The translocation of calcium from oat roots infected by the cereal cyst nematode *Heterodera avenae* (Woll.)

Nigel S. PRICE * and John SANDERSON

Dept. of Zoology, University of Reading, Reading, Berks. RG6 2AT, U.K.
and A.R.C. Letcombe Laboratory, Wantage, Oxon, OX12 9JT, U.K.

SUMMARY

Oat seedlings were grown in sand, a third of the plants were inoculated with second-stage juveniles of *H. avenae*. Plants were transferred to hydroponic culture where lengths of seminal root axes, without nematodes or with established J3 *H. avenae*, were labelled with $^{45}Ca^{++}$. Translocation of calcium to the shoots was eleven times greater from the labelled nematode infected roots than from the labelled uninfected roots. Histological examination showed nematodes caused major disruption of the endodermis. It was concluded that nematode invasion and establishment maintened the apoplastic pathway for calcium in regions of the root where it is normally blocked by the development of the endodermis.

RÉSUMÉ

Le transport du calcium des racines d'avoine infestée par le nématode à kystes des céréales Heterodera avenae *(Woll.)*

Des plantules d'avoine sont cultivées dans du sable et un tiers d'entre elles sont inoculées avec des juvéniles du second stade d'*H. avenae*. Les plantes sont transférées en culture hydroponique où des portions de racines seminales, avec ou sans nématodes, sont marquées avec du $^{45}Ca^{++}$. Le transport de calcium vers les parties aériennes est onze fois plus élevé à partir des racines infestées qu'à partir des racines non infestées. L'examen histologique révèle que les nématodes provoquent une rupture importante de l'endoderme. On en conclut que la pénétration et l'établissement des nématodes maintient la voie apoplastique de pénétration du calcium dans les régions où elle est normalement empêchée par le développement de l'endoderme.

Characteristic aerial symptoms of plant damage by root endoparasitic nematodes include those of nutrient deficiencies. In oats (*Avena saliva* L.) attacked by the cereal cyst nematode *Heterodera avenae* Woll., leaves may show symptoms typical of nitrogen and phosphorus deficiencies (Kort, 1972). It has been suggested that deficiences result from reduced root growth caused by nematode invasion (Evans, Trudgill & Brown, 1977) rather than impaired root function, Price, Clarkson and Hague (1982) finding the uptake of phosphorus and potassium in oats infested with *H. avenae* unaffected or enhanced. However calcium deficiencies are not characteristic of nematode damaged plants, workers even finding increased amounts of calcium in both potato infested with potato cyst nematodes (Trudgill, Evans & Parrott, 1975 ; Trudgill & Cotes, 1983) and wheat infested with *H. avenae* (Rovira, 1979).

We report here an experiment examining the effect of established *H. avenae* on the uptake of calcium by oat roots. Histological studies are presented to explain our results and their implications discussed.

Materials and methods

PREPARATION OF PLANTS

Sixty seeds of oats cv. Maris Huntsman, were germinated and sown individually in pots (d. 7.5 cm) containing coarse sand. Plants were grown for eight days in the glasshouse and lightly watered as necessary. Second-stage juveniles of *H. avenae* were obtained from cysts extracted from field soil (Trudgill, Evans & Faulkner, 1972) and hatched on trays (Whitehead & Hemming, 1965). Twenty of the pots had nematodes added on three occasions, receiving a total of approximately 400 second-stage juveniles each over the eight days of sand growth.

After eight days all seedlings were carefully washed from pots and transferred to hydroponic culture jars containing nutrient solution (mM : KNO_3 : 0.5 ; K_2SO_4 : 2.25 ; $MgSO_4$: 0.5 ; KH_2PO_4 : 0.25 ; $CaCl_2$: 1.0 ; together with micronutrients and iron-EDTA). Plants were grown in nutrient solution in the glasshouse for an additional eight days.

* Present address, Dept. of Plant Pathology, University of Georgia, Athens, GA 30602, U.S.A.

Radio-active labelling

After eight days of hydroponic growth (sixteen days from initial sowing) twelve control plants (that had not received nematodes) and six nematode inoculated plants were selected to be placed in six replicate sets of the apparatus shown on Fig. 1.

A plastic container (approximately 20 cm × 10 cm × 5 cm) had two control plants and one inoculated plant attached with tape to an end wall, with roots lying out in 0.1 normal strength nutrient solution. Two plastic vial caps (d. 1.5 cm) with two slots cut in their walls were placed in the tanks to form small compartments separate from the main body of nutrient solution. Through the slots and across each cap was passed a seminal root, one from a control plant, the other a length of seminal root of an inoculated plant that displayed symptoms of nematode damage (a slight thickening and some discoloration). The length of seminal root passing through a cap was sealed and separated from the main solution with a wax-resin mixture after air drying the surface of the root (Scott-Russell & Sanderson, 1967). The caps were also filled with 0.1 strength nutrient solution. The third control plant was left entirely in the main body of nutrient solution.

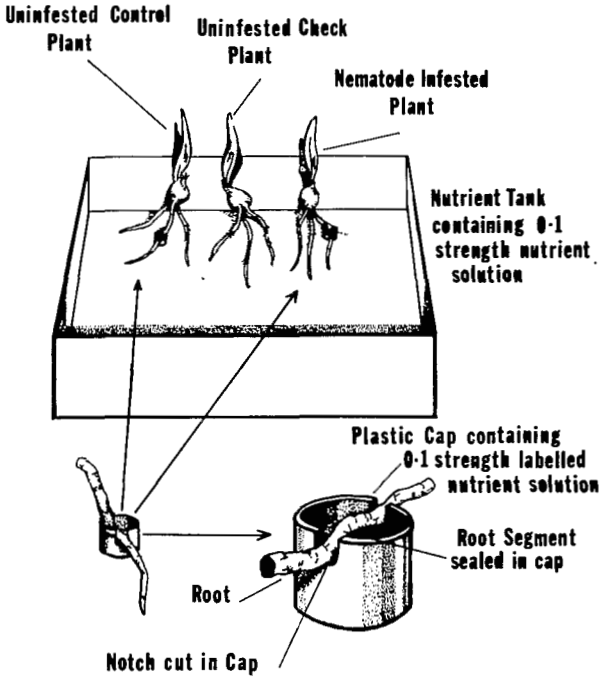

Fig. 1. A diagrammatic representation of the apparatus used in the radio-labelling experiment.

The apparatus was left overnight after which the solution in the tank was replaced with fresh 0.1 strength nutrient solution and the solution in the caps removed (by syringe) and replaced by 0.1 strength nutrient solution labelled with $^{45}CaCl_2$ to give an activity of $^{45}Ca^{++}$ of 25 μCi ml^{-1}. The apparatus was left for a treatment period of 24 hours.

Examination of material

After 24 h the apparatus was dismantled and shoot and root fresh weights taken. Lengths of root within the caps were retained and individually preserved in F.A. 4 : 1 (Southey, 1970). Shoot and remaining root material was prepared for activity counting. Tissue was wet ashed to dryness and made up to 1 ml with 10% nitric acid and 0.1 ml of each sample was transferred to planchettes and counted on a Beckman Lowbeta II Planchette Counter (Price, Clarkson & Hague, 1982). Retained lengths of root were stained and cleared in lactophenol acid-fuchsin (Southey, 1970) and examined for nematodes.

Histological examination

Additional oat plants were sown, inoculated and later transferred to hydroponic culture as described above. Root segments showing damage symptoms were removed, dipped into methyl-cellulose and frozen in liquid nitrogen. Twelve μm sections were cut at — 20° in a cryostat (Sanderson, 1972). The ribbons of sections were melted on glass slides and quickly refrozen before being transferred to acetic alcohol (3 ml acetic acid in 100 ml ethanol) at — 20° and then allowed to warm to room temperature and immersed in ethanol for ten minutes.

Sections illustrated in Figure 2 A and C were dehydrated in absolute alcohol followed by xylene for 24-48 h to remove soluble fats. Sections were then hydrated to 70% alcohol and immersed for ten minutes in a saturated solution of Sudan Black B in 70% alcohol, hydrated through an alcohol series to water and mounted in Hydromount. The section illustrated in Figure 2 C was dehydrated through an alcohol series and overstained in a 0.03% safranin solution for 4-5 h before washing in water, then dehydrated and stained in Fast Green for 2-5 min. The section was then passed through an ethanol-xylene 1 : 1 mixture into three changes of xylene and mounted in Euparol.

Results

In the labelling experiment all root lengths of inoculated plants retained for examination (that

had been within the cap and exposed to $^{45}Ca^{++}$) contained nematodes, between two and seven J3 *H. avenae* being found in each 1.5 cm length of root.

Activity counts in the shoots of the two treated plants per apparatus were adjusted by subtracting the count found in the shoot of the third control plant. This ensured that the shoot counts represented calcium translocated from the labelled length of root of each plant, and was not due to any leakage of isotope from the caps with uptake elsewhere in the root system.

When counts were adjusted to nanomoles Ca^{++} and expressed on a fresh weight basis, calcium translocation to the shoot (Ca^{++} nM g^{-1} fresh weight of shoot $\pm$ 95% confidence limits) had a mean value of 1.46 $\pm$ 0.69 nM from the uninvaded 1.5 cm lengths of root axes and 16.12 $\pm$ 4.48 nM from the nematode invaded 1.5 cm lengths of root axes, a difference significant at $P > 0.001$.

Prepared sections (Fig. 2) show that *H. avenae* can cause disruption to the internal organization of the root. Figure 2 A illustrates an enlargement and breakdown of the endodermis, which was unaffected away from the area of nematode establishment. Figure 2 B illustrates that the syncytial feeding structures formed by the nematode may

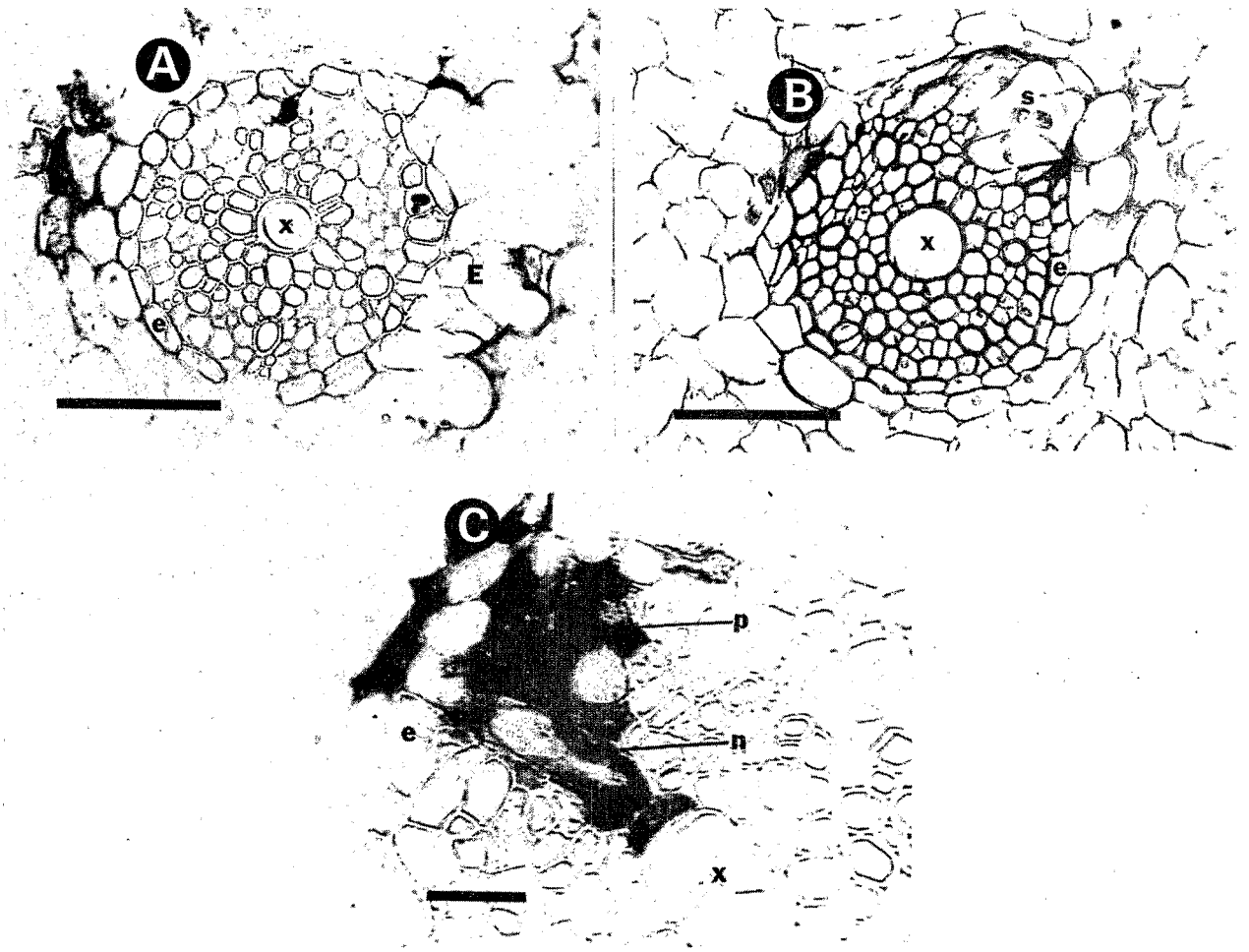

Fig. 2. Transverse section of oat seminal roots. A : Showing an enlargement and breakdown of the endodermis caused by *H. avenae*. (E = enlarged endodermis ; e = normal endodermis ; x = central xylem vessel ; bar represents 50 μm). B : showing syncitial feeding structures (s) traversing the endodermis (e). (Bar represents 50 μm). C : showing the anterior portion of a J3 *H. avenae* (n) lying within the vascular tissues and endodermis (e). Heavily staining phenolic substances (p) have been produced in response to this damage. (Bar represents 100 μm).

traverse the endodermis, lying in both the stele and cortex, and this may also occur with the nematode itself (Fig. 2 C).

Discussion

There are considered to be two pathways for the radial movement of ions across the root to the xylem. The symplastic pathway, through the cytoplasm, from cell to cell, through interconnecting plasmodesmata, and the apoplastic pathway through free space in cell walls (Scott-Russell, 1977). Ions such as potassium and phosphate are transported through the symplasm and may be absorbed along the whole length of a root (Scott Russell, 1977). Calcium is taken up apoplastically, a pathway effectively blocked by the development of the endodermis (Clarkson & Robards, 1975) and uptake, especially from dilute solutions (likely to occur in soil), is generally restricted to the apical parts of the root (Robards *et al.*, 1973).

A major effect of nematode invasion is on plant root growth. Reduced root extension resulting from mechanical impedence may shorten the extension zone of the root (Goss & Scott-Russell, 1980), and the endodermis has been observed to mature much closer to the root apex in nematode damaged roots (Christie, 1959 ; Wyss, 1978). This earlier maturation, by reducing the apoplastic zone of the root would be expected to lead to reduced calcium uptake (Drew, 1979).

Figure 2 shows that *H. avenae* endoparasitism may cause considerable disruption to the endodermis. In the radio-labelling experiment the amount of calcium translocated from lengths of root containing established J3 *H. avenae* was over eleven times that translocated from lengths of root on uninoculated control plants labelled in the same manner.

We conclude that the disruption to the endodermis caused by the nematode and its associated feeding structures maintains an apoplastic pathway for the uptake of calcium in regions of the root where such a pathway is normally closed off. Jones (1981) has made the same suggestion based on consideration of reports of leakage of metabolites from, and pre-disposition to secondary infection of nematode parasitized roots.

This work suggests that in addition to changes in root system size and morphology, endo-parasites such as *Heterodera* may alter the functional histology of roots. It may be necessary to consider both aspects in interpreting both the mineral nutrition and water relations of nematode infested plants (Evans & Franco, 1979) as water itself is taken up both apoplastically and symplastically, but at much greater rates through the apoplast (Clarkson & Robards, 1975 ; Sanderson, 1983).

ACKNOWLEDGEMENTS

The senior author wishes to acknowledge the financial support of the Agricultural Research Council and thank Lydia Nolan-Davis for preparing Figure 1.

REFERENCES

CHRISTIE, J.R. (1959). *Plant nematodes, their bionomics and control.* Gainesville, Univ. Fla. Agr. Exptl Sta., 256 p.

CLARKSON, D.T. & ROBARDS, A. W. (1975). The endodermis, its structural development and physiological rôle. In : Torrey, J.G. & Clarkson, D.T. (Eds). *The development and function of roots.* Proc. 3rd Cabot Symp. London, Academic Press : 415-436.

DREW, M.C. (1979). Properties of roots which influence rates of absorption. In : Harley, J.L. & Russell, R.S. (Eds). *The soil-root interface.* London, Academic Press : 23-28.

EVANS, K. & FRANCO, J. (1979). Tolerance to cyst nematode attack in commercial potato cultivars and some possible mechanisms for its operation. *Nematologica*, 25 : 153-162.

EVANS, K., TRUDGILL, D.L. & BROWN, N.J. (1977). Effects of potato cyst-nematodes on potato plants V. Root system development in lightly and heavily infested susceptible and resistant varieties, and its importance in water and nutrient uptake. *Nematologica*, 23 : 153-164.

GOSS, M.J. & SCOTT-RUSSELL, R. (1980). Effects of mechanical impedence on root growth in barley (*Hordeum vulgare* L.) III. Observations on the mechanism of response. *J. exp. Botany*, 31 : 577-588.

JONES, M.G.K. (1981). Host cell responses to endoparasitic attack : structure and function of giant cells and syncytia. *Ann. appl. Biol.*, 97 : 353-372.

KORT, J. (1972). Cereals of temperate climates. In : Webster, J.M. (Ed.). *Economic Nematology.* London, Academic Press : 97-126.

PRICE, N.S., CLARKSON, D.T. & HAGUE, N.G.M. (1982). The uptake of potassium and phosphorus in oats infested with the cereal cyst nematode, *Heterodera avenae* Woll. *Revue Nématol.*, 5 : 321-325.

ROBARDS, A. W., JACKSON, S.M., CLARKSON, D.T. & SANDERSON, J. (1973). The structure of barley roots in relation to the transport of ions into the stele. *Protoplasma*, 77 : 291-312.

ROVIRA, A.D. (1979). Biology of the soil-root interface. In : Harley, J.L. & Russel, R.S. (Eds). *The soil-root interface.* London, Academic Press : 145-160.

SANDERSON, J. (1972). Micro-autoradiography of diffusable ions in plant issues : problems and methods. *J. Microsc.*, 96 : 245-254.

SANDERSON, J. (1983). Water uptake by different regions of the barley root. Pathways of radial flow in relation to development of the endodermis. *J. exp. Botany*, 34 : 240-253.

SCOTT-RUSSELL, R. (1977). *Plant root systems : Their function and interaction with the soil.* London, McGraw Hill, 298 p.

SCOTT-RUSSELL, R. & SANDERSON, J. (1967). Nutrient uptake by different parts of the intact roots of plants. *J. exp. Botany,* 18 : 491-508.

SOUTHEY, J.F. (1970). *Laboratory methods for work with plant and soil nematodes.* Techn. Bull. No. 7, London, H.M.S.O., Ministry of Agriculture, 148 p.

TRUDGILL, D.L. & COTES, L.M. (1983). Tolerance of potato to potato cyst nematodes *(Globodera rostochiensis* and *G. pallida)* in relation to the growth and efficiency of the root system. *Ann. appl. Biol.,* 102 : 385-397.

Accepté pour publication le 2 septembre 1983.

TRUDGILL, D.L., EVANS, K. & FAULKNER, G. (1972). A fluidising column for extracting nematodes from soil. *Nematologica,* 18 : 469-475.

TRUDGILL, D.L., EVANS, K. & PARROTT, D.M. (1975). Effects of potato cyst-nematodes on potato plants II. Effects on haulm size, concentration of nutrients in haulm tissue and tuber yield of a nematode resistant and a nematode susceptible variety. *Nematologica,* 21 : 183-191.

WHITEHEAD, A.F. & HEMMING, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. appl. Biol.,* 55 : 25-38.

WYSS, U. (1978). Root and cell response to feeding by *Xiphinema index. Nematologica,* 24 : 159-166.