Early detection of root-knot nematode (*Meloidogyne incognita*) infection by monitoring root dielectric response non-destructively**

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Abstract. The early recognition of root-knot nematode injury belowground is essential in order to avoid serious crop losses. The measurement efficiency of the root dielectric response for detecting *Meloidogyne incognita* infection non-destructively was tested in potted cucumber and tomato. The electrical capacitance, dissipation factor and electrical conductance of the root, and also the leaf chlorophyll concentration were measured instrumentally three times during plant growth, this was followed by an evaluation of the root galling intensity after harvest. The electrical capacitance and conductance increased significantly shortly after *Meloidogyne* infection, this was likely due to the substantially enhanced surface area and electrolyte permeability of the root membranes during giant cell formation. The dissipation factor and electrical conductance (related to hydraulic conductance) markedly decreased at the late stage of nematode infection, this was due to restricted root growth and solute uptake caused by the intrusion of giant cells into the root vascular tissues. No serious aboveground pest symptoms were visible in the plants studied owing to the low inoculum density. The results demonstrated the potential of dielectric measurement for the early detection of root-knot nematode infection without plant damage, before the appearance of obvious disease symptoms. This diagnostic tool has the potential to contribute to the improved selection of *Meloidogyne*-resistant crop genotypes, as well as more efficient nematode control to mitigate economic losses.

Keywords: chlorophyll, electrical capacitance, electrical conductance, *in situ* root methods, plant-parasitic nematode detection, soil-borne pest

INTRODUCTION

Root-knot nematodes (RKN), *i.e. Meloidogyne* species, are notorious phytopathogens worldwide (Bernard *et al*., 2017). These obligate endoparasites damage the majority of agricultural crops, leading to huge economic losses and putting global food security at risk (Abd-Elgawad and Askary, 2015). RKN infestation also facilitates root invasion by secondary pathogens, such as fungi and bacteria. The entry and feeding process of second stage juveniles (J2) of RKN, is associated with giant cell development and gall formation, it impedes host root growth, and severely damages vascular tissues and thus root uptake function. This results in insufficient water and nutrient supplies, and also hampers photosynthate translocation, which is manifested in aboveground symptoms, such as wilting, chlorosis, foliage loss, stunted growth (even plant death), leading to reduced biomass, crop yield and quality (López-Gómez *et al*., 2015). It is a challenging problem that shoot damage in RKN-infected plants usually appears in the late stage or during high infection levels. In addition, aboveground symptoms are nonspecific, and can barely be differentiated

from injuries caused by nutritional deficiencies or other root pathogens (Sikandar *et al.*, 2020). Visible root galls are a decisive sign of infection, but they remain hidden in the soil, and are thus unobservable without plant removal or destructive root sampling (Lu *et al.*, 2020). A rapid, non-intrusive method with the potential to detect RKN attack in the early stages could help in the effort to recognize the affected plants sooner, and establish an effective pest control strategy for growers.

In situ root methods have been gaining an increasing level of attention in recent times (Liu *et al.*, 2021). A part of these techniques is based on measuring the components of the dielectric (impedance) response generated in the root system by an external energy source (Ehosioke *et al.*, 2020). When a low-frequency (1 kHz) alternating current (AC) is applied to the plant-soil system between the ground and plant electrodes, both the amplitude and the phase of the sine wave signal changes due to the polarization of the cell membranes and the apoplastic and symplastic compartments. Biophysical models consider roots to be analogous to cylindrical capacitors in which the membranes act as a dielectric material by storing electrical charges, whereas the root sap and the soil solution form the conductive inner and outer capacitor plates, respectively (Dalton, 1995). The measured root electrical capacitance (C_R) is directly proportional to the membrane surface area and thus to the root system size, but it is also influenced by the properties of the tissue (*e.g.* water and ion contents, suberization, tissue density), which in turn depend on the plant phenology stage and the environmental conditions (Ellis *et al.*, 2013; Peruzzo *et al.*, 2020). Therefore, the C_R value obtained represents not only the geometrical surface of the root system but also its physiological status linked to water and nutrient uptake activity.

Unlike physical capacitors, roots are leaky (lossy); their dielectric properties are a combination of electrical polarization on the membrane surface and concurrent electrical conduction (electrolyte movement) through the membranes (Prasad and Roy, 2020). The ratio of conductive energy loss (due to free charge carriers) to energy storage (by polarization) is known as the dissipation factor (D_R), which depends on the composition, physicochemical properties, structural integrity and permeability of the root membranes (Li *et al.*, 2017). The electrical conductance of the whole root system (G_R), as measured between the ground and plant electrodes, may be expressed as: $G_R = C_R \times D_R \times \omega$, where ω is the angular frequency. G_R is assumed to be mainly determined by ion movement through low-resistance pathways, *i.e.* xylem and phloem vessels (Aubrecht *et al.*, 2006). Several studies using hydroponics or very wet soil showed substantial current leakage from proximal root segments and an uncertain contribution from the distal roots to the impedance response (Dietrich *et al.*, 2012; Peruzzo *et al.*, 2020). Other studies, however, further supported the validity of the method, by providing evidence that AC could penetrate deep and was distributed throughout the root system

(including the distal parts), especially when the topsoil was relatively dry and therefore less conductive than the roots (Ellis *et al.*, 2013; Mary *et al.*, 2018; Gu *et al.*, 2021). It should be noted that it is only possible to compare dielectric properties when the same plant species is measured under the same substrate conditions (*i.e.* water content) with the same electrode parameters (Středa *et al.*, 2020).

Under standardized conditions, the dielectric (C_R, D_R and G_R) measurements of the intact root-substrate systems can provide relevant information about growth dynamics, as well as the physiological status and stress-related changes in the root system (Cseresnyés *et al.*, 2018, 2019; Ehosioke *et al.*, 2020; Liu *et al.*, 2021). Host roots are known to respond to RKN infection at various scales, this includes modifications to the membrane functions, cell-wall composition and structure, xylem anatomy, fine-root formation, root morphology and root system architecture (Jones and Goto, 2011; Bernard *et al.*, 2017). Therefore, it was surmised that the dielectric approach was suitable for the detection of RKN injury at the early stages, prior to the onset of visible aboveground symptoms. A pot study was undertaken in order to test this hypothesis by infecting the cucumber and tomato plants with *Meloidogyne incognita* (Kofoid and White, 1919). In this way, it was hoped to provide a rapid diagnostic tool to identify nematode-attacked plants without performing destructive root investigations.

MATERIALS AND METHODS

RKN-sensitive cultivars of cucumber (*Cucumis sativus* L., *cv*. Monolit F1) and tomato (*Solanum lycoprsicum* L., *cv*. Moneymaker) were used for the two pot experiments. The seeds were germinated on wet paper towels in Petri dishes at 24°C for 3 days. The plants were grown in 1.45 L rectangular plastic pots (11 × 11 cm in width and length, 12 cm in height) and containing 800 g of a vermiculite (0-2 mm) and quartz sand (0-0.6 mm) mixture in a 2:1 volume ratio, with a pH of 7.86, a cation exchange capacity of 8.87 mmol 100 g^{-1}, <150/699/5 740 mg kg^{-1} of total N/P/K content and 0.28 cm^3 cm^{-3} water content at field capacity. Two seedlings were planted per pot, and thinned to one a week later to obtain a uniform plant population. At the time of sowing, a plastic tube (5 cm in length and 3 mm i.d.) was placed vertically in the substrate to facilitate later nematode treatment. The plants were cultivated in a growth chamber in a randomized design at 26/20°C (light/dark) with a 16-hour photoperiod, ~600 μmol m^{-2} s^{-1} PAR and 50-70% relative humidity. The illumination was supplied by 450-460/660-665 nm seedling light (LED) panels (QS-2021; Q-Cig Microelectronics, Košice, Slovakia). All pots were irrigated weekly with tap water to field capacity using a weight basis. Each plant was fertilized biweekly with 200 mL of 0.1 V/V% Vitaflóra® vegetable nutrient solution, consisting of 3.3% N, 2.3% P, 5.6% K, 0.014% B, 0.007% Cu, 0.02% Fe, 0.01% Mg, 0.01% Mn, 0.001% Mo and 0.007% Zn.

Sixty replicate pots were used per treatment, with the non-inoculated plants serving as the control, and the others being treated with *M. incognita* three or four weeks after sowing, for cucumber and tomato, respectively. Before inoculation, the plastic tube was removed from the substrate in order to leave a hole near the roots, two egg masses of *M. incognita* in 2 mL of water were added by pipette using this hole (the control plants received 2 mL of pure water). The population of *M. incognita* used in this experiment was initially collected from the galled roots of the field-grown cucumber *cv.* Monolit F1, and was identified by examining the perineal pattern of female individuals microscopically. The isolated nematodes were propagated in a potted cucumber; the egg masses were collected after root washing, and then placed in pairs in Eppendorf tubes filled with 2 mL of water.

The root dielectric measurements were taken three times, namely 2, 4 and 6 weeks after inoculation (WAI). The impedance response (modelled by a parallel equivalent circuit) was detected using a U1733C handheld LCR meter (Agilent Co. Ltd., Penang, Malaysia) at a 1 kHz AC frequency and a 1 V terminal voltage. The ground electrode was an acid-free steel rod, 15 cm in length and with a 6 mm i.d., inserted vertically into the growing medium 4 cm from the stem base to a depth of 12 cm. In all cases the plant electrode consisted of a 4 mm wide, 25 μm thick aluminium strip, which was smeared with conductivity gel and clamped, it was bent round the stem, 15 mm above the substrate surface. The C_R and corresponding D_R parameters were recorded using the LCR instrument, from which a G_R ($= C_R \times D_R \times \omega$) value was calculated for each plant. The impedance measurement was always carried out on the day after irrigation. This period allowed for surface drying (to minimize current leakage near the stem-substrate interface) without leading to any considerable variance among the pots in terms of the water status of the bulk substrate. Immediately before the measurements took place, the moisture content of the 0-12 cm layer was checked with a HS2 meter attached to a CS659 TDR probe (Campbell Inc., Logan, UT, USA).

Thereafter, the chlorophyll (Chl) content of the leaves was detected *in situ*. As the Chl quantity is known to be closely related to the nitrogen status of the plant, it is considered to be a reliable indicator of the nutrient deficiencies caused by RKN infestation before the appearance of serious aboveground disease symptoms (López-Gómez *et al.*, 2015). Chl was measured on each fully expanded leaf (numbered with reference to their order of appearance, from the oldest to the youngest) excluding the first ones because of their natural early senescence. A portable MC-100 instrument (Apogee Inc., Logan, UT, USA) was used to record the total Chl concentration (μmol m^{-2}) on the adaxial (upper) leaf surface, avoiding margins and main veins. Three readings were taken per leaf and then averaged.

After each of the three sets of dielectric and Chl measurements were conducted, twenty plants per treatment were selected randomly for destructive sampling. The shoots were cut at the substrate surface, and the roots were gently washed out of the medium with running water over a 0.5 mm mesh sieve to retain the fine roots. The severity of RKN disease was assessed by counting the root galls that had developed on the inoculated plants. The root systems were rated according to Mukhtar *et al.* (2013) with a galling index (GI) on a 0-6 scale, where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-70 galls, 5 = 71-100 and 6 = >100 galls. The roots and shoots were then oven-dried at 70°C to a constant weight in order to determine the root dry mass (RDM ±0.001 g) and shoot dry mass (SDM).

A data analysis was performed using *Statistica* ver. 13 software (StatSoft Inc., Tulsa, OK, USA). The effect of *Meloidogyne* infection on C_R, D_R, G_R, leaf Chl concentration, SDM and RDM was evaluated using an unpaired t-test. Welch's correction was applied when the F-test indicated significantly different variances between study groups. The normality of the data groups was proven using the Kolmogorov-Smirnov test. The statistical significance was assessed at $p < 0.05$ in each case.

RESULTS

The mean C_R increased over time in each treatment group due to root system development. The *Meloidogyne* infection in cucumber roots led to a significant increase in C_R at 2 WAI (7%) and at 4 WAI (6%), but the effect ceased at 6 WAI (Fig. 1a). A relatively greater RKN effect was observed in tomato at 2 WAI (16%) and at 4 WAI (22%), but the difference was again statistically non-significant at the last dielectric measurement date (Fig. 1b). Nematode infection had no significant influence on the D_R value in cucumber at 2 and 4 WAI, whereas it induced a substantial (15%) decrease in D_R at 6 WAI (Fig. 1c). As for tomato, the reduction in D_R caused by RKN was significant even at 4 WAI (5%), and was considerably greater (16%) at the last measurement date as compared to the control (Fig. 1d). The calculated G_R increased over time for the control plants of both species due to the enlargement of the root system, but it decreased slightly for the inoculated cucumber between 4 and 6 WAI. The *Meloidogyne* infestation in the cucumber hosts provoked a significant increase in G_R at 2 and 4 WAI (9% and 7%, respectively), whereas it induced a significant decrease (15%) at 6 WAI (Fig. 1e). Similar results were obtained for tomato: the relative change in G_R caused by the RKN treatment was +19, +16 and −12% at 2, 4 and 6 WAI, respectively (Fig. 1f).

The total Chl concentration detected in the cucumber leaves was not significantly affected by root infection at 2 WAI (Fig. 2a). However, it proved to be slightly (4%), but significantly lower in statistical terms in the oldest measured leaf (*i.e.* leaf 3) at 4 WAI (Fig. 2b), when the
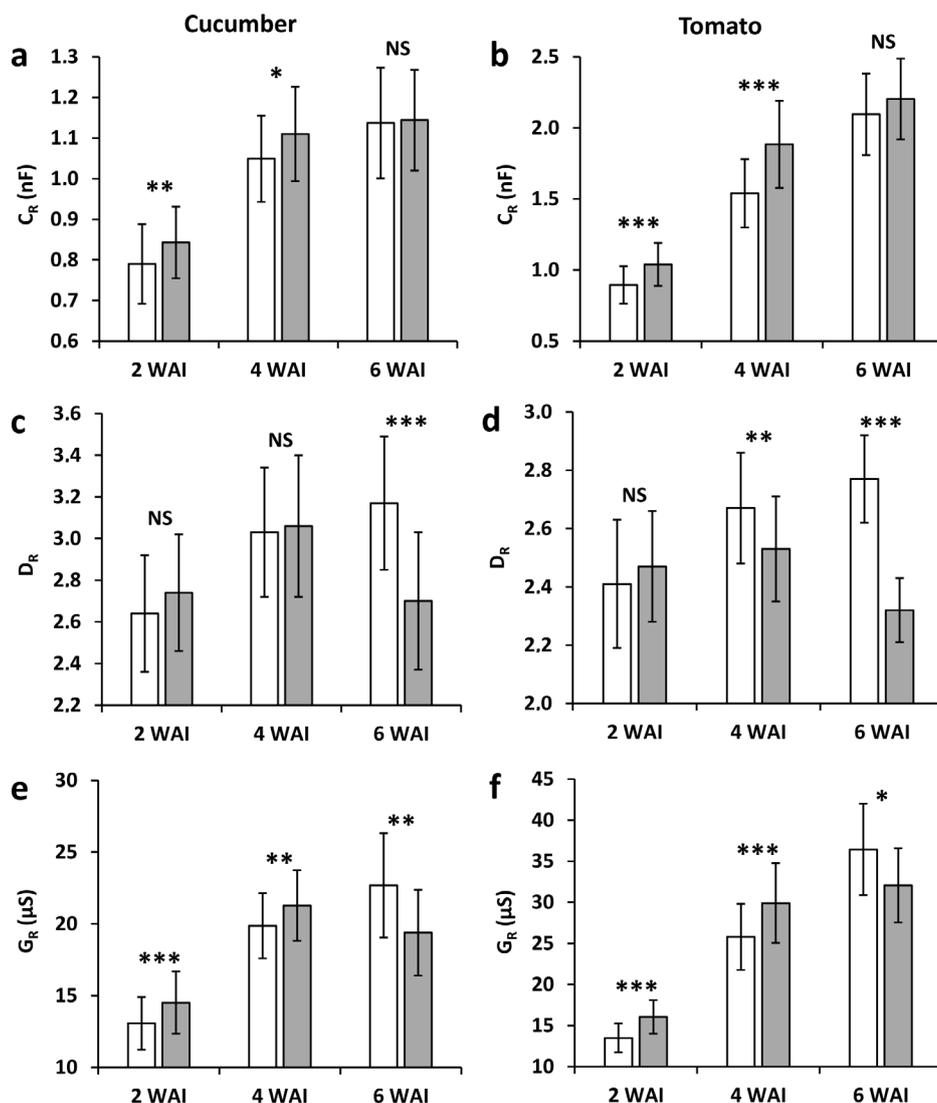
Fig. 1. (a, b) Root electrical capacitance (C_R), (c, d) dissipation factor (D_R) and (e, f) electrical conductance (G_R) for the non-infected control (empty bars) and the *Meloidogyne incognita*-infected (grey bars) cucumber and tomato plants at 2, 4 and 6 weeks after inoculation (WAI). The values are the means ± SDs of n = 60, 40 and 20 for 2, 4 and 6 WAI, respectively. Asterisks represent significant differences between the control and the infected plants (paired *t*-tests). *p < 0.05, **p < 0.01, ***p < 0.001, NS not significant.

aboveground symptoms were not yet visible. According to the last measurement which was conducted at 6 WAI, the Chl concentration in each investigated leaf of the RKN-infected cucumber plants was significantly lower: the relative change was 12% for leaf 3, and 7% for leaves 4 and 5, as compared to the control (Fig. 2c). By this time the oldest leaves were flavescent. In the tomato hosts the *Meloidogyne* infection had no significant influence on leaf Chl concentration at 2 WAI (Fig. 2d), but it resulted in significant 16, 13 and 6% reductions in leaves 2, 3 and 4, respectively, at 4 WAI, as compared to the control (Fig. 2e). An accelerated senescence of the oldest leaves was observed in several plants from the RKN-treated group. The last measurement at 6 WAI showed a significant, 25, 24 and 18% relative decrease in Chl content

for leaves 3, 4 and 5, respectively (Fig. 2f). By this time, leaf 2 had dried up and could not be measured, furthermore, leaves 3 and 4 were obviously chlorotic.

The destructive measurements showed that the effect of *Meloidogyne* infestation on the RDM of cucumber was not significant at any of the measurement dates, although a 22% increase was observed at 6 WAI (Fig. 3a). By contrast, the infected tomato plants exhibited a significantly higher RDM at 2 WAI (42%) and at 4 WAI (35%), whereas the difference was insignificant at 6 WAI (Fig. 3b). There were no significant differences in SDM between the cucumber treatment groups at 2 and 6 WAI, but the *Meloidogyne*-infected plants exhibited a significantly lower SDM value (14%) at 4 WAI (Fig. 3c). In the case of tomato, no significant RKN effect
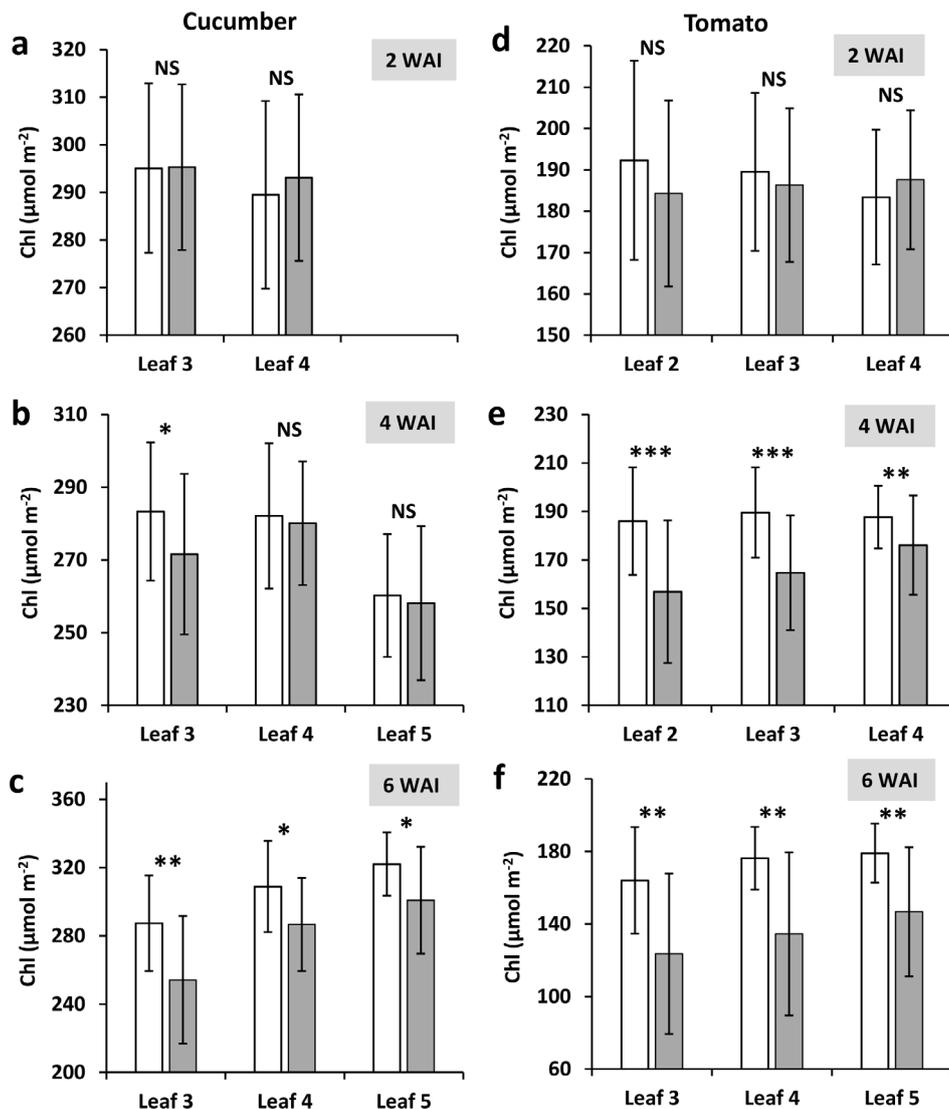
Fig. 2. The chlorophyll (Chl) content in various leaves (identified according to their age) for the non-infected control (empty bars) and *Meloidogyne incognita*-infected (grey bars) cucumber (a-c) and tomato (d-f) plants at 2, 4 and 6 weeks after inoculation (WAI). The values are means ± SDs of n = 60, 40 and 20 at 2, 4 and 6 WAI, respectively. The asterisks represent significant differences between the control and the infected plants (paired *t*-tests). *p < 0.05, **p < 0.01, ***p < 0.001, NS not significant.

was observed for SDM at 2 or 6 WAI (Fig. 3d), however, it is interesting to note that the infected plants had a significantly higher SDM value (41%) than the control at 4 WAI. Root galls were observed on all RKN-treated plants of both species. Initially (at 2 WAI), small discrete galls appeared on the roots, but in the later stages of infestation larger coalescent galls frequently occurred. The GI established for the root systems progressively increased over time and reached up to values of 5.0±1.6 (mean ± SD) for cucumber (Fig. 3e) and 4.7±1.7 for tomato (Fig. 3f) at 6 WAI.

DISCUSSION

The root dielectric response proved to be a sensitive way of detecting the various stages of RKN infection. The significant increase in C_R observed after RKN attack may be attributed to root thickening and giant cell formation with a considerable degree of confidence. Giant cell development is accompanied by extensive cell wall and plasma membrane convolutions opposite the root vascular tissues, it takes place in order to enhance nutrient uptake for the nematode, and results in a substantial, up to 15-20-fold amplification of the polarizable (electrically capacitive) membrane surface area (Jones and Goto, 2011). After some time, this membrane proliferation appears to be counterbalanced by a reduction in the total root length, also, there is a decrease in the proportion of fine roots, and in addition, there is a lower root-hair density caused by RKN (Lu *et al*., 2020), which eliminates the treatment effect on C_R, as detected at 6 WAI for both species.
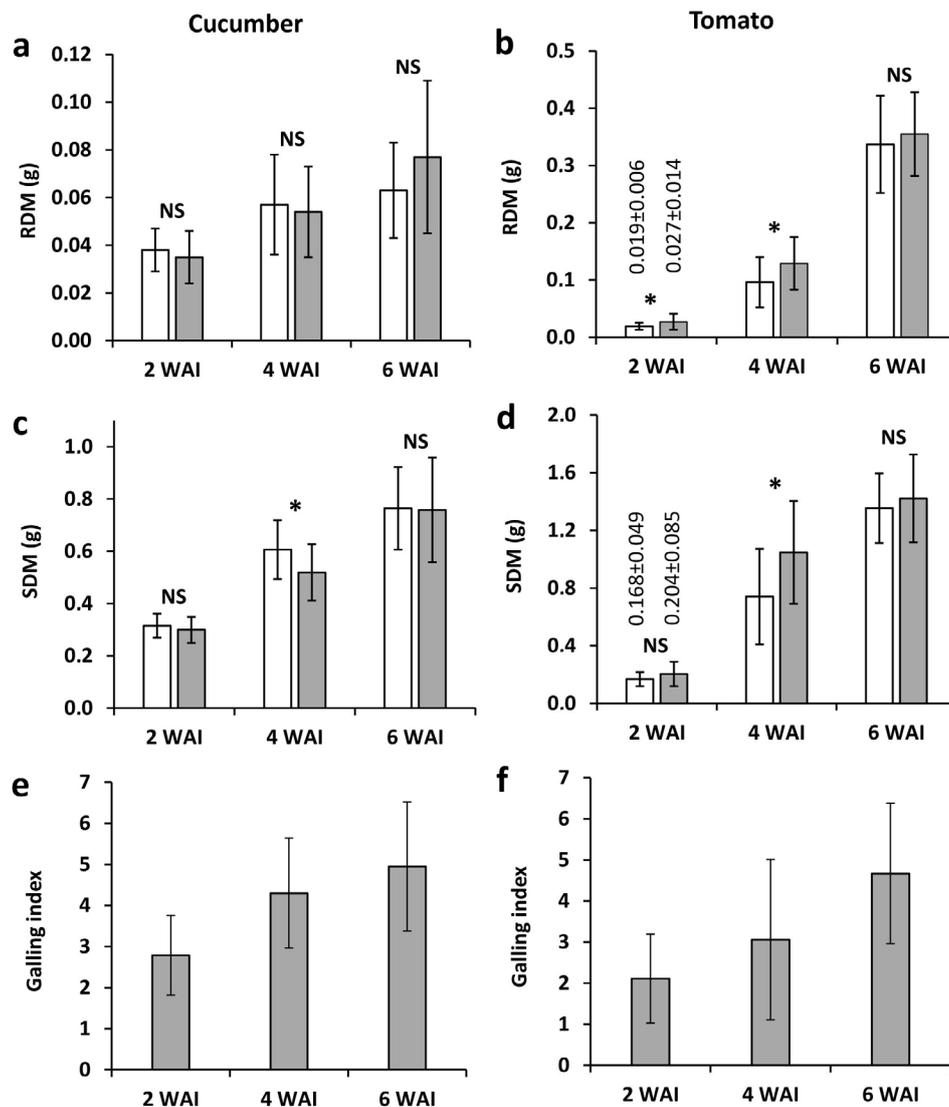
Fig. 3. (a, b) Root dry mass (RDM) and (c, d) shoot dry mass (SDM) for the non-infected control (empty bars) and *Meloidogyne incognita*-infected (grey bars) cucumber and tomato plants at 2, 4 and 6 weeks after inoculation (WAI). (e, f) is the Galling index (according to Mukhtar *et al.*, 2013) for infected plants. Values are means ± SDs of n = 20. The asterisks represent significant differences between the control and the infected plants (paired *t*-tests). *p < 0.05, NS not significant.

A significant increase in G_R value was also observed shortly after *Meloidogyne* inoculation. RKN juveniles secrete an assortment of enzymes to degrade the polysaccharide components of host cell walls during their intercellular migration through the cortex (Bernard *et al.*, 2017; Meidani *et al.*, 2019). The impaired cell integrity and enhanced membrane permeability facilitates a solute influx from the plant vascular tissues to the giant cell to support nematode feeding. The increased rate of electrolyte leakage through the root membranes was suspected to be responsible for the higher G_R detected in the RKN-treated plants. Additionally, the generation of reactive oxygen species (ROS) is stimulated as a host defence mechanism against pathogen invasion, which induces the peroxidation of cell membrane constituents, and thus interrupts the membrane

potential (Maqsood *et al.*, 2020). Previous studies have shown that root stress (*e.g.* heavy metals, alkalinity) caused an increase in conductive energy loss (D_R) and a decrease in intracellular resistance by altering the selective permeability of the cell membranes (Cseresnyés *et al.*, 2018; Xiang *et al.*, 2018). This effect was also observed in the present study at 2 WAI, although the increase in D_R induced by RKN proved to be only marginally significant for cucumber (p = 0.06) and was insignificant for tomato (p = 0.14).

Nevertheless, both D_R and G_R were reduced markedly in the infected plants at 6 WAI. This result was likely due to the seriously hampered solute transport in plant vessels which were extensively destroyed by the developing giant cells (Strajnar *et al.*, 2012; Al Abadiyah Ralmi *et al.*, 2016). As the applied electric current flows across

the root-substrate interfaces (which are the predominant water absorption zones) and within the roots through ion fluxes (electron conductivity is negligible), G_R is closely related to the hydraulic conductance of the root system (Weigand and Kemna, 2019). Accordingly, previous studies reported a decreased root hydraulic conductance due to *Meloidogyne* infection owing to the considerably increased axial resistance of the galled root segments to water flow, and this effect was associated with a reduced stomatal conductance and transpiration rate (Dorhout *et al*., 1991; Maqsood *et al*., 2020). Restricted water transport and a low leaf water potential result in a reduced photosynthetic rate and a progressively decreasing leaf Chl content over time (López-Gómez *et al*., 2015), this was detected in the present experiment during RKN infection for both species, initially in the older but later also in the younger leaves.

A lower SDM and total root length, and a higher RDM due to gall formation are typical consequences of *Meloidogyne* infection. However, the severity of plant damage depends on various factors, such as nematode density, the timing of the infection, soil conditions and host susceptibility (Kamran *et al*., 2013; Mukhtar *et al*., 2013). The current study has also revealed an increased root biomass for tomato, and a reduced aboveground biomass for cucumber due to RKN infection at some of the measurement dates. Nevertheless, the higher SDM value detected in RKN-treated tomato plants at 4 WAI was an unexpected finding, although it was reported that low or moderate *Meloidogyne* pressure could result in an enhancement in host growth by inducing adventitious root formation to compensate for those destroyed (Olthof and Potter, 1972; Poll *et al*., 2007; Mukhtar and Kayani, 2020). In the present study, the small changes found in plant biomass in response to the nematode, along with the numerous but small root galls implies a relatively low degree of RKN disease. Accordingly, there was no plant mortality, and accelerated senescence was only visually apparent in the oldest leaf of the tomato plant. The low level of plant disease was probably due to the artificial soil-less growing medium and the low inoculum density applied.

CONCLUSIONS

1. Root-knot nematodes infection could potentially be reliably detected by measuring the root dielectric properties non-destructively. Increased values of root electrical capacitance and root electrical conductance in the early stages of infection, followed by a decrease in root electrical conductance proved to be valid indicators of dynamic changes in the infected roots in response to nematode invasion, even before the appearance of obvious visible symptoms aboveground.

2. The strength of our study is that we managed to show detectable differences in the early stages of infection, even with a low number of galls. Although some infected juveniles were already inside the roots at that stage, there was still an opportunity to apply a more targeted plant protection strategy. During root-knot nematodes-identification based on the soil, misleading information and erroneous conclusions may be drawn from the number of juveniles depending on various factors (how infective they are, their heterogeneous distribution in the field, sampling at a focal point, or if they remain at a dormant stage concerning their egg mass).

3. The presented simple electrical method may be considered as a novel diagnostic tool designed to indirectly reveal the entry of root-knot nematodes and assess the extent of the damage it has caused belowground, thereby providing rapid pest recognition and control to avoid serious crop losses (*i.e.* by the removal of infected plants from the growing system). As the measurement does not affect plant life functions, numerous plants may be investigated repeatedly in different growth stages, and used for further reproduction. Therefore, this approach is a potential alternative for the selection and screening of root-knot nematodes-resistant crop varieties (which is the most sustainable and economic practice for nematode control), and also for the development and testing of nematicide agents.

4. The dielectric measurement technique was successfully applied under field conditions to estimate root traits for various specified purposes. However, the evaluation of the efficiency and sensitivity of the method for detecting root-knot nematodes infection in spatially and temporally heterogeneous, structured field soils is undoubtedly an important future task.

Conflict of Interest: The authors declare that they have no conflict of interest.

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