RESEARCH PAPER

Early responses of resistant and susceptible potato roots during invasion by the potato cyst nematode Globodera rostochiensis

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Abstract

Signals from roots of resistant (cv. Maris Piper) and susceptible (cv. Désirée) potato cultivars during invasion by second stage juveniles (J2s) of the potato cyst nematode, Globodera rostochiensis, were investigated. Novel experimental chambers enabled the recording of electrophysiological responses from roots during nematode invasion. The root cell membrane potentials were maintained throughout the 3 d required to assess invasion and feeding site development. The steady-state resting membrane potentials of Désirée were more negative than those of Maris Piper on day 1, but the reverse on day 3. After 5 d there was no difference between the two cultivars. Intracellular microelectrodes detected marked spike activity in roots after the application of J2s and there were distinct and reproducible differences between the two cultivars, with the response from Désirée being much greater than that from Maris Piper. The responses to mechanical stimulation of roots by blunt micropipettes and sharp electrodes were consistent and similar in both cultivars to the responses in Maris Piper obtained after nematode invasion, but could not account for the marked response found in Désirée. Exogenous application of exoenzymes, used to mimic nematode chemical secretions, resulted in a distinct depolarization pattern that, although similar in both cultivars, was different from patterns obtained during nematode invasion or mechanical stimulation. The pH of homogenates prepared from roots of both cultivars was measured and a Ca2+ channel blocker was used to assess the role of Ca2+ in nematode invasion. The results indicated a role for Ca2+ in the signalling events that occur during nematode invasion.

Key words: Cell signalling, electrophysiology, plant responses, potato cyst nematode.

Introduction

The potato cyst nematodes (PCN), Globodera rostochiensis and G. pallida, are economically important pests of seed and ware potatoes and pose considerable control problems (Whitehead and Turner, 1998). The use of chemical nematicides is environmentally undesirable and alternatives to nematicides are being sought. Novel control strategies may arise through an understanding of the nature of resistance and the plant responses at the cellular level to nematode invasion.

Once hatched, infective second stage juveniles (J2s) of Globodera spp. are attracted to host roots, the root tip being the preferred site for invasion (Steinbach, 1972). On reaching the root tip, the J2 explores the surface of the root by lip rubbing and stylet probing. When a suitable site for cell wall penetration is located, the stylet is thrust vigorously to penetrate the cell and allow entry of the J2 (Wyss, 2002). After entering the root, intracellular migration occurs, with the J2 cutting through successive cells to move towards the vascular cylinder where a feeding site (syncytium) is initiated. These processes involve both physical probing by and chemical release from the migrating nematode (Wyss, 2002). The host response to invasion of J2s is clearly seen in the incompatible interaction, which involves localized host cell necrosis and browning, followed by the disorganization and lysis of
the syncytium. A fluorescent hypersensitive response in roots to the invasion of *Globodera* J2s was correlated with the degree of compatibility of the interaction (Robinson *et al*., 1988). The biochemistry of plant defence responses to nematode invasion has been discussed by Zacheo *et al.* (1997), but there is a paucity of information on signalling responses of plant cells to nematode invasion.

Transient electrical signals have been recorded in many different types of plants and algae using electrodes placed both intra- and extracellularly and changes in cytosolic Ca$^{2+}$ have a role in these responses (Beilby, 1984; Davies, 1987). These electrical transients are much slower than their animal counterparts and they have been recorded from many different types of plant tissues, including the syncytium developed at the feeding site of an invading nematode (Jones *et al*., 1974, 1975). The stimulus for these electrical signals can be environmental triggers such as temperature changes (Minorsky and Spanswick, 1989) or wounding (Meyer and Weisenseel, 1997). Long-distance electrical signals have been measured in whole plants in response to wounding, with the signal propagated throughout the plant via the phloem sieve-tube companion cell complex (Wildon *et al*., 1992; Rhodes *et al*., 1996). Later measurements suggested an apoplastic route for these signals and the use of ABA-deficient mutants implicated a role for plant hormones in the response (Herde *et al*., 1998). Wounding has also been shown to induce localized transient changes in membrane voltage and ion fluxes in maize roots (Meyer and Weisenseel, 1997). The application of electrical stimuli to plant tissues also generated electrical transients that could be inhibited by Ca$^{2+}$ channel blockers (Fromm and Spanswick, 1993). To date, no direct link between attack and invasion by a pathogen and an in planta electrical signal has been demonstrated. The present work investigated signals from roots during invasion by *G. rostochiensis*. Intracellular microelectrodes were used to detect if invasion of J2s elicited electrical activity in the roots of resistant and susceptible potato cultivars; transient electrical changes were also compared. The responses of roots of both cultivars to mechanical probing and to exoenzymes, used to mimic nematode chemical secretions (Yan *et al*., 1998; Smant *et al*., 1998), were also examined. The pH of homogenates prepared from roots of resistant and susceptible cultivars was measured and a Ca$^{2+}$ channel blocker was used to assess the role of Ca$^{2+}$ in nematode invasion.

**Materials and methods**

**Nematodes**

Single generation cysts, at least one year old, of *G. rostochiensis* pathotype Ro1 were stored dry at room temperature until required. J2s were obtained by presoaking cysts in glass distilled water (GDW) at room temperature for 5–7 d. The cysts were then placed in potato root diffusate, obtained using standard methods (Fenwick, 1949). Fresh J2s were harvested daily, rinsed in GDW and used for experimentation.

**Tissue and hydroponic culture of potato plants**

Potato (*Solanum tuberosum*) plants, cvs Désirée and Maris Piper, were grown from chitted tuber pieces in steam-sterilized sand/loam mix in 100 mm diameter plastic pots under glasshouse conditions (min. 12 °C, max. ambient). The hours of light varied depending on the time of year, but 12 h of daylight was supplied, even in winter. Nodes were cut from plants (4–6-week-old) and sterilized by soaking in 70% ethanol for 30 s followed by 10 min in 10% sodium hypochlorite solution. After three rinses in sterile distilled water, the nodes were trimmed using a scalpel and cultured on Murashige and Skoog basal medium with 0.1 mg l$^{-1}$ indole acetic acid (both Sigma-Aldrich, Poole, UK) and buffered to pH 5.7. Both cultivars were grown for approximately 4 weeks (16 h light, 22 °C) and subsequently subcultured weekly.

Plants were transferred from tissue culture after 14–21 d into hydroponic culture. Plants were removed from Magenta boxes (Sigma-Aldrich, Poole, UK) and the roots were washed in artificial pond water (APW) that was suitable for plant growth and nematode activity (Miller and Zhen, 1991) to remove any agar adhering to the roots. Four plants were then transferred into individual pots containing approximately 800 ml of APW, aerated and placed in controlled environment conditions. The temperature was maintained at 20 °C, 16 h of light and 75% relative humidity. The nutrient solution was changed once after 24 h and plants were used after 4–8 d for experiments in either agarose chambers or perfusion chambers.

**Experimental root chambers**

A potato plant was placed on top of absorbent paper saturated with nutrient solution in one half of a 90 mm diameter Petri dish. One or more roots were positioned onto a 5 mm layer of 1% seaplaque agarose (Bionwittaker Molecular Applications, Rockland, Maine, USA) prepared in APW (volume 3 ml) just at the point of setting so the root was partially embedded and held securely. The agarose was contained in a 40 mm diameter Petri dish with a groove cut in the side to prevent damage to the root (Fig. 1). This Petri dish was elevated by approximately 10 mm above the level of the plant to reduce excessive wicking of the nutrient solution along the length of the root, which may have prevented nematode invasion. Potato seedlings could be maintained successfully in these chambers for 5 d, sustaining root elongation growth rates that were comparable to similar aged plants in hydroponic culture (data not shown).

To confirm that resistance was maintained in Maris Piper roots when set up on the agarose root chamber, invasion tests were carried out. One root from each of five individual plantlets of Désirée and five of Maris Piper was inoculated with J2s of *G. rostochiensis* (n=10). Nematodes inside roots were visualized using standard techniques (Hooper, 1986) by boiling excised roots in 0.05% acid fuchsin (Sigma-Aldrich, Poole, UK) for 2 min. Numbers of J2s that had invaded roots after 24 h were recorded and also the number of nematodes that had set up feeding sites within 3 d. The numbers of nematodes invading were similar in both cultivars but, after 3 d, no feeding sites had been established in Maris Piper (resistant), whereas 50% of the invading juveniles had successfully formed feeding sites in Désirée (susceptible). This demonstrated that the resistant attributes of Maris Piper were retained in the experimental chambers and, thus, the chambers could be used to examine the electrophysiological responses of resistant and susceptible cultivars.

For experiments using flowing nutrient solution, a single plant was held securely in a 50 mm Petri dish attached to a perfusion chamber prepared from Perspex, which enabled electrophysiological recordings to be made from individual roots maintained in an aqueous environment. A gravity-fed perfusion system was set up to allow
perfusion of test solutions over the root surface during recording. Test solutions were removed from the perfusion chamber by suction via a vacuum pump. Using this system, different test solutions could be applied sequentially.

Electrophysiological techniques

Single-barrelled micropipettes were made from filamented borosilicate glass tubing (external diameter 1.0 mm, internal diameter 0.58 mm; Hilgenberg GmbH, Malsfeld, Germany). Lengths of glass (50 mm) were pulled using a vertical single-stage micropipette puller (model PE-2, Narishige Scientific Instrument Laboratory, Tokyo, Japan) and these had final tip diameters of approximately 0.3 µm. These micropipettes were back-filled with 100 mM KCl and fitted into a microelectrode holder (model ESP-F10N, Harvard Apparatus Ltd, Edenbridge, UK), filled with the same electrolyte. The microelectrodes were manipulated into position and the output voltage from the electrometer (model FD223, World Precision Instruments Inc. (WPI), Stevenage, UK) was recorded as described previously (Miller and Zhen, 1991; Miller and Smith, 1992). A reference half-cell electrode (Flex-Ref, WPI) provided the connection between the ground of the electrometer and the sample solution/agarose.

Resting membrane potential recordings: Membrane potential recordings were measured from epidermal cells of Désirée and Maris Piper roots approximately 20–50 mm behind the root tip. Steady-state resting membrane potentials were recorded 1, 3, and 5 d after the roots had been on the agarose root chamber apparatus.

Membrane potential recordings in the presence of nematodes: Individual roots which had been on agarose for 1–1.5 h were inoculated with J2s of *G. rostochiensis* (*n*=10, <24-h-old) close to each root tip. Roots were impaled in the same region behind the root tip as above and recordings made within 24 h post-inoculation. At
least five recordings were made from each cultivar in the presence of nematodes.

Artificial probing—rubbing on epidermal root surface without impalement: Holding the sharp ends of previously pulled microelectrodes close to the hot element of the electrode puller for several seconds made blunt micropipettes for artificial probing. Roots of Désirée and Maris Piper set up on the agarose root chamber were artificially probed. Blunted micropipettes mounted on a micro-manipulator were used to rub and probe the surface of the root in the zone of elongation, approximately 2.5 mm behind the root tip, which is the preferred region of invasion. This probing was carried out without piercing the epidermal cells while membrane potentials were recorded. At least five recordings were made from each cultivar.

Artificial impalement of root epidermal cells: Roots were set up as before but a second electrode, filled with electrolyte solution and attached to the electrometer, was used briefly to impale the epidermal cells approximately 2.5 mm behind the recording electrode. This repeated brief insertion and withdrawal of a second electrode was used to mimic stylist insertion of an invading J2. Membrane potential measurements from both electrodes were recorded using two channels of the electrometer and root cell impalements were detected by the voltage changes that occurred as the electrode made contact with the tissue. At least five recordings were made from each cultivar.

Chemical stimulus—bacterial culture

Single colonies of the bacterial phytopathogen Erwinia carotovora atroseptica were cultured overnight in liquid Luria Bertani (LB) (25 ml) at 30°C with continuous shaking. After the medium and bacteria were centrifuged for 10 min at 6000 g, the supernatant containing the exoenzymes was collected and stored at ~20°C until use. Tubers were inoculated with the supernatant plus LB or LB alone and incubated at 30°C for 5 d. The appearance of soft rot in the tubers injected with the supernatant confirmed that the bacterial culture was viable and producing exoenzymes when grown in LB media. Roots of both cultivars were set up in the perfusion chamber. They were exposed, firstly, to a 1 in 10 dilution (in APW) of LB alone and then to a 1 in 10 dilution of the supernatant. The exposure period for both solutions was 2 min. At least five recordings were made from each cultivar in the presence of the diluted supernatant. A Bradford assay (Bradford, 1976) confirmed the presence of protein in LB supernatant.

Examination of the role of Ca²⁺ and pH in plant responses

Roots of both cultivars arranged on agarose root chambers for 24 h were excised from plantlets and homogenates prepared. Root tissue from five plants of each cultivar was used and homogenates were prepared by placing the fresh root material into a 5 ml syringe with a small disc of filter paper blocking the aperture. The roots were plunged into liquid nitrogen 2–3 times and this was interspersed with macerating the root tissue by depressing the syringe plunger several times. After centrifugation at 4°C (150 g for 10 min) the liquid homogenate was stored at ~20°C until pH measurements were made. These measurements of five replicate 5 µl samples of each homogenate were made under paraf®n oil saturated with water using pH microelectrodes (Miller and Smith, 1992).

Butyric acid treatment: Acid load treatments are known to change intracellular pH (Sanders et al., 1981) and the acidification of root cell cytosolic pH by treatment with 10 mM butyric acid at pH 6 has been demonstrated (Walker et al., 1998). The aim of the present work with butyric acid was to interfere with any possible change in cytosolic pH that may be part of the signalling process. Désirée plants were soaked in aerated butyric acid (10 mM) prepared in APW for 2 h. The roots were then rinsed in APW and laid on the surface of 1% agar plates (140 mm diameter). J2s (n=5) were applied <10 mm from individual root tips (n=10). Control plants were soaked in APW for 2 h prior to inoculation. All roots were stained after 24 h to detect nematodes inside roots. The experiment was repeated with Maris Piper roots. The experiment was also repeated using excised roots. Roots of Désirée plants were excised while in APW and soaked for 2 h in butyric acid (10 mM) or APW with aeration. The roots were then rinsed in APW, dried off using tissue and placed on 1% agar plates (140 mm diameter). J2s (n=10) were applied <10 mm from root tips (n=6). Roots were stained after 24 h.

Examination of the role of Ca²⁺ in plant responses: Intracellular Ca²⁺ signalling has been implicated in the transmission of plant responses. To examine whether this mechanism was involved in root cell responses to nematode invasion, roots were treated with LaCl₃, which is known to inhibit changes in cytosolic Ca²⁺ in plant cells (Felle and Hepler, 1997; Plieth et al., 2000). Agarose root chambers were prepared as above except that 0.2 mM LaCl₃ was added to the agarose prior to setting. J2s (n=8) were applied in two batches of four on either side of individual roots, 4 mm away from the root and 5 mm back from the root tip. Orientation and movement of J2s towards the root was examined after 1, 6, and 24 h and then roots were stained as above.

Invasion assays were carried out as before using Désirée roots (n=10) inoculated with freshly hatched G. rostochiensis J2s (n=10, 180 to 210 mm from the root tip, with four replicates of 6 mm from each side of the root. Values shown are the means ±SE (minimum and maximum values) [sample size]. Membrane potentials reported by microelectrodes inserted into the root epidermal cells of the two potato cultivars growing in the agarose root chamber for 1–5 d.

Values shown are the means ±SE (minimum and maximum values) [sample size]. The electrode measurements were all made in a region 20–30 mm from the root tip and none of the plants had been exposed to nematodes.

<table>
<thead>
<tr>
<th>Potato cultivar</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
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</thead>
<tbody>
<tr>
<td>Désirée</td>
<td>−122±4</td>
<td>−109±6</td>
<td>−98±7</td>
</tr>
<tr>
<td></td>
<td>(−80, −172) [52]</td>
<td>(−76, −140) [14]</td>
<td>(−70, −171) [19]</td>
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<tr>
<td>Maris Piper</td>
<td>−110±3</td>
<td>−125±6</td>
<td>−106±6</td>
</tr>
<tr>
<td></td>
<td>(−75, −155) [52]</td>
<td>(−97, −170) [14]</td>
<td>(−80, −125) [19]</td>
</tr>
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respectively; \( n \) between the two cultivars (Table 1).

There were differences in the resting membrane potential between the two cultivars (Table 1). After 1 d, the values for steady-state resting membrane potentials from roots of Désirée were significantly more negative (\(-122 \pm 4 \text{ mV}\)) than those from Maris Piper (\(-110 \pm 4 \text{ mV}; n=52 \) for each cultivar). However, within this large sample values actually ranged from \(-80 \) to \(-172 \text{ mV}\) for Désirée and \(-75 \) to \(-155 \text{ mV}\) for Maris Piper. The reverse occurred after 3 d, with Maris Piper being significantly more negative than Désirée (\(-125 \pm 6 \text{ mV}\) and \(-109 \pm 6 \text{ mV}\), respectively; \( n=14 \) for each cultivar). After 5 d there was no significant difference in resting membrane potentials between the two cultivars (Table 1).

Application of nematodes to the roots caused marked spike activity in the electrophysiological recordings, but there were distinct and reproducible differences between the two cultivars. Figure 3A shows a typical recording from epidermal root cells of Désirée during nematode invasion, where conspicuous, irregular spike activity, lasting about 10 min, led to a fall in membrane potential (depolarization) down to a few millivolts. These episodes were characterized by a spontaneous recovery of the resting membrane potential. By contrast, the electrical activity caused by nematodes in the resistant cultivar, Maris Piper, was far less marked, with more regular spike activity that, at maximum, altered the membrane potential to about \(-50 \text{ mV}\) (Fig. 3B). The duration of these transient electrical changes was between 30 and 60 s. The mean ±standard error values for the maximum depolarizations were \(118 \pm 3.4\) (\( n=10 \)) and \(19 \pm 2.6 \text{ mV}\) (\( n=8 \)) for Désirée and Maris Piper, respectively. These events occurred at mean intervals of \(31 \pm 7.0\) and \(13 \pm 3.0\) min for Désirée and Maris Piper, respectively.

Mechanical stimulation of both cultivars by probing with blunt micropipettes without penetrating the epidermal cells resulted in regular depolarizations each time the root was probed. Figure 4A and B show representative recordings from Désirée and Maris Piper, respectively, and it is clear that both cultivars responded in a similar manner, with depolarizations rarely below \(-100 \text{ mV}\). The responses were very different from the spike activity obtained from Désirée during nematode invasion (Fig. 3A), but corresponded more closely to the spike activity from Maris Piper (Fig. 3B).

Using a second electrode repeatedly to impale the epidermal cells resulted in a similar pattern of membrane potential depolarizations to that obtained using single blunt electrodes to probe the roots. Figure 4C and D shows representative recordings from Désirée and Maris Piper, respectively, with the upper part of each showing the spike activity from the root and the lower part showing the recordings of voltage change from the second electrode as it briefly penetrated the root. The voltage changes correspond to the spike activity generated, indicating that the upper recordings were from root impalement. The voltage returns to zero because, on withdrawing the electrode from a cell, the electrode remained in wet contact with the root surface. The narrow tip of the electrode, used to simulate repeated brief stylet penetration, and the possible occlusion of the tip by accumulated cell wall material resulted in extra noise in the recordings. Both cultivars responded to epidermal cell impalement in a similar manner, which differed from that obtained from Désirée during nematode invasion.

The mechanical probing and impalement of potato roots was an attempt to imitate the effects of nematode stylet probing and insertion into the root cell. Experiments using bacterial supernatant containing exoenzymes, used to
mimic nematode secretions, aimed to examine the effects on plant cell responses. Figure 4E and F shows typical results when Désirée and Maris Piper, respectively, were exposed to the bacterial supernatant. A distinct depolarization pattern, usually between 10 and 40 mV, was observed, which was similar in both cultivars. Depolarization in response to LB alone was very slight; thus, the distinct and much greater depolarization that was observed in the supernatant was due to the presence of the exoenzymes. These responses differed from those obtained after nematode invasion or mechanical stimulation (Figs 3, 4A–D).

The mean pH values of root homogenates prepared from the two cultivars were not significantly different (pH 4.8 and 5.0 for Désirée and Maris Piper, respectively). Pretreatment of potato roots with 10 mM butyric acid for 2 h did not affect the number of nematodes invading either cultivar within 24 h, compared with untreated controls (data not shown). Exposure of roots to 10 mM butyric acid for periods greater than 6 h proved to be phytotoxic, so it was not possible to study long-term effects of butyric acid–induced cytosolic acidification on the development of nematode feeding sites. Therefore, further experiments on the possible role of pH in the signalling process were not undertaken.

Experiments on the possible role of Ca²⁺ in the root response to nematodes involved the use of 0.2 mM LaCl₃, which is known to inhibit changes in cytosolic Ca²⁺. In behavioural assays, LaCl₃, prepared in agarose, did not affect the number of J2s that located host roots after 1, 6, and 24 h compared with controls with APW in agarose (Fig. 5). However, very few J2s invaded roots on agarose containing LaCl₃ compared with the numbers invading roots on control plates, and those few that did invade were unable to set up a feeding site and develop to third stage juveniles within 96 h (Fig. 6).

Fig. 4. Membrane potential recordings from root epidermal cells of Désirée (left) and Maris Piper (right). Recordings obtained from roots touched with blunt micropipettes to simulate nematode stylet probing (A, B); roots penetrated by a second electrode, the upper panels show recordings from the roots, the lower panels show recordings made from the second electrode used for repeated brief impalement of the roots (C, D); recordings obtained from potato root epidermal cells treated with bacterial supernatant (E, F).
Discussion

Cyst nematodes usually invade host roots at the zone of elongation and then cut their way through cells to reach the vascular system (Hussey and Grunler, 1998). Here they set up a feeding site (a syncytium) and commence feeding. Most of the research on plant responses to nematode invasion has focused on events occurring during initiation and development of the syncytium (Wyss, 2002); far less is known about events during the invasion and movement through the roots (Atkinson, 2002). Several secondary metabolites are known to have anti-nematode effects (Huang, 1985). Immunoblotting using specific antibodies demonstrated that roots of potato infected with *G. pallida* had an increased presence of chitinase (Rahimi et al., 1998a, b) but not β-1,3 glucanase activity (Rahimi et al., 1996). This work, and studies by Hammond-Kosack et al. (1989) on the systemic induction of PR-proteins in potato leaves by *G. rostochiensis*, indicated that the induced proteins corresponded to those described by White et al. (1987) and were part of a general response to pathogen invasion. It is known that the plant response to nematode invasion can be rapid. Fluorescence under UV light occurred in roots within 3 h of invasion by *G. rostochiensis* and *G. pallida* and was more extensive in the incompatible reaction of *G. rostochiensis* with the resistant cultivar Maris Piper (Robinson et al., 1988). The fluorescence was interpreted by Robinson et al. (1988) as a hypersensitive response and preliminary analysis indicated that the compounds involved were phenylpropanoid.

Previous work on plant electrophysiological responses to nematodes in roots related to investigations of syncytium development caused by *G. rostochiensis* in potato roots (Jones et al., 1974, 1975). The present work includes the first studies using electrophysiology to examine early root responses to nematode invasion. In the experimental chamber developed for this work the differential responses of the two potato cultivars have been demonstrated and the root cell membrane potentials were maintained throughout the 5 d required to assess invasion and feeding site development. The differences in resting membrane potential between the two cultivars on days 1 and 3, although significant (Table 1), did not affect the numbers of nematodes invading. The large range in resting potentials may reflect the local environment in this new purpose-built chamber, where the root is partially embedded in agar. Bird (1959) was the first to suggest that redox potential may have a role in invasion site location by endoparasitic nematodes, but it is unclear whether the differences found in the present work would influence host selection and nematode invasion. Application of nematodes to the roots caused marked spike activity in the electrophysiological recordings and there were distinct and reproducible differences between the two cultivars, with the response from the susceptible cv. Desireé being much greater than that from Maris Piper (see Fig. 3). This was unexpected, and may indicate the concept of a susceptible response, relating to signal molecules. The responses to mechanical stimulation of roots by blunt micropipettes and to repeated brief insertion of electrodes into the root epidermal cells were consistent and similar in both cultivars to the responses obtained from Maris Piper after nematode invasion, but clearly could not account for the marked response found in Desireé. It appears unlikely that the responses found during nematode invasion are due to mechanical factors such as wounding.

Similarly, the electrophysiological responses to exogenous, inundative application of a bacterial supernatant containing exoenzymes, used to mimic nematode secre-
tions (Smant et al., 1998; Yan et al., 1998), resulted in a distinct depolarization pattern that, although similar in both cultivars, was different from those obtained after nematode invasion or mechanical stimulation. It appears that probing and wounding and preliminary studies on nematode secretion mimics indicate that the responses to nematode invasion are associated with other factors. The artificial probing of roots with both sharp and blunt micropipettes elicited similar electrical responses in both potato cultivars (comparing Fig. 4A, B, C, D). The results for the resistant cultivar Maris Piper suggest that the root cells show a very similar pattern of electrical activity during actual probing by an invading nematode (comparing Figs 3B, 4B, D). The combined effects of mechanical probing and nematode secretion mimics may lead to the distinct pattern of electrical activity demonstrated by the susceptible cultivar Désirée. However, it is clear that inundative application of exoenzymes is not directly comparable with the localized secretion of small quantities of enzymes by the invading nematode. Future work will need to examine the localized application of secretion mimics and, more generally, determine the extent to which enzymes are secreted, if at all, during movement of G. rostochiensis through root tissue. Two β-1,4-endoglucanases have been cloned from the soybean cyst nematode, Heterodera glycines, and are expressed in the J2 during root invasion (de Boer et al., 1999).

The electrophysiological responses associated with wounding always show a transient decrease in the resting membrane potential (depolarization) and the nematode-elicited electrical signals reported here follow this pattern. The magnitude and duration of wound-elicited electrical transients in roots have been shown to be dependent on the location of the recording electrode relative to the wound site (Meyer and Weisenseel, 1997). In this work, although the location of the recording microelectrode was fixed, the site of nematode invasion was variable and yet the electrical responses were larger than those reported for maize roots wounded with a scalpel blade (Meyer and Weisenseel, 1997). The probing with blunt and sharp probes in this work may elicit a different type of wound response, or this may reflect species differences. The different type of electrical transients shown in resistant and susceptible potato cultivars (Fig. 3) suggests that there may be different types of electrical signal associated with wounding and that, in Maris Piper, this can lead to nematode resistance. One testable hypothesis is that the type of electrical spikes elicited by artificial probing in Désirée (Fig. 4A, C) that resemble the artificial probing and nematode invasion responses in Maris Piper (Figs 3B, 4B, D), will subsequently lead to the initiation of a ‘resistance’ response, such as the systemic induction of PR-proteins, that will block the establishment of syncytia in the plant. There is molecular evidence that the dissimilar types of electrical transients associated with wounding and non-injurious stimuli can elicit different patterns of expression of calmodulin genes (Vian et al., 1996).

Subsequent experiments investigated whether some of the known signalling processes in plants, identified in other host–pathogen interactions, were involved in invasion by G. rostochiensis. It is known that changes in cytoplasmic pH and Ca²⁺ are signalling responses of plant cells (Felle and Hepler, 1997) to stress conditions. Vascular pH may be changing the sensory information required by the nematode for successful migration through the host tissue, especially as the invading nematode will cut through cells and release the vacuolar contents. Butyric acid treatment was used to examine the effect of pH on invasion, as it is known to acidify the cytoplasmic pH of plant cells (Walker et al., 1998). The pH values of root homogenates prepared from the two cultivars were not significantly different and pretreatment of potato roots with 10 mM butyric acid for 2 h did not affect nematode invasion. Longer periods of exposure to butyric acid were phytotoxic and, therefore, prevented examination of the role of cytosolic pH in syncytial initiation. Conclusions on the role of pH can only be tentative, but it appears unlikely that pH of these two cultivars affect nematode invasion, although measurements comparing cytosolic pH in the two potato cultivars may be worthwhile.

The possible role of Ca²⁺ fluxes in successful parasitism was investigated. Initial work with EGTA, which binds extracellular Ca²⁺ and has been used previously to demonstrate that hatching of G. rostochiensis is a Ca²⁺-dependent process (Clarke and Perry, 1985), were inconclusive (data not shown) because of the detrimental effects on root growth and appearance. However, the Ca²⁺ channel blocker, LaCl₃ was used successfully to assess the role of Ca²⁺ in nematode invasion of susceptible and resistant hosts. La³⁺ has a relatively high affinity for specific sites in Ca²⁺ channels and can displace Ca²⁺ from the cell wall, but cannot penetrate the cell membrane via the channel pore (Shiina and Tazawa, 1987). Thus, it acts as an inhibitor of Ca²⁺ channel activity and regulation (Felle and Hepler, 1997) and has been shown to inhibit plant action potentials (Fromm and Spanswick, 1993), but not wound-induced electrical currents (Meyer and Weisenseel, 1997). The results from the invasion assays with LaCl₃ indicated that Ca²⁺ channels play a role in cellular signalling during nematode invasion. Treatment of roots with LaCl₃ did not modify the behaviour of the nematodes in invading the roots, but did affect their ability to form feeding sites. This result resembles the resistant versus susceptible responses of the two potato cultivars, implying a key role for Ca³⁺ signalling in the response. However, it is possible that the LaCl₃ affects the ability of the J2 to execute the behavioural and chemical processes required for feeding site induction. Results from the LaCl₃ treatment are exciting and further experiments will be required. Patch clamp analysis can be used to compare Ca²⁺ channel
activity in root cell protoplasts of the two cultivars, in order to substantiate the role of Ca\(^{2+}\) in relation to the electrophysiological responses of the roots during nematode movement through root tissue and initiation of the syncytium. Molecular approaches can be used to identify Ca\(^{2+}\) channel differences in the two cultivars, for example, and in Désirée a comparison of the patterns of gene expression that are elicited by nematode attack and artificial wounding is likely to reveal some key aspects of the early signalling pathways.

Plant and nematode physiology are research areas of increasing importance in explaining gene function and defining control mechanisms. Understanding mechanisms of host resistance to PCN will be enhanced by utilizing novel electrophysiological approaches to analyse factors involved in host root location (Perry, 1996) and to determine plant responses at the cellular level to nematode invasion and feeding. The present work has indicated that Ca\(^{2+}\) channels may be involved in the signalling process of invasion and feeding. The present work has indicated that Ca\(^{2+}\) channels may be involved in the signalling process of invasion and feeding. The present work has indicated that Ca\(^{2+}\) channels may be involved in the signalling process of invasion and feeding. The present work has indicated that Ca\(^{2+}\) channels may be involved in the signalling process of invasion and feeding.

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