

Characterization of attractiveness of excised root tips of resistant and susceptible plants for *Meloidogyne naasi*

Pascale BALHADÈRE and Adrian A. F. EVANS

Department of Biology, Imperial College, Silwood Park, Ascot, Berks., SL5 7PY, Great Britain.

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Summary – The numbers of second-stage juveniles (J2) of *Meloidogyne naasi* attracted to excised root tips of the susceptible cultivars of barley *Hordeum vulgare* cv. Doublet and wheat *Triticum aestivum* cv. Chinese Spring were measured in a root-tip bioassay. These were compared to numbers attracted by root tips from the partially resistant barley *H. vulgare* cv. Morocco and from selections of two other fully resistant grass species, *Hordeum chilense* and *Aegilops variabilis* (accession n° 1). Root tips of three accessions from *H. chilense* and from cv. Morocco showed the same attractiveness for juveniles at 1 and 24 h as tips of susceptible plants. In contrast, root tips of *A. variabilis* were less attractive at 1 and 24 h; but when sucrose was added in the medium, the retention of the J2 at 24 h was the same as for controls of susceptible plants, suggesting a “kairomone” action of sucrose on nematode behaviour. J2 were attracted to sources of CO₂ supplied in the agar. The pattern of pH changes around root tips of susceptible and resistant hosts was studied, using pH indicators in agar. Root tips of *A. variabilis* differed from other hosts by creating a less acidic pH around the surface of the roots, a lower gradient of pH on agar and differences in relative sizes of the developmental regions of the root. Reduced attraction of this grass may be associated with: *i*) a small meristematic region; *ii*) a lower production of exudates; *iii*) a reduced retention of nematodes near root tips; *iv*) the presence of long root hairs, which may interfere with J2 locating the appropriate invasion site.

Résumé – *Caractéristiques de l'attractivité de racines excisées pour Meloidogyne naasi* – L'attraction des juvéniles de second stade du nématode à galle *Meloidogyne naasi* par des apex radiculaires excisés a été comparée entre les cultivars sensibles d'orge *Hordeum vulgare* cv Doublet et de blé *Triticum aestivum* cv Chinese Spring, le cultivar d'orge partiellement résistant *H. vulgare* cv. Morocco et deux espèces de graminées sauvages totalement résistantes *Hordeum chilense* et *Aegilops variabilis* (lignée n° 1). À l'exception d'*A. variabilis*, les hôtes résistants ne se démarquent pas des hôtes sensibles. En présence de saccharose dans le milieu gélosé, la rétention des J2 à 24 h est la même que celle obtenue pour les témoins sensibles, suggérant une action de kairomone du saccharose sur le comportement d'exploration des J2. Il a aussi été montré que les J2 étaient attirées par des sources de CO₂ dans l'agar. La distribution du pH autour des apex radiculaires d'hôtes sensibles et résistants a été étudiée, avec des indicateurs de pH dans l'agar. Les apex d'*A. variabilis* différaient de ceux des autres hôtes par un pH moins acide, à la surface des racines, un gradient de pH plus faible sur l'agar et des différences de taille relative des régions en développement de la racine. Dans le cas d'*A. variabilis*, la plus faible capacité d'attraction tient probablement à *i*) une région méristématique limitée; *ii*) une faible production d'exsudats; *iii*) une faible rétention des nématodes sur les racines; *iv*) la présence de longs poils absorbants qui peuvent limiter l'accès au site d'invasion.

Key-words : *Meloidogyne naasi*, root attraction, CO₂, nematodes.

The ability of infective second-stage juveniles of root-knot nematodes to locate host roots is a complex process, involving orientation along CO₂ and thermal gradients in soil (Croll & Viglierchio, 1969; Croll & Matthews, 1977; Pline & Dusenbery, 1987; Dusenbery, 1989). Such non-specific clues could be particularly useful to nematodes with a wide host range (Dusenbery, 1987). Nematodes seem only to respond to molecular CO₂ (Bird, 1959, 1960; Klingler, 1963) and not to the soluble forms like the bicarbonate ion. Pline and Dusenbery (1987) discussed the range of possible functions of the attraction of J2 to CO₂: the attraction to roots, the downward movement of juveniles towards an optimal depth in the soil profile, or as a directional reference for nematodes to maintain a straight path in a gradient.

Once in the vicinity of the root tips, the localization by the infective J2 of the appropriate invasion site (meristematic tissue; Bird, 1962) is particularly difficult as the root tip is a complex structure composed of different parts (cap, apical meristem, elongation zone, differentiation zone, hair zone) and surrounded by products of rhizodeposition (soluble exudates, secretions, lysates and gases; Whipps, 1990). The localization is thought to involve more specific clues, such as attractants and repellents in the root exudates (e.g. glutamic acid was found to be attractant for *M. javanica* by Bird, 1959), but their nature and their balance is still speculative (Diez & Dusenbery, 1987). Recent work by Weisenseel *et al.* (1979), Behrens *et al.* (1982) and Miller and Gow (1989), which elucidated the pattern of currents of pro-

tons flowing inside the root cells (from cytosol to walls) and around roots (inward and outward currents depending on the part of the root tip complex), suggested that patterns of proton currents could be the result of indole-3-acetic acid gravitational distribution in roots and part of the "acid-growth" mechanism (Salisbury & Ross, 1992), and thus causally linked to root development.

This common pattern of polarizing currents on the surface of roots could also be detected and then used by root-knot nematodes for orientation or discrimination. There is strong evidence that, as with other water creatures which profit by the good electrical conductivity of water to develop a system of navigation, plant-parasitic nematodes could actively orientate in gradients of electrical potentials. They could align themselves to the current generated at the surface of the root, responding to changes in direction of the current, as they do *in vitro* when they show migration towards electro-negative poles (Croll, 1970). Any roots in which these currents or potentials were small, highly localised, or difficult to discriminate between, may appear less attractive to nematodes.

The accession n° 1 of the wild grass *Aegilops variabilis* has full resistance to the cereal root-knot nematode *Meloidogyne naasi* depending on a dominant gene "Rkn-mn1" (Yu *et al.*, 1990), which suppresses the development of galls and of nematodes; and one recessive gene "Rkn-mnAv" (Yu *et al.*, 1992), which suppresses the development of nematodes but allows the development of a few galls. Fewer nematodes were found in roots of *A. variabilis* n° 1, 1 day after inoculation on 2 % water-agar, than in roots of susceptible cultivars of the wheat "Chinese Spring" and the barley "Doublet" (Balhadère, 1993), suggesting a correlation between nematode compatibility and host attractiveness, as has already been reported by Viglierchio (1961) and Lee and Evans (1973).

The process of attraction and the mechanisms of resistance in grasses to the root-knot nematodes are poorly studied and understood (Lewis, 1987). Observations were made and experiments subsequently designed, to examine further some aspects of the link between *M. naasi* attraction and the host compatibility in several susceptible and resistant cereals and selected breeding accessions of their grass-like relatives. Because of the importance to infection of the root tip complex, experiments were done using excised tips in small amounts of agar, to follow the behaviour of juveniles near tips, with time and under the stereoscopic microscope.

Attractiveness of excised root tips for J2 of *M. naasi* was compared between the hosts with different compatibilities. The existence of an invasion-enhancing effect of gaseous CO₂ on *M. naasi* was sought. The effect of sucrose, a common root exudate (Whipps, 1990), on the attraction of *M. naasi* to the tips from different host roots was also studied. Finally, the pattern of pH and

proton flows around root tips of susceptible and resistant hosts was studied with pH indicators (Weisenseel *et al.*, 1979) using Bromocresol Purple and intact root tips of *H. vulgare*.

Materials and methods

SOURCE OF NEMATODES AND PLANT HOSTS

Soil infested with *M. naasi* was collected from the Institute of Grassland and Animal Production (Aberystwyth) and stored at 5 °C before use. Fresh J2 of *M. naasi* were extracted into water after incubation of infested soil at 20 °C (Whitehead & Hemming, 1965).

The following plant hosts were tested: the susceptible barley cv. Doublet (tip diameter, diam = 2 mm), the susceptible wheat cv. Chinese Spring (diam = 2 mm), the partially resistant barley cv. Morocco (diam = 2 mm), the fully resistant accession of *A. variabilis* n° 1 (diam = 1 mm), a recombinant wheat "x⁸" with the resistance gene "Rkn-mn1" from *A. variabilis* n° 1 (diam = 2 mm; tested only for the pH pattern study) and 3 selections of *Hordeum chilense*, the accessions PI283374, PI283375 (from the Institute of Grassland and Animal Production, Aberystwyth) and one accession from PBI Cambridge Laboratory (diam = 1 mm) and finally a local population of *Stellaria media* (diam = 1 mm), a weed host of *M. naasi*.

Root tips were all excised from the point where root hair elongation reached its maximum (Fig. 5).

MINIATURE OBSERVATION CHAMBERS ON SLIDES

Microscope observation slides were prepared based on Bird's (1959) design, but scaled down to only two rings on the slide instead of four. Using an ordinary glass microscope slide, some melting Paraffin wax was poured from a Pasteur pipette and molded around a glass tube (diam = 20 mm; previously dipped in 100 % ethanol). This created a cylindrical chamber (diam = 18 mm, which corresponded to the outer ring, i.e. the border of the chamber) for observation (Fig. 1). The tube was removed and a glass plate (coated with ethanol) was used quickly to flatten the upper surface of the wax. An inner ring (diam = 5 mm) was then marked on the under surface of the slide.

An inoculum of thirty J2 was deposited with a fine needle into 2 ml of distilled water in the inner ring and 2 ml of cooled 2 % water-agar was gently poured over the suspension of nematodes. This had the effect of minimizing the mechanical dispersion of nematodes away from the marked ring and leaving five to ten nematodes on average inside the ring.

The following procedure was adopted for all experiments unless otherwise noted. Before the agar set, the specimen root tip to be tested for attractiveness was inserted vertically into the agar at the center of the marked ring. A coverslip was carefully added on top of the setting agar, in order to prevent the formation of any

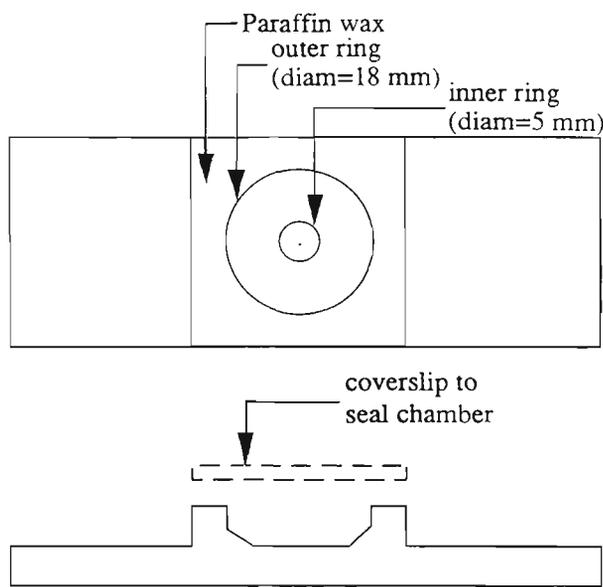


Fig. 1. Microscopic observation chamber slide (slightly modified from Bird, 1959).

air bubble or the desiccation of the medium. In these conditions, the preparation was air-tight.

Contrary to Bird's (1959) procedure, the initial mixing failed to dispense nematodes uniformly in the agar and initial numbers in the inner ring were more frequent than the expected random distribution of mean numbers (i.e. 2.3 J2 for experiments with agar only; 1.7 J2 for experiments with specimens of 1 mm diam; 1.1 J2 for experiments with specimens of 2 mm diam). Therefore all experiments started with a bias.

Every 5 min following preparation from $t = 0$ to $t = 1$ h, the number of J2 inside the inner ring was recorded. A last count was made at $t = 24$ h for slides which remained intact (in which no air bubble had developed).

The numbers of J2 inside the inner ring and this number as a percentage of the initial numbers of J2 in the inner ring, at both $t = 1$ h and 24 h were calculated and analysed for statistical significance, using the Kruskal-Wallis test (Pearson & Hartley, 1972). In the case of significant differences, mean numbers were further classified using the Wilcoxon rank-sum test (Bradley, 1968). For each of the treatments, the time-evolution of the mean number of J2 in the inner ring was studied and individual means compared to the expected mean in case of a random-distribution by using a t-test (Mood *et al.*, 1974).

EXPERIMENTAL DETAILS

Root attraction

The attractiveness of freshly excised root tips (5 mm long) were compared with control treatments of: *i*) 1 % agar containing nematodes but no root tip, to check the

usual distribution of nematodes on the slides (thirteen replicates), *ii*) 1 % agar with a small stick of wood (5 mm \times 2 mm) instead of a root tip to check the effect of a mechanical disturbance on nematode distribution (eight replicates); *iii*) 1 % agar with root tips of "Doublet" treated for 2 min in boiling water (and carefully wiped dry twice) to check the effect of inactive compared to active metabolism of living roots on nematodes (eight replicates). Numbers of replicates used for tested root tips were 19 for cv. Doublet, 19 for cv. "Chinese Spring", 20 for cv. Morocco, 22 for *A. variabilis* n° 1, 8 for the accession of *H. chilense* from PBI Cambridge Laboratory, 8 for *H. chilense* PI283374, 7 for *H. chilense* PI283375 and 8 for *S. media*.

Attraction to CO₂

A device simplified from Robinson and Heald (1990) and Klingler (1972), was set up to regularly supply the nematode environment with gaseous CO₂ to study the behaviour of nematodes in the absence of roots. After removing the coverslip, air charged with 5 % CO₂ was introduced with a fine needle (40 \times 0.8 mm) 2 mm into the agar from a 1 ml syringe held obliquely at the centre of the ring. At $t = 0$, 0.2 ml was slowly delivered, then at 5 min intervals, 0.1 ml were delivered the same way until 1 h. After each delivery, the coverslip was replaced to prevent the desiccation of the medium. Sixteen replicates were done. Controls consisted of: *i*) 1 % agar alone (no added gas) (thirteen replicates); *ii*) 1 % agar into which atmospheric air was dispensed following the same procedure as for the dispensing of CO₂ (eight replicates).

Effect of sucrose on attractiveness of roots

Only two hosts were tested: "Doublet" and *A. variabilis* n° 1. Three treatments were applied: *i*) tips in 1 % agar (19 replicates for cv. "Doublet" and 22 replicates for *A. variabilis* n° 1; *ii*) tips into 0.5 % sucrose in 1 % agar (9 replicates for cv. Doublet and 20 replicates for *A. variabilis* n° 1); and *iii*) tips in 1 % agar, originating from plants grown over 1 % agar containing 0.5 % sucrose (11 replicates for cv. Doublet and 23 replicates for *A. variabilis* n° 1). Controls consisted of: *i*) 1 % agar alone (13 replicates); *ii*) 1 % agar with a cylinder (2 mm \times 5 mm) of 0.5 % sucrose in 1 % agar at the center of the slide to check the effect of sucrose alone on nematode attraction (12 replicates); *iii*) 0.5 % sucrose in 1 % agar with a root tip of cv. Doublet heat-treated as previously described (8 replicates).

Additional qualitative observations were made on nematode behaviour using excised tips of both cultivars on either 0.5 % agar or 0.5 % sucrose in 0.5 % agar to study the behaviour of nematodes for a continuous period of 30 min after being placed near the tip (distance 1 mm), or on the tip. In the first case, tips had been plunged into melting agar vertically, with cap cells emerging at the upper surface of the agar, and left at least 10 h to build up a diffusion gradient of root exudates. In the second

case, tips were put on top of cold agar and nematodes added without any delay.

Four replicates of each cultivar were observed for each treatment. A small inoculum of four J2/root was used so that individuals could be monitored over time. Different parameters were recorded: in the first experiment, the minimum time for the first J2 to reach the root, the number of J2 on root and the distance between the J2 in agar and the root at the end of the observation, the number of contacts between the J2 and the root for 30 min, the minimum and maximum times of contact; in the second experiment, the minimum time for the first J2 to leave the root, the number of J2 on the root and the distance between the J2 in agar and the root at 30 min and the number of departures from and returns to the root for 30 min.

A study of the pH changes around root tips

Using the observation chamber but without added nematodes, a solution of a pH indicator, either Bromothymol Blue (0.001%) or Haematoxylin (Ehrlich-0.001%) was mixed (v/v) with 2% melting agar. An excised root tip of either "Doublet", "Chinese Spring", *A. variabilis* n° 1 or wheat accession "x⁸" was placed in the observation chamber and the indicator-agar was poured over it. Initial mixing of Bromothymol Blue (pK_a = 7) with the melting agar gave a blue-green colour, revealing a pH of 7-8. This indicator detected a change of acidity due to roots below pH 6 (change of colour to yellow). Haematoxylin (pK_a = 5) was also used; it was initially purple-red in agar, but below pH 5 changed colour to pale yellow.

Table 1. Attraction of second-stage juveniles (J2) of *Meloidogyne naasi* to excised root tips and to CO₂

Experiment	Initial numbers	Numbers at T = 1 h	Numbers at T = 24 h	N
CONTROLS				
Agar alone	8.5 ± 3.8	2.2 b ± 1.3	0.5 c ± 0.8	13
Mechanical disturbance	5.9 ± 2.6	0.5 a ± 0.8	0.1 a ± 0.4	8
Heat-treated root of Doublet	9.4 ± 4.3	3.3 b c d ± 2.1	0.3 b ± 0.5	8
Cylinder of sucrose	6.3 ± 2.9	0.8 a ± 0.8	0.5 c ± 0.8	12
Heat-treated root of Doublet + suc	6.8 ± 3.0	1.1 a ± 1.7	0.4 b c ± 0.5	8
Bubbling of air	8.3 ± 4.2	2.2 b ± 1.4	-	8
ROOTS TIPS				
Doublet (S barley)	6.5 ± 2.1	3.8 c d ± 2.9	7.0 f ± 6.4	19
Chinese Spring (S wheat)	7.0 ± 3.3	5.4 d e ± 4.4	9.8 f ± 4.4	19
Morocco (R barley)	6.4 ± 2.3	7.7 f ± 3.9	7.6 f ± 6.2	20
<i>A. variabilis</i> n 1 (R grass)	6.9 ± 3.5	3.1 b c ± 3.0	2.3 d e ± 2.7	22
<i>S. media</i> (S weed)	6.4 ± 2.1	2.1 b ± 1.7	1.8 d ± 1.8	8
<i>H. chilense</i> (1) (R grass)	7.1 ± 2.0	4.8 d ± 2.8	3.1 e f ± 2.4	8
<i>H. chilense</i> (2) (R grass)	7.5 ± 4.2	3.0 b c ± 1.9	3.0 e f ± 2.6	8
<i>H. chilense</i> (3) (R grass)	7.7 ± 4.5	3.1 c ± 3.8	4.0 f ± 5.8	7
EFFECT OF SUCROSE ON ATTRACTION				
Doublet root on suc	6.3 ± 2.9	7.0 f ± 1.6	-	9
Doublet grown on suc	7.9 ± 3.8	6.2 e f ± 3.7	7.4 f ± 6.7	11
<i>A. variabilis</i> on suc	7.2 ± 3.0	3.9 c d ± 2.5	6.1 f ± 5.0	20
<i>A. variabilis</i> grown on suc	6.9 ± 3.5	4.9 d e ± 3.5	4.2 f ± 2.3	23
ATTRACTION TO CO₂				
Bubbling of CO ₂ (5% in air)	9.5 ± 3.9	5.0 d e ± 2.9	-	16

Inoculum = 30 J2/replicate in 1% water-agar (inside a cylindrical observation chamber on slide with a diameter of 18 mm and a height 5 mm).

All root tips were inserted vertically in agar at the center of the inner ring of the slide (5 mm diameter) at t = 0.

For treatments with gas (bubbling of air or air charged with 5% CO₂), the gas was dispensed into agar at the center of the inner ring at t = 0 (0.2 ml) then every 5 min until 1 h (0.1 ml).

All numbers represented numbers of J2 present inside the inner ring of the slide and were given as mean ± standard deviation.

N = number of replicates; S = susceptible; R = resistant; suc = 0.5% sucrose.

(1) = accession from PBI Cambridge Laboratory; (2) = accession PI283374; (3) = accession PI283375.

- = missing data.

Means with same letter were not significantly different at p = 0.05 (Wilcoxon test).

Results

ATTRACTION OF J2 TO ROOT TIPS

Analyses of both numbers of J2 and percentages of initial numbers gave similar results. In all controls (except for heat-treated roots), the mean numbers of J2 in the inner ring were not significantly different ($p = 0.05$) from the random distribution means, after a period of time: 25 min for the agar alone treatment and 30 min for the mechanical disturbance treatment. This tendency was maintained up to 1 h, but by 24 h, all means recorded for controls were very low (See Table 1). For heat-treated roots, the mean numbers of J2 in the inner ring remained above the random distribution mean up to 55 min, then decreased below this threshold.

For the tested tips on the contrary, after an initial decrease in the mean numbers of J2 attracted to the tip (usually from $t = 0$ to $t = 15$ min, but only 10 min for cv. Morocco), the numbers remained stable until 1 h (Fig. 2). By 24 h, significantly more J2 were attracted to root tips than in controls ($p = 0.001$) and occasionally nematodes were found inside "large" root tips of cvs Doublet, Chinese Spring and Morocco. Except for small root tips (i.e. those from *H. chilense*, *A. variabilis*, *S. media*), more nematodes were close to the root tip than would be expected by random distribution.

From $t = 25$ min to $t = 1$ h, cv. Morocco had significantly more nematodes around its tips ($p = 0.05$) than other cultivars. At both $t = 1$ h and 24 h, the two hosts *A. variabilis* and *S. media* were significantly less attractive ($p = 0.05$) than all cultivars (See Table 1). Com-

pared with root tips of cvs Doublet, Chinese Spring and Morocco, those of *A. variabilis* n° 1 did not seem to retain nematodes in their vicinity over prolonged periods of times.

ATTRACTIONS TO CO₂

When air charged with 5% CO₂ was introduced into the agar with nematodes, but in the absence of root tips, significantly more J2 were retained near the point of origin ($p = 0.05$) than either of the controls (Fig. 3 and Table 1). Numbers of J2 around the source of CO₂ initially decreased up to 25 min, but remained stable and significantly above the random distribution mean up to 1 h ($p = 0.05$).

EFFECT OF SUCROSE ON ATTRACTION TO ROOT TIPS

The presence of sucrose in the agar medium of the chamber or in the medium of culture of roots before excision, had two common effects:

i) in the controls, it significantly shortened the period of time before which the threshold of random distribution was reached ($p = 0.05$): 5 min for agar + sucrose against 25 min for agar alone and 15 min for heat-treated roots in agar + sucrose against 55 min for heat-treated roots in agar alone.

ii) in experiments with root tips of the susceptible cv. Doublet or the resistant accession *A. variabilis* n° 1, it shortened the period during which an initial decrease in J2 numbers was observed (Fig. 4): for cv Doublet this was 5 min when sucrose was still present; 10 min when

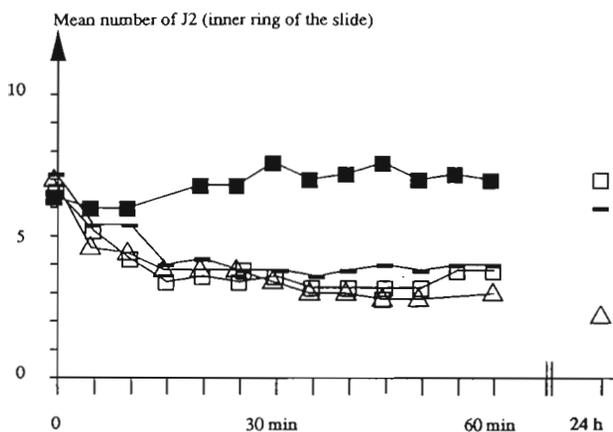


Fig. 2. Comparison of attraction of second-stage juveniles (J2) of *Meloidogyne naasi* for excised root tips. □: control agar alone ($n = 13$); △: root tip of cv. Doublet ($n = 19$); ■: root tip of cv. Morocco ($n = 20$); ▲: root tip of accession n° 1 of *Aegilops variabilis* ($n = 22$). Inoculum = 30 J2/replicate in 1% water-agar (inside a cylindrical observation chamber on slide with a diameter of 18 mm and a height of 5 mm). For each observation, the root tip (5 mm long) was inserted vertically in agar at the center of the inner ring of the slide (5 mm diameter) at $t = 0$. ($n =$ number of replicates.)

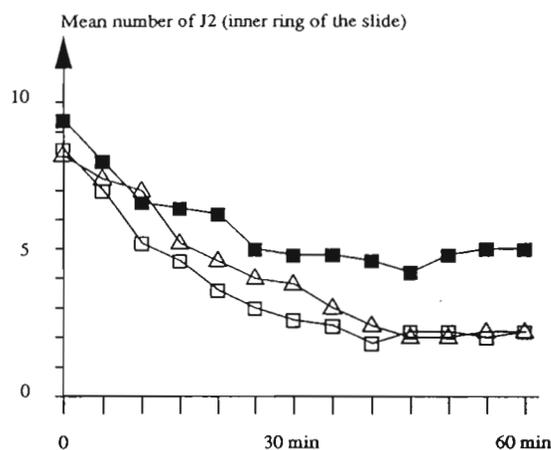


Fig. 3. Attraction of second-stage juveniles (J2) of *Meloidogyne naasi* for sources of CO₂. □ = control agar alone ($n = 13$); △ = control air bubbled ($n = 8$); ■ = air charged with 5% CO₂ bubbled ($n = 16$). For details of inoculum preparation, refer to Fig. 2. In both treatments with gas, the gas was delivered into the agar at the centre of the inner ring (5 mm diameter) at $t = 0$ (0.2 ml) then every 5 min until 1 h (0.1 ml). ($n =$ number of replicates.)

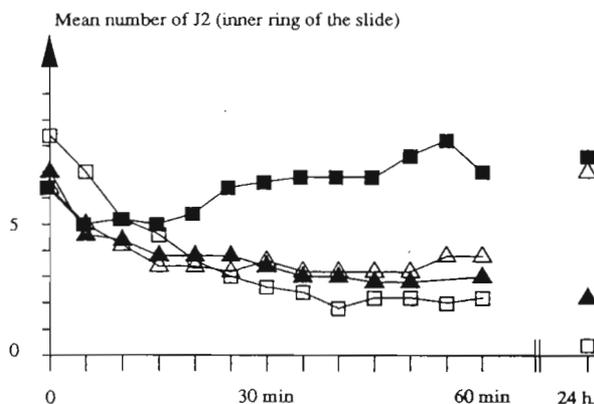


Fig. 4. Effect of sucrose in agar on attraction of second-stage juveniles (J2) of *Meloidogyne naasi* for excised root tips. □ = root tip of cv. Doublet on agar ($n = 19$); ■ = root tip of cv. Doublet on agar + 0.5% sucrose ($n = 9$); △ = root tip of accession n° 1 of *Aegilops variabilis* on agar ($n = 22$); ▲ = root tip of *A. variabilis* n° 1 on agar + 0.5% sucrose ($n = 20$); for details of inoculum and root tip preparation, please refer to Fig. 2; (n = number of replicates.)

root tip had been grown on sucrose, and 15 min when on agar alone. For *A. variabilis*, a similar pattern was observed, but over longer times, i.e. 25 min for tips both with sucrose still present and those grown on sucrose, compared with 35 min when on agar alone.

The presence of sucrose in the chamber medium also shortened the time during which maximum aggregation of J2 around roots of cv. Doublet was reached (1 h instead of > 1 h), resulting in a bigger mean at $t = 1$ h, but the same mean at $t = 24$ h ($p = 0.05$; Table 1). With *A. variabilis* this effect at $t = 1$ h was not seen (same mean as roots on agar alone), but by 24 h, the mean was significantly larger ($p = 0.05$; Table 1).

When sucrose had been used in the growing medium of roots, its effect was seen mainly before 1 h by increasing the numbers of J2 (same means found on both hosts, significantly bigger than means obtained for root tips on agar alone). At 24 h, means of both hosts were not significantly different from those obtained with sucrose on agar or cv Doublet on agar alone (Table 1).

The additional qualitative observations showed that when sucrose was added into agar,

i) nematodes were orienting to the root tips of both cv. Doublet and *A. variabilis* sooner than controls of root tips in agar without sucrose.

ii) more nematodes were attracted to the root tips of both cultivars, but fewer nematodes were attracted to *A. variabilis*.

iii) more J2 were retained on the surface of roots.

iv) the proportion of total movements (departures, returns)/inoculum was decreased.

A STUDY OF THE pH CHANGES AROUND ROOT TIPS

Although pH change was visible within 5 min, root tips were nevertheless observed up to 30 min to see the extent of pH gradient on agar and the pattern of absorption of dye inside the root tissues, which reflected differences in staining between cultivars. The indicator was progressively absorbed until all cells became heavily stained.

For all the root tips tested, pH indicators showed that the root surface was acidic and generated a gradient of pH ($5 < \text{pH} < 7$) on the agar which intensified with time. Both indicators were useful: Bromothymol Blue to show the size of the pH gradient on agar; Haematoxylin to show precisely the regions of lowest pH on the root.

Two areas of greater acidification (pH 5) could be distinguished (Fig. 5). One strictly corresponded to the root exudates, secretions and lysates (associated with the polysaccharidic mucigel around the cap, the meristem and the region of elongation). It was clearly visible on agar and more prominent in tips of larger dimension (i.e. cvs Doublet, Chinese Spring and accession "x⁸") than in the tips of *A. variabilis* n° 1. The second area was associated with the root hairs in all roots, but in *A. variabilis* n° 1 and accession "x⁸", these root hairs were longer, more numerous and closer to the elongation zone.

Furthermore, the extent and the intensity of pH gradient ($6 < \text{pH} < 7$) on agar was also lower in *A. variabilis* than in the bigger tips of other cultivars.

Observations over 30 min on the absorption of dyes into root tissues (greenish colour with bromothymol blue; red colour with haematoxylin), showed that Haematoxylin was absorbed more quickly than bromothymol blue in all root tissues. With both indicators however, three separate regions were revealed:

region (1) comprised the cap, the meristem and the elongation zones; absorption was slow and incomplete at 30 min with bromothymol blue;

region (2) the zone of differentiation, where the dyes were absorbed quickly and massively;

region (3) the root hair zone, where absorption was slower than in region (1) and incomplete at 30 min.

The specimens tested here varied in the relative size of regions 1 and 2: 56%–44% in the wheat cv. Chinese Spring, 71%–29% in *A. variabilis* n° 1 and wheat accession "x". More replicates would however be necessary for a statistical analysis. Resistant accessions of *A. variabilis* n° 1 and wheat "x⁸" were also distinguished by the presence of a darker and more clearly defined endodermis in region 1, compared with cvs Doublet and Chinese Spring.

Discussion

The miniaturized microscope slide observation chamber was adequate to show attraction to all

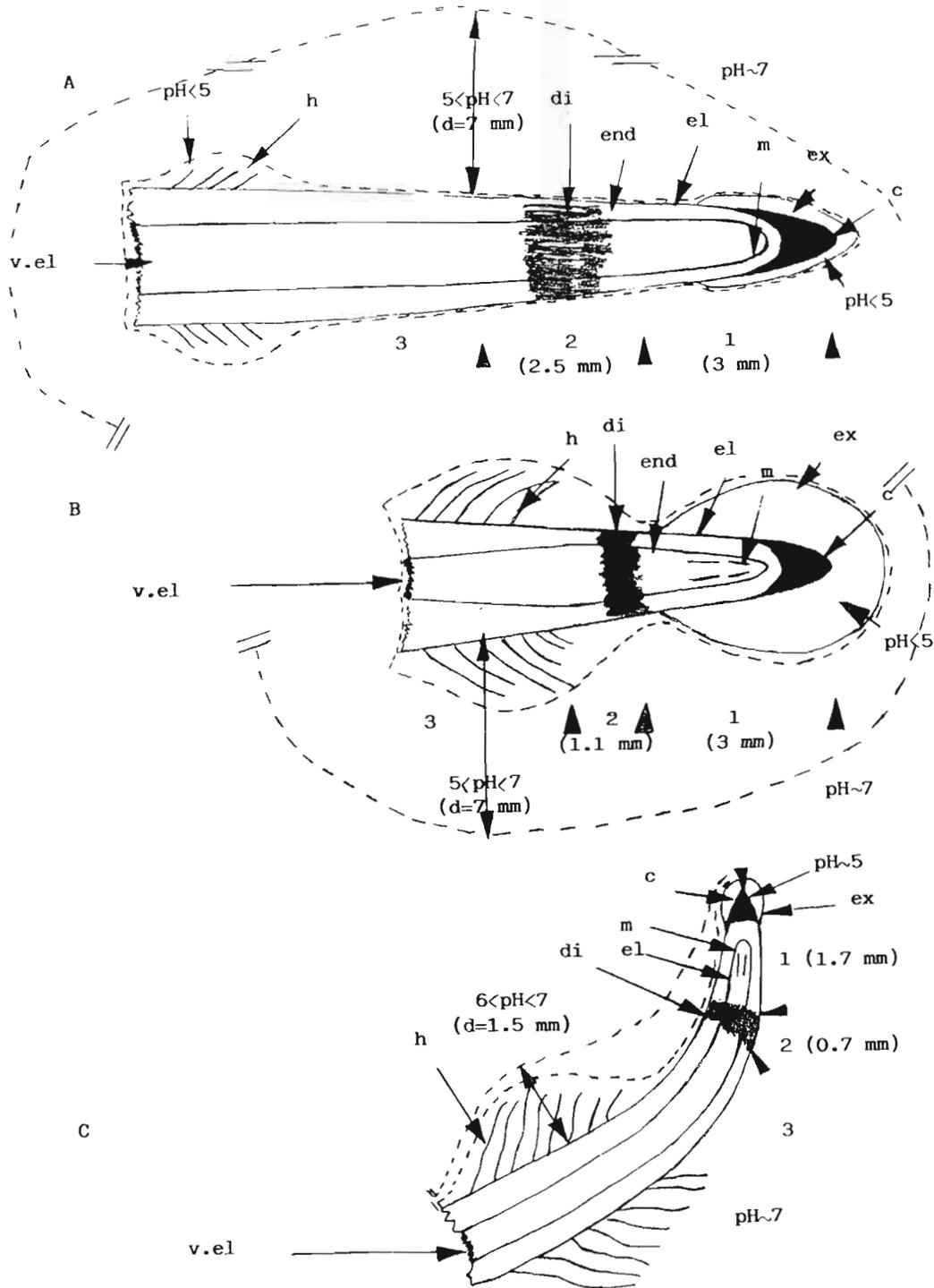


Fig. 5. Pattern of pH around excised root tips of Graminae spp. in 1% water-agar, as indicated by the use of Bromothymol Blue or Haematoxylin (Ehrlich). A: susceptible wheat cv. "Chinese Spring"; B: resistant wheat accession "x⁸"; C: resistant grass accession *Aegilops variabilis* n° 1. The curvature of the root tip of *A. variabilis* n° 1 is a general feature and therefore has been represented on the drawing. c = cap; d = maximum distance of pH gradient on agar; di = differentiation zone; ex = exudates; el = elongation zone; end = endodermis; h = hairs; v.el = vascular elements). Dotted lines indicate limits between minimum and maximum pH on agar. The scale (10 : 1) is respected for representation of root tips but not for distances of pH gradients in agar. Changes are symmetrical about the root axis.

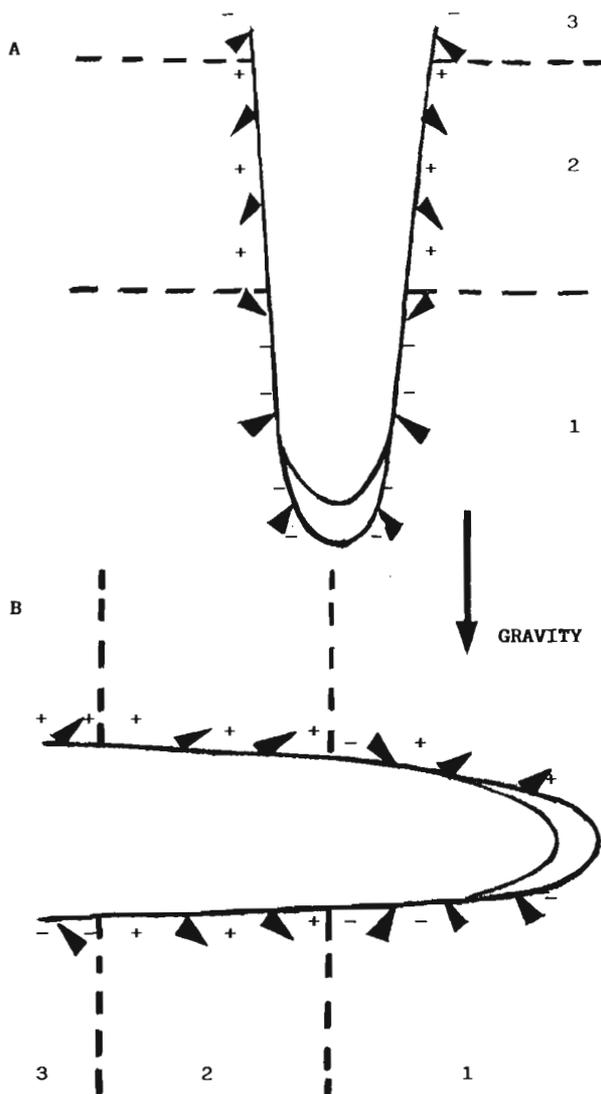


Fig. 6. Qualitative pattern of proton currents in grass roots e.g. *Triticum aestivum* (adapted from Behrens *et al.*, 1982; Miller & Gow, 1989). *A*: The root is vertical, as occurs in the soil. *B*: The root is horizontal, as artificially occurs in the agar experiment. The small arrows indicate the direction of movement of H⁺ ions. Depending on this direction, three regions can be distinguished; 1 = cap, meristem, elongation zone; 2 = differentiation zone; 3 = hair zone. + = electropositive charges; - = electronegative charges on the surface of the root. Some variations can exist, concerning the currents associated with the root cap; i.e. in *Avena sativa* (Miller & Gow, 1989) some low outward currents and in *Hordeum vulgare* (Weisenseel *et al.*, 1979) no currents at all have been recorded. The pattern is not known for *Aegilops variabilis*.

root tips tested and to the introduced CO₂. The small volume of the chamber allowed all nematodes to be retained within effective range of the tip for 24 h

rather than to become “lost” as reported with bigger volumes of agar (Bird, 1959; Lee & Evans, 1973).

Root tips of the partially resistant cv. Morocco appeared more attractive to nematodes than tips of susceptible cultivars up to 60 min, but by 24 h, the aggregation was similar. Thus the non-specific attractiveness of cv. Morocco may be higher but the retention less, perhaps due to the presence of some later acting “inhibitory” substances. The fully resistant accessions of *H. chilense* were also very attractive to nematodes. Tips of both the resistant accession n° 1 of *A. variabilis* and the wild susceptible population of *S. media* were less attractive than other hosts, yet remained more attractive than the negative controls. These observations confirm the truth of Viglierchio’s observation (1961) that “correlation of attraction with host preference or specificity was unwarranted” and might only be the result of “a fortuitous selection”. The attraction of J2 to the exogenous source of CO₂ confirms several observations made previously that this gas is probably a general non-specific attractant acting at a distance.

Observations on root tips in agar with pH indicators revealed that the changes of pH were rapid (< 5 min). They were thus considered to express the normal processes involved in root tip metabolism. The acidification of agar by the root tips probably results from two distinct phenomena: *i*) the release of CO₂ in respiration and the formation of carbonic acid present in root exudates; *ii*) the existence of proton current flows.

It is interesting to interpret the responses of roots in indicator agar in the light of the proton current theories of Behrens *et al.* (1982) and Miller and Gow (1989), and to compare the three regions found here (Fig. 5) with the qualitative pattern of proton current in root tips of grasses (Fig. 6).

In roots growing horizontally (Fig. 6), region (1) is crossed by a flux of protons (influx at the bottom, efflux at the top). This would explain the acidification around the upper surface of the root.

In the wheat accession “x⁸”, the dark color obtained at the site of the endodermis in bromothymol blue-treated agar, could be explained by the fact that the dye was accumulating into the differentiating endodermis. As the cytosol pH increased with time (with excised roots, the system no longer functioned in homeostatic conditions), the dye became blue-green. The absence of such a phenomenon in cv. Chinese Spring could be due to the absence of differentiation of any cells at that stage, as suggested by the more uniform and lower distribution of the dye in the tissue. With haematoxylin, due to a lower pK_a, the process might be seen even sooner on *A. variabilis*, resulting in a rapid saturation and staining of all the region.

The second region is characterized by a leakage of protons out of all cells, leading to an alkaline cytosol pH,

explaining the darker color obtained in this zone after absorption of the dye and the even darker coloration at the endodermis layer for both Chinese Spring and "x⁸".

The third region behaves in a similar way to the first zone, except that the proton flows are less intense. The greater vacuole/cytoplasm ratio could act as a buffer for the slow process of alkalization, resulting in the absence of coloration of all tissues.

The reduced acidification and rhizodeposition shown by *A. variabilis* compared with other plants could explain the poor attractiveness of its tips to nematodes. The susceptible cvs Doublet and Chinese Spring, as well as in resistant accession "x⁸" had similar acidification and rhizodeposition, which possibly explains the similar attractiveness (Balhadère, 1993).

These results allow the formulation of a theoretical sequence of signals and responses from roots and nematodes respectively. In soil both CO₂ at a distance, then electrical potential gradients on the root tip, could draw nematodes towards the favorable zone for invasion, i.e. the region of meristematic and elongation zones, both sites of maximum CO₂ release and electronegative charges (Croll, 1970; Whipps, 1990). Together with some putative kairomone substances, the trigger for penetration could be the reversal of the electrical potential gradient between the regions 1 and 2, which would lead to concentration of J2 in region 1. The same would occur in agar, except that with a root tip placed horizontally, negative charges would be located at the bottom surface of the root (Fig. 6). The implications of negative charges in attraction of nematodes would be consistent with the observations in agar, where horizontal roots were invaded by J2 of *M. naasi*. Here, the J2 were observed accumulating mostly underneath the root tips (Balhadère, 1993).

It was observed that the relative sizes of regions 1 and 2 differed between cultivars and that the reduction in size of region 2 relative to region 1 in *A. variabilis* and accession "x⁸" may produce some difficulty for the J2 in discriminating between the narrowly separated regions of inward and outward proton current flows. It could also explain how some trapping of nematodes inside the root hair zone is possible (Siddiqui, 1971). Both of these mechanisms may contribute to the lower penetration observed in the resistant accessions (Balhadère, 1993). The earlier differentiation of tissues might therefore have been genetically linked to the expression of resistance or to some other genes associated with the resistant gene "Rkn-mn 1" and introgressed with it.

A better understanding of the effect of root tip size on resistance might be obtained after characterization of the currents generated by roots of susceptible and resistant cultivars following the technique of vibrating probe examination described by Miller and Gow (1989).

The effect of sucrose (a non-attractive molecule itself, as shown by the absence of attraction of J2 to cylinders of sucrose and former experiments of Bird, 1959 on *M. javanica*), has already been questioned by Loewenberg *et al.* (1960) and McClure and Viglierchio (1966). It could act by inducing in juveniles a change from migratory behaviour into exploratory behaviour, as had been reported with *Aphelenchus avenae* (Fisher, 1975), *Pratylenchus scribneri* (Tsai & Van Gundy, 1990) or *Heterodera schachtii* (Grundler *et al.*, 1991). By stating the stylet thrusting sequence and enhancing the cumulative effect of root exudates (Grundler *et al.*, 1991), it may lead to a higher retention of nematodes on roots of *A. variabilis*.

This hypothesis of induction of behaviour change is an attractive way in which to interpret the results reported here. It is supported by the observations that in already attractive cv. Doublet tips, sucrose in agar acts at an early stage to retain nematodes around tips, an effect only seen after 24 h for the root tips on agar alone. Moreover in the controls treatments (sucrose cylinders or heated roots on sucrose), there was an earlier negative response of nematodes, expressed by a departure away from the inner ring (compared with agar alone or heated roots on agar).

Other chemicals have been found to induce nematode stylet thrusting and salivation, among which are some plant compounds such as catechol and caffeic acid (McClure & von Mende, 1987) but their effect was not examined in this study.

In addition to its direct effects on nematodes, sucrose probably has a stimulatory effect on root tip metabolism, in enhancing the production of CO₂ and organic acids (therefore increasing the proton efflux; Scott, 1967).

In conclusion, it is interesting to contrast the root tip complex of the resistant wild grass *A. variabilis* to the root tip complex of selected cultivars of cereals, which are both invaded by J2 of *M. naasi*. The former presents a set of physical and physiological barriers which efficiently limit the invasion of its parasite and ensure the survival of the root and the plant; many selected cultivars do not possess these mechanisms, possibly as a result of selection for root vigour as a contributory factor to grain yield.

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