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Electrical signalling in tomato — *Oidium neolycopersici* pathosystem for detection of powdery mildew

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ABSTRACT

Plants are subjected to a plethora of biotic stresses caused by various pathogens; among them, fungal pathogens represent the most destructive ones. In order to preserve the health status of plants, especially under the influence of climate change, the need to develop new sustainable, inexpensive, in-field and non-destructive diagnostic methods for plant pathogens is of great importance. In this direction, spectroscopic and molecular methods have made progress, while others, such as electrical diagnostic methods are still in the early stages of development. In this work, electrical signals in tomato plants infected with the fungal pathogen *Oidium neolycopersici*, the causative agent of powdery mildew, were measured. Differences in electrical responses were observed between healthy and infected plants during the entire monitoring period, and infected plants showed overall lower values of the electrical potential in comparison with healthy plants. Measurement of electrical potential allowed the successful differentiation between infected and healthy plants before the onset of symptoms (3.2 days in advance). A significant difference in electrical signals was obtained not only between infected and healthy plants, but also concerning the growing substrate: A stronger electrical potential was measured in plants grown in the peat-based substrate compared to those cultivated in the water substrate allowing a 97.5% discrimination. Based on the results of this study, measurements of electrical signals may become the basis for an alternative non-destructive diagnosis of tomato powdery mildew and other plant diseases. With the possibility of directly applying the technique in the field followed by remote monitoring of electrical signals, it may become useful for supporting timely disease control.

1. Introduction

Given their sedentary nature, plants cannot escape adverse environmental factors, either biotic (pathogens and pests) and/or abiotic (drought, extreme temperatures, hail, nutrient deficiency), or their combination. Plants respond to these environmental stressors through various mechanisms, including signalling. Locally generated signals in the plant need to be propagated throughout the plant over long distances either by low-speed chemical signals or high-speed electrical and hydraulic signals (Mudrilov et al., 2021; Wang et al., 2021). Various studies have demonstrated that electrical signalling plays an important role in plant systemic communication. Electrical signals may propagate in plants with speeds in the range of $0.001\,m\,s^{-1}$ – $30\,m\,s^{-1}$ upon mechanical stress or ionophore action reaching the velocity of those in animal nerves (Huber and Bauerle, 2016; Volkov et al., 2000).

Beginning in the 1870s, studying the response of the carnivorous plant *Venus flytrap* to mechanical stimulation Charles Darwin observed that the action potential changed importantly during the plant's contact with prey and successively during the closure of the plant's capture organ (Burdon-Sanderson, 1873; Burdon-Sanderson and Page, 1877; Darwin, 1875). In 1926, the Indian scientist Bose demonstrated that plants transmit electric signals with mechanisms similar to nerve cells in animals (Bose, 1926). The first plant cell for which single channels were recordable was the guard cell thanks to the invention of the patch-clamp technique (Neher and Sakmann, 2003; Hedrich et al., 1987). Subsequently, measurements of the plasma membrane potential containing different ion channels, together with the exploration of the molecular mechanisms regulating these channels, allowed single-channel studies of electrical signalling in plants (Hedrich, 2012).

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Four types of electrical signals are found in plants: action potentials (APs), slow wave or variation potentials (SWPs), wound potentials (WPs), and system potentials (SPs). APs and SWPs are elicited by both biotic and abiotic stimuli, and due to the orchestrated mechanisms of various ion channels, they propagate rapidly (APs) or slowly (SWPs) in vascular bundles, while WPs and SPs are mainly induced upon wounding (Hedrich, 2012; Huber and Bauerle, 2016). The 'ome' era, including names for large sets of biological units such as *genome* for genes and *proteome* for proteins, prompted the proposal of the term *electrome*. This term refers to the totality of electrically charged ion currents in any living entity, from the cell to the whole-organism level, as well as the electric fields formed by these charges (De Loof, 2016; Souza et al., 2017). '*Plant electrome*' studies indicate that environmental biotic and abiotic stimuli alter various features of electro-temporal dynamics. In animals, especially in humans, the temporal dynamics of electrical signals measured by electroencephalography (EEG) permits the evaluation of the health status of patients. Interest in the study of plant electrical activity has only recently increased and it seems that it may become one of the methods for monitoring the health status of plants. There are studies of the electrical activity of plants over both short times periods (seconds to hours) and longer ones (days), with some offering real-time monitoring.

Electrophysiology is increasingly used to study plant responses to environmental stimuli mainly related to abiotic stressors such as cold, mechanical injury, and water stress (reviewed in Huber and Bauerle (2016), Mudrilov et al. (2023), Tran et al. (2019)) and biotic pest injury (reviewed in Pachú et al. (2023), Sukhov et al. (2024)). On the other hand, the development of methods based on the monitoring of electrical signals in plants affected by pathogens is still in its early stages. Only a limited number of scientific publications have investigated electrical signals in plants under biotic stress caused by pathogens. These studies are consistently based on short-term observation periods (seconds to hours) and focus on measuring of electrical potentials typically within medium frequency ranges, as for the detection of alfalfa mosaic virus in tobacco (Ghasemi et al., 2024), *Blumeria graminis* and *Bipolaris sorokiniana* in barley (Zaiosc Simmi et al., 2023), and *Oidium neolycopersici* (*On*), the causal agent of powdery mildew, in tomato (Simmi et al., 2020). To our knowledge, there is no available long-term monitoring of electrical potential in plants during the course of infection.

Powdery mildew is one of the most important tomato diseases, which is not easy to monitor due to subtle symptoms and rapid spread (Jones et al., 2001; Lebeda et al., 2015). Additionally, its control is difficult as it can be spread by wind over very long distances (several kilometers) and can invade greenhouses very easily transported by personnel and machinery (Panno et al., 2021). When present, it must be controlled with multiple fungicide treatments throughout the tomato's life cycle; otherwise, it can lead to a significant reduction in yield and product quality (Jones et al., 2001).

Due to this, great importance has recently been dedicated to developing innovative diagnostic methods that can detect disease in the early stages of development, before the appearance of symptoms (Buja et al., 2021). In addition, priority is given to techniques that are economical, sustainable and can quickly lead to results even in remote. Along these lines, few non-destructive and non-contact techniques have been developed, particularly for the detection of tomato powdery mildew, including thermal/light imaging, RGB imaging, machine and deep learning systems (Raza et al., 2015; Wspanialy and Moussa, 2016; Osokin et al., 2024; Liu et al., 2024a,b; Zhang et al., 2025). Notably, only one study has focused on electrical signal measurements (Simmi et al., 2020). All of these techniques have a common environmentally friendly feature in their non-destructive action. Differences of electrical signalling over chemical and spectroscopic techniques consist in nature of measurement, real-time monitoring, and cost of analyses. Moreover, while all imaging methods are based on the evaluation of symptoms from the plant surface, electrical methods can detect changes in the ionic balance inside the plant, possibly before symptoms manifest. Thus, electrical signals provide a solution in between chemical and non-contact analyses since they provide, at the same time, analysis at microscale level and simplicity similar to imaging methods.

Due to these advantages and the lack of electrical measurement studies in plant disease diagnostics the objective of this study was to measure the electrophysiological status of tomato infected with powdery mildew, one of the most economically important tomato diseases in Italy and worldwide during the long-term course of infection (15 days), and to compare it with non-inoculated control plants. Another objective was to evaluate whether the electrical potential was conditioned by the different type of substrate, water and peat, in which tomato plants were grown. Finally a correlation model connecting the electrical potential in plants to the disease symptoms is presented (Cornelissen et al., 2006; Masoero and Giovannetti, 2015; Giorgio and Alberto, 2018).

2. Materials and methods

2.1. Fungal and plant maintenance

The MB1 isolate of the phytopathogenic fungus *On* D'Errico et al. (2023) was maintained on tomato cv. Marmande in a greenhouse at 23 °C (day) and 19 °C (night) and 70% relative humidity. Tomato seeds were sown in small plug trays, and after 2 weeks individual plants were transplanted in a 1 L pot filled with a substrate composed of peat-based mixture (peat/coconut granules/volcanic pumice; 6:2:1 by volume) containing a NPK + microelements slow release fertilizer (Vigorplant, Fombio (LO), Italy) and a 1 L flask filled with unsupplemented tap water (6 plants per each growing substrate).

2.2. Inoculation with On and disease index evaluation

One month after sowing, three out of six plants were artificially inoculated by *On* as described by D'Errico et al. (2023). In detail, two primary leaflets of three randomly selected leaves were sprayed with 10 µL of conidial suspension (5×10^{-4} spores/mL) made by detaching conidial spores from heavily infected leaves in the sporulation stage. Following inoculation, the plants were cultivated for an additional 22 days and monitored for powdery mildew disease. The three remaining plants were used as non-inoculated (healthy) controls. The experiment was repeated twice and the results presented were mediated between the two repetitions.

The *On* symptoms were evaluated by visual inspection at 8, 15, and 22 days post-inoculation (dpi) assessing the disease index (DI) based on the estimated percentage of the affected leaf area. . The DI was evaluated at the same time points observing the same leaflets and was observed till 22 dpi to ensure full development of symptoms. The measured DI was then linearly regressed on the dpi to obtain daily a disease rate (DR) expressed as % d^{-1} for each plant.

To confirm the infection on inoculated plants by *On*, the ITS sequence was amplified through PCR using ITS4 and ITS5 primers (White et al., 1990) on DNA extracted from symptomatic leaves of tomato plants. The PCR amplicons (595–596 bp) were sequenced by the Sanger method (BMR Genomics, Padova, Italy) and identified as *On* (Supplementary File S1).

2.3. Electrical measurements setup in stems

The choice of the material for electrodes is essential for reliable results: different metals as pure copper and 750 ‰ Gold showed prone to contamination of the surface and oxidation in experiments lasting several days (data not shown), then pure gold was finally adopted to overcome electrode degradation. Thus, for detection of electrical signals in the plants, pure gold custom-made electrodes with 0.1 mm diameter and 10 mm length were inserted into plant stems down to a depth of 3 mm, in order to reach the conducting bundles. To acclimate

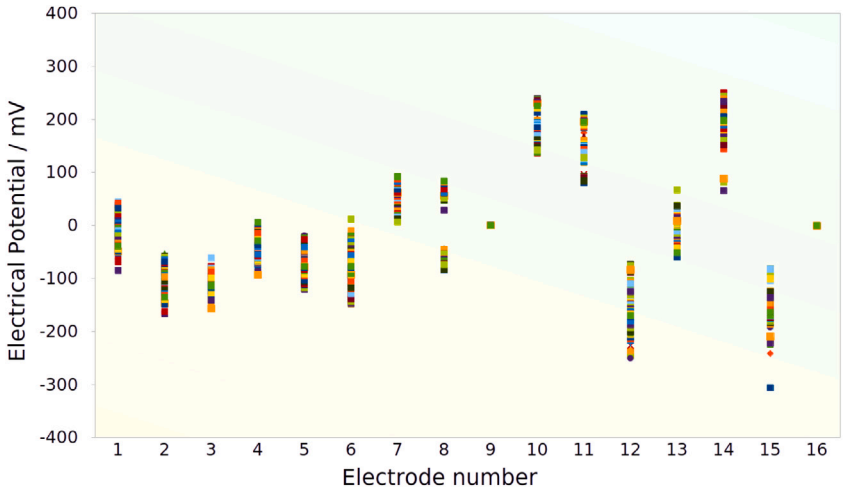

Fig. 1. Measurements of voltages along the stem of a non-inoculated tomato plant showing dependence of values from distance to ground level. In abscissa the 1 cm spaced electrodes are enumerated from the basal to apical part of the stem. The coloured points above each abscissa value, corresponding to a single measurement of voltage, show the spread of data.

(Volkov et al., 2000), the electrodes were placed one day before to the electrical signal recording. Cables with 4 wires, soldered to 4 separate electrodes, provided the electrical connection to the voltmeter (Agilent 34970 A) for measuring the electromotive force on two channels, each with independent grounding. In the measurements, the voltmeter is set to "auto" range to provide full adaptability for changes in the potentials, and the integration time set at 200 Power-Line-Cycles allows to reduce noise without the need of a Faraday cage (Tran et al., 2019), providing sensitivity at mV level. To further minimize interference effects in the measurements, the internal prefilter of the voltmeter was set to "low" to operate as low-pass with 3 Hz cutoff frequency. This allowed us to observe all frequencies relevant for AP and SWPs, excluding electrical disturbances and unwanted effects from WP and SP signals.

Overall, the setup used 15 cables (30 electrically fully independent bipolar lines) that could be automatically switched to separately monitor the line voltages. Three electrodes per plant were distributed throughout the stem, to detect a possible dependency of voltages *vs.* distance from top. Three electrodes were also placed in each growing substrate (peat and water) without the plants as reference control electrodes. The last two lines (cable 15) were not soldered to electrodes but connected to sensors to monitor temperature and illumination during the experiment.

Preliminary experiments showed that the voltage increased with distance from ground level, though without a clear linear relationship with height (Fig. 1). To minimize all effects related to the position of the electrode, the mean or aggregated data from three (positive) electrodes, distributed at 2–2.5 cm distance over the stem, were used in successive experiments.The ground (negative) electrodes were inserted into the basal part of the plant stem (water substrate) or directly into the peat (peat substrate). This arrangement was due to the fact that leaving the ground electrodes freely in the water produced a lot of noise preventing proper measurement of electrical signals, which was not the case for the peat (solid) substrate, which allowed a fixed position of the electrodes. The same electrode placement was performed in non-inoculated control plants in both growing media.

Voltages were acquired periodically, scanning repeatedly in sequence all lines in use, with acquisitions separated from each other by approximately 200 s, and were recorded for 15 dpi by means of a dedicated custom Python program run by a Raspberry Pi board. Measurement data are saved into a file in *comma-separated-values* format for post-processing with various software tools, and stored into a USB key for easy data transfer from the working location. The apparatus is very compact and can operate collecting data autonomously, without the intervention of an operator for a long time, requiring only a supply with moderate power requirements (Fig. 2). Data from electrical measurements were recorded on PC through USB or via direct wifi connection to a smart phone.

2.4. Measuring foliar pH in petioles

The in-vivo raw pH measurements were conducted using a BORMAC "XS pH 70" pH meter (www.giorgiobormac.com), range 0 pH – 14 pH, provided with a combined plastic-glass electrode Hamilton Peek DoubleporeF, / Knick, dimensions (L * W) 35 mm * 6 mm, terminating with a very small and sensible tip sensor. The insertion of the tip in the tomato petiole was facilitated by a wood screw and pH measurements were done at the end of electrical measurement tests.

2.5. Fast fourier transform analysis

To analyse the periodicity and harmonic content of the measured signals, Fast Fourier Transform (FFT) analysis was performed using the data analysis software program Origin by OriginLab, Northampton USA. The FFT analysis was performed on a data portion; specifically, from the entire database of the available time series representing the electric signals acquired during 2 weeks of data collection, the cleanest ones were isolated and processed. These signals were then averaged for healthy and inoculated plants per each substrate (water and peat). The elaboration of selected data improved the quality of the analysis and reduced noise induced by unwanted fluctuations of the signals due to unpredictable experimental conditions. The acquisition frequency (≈1 value each 226 s) and the spectral resolution (≈ 0.09 days^{-1}) of the temporal series were sufficient to achieve good reliability of the results obtained in a way compatible with the life cycle of the plants.

2.6. Statistical analyses

2.6.1. ANOVA model

One-way ANOVA was used for analysis of the disease rate, whereas the four-way ANOVA was implemented when four variables were considered; Replicates (R), Substrate (S), Healthy status (H) and Photoperiod (P). The data were subdivided according to the Photoperiod as Day (1-D; No=1452) or Night (2-N; No=1452). The D and *N* line averages, as well the line standard deviations (No=197472) were examined with four fixed effects(weight 1452) , namely R (1,2), S (1-Peat, 2-Water), H(1-Healthy, 2-Infected), and P with their two-level interactions using the XLSTAT software (Lumivero, Denver, CO, USA).

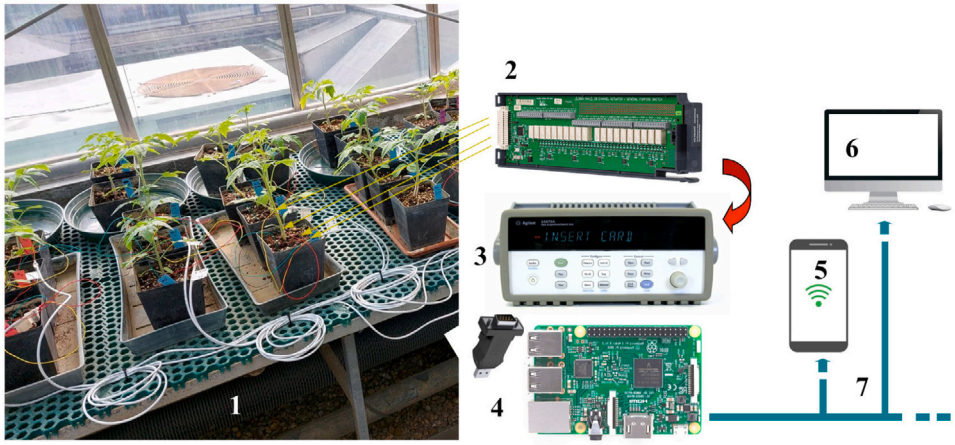

Fig. 2. Electrical measurement setup. (1) tomato plants under test, (2) 20-channel scanner, (3) 6 1/2 digit multimeter, (4) Raspberry P3 controller, (5–6) control and acquisition devices, (7) LAN/wireless connection lines,.

2.6.2. Discriminant analyses

In order to assess the sensitivity (true negative) and the specificity (true positive) for the healthy status, the data (disregarding the replicates and the photoperiod) were partitioned according to the substrate. Thus, the 2904 electric points, half from daylight and half from night, available for each plant pertaining to the 44 cases were submitted to a binary Partial Least Squares Discriminant (PLS-D) analysis separately according to the photoperiod, using the PLS procedure of the chemometric software WinISI-III (Infrasoft International, Port Matilda, PA, USA) coupled to a discriminant analysis with cross-validation provided from the XLSTAT software. To quantify the minimum period of observation that requires significant discrimination, ignoring the photoperiod, the whole interval of measurements (2904 points) was subdivided in eight steps (ca.1.9 days), and for each step two PLS-D analyses were performed separately in the peat and water substrates. PLS-D was adopted due to its specific capability to handle data, particularly in the context of high-dimensional and complex datasets included in this study, with many samples acquired over the long observation periods. Additionally, some correlation in our results coming from the common growing environment of plant under observation was suspected. PLS-D is frequently used in plant science for genomics, metabolomics, or spectroscopic datasets, owing to its capability of handling correlation and multicollinearity. PLS-D integrates information from multiple variables to improve classification and prediction accuracy and reduces the data into latent variables, which are combinations of the original variables, using these for classification or prediction. Finally, PLS-D fitted well to our need to classify samples into groups (in our case healthy vs. diseased plants and peat vs. water substrates) without tight assumptions (like ANOVA) about the data distribution, while being robust to non-normal distributions, which are common in plant science data.

Ultimately, confusion matrix was employed to assess how well the electrical signals can correctly identify healthy and diseased plants and, in general, the accuracy and reliability of our method. In parallel, a fitting of the DR data on the electrical traces elaborated as raw or after math-1th derivation was performed using the Modified PLS regression procedure.

3. Results

3.1. Disease index

Specific powdery mildew symptoms were observed 5 dpi with few initial fungal lesions on leaves of inoculated plants grown in both substrates, water and peat. The mature leaves showed more severe powdery mildew symptoms than the young leaves of the infected plants that progressively expanded with the development of the infection (Fig. 3). Thus, powdery mildew spots randomly observed on leaves at 8 dpi became more evident at 15 dpi, when chlorosis and necrosis were also observed on the apical part of the leaves. At 22 dpi, necrosis was observed in most leaves during plant growth. The evaluation of DI based on percentage of the affected leaf area resulted in the development of powdery mildew disease in both substrates (Supplementary Figure S1) allowing carrying out the electrical measurement experiment in both growing conditions.On average, 37.9% of infected tomato plants grown in water showed necrosis during the 1–22 dpi interval, whereas symptoms were less pronounced in plants grown in peat (29.8%, −21%). When the disease rate was considered (Table 1) the daily increase of the symptoms were 2.7% d^{-1} and 3.3% d^{-1} in peat and water substrate, respectively.

Table 1
One-way ANOVA of the disease rate. Pr > F(Model) < 0.0001; a>b>c>d P < 0.05.

Category	LS means d^{-1}	Standard error	
Healthy	0.000	0.075	c
Infected-Peat	2.726	0.097	b
Infected-Water	3.328	0.097	a

3.2. FFT analyses

FFT analyses of all the available series revealed the presence of two clear peaks in both healthy and infected plants allowing the periodicity of measured electrical signals. Thus, two peaks were observed in the two distinct time periods of 12 and 24 h in both growing substrates (Fig. 4); where the second peak is characterized by an intensity double that of the first one.

3.3. Electrical monitoring of infected and control plants grown in water and peat

The electrical activity of tomato - *On* pathosystem, in both water and peat, was observed during 15 days of the infection period (Fig. 5).

The overall trend showed a lower electrical potential in infected plants than in control plants (Fig. 6), but with a marked difference in the intensity: The signals in water-grown plants were always much weaker than those in peat-grown plants. In all cases, the values of electrical potential of control as well as infected plants in water were so small to be considered indistinguishable from system noise, thus substantially negligible.

These observations were confirmed by ANOVA analysis, which showed a significant difference in relation with the infection status of

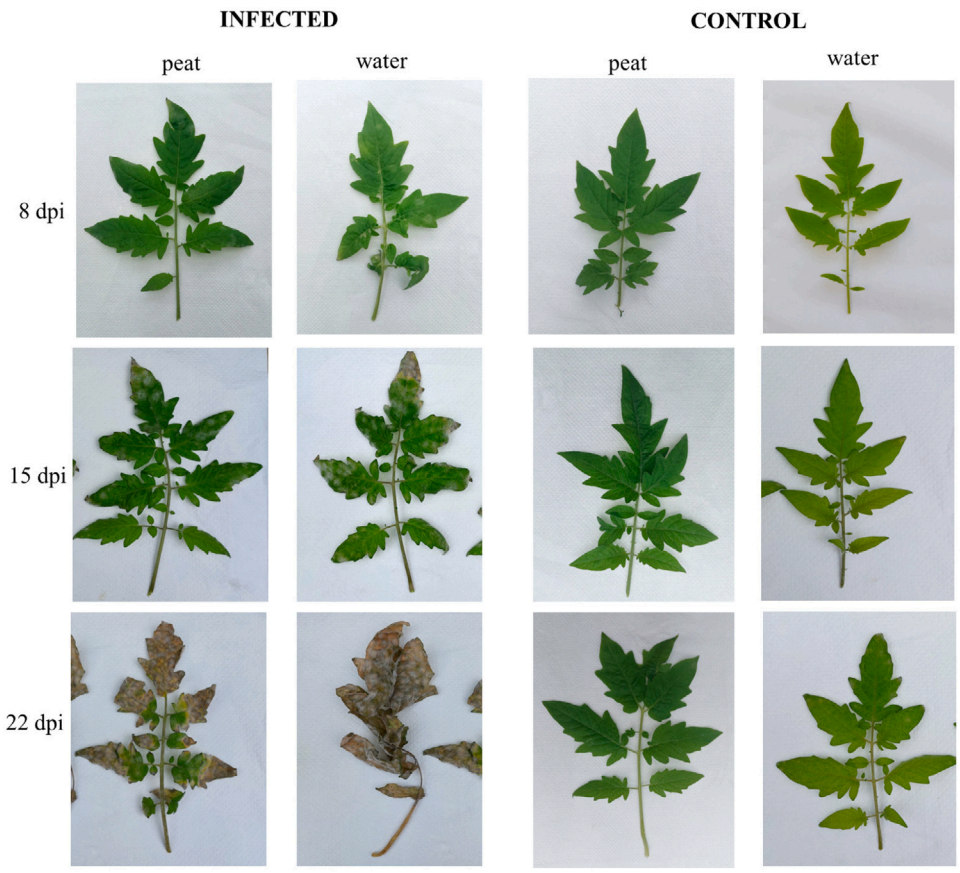

Fig. 3. Powdery mildew symptoms on tomato plants artificially inoculated with *Oidium neolycopersici* at 8, 15, and 22 dpi grown in water and peat-based substrates. Non-inoculated tomato plants were used as controls.

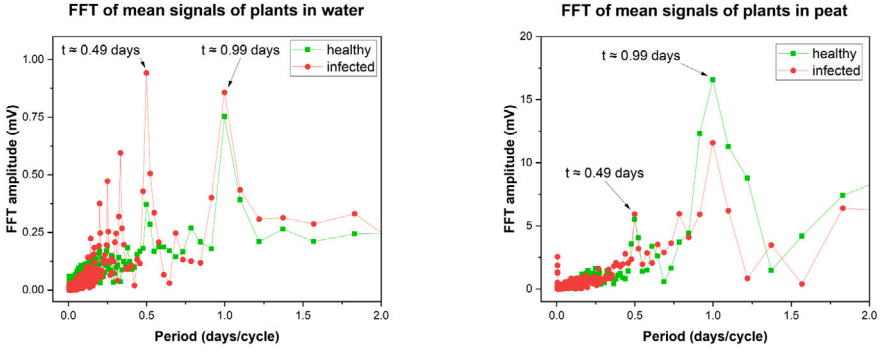

Fig. 4. FFT spectrum of averaged selected data from measured electrical signals in healthy non-inoculated and infected plants, grown in peat and water.

Table 2
Four-way ANOVA summary for trial, infection, substrate, and photoperiod.

Source	DF	Mean		Standard deviation	
		F	Pr > F	F	Pr > F
T-Trial (1,2)	1	0.58	0.448	31.54	<0.0001
I-Infection (1-Uninfected,2-Infected)	**1**	**12.44**	**0.001**	**14.74**	**0.000**
S-Substrate (1-Peat,2-Water)	**1**	**194.95**	**<0.0001**	**162.90**	**<0.0001**
P-Photoperiod (1-Day,2-Night)	1	0.16	0.695	0.34	0.564
Interaction T * S	1	0.08	0.774	3.69	0.058
Interaction T * I	1	2.72	0.103	0.91	0.344
Interaction T * P	1	0.00	0.985	0.03	0.855
Interaction S * I	**1**	**20.60**	**<0.0001**	**0.30**	**0.584**
Interaction S * P	1	0.06	0.804	0.23	0.636
Interaction I * P	1	0.00	0.969	0.38	0.541

the plant during both experiments (Table 2). Moreover, significant differences were observed in relation with the condition of substrate and the interaction between substrate and infection. However, when the growth period (day and night) was taken into account, no significant differences were observed (Table 2).

When the symptoms and electrical signals were compared, the fitting of the DR from the electric traces (Table 3) was significant 2 days from infection onwards in peat and from 6th day in water (after math derivation). Statistical analyses of least squares means, confirming the overall trend, showed that within the substrate condition, peat had a significant signal, while potentials in water substrate were not significantly different (Suppl Table S1).

Likewise, when the data were averaged, different electrical responses were obtained between infected plants (−155 mV) and healthy plants (−278 mV), as well as between two substrates (Fig. 7). Similar

Fig. 5. Measurements of electric signals in tomato plants inoculated with *Oidium neolycopersici* at 15 dpi grown in water and peat substrates. (a) Electrode placement in tomato plants for each growing medium: red/yellow generic electrodes and black/green ground electrodes. (b) Lateral and up-ward images of tomato plants during the measurements. Non-inoculated tomato plants were used as controls.

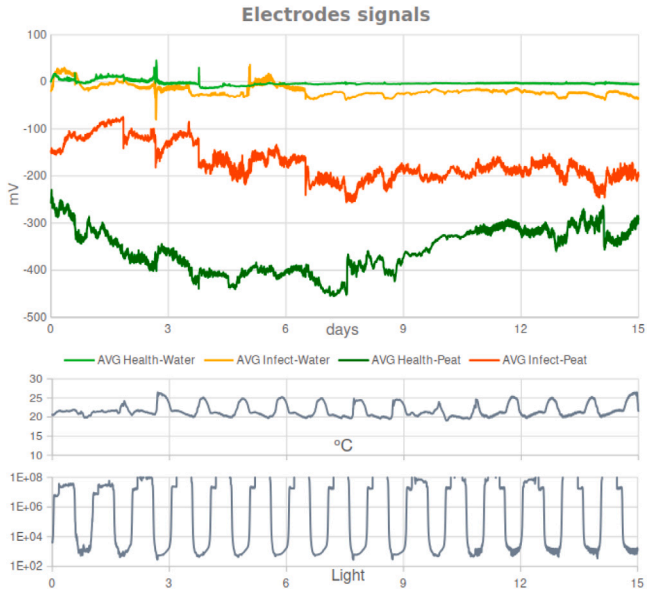

Fig. 6. Electrical measurement of healthy tomato plants and infected with *Oidium neolycopersici*, grown in water and peat during the 15 days course of infection. Healthy (non-inoculated) plants were used as control experiments. The measurement was represented by the average (AVG) electrical response of plants during all experiments concerning different growing substrates (water and peat).

Table 3
Fitting of disease rate from electric traces. RSQ = R^2 calibration Disease Rate; 1-VR = R^2 cross-validate Disease Rate. In bold significant values (P<0.05)

Substrate	Math	Status	RSQ	1-VR	RSQ	1-VR
			2 days		6 days	
Peat	None	Infected	**0.71**	**0.63**	**0.72**	**0.62**
	Derivated	Infected	**0.41**	0.10	**0.50**	0
Water	None	Infected	0.07	0	0.14	0
	Derivated	Infected	**0.61**	0	**0.58**	0

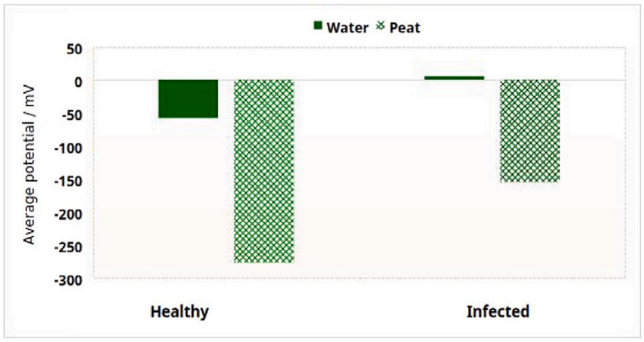

Fig. 7. Electrical measurement of tomato plants infected with *Oidium neolycopersici* grown in water and peat. Non-inoculated tomato plants were used as healthy (control) plants. The measurement was represented as an average electrical response of plants of two experiments with respect to different growing substrates (water and peat).

mean values of electrical potential were obtained healthy and infected plants concerning the night and day periods (Supplementary Figure S2).

Cross-validated discriminant analyses based on classification of groups according to: the health status of tomato plants (infected and healthy); the growth period (day and night); and growing substrate (water and peat) showed a good discrimination of 97.5% between infected and healthy plants in peat (Table 4). In the growing water medium no discrimination was possible (46%), confirming the previous results of overall electrical trend.

Moreover, progressive accuracy analyses of electrical signals permitted satisfactory sensitivity and specificity in the peat substrate (Table 5). In peat, the average sensitivity was 71%, but in three significant

Table 4
Confusion matrix deriving from PLS-Discriminant Analysis applied on categorial data set (1, uninfected; 2, infected) according to the Substrate of cultivation (Peat vs Water) and the Photoperiod (Daylight vs Night) pooling all periods.

		Daylight					Night					Average
Substrate	from \to	1	2	Total	% correct	P	1	2	Total	% correct	P	
Peat	1	8	0	8	100%	0.0047	8	0	8	100%	0.0047	100
	2	1	11	12	92%	0.0036	0	12	12	100%	0.0047	96
	Total	9	11	20	95%		8	12	20	100%		97.5
Water	1	8	4	12	67%a	0.2389	8	4	12	67%a	0.2389	67
	2	9	3	12	25%b	0.0833	9	3	12	25%b	0.0833	25
	Total	17	7	24	46%		17	7	24	46%		46

Table 5
Progressive accuracy based on the electric signal, raw or mathematical first derivative, according the type of substrate, during eight periods, each of about 15 days post infection. In bold P<0.05.

Signal	Substrate	Accuracy	N	Days post-inoculation							
				1.9	3.8	5.6	7.5	9.4	11.3	13.1	15.0
Raw		Sensitivity	12	67%	**83%**	**92%**	67%	**83%**	50%	58%	67%
1st der.		Sensitivity	12	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
Raw	Peat	Specificity	8	75%	**100%**	**100%**	**88%**	75%	**100%**	**88%**	**88%**
1st der.		Specificity	8	63%	75%	**88%**	**88%**	**88%**	**88%**	**88%**	**88%**
Raw		Sensitivity	12	75%	42%	33%	33%	33%	33%	25%	25%
1st der.		Sensitivity	12	50%	67%	58%	58%	42%	42%	42%	58%
Raw	Water	Specificity	12	**83%**	67%	67%	67%	75%	**92%**	**83%**	**83%**
1st der.		Specificity	12	**92%**	**92%**	**92%**	**83%**	**92%**	**92%**	**92%**	**75%**

Table 6
Differentiation of infected and control plants grown in two substrates 1.3 days post inoculation with *Oidium neolycopersisci*.

from \ to	Peat % correct	Water
Sensitivity	100.00%	50.00%
Specificity	63.00%	92.00%
Average	85.00%	70.83%

intervals (2,3,5), it reached up to 92%. Using the derivative of the signals the sensitivity increased up to 100% from the first period. The healthy status is recognized at 94% in 6 intervals out of 8. In water, the sensitivity was not significant with any data sequence, either in raw data or in the 1st derivative. On the other hand, specificity was partially significant in half of the raw data sequences, and totally significant in the 1st derivative.

When the first derivatives of all measurements were considered, the minimum significant period allowing discrimination between healthy and infected plants was 1.9 days, comprising 500 data points. This indicates that successful distinction can be achieved before the onset of symptoms (Table 6).

3.4. pH measurement of leaves

The average pH value of leaves of infected plants grown in peat was 6.10 compared to those of healthy plants whose value was 6.40. A lower average pH value of infected plants compared to healthy plants was also obtained in the water substrate (6.15 vs. 6.38).

4. Discussion

Regarding electrophysiology, as a newly developed technique that is increasingly used to monitor plant responses to environmental stimuli (Sukhov et al., 2024), there is only one publication for the detection of *On* in tomato based on measuring electrical signals during short-term period of powdery mildew infection (96 h) (Simmi et al., 2020).

In this study, it was shown that it is possible to discriminate the electrical response of powdery-mildew infected tomato plants from healthy ones grown in peat. It should be noted that, differently from what reported in Simmi et al. (2020), significant electrical responses are observed not only during the first-day post-inoculation, but confirmed by results throughout the whole 15-day infection period. Specifically, different electrical potentials in infected plants (−155 mV) compared to healthy plants (−278 mV) were obtained when grown in peat and the average electrical signals of healthy and infected plants never intersected. The advantage of this study over spectral imaging remote systems is that the measurement of electrical potential allowed the differentiation between infected and healthy plants before the appearance of visible symptoms of powdery mildew infections; e.g. 1.9 dpi *vs.* 9 and 13 dpi, respectively (Raza et al., 2015; Liu et al., 2024a). Electrical signals have a very high propagation speed in the range of centimeter to meter per second during various abiotic stresses (wounding, ice shock, etc.) (Huber and Bauerle, 2016). Their high speed also applies during biotic stress (Volkov and Haack, 1995), which allowed in the case of this study to diagnose powdery mildew of tomato 3.2 days before symptoms onset.

In the previous three studies of electrical signals in plants infected with the pathogens (Ghasemi et al., 2024; Zaiosc Simmi et al., 2023; Simmi et al., 2020), no healthy control plants have been included; that does not allow their use for data comparison. However, some studies on abiotic stresses, such as water stress, reported a decrease in electrical response in stressed plants that is in line with our study (Tran et al., 2019; Zhou et al., 2022).

Another advantage in this study is a simplification of the measurement procedure without having the necessity to include the Faraday cage, without important impact on the quality of recorded signals. These results are consistent with other studies measuring electrical signals in a plant context without a Faraday cage allowing good quality of signals without limits of the technique application in the standard plant

cultivation environment (Tran et al., 2019; Camps et al., 2020; Zhou et al., 2022). All these studies indicate that the electrical measurements of plant status without a Faraday cage could present a tool for continuous and stable long-term recordings of plant electrical signals. Thus, tomato health status may be monitored over the whole production cycle in tunnel farming by inserting electrodes into the plants and connecting them to a multimeter equipped with rechargeable batteries.

Regarding FFT analysis, the electrical response of the tested plants in both substrates (water and peat) showed periodicity possibly related with the periodic variation of light, known as the photoperiod as already observed in tomato (Xiang et al., 2022).

Furthermore, in this study, electrical signals were compared between infected and healthy tomato plants cultivated in two substrates, peat and tap water, to determine if the electrical potential of the plant was influenced by the substrate. Based on the results obtained, ions present in the peat are necessary for obtaining a significant electrical response of both groups of plants, because a plant growing only in water (with only traces of ions) has a very low electrical potential close to the detection threshold (Jin et al., 2022). With this, the peat is good for the transmission of electrical signals not only between the plants (Volkov et al., 2000), but it also provides ions supporting the electrical currents inside the plant. In our study, the pH of peat was constant (ca. 6.4) and its value allowed the production of good quality of electrical signals as reported by Love et al. (2008).

This study provided electrical measurements in plants that are cheap, and do not require sample collection and extraction as many chemical and spectroscopic techniques (Badawy et al., 2022). It may provide this method more attractive for in-field application and large-scale analyses of powdery mildew and other plant diseses.

Powdery mildew is a parasitic biotrophic pathogen that extracts food and ionic elements through the haustoria (Kwaaitaal et al., 2017). This may also contribute to a decrease in the electrical response of infected plants compared to healthy plants, similar to the response obtained with the fungus *Fusarium oxysporum* in Arabidopsis plants (Kesten et al., 2019). A possible explanation for the reduced electrical potential in infected tomato plants is that after pathogen attack, there is a change in ion flux, such as an increase in the influx of Ca cations into the cytoplasm and the efflux of anions (Cl, NO3) and cations (K) passing through the plasma membrane channels necessary for the fungus metabolism but involved in generating plant electrical signals (Yue et al., 2023; Köster et al., 2022; Mudrilov et al., 2023; Schenk and Bettin, 1990). Given that powdery mildew extracts plant ions through the haustoria necessary for its nutrition and metabolism, a disturbed balance of ions in diseased plants compared to healthy ones necessary for developing plant electrical signals may be a plausible explanation.

In addition, the lower electrical potential may be related to the pH of plant tissue infected with *O. neolycopersici*. In our work, the decrease of pH value in infected plants compared to healthy plants may affect the reduced electrical signal. This is in line with previous studies where it was observed that with a decrease in substrate pH (from alkaline to acidic) the electrical potential of plants also decreases (Love et al., 2008; Lu et al., 2020). In numerous experiences of effective use of complex microbial consortia with arbuscular mycorrhizal fungi, an acidification of the leaf pH has been observed (Masoero and Giovannetti, 2015; Giorgio and Alberto, 2018). Thus the plant pH and H+ (proton) concentration through the changes in plasma membrane potential and activity of ion channels may also contribute in electrical signal profiling of powdery mildew infected tomato plants (Miedema et al., 1992; Lehmann et al., 2021).

In addition to pH and phytopathological measurements, in order to elucidate the mechanism of the difference in electrical potential between infected and healthy plants, future studies will be necessary including variations in ion concentrations (e.g. K^+, Ca^{2+}, Na^+, Cl^-) within plant cells and tissues, gene expression associated to ion channels, and plant hormones (e.g., auxins, abscisic acid and kinetin) and signalling molecules involved in the regulation of ion transport and electrical responses (Karmoker, 1985; Lee and Calvo, 2023; Mudrilov et al., 2021). These additional data will contribute to a better understanding of the electrical potential activity in tomato-powdery mildew pathosystem and to more easily implement its use for early non-destructive diagnosis of tomato powdery mildew.

5. Conclusions

In this study the detection of tomato powdery mildew based on electrical signalling was described which may present an alternative for innovative, non-destructive and remote diagnosis of the disease. Healthy and infected plants were successfully distinguished by measuring the electrical potential before symptoms onset, specifically 3.2 days in advance. Discrimination between infected and healthy plants was observed in the following period as well, with stable results over time, allowing to confirm diagnosis and improve reliability by proper setting the duration of the experiment. A more efficient distinction between infected and healthy plants was obtained when they were grown in peat compared to water, highlighting the importance of the substrate on the plant electrical signal response. Electrical signalling appears to have an important influence on plant-pathogen interactions, in addition to molecular and chemical signalling. In particular, the possibility to observe modifications in the microscopic ionic phenomena inside the plant vascular tissue is promising for early and accurate diagnosis, without the complexity of chemical methods.

For future applications of the technique, the multimeter instrument should be adapted with additional batteries that will enable point-of-care plant pathogen detection during the crop cultivation and direct application in the field by agricultural operators and farmers.The main drawback that will have to be addressed when moving from greenhouse and tunnel crop production to field cultivation is the long-term fixation of electrodes in plants and their resistance to air movement, rain and plant growth.

CRediT authorship contribution statement

Slavica Matić: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giorgio Masoero:** Writing – review & editing, Methodology, Data curation. **Andrea Egidi:** Writing – review & editing, Software, Data curation. **Claudio Francese:** Methodology. **Pier Paolo Capra:** Writing – review & editing, Visualization, Software. **Andrea Sosso:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.compag.2025.110585.

Data availability

Data will be made available on request.

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