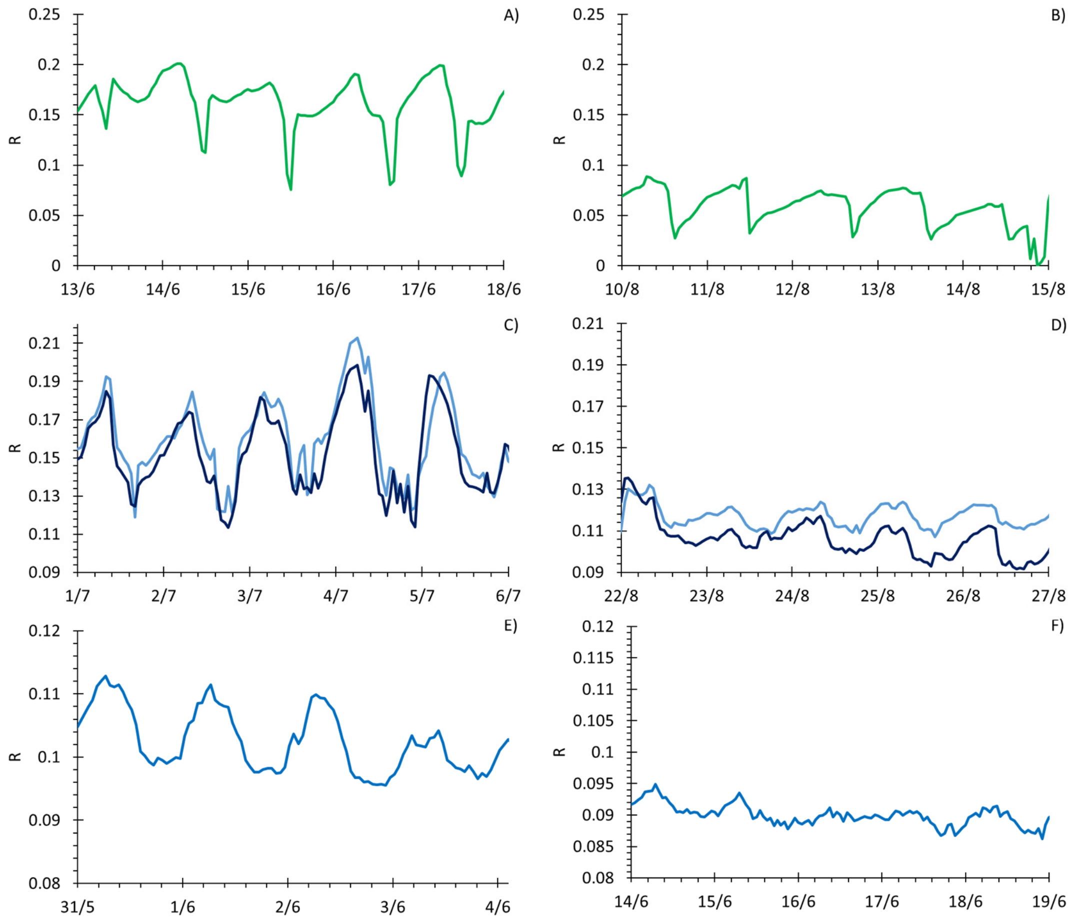

Figure 5. Daily R trend for circadian rhythm. (**A**) Apple, (**C**) kiwi, and (**E**) grape, and under drought stress conditions in (**B**) apple, (**D**) kiwi, and (**F**) grape.

The overall R circadian pattern showed a decrease in the amplitude during drought stress where a reduction in the diurnal cycle was observed.

In kiwi, the R trend varies cyclically every day (Figure 5), showing a maximum during midnight when the transpiration is almost stopped and showing a daytime minimum at 16:00, in accordance with previously reported data of transpiration in kiwi of a maximum value around the middle of each day and reducing to a minimum at night [32]. The R trend that decreased during the progress of the fruit maturation suggests a reduction in the ion accumulation according to ripening progression. In addition, all plants highlight a variation in the dynamic of day–night oscillation that becomes more variable according to the drought stress occurring.

The day–night pattern of the sensor response observed in fruit trees confirms the inverse relation between the transpiration and the sensor response trend, as reported in Coppedè et al. (2017) [21]. An overall increase in the R amplitude, and thus in ion concentration, was observed during the night between 23:00 and 7:00 h when transpiration decreased, leading to a reduction in the sensor response, supporting the previously reported increase in ion concentration in the plant sap as a result of the low transpiration rate and increased translocation of daily produced photosynthates in the vascular tissues [33].

In apple, the R values reached their maximum at 2:00 a.m. and remained constant till 7:00 a.m. (Figure 6A). In kiwi, a different behavior in the daily sensor response was observed. In the 120% water regime, a continuous increase in the R was observed from 18:00, reaching a maximum at 09:00, while in the 100% water regime, R reached a maximum at 0:00 and remained stable till 09:00, although at lower values in comparison with the 120% regime (Figure 6B).

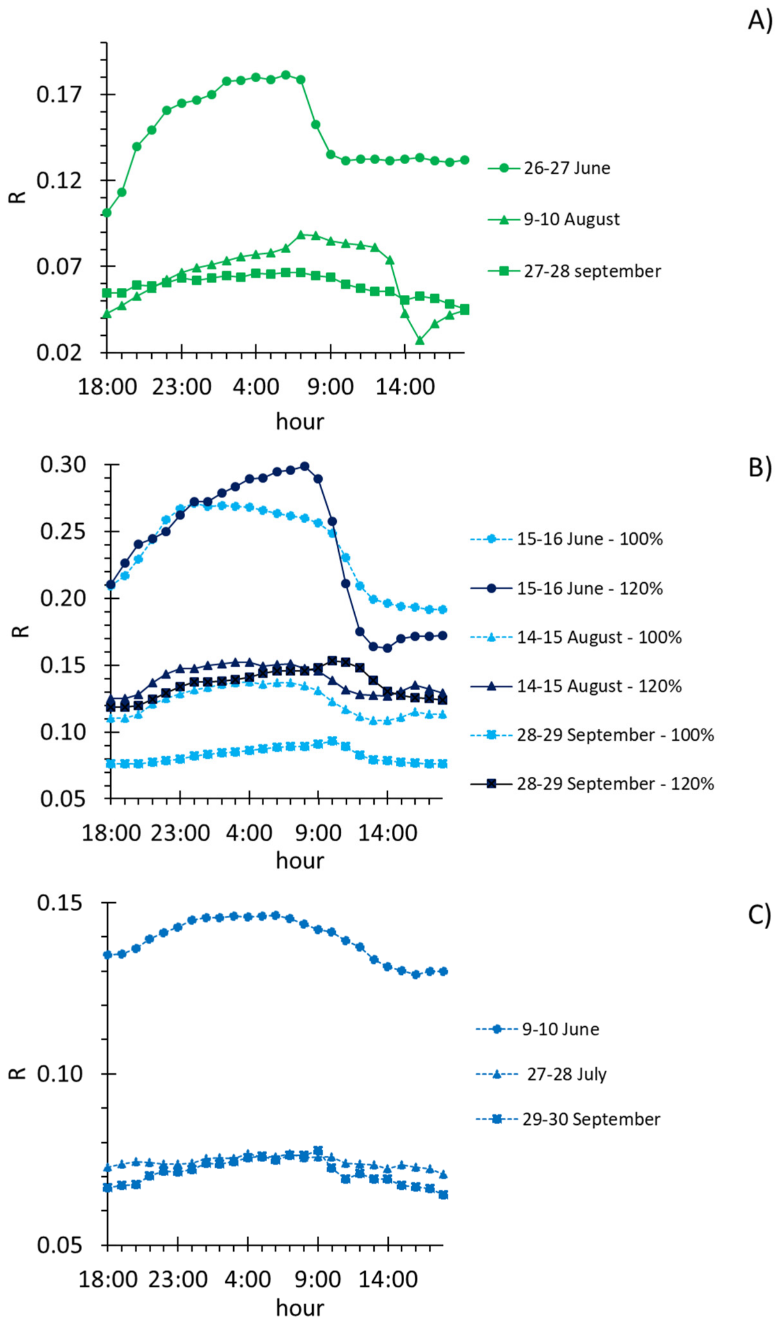

Figure 6. Circadian trends of the sensor response. (**A**) Apple, (**B**) kiwi, (**C**) grape.

In grapes, the day/night trend of the R reached a maximum at 02:00 and remained constant till 06:00 when it gradually decreased (Figure 6C).

4. Discussion

In this study, the bioelectronic OECT-based biosensor, named Bioristor, was applied to continuously monitor fruit tree crops. Bioristor demonstrates its ability in detecting physiological plant status and the changes in the physiological processes such as the transpiration rate and environmental conditions. It reports the occurrence of water stress in a timely manner.

The implementation of an OECT-based sensor allows the tracing of dynamic changes in ion sap content (R) and saturation (Igs) for the entire vegetative and productive season in fruit trees.

The sensor response (R) in this case study is modulated by environmental changes such as heavy rain. As reported in Vurro et al. (2019) [26], rain alters the environmental VPD values, and, in turn, the transpiration process, the concentration of ions, and the plant water content (saturation, Vurro et al., 2019). This is further supported by the rapid increase in the R trend observed during and immediately after rain (apple tree, 13–112%; kiwi fruit, 10–35%; grapevine, 13–130%).

Also in trees, the sensor response was reported to be inversely correlated with stomatal conductance [22], demonstrating that, under stress, the reduction in transpiration is triggered to prevent the loss of water [34].

In kiwi fruit, the different irrigation regimes imposed do not influence the slope of the R, leading to the hypothesis that the constant administration of an additional 20% more water does not change the ion concentration or movement in the plant. On the contrary, increased irrigation seems to be fundamental in key time windows characterized by the decrease in the Bioristor response (red squares in Figure 3A–C).

In apple, a moderate irrigation was performed; however, the occurrence of drought stress was identified by Bioristor. The R trend decreased for the entire month of August, and on the basis on Bioristor data, a deficit irrigation strategy can be used to manipulate fruit size and sugar content for a premium price in specific markets.

From the physiological point of view, the results obtained with Bioristor in fruit trees can significantly improve the knowledge on the diurnal changes in ion dynamics occurring in the plant sap, both during the plant and fruit development, but also during the drought stress occurrence.

The sensor response showed a trend in accordance with previously reported data in kiwi where the fruit transpiration rates are generally high during early fruit growth, but decline to much lower values towards maturity [32]. This study examines the regulation of the R index and its dynamic changes across the day. An increase during the night and pre-dawn for most days and species were observed and associated with increased or decreased transpiration in fruit trees.

In drought conditions, plants close the stomata, leading to a strong reduction in the ions flowing in the transpiration stream and indirectly in a decompartmentalization of ions, resulting in a strong reduction in the sensor response and of the Igs values [22,25,35–37].

As shown in Figure 3, rainy events cause a strong increase in the R values as a result of the leaves wetting, causing a block in the transpiration process. The strong increases in the R value can thus be used to exactly calculate the overall time of the wetting of the leaves and to correctly plan the use of actions to avoid the development of fungal diseases.

In all species analyzed, the sensor response decreased both under drought and during fruit maturation, as previously reported by Boini et al. (2022) [38]. The high transpiration rates observed in the early stages of G3 fruit growth and maturation and the subsequent decrease in plant water transport are consistent with the dynamics observed in the sensor response.

The analysis of the daily modulation of the sensor response provides insights into the circadian regulation of the ion allocation as a result of the effects of the environmental changes on the plant transpiration [22]. Here, we observed that the phase of the circadian oscillator of the sensor response has been adjusted continuously to match the phase of the environment for all species. Our results are consistent with those reported by Montanaro et al. (2012) [32] that described the fruit transpiration rate in kiwi. The lowest transpiration measured at 18:00 (h) the maximum of the sensor response was observed. Boini et al. (2022) [38] describes, in cv G3 of kiwi, a maximum of transpiration at 12:30 both in optimal and reduced water conditions; the transpiration remains high at 15:30, although it is reduced in both water conditions. Our data are in line with this observation showing a minimum of ion content at 13:00 that is more marked in 120% water condition.

In grapes, the daily trend in ion accumulation is in line with the previously observed results on the transpiration cycle in several grape types [39].

Under drought, the R circadian pattern showed a decrease in the slope that is pronounced in apple and kiwi, while the flattening of the R is reduced in grapes, indicating an increased tolerance to drought.

The reduction in the daily oscillation during fruit growth development is consistent with the observation that daily and seasonal changes in fruit development are influenced by variations in stem and fruit water potentials. In 2002, Rossi et al. [33] observed that water potentials tend to decrease at midday with a recovery during the afternoon and night hours [40,41]. A difference among and within the fruit species' transpiration water losses that lead to a reduction in the fruit water balance was also observed, concluding that there is a positive influence on the fruit's ability to attract xylem and phloem flows, since they reduce fruit pressure potential, thus potentially increasing stem-to-fruit water potential [33] and supporting the trend of the sensor response.

One of the biggest challenges for scientists and farmers to increase water use efficiency worldwide is to develop better sensors and methods for measuring plant water status to use for irrigation scheduling [8,42]. Indeed, several methods for plant water status determination are available and can be universally applied, as reviewed in Jones et al. (2007) [43].

Thus, the possibility of determining the circadian phenotypes through in vivo monitoring can be important to identify superior water use efficiency genotypes and superior irrigation practices.

Modern drip irrigation systems can be very accurate, precise, and efficient at delivering the optimum amount of water to the root zone of fruit trees to achieve the desired crop production requirements. However, the full potential of drip irrigation can only be achieved with good management decisions that can only be made with good plant water status. On these bases, the real-time in vivo detection of plant health and of the plant water status acquired by Bioristor can be an optimal solution for precision agriculture in fruit trees.

5. Conclusions

Irrigation is one of the major agricultural practices that strongly impact plant production. The current decrease in available water resources is leading to an urgent need to adopt a strategy to efficiently utilize water. In the case of fruit trees, water is often required for frequent irrigation during fruit development, and the mismanagement of the water supply to trees at critical stages leads to fruit drop, reduced fruit size, and poorer quality.

In this study, Bioristor was used as a novel tool for fruit tree monitoring in view of a precision agriculture approach. Bioristor is biocompatible and able to work for the entire fruit tree production season.

The Bioristor indices of R and Igs allowed us to trace the water and ion movements in the transpiration stream of plants during the day/night cycle, but also during drought stress. The possibility of precisely estimating the time and amount of the drought stress can optimize the irrigation management in fruit trees, for example, identifying the timing of a deficit irrigation, where allowed [44,45].

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References

1. Devin, S.R.; Prudencio, Á.S.; Mahdavi, S.M.E.; Rubio, M.; Martínez-García, P.J.; Martínez-Gómez, P. Orchard Management and Incorporation of Biochemical and Molecular Strategies for Improving Drought Tolerance in Fruit Tree Crops. *Plants* **2023**, *12*, 773. [CrossRef]
2. Fader, M.; Shi, S.; von Bloh, W.; Bondeau, A.; Cramer, W. Mediterranean Irrigation under Climate Change: More Efficient Irrigation Needed to Compensate for Increases in Irrigation Water Requirements. *Hydrol. Earth Syst. Sci.* **2016**, *20*, 953–973. [CrossRef]
3. Tombesi, S.; Frioni, T.; Poni, S.; Palliotti, A. Effect of Water Stress "Memory" on Plant Behavior during Subsequent Drought Stress. *Environ. Exp. Bot.* **2018**, *150*, 106–114. [CrossRef]
4. Wang, X.; Xing, Y. Evaluation of the Effects of Irrigation and Fertilization on Tomato Fruit Yield and Quality: A Principal Component Analysis. *Sci. Rep.* **2017**, *7*, 350. [CrossRef]
5. Anderson, R.G.; Girona, J.; Gucci, R. Using Water for Best Product Quality in Fruit and Nut Trees and Vines. *Irrig. Sci.* **2023**, *41*, 449–452. [CrossRef]
6. Du, T.; Kang, S.; Zhang, J.; Davies, W.J. Deficit Irrigation and Sustainable Water-Resource Strategies in Agriculture for China's Food Security. *J. Exp. Bot.* **2015**, *66*, 2253–2269. [CrossRef]
7. Chai, Q.; Gan, Y.; Zhao, C.; Xu, H.-L.; Waskom, R.M.; Niu, Y.; Siddique, K.H.M. Regulated Deficit Irrigation for Crop Production under Drought Stress. A Review. *Agron. Sustain. Dev.* **2016**, *36*, 3. [CrossRef]
8. Scalisi, A. Continuous Determination of Fruit Tree Water-Status by Plant-Based Sensors. *Italus Hortus* **2018**, *24*, 39–50. [CrossRef]
9. Costa, C.; Schurr, U.; Loreto, F.; Menesatti, P.; Carpentier, S. Plant Phenotyping Research Trends, a Science Mapping Approach. *Front. Plant Sci.* **2019**, *9*, 1933. [CrossRef]
10. Dufil, G.; Bernacka-Wojcik, I.; Armada-Moreira, A.; Stavrinidou, E. Plant Bioelectronics and Biohybrids: The Growing Contribution of Organic Electronic and Carbon-Based Materials. *Chem. Rev.* **2022**, *122*, 4847–4883. [CrossRef]
11. Valentini, R.; Belelli Marchesini, L.; Gianelle, D.; Sala, G.; Yarovslavtsev, A.; Vasenev, V.; Castaldi, S. New Tree Monitoring Systems: From Industry 4.0 to Nature 4.0. *Ann. Silvic. Res.* **2019**, *43*, 84–88. [CrossRef]
12. Blanco, V. Microtensiometers: A New Tool to Monitor Your Apple Trees for Deciding When and How Much to Irrigate. Ph.D. Thesis, WSU Tree Fruit, Washington State University, Pullman, WA, USA, 2023.
13. Gontia, N.K.; Tiwari, K.N. Development of Crop Water Stress Index of Wheat Crop for Scheduling Irrigation Using Infrared Thermometry. *Agric. Water Manag.* **2008**, *95*, 1144–1152. [CrossRef]
14. Gracia-Romero, A.; Kefauver, S.C.; Fernandez-Gallego, J.A.; Vergara-Díaz, O.; Nieto-Taladriz, M.T.; Araus, J.L. UAV and Ground Image-Based Phenotyping: A Proof of Concept with Durum Wheat. *Remote Sens.* **2019**, *11*, 1244. [CrossRef]
15. Blaya-Ros, P.J.; Blanco, V.; Domingo, R.; Soto-Valles, F.; Torres-Sánchez, R. Feasibility of Low-Cost Thermal Imaging for Monitoring Water Stress in Young and Mature Sweet Cherry Trees. *Appl. Sci.* **2020**, *10*, 5461. [CrossRef]
16. Ahmad, U.; Alvino, A.; Marino, S. A Review of Crop Water Stress Assessment Using Remote Sensing. *Remote Sens.* **2021**, *13*, 4155. [CrossRef]
17. Alizadeh, A.; Toudeshki, A.; Ehsani, R.; Migliaccio, K.; Wang, D. Detecting Tree Water Stress Using a Trunk Relative Water Content Measurement Sensor. *Smart Agric. Technol.* **2021**, *1*, 100003. [CrossRef]
18. Noun, G.; Lo Cascio, M.; Spano, D.; Marras, S.; Sirca, C. Plant-Based Methodologies and Approaches for Estimating Plant Water Status of Mediterranean Tree Species: A Semi-Systematic Review. *Agronomy* **2022**, *12*, 2127. [CrossRef]
19. Simbeye, D.S.; Mkiramweni, M.E.; Karaman, B.; Taskin, S. Plant Water Stress Monitoring and Control System. *Smart Agric. Technol.* **2023**, *3*, 100066. [CrossRef]
20. Simon, D.T.; Gabrielsson, E.O.; Tybrandt, K.; Berggren, M. Organic Bioelectronics: Bridging the Signaling Gap between Biology and Technology. *Chem. Rev.* **2016**, *116*, 13009–13041. [CrossRef]
21. Coppedè, N.; Janni, M.; Bettelli, M.; Maida, C.L.; Gentile, F.; Villani, M.; Ruotolo, R.; Iannotta, S.; Marmiroli, N.; Marmiroli, M.; et al. An in Vivo Biosensing, Biomimetic Electrochemical Transistor with Applications in Plant Science and Precision Farming. *Sci. Rep.* **2017**, *7*, 16195. [CrossRef]
22. Janni, M.; Coppede, N.; Bettelli, M.; Briglia, N.; Petrozza, A.; Summerer, S.; Vurro, F.; Danzi, D.; Cellini, F.; Marmiroli, N.; et al. In Vivo Phenotyping for the Early Detection of Drought Stress in Tomato. *Plant Phenomics* **2019**, *2019*, 6168209. [CrossRef]
23. Janni, M.; Claudia, C.; Federico, B.; Sara, P.; Filippo, V.; Nicola, C.; Manuele, B.; Davide, C.; Loreto, F.; Zappettini, A. Real-Time Monitoring of Arundo Donax Response to Saline Stress through the Application of in Vivo Sensing Technology. *Sci. Rep.* **2021**, *11*, 18598. [CrossRef]

24. Amato, D.; Montanaro, G.; Vurro, F.; Coppedé, N.; Briglia, N.; Petrozza, A.; Janni, M.; Zappettini, A.; Cellini, F.; Nuzzo, V. Towards In Vivo Monitoring of Ions Accumulation in Trees: Response of an in Planta Organic Electrochemical Transistor Based Sensor to Water Flux Density, Light and Vapor Pressure Deficit Variation. *Appl. Sci.* **2021**, *11*, 4729. [CrossRef]
25. Gentile, F.; Vurro, F.; Janni, M.; Manfredi, R.; Cellini, F.; Petrozza, A.; Zappettini, A.; Coppedè, N. A Biomimetic, Biocompatible OECT Sensor for the Real-Time Measurement of Concentration and Saturation of Ions in Plant Sap. *Adv. Electron. Mater.* **2022**, *8*, 2200092. [CrossRef]
26. Vurro, F.; Janni, M.; Coppedè, N.; Gentile, F.; Manfredi, R.; Bettelli, M.; Zappettini, A. Development of an In Vivo Sensor to Monitor the Effects of Vapour Pressure Deficit (VPD) Changes to Improve Water Productivity in Agriculture. *Sensors* **2019**, *19*, 4667. [CrossRef]
27. Manfredi, R.; Vurro, F.; Janni, M.; Bettelli, M.; Gentile, F.; Zappettini, A.; Coppedè, N. Long-Term Stability in Electronic Properties of Textile Organic Electrochemical Transistors for Integrated Applications. *Materials* **2023**, *16*, 1861. [CrossRef]
28. Coppedè, N.; Villani, M.; Gentile, F. Diffusion Driven Selectivity in Organic Electrochemical Transistors. *Sci. Rep.* **2014**, *4*, 4297. [CrossRef]
29. Bernards, D.A.; Malliaras, G.G. Steady-State and Transient Behavior of Organic Electrochemical Transistors. *Adv. Funct. Mater.* **2007**, *17*, 3538–3544. [CrossRef]
30. Gentile, F.; Vurro, F.; Picelli, F.; Bettelli, M.; Zappettini, A.; Coppedè, N. A Mathematical Model of OECTs with Variable Internal Geometry. *Sens. Actuators Phys.* **2020**, *304*, 111894. [CrossRef]
31. Finco, A.; Bentivoglio, D.; Chiaraluce, G.; Alberi, M.; Chiarelli, E.; Maino, A.; Mantovani, F.; Montuschi, M.; Raptis, K.G.C.; Semenza, F.; et al. Combining Precision Viticulture Technologies and Economic Indices to Sustainable Water Use Management. *Water* **2022**, *14*, 1493. [CrossRef]
32. Montanaro, G.; Dichio, B.; Xiloyannis, C.; Lang, A. Fruit Transpiration in Kiwifruit: Environmental Drivers and Predictive Model. *AoB Plants* **2012**, *2012*, pls036. [CrossRef]
33. Rossi, F.; Manfrini, L.; Venturi, M.; Corelli Grappadelli, L.; Morandi, B. Fruit Transpiration Drives Interspecific Variability in Fruit Growth Strategies. *Hortic. Res.* **2022**, *9*, uhac036. [CrossRef]
34. Oliveira, C.P.M.D.; Simões, W.L.; Silva, J.A.B.D.; Faria, G.A.; Lopes, P.R.C.; Amorim, M.D.N. Physiological and Biochemical Responses of Apple Trees to Irrigation Water Depth in a Semiarid Region of Brazil. *Ciênc. E Agrotecnol.* **2020**, *44*, e015620. [CrossRef]
35. Tombesi, S.; Nardini, A.; Frioni, T.; Soccolini, M.; Zadra, C.; Farinelli, D.; Poni, S.; Palliotti, A. Stomatal Closure Is Induced by Hydraulic Signals and Maintained by ABA in Drought-Stressed Grapevine. *Sci. Rep.* **2015**, *5*, 12449. [CrossRef]
36. Danzi, D.; Briglia, N.; Petrozza, A.; Summerer, S.; Povero, G.; Stivaletta, A.; Cellini, F.; Pignone, D.; De Paola, D.; Janni, M. Can High Throughput Phenotyping Help Food Security in the Mediterranean Area? *Front. Plant Sci.* **2019**, *10*, 15. [CrossRef]
37. Liu, H.; Song, S.; Zhang, H.; Li, Y.; Niu, L.; Zhang, J.; Wang, W. Signaling Transduction of ABA, ROS, and Ca2+ in Plant Stomatal Closure in Response to Drought. *Int. J. Mol. Sci.* **2022**, *23*, 14824. [CrossRef]
38. Boini, A.; Cavallina, L.; Perulli, G.; Bresilla, K.; Bortolotti, G.; Morandi, B.; Corelli Grappadelli, L.; Manfrini, L. Actinidia Chinensis: Physiological and Productive Performance under Water Stress Condition. *Acta Hortic.* **2022**, 43–50. [CrossRef]
39. Dayer, S.; Herrera, J.C.; Dai, Z.; Burlett, R.; Lamarque, L.J.; Delzon, S.; Bortolami, G.; Cochard, H.; Gambetta, G.A. Nighttime Transpiration Represents a Negligible Part of Water Loss and Does Not Increase the Risk of Water Stress in Grapevine. *Plant Cell Environ.* **2021**, *44*, 387–398. [CrossRef]
40. Morandi, B.; Manfrini, L.; Losciale, P.; Zibordi, M.; Corelli Grappadelli, L. Changes in Vascular and Transpiration Flows Affect the Seasonal and Daily Growth of Kiwifruit (*Actinidia deliciosa*) Berry. *Ann. Bot.* **2010**, *105*, 913–923. [CrossRef]
41. Morandi, B.; Losciale, P.; Manfrini, L.; Zibordi, M.; Anconelli, S.; Pierpaoli, E.; Corelli Grappadelli, L. Leaf Gas Exchanges and Water Relations Affect the Daily Patterns of Fruit Growth and Vascular Flows in Abbé Fétel Pear (*Pyrus communis* L.) Trees. *Sci. Hortic.* **2014**, *178*, 106–113. [CrossRef]
42. Ortega-Farias, S.; Meza, S.E.; López-Olivari, R.; Araya-Alman, M.; Carrasco-Benavides, M. Effects of Four Irrigation Regimes on Yield, Fruit Quality, Plant Water Status, and Water Productivity in a Furrow-Irrigated Red Raspberry Orchard. *Agric. Water Manag.* **2022**, *273*, 107885. [CrossRef]
43. Jones, H.G. Monitoring Plant and Soil Water Status: Established and Novel Methods Revisited and Their Relevance to Studies of Drought Tolerance. *J. Exp. Bot.* **2007**, *58*, 119–130. [CrossRef]
44. Canaj, K.; Parente, A.; D'Imperio, M.; Boari, F.; Buono, V.; Toriello, M.; Mehmeti, A.; Montesano, F.F. Can Precise Irrigation Support the Sustainability of Protected Cultivation? A Life-Cycle Assessment and Life-Cycle Cost Analysis. *Water* **2022**, *14*, 6. [CrossRef]
45. Buono, V.; Mastroleo, M.; Lucchi, C.; D'Amato, G.; Manfrini, L.; Morandi, B. Field-Testing of a Decision Support System (DSS) to Optimize Irrigation Management of Kiwifruit in Italy: A Comparison with Current Farm Management. *Acta Hortic.* **2022**, 355–362. [CrossRef]