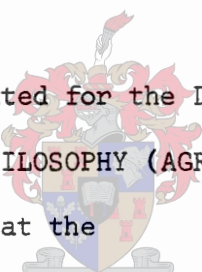


AN ANATOMICAL AND EXPERIMENTAL STUDY ON CHANGES INDUCED
BY *MELOIDOGYNE HAPLA* CHITWOOD, 1949 IN *VITIS* ROOTS

by

D.J. Joubert

Thesis presented for the Degree of
DOCTOR OF PHILOSOPHY (AGRICULTURE)
at the
University of Stellenbosch

The crest of the University of Stellenbosch is centered behind the text. It features a shield with various symbols, including a book and a scale, topped with a crown and surrounded by decorative flourishes.

Promoter: Prof. Dr. C.J. Orffer

Co-Promoter: Dr. M.P. de Vos

External Examiner: Dr. J. Heyns

December 1971

ACKNOWLEDGEMENTS

Gratitude and appreciation are expressed to the following:

Prof. Dr. C.J. Orffer, Head of the Department of Viticulture of the University of Stellenbosch, who suggested this study, for his interest and constructive criticism during the compilation of the manuscript.

Dr. M.P. de Vos, Senior Lecturer in the Department of Botany of the University of Stellenbosch, for her interest, invaluable advice and guidance during the course of this study; her most constructive criticism, moral support and unselfish assistance during the compilation of the manuscript.

Dr. J. Heyns, Senior Lecturer in the Department of Zoology of the Rand Afrikaans University, Johannesburg, for invaluable advice and guidance, especially in the initial stage of this study; his identification of the nematode concerned and his most valuable criticism of the manuscript.

Dr. M.L. Milne of the Research Institute for Citrus and Subtropical Fruit, Nelspruit, for helpful advice in connection with staining techniques of nematodes in roots and for the loan of valuable literature.

Prof. Dr. P.G. Jordaan, Head of the Department of Botany of the University of Stellenbosch for placing indispensable facilities of his Department at my disposal.

The Department of Agricultural Technical Services for facilities placed at my disposal.

Mrs. J.M. Serdyn who typed this thesis, for keen interest taken in the typing.

Finally I would like to express my gratitude to my wife and children for their immeasurable sacrifices and continuous enthusiasm they engendered during the course of this study.

A B S T R A C T

The object of this anatomical study was to collect scientific data on the effect of *Meloidogyne hapla* Chitwood, 1949, on the roots of the following grapevine cultivars viz: *Vitis vinifera* L. cvs. Steen and White French and the root-stocks, Jacquez, 1202 C, 99 R, Salt Creek and Dogridge.

These cultivars differed widely in their resistance to *M. hapla* attacks. In the roots of Steen, White French, Jacquez and 1202 C the formation of multinucleate syncytia by the destruction of the walls of groups of cells often occurred. In Salt Creek, Dogridge and 99 R roots, syncytia were observed in the stele only. The formation of abnormal xylem as a result of nematodal activities was a common occurrence. In the roots of these latter three cultivars, *M. hapla* could not complete its life cycle.

Salt Creek, Dogridge, 99 R and often Steen formed a wound periderm which prevented the nematodes from reaching the xylem. Histological changes were often induced in advance of the invading nematodes.

UITTREKSEL

Die doel met hierdie anatomiese studie was om wetenskaplike inligting te versamel aangaande die uitwerking van *Meloidogyne hapla* Chitwood, 1949, op enkele cultivars van wingerdstokke, te wete Steen en Fransdruif van *Vitis vinifera* L. en die onderstokke Jacquez, 1202 C, 99 R, Salt Creek en Dogridge.

Hierdie cultivars het onderling baie verskil in hul weerstandvermoë teen *M. hapla*. Ten gevolge van die vernietiging van die wande van selgroepe, in die wortels van Steen, Fransdruif, Jacquez en 1202 C, is veelkernige sinsiete (Eng. syncytia) gevorm. In die wortels van Salt Creek, Dogridge en 99 R, is sinsiete net in die sentrale silinder waargeneem. Die vorming van abnormale xileem weens nematodiese bedrywighede was 'n baie algemene verskynsel. *M. hapla* kon in laasgenoemde drie cultivars nie sy lewenskringloop voltooi nie.

In Salt Creek, Dogridge, 99 R en dikwels ook in Steen, is wondperiderm gevorm, waardeur die nematodes verhinder was om die xileem te bereik. Die indringende nematodes het voor hulle uit dikwels histologiese veranderinge in die wortels teweeggebring.

- - - - -

C O N T E N T S

	<u>Page</u>
ACKNOWLEDGEMENTS	i
ABSTRACT	ii
UITTREKSEL	iii
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
2.1 Anatomy of normal roots	4
2.2 Influence of <i>Dactylosphaera vitifolii</i> Shimer on <i>Vitis</i> roots	4
2.3 Reaction of <i>Vitis</i> species and cultivars to nematodes	4
3. MATERIAL AND METHODS	12
3.1 Plant material used	12
3.1.1 <i>Vitis</i> cultivars	12
3.1.2 <i>Solanum lycopersicum</i> L. cv. Pearce and <i>Nicotiana tabacum</i> L. cv. Elsenburg Soulook	12
3.2 Growth medium for plants	12
3.3 Containers for cultivation of plants	13
3.3.1 Containers with corrugated iron sides	13
3.3.2 Earthen-ware and plastic pots	13
3.4 The handling of plants	13
3.5 Nutrition and watering of plants	15
3.6 Microtechnical techniques	16
3.6.1 Isolating and handling of <i>Meloidogyne hapla</i> Chitwood, 1949	16
3.6.2 Staining nematodes in rootlets	17
3.6.3 Sampling, killing and fixing of plant material	17
3.6.4 Dehydration, infiltration and embedding in paraffin wax	18
3.6.5 Microtome sectioning of material in wax	18
3.6.6 Staining paraffin sections	18
3.6.7 Microchemical tests	18
3.6.8 Photography	18
4. THE EXTERNAL MORPHOLOGY OF <i>VITIS</i> SPP. ROOTS	23

5.	THE INFLUENCE OF <i>M. HAPLA</i> ON THE PRIMARY STRUCTURE OF							
	<i>VITIS</i> ROOTS	22
5.1	Root cap and epidermis		22
5.2	Cortex	23
5.3	Endodermis		24
5.4	Pericycle		24
5.5	Primary xylem		24
5.6	Primary phloem and procambium			25
5.7	Pith parenchyma	25
5.8	Syncytia in the central cylinder			25
5.8.1	Formation of syncytia		25
5.8.2	Cell-walls of syncytia and cells in the immediate vicinity						26
5.8.3	Contents of syncytia		27
6.	THE CHANGES INDUCED BY <i>M. HAPLA</i> IN SECONDARY GROWTH OF <i>VITIS</i> ROOTS							29
6.1	Nematode eggs and development of larvae			29
6.2	The penetration of <i>M. hapla</i> in roots with secondary growth and the formation of syncytia				31
6.3	Periderm formation in roots and its effect on nematodes						32
6.4	The penetration of nematodal products towards the root pith						35
6.4.1	1202 C		35
6.4.2	Jacquez		36
6.4.3	Steen		37
6.4.4	99 R		38
6.4.5	Dogridge and Salt Creek		39
6.4.6	Partial obliteration of vessels			40
6.4.7	The formation of abnormal tracheary elements					40
6.4.7.1	In central cylinder		40
6.4.7.2	In the secondary xylem of two-year-old roots					40
6.4.8	Cambial cells affected by nematode attacks				41
6.4.9	Pericycle and secondary phloem affected by nematodes						42
7.	DISCUSSION		43
7.1	The influence of soil and climatic conditions on the reactions of nematodes				43

	<u>Page</u>
7.2 Physiological races	43
7.3 Resistance to nematodes	43
7.4 Wound periderms	46
7.5 Necrosis	47
7.6 Occlusion of vessels	48
7.7 Tyloses	48
7.8 Gums in vessels	50
7.9 Hypersensitivity	51
7.10 Nematodes and the production of growth regulators	52
7.11 External morphology of roots and root-knots	54
7.12 Nematode eggs and larvae	55
7.13 Influence of the nematode on the internal structure of the root	58
7.13.1 Root cap	58
7.13.2 Cortex	58
7.13.3 Endodermis	59
7.13.4 Pericycle	60
7.13.5 Phloem	61
7.13.6 Xylem	63
7.13.7 Syncytia	66
7.13.7.1 The initiation of syncytia	66
7.13.7.2 Cell contents of syncytia	71
8. SUMMARY	75
ABBREVIATIONS	77
FIGURES AND LEGENDS	81-195
FIGURE INDEX	196
LITERATURE CITED	197

INTRODUCTION

No organ or tissue can be properly understood unless its ontogeny, anatomy and physiology are well known.

This statement is the basic principle on which the clinical tests and therapeutic treatments, of the medical profession is based. No wonder that this profession makes such phenomenal strides.

The above-mentioned basic principle should also be applied to the organs of *Vitis* species including their roots.

The ontogeny, anatomy and physiology of the *Vitis* roots should be known in order to understand the vine with respect to the following:

- 1.1. Histogenesis and initial vascularisation of the roots
- 1.2. Root development under different conditions e.g. soil texture and water content of the soil
- 1.3. The absorption of plant nutrients as well as other compounds through the roots and leaves in their effect on the roots and on harmful nematodes.

Some insecticides, fungicides and herbicides applied to the aerial or subterranean parts of the vine may directly or indirectly have a harmful or beneficial effect on the roots.

According to Davide and Triantaphyllou (1965) foliar application of maleic hydrazide on various host plants has an influence on sex differentiation of *Meloidogyne javanica* (Treub, 1885) and *M. incognita* (Kofoid and White, 1919) Chitwood, 1949.

According to Nusbaum (1958) maleic hydrazide applied to shoots of susceptible tobacco plants growing in soil infested with *M. incognita* caused inhibition of gall development and nematode reproduction.

It was found that the herbicide, 2,4-dichlorophenoxy acetic acid (2,4-D) may cause a nematode infection to increase fourfold in the case of some plants resistant to *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, and fivefold in the case of susceptible plants (Krusberg, 1967).

It is thus essential that the effect of foliar sprays on the anatomy, cytology and physiology of plant roots, including those of *Vitis* should be known.

Abnormal plant nutritional conditions may cause abnormal roots e.g. root tips of zinc deficient tomato plants may show a series of three to six swellings at one or three millimetre intervals behind the apex (Steward, 1963):

1.4 The influence of harmful and beneficial soil organisms on *Vitis* roots.

A synergistic fungus root-knot nematode interaction in some plants, e.g. *Gossypium* spp., *Nicotiana* spp., *Medicago sativa*, *Vigna* spp. and *Citrus* spp. is well known. (Birchfield and Jones, 1966; Bowman and Bloom, 1966; Feder and Feldmesser, 1961; Lucas, Sasser and Kelman, 1955; Martin, Newson and Jones, 1956; McGuire, Walters and Slack, 1958; Miles, 1939; Mountain, 1954; Powell and Nusbaum, 1960; Powell, 1963; Schindler, Stewart and Semenink, 1961; Smith, 1954; Taylor, Barker and Owens, 1939; van Gundy and Tsao, 1963; Walters and Slack, 1956).

It is well-known that species of *Meloidogyne* are very well represented in South African soils. Smith (1967) found *Meloidogyne* in 77 out of 100 soil samples taken from South African vineyards.

As a very large number of plant species, including weeds and cultivated plants, on South African wine farms are susceptible to attacks by *Meloidogyne* spp., we should have detailed information on the reaction of vines to these nematodes.

Nematodes in plant roots, e.g. strawberries, may survive the winter months with temperatures of 10-25°F below zero and even when the soil freezes to a depth of 1,25 m (Thorne, 1961).

In the Western Cape of the Republic of South Africa, with its moderate climate, *M. hapla* Chitwood, 1949 can thus easily winter in plant roots to re-infest plants during spring. Thorne also recorded that this nematode was widely distributed by seed potatoes and other plant material. It has also been reported from several continents, including Africa.

In the Republic of South Africa, vines are frequently grown on old potato fields or potatoes are grown as catchcrops in vineyards between one- and two-year-old vines. Consequently it was decided to investigate to what extent vines are attacked and damaged by this nematode.

- - - - -

2. LITERATURE REVIEW

2.1 Anatomy of normal roots

Since the middle of the previous century different parts of undamaged *Vitis* roots of different grapevine cultivars have been examined by several investigators: Foëx, 1877; Cornu, 1878; Millardet, 1897; Ravaz, 1897; Kövessi, 1903; Szigeti-Gyula, 1905; Guillon, 1905; Weber, 1909; Viala, 1910; Kroemer, 1923; Alexandrov, 1926; Perold, 1926; Staehlin, 1927; Abesadze, Makarevskaja, Zchakaja, 1930; Turner, 1935; Rodriques, 1942; Baranov, 1946; Esau, 1948a; Esau, 1948b; Metcalfe and Chalk, 1950; Flerov and Kovalenko, 1952; Manzoni, 1952; Becker, 1957; Schanderl, 1957; Parniewski, 1962; Hegedüs, Kozma, Németh, 1966; Britz, 1968 and Pongrác, 1969.

The above-mentioned investigators gave special attention to one or more of the following: pith cells, primary and lateral roots, pericycle, xylem rays, raphides, tracheary elements, annual rings, cambium, phloem and phloem rays and periderm.

2.2 Influence of *Dactylosphaera vitifolii* Shimer on *Vitis* roots

Since the disastrous attacks on vines by phylloxera, the following research workers have given attention to the influence of this insect on the anatomy of *Vitis* roots: Foëx, 1877; Despeissis, 1895; Ravaz, 1897; Petri, 1909; Perold, 1926; Abesadze, Makarevskaja, Zchakaja, 1930; Breider and Husfeld, 1938; von Börner, 1942; Anders, 1955, 1957a, 1957b, 1958, 1960a, 1960b, 1961; Becker, 1957; Niklowitz, 1955; Hofmann, 1957; Henke, 1958; Parniewski, 1962; Coombe, 1963 and Britz, 1968.

2.3 Reaction of *Vitis* species and cultivars to nematodes

Serious damage by root-knot nematodes to vines of the *vinifera* and *aestivalis* species was reported by Neal (1889). He also stated that vines grafted onto *cordifolia* and *riparia* species grew very well and were free from these nematodes. According to Bessey (1911), Lavergne (1901) pointed out that in Chile *Vitis vinifera* Linnaeus, was seriously attacked by root-knot nematodes. Lavergne also reported that cultivars which were resistant to phylloxera, were also resistant to root-knot nematodes and that *Vitis riparia* Michaux and *Vitis rupestris* Scheele, were at that time very important.

A "slow decline" or "die back" phenomenon which was noticed at the beginning of this century in South African vineyards, sparked off a series of investigations into the cause of many weakly growing and dying vines.

The fibrous roots of 1202 C (= *V. vinifera* x *V. rupestris*, Couderc, 1883) in moist and low lying soils of nurseries in the Constantia area, near Cape Town, revealed symptoms of decay and the principle ramifications dotted with nodules caused by "gall worms". Hybrids 3309 C (= *V. riparia* x *V. rupestris*, Couderc, 1881) were to a lesser extent affected. In drier soils these root-stocks were only slightly infected by this nematode (Watermeyer, 1908).

Although *V. rupestris* cv. Constantia Rupestris Metallica root-stock did well in cool, deep sandy soils, it was easily killed by nematodes in these soils (Perold, 1912). He also mentioned the destruction by nematodes of 1202 C in sandy soils, but in other soils these nematodes were not a serious problem. He found several cases where Jacquez (a natural hybrid probably of *V. vinifera* x *V. cinerea* Engelman x *V. aestivalis* Michaux) was attacked by phylloxera but did not state whether Jacquez was also attacked by nematodes.

In their report about grafted vines in the Cape Province of South Africa, Perold and Wagner (1914) stated that Constantia Rupestris Metallica, Greengrape and Hermitage (cultivars of *V. vinifera*) on sandy soils were attacked by nematodes. These two authors did not specify which nematodes attacked the vines. As they often found nematodes in sandy soils and did not mention "root-lesion nematodes", they most probably meant root-knot nematodes.

The decline of Aramon noir x Rupestris Ganzin nos. 1 and 2, Ganzin, 1879, was caused not so much by phylloxera but by nematodes (van Niekerk and Theron, 1927). Neither did these two investigators state to which nematodes they referred.

The following root-stocks gave "profitable crops even when infested by root-knot nematodes" (Tyler, 1933):

Dogridge (*V. champini*, Planchon)

Salt Creek (*V. champini*)

106-8 Mgt (= *V. riparia* x (*V. cordifolia* Michaux x *V. rupestris*) Millardet and de Grasset, 1882)

101-14 Mgt (= *V. riparia* x *V. rupestris*, Millardet and de Grasset, 1882)

1616 C (= Solonis x *V. riparia*, Couderc, 1881)

1613 C (= Solonis x Othello, Couderc, 1881)

120 A (= *V. berlandieri* x *V. riparia*, Couderc, 1881)

420 A (= *V. berlandieri* x *V. riparia*, Millardet and de Grasset, 1887)

According to Tyler, *V. rupestris* cv. St. George is not sufficiently resistant to nematodes. He also thought that resistance of a root-stock depended on local soil and climatic conditions as well as "congeniality of stock and scion". He recommended the breeding of resistant plants, but warned that it is a long and difficult undertaking.

In the United States of America root-knot nematodes were the most common nematodes that attacked vines. Although apparently all *viniferas* were susceptible to nematodes, they differed in the extent of nematode injury (Jacob, 1947). He also warned against the susceptibility of *V. rupestris* cv. St. George to nematodes and stated that in sandy nematode-infested soils in the San Joaquin Valley Aramon noir x Rupestris Ganzin no. 1 was practically worthless. According to Jacob, 1613 C is highly resistant to root-knot nematodes, whereas Dogridge and Salt Creek are extremely nematode resistant root-stock cultivars.

Snyder (1933) stated that 154 cultivars of *V. vinifera* studied by him were very susceptible to nematodes. Even during one season heavy infestation could take place and no cultivar can be said to be immune. He further reported that vines like Dogridge, Salt Creek, 1616 C and 1613 C can be attacked by nematodes. These vines are also important to the Republic of South Africa.

According to Allen (1952b) several root-stocks possessed some degree of resistance to root-knot nematodes. These included Dogridge, Salt Creek, 1616 C, but their performance varied in different localities or even in vineyards in the same locality. He also warned against continued cropping of infested land with susceptible crops, as it caused a building up of a high nematode population with disastrous results. He also noticed that root-lesion nematodes caused

poor growth, yellow foliage, die-back of leaves, "necrotic areas or lesions of varying size on larger roots and the presence of dead feeder-roots".

The majority of root-knot nematodes on *Vitis* roots in California were identified as *Meloidogyne incognita* var. *acrita* Chitwood, 1949, while *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 was found infrequently. In California damage to vines tended also to be more severe on light sandy soils. *Pratylenchus vulnus* Allen and Jensen, 1951, was not encountered as frequently as root-knot nematodes but is considered to be as serious a pest (Raski, 1955).

In California, 1613 C was recommended for heavily nematode infested soils which were irrigated and Salt Creek for coarse sandy soils with low fertility (Smith and Stafford, 1955). These authors thought that grapes from vines on nematode resistant stocks were usually somewhat inferior in quality to those produced by own-rooted vines in non-infested soils. They did not mention any particular cultivar in this respect and recommended the use of grafted vines only where they were required. Although these two research workers stated that all American grape cultivars and hybrids were believed to be susceptible to nematode attacks they reported that *V. doaniana* Munson, *V. champini*, *V. longii* Prince and *V. cinerea* Engelman, showed resistance to nematode attacks. They further stated that Salt Creek, Dogridge, 1613 C and 1616 C showed good resistance to nematodes.

When referring to *P. vulnus*, Smith and Stafford mentioned that root-stocks resistant to root-knot nematodes did appear to be resistant to root-lesion nematodes.

Although *M. incognita* var. *acrita* was the most common root-knot species in Californian vineyards, *M. javanica* and *Meloidogyne arenaria thamesi* Chitwood, 1952, were usually equally injurious where they occurred (Raski and Lider, 1959).

According to Lider (1959) 1613 C was the most useful nematode-resistant root-stock for grapes in California and Dogridge was recommended for sandy soils where nematode infestations may be high. He also stated that 1616 C was mildly resistant to phylloxera as well to nematodes and that 5-A (= *V. berlandieri* x *V. riparia* Teleki, 1896), was recommended for further use in tests in nematode-

infested soils, but 420 A, owing to its low vigour and insufficient resistance to nematodes was not recommended for further testing in California.

Feeder roots of grapevines might be almost completely lacking when heavily infested with nematodes (Raski and Lider, 1960). These two investigators stated that *M. incognita acrita* Chitwood, 1949 was the most common root-knot nematode in California.

When *M. incognita* and *M. javanica* fed on vine roots, the cell-walls "collapsed" and syncytia (giant cells)* were formed (Raski, Hart and Kasimatis, 1966). These authors also noticed root-decay caused by root-knot species and the formation of an abnormally branched "hairy root" condition. They reported the formation of lesions and girdling of larger roots caused by root-lesion nematodes. As far as root-knot, root-lesion and dagger nematodes are concerned, these authors classified the following root-stocks in California as follows:

Dogridge was resistant to root-knot, lesion and dagger nematodes.

1613 C was resistant to some root-knot strains and susceptible to lesion and dagger nematodes.

Salt Creek was resistant to root-knot, lesion and dagger nematodes.

St. George was susceptible to all three of the above-mentioned species.

Aramon noir x Rupestris Ganzin no. 1 was susceptible to all three the above-mentioned nematode species.

In 1954 Boubals reported that damage caused by nematodes to cultivated plants was becoming more and more important. He observed that *Meloidogyne marioni* Cornu, 1879, was almost exclusively located in the sandy soils of Southern France, where in general, *V. vinifera* is cultivated ungrafted. He also stated that very sensitive *V. vinifera* cultivars, including Ugni blanc, produced fewer, slenderer and shorter canes without showing the classic irregularities of infectious degeneration, especially double nodes. In this case the infected vines only bear a reduced quantity of grapes and sometimes none at all.

* "Giant cell" is a recognised term amongst nematologists for "syncytium".

Finally, in autumn the apical parts of the canes did not ripen to a length of 10-20 cm. The plants infected with the parasite weakened very rapidly and finally died. He ascribed it to the destruction of the root-systems by nematodes. The principle roots, i.e. those which were established at the normal depth of the root-system (c.a. 30 cm) were destroyed first. New roots developing higher up the trunk were soon infected and destroyed, resulting in the death of the plant. He also noticed that in the case of very sensitive vines nematodes attacked the medium-sized roots as well, and that when there were a number of nematodes at these particular sites, the roots were partly or totally destroyed. He thought that it was the result of the intervention of saprophytes. He observed that when nurseries were established in marshy areas infected by nematodes, galls on the roots appeared smaller than those found in sandy soils.

Boubals (1954) stated that the development of *Heterodera marioni* began in spring when the temperature was about 15°C. Several generations were produced during the year. The larvae had a high resistance to drought and moisture. According to this author it was thought, especially in the United States of America, that there were two genera and four species which parasitised vines, i.e. *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 called *H. marioni* until 1949, *M. hapla* and *P. vulnus* which caused necrosis in the roots without forming nodules. He observed that *V. vinifera* cultivars were more sensitive to root-knot nematodes than others and recommended the following root-stocks for nematode infested soils:

SO₄ (= *V. riparia* x *V. berlandieri*, Téléki, Oppenheim).

5 BB (= *V. riparia* x *V. berlandieri*, Téléki, 1896).

8 BB (= *V. riparia* x *V. berlandieri*, Téléki, 1896).

4010 C1 (= *V. riparia* x (Aramon noir x *Rupestris* Ganzin no. 1) Castel).

1616 C (= *Solonis* x *V. riparia*, Couderc, 1881).

44-53 M (= *V. riparia* x 144 Malègue (*V. cordifolia* x *V. rupestris*) Malègue.

99 R (= *V. berlandieri* x *V. rupestris*, Richter, 1889).

M. javanica was the only root-knot nematode found, that attacked vines in Australia (Seinhorst and Sauer, 1956). These authors reported that:



1. Root-knot nematodes were widely spread throughout the irrigation area where vegetables and certain weeds were attacked.
2. Different nematodes caused 'slow decline' of vines.
3. Symptoms on the roots differed widely in different cultivars and the most common symptoms were small swellings on strongly branched roots.
4. One cultivar, e.g. Gordos, showed hardly any galls but many egg masses about 0,5 mm in diameter, were observed.
5. Normal galls appeared on Walthams, while Riesling showed quite different symptoms. The fine roots of this cultivar were comparatively lightly infested, but on the older roots galls of up to about 2,5 cm were observed. These authors thought that it might be a cultivar characteristic, but it is possible that this plant was infested in the nursery and later transplanted to uninfested soil.
6. Heavy attacks might kill many roots which might lead to the ultimate death of the vines.
7. *P. vulnus* was also found on vines. The aerial parts showed the same poor growth as vines attacked by *Meloidogyne* spp., but the symptoms in the roots differed from those attacked by *Meloidogyne* spp. *P. vulnus* caused lesions in the cortex of the roots which were girdled. The part distal to the lesion was ultimately killed.
8. In contrast to *Meloidogyne* spp., *P. vulnus* caused serious root-rot which lead to severe reduction of root numbers. With heavy attacks only thick, relatively undamaged roots remained. Sauer (1962) drew attention to the damage caused in vineyards by using infested nursery material, and the presence of *Meloidogyne* spp. where vegetables were grown between vine rows.

The most dangerous plant parasitic nematodes in Chilean vineyards were *Meloidogyne* spp. and *Xiphinema index* Thorne and Allen, 1950, (Schäfer, 1966). No *Meloidogyne* spp. were present in Germany. He recommended 1613 C, Dogridge, Salt Creek and Carignan to control the nematode problem in Chile.

A survey was made in a number of German vineyards to determine the pre-

sence of harmful nematodes (Weischer, 1960) but no *Meloidogyne* spp. were found. He attributed it to heavy soils.

Since the end of the nineteenth century many publications have appeared on the danger of nematodes to vines, including *Meloidogyne* species, as well as reports on experiments carried out to control these organisms in vineyards with the aid of soil treatments and resistant root-stocks.

Contrary to the many publications on the influence of nematodes on the structure of roots of annuals, no publications could be found dealing with a detailed study on the effect of nematodes on the structure of *Vitis* roots.

- - - - -

3. MATERIAL AND METHODS

3.1 Plant material used

3.1.1 Vitis cultivars

The following cultivars which are well known in the Republic of South Africa (R.S.A.) were grown from cuttings 30 cm in length.

V. vinifera cvs. White French and Steen. Both are white cultivars and are very popular for wine production.

Root-stocks.

Jacquez, 99 R, 1202 C, Salt Creek and Dogridge.

3.1.2 Solanum lycopersicum L. cv. Pearce and Nicotiana tabacum L. cv. Elsenburg Soulook

The above-mentioned tomato and tobacco plants which are highly susceptible to root-knot nematodes, were grown from seed in sand which was made nematode-free either by fumigation or steam sterilisation.

These plants were used for building up the necessary *M. hapla* population (cf. 3.6.1).

3.2 Growth medium for plants

Pure white sand with a pH of 7,4, free of compounds, harmful to either vines or nematodes, was used.

Fumigation of sand. The sand was spread out on a concrete floor in strips of 30 m x one metre x 15 cm. Two pints of a mixture, containing 98% w.w. methylbromide and 2% chloropicrin, in special containers from which the fumigants could be released by manual manipulation were used. The sand and containers were then enclosed in plastic sheets, the edges of which were carefully covered with sand to avoid the loss of vapours. Fumigation proceeded for 48 hours, after which the sand was uncovered and left for 24 hours for the fumigants to escape.

The effectiveness of the fumigation was tested by growing tomato and tobacco

plants cultivated in the steam sterilised sand, in the fumigated sand. No live nematodes were ever detected.

3.3 Containers for cultivation of plants

3.3.1 Containers with corrugated iron sides

Corrugated iron 60,0 cm wide was used for making the sides of the containers 240 cm long, 60,0 cm deep and 120 cm wide. The floors of these containers were level and of concrete.

The containers were built in the open, on a hill-side with an eight percent slope and placed lengthwise downhill in order to facilitate proper draining of excessive water.

To improve drainage, strips (3 cm high by 5 cm wide) of crushed stones (1,25 cm in diameter) were placed inside the containers where the corrugated iron rested on the concrete floor. The necessary outlet pipes were installed.

The containers were lowered into the ground so that the sides could be covered with soil in order to limit temperature fluctuations to a minimum.

3.3.2 Earthen-ware and plastic pots

Earthen-ware pots of 45 cm x 50 cm, as well as plastic pots of 15 cm x 17,5 cm were used.

The earthen-ware pots were also lowered into the soil but the plastic ones were kept in a hot house at 25°C.

The temperature in the pots, during the growth season (1st September to 30th April) was recorded by means of soil thermographs with mercury recording systems. At a depth of 15 cm the maximum temperature fluctuations per day, were from 17°C - 24°C and at 30 cm, 20°C - 21°C. The maximum fluctuations at a depth of 15 cm during the whole growth season, were 14°C - 28°C and at 30 cm 19°C - 22°C.

3.4 The handling of plants

To get enough rooted vines, three of the containers, as described under 3.3,1 were filled with fumigated sand.

Vine cuttings, 30 cm long, of the cultivars mentioned were planted 15 cm x 7,5 cm apart in these containers, on the 1st August, 1965.

To prevent sucker growth below soil level, all buds were removed, with the exception of two near the apical end. The plants received their nutrients and water as described under 3.5.

On the 1st September, 1966 the containers were opened on the downhill side and the plants removed by washing out the sand by means of water under low pressure. By making use of this technique the minimum number of rootlets were damaged.

The vines were transplanted (7 plants per pot) into the earthen-ware pots mentioned. There were 10 replications and 10 control pots per cultivar.

Tomato roots severely infected with *M. hapla*, were chopped up and placed next to the roots of the vines. The shoots of these vines were pruned back but not the roots. The roots were covered with sand, the plants watered and fertilized as described under 3.5.

At the end of the growth season (30th April, 1967) the roots of the plants were again washed free from sand, sampled, killed and fixed as described under 3.6.3.

In order to determine the speed with which the nematodes entered the roots and completed their life cycles, cuttings of Steen, Jacquez, 99 R and Salt Creek were disbudded as described and planted in this sterilized sand (four cuttings per pot). The plants were watered and fertilized as described under 3.5. After three months when many fine rootlets were formed, the upper 10 cm of sand was washed out and 1 000 larvae per pot placed amongst the rootlets, after which the pots were again filled with sterile sand. There were five replications. The breeding and handling of the larvae are described under 3.6.1.

After 12 hours all the sand was washed out of the pots and replaced with steam sterilized sand. With this technique no larvae were left to enter the roots after 12 hours.

15.

Root samples were taken after 12 hours, and consecutively on the 3rd, 15th, 10th, 15th, 20th, 30th and 35th days.

Samples were taken by washing out the sand, removing some rootlets and filling the pots again with sterile sand.

The handling of the roots as well as the staining of nematodes etc. are described under 3.6.

3.5 Nutrition and watering of plants

The following solutions, based on Hoagland's nutrient medium were used:

	<u>Solution A</u>	<u>Solution B</u>
		The following quantities of A were added to 25 litres of water.
$\text{Ca}(\text{NO}_3)_2$	164 g/l	125 ml
KNO_3	101 g/l	125 ml
MgSO_4	120 g/l	50 ml
KH_2PO_4	136 g/l	25 ml

Solution C

Trace element stock solution

To 10 litres of water the following compounds were added:

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	8 g
MnSO_4	154 g
ZnSO_4	22 g
H_3BO_4	286 g
$\text{H}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$	9 g

Solution D

16,6 g Iron Chelate per l

Solution E

This was the final solution added to the plants and consisted of:

Solution B 325,0 ml as specified above

Solution C 2,5 ml as specified above

Solution D 125,0 ml as specified above

Water 25,0 l

Total 25 452,5 ml

Every second day 2 l of the above-mentioned solution "E" were used on 80 l of sand. The earthen-ware pots mentioned, had a capacity of about 45 litres. Solution E had a pH of 6,8.

On the days when no nutrient solutions were added to the containers they were well watered in order to keep the sand saturated. They were watered until free water drained off through the outlets.

3.6 Microtechnical techniques

3.6.1 Isolating and handling of *Meloidogyne hapla*, Chitwood 1949

Solanum tuberosum L. cv. King George infested with nematodes were cut up into small pieces and distributed among the roots of tomatoes cultivated as described.

After about 30 days the roots were inspected and one of the egg sacs removed under a stereo-microscope and placed next to the roots of an uninfected tomato plant. After 30 days a large number of egg sacs on the roots were available for further propagation.

Within a couple of months a large number of infested tobacco and tomato plants were available for infestation of vine roots. Eggs were incubated, to obtain larvae for placing next to the roots of the vines.*

When egg sacs were placed in water at 25°C to incubate, fungi were very troublesome and consequently all the egg sacs had to be disinfected. This was done according to Bird's (1962) technique.

Sterilization of eggs

Staining wells of 7 cc and with lids were used as containers and a hypodermic syringe was used to replace the different disinfecting solutions.

The egg sacs were placed successively in the following solutions:

1. 20 minutes in 3,0% hydrogen peroxide
2. 5 minutes in 0,1% "cetavlon"
3. Washed in distilled water
4. 20 minutes in 0,5% "hibitane"

* Dr. J. Heyns kindly identified the nematode as *M. hapla*, Chitwood, 1949.

5. Washed in distilled water
6. 5 minutes in 50 mg per litre tetracycline
7. Washed in distilled water.

This technique kept the egg sacs free from fungi during the incubation period.

A water suspension of young larvae was made and counts of larvae made under the stereo-microscope in a petri dish of which the bottom was divided into squares.

3.6.2 Staining nematodes in rootlets

Nematodes in rootlets were stained with the following solution as recommended by Dr. D.L. Milne, (personal communication):

Phenol	400 g
Lactic acid	400 ml
Glycerol	800 ml
Distilled water	400 ml
Cotton blue	0,1 g

Smaller roots were boiled for three minutes in this solution and large roots for five to eight minutes. The solution was quickly cooled down and the rootlets examined after 24 hours. The nematodes were stained blue-black (Figs. 58-60).

3.6.3 Sampling, killing and fixing of plant materials

Immediately after the roots had been washed out of the sand, they were fixed in FAA as described by Sass (1961, p.15).

Since many vine roots did not form knots or only very small ones when attacked by *Meloidogyne* it was not possible to collect infested roots only. A large number of roots had to be examined under the stereo-microscope for many hours on end in an effort to detect abnormalities in their external appearance. Very often roots which appeared normal were very badly damaged internally, as revealed by photomicrographs discussed in this treatise.

The cut lengths were numbered with slit paper dipped in wax or the wax blocks were numbered. This technique facilitates orientation serial sectioning.

3.6.4 Dehydration, infiltration and embedding in paraffin wax

Dehydration was carried out in progressively higher concentrations of ethyl alcohol.

Infiltration with wax was carried out by gradually replacing the absolute alcohol with chloroform and then by adding small pieces of paraffin wax (melting point 52°C) over a number of days, after which the chloroform was allowed to evaporate in a thermostat.

Before embedding the root tips, a very small quantity of erythrosin powder was added to the wax and chloroform mixture to stain the root tips in order to make them clearly visible for embedding and cutting.

3.6.5 Microtome sectioning of material in wax

A rotary microtome was used and the sections cut to a thickness of 12-15µm.

3.6.6 Staining paraffin sections

To illustrate cell-walls, nuclei and nucleoli, sections were stained with Dalafield's haematoxylin and with safranin O as a counterstain, as described by Peacock (1966) and with Heidenheim's iron haematoxylin and aniline blue as used by Vaughan (1955). The latter stain combination gave excellent results.

3.6.7 Microchemical tests

The following tests were carried out:

Tannin: Safranin stains tannin dark red.

A 10 percent solution of potassium dichromate gives a red-brown deposit in tannin containing cells.

Lignin:

Anilin chloride was used to test for the presence of lignin.

Gum:

Neutral violet, as described by Peacock (1966), was used to test for the presence of gums.

3.6.8 Photography

The following filters and film were used for the production of the photomicrographs: Kodak Wratten K2 filter no. 8, Kodak Wratten X1 filter no. 11 and Kodak Panatomic-X 35 mm.

4. THE EXTERNAL MORPHOLOGY OF VITIS ROOTS

When examining *Vitis* roots for abnormalities one should know what a normal *Vitis* root looks like.

The young actively growing *Vitis* roots are light in colour. The root hairs are not as plentiful and well developed as in the case e.g. the *Gramineae*.

When the vine grows in fine sand or other porous material, free of pebbles, the young roots are very smooth. If they grow in a gravelly or dense soil the roots may have many bends and dents caused by the sand grains. Even in sandy soil the older roots often have many bends and their outer surfaces are not quite smooth; consequently it is difficult to judge by their outer appearance whether they have been attacked by nematodes or not.

Figure 1 illustrates the appearance of a normally developed active growing Jacquez root. The initiating lateral roots were about 150 mm from the root cap. Normally they are more or less distributed along the main root.

The tips of the *Vitis* roots studied were not always nearly tapered but often suddenly decreased in thickness, as illustrated in Fig. 2 which shows the root of a Jacquez cutting grown in sawdust, and with the aid of Hoagland's culture medium. This type of growing point was often found when vines were grown in sandy soil.

When badly infected with *Meloidogyne*, the latter roots were closer together and some were branched, thus forming brush or broom-shaped clusters or groups.

Of the cultivars examined in this study, Jacquez normally had thick, actively growing root parts, whereas those of Dogridge were very slender. No other difference in outer appearance was noticed amongst these root parts of the seven cultivars examined.

Experience has taught that when lateral roots form a brush or broom, there was reason to suspect nematode infection, especially when all or the majority of the lateral roots were stunted or their tips damaged (Figs. 3 and 4).

Sometimes the only indication of *Meloidogyne* infection of *Vitis* roots were,

that, when the tender vine roots had been washed in water, little heaps of sand particles were found sticking together on the thinner roots, especially near the tips. The posterior parts of the females were covered with a gelatinous matrix into which eggs were deposited. The sand particles mentioned stuck to these gelatinous masses (Fig. 5). When the sand particles were removed under a stereo-microscope the eggs were clearly visible (Fig. 6).

Under the gelatinous matrix, known as "egg sacs" in nematological literature, the flask-shaped nematode females occurred (Fig. 10). These females were often found in groups (Fig. 8) or in rows neatly spaced (Fig. 52). Their necks were short (Figs. 10, 66 and 68) or long (Figs. 50 and 91).

In roots one-year-old and older, infected with *Meloidogyne*, many holes or sockets, resembling the sockets of teeth were observed. These sockets are old female nests (Fig. 11). No indication was found that these cavities or sockets were being filled with new root tissue.

Even if the nematodes were still active in *Vitis* roots, by forming egg sacs etc., their activities could not always be detected with the naked eye. As they did not always form knots (Fig. 7) it was not always possible to detect the infection without a magnifying glass or even a research microscope.

Even if there was a concentration of females at one particular site on the root (Fig. 8), their presence could not always be detected without washing the roots free from soil or using a magnifying glass, or both. Often clearly visible knots were seen (Fig. 9).

Jacquez root-stock formed very small knots or none at all (Fig. 7), whereas on White French knots were fairly easily formed, but they were small (Fig. 9). 99 R, Dogridge and Salt Creek were very severely attacked by *M. hapla*, but did not form knots (Figs. 104, 108 and 110). As will be discussed later, the nematodes did a certain amount of damage, but perished before they reached the egg-laying stage. When the latter three root-stocks were infected with *M. hapla* the only indication of infection was a soft spongy appearance of the surface of the one and two-year-old roots. It takes a long time and considerable experience to detect spots of infection on the roots of 99 R, Dogridge and Salt

Creek. Internal infections of the roots of these three root-stocks could be detected only with the aid of a research microscope.

When one and two-year-old Jacques, Steen and White French roots were attacked by *Meloidogyne* nematodes, the outer cell layers of the root sometimes loosened up and formed a loose mantle over their egg sacs. Only when this loose mantle of dead cells was removed could the females and egg sacs be seen.

- - - - -

5. THE INFLUENCE OF *M. HAPLA* ON THE PRIMARY
STRUCTURE OF *VITIS* ROOTS

Britz (1968) made a detailed study of the root anatomy of White French, Jacquez, 1202 C and 99 R. Pongrácz (1969) compared the anatomy of a number of one-year-old and two-year-old roots of *Vitis* cultivars including the following: White French, 1202 C, Jacquez, 99 R and Salt Creek.

The vines studied by these research workers were grown under conditions quite different from those in the present study. It was, therefore, necessary to examine control plants to compare undamaged roots with roots damaged by *M. hapla*.

In this treatise tissues of undamaged roots will be discussed in detail only where observations in this study differ from those of other research workers, or where the tissue concerned was of importance when roots were attacked by nematodes.

5.1 Root cap and epidermis

The root cap consisted of 5 to 6 layers of cells which decreased in number towards the secondary growth (direction opposite to the root tip). As the nematodes attacked the root behind the root cap and further away, the root cap was of no practical value in preventing nematodes from entering the root.

The epidermis, being one cell layer in thickness, could not prevent the nematodes from entering; neither did these cells form a wound periderm to stop the nematodes from entering the roots.

There were normally many raphides and raphide sacs in the root tip area (Fig. 13), but no indication was found that they had any influence on nematodes.

Figure 12 shows a cross-section 600µm from the root tip of an uninfected 99 R root. Normal large nuclei in the cells of this part of the root were clearly visible.

About 630µm to 660µm from the tip of the root cap the first indications of sieve-tube members were found (Figs. 13 and 16).

When Dogridge roots were infected with *M. hapla* 600 μ m to 700 μ m from the root tip, necrosis and cracks of the outer cell layers were often observed as well as cracks across the root (Figs. 15 and 16). No other abnormalities were found in this part of Dogridge roots. The cortical cells always appeared normal (Fig. 17).

At 600 μ m and 630 μ m from the root cap, the stage of root development of 99 R and Dogridge respectively was the same, although Dogridge was badly cracked (Figs. 12 and 15).

Roots of other cultivars examined in the present study did not show any cracking of root tips.

5.2 Cortex

The cortical cells were normally not radially arranged, although there was one instance viz. 99 R where this was noticed near the endodermis (Fig. 26). Since the cortical cells consisted of thin-walled parenchymatous cells (Fig. 14), they rendered very little protection to the vine root against nematode attacks.

Of several hundred cross-sections examined where the central cylinders were invaded by nematodes, only a few instances were encountered where slight necrosis occurred in the cortex (Fig. 18). In most other cases no necrosis was observed in the cortex and no indication was found that it retarded the movement of the nematodes. Since intercellular spaces in the cortex were up to 30 μ m wide and since nematode larvae with a diameter of only 17 μ m were observed 24 hours after invasion of the roots, it stands to reason that the larvae could move through intercellular spaces in the cortex without damage to cortical cells (Figs. 13 and 14).

In contrast to other parts of the roots, no indication was found that the nematodes formed syncytia in the cortex.

As the central cylinder developed, the cortical cells elongated and consequently the nuclei and raphides in the cortical cells diminished in number, per unit area of the section. Eventually the cortex was cast off, after formation of periderm in the pericycle.

5.3 Endodermis

The pro-endodermis was observed in the vicinity of 660 μ m (Fig. 13), from the root tip.

Granulated tannin in the endodermis was observed about 12 mm from the root tip. The endodermal cells, 30 mm and more from the tip, were filled with tannin, whether the root was attacked by nematodes or not (Figs. 21-23; 29 and 46).

No indication was found that the endodermis retarded or prevented the nematodes from entering the central cylinder. In zones where a proliferation of pericycle cells was stimulated by a nematode attack, the endodermis formed a bulge in a centrifugal direction. The cell-walls of the endodermal cells and even their cell contents sometimes disappeared (Figs. 28 and 29).

If nematodes' attacks were limited only to the vascular bundles and the pericycle was not seriously affected, no indication was found that the normal arrangement of the endodermal cells was altered (Fig. 46).

5.4 Pericycle

In the majority of cases the pericycle consisted of 4 to 6 parenchymatic cell-layers (Figs. 21-23). Since this is the tissue in which lateral roots were formed (Fig. 42), it is an important part of the stele. In this investigation it was found that *M. hapla* damaged the pericycle and could even destroy it in the case of Salt Creek, Dogridge and 99 R (Fig. 27). The pericycles of the other four cultivars studied were not destroyed when attacked by nematodes (Figs. 28-29; 46 and 47-49).

When the central cylinders of Jacquez, Steen, White French and 1202 C were attacked by *M. hapla*, proliferation of the pericycle sometimes occurred (Figs. 28-29).

5.5 Primary xylem

The first protoxylem elements were formed about 6 mm from the root tip (Fig. 19). At this stage the pith cells were still free of tannin. At 10 mm from the root tip protoxylem and metaxylem elements were clearly visible (Fig. 20). At this stage tannin in the xylem parenchyma cells was a normal occurrence (Fig. 20).

Secondary walls of the xylem elements developed at different distances from the root tips.

As the nematodes preferred to feed on cells adjacent to xylem bundles, the roots were very vulnerable to nematode attacks at 10 mm from the root tip and towards the stem. This is shown by the occurrence of syncytia next to xylem bundles (Figs. 27-29, 36, 38, 46 and 47-49).

Although the nematodes caused the formation of syncytia near xylem elements, and sometimes even caused the formation of abnormal xylem elements, no occlusion of vessels with tyloses was observed.

5.6 Primary phloem and procambium

At 30 mm from the root tip the phloem and vascular cambium were already fairly well developed (Figs. 22, 23 and 25). No crushing or collapsing of cambial cells was noticed at this stage (Figs. 22 and 25).

5.7 Pith parenchyma

At 6 mm from the root tip the uninfected pith parenchyma cells were still free of tannin but at about 10 mm and upwards many of these cells contained tannin (Figs. 20-23 and 27). No indication was found that nematode attacks increased the tannin content of pith cells. Although a number of pith cells were destroyed no syncytia were observed in the centre of the pith (Figs. 27, 47-49).

When a root was badly attacked, as in the case of Jacquez (Figs. 47-49), abnormal parenchyma cells and abnormal xylem were formed in the place of the pith. A vascular cambium encircled the abnormal xylem (Figs. 44 and 45).

5.8 Syncytia in the central cylinder

5.8.1 Formation of syncytia

Five days after inoculation with *M. hapla* the first syncytia were observed at 2 mm - 12 mm and 50 mm, respectively, from the root tip (Fig. 26). The measurements of the syncytia observed at this stage varied from 120µm x 125µm to 70µm x 75µm. Observations made after the fifth day showed that syncytia were formed from 2 mm from the root tip and further at irregular intervals, as

well as in the region where secondary root growth took place (Figs. 28, 34, 45, 47 and 49).

In addition to syncytium formation in 99 R, the pericycle was also destroyed (Fig. 27). Destruction of the pericycle was also observed in Dogrigde and Salt Creek. After the destruction of the pericycle and the forming of syncytia, the nematodes in these three cultivars disappeared. No dead or partly disintegrated nematodes were found in the central cylinder of these three cultivars.

No indication was found that force was used to form the syncytia (Figs. 27, 34, 38 and 43). No indication was found that syncytia were formed by compressing the neighbouring cells. Many of the cells bordering the syncytia or in their vicinity were badly distorted (Figs. 34-35, 37-38, 43 and 47-49).

The number of syncytia at one particular feeding site of a nematode varied from a few to as many as ten (Figs. 41 and 94). These cells differed in shape from almost round to almost square (Figs. 36 and 43).

As found by other investigators, the nematodes' heads were often near the syncytia (Figs. 40-41, 48-50, 66, 68 and 85).

M. hapla caused the formation of syncytia of various sizes e.g. 1,04 mm x 90µm, 590 x 190µm and 125 x 125µm in *Vitis* roots.

5.8.2 Cell-walls of syncytia and of cells in the immediate vicinity

Ten days after inoculation with nematodes, a very marked difference was often observed between the thickness of the syncytia-walls and walls of bordering cells, as well as walls, of cells in the immediate vicinity (Figs. 31, 33-36, 37, 41, 44 and 45). In some cases the difference was not so marked (Fig. 43).

TABLE 1. Wall thickness of syncytia and that of cells in the immediate vicinity.

Syncytia-walls	4µm to 24µm
Cell-walls in the immediate vicinity of syncytia	2µm to 3µm

The walls of syncytia can thus be two to eight times as thick as those of adjacent cells.

It was often observed that the walls of cells in the immediate vicinity of syncytia were in a state of dissolution (Figs. 31, 33-37, 43, 85 and 86).

Broken down cell-walls in developing syncytia were often observed (Figs. 75-84) as well as an opening or perforation between two syncytia (Fig. 88).

As no indication was found that the walls were mechanically broken down by the nematodes, it must be ascribed to substances produced (i.e. substances injected or excreted or secreted or both) by the nematodes in the roots.

Syncytia with discontinuous thick cell-walls were often observed (Figs. 31 and 89). Stained cell material appressed to the walls of syncytia was a common sight (Figs. 75-82, 85-88, 94-95, 115 and 116). No lignin was detected in the walls of syncytia.

5.8.3 Contents of syncytia

Five days after inoculation with nematodes the syncytia in all the cultivars appeared empty.

Ten days after inoculation many multinucleate syncytia, containing granulated cytoplasm, were found (Figs. 28 and 31-38). The granulated cytoplasm often varied in appearance, e.g. in a cross-section it either covered almost the whole surface of the cell or formed a thick layer around a large central vacuole (Figs. 31, 36-38).

The enlarged nuclei in syncytia were spherical to slightly oval, as shown in the above-mentioned Figures. Lobed nuclei were observed only once (Fig. 32). Enlarged nuclei and nucleoli were often observed in cells bordering syncytia (Fig. 35), and cells adjacent to the syncytia, but they were much smaller and more oval shaped than in the syncytia (Figs. 33, 35, 37 and Table 2).

TABLE 2: Diameters of nuclei and nucleoli in syncytia and in adjacent cells.

Nuclei in syncytia	15 μ m to 20 μ m
Nuclei in adjacent cells	5 μ m to 10 μ m
Nucleoli in syncytia	4 μ m to 5 μ m
Nucleoli in cells near syncytia ..	2 μ m to 2,5 μ m

As the syncytia grew older the granulated cytoplasm, the nuclei and nucleoli disintegrated and disappeared.

6. CHANGES INDUCED BY *M. HAPLA* IN SECONDARY GROWTH
OF *VITIS* ROOTS

6.1 Nematode eggs and development of larvae

The influence of the nematodes on the secondary growth was studied by making tangential and radial longitudinal microtome sections, 25µm thick, of the different cultivars as well as cross-sections.

The eggs of *M hapla* when placed in water at 25°C hatched within 2-4 days. Young larvae, when left in distilled water at 25°C, died within a few days.

The larvae of a few days old, entered the roots of all the cultivars behind the root cap within 24 hours. The larvae entered Jacquez, Steen, 99 R and Salt Creek within 12 hours after inoculation. The other three cultivars were not examined within the first 24 hours. In the case of 99 R, Dogridge and Salt Creek, penetration into the xylem was prevented by a second periderm. The nematodes penetrated the xylem of Steen, White French, Jacquez and 1202 C to feed near the tracheary elements. Steen, however, sometimes managed to form a wound periderm, the nematodes thus being prevented from penetrating the root any further (Fig. 99).

The nematodes invaded the primary root tissue in all the cultivars studied, but in 99 R, Salt Creek and Dogridge they disappeared some time after the fifth day of invasion. In the secondary tissue of the roots of these latter cultivars they penetrated only up to the oldest layers of phloem, where they were stopped by the formation of a second periderm or a wound periderm and consequently perished.

The female nematodes which were enclosed between the two periderms grew until they reached a spherical shape and then perished (Figs. 143 and 144). Even under such unfavourable conditions they sometimes managed to form nematode nests (Fig. 57).

Once the nematodes invaded the roots, they might cause, even at an early stage, a certain amount of necrosis of root tissue. When the nematodes migrated in the root, they often left traces or "nematode paths" behind in the form of necrotic and obliterated cells. This was seen in Jacquez and Steen (Figs. 61 and 101).

The nematodes always moved through soft parenchyma tissue of the phloem and vascular rays (Figs. 61 and 64). It was never observed that they penetrated tracheary elements.

In the secondary tissues of the stele the nematode larvae always moved up to the tracheary elements (Figs. 62 and 63).

Once the larvae had settled down to feed at a particular spot, they started to change into the well known flask shape (Fig. 10). Before they reached this final stage, they passed through several moults.

The so-called "spike" stage of the larva, feeding on cells adjacent to the tracheary elements was observed (Fig. 63). There was a further stage of development where only a small part of the original tail tip was left (Fig. 65), as well as a more advanced stage of an almost full grown female feeding near the tracheary elements. The vulva of the female was often visible (Fig. 66).

After about 30-35 days at 25°C the females completed their life cycles by depositing their eggs in a gelatinous matrix, on the surface of the root or in the pericycle (Figs. 54-57). When the eggs started to hatch their nuclei enlarged and the eggs divided into segments (Fig. 67).

Nematode nests with nematode eggs and the remains of the female nematodes were observed in the phloem (Figs. 52 and 67). Nematode nests were sometimes spaced at fairly regular intervals (Fig. 52), in the phloem and very close to the surface of the root. The diameter of the nest was about 206µm and the four nests in the row were from 270µm to 420µm apart. Figures 53 and 54 are enlargements of some of these nematodes' nests. In Fig. 54 the small compartments of the gelatinous matrix in which the eggs were embedded are clearly visible. According to the stage of development of the larvae the diameter of the compartments in the gelatinous matrix varied from 70µm to 125µm. Figure 55 shows a number of eggs and the nematode's vulva 400µm from the surface of the root.

Although nematodes often had a detrimental effect on cells near their nests, as well as on cells near the syncytia (Fig. 66), necrosis of root tissues was

not observed near females in the "spike" stage (Fig. 63), nor near almost full-grown females (Fig. 65).

A slight necrosis of practically all the cells bordering the nematode nest sometimes occurred (Fig. 69-71).

Where mature females were embedded in root tissue, different grades of necrosis of parenchyma cells were observed bordering the nematodes' nests (Figs. 68-71). While feeding on syncytia the posterior part of the female was often embedded in parenchyma tissue of the pericycle and phloem rays (Fig. 68).

When necrosis occurred in cells bordering the nematode's nest, cell-walls near the necrotic area were sometimes in a state of dissolution as shown in the right hand corner of Fig. 70.

The shape of the cells bordering a nematode's nest may be normal (Fig. 69), but sometimes the cells were abnormally stretched in the radial direction (Fig. 68). The cells of the vascular rays were often radially (Figs. 113-115) as well as semi-tangentially stretched (Figs. 117-119, 121 and 122).

6.2 The penetration of *M. hapla* in roots with secondary growth and the formation of syncytia

Nematodes often attacked roots simultaneously from various directions (Fig. 72). The females arranged themselves in such a way that they could feed on the tracheary elements, and at the same time deposit their eggs on or near the surface of the root (Fig. 72). Nematodes were not seen feeding on the pith cells of the root (Fig. 72). The syncytia were of many shapes and sizes (Figs. 74-75, 94, 115 and 116). They were gradually formed by the dissolution of cell-walls, with the result that some of their cell-walls were extraordinarily thick, while others were very thin (Fig. 75). As in the primary root growth the syncytium-wall was sometimes much thicker than those of adjacent cells (Figs. 94-95 and 115-116). A distortion of the tissues in the vicinity of syncytia was often observed (Fig. 73).

The measurements of the largest syncytium in Fig. 74 are 1,04 mm x 90µm and in Fig. 94, 590µm x 190µm.

The number of syncytia in a syncytia group differed very much. Up to 10 syncytia in one group were observed (Fig. 94).

Nematode infection often caused proliferation of the pericycle. Consequently the posterior part of the female and her egg sac were embedded amongst these cells (Fig. 80).

The females sometimes had short necks (Fig. 68) but sometimes they developed long necks in order to reach the xylem tissue and at the same time be able to deposit their eggs near or on the surface of the root. A female's neck can be as long as the rest of her body (Figs. 50 and 51).

6.3 Periderm formation in roots and its effect on nematodes

As in other plants the tangentially dividing vascular cambium cells of the young *Vitis* roots caused the cortex to become successively smaller. Ultimately the epidermis, exodermis and cortex flaked off at the periphery, and were completely lost (Figs. 47-49). The external layer left, known as periderm, derives from the phellogen. The phellogen arises in the pericycle and forms phellem towards the outside, and it may also produce phelloderm towards the inside. In very young roots the phellogen may also be formed in the epidermis or cortex. Later it may arise direct from the phloem and even near the vascular cambium.

Wound periderm is often formed when cells are damaged mechanically or by parasites e.g. roots of some *Vitis* cultivars, when attacked by *M. hapla* formed wound periderm in the pericycle and in the axial elements of the phloem and phloem ray.

Natural and wound periderm have the same origin and growth, and their cells might have the same cellular composition.

Steen (Figs. 96-101 and 131-133).

This is a most remarkable cultivar, as nematodes were sometimes able to develop normally in its roots, but occasionally wound periderm was initiated and thereby stopped the nematodes from penetrating further (Figs. 97 and 99). The dead nematodes trapped between the normal periderm and the wound periderm

were usually malformed (Fig. 99).

It sometimes happened that a nematode was prevented from reaching the xylem, by the formation of wound periderm at a particular zone, but just next to the latter a nematode might have succeeded in penetrating the root further, forming syncytia and causing severe necrosis of adjacent cells (Fig. 100).

The nematodes occasionally penetrated the xylem of the root via a vascular ray, forming syncytia and leaving a nematode path and severe necrosis behind (Fig. 101).

White French and 1202 C

It was not observed that these two cultivars formed effective wound periderms.

Jacquez (Figs. 102-103 and 126-130)

This cultivar was a very good host for *M. hapla*, notwithstanding the fact that wound periderms were formed.

Soon after the nematodes attacked the root, wound periderm was formed at that site followed by proliferation of the pericycle cells, which caused a spongy external appearance to the root (Figs. 102 and 103).

The older part of the secondary phloem was often cut off by wound periderm (Figs. 126-128), and consequently it deteriorated and was ultimately sloughed off.

Wound periderm can be very effective in protecting the xylem against the harmful effect of the nematodes (Fig. 128). Occasionally, however, it was only partly effective when it did not form a continuous layer. Consequently where the periderm was present the tissues on the inside were normal, but where the periderm was absent, they were abnormal (Figs. 129 and 130).

The wound periderm may be initiated at different distances from the vascular cambium on the centrifugal side. It often arises in discontinuous layers located in the pericycle or secondary phloem or both. These layers may intersect and in a cross-section have the appearance of a "x" (Figs. 102 and 128).

Dogridge (Figs. 104 and 105), Salt Creek (Fig. 106) and 99 R (Figs. 107-110 and 143-147)

These cultivars were hypersensitive to the attacks of *M. hapla* and when attacked by these nematodes they reacted by initiating wound periderms. No indication was found that the nematodes themselves penetrated the wound periderms or by-passed them. Damage done in the xylem is ascribed to substances produced by the nematodes which were harmful to the root tissues (Fig. 110).

As far as the forming of wound periderm is concerned, there was no notable difference among these three cultivars. They will therefore be discussed together.

The formation of periderm was preceded by the occurrence of one or two rows of cells with enlarged nuclei. Especially in the case of 99 R the formation of wound periderm could be predicted by looking for these rows of cells. Cells with enlarged nuclei in an unexpected area were always an indication that infection had taken place.

Several periderms were formed within a root (Fig. 106) and as many as four layers were counted at one particular area. In the case of 99 R the nematodes were enclosed between two periderms where they ultimately died (Figs. 143 and 144).

As with Jacquez, older parts of the phloem, including phloem fibres, were sloughed off by the wound periderm (Fig. 106).

The wound periderm often formed an unbroken layer at varying distances from the outer periderm (Fig. 105). Scale bark was formed locally where short segments of periderm were formed inside the root (Fig. 106). As with Jacquez in this way small pockets of dead tissue occurred between two periderms (Figs. 104, 110 and 128).

Wound periderm was formed up to slightly more than 1,1 mm from the surface of a two-year-old root with proliferated pericycle cells between the periderms. These proliferated cells give the pericycle a spongy appearance in a cross-section (Fig. 110).

Although the wound periderm in these three cultivars was very effective in protecting the roots against nematode invasion, it could not prevent serious necrosis or the forming of lesions in the first annual ring (Figs. 108-110 and 151-154).

6.4 The penetration of nematodal products towards the root pith

There is still uncertainty as to the exact nature of the nematodal products affecting plant growth, however, the term "growth regulators" will be used in this treatise to describe such products. Another factor which must also be borne in mind, is the mechanical disruption of the cells by the nematode and the possibility of such growth regulators "produced" by the affected cells.

It was obvious that cultivars like White French, Steen, Jacquez and 1202 C were fairly slow in their reactions, when attacked by nematodes as compared with cultivars like 99 R, Dogridge and Salt Creek.

With the object of studying the penetration of possible growth regulators and their effect on *Vitis* roots, the vines were cultivated in sand in earthenware pots as described under 3.3 (p.13) and subjected for eight months to the attacks of *M. hapla*.

6.4.1 1202 C (Figs. 112-116)

After eight months it was observed that the pericycle cells had proliferated and syncytia were formed (Figs. 112 and 113). The substance(s) produced by the nematode probably moved along the vascular rays in a centripetal direction. The cell-walls were not completely dissolved but were often hardly visible with a light microscope. Near the pith there was apparently a slower movement of the substances mentioned - perhaps growth regulators - and consequently a higher concentration of the growth regulator(s), resulting in the formation of syncytia with U-shaped wall thickenings (Figs. 115 and 116). As with the other *viniifera* cultivars, the pith cells remained undamaged.

The U-shaped wall of the syncytium shown in Figs. 115 and 116, was 17 μ m thick compared with 2,0 μ m of the adjacent cells. Bordering this U-shaped cells-

wall, abnormal xylem was visible (Fig. 116).

Although no tyloses occurred in vessels of control plants, many vessels were occluded with tyloses in the infected roots (Figs. 158-159).

6.4.2 Jacquez (Figs. 117-125 and 128-130).

As with 1202 C, proliferation of the pericycle cells was induced. Wound periderm cut off the older part of the phloem, including normal ray parenchyma cells (Fig. 118). A large cigar-shaped syncytium was observed in the adjacent ray parenchyma (Fig. 118).

The ray parenchyma cells were abnormally widened in a tangential direction. The abnormal ray parenchyma cells extended up to the primary xylem. Figure 120 shows penetration from at least four directions by growth regulator(s) via the ray parenchyma cells. The substance(s) stopped in the primary xylem, but still caused disorganisation of the parenchyma cells.

A slight necrosis of the primary xylem elements occurred where the infection stopped. The pith cells appeared normal.

The movement of the growth regulator(s) was no doubt retarded and even stopped by xylem tissue and consequently the syncytium (Fig. 119) did not reach the pith.

Figures 121 and 122 are photomicrographs of the same cross-section as shown in Figs. 117-120, but they were taken more to the left to illustrate the wound periderm and cambial cells. On the left-hand side of Fig. 122 phloem cells cut off by wound periderm, are clearly visible. The left part of Fig. 122 shows the vascular cambial cells. At their centrifugal side, the phloem cells appear normal, but to the centre of the photomicrograph where cambial cells are normal or absent, the phloem and xylem cells are also abnormal. Figure 130 illustrates normal vascular cambium (left half of Figure), to the far right in this Figure the cambium cells are either abnormal or absent. Where the cambium cells were normal the adjacent xylem and phloem were also normal, but if the cambium cells were abnormal the xylem and phloem were also abnormal.

Figures 118 and 123 are additional illustrations of the effect of growth regulator(s) produced by the nematodes, on the ray parenchyma cells. In both cases the syncytia were formed centripetally to the wound periderm.

In Fig. 123 the infected zone is wedge-shaped. In the left half of Fig. 123 where cambial cells are absent, the xylem and phloem are abnormal; but in the right half, where the cambium is more or less normal, the phloem and xylem cells are also apparently normal. The largest syncytium illustrated in Fig. 123 is 325µm long. It is obvious that its wedge-shaped form is the result of the shape of the vascular ray.

Obliteration of cells as a result of nematode infection was a very common occurrence (Fig. 124). In this Figure abnormally shaped parenchyma cells and cell-walls in a state of dissolution are visible.

Intrusion of new cell growth into a syncytia sometimes occurred but this was rare (Fig. 125).

If wound periderm was well formed, the nematodes were prevented from moving centripetally and the cells and tissues on the inside of the wound periderm were sometimes quite normal (Fig. 128).

Even if there was no necrosis in the immediate vicinity of the nematode's nest, cells, including cambial cells, were sometimes seriously affected (Figs. 129-130). On the left of the nematode nest, wound periderm was formed (Figs. 129 and 130). On the centripetal side of the wound periderm and of a part of the nematode's nest, the cells were apparently normal. To the right (Fig. 130), however, where there was no wound periderm, the cells were abnormal.

6.4.3 Steen (Figs. 131-138)

Figures 131 and 132 are a further illustration of a well-formed wound periderm, with the cells on its centripetal side well protected.

If the growth regulator(s) produced by the nematodes bypass the wound periderm, these substances can move via the xylem rays up to the primary xylem bordering the pith cells, where, as a result of serious necrosis, a slit was often formed (Fig. 134).

As with Jacquez (Fig. 120), necrosis often occurred in several places at the centripetal extremities of the xylem rays (Fig. 135), while the pith parenchyma cells remained intact (Figs. 134 and 135). Few pith cells contained tannin and many contained starch (Fig. 138). The primary xylem at the centripetal end of the xylem rays seems to stop the growth regulator(s) from entering the pith parenchyma cells. Necrosis and cells containing tannin were observed in the centripetal end of damaged vascular rays (Fig. 138).

Although the lateral roots developed tracheary elements, necrosis often set in at a very early stage. Many cells were filled with tannin. From the mummified appearance of the lateral root it is obvious that it was of doubtful value to the plant (Figs. 136-138).

6.4.4 99 R (Figs. 107-111, 139-145 and 151-156)

The reactions of 99 R to growth regulator(s) produced in the roots by *M. hapla*, were almost the same as those of Dogridge and Salt Creek.

The proliferated pericycle which had a spongy appearance and the outer periderm, were ultimately sloughed off by the wound periderm (Figs. 110 and 145).

Lateral roots were formed but necrosis often set in, with the result that it is doubtful whether those particular roots were of any value to the plant (Figs. 139-142).

99 R formed a second periderm by which the phloem and xylem were well protected and the nematodes enclosed between the periderm layers (Figs. 143 and 144). Very often, however, this cultivar was unsuccessful in preventing the nematodes from producing harmful substance(s) in the roots, with the result that they were severely damaged.

If 99 R is unsuccessful in forming a wound periderm, the growth regulator(s) may penetrate in a one-year-old root to the summer wood, causing serious damage (Figs. 110 and 111).

A series of cross-sections (Figs. 107-110) through a two-year-old root, shows a large lesion or slit (Fig. 110) in the secondary xylem of the previous

year's summer wood. In the middle of the lesion a slit has been formed (Figs. 110 and 111). Above and below the slit necrosis in the previous year's summer wood is evident (Figs. 107-109). On the centrifugal side of the lesion i.e. in the wood of the second annual ring, only undifferentiated tissue is often formed (Fig. 110). Figures 151-154 illustrate the same phenomenon.

Many vessels in the above-mentioned necrotic area were occluded by discoloured tyloses and gums (Figs. 107-109, 111 and 151-154). The spring wood, as seen in Fig. 156 was apparently very sensitive to the plant stimulus caused by *M. hapla*. The occlusion of vessels was never observed in undamaged cultivars in this study.

Although the growth regulator(s) apparently moved via the vascular rays, it is remarkable that the necrosis was first visible near the tracheary elements of the secondary xylem and not in the xylem rays (Figs. 108-109 and 151-154). Where the xylem was badly torn the parenchyma cells of the xylem rays were apparently still undamaged. The cambium derivatives in the xylem rays formed abnormal tracheary elements (Figs. 154 and 160-161), which indicates that the metabolism of the former cells was badly upset. If the root was badly affected serious necrosis of the entire region sometimes occurred while the parenchyma cells of the vascular rays became rich in tannin.

The primary xylem which was apparently sensitive to the nematode's growth regulator(s) was often damaged. Necrosis and lesions were often observed in this tissue (Fig. 155).

6.4.5 Dogridge and Salt Creek (Figs. 162-165)

Basically the reactions of these two cultivars to *M. hapla* were the same. When the summer wood area was very seriously affected, a lesion in the form of a slit was formed (Figs. 162-165).

A cone-shaped necrotic mass often occurred in the second annual ring (Figs. 162 and 163).

6.4.6 Partial obliteration of vessels

Salt Creek (Figs. 106 and 157)

Two wound periderms and partially obliterated vessels were observed in two-year-old Salt Creek roots (Fig. 106 and 157). Only in Salt Creek did part of the walls of the bigger vessels near the previous year's summer wood, collapse. It is remarkable that only the cell-wall in the centrifugal direction collapsed. The collapsing was accompanied by an intrusion of adjacent cells into the vessel, resulting in its obliteration (Fig. 157).

6.4.7 The formation of abnormal tracheary elements

6.4.7.1 In central cylinder (Figs. 44-45 and 47-49)

Abnormal xylem was observed in the central cylinder 20-30 days after the roots had been infected with *M. hapla* (Figs. 44-45 and 47-49). The abnormal xylem occurred near syncytia (Figs. 44 and 45) and consisted of xylem parenchyma with tannin and tracheary elements. No fibres were observed. A zone of cambium encircled a mass of abnormal xylem (Fig. 45). The diameter of the abnormal xylem mass or bundle in Fig. 45 was 172 μ m.

When the central cylinder was seriously attacked by nematodes, as seen in Figs. 47-49, there was no orderly arrangement of the different tissues, e.g. the pith was absent and the primary xylem bundles were abnormally arranged. The damaged pericycle had a lattice-like appearance (Fig. 27) or showed proliferation (Figs. 28 and 29). Tracheary elements of the abnormal xylem were tangentially arranged without forming anastomoses with the normal xylem.

6.4.7.2 In the secondary xylem of two-year-old roots

Localised masses of abnormal tracheary elements near syncytia were observed in the roots of Jacquez, White French, Steen and 1202 C (Fig. 116).

Dogrigde, Salt Creek and 99 R did not form syncytia but formed abnormal tracheary elements (Figs. 160-163). The latter elements originated in the vascular rays from cambium derivatives near the summer wood of the first year. These vessels with scalariform thickenings and simple perforation plates were tangentially elongated (Figs. 160 and 161).

The above-mentioned elements may be short, stretching from one vascular ray to the next, or over a much longer distance e.g. the total length of the cambium circle in the Dogridge root, which is partially shown in Fig. 162, is 5,0 mm. The abnormal xylem and abnormal parenchyma in the second annual ring, as illustrated in the same Figure, stretched over 0,6 mm in a tangential direction. This abnormal tissue thus covered almost $\frac{1}{8}$ th of the total surface of the second annual ring.

A second necrotic mass (Fig. 165) in the same cross-section, also near the summer wood of the first annual ring, stretched over 0,74 mm tangentially. On the centrifugal side of the latter necrotic mass, abnormal parenchyma cells with hardly any tracheary elements, stretched to the vascular cambium. These two necrotic masses with the abnormal tissues covered almost $\frac{1}{6}$ th of the total area covered by the second annual ring.

Where the xylem was seriously affected by substances produced by nematodes, a dome-shaped necrotic mass was often formed near the summer wood of the first year (Figs. 110 and 162). Occasionally no differentiation of tracheary elements occurred between this dome-shaped necrotic mass and the vascular cambium; but only xylem parenchyma cells developed (Figs. 110 and 165). Although the tissues adjacent to the vascular cambium were affected, the latter retained its meristematic activity, and continued forming cells which sometimes differentiated into the different types of xylary elements. In other regions cambial cells and their derivatives were sensitive to nematode attacks and could be damaged or destroyed by them. If the derivatives are damaged it stands to reason that the necessary cell differentiation cannot take place.

6.4.8 Cambial cells affected by nematode attacks

One-year-old vines were subjected to nematode attacks for eight months from the month of September. Hence the favourable conditions for the nematodes to affect the cambial cells.

The cambial cells of 99 R, Salt Creek and Dogridge were very sensitive to nematode attacks. The comparatively thick radial walls of the cambial cells were compressed while the comparatively thin tangential walls appeared

intact (Figs. 148-150). This was probably caused by a centripetal pressure exercised by the proliferating pericycle and the increase of the secondary xylem.

This phenomenon was observed only in the case of Dogridge, 99 R and Salt Creek cultivars, and on those sides of the roots where the attacks were most severe.

Although the nematodes were not in direct contact with the cambial cells, their metabolism was upset by substances produced by the nematodes, causing the cambial cells to form abnormal xylem and phloem tissues, which were almost free of tracheary and sieve-tube elements. Abnormal phloem elements and a muriform phloem parenchyma tissue on the centrifugal side of the vascular cambium sometimes occurred (Figs. 145-147).

6.4.9 Pericycle and secondary phloem affected by nematodes

As the female nematodes penetrated the pericycle and phloem on their way to the xylem, and settled in the pericycle and phloem, to complete their life cycle, it stands to reason that the latter two tissues must be damaged.

Nematode nests were observed in the pericycles and phloem of Jacquez, White French, Steen, 1202 C and Salt Creek (Figs. 57, 72, 102-103, 129 and 130), but not in Dogridge and 99 R. The proliferation of the pericycle was observed in all seven cultivars (Figs. 90-91, 96-97, 102-104, 112, 117, 121, 126 and 145). The wound periderm often cut the secondary phloem in two (Figs. 126-128) and the part of the phloem on the centrifugal side of the wound periderm sloughed off at a later stage.

The vascular cambium in Dogridge, Salt Creek and 99 R often formed muriform phloem parenchyma tissues instead of the normal secondary phloem (Figs. 145-147), and their phloem elements became tangentially elongated (Fig. 162). These abnormalities did not occur in the other cultivars.

7. DISCUSSION

Many factors influence both the reaction of plants towards the attacks of harmful nematodes and the reactions of nematodes towards plants.

7.1 The influence of soil and climatic conditions on the reactions of nematodes

When discussing the influence of nematodes on plants, the conditions in the particular soil as well as the climatic conditions under which the plants are grown, should be considered e.g.

Meloidogyne javanica may be attracted to root meristems by root exudates (Bird, 1962b) and the latter are detectable by larvae even over long distances (Bird, 1960). These exudates may quickly become ineffective by being absorbed on clay colloids in the soil or inactivated by bacteria (Rovira, 1956a, 1956b).

Plants under stress as a result of high temperatures and improper fertilization may be stunted and even killed by relatively low numbers of nematodes (Brodie and Cooper, 1964a).

Temperature, light intensity and photoperiod have an influence on variations with experiments (Burdett, Bird and Fisher, 1963).

Plant parasitic nematodes respond to electric potentials (Caveness and Panzer, 1960; Bird, 1962b; Jones, 1960).

Poor hosts are presumably poor sources of nutrition of nematodes and thus favour the development of males (Dropkin, 1959). Nitrogen, potassium and phosphate have an influence on population levels of nematodes, (Kirkpatrick, Mai and Fischer, 1964).

7.2 Physiological races

Root-knot nematodes are known to consist of different races or biotypes (Allen, 1952a; Jones, 1957; Martin, 1954). Thus, when dealing with a nematode species, it must be remembered that these different physiological races may lead to different results.

7.3 Resistance to nematodes

Resistance to nematodes can be defined as the properties which make an organism unsuitable for initial or continued growth of a parasite, but resis-

tance sometimes takes the form of reduced susceptibility (Wood, 1967). The converse to susceptibility is "immunity". There is no sharp line of differentiation between immunity and susceptibility, which are two relative terms (Fairbrother, 1943). One or more of the following characteristics may be present in resistant plants:

1. lack of attraction
2. lack of larval entry
3. nutrient deficiency for nematodes in plants
4. failure of plant cell to respond to larval secretions
5. non-occurrence of syncytium development
6. necrosis of root tissues
7. release of nematotoxic substances
8. hypersensitive reactions
9. endodermal barrier
10. formation of wound periderm.

(Christie, 1949; Rohde, 1965; Riggs and Winstead, 1969; Cohn, 1964a and b; Cohn, 1965; Baxter and Blake, 1968; Pitcher, Patrick and Mountain, 1960; Davis and Jenkins, 1960; Sembner, 1963; van Gundy and Kirkpatrick, 1964; Dropkin and Webb, 1967; Gialmalva *et al*, 1963).

Several writers postulated that nematodes in roots often died of starvation, as they could not consume the cell contents (Barrons, 1939; Christie, 1949; Dean and Struble, 1953; Liao and Dunlap, 1950; Mizogani, 1947 and Shibuya, 1952).

When the cells immediately around the nematode die, the nematode also ultimately dies, but if the syncytia are formed, the nematode begins to develop (Dropkin, 1969). He also mentioned that the finding that genes for resistance to nematodes may be "temperature-sensitive or temperature-indifferent complicated the breeders' problems", and that the formation of necrosis can be influenced by the temperature. He also stated that the host tissues reacted to nematodes according to the general physiology of the plant.

The roots of all the cultivars studied were to a greater or lesser degree attacked by *M. hapla*.

Studies by Riggs and Winstead (1959) on resistance to root-knot nematodes revealed that:

The factors for susceptibility to *Meloidogyne incognita* in tomato plants were found to be located in both the tops and roots of plants. They were not translocated across a graft union. There are indications that the factors for resistance or susceptibility were inherent within individual cells. The factors for resistance or susceptibility are not translocated, nor do they cross graft unions.

No vine grafting was done in this study. If grafted vines with a heavy crop are grown under very poor soil conditions, it may be that the *viniferas* have indirectly a detrimental effect on the resistance of the root-stock to nematodes. It is a well known fact that if vines with a very heavy crop, and grafted on a root-stock insufficiently resistant to phylloxera, are grown under unfavourable conditions they are more liable to be destroyed by phylloxera. Under these conditions the *vinifera* has an indirect influence on the resistance of the root-stock.

During their studies on the resistance to root-knot nematodes in tomatoes, Riggs and Winstead (1959) also observed that:

1. Larvae penetrated the roots of resistant plants as freely and as rapidly as they penetrated roots of susceptible plants.

M. hapla penetrated the roots of all seven *Vitis* cultivars within 24 hours of being subjected to infection. This indicates that pre-invasion factors, e.g. nematocidal secretions by roots of the hosts either did not exist or were unable to prevent nematodal entry into the roots.

2. The invasion of resistant tissues resulted in the death of tissues around the invading larvae and the subsequent death of the larvae.

Necrosis in some instances caused the ultimate death of the larvae (cf. Steen, Fig. 98). Somestimes a wound periderm was formed with the same result (Figs. 97-99, 143 and 144).

3. Larvae were dead within 96 hours after inoculation. Five days

after infection no indications of live larvae were found in the roots of 99 R, Salt Creek and Dogridge.

Roots of Jacquez and Steen showed signs of resistance by forming wound periderms, but nevertheless were very susceptible. The very small galls occasionally formed on these two cultivars and those on White French and 1202 C, which are also very susceptible were indistinguishable. The larvae did not reach the egg laying stage in 99 R, Salt Creek and Dogridge. Although all the roots of the latter three cultivars were thoroughly examined microscopically, no root-knot galls were observed.

A few larvae occasionally developed to maturity in the roots of resistant tomatoes (Riggs and Winstead, 1969).

Nematode nests were occasionally formed in infected Salt Creek roots, but no eggs were observed.

7.4 Wound periderms

Several investigators observed wound periderms in the roots of plants attacked by nematodes e.g. in roses, (Davis and Jenkins, 1960), tomatoes (Sembner, 1963), citrus (van Gundy and Kirkpatrick, 1964 and Cohn, 1964a), orange roots (Schneider and Baines, 1964), peony roots (Eversmeyer and Dickerson, 1966) and edible ginger (Huang, 1966).

The formation of more than one periderm, including wound periderms in roots is well known. The formation of several periderms is considered by some research workers as being characteristic of the American root-stocks which are resistant to phylloxera e.g. (Foëx, 1977; Millardet, 1897; Ravaz, 1897 and Becker, 1957). According to Becker (1957) the *V. riparia* x *V. berlandieri* hybrids, have a better ability to form wound periderms than other *Vitis* cultivars. No literature is available as to what extent *Vitis* roots are able to form wound periderms when attacked by nematodes.

When discussing wound periderms of *Vitis* roots it must be remembered that undamaged roots of some cultivars form more than one periderm during the first year, e.g. Manzoni (1952) reported three periderms in one-year-old *V. riparia*

roots, but no second periderm in *V. vinifera* roots.

In this study it was observed that 99 R, Dogridge and Salt Creek formed two periderms during the first year, as confirmed by Britz (1968) and Pongráscz (1969).

As some of the above-mentioned cultivars form two periderms within the first year, care must be taken not to confuse a normal second periderm with a wound periderm. It is not possible to distinguish between a wound periderm and a second periderm with any degree of certainty (Figs. 107, 143 and 144). Observations made in this study confirm the statement made by Morris and Mann (1955) that natural and wound periderms were basically alike in method of origin and growth and might have the same cellular composition.

The nematodes penetrated the outer periderms of all seven cultivars studied. White French, 1202 C and Jacquez were not able to stop the invasion of the nematodes by forming wound periderms. It appeared that wound periderms formed by Jacquez hampered the movements of the nematodes but could not always prevent them from damaging the roots (Figs. 102-103, 128-130).

Steen sometimes managed to form an effective wound periderm at certain sites in the root, which prevented deeper penetration by nematodes at these spots. Invasion next to these particular spots sometimes took place, however, causing serious damage to the roots (Figs. 99 and 100).

Dogridge, 99 R and Salt Creek roots formed very effective second periderms by which the nematodes were prevented from further penetration (Figs. 143 and 144).

7.5 Necrosis

Resistant plants do not always develop cell necrosis in response to nematode attacks. The reduction of the necrosis at elevated temperatures, when tomatoes were attacked by *Meloidogyne* spp. suggest that high temperatures may effect browning of cells after injury (Dropkin, 1969).

The occurrence of necrosis in nematode susceptible roots as well as in roots resistant to nematodes is well known, e.g.

Axenic tomato seedlings resistant to *M. incognita* showed brown coloration when infected with this nematode. This response was absent in susceptible tomatoes. *M. hapla* caused necrosis in some tomato varieties (Dropkin and Webb, 1967).

Sweet potatoes showed two types of resistance to *Meloidogyne* spp. i.e. those associated with root necrosis and that type with reduced galls and no necrosis (Gialmalva *et al.*, 1963).

When *Heterodera glycines* Ichinohe, 1952, penetrated the protophloem of soybean roots, the latter became necrotic (Endo, 1964).

According to Endo (1965), Bergmann (1958) found that *Heterodera schachtii* Schmidt, 1871, caused root necrosis in resistant plants soon after penetration and that the larvae showed practically no further penetration of the roots.

It is remarkable that in those *Vitis* roots studied which formed effective wound periderms and showed hypersensitivity when invaded by larvae, necrosis is a more common occurrence, than in those which are more susceptible to the nematodes (Figs. 98, 100-101, 107-110, 136-142, 151-154 and 162-165).

7.6 Occlusion of vessels

Occlusion of vessels may be the result of:

- (a) hyperplastic crushing of vessels (Gardner, 1925). This phenomenon is of no practical value in stopping or retarding the spread of a disease through the vessels as in the case of tyloses or gums in vessels
- (b) tyloses
- (c) gums in vessels.

7.7 Tyloses

Gum deposition and tylosis development in the tracheary elements of the grapevine may occur in roots and shoots under apparently normal conditions (Mangin, 1895; Ráthay, 1896 as quoted and reaffirmed by Esau, 1948b) but particularly when the tissues are affected by various pathogens and physiological disturbances (Esau, 1948b).

Vascular occlusion is a general mechanism by which plants, once infected, protect themselves from systemic invasion. According to Eames and MacDaniels (1947), tyloses are a common occurrence in angiosperm wood, and are characteristic of certain species, but always absent in others.

Tyloses were observed in the following *Vitis* cultivars which were, as far as could be ascertained, healthy (Britz, 1968): White French, Jacquez, 1202 C, 101-14 Mgt. and 99 R. Britz also noticed:

- 1) more tyloses in 99 R than in the other above-mentioned cultivars;
- 2) the large vessels of 99 R formed during the late spring, also contained tyloses, whereas in the other cultivars mainly the small vessels of the early spring wood contained tyloses;
- 3) many tyloses contained small amounts of tannin and small starch grains. He did not observe when the tyloses were formed.

Pongrácz (1969) made a comparative anatomical study of a number of *Vitis* cultivars, including the following, but did not observe tyloses (in a personal communication): White French, Jacquez, Salt Creek, 1202 C and 99 R.

Tyloses were observed only in the infected roots of 1202 C (Figs. 158 and 159), Salt Creek (Fig. 157), Dogridge (Figs. 164-165) and 99 R (Fig. 156).

In the case of 1202 C tyloses were formed in the absence of any form of necrosis, but in the hypersensitive cultivars they were always accompanied by discoloration of adjacent tissue and serious necrosis (Fig. 157). Beckman (1966) in his studies on *Fusarium oxysporum* f. *cubense* in banana roots observed permanent sealing off of the infected vessels by tyloses. He found that the entire process involved two or four days and that vascular occlusion by tyloses occurred just above infection sites. In this study tyloses were observed opposite infection sites (Figs. 109, 157 and 161).

Blair and Darling (1968) who studied the influence of *Rhadinaphelenchus cocophilus* (Cobb) on coconut, *Cocos nucifera* L., and cabbage palm trees, *Roystonea oleracea* F.O. Cook, stated, that although the nematode was not found in the vascular tissue, xylem vessels in the discoloured area were found to be occluded and tyloses were not found in corresponding areas in healthy tissues.

Tyloses and discoloured tissues were observed in the tissues of 99 R, Salt Creek and Dogridge, although the nematodes were not present (Figs. 107-109 and 110).

Powers (1954) as quoted by Akai (1959) observed that tyloses and gums in the vessels of diseased stems are caused primarily by toxic effects of decomposition products of invaded cells and that these toxic substances are not systemic in nature but affect primarily a restricted region in the xylem.

Observations made during the present study are in agreement with those of Powers. In the infected roots of 99 R, Salt Creek and Dogridge occlusion of vessels and browning of tissues were observed, although the nematodes themselves were never in direct contact with the affected cells. I did not find any proof that toxic substances move in tracheary elements over appreciable distances.

Struckmeyer *et al* (1954) observed tyloses in the large vessels of the spring wood of oaks, especially in the last annual ring but less so in the small vessels of the summer wood.

The majority of tyloses in the infected *Vitis* roots were in the large vessels of the spring wood and to a lesser degree in the vessels of the summer wood (Figs. 151-157).

7.8 Gums in vessels

Rathay (1896) reported that gum development in *Vitis* roots appeared to be less common and less regular than in the stem.

Pierce's disease of *V. vinifera* induced the formation of gums in various xylem cells of the canes, including the vessels (Esau, 1948b).

Gum substances were observed only in the vessels of the hypersensitive cultivars i.e. 99 R, Salt Creek and Dogridge (Figs. 109, 111 and 151). According to Akai (1959) Hemmi (1916) made similar observations where cherry stems were attacked by *Valsa japonica*.

7.9 Hypersensitivity

Hypersensitivity is usually regarded as an acute local reaction of a resistant or highly resistant plant to infection. It is normally recognised by dead tissue around the entry of the parasite, which is visible to the naked eye, but the term is also applicable to less obvious local reactions detectable in other ways (Wood, 1967).

The phenomenon of hypersensitivity has been known since at least 1902. For many years this subject has been thoroughly discussed by plant pathologists.

Hypersensitive reactions are considered to encompass "all morphological and histological changes that, when produced by an infectious agent, elicit the premature dying off of the infected tissue as well as inactivation and localization of the infectious agent" (Müller as quoted by Horsfold and Dimond, 1959).

The resistance of the *Vitis* cultivars studied varied from highly susceptible, e.g. White French, Steen, Jacquez and 1202 C, to highly resistant, e.g. Salt Creek, 99 R and Dogridge.

These seven cultivars differ in grades of resistance as shown clearly in Figures 72, 90-92, 104-123, 126-130, 131-135, 136-138, 139-142, 143-144, 145-147, 148-150, 151-154, 160-163, 164 and 165).

Different grades of resistance are found in the Steen cultivar. Steen (Figs. 99-101) for instance, formed wound periderm in a discontinuous layer. Where the wound periderm was formed, the nematodes were kept out, but next to the wound periderm the nematodes succeeded in penetrating the root. Severe necrosis which is found in hypersensitive hosts is illustrated in Fig. 101.

The following cultivars clearly show hypersensitivity:

Steen (Figs. 99-101 and 131-135)

99 R (Figs. 107-111, 139-141, 143-146, 148-157, 160 and 161)

Dogridge (Figs. 104-105, 162-165)

Salt Creek (Fig. 106).

7.10 Nematodes and the production of growth regulators

When *Gossypium hirsutum* was infected by *Rotylenchus reniformis* Linford and Oliveira, 1940, Birchfield (1962) noticed that cells near the head of the nematode were stained darker than surrounding tissue. These darkened necrotic areas extended into several cells in the phloem on each side of the nematode's head, along the axis of the root. He ascribed it to the translocation from the point of injection of a toxin produced by the nematodes.

Although *M. hapla* was not in direct contact with the *Vitis* root cells concerned, necrosis occurred (Figs. 98 and 101). Necrotic cells, were observed adjacent to tracheary elements, without *M. hapla* being in the xylem itself (Figs. 104, 106 and 107). The xylem of 99 R, Salt Creek and Dogridge was very severely damaged without the nematodes having entered it.

In 1202 C long syncytia which nearly reached the primary xylem without the nematode having penetrated so far, were formed (Figs. 112-116).

In Jacquez the long syncytia reaching almost up to the pith without the nematode having penetrated so far, is also an example of severe damage done to cells without the nematodes being in direct contact with them (Figs. 117-122). The abnormally long parenchyma cells in the vascular rays are an indication that the substances produced by the nematodes probably move along the vascular rays.

It would appear that harmful substances are not injected by the nematode's stylet alone, but are probably also excreted through its anus and secreted by the gelatinous matrix and perhaps by the rest of its body as well.

Damage is sometimes done to cells adjacent to a nematode nest (Fig. 129 and 130). A nematode nest might be surrounded by periderm (Figs. 131 and 132). The absence of starch-containing cells adjacent to the nematode's nest is clearly seen in Fig. 132, but beyond the wound periderm the cells contain much starch.

Severe necrosis occurred in the roots of Steen and 99 R (Figs. 133-142). Although *M. hapla* was prevented, by wound periderm, from penetrating the

root, the nematodes probably managed to produce a toxin in the roots before a wound periderm was formed, with the result that the damage done spread to the primary xylem.

Damage was done to xylem elements, including necrosis of tracheary elements. The formation of gums and tyloses, was observed although the nematodes were not in direct contact with the cells concerned (Figs. 151, 153 and 157).

Goodey (1948) was most probably the first person who suggested that nematode galls were caused by excretions similar to auxins.

Despite Bird's inability to detect tryptophane or IAA in significant amounts in galled tissue (Bird, 1962a), he still thought that Krusberg's observations (Krusberg, 1961) supported the idea that plant growth hormones are associated with gall formation by plant parasitic nematodes.

The formation of dense cytoplasm by plant cells in response to nematode stimuli seems to be a factor associated with the host-parasite relationship (Bird, 1961, 1962a). He detected neither amylase activity nor phenolic compounds in exudates (Bird, 1966). In this connection it is interesting to note that the cells adjacent to a female were almost depleted of starch (Fig. 132).

When studying the feeding and histopathology of the citrus nematode, Cohn (1965) observed the depletion of starch granules in the feeding zone. Starch breakdown occurred in the presence of homogenates of larvae males of *Tylenchulus semipenetrans* Cobb, 1913.

Pyrenchyma cells of peony roots were void of starch when attacked by *M. hapla* and *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 (Eversmeyer and Dickerson, 1966).

There is evidence that auxins are a normal product of autolysing cells, and that dying cells are an important source of auxin in plants (Sheldrake and Northcote, 1968). Damaged cells provide substances which stimulate cell division (Bloch, 1941). If one assumes that there may be two sources of auxins i.e. from *M. hapla* and from dying or autolysing cells, then immediately

the question arises, what role did these auxin sources play in the serious damage done and in the formation of new cells, the formation of parenchyma cells and abnormal tracheary elements, as illustrated by Figs. 110, 151-154 and 160-163.

One plant species will suffer much from an attack and even be killed, whereas another species will show no signs of real suffering even in a heavy degree of infestation (Steiner, 1925).

Although 99 R, Dogridge and Salt Creek are hypersensitive to attacks by *M. hapla*, no indication was found that these cultivars were easily killed by this nematode. Jacquez which is very susceptible to attacks by *M. hapla* no doubt tolerates serious infestations.

7.11 External morphology of roots and root-knots

Several investigators have reported that roots infected with nematodes show certain external aberrations e.g. stunting, branching of rootlets and root-knots.

Meloidogyne spp. differ as to their ability to form galls on the roots of the hosts concerned.

Swollen *Vitis* root tips were very seldom seen and excessive lateral root development of roots was occasionally observed.

M. hapla caused extensive lateral root development on cotton (Brodie and Cooper, 1946b).

The number of galls and their size were no reliable indication of the resistance of the vines to *M. hapla*. Gall formation on Jacquez roots was either small or absent (Fig. 7). Salt Creek, 99 R and Dogridge formed no galls at all.

With *M. hapla* infection of soybeans individual galls were too small to score the root (Dropkin, 1959). He considered egg mass production as "the most sensitive indicator of the parasite's welfare". The development rate and egg-mass production of *Meloidogyne* is considerably more accurate measures

than visual rating. With some hosts, galls are produced entirely by root surface feeding. Gallling and syncytia formation in response to *Meloidogyne* are two quite different phenomena. Host susceptibility is not necessarily related to galling (Burdett, Bird and Fischer, 1963).

Poor egg mass production of root-knot nematodes in both heavily and lightly galled soybean roots was observed (Dropkin, 1959). On tomato roots *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 induced large multiple galls, whereas *M. hapla* induced small individual galls with numerous side roots (Dropkin and Boone, 1966).

M. hapla and *M. incognita* caused galls at many points on peony roots even though the favoured place of nematode entrance was near or at the root tip (Eversmeyer and Dickerson, 1966).

The pseudo root-knot nematode *Hypsoperine ottersoni* Thorne caused sickle or ring-shaped galls on the roots of *Phalaris arundinacea* (Webber and Barker, 1967). They were of the opinion that "gall-like swellings on crops which were not penetrated by *H. ottersoni* may have been induced by surface feeding of larvae, since no nematodes were present in some swellings". They referred to the same phenomena as reported by Loewenberg *et al* (1960).

A number of small gall-like swellings, not containing nematodes, were observed on the roots of all seven *Vitis* cultivars. Before a definite opinion can be given as to whether this phenomenon is in fact due to surface feeding by *M. hapla*, more detailed research should be done.

7.12 Nematode eggs and larvae

When *M. hapla* developed close to the xylem tissues the female died before eggs were produced (Fig. 68).

Larvae of *M. incognita* which were in the inner tissues of the tubers of *Dioscorea composita*, *D. spiculiflora* and *D. floribunda* died before viable eggs were produced (Bruhn and Koch, 1962).

Daulton and Nusbaum (1961) pointed out that the ability of *M. hapla* eggs

to survive the winter is marked. Especially in the Western Cape with its cool wet winters this ability of *M. hapla* is of great practical value to the grape grower and scientist.

Egg mass production by *Meloidogyne* spp. seemed to be independent of gall size (Dropkin, 1959).

M. hapla formed many egg sacs without root-knots being present (Figs. 7, 47-49 and 102).

Observations made with *Vitis* roots, confirm Dropkin's (1959) statement that "egg mass production is the most sensitive indicator of the parasite's welfare". He observed poor egg masses in both heavily and lightly galled cultivars. He also noticed in histological sections a correlation between syncytium structure and superficial egg mass production. The same observations were made where *M. hapla* attacked Steen, White French, Jacquez and 1202 C.

Root-knot nematodes penetrated pineapple and cowpea roots within six hours of inoculation (Godfrey and Oliveira, 1932). *M. hapla* entered Jacquez, Steen, 99 R and Salt Creek within 12 hours of inoculation. The other three cultivars were not examined within the first 24 hours.

The penetration of the sweet potato by root-knot nematodes was primarily intercellular in the cortex and intracellular in the vascular cylinder (Krusberg and Nielsen, 1958).

When a female larva is at an advanced stage of development she is too big to move in the intercellular spaces (Figs. 63-64). At this stage she must migrate by means of physical or chemical destruction of cell-walls.

Only once was there an indication that the nematodes had probably broken the cell-walls by force (Fig. 18). Many indications were found that the cell-walls had been dissolved (c.f. Syncytia p.66).

Mankau (1956) as quoted by Endo (1964) stated that the migration of larvae of *Heterodera trifolii* (Goffart) Oostenbrink, in the cortex of clover

roots, were entirely intracellular (Mankau, 1956).

The egg matrix of *Meloidogyne* spp. in edible ginger was destructive to parenchyma cells (Huang, 1966). Similar observations were made where *M. hapla* infected 1202 C and White French (Figs. 69-71). Huang further reported that egg masses were discharged from the fibrous roots, whereas they were mostly retained within the cortex of the fleshy roots. Egg masses in *Vitis* roots were always produced outside the xylem (Figs. 47-49, 72, 90-91, 102 and 103).

Larvae development of *M. incognita* was retarded under conditions of crowding (Triantaphyllou, 1960). No observations in this connection were made in the present study. *M. hapla* larvae penetrated 99 R, Salt Creek and Dogridge but were unable to develop up to the egg laying stage. Most probably they died of starvation, as these three *Vitis* cultivars were poor hosts for *M. hapla*.

Five days after inoculation, the larvae could not be detected in 99 R, Salt Creek and Dogridge. *M. incognita* appeared to be dead after 96 hours in resistant plants (Riggs and Winstead, 1969).

It has been previously postulated that larvae which penetrated resistant roots died of starvation (Barrons, 1939; Christie, 1949; Dean and Struble, 1953; Frazier and Dennett, 1949; Gilbert and McGuire, 1952; Mizogami, 1947 and Shibuya, 1952).

Steiner (1925) stated that when larvae were driven by starvation they attacked a host which they completely ignored if a preferable one was present. This may be the explanation why *M. hapla* attacked 99 R, Salt Creek and Dogridge in such large numbers, although some of these cultivars formed up to four periderms in succession (Figs. 106, 143 and 144).

M. hapla females in *Allium cepa* roots did not increase in diameter after 35 days (Smith and Mai, 1965). Similar observations were made with *M. hapla* in *Vitis* roots. These two research workers always found *M. hapla* larvae within 1,2 mm of the root tip one day after inoculation, but never in

or below the apical initials. *M. hapla* attacked *Vitis* roots also above the apical initials, from 650µm to 10 mm from the root tips (Figs. 15-16 and 18). They settled down to produce eggs at 10 mm from the root tips (Figs. 47-49). They also noticed that "few of the nematode bodies were straight in roots harvested one day after inoculation. Instead, the bodies were frequently greatly curved". Similar observations were made with *M. hapla* larvae in *Vitis* roots (Figs. 58-60).

These research workers also observed that the larvae "became sedentary and began to feed on the immature vascular tissues surrounding their heads". In vines *M. hapla* preferred to feed on immature vascular tissues (Figs. 27, 34, 36, 38, 42, 45 and 47-49). This nematode often developed a long neck to reach the vascular tissues (Fig. 49-51). Sometimes, however, it remained short (Figs. 66-68).

7.13 Influence of the nematode on the internal structure of the root

7.13.1 Root cap

Meloidogyne larvae entered the roots of *Portulaca oleracea* just behind the root cap (Linford, 1939). *M. hapla* also attacked *Vitis* roots behind the root cap (Fig. 18).

7.13.2 Cortex

The penetration of the cortex by nematodes, breaking of cortical cell-walls, hypertrophied cortical cells and necrotic cells, in several plant species other than *Vitis* roots, are known (Baxter and Blake, 1968; Troll and Rohde, 1966; DuCharme, 1959; Blake, 1966; Krusberg and Nielsen, 1958; Mountain and Patrick, 1959; Townshend, 1963a and b; Ross, 1958; Eversmeyer and Dickerson, 1966; Huang, 1966; Webber and Barker, 1967).

In contrast to the above-mentioned observations, only two instances of damage to the cortex of *Vitis* roots by *M. hapla* were observed (Figs. 15-16 and 18). A possible explanation for this phenomenon is that *Vitis* roots in general form the first periderm at an early stage of their development. It was formed at about 10 mm from the root tip (Figs. 47 and 49). The cortex is thus sloughed off and is of no further value to the plant.

7.13.3 Endodermis

It is known that the different tissues of *Vitaceae* are rich in tannin, nevertheless the high tannin content of the endodermis in *Vitis* roots is remarkable, as already pointed out by Britz (1968).

According to Van Fleet (1961) the endodermis contains several organic compounds including large quantities of phenols, naphthols, anthrols etc.

There are also enzyme systems in the endodermis. The endodermis may also play a role in the selection of ions and the accumulation of salts in the xylem (Van Fleet, 1961).

Taking these observations into consideration it is obvious that the endodermis might have an effect on the penetration and feeding of nematodes, and that different nematode species and physiological races may react differently when they reach the endodermis. In fact this is what happened. For example, when the citrus nematode *Tylenchulus semipenetrans* penetrated the fine roots of citrus, it often reached the endodermis. Although the cortex was badly damaged, the stele was intact (Cohn, 1964_a and _b and Cohn, 1965).

Pratylenchus thornei (Sher and Allen) invaded wheat roots. While it caused extensive destruction of the cortex, the endodermis and stele remained intact (Baxter and Blake, 1968).

Heterodera glycines extended its stylet through the inner endodermal wall of a soybean root, into the stele (Endo, 1964). In connection with his observations on the influence of this nematode on the endodermis, he reported that:

Within two days of infection cell-wall dissolution of endodermal cells occurred and multinucleate conditions of endodermal cells, and clumped and disintegrating nuclei were observed. Endodermal cells were greatly enlarged during syncytial development. The endodermal layer, however, was compressed or crushed when the nematode stylet extended through the outer wall and into the inner tangential wall to a healthy pericycle cell-wall.

M. incognita ruptured the endodermis and the pericycle and penetrated vascular elements of edible ginger (Huang, 1966). He also observed extensive wall thickenings of the endodermis. Nematode infection was found in the cortex as well as in the central cylinder.

Radapholus similis (Cobb, 1893) Thorne, 1949 and *Helicotylenchus multincinctus* Cobb, 1893; Golden, 1956, unlike *R. similis* in citrus (Du-Charme, 1959), did not enter the stele of banana roots through passage cells in the endodermis, nor was hyperplasia or tumour formation evident in the pericycle (Blake, 1966).

P. penetrans (Cobb, 1917) Filipjev and Stekhoven, 1941, was also unable to enter the stele of apple roots (Pitcher, Patrick and Mountain, 1960).

In *Vitis* roots the endodermis was definitely unable to stop *M. hapla* from entering the stele (Figs. 26-29). Typical, intact endodermal cells were absent in the infected area, but the tannin masses of the endodermal cells were still visible (Fig. 28). Apart from the endodermal cell-walls being affected, no other effect of the nematode on the contents of the endodermal cells was noticed.

From the above it is clear that in some plants the endodermis was an effective barrier against invading nematodes, but in other cases the endodermis, as well as the stele, was badly damaged.

7.13.4 Pericycle

Since the pericycle can become meristematic, its reaction to nematode attacks is of great importance.

Endo (1964) studied the influence of *H. glycines* on the pericycle of soybeans and observed, inter alia, the following:

1. pierced pericycle cells and limited hyperplasia of surrounding tissue ;
2. the dissolution of pericycle cell-walls within two days of infection, leaving a lattice work of cell-wall fragments ;

3. clumped nuclei and multinucleate conditions. Syncytia were first restricted to the pericycle, but later they extended to other tissues.

In *Vitis* the multiseriate pericycle (Fig. 23) consisted of thin-walled parenchyma cells (Figs. 21 and 22), which were easily destroyed by *M. hapla* (Fig. 27).

M. incognita acrita caused hyperplasia in the pericycle of the roots of *Hibiscus esculentus* (Littrell, 1966).

In *Vitis* roots different degrees of stele destruction by *M. hapla* were observed (Figs. 26 and 27). The pericycle cells (Fig. 27) were destroyed by the nematodes, leaving cell-wall fragments behind. The nuclei in broken and intact cells were still visible. The pericycle cells in the cultivar group exhibiting high resistance viz 99 R, Salt Creek and Dogridge suffered badly whereas the pericycle cells of the susceptible group e.g. Steen, 1201 C, Jacquez and White French escaped injury to a remarkable degree (c.f. 5,4). Syncytia next to the protoxylem replaced the metaxylem (Fig. 27).

A. cepa roots infected with *M. hapla* formed lateral roots in the pericycle, in the immediate region of syncytia (Smith and Mai, 1965).

Syncytia were observed in the pericycle and at the basis of lateral roots in the infected Jacquez roots (Fig. 42).

7.13.5 Phloem

The nematodes apparently prefer to settle in the phloem rays which consist of living thin-walled parenchyma cells. In this tissue wound periderms are formed thus, preventing the immigrating nematodes from further penetration.

According to Bird (1962a) results with *M. javanica* in tomatoes and beans indicate that syncytia development and maintenance depend on a continuous stimulus from the nematodes and that once this stimulus is removed the cytoplasm of the syncytia cells become vacuolated, break down and is eventually encroached upon by the surrounding cells of the host.

In soybeans, infested with *Heterodera glycines*, Endo (1965) found that many syncytia degenerated within five days of inoculation and were associated with dead second stage larvae. Rejuvenated parenchyma cells had invaded regions of the roots vacated by degenerated syncytia within 8-10 days of inoculation.

In *Vitis* roots vacuolation of syncytia occurred where the nematode were old or dead (Figs. 41, 87, 92, 94-95, 112-116 and 118). The encroachment of surrounding adjacent root tissue into old vacuolated syncytia were often observed (Figs. 115 and 125).

Where *H. glycines* attacked soybean roots, some cortical parenchyma cells enlarged and elongated to fill gaps formed when cells were destroyed by invading larvae. The partial filling of cavities could restrict the entry of other micro-organisms and thus reduced possibilities for a disease complex. The protophloem of soybean roots became necrotic when attacked by *H. glycines* (Endo, 1964). No necrosis of the protophloem was observed where *M. hapla* attacked *Vitis* roots. Syncytia were formed next to the primary xylem of Steen, White French and Jacquez (Fig. 28).

In soybeans attacked by *H. glycines* deteriorated syncytia in cortical cells maintained their original cell boundaries, whereas syncytia consisting of vascular tissues were compressed by rejuvenated tissues surrounding syncytia and by the secondary growth of the roots (Endo, 1965). He also observed that the invasive growth of parenchymatous tissue was more gradual in susceptible plants when the tissues were associated with enlarged syncytia associated with adult egg-producing females.

In peony roots infected with *M. hapla* and *M. incognita* the parenchyma cells were nearly void of starch (Eversmeyer and Dickerson, 1966). These two research workers also observed that some cells appeared compressed which they ascribed to pressure from development of adult nematodes, large syncytia and egg masses.

Figures 73 and 92 illustrate cells which were compressed and deformed adjacent to large syncytia. Even cambial cells were crushed probably as a

result of abnormal phloem growth, thus pressure was exercised on them (Figs. 148-150).

Elongated parenchyma cells developed between syncytia, where *Gardinia jasminoides* (Veitchi) was infected with three *Meloidogyne* species (Davis and Jenkins, 1960).

Narrow, elongated parenchyma cells were of common occurrence in vascular rays where *M. hapla* infected Steen, White French, Jacquez and 1202 C (Figs. 117-119, 121 and 122). This phenomenon was not observed with 99 R, Salt Creek and Dogridge. This is probably due to the fact that the nematodes never came into direct contact with vascular rays as they were prevented from doing so by means of periderms (Figs. 106, 143, 144 and 146).

Shoots of sweet orange (*Capensis sinensis* (L) Osbeck) were infected by a virus which often caused the cambium to be hyperactive and disorganized. Hyperactive cambium resulted in formation of wide bands of phloem called "replacement phloem". The cells in this replacement phloem varied from almost normal to "wild and irregular in form". Sieve-tubes and some parenchyma cells were necrotic, and other parenchyma cells contained thick cytoplasm and enlarged nuclei (Schneider, 1968).

A wide band of pericycle was formed in all seven cultivars studied, when attacked by *M. hapla* (Figs. 90, 112, 121, 126 and 145). The organisation of cells in the "replacement phloem" can be from almost normal (Fig. 148) to "wild" and irregular in form (Figs. 112, 117 and 145). Some cells contained dense cytoplasm and others were necrotic (Figs. 121 and 122). Hyperactive cambium that can be recognized by the formation of secondary phloem in the form of muriform tissue was observed (Figs. 145 and 146).

7.13.6 Xylem

Since the xylem is the principal water-conducting tissue of a vascular plant, its reactions to infections of different organisms, are of the utmost importance.

Huang (1966) observed abnormal xylem in all infected tissues except

rhizome meristems, of edible ginger infected with *Meloidogyne* spp. He also observed that malformed xylem was always associated with syncytia. This was almost always the case where *M. hapla* attacked Steen, White French, Jacquez and 1202 C (Figs. 38-45).

Meloidogyne incognita acrita caused abnormal xylem in *Hibiscus esculentus* (Littrell, 1966). Littrell observed that syncytia were also bordered by abnormal xylem on at least one side, which prevented a further increase of syncytia in that direction. Similar results were obtained with *M. hapla* in *Vitis* roots (Figs. 45, 115 and 116).

Hypoperine ottersoni caused syncytia to form in *Phalaris tuberosa* and *Hordeum vulgare* which were encircled by xylem elements except for the area around the head of the nematode (Webber and Barker, 1967). Esau (1933) also observed xylem elements which differentiated in hyperplastic tissue. Salt Creek, 99 R and Dogridge formed abnormal xylem elements as a result of *M. hapla* attacks (Figs. 154 and 160-163).

When soybeans are attacked by *H. glycines*, direct damage of xylem elements may occur after penetrations by second stage larvae (Endo, 1964). Similar observations were not made where *M. hapla* attacked *Vitis* roots.

Endo (1964) also observed the crushing or incorporation of xylem elements by indirect action of enlarging syncytia. This was also observed where *M. hapla* attacked *Vitis* roots (Figs. 27, 74, 96, 101, 113-116, 118 and 123).

According to Eversmeyer and Dickerson (1966), Christie (1936) found that the xylem often failed to differentiate on the side of the root where infection occurred, and that short irregular and unorganized elements were often formed "from parenchyma cells in older galls".

The failure of differentiation of tracheary elements when *M. hapla* attacked 99 R, Salt Creek and Dogridge were often observed. Figure 110 illustrates the absence of vessel formation in the second annual ring. This is the region from where the nematodes attacked the root. The formation of

unorganized xylem often occurred (Figs. 160-163).

M. hapla and *M. incognita* caused syncytia, surrounded by irregularly shaped tracheary elements, in peony roots. As the xylem on the affected side of the root continued to develop and by-passed the affected area, a wedge-shaped area of parenchymatous cells void of tracheary elements, radiated from the centre of the xylem (Eversmeyer and Dickerson, 1966).

In infected *Vitis* roots, tracheary elements often by-passed syncytia, and formed in some places wedge-shaped areas of parenchymatous cells (Fig. 73).

Christie (1936), Davis and Jenkins (1960), Krusberg and Nielsen (1958) reported the formation of tracheary elements from xylem parenchyma, near syncytia. These tracheary elements had no definite arrangements and were short, reticulate and probably functionless.

Gardner (1925) who studied the "Hyperplastic crushing of tracheal tubes in mosaic tomato stems" reported that necrosis accompanied the above-mentioned disease. There was frequently a vigorous hyperplastic or proliferated growth which was so directed as to "crush the tracheal tubes". He stated that the necrosis was frequently accompanied by hyperplasia or proliferation of the adjacent cells. Zones or cushions of muriform tissue were formed which pushed in against necrotic tissue.

The crushing of tracheary elements in *Vitis* roots was often observed (Figs. 106 and 157). The vessels of the spring wood suffered more than those of the summer wood (Fig. 157). Muriform phloem parenchyma tissue pressing against the vascular cambium was observed when 99 R, Salt Creek and Dogridge were attacked by *M. hapla* (Figs. 145-147). This phloem tissue exercised severe pressure on the radial walls of the cambial cells, thus partly crushing them.

In soybean roots infected with *H. glycines*, cambium activity ceased almost entirely in those portions of lateral roots, distal to the disturbances incited by the nematode (Endo, 1964).

In Steen, White French, Jacquez and 1202 C roots infected with *M hapla*, the absence of vascular cambium was often noticed in regions where syncytia stretched from the xylem into the phloem (Figs. 112, 113 and the left part of Fig. 123).

When soybeans were attacked by *H. glycines*, the destruction of cambial cells, deprived large regions of the expanding root of normal secondary xylem and phloem tissue (Endo, 1965).

Abnormal xylem and phloem caused by damage to cambial cells were often observed in the roots of 99 R, Salt Creek and Dogridge (Fig. 110).

7.13.7 Syncytia

7.13.7.1 The initiation of syncytia

There are two schools of thought as to how syncytia are formed, i.e.:

- 1) syncytia are formed as a result of cell-wall breakdown;
- 2) cell-wall breakdown in a root-knot infected plant, plays no part in syncytia formation, but is initiated by repeated mitoses of the infected diploid cells without subsequent cytokineses.

Several research workers reported that syncytia are formed as a result of cell-wall dissolution (Birchfield, 1964) Bird, 1961; Christie, 1936; Crittenden, 1962; Dropkin and Nelson, 1960; Littrell, 1966; Owens and Specht, 1964).

According to Dropkin (1969), Nemeč (1910) presented some of the first detailed studies on the formation and structure of syncytia in plants infected with root-knot nematodes. Bird (1962a) stated that from the results with *M. javanica* in tomatoes and beans it appears that syncytia development and maintenance depends on a continuous stimulus from the nematode and once this stimulus is removed the cytoplasm of the syncytium becomes vacuolated, breaks down and is eventually encroached upon by the surrounding cells of the host.

Such vacuolation and encroachment was observed in infected *Vitis* roots after the nematode was dead or its life cycle completed (Figs. 41, 74-75, 91-92,

94-95 and 116-118).

The size and character of syncytia vary amongst different hosts exposed to the same nematode species. Similar variations occurred when the same nematode species attacks different hosts (Dropkin, 1959).

Syncytia in Steen were smaller than in Jacquez, White French or 1202 C. In 99 R, Salt Creek and Dogridge syncytia were observed only in the young stele.

Dropkin and Boone (1966) observed development of syncytia around the larval head. This often occurred in Steen, White French, 1202 C and Jacquez (Figs. 40, 41, 48-49, 66, 68, 85, 91 and 92). These two researchers further observed a brief period of cell-wall destruction during the enlargement of the syncytium which is surrounded by thick cell-walls. Cell vacuoles disappeared and the cytoplasm approached meristematic cytoplasm in appearance. *M. hapla* also caused cell-wall destruction in *Vitis* roots (Figs. 27, 43, 75-88, 95-96, 100-101 and 103) as well as thickening of cell-walls (Figs. 31-35, 37, 41, 44-45, 75-89 and 91-92).

When the roots of Lee soybeans (*Glycine max* (L) Merr.) were infected by the soybean cyst nematode *H. glycines*, Endo (1964) observed that the "thin and heavy wall appearance" in cross-sections and the scalariform-like perforations of cells in longitudinal sections, indicated cell-wall breakdown.

"Thin and heavy wall appearance" of syncytia in Steen, White French, Jacquez and 1202 C caused by *M. hapla* is illustrated in Figs. 75-89 and 101.

Endo (1964) also observed:

- 1) the dissolution of cell-walls adjoining syncytia;
- 2) the dissolution of cell-walls two days after infection;
- 3) no apparent change in cell-wall thickness of the syncytium was found as it developed in the pericycle;
- 4) the relative suitability of plant tissue for syncytial development varies according to the character of the tissue invaded;
- 5) syncytia developed readily in the pericyclic tissue;
- 6) the walls of the pericycle tissue were partially dissolved within

two days, leaving a lattice work of cell-wall fragments

(c.f. Fig. 27);

- 7) at and near the feeding sight, the syncytial-wall was slightly thickened;
- 8) the reduction in syncytial volume and width, especially in elongated syncytia, resulted in the receding of the syncytial-wall. The surrounding parenchymatous tissue occupied the resulting space.

The dissolution of cell-walls adjoining syncytia in *Vitis* roots was often observed (Figs. 31, 33, 37, 51, 85-86 and 115). The total destruction of cell-walls five days after infection was observed. The thickness of the walls of syncytia and the adjacent pericycle cells varied (Fig. 31).

The syncytial-wall at and near the feeding site of *M. hapla* in *Vitis* roots was sometimes slightly thickened (Fig. 41). In other instances there was no noticeable difference in thickness between the syncytial-wall and the walls of adjacent cells (Fig. 51).

The reduction of syncytial volume and the receding of syncytial-walls in *Vitis* roots, resulted in the encroachment of syncytia by parenchymatous tissue in Jacquez, Steen, White French and 1202 C (Figs. 115 and 125).

M. incognita caused syncytia in all infested tissues of edible ginger roots (Huang, 1966). In *Vitis* roots syncytia were observed:

- 1) only in the stele of 99 R, Salt Creek and Dogridge;
- 2) in all the tissues of Jacquez, White French, Steen and 1202 C, except in the pith.

Huang (1966) also found that malformed xylem was always associated with syncytia as in *Vitis* roots (Figs. 74-75, 100, 112-114, 118-119 and 123).

Huang and Maggenti (1969b) who studied "Wall modifications in developing cells of *Vicia faba* and *Cucumis sativus* induced by root-knot nematode, *M. javanica*", concluded:

- 1) The penetration and the subsequent migration of root-knot nematode larvae caused mechanical breakdown of numerous cell-walls. The growth of syncytia caused the collapse of broken cells. Wall breakdown played no part in

the syncytia formation. The breakdown of cell-walls have never been observed.

2) Although many workers reported that root-knot nematodes caused cell-wall breakdown, they failed to explain (i) how the walls were broken down, mechanically or enzymatically and (ii) when the breakdown took place, before or after the nematodes became sedentary.

3) The fusion of two neighbouring protoplasts were never observed, but on the contrary their evidence indicated that cells whose walls broke down eventually, collapsed.

As pectinase and cellulase have been demonstrated in *Pratylenchus penetrans* and *Heterodera trifolii* (Goffart, 1932) Oostenbrink, 1949 and hydrolytic enzymes in *P. penetrans* (Morgan and McAllan, 1962), there is a possibility that *Meloidogyne* species are also able to secrete such enzymes and dissolve cell-walls causing the formation of syncytia.

Syncytia in *Vitis* roots infected with *M. hapla* were no doubt formed as the result of cell-wall dissolution or breakdown (Figs. 27, 31, 33, 35, 37, 43, 75-89). *M. hapla* is able to dissolve cell-walls in the immediate vicinity of its head (Fig. 40). The lower left hand corner of Fig. 31 illustrates the dissolution of the syncytium-wall and walls of adjacent cells. The fusion of neighbouring protoplast was often observed (Fig. 86). The engulfing of adjacent parenchyma cells through cell-wall dissolution and the ultimate breaking of the wall was a very common occurrence (Figs. 31, 33, 35, 37, 43, 85 and 86).

Syncytium formation is normally accompanied by hypertrophy (Christie, 1936 and Huang, 1966). With *M. hapla* in *Vitis* roots, hypertrophy was often observed in the pericycle (Figs. 28 and 29).

Littrell (1966) studied the "Cellular responses of *Hibiscus esculentus* to *Meloidogyne incognita acrita*" and reported:

- 1) Syncytium development by engulfing of adjacent parenchyma cells until a maximum size of 250-400 μ m x 100-250 μ m was obtained.
Abnormal xylem and parenchyma accompanied all infections ;
- 2) walls of syncytia were from 3 μ m to 23 μ m thick ;

- 3) characteristic apertures in syncytium-walls 17 days after inoculation;
- 4) walls consisted of two portions, a comparatively thin external layer and a thicker layer that often protruded into the cytoplasm;
- 5) four to seven syncytia were associated with each individual nematode.

In *Vitis* roots infected with *M. hapla* the following observations were made:

- 1) Syncytia varied from 1,04 mm x 90,0 μ m, 590 μ m x 190 μ m and 125 μ m x 125 μ m in *Vitis* roots;
- 2) apertures in syncytia-walls were often observed which are ascribed to cell-wall dissolution and ultimate breaking (Figs. 43, 75-82 and 88);
- 3) cell-walls of syncytia in susceptible cultivars consisted of a comparatively thin external layer and a layer which often protruded into the cytoplasm (Figs. 31 and 88). The walls of the syncytia were from 2 μ m to 25 μ m thick;
- 4) four to ten syncytia per individual nematode were observed (Figs. 34, 40-41, 47-49 and 94).

Heterodera rostochiensis Wollenweber, 1923 caused the dissolution of cell-walls in potato roots (Piegat and Wilski, 1963). They very seldom observed cells mechanically damaged by larvae.

M. hapla caused "rosette-appearing" syncytia in onion, carrot, tomato and geranium (Riffle and Kuntz, 1967).

Syncytia in *Vitis* roots, as seen in microtome sections, were of different shapes, e.g. circular (Fig. 36), ovate (Fig. 38), almost square (Fig. 43), boomerang-shaped (Fig. 74), kidney-shaped (Fig. 101), cigar-shaped (Fig. 118), wedge-shaped (Fig. 123) etc.

The volume of syncytia in *A. cepa* roots infested with *M. hapla* increased

up to 14 days after inoculation but the walls of these syncytia were not thicker than the walls of normal parenchyma cells of onion roots (Smith and May, 1965). The volume of syncytia in *Vitis* roots attacked by *M. hapla* also increased up to about 14 days after inoculation.

Hypsoperine ottersoni caused cell-wall dissolution in canary grass (*Phalaris arundinacea*) and Moore barley (*Hordeum vulgare*). (Webber and Barker, 1967).

According to Steiner (1925), Vuillemin and Legrain (1894) suggested that *H. radicicola* might be useful for some crop plants in the Sahara. When these plants are attacked by this nematode they lignify the syncytia-walls and use the so-formed cavities as water reservoirs, thereby enabling them to accumulate water for the hot part of the day, whereas specimens not harbouring *H. radicicola*, perish.

No lignification of syncytia was observed in *Vitis* roots infected with *M. hapla*.

The above-mentioned discussion indicates that the majority of research workers believe that syncytia are formed by cell-wall dissolution, caused by the activities of nematodes.

The present study furnishes supplementary proof to this effect.

7.13.7.2 Cell contents of syncytia

Many publications appeared dealing with the occurrence of multinucleate syncytia and enlarged nuclei when plant roots are attacked by nematodes e.g.:

M. incognita acrita caused the formation of multinucleate cells in *Hibiscus esculentus* as a result of cell-wall dissolution (Littrell, 1966).

M. javanica caused multinucleate syncytia in tomato roots (Bird, 1961, 1962a). *H. ottersoni* caused multinucleate and vacuolated syncytia in canary grass (Webber and Barker, 1967). They attributed this multinucleate state to cell-wall dissolution and karyokinesis.

Heterodera glycines caused clumped nuclei in soybean roots, the migration of nuclei through dissolved cell-walls and "hyperchromatic material extending from the nematode feeding site to nuclear clusters appeared to coalesce" (Endo, 1964).

M. hapla and *M. incognita* caused clustered nuclei in peony roots (Eversmeyer and Dickerson, 1966).

M. incognita acrita caused enlarged nuclei, clumping of nuclei and distorted nuclear membranes in *Hibiscus esculentus* (Littrell, 1966). He also noticed mitoses without cell division.

Nuclei in syncytia of Steen, White French, 1202 C and Jacquez roots infected with *M. hapla* were sometimes also clumped, lobed or spread in gray masses of cytoplasm (Figs. 31-32, 34-38 and 79). Vacuolated syncytia were often observed in the roots of White French, Steen, Jacquez and 1202 C (fig. 87).

M. javanica in *Cucumis sativus* caused some nuclei in the hyperplastic tissues around cells to be lobed. They were, however, definitely smaller than those of the syncytium (Huang and Maggenti, 1969a).

In *Vitis* roots no lobed nuclei were observed in cells adjacent to syncytia.

When *H. rostochiensis* attacked potatoes the nuclei became irregularly elongated. The nuclear contents flowed out into projections formed by nuclei, though no mixing of the contents with the cytoplasm occurred (Piegat and Wilski, 1963).

Tylenchulus semipenetrans females feeding on cortical cells, caused vacuoles to disappear, the nuclei and nucleoli to be enlarged and the nuclei to appear amoeboid. The nuclear membrane was sometimes difficult to detect. The enlarged nuclei were thought to be a form of toxification (Schneider and Baines, 1964).

In *Vitis* roots enongated nuclei in syncytia were often observed (Figs.

32 and 37). The nuclear membrane was sometimes also difficult to detect (Fig. 79). Amoeboid nuclei were not observed.

Collapsed and distorted nuclei were embedded in thick, granular, grey stained cytoplasm of 20-30 day old syncytia in soybean roots infected with *H. glycines* (Endo, 1964).

Distorted and disintegrating nuclei embedded in gray cytoplasm were often observed in *Vitis* roots (Fig. 40).

M. incognita acrita caused the formation of densely staining cytoplasm in syncytia of *Hibiscus esculentus* roots (Littrell, 1966).

The formation of densely staining cytoplasm was a very common occurrence where *M. hapla* attacked Jacquez, Steen, White French and 1202 C (Figs. 31-38, 40, 42, 45, 68, 73, 76, 80 and 85-88).

In soybeans attacked by *H. glycines*, the nuclei in syncytia varied widely in size (Endo, 1964).

In *Vitis* roots also the nuclei in syncytia varied widely in size (Figs. 31-36, cf. Table 2). Nuclei in cells adjacent to the syncytia were always smaller than those in the syncytia and were sometimes elongated (Figs. 33 and 37, cf. Table 2), whereas nuclei and nucleoli in tissues not in close contact with syncytia were, as a rule, round and also smaller than those in the syncytia (Figs. 142, 147 and 149). Lobed nuclei were observed only in syncytia (Fig. 32) but not in hyperplastic tissues.

In infected tissue of hypersensitive cultivars e.g. 99 R, Salt Creek, Dogridge and the partly hypersensitive cultivar, Steen, the cell contents of cells, including their nuclei, stained more deeply red with safranin, than in uninfected tissues (Figs. 133-141). Much of the red colour was due to tannin in the cells and the necrotic condition of some cells.

A root of 99 R (Figs. 139-141) was 4,5 mm in diameter and although the base of the lateral root was 33 ray parenchyma cells from the periphery of the root, the entire lateral root, including the nuclei at its base, stained dark

red with safranin.

The Steen root (Figs. 133-138) was 2,3 mm in diameter and as with 99 R, although the base of the lateral root was 25 parenchyma cells from the epidermis of the root, the entire lateral root stained deep red with safranin. The nuclei (not visible in Figs. 137-138) also stained red. The vascular ray illustrated in Figs. 133-135 was necrotic over its entire length and stained dark red with safranin. No nuclei were noticed in the necrotic cells. In none of the above-mentioned *Vitis* roots was any indication found that the nematodes migrated beyond the pericycle.

= = = = =

8. S U M M A R Y

1. Many publications appeared since the last century in connection with the anatomy of undamaged *Vitis* roots and on the influence of *Dactylospira vitifolia* Shimer on the anatomy of these roots.
2. The object of this study was to collect scientific data as to the effect of *Meloidogyne hapla* Chitwood, 1949 on *Vitis* roots, without losing sight of the fact that these observations should as far as possible be of practical value.
3. *M. hapla* isolated from *Solanum tuberosum* L. cv. King George obtained from the Western Cape in the Republic of South Africa, was used to study the plant-host reaction of the following grapevine cultivars, namely Steen, White French, 1202 C, Jacquez, 99 R, Salt Creek and Dogridge, to a *Meloidogyne* species.
4. An inorganic nutrient medium based on Hoagland's medium was used for growing the ungrafted vines in sand. No effort was, however, made to study the effect of plant nutrients on the nematode-host reaction.
5. The method of macroscopically examining *Vitis* roots in order to determine the severity of infection with *M. hapla* proved unreliable, as root-knot or galls were small or absent. Negative correlations were recorded on root-knot forming ability and susceptibility.
6. Susceptibility of roots of the various cultivars differed widely.
7. Although *M. hapla* larvae entered the roots of all cultivars studied, they were unable to complete their life cycle in the roots of 99 R, Salt Creek and Dogridge.
8. The roots of 99 R, Salt Creek and Dogridge formed several periderms by which the nematodes were prevented from reaching the xylem. Jacquez and Steen also formed wound periderm which was only partly effective in protecting the xylem against the invading nematodes. A similar phenomenon was absent in the roots of White French and 1202 C, thus enabling the parasitic organisms to reach the xylem tissues and inflict severe damage.

9. Roots of Jacquez, Steen, White French and 1202 C are regarded as susceptible. Only in these cultivars was *M. hapla* capable of surviving, and its life span was apparently similar to that of other *Meloidogyne* spp. in a wide range of susceptible plant species.
10. The formation of multinucleate syncytia with enlarged nuclei and nucleoli resembles that observed in other plants susceptible to *Meloidogyne* spp.
11. The conclusions of various investigators that syncytia were formed by the destruction of the walls of groups of cells, by nematodal effects, were confirmed.
12. The differentiation of abnormal xylem and phloem tissue was a common occurrence in *Vitis* roots attacked by *M. hapla*.
13. The crushing of xylem vessels and the formation of atypical new xylem elements in 99 R, Salt Creek and Dogridge as a result of *M. hapla* infection were observed.
14. Observations point to the possibility that substances of a plant regulatory, toxic and enzymatic nature were associated with the activities of *M. hapla* in grapevine roots. Histological changes were frequently induced in advance of the invading nematodes.

~ ~ ~ ~ ~

ABBREVIATIONS

ab. camb.	abnormal cambium
ab. p.	abnormal parenchyma
ab. phl.	abnormal phloem
ab. x.	abnormal xylem
ant.	anterior
b,c.w.	broken cell-wall
b.n.n.	border of nematode nest
cam.	cambium
cw.	cell-wall
c.w.d.	cell-wall dissolution
c.p.	cortex parenchyma
cr.	crack
d.c.w.	dissolved cell-wall
d.n.f.	deformed female nematodes
e.	egg
end.	endodermis
ep.	epidermis
ex.	exodermis
g.	gall
g.c.	syncytium
g.c.w.	syncytium-wall
g.e.s.	gelatinous egg sac
g.m.	gelatinous matrix
gm.	gums
g.z.o.	growth zone of ovary
i.a.	infected area
i. par.	infected parenchyma
i.s.	intercellular space
it.	intrusion

j.	gelatinous material
l.	larva
l.r.	lateral root
l. rn.	lip region
m.b.	median bulb
m.t.	muriform tissue
mx.	metaxylem
n.	nucleus
nec.	necrosis
nem.	nematode
n.f.	female nematode
n.h.	nematode head
n.n.	nucleoli
n. nt.	nematode nest
n.p.	nematode path
ob.	obliteration
o.n.n.	old nematode nest
o.p.	opening between syncytia
o. s. pl.	old secondary phloem
o.x.	obliterated vessel
p.	granulated cytoplasm
par.	parenchyma
par. r.	vascular ray
pb.	sand particles
p. end.	pro-endodermis
perc.	pericycle
perd.	periderm
p.f.	perforation plate
phl.	phloem
phl.r.	phloem ray
p.i.	point of infection

p.m.	pith membrane
p.o.	partial occlusion
post.	posterior
p. perc.	proliferated pericycle
p. phl.	primary phloem
pr. x.	primary xylem
pth.	pith
px.	protoxylem
r.	root
ra.	raphide
r.c.	root cap
r.t.	root tip
r.w.	radial cell-wall
sc. t.	scalariform tracheid
sl.	slit
s.p.	second periderm
s. phl.	secondary phloem
sta.	starch
st. c.m.	stained cell-material
s.t.m.	sieve-tube member
s.x.	secondary xylem
tan.	tannin
tp.	transparent
tra.	tracheary elements
t.t.	tail tip
ty.	tyloses
un. x.	undifferentiated xylem
vac.	vacuole
vul.	vulva
w.p.	wound periderm

x. xylem
x.p. xylem parenchyma
x. pl. xylem pole

y.s. phl. young secondary phloem

Fig. 1: Jacquez. Active growing root (r) with initiating rootlets (l.r.). X 1,5.

Fig. 2: Jacquez. Growing point of root. Note the sudden increase in thickness just behind the growing point, a phenomenon which has often been observed but, as far as could be ascertained, was not related to any pathogenic condition. X 4,0.

Fig. 3: White French. Stunted lateral roots (l.r.) attacked by *Meloidogyne hapla*; root (r.). X 10.

Fig. 1.

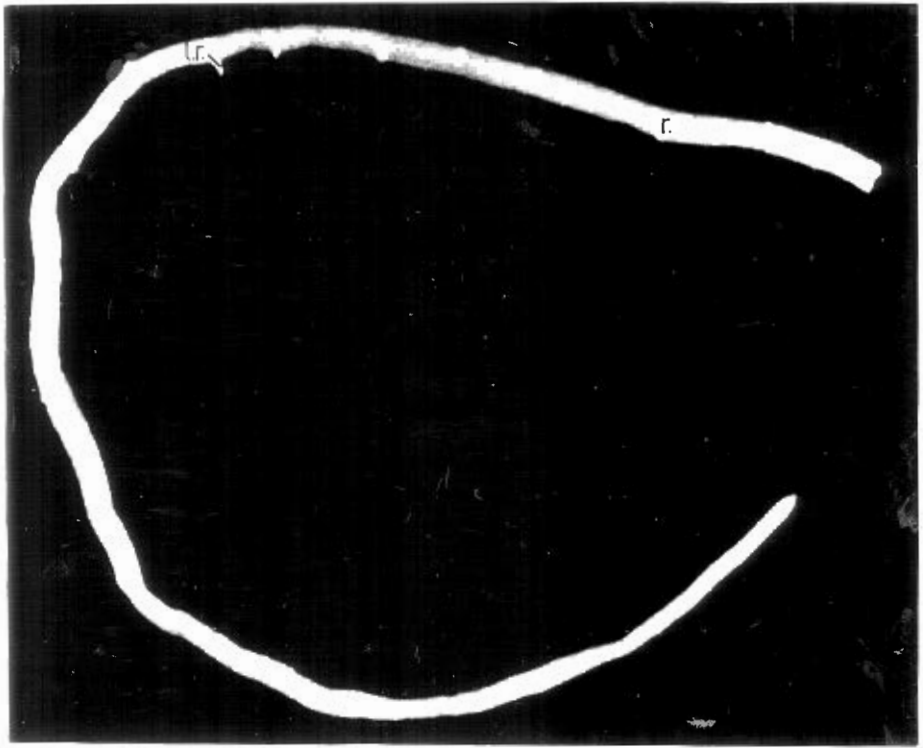


Fig. 2.

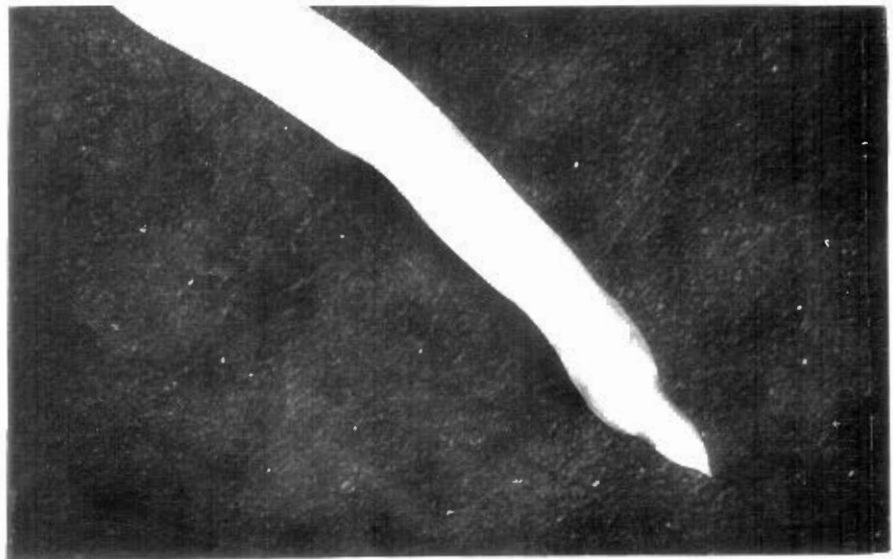


Fig. 3.

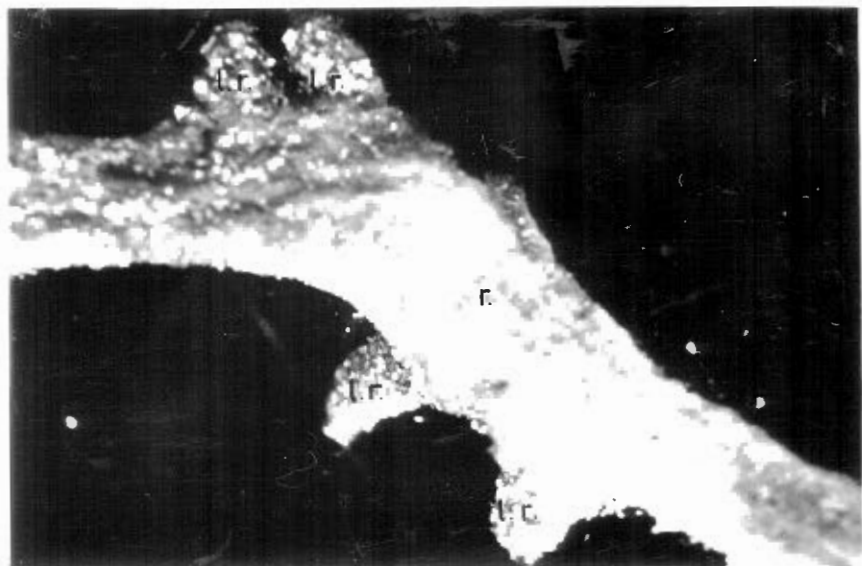


Fig. 4: White French Lateral roots (l.r.) infected with *M. hapla*. Sand particles (pb.) removed from tips of lateral roots (l.r.) to expose tips damaged by nematode attacks. X 10.

Fig. 5: White French. Root infected with *M. hapla*. Female in gall (g.) with her gelatinous matrix, "egg sac", on surface of root (r.) and covered with sand particles (pb.) which have adhered to the gelatinous matrix. X 10.

Fig. 6: White French. Root (r.) infected with *M. hapla*. Sand particles removed to expose eggs (e.); gall (g.). X 10.

Fig. 4.

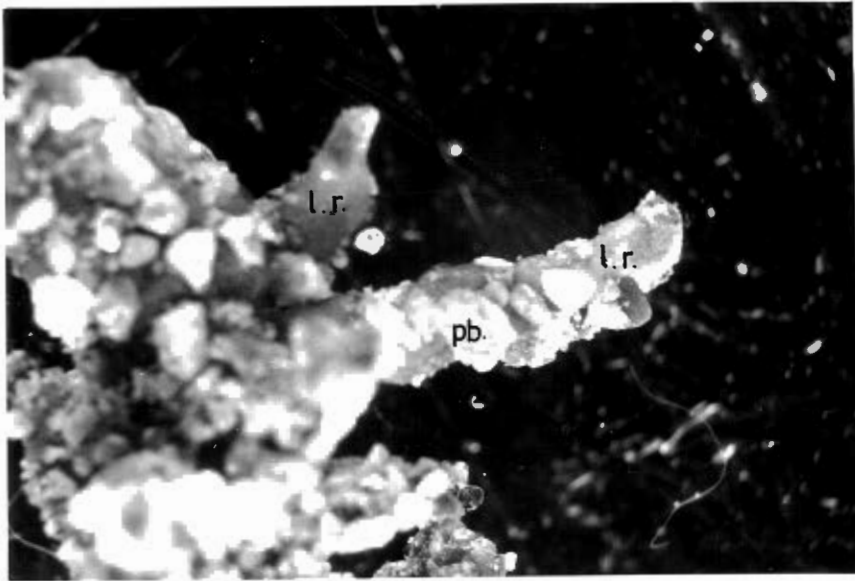


Fig. 5.

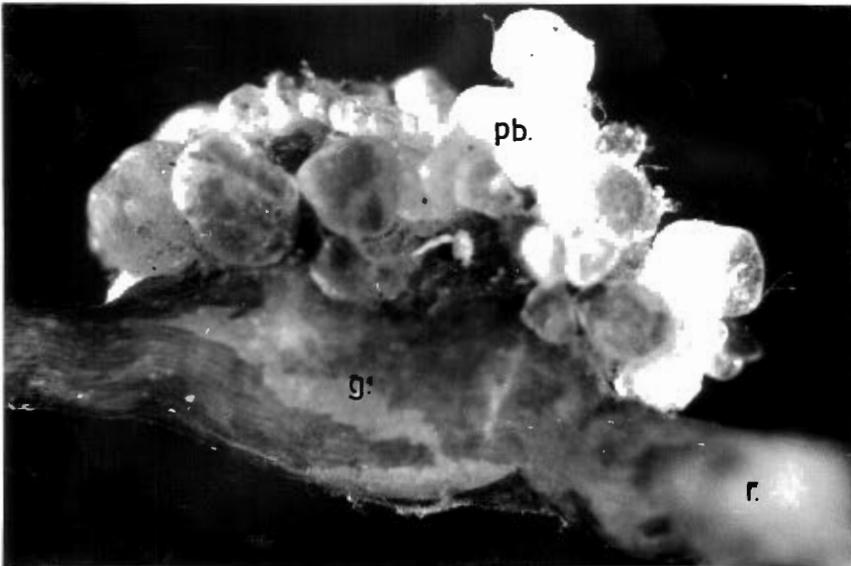


Fig. 6.

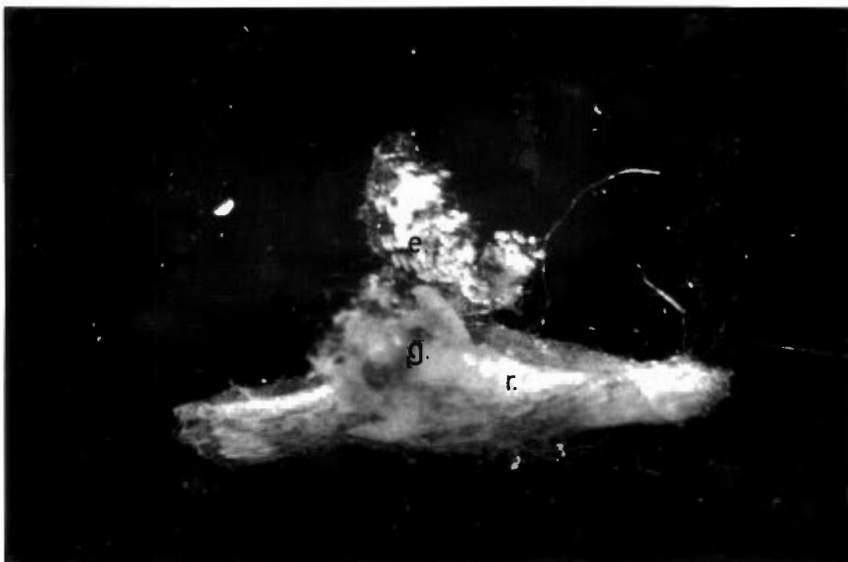


Fig. 7: Jacquez root (r.) with lateral roots (l.r.) infected with *M. hapla*. Gelatinous matrixes removed to expose posterior parts of female nematodes (nem. f.). Note absence of galls. X 10.

Fig. 8: Jacquez root (r.) inoculated with *M. hapla*. Gelatinous matrixes removed to expose four female nematodes (nem. f.). X 10.

Fig. 9: White French roots (r.) inoculated with *M. hapla*, showing surface of galls (g.). Part of gelatinous matrix (g.m.) can be seen; sand particles (pb). X 10.

Fig. 10: *M. hapla* females removed from Jacquez root, which was stained with lacto-phenol-fuchsin. The females were also stained in this process. X 30.

Fig. 7.

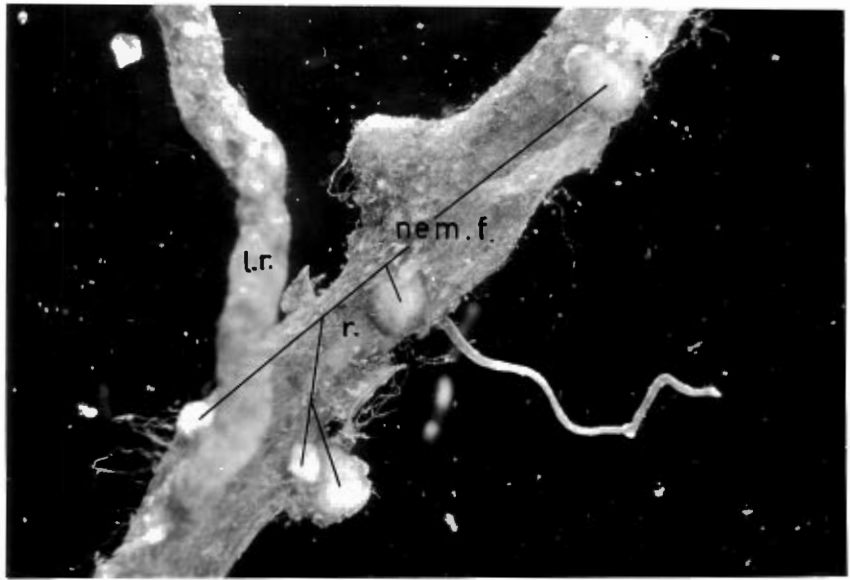


Fig. 8.

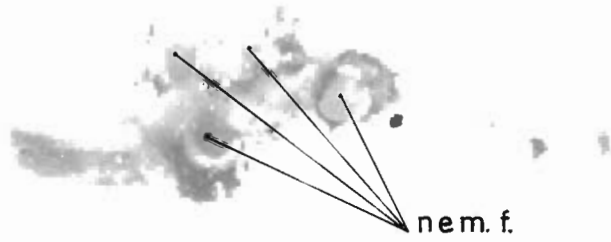


Fig. 9.

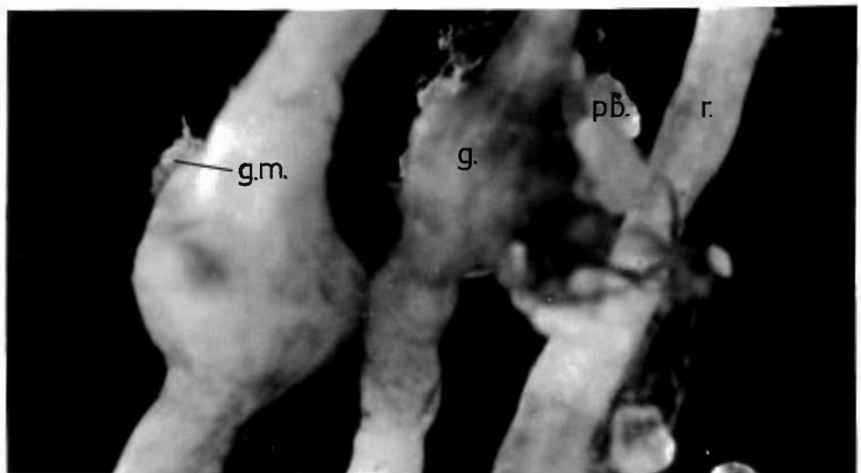


Fig. 10.



Fig. 11: Jacquez root (r.) with lateral root (l.r.), showing four old *M. hapla* nests (o.n.n.), which resemble tooth-sockets.
X 20.

Fig. 12: 99 R. Cross-section 600µm from root tip, of an uninfected root, showing root cap cells (r.c.), epidermis (ep.); cortex parenchyma (c.p.); sieve-tube members (s.t.m.); pro-endodermis (p. end.) and raphide sacs (ra.).
X 30.

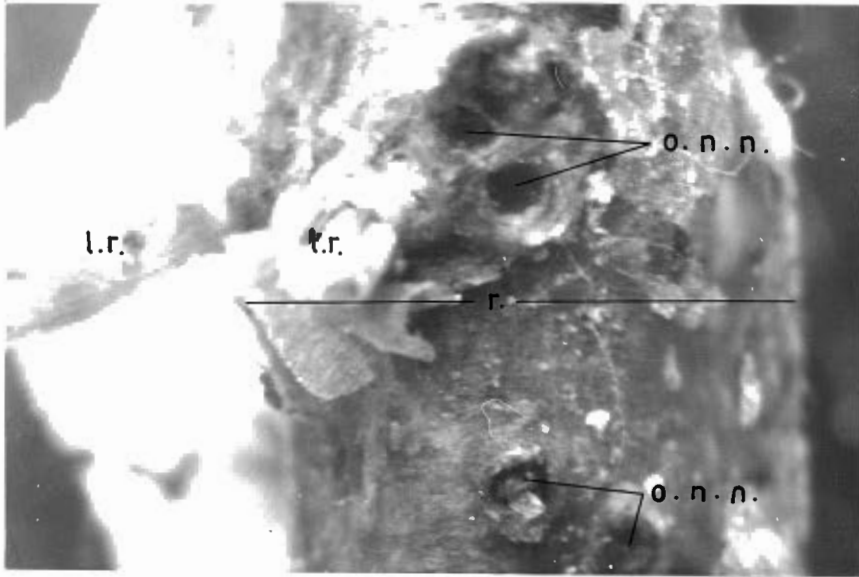


Fig. 11

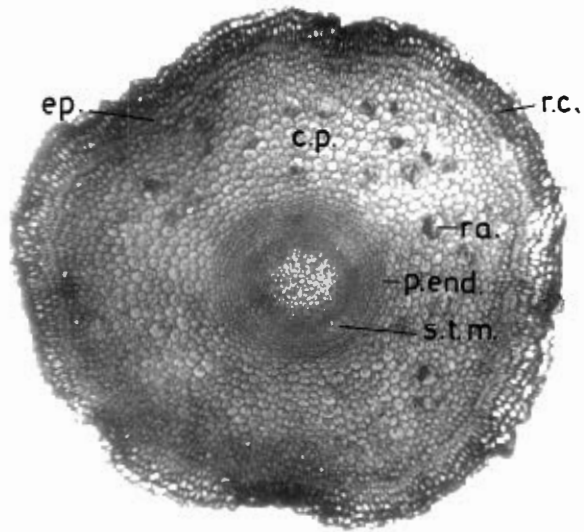


Fig. 12

Fig. 13: 99 R. Cross-section, 660 μ m from uninfected root tip, showing sieve-tube members (s.t.m.); xylem poles (x. pl.); pro-endodermis (p. end.); cortex parenchyma (c.p.) with nuclei (n.) and raphide sac (ra.). X 95.

Fig. 14: Steen. Cross-section, 10 mm from uninfected root tip, showing epidermis (ep.); cortex parenchyma (c.p.); intercellular spaces (i.s.) and tannin (tan.). X 165.

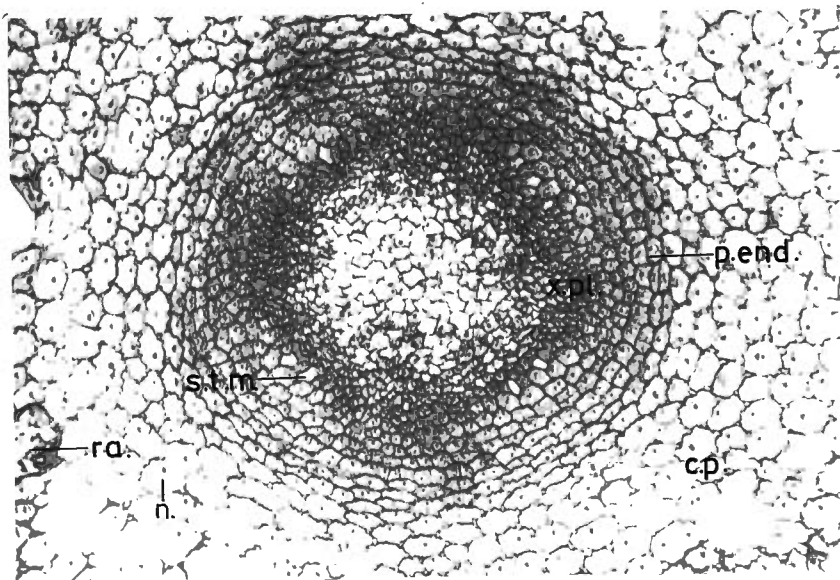


Fig. 13

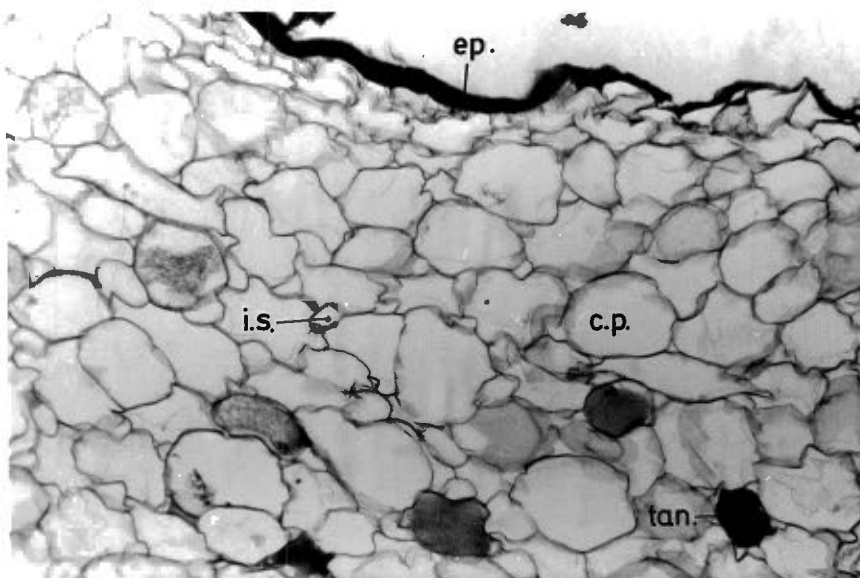


Fig. 14

Fig. 15: Dogridge. Cross-section, 630 μ m from root tip.
Note cracks (cr.) caused by *M. hapla*. X 90.

Fig. 16: A magnification of Fig. 15.
Sieve-tube members (s.t.m.) and raphide sacs (ra.)
are clearly visible. Fig. 1 in Figs. 15 and 16
corresponds. X 200.

Fig. 17: Dogridge. Part of cortex in Fig. 16, enlarged to
show cortical cells (c.p.) with nuclei (n.) and
nucleoli (n.n.) 700 μ m from root tip. X 1 670.

Fig. 15

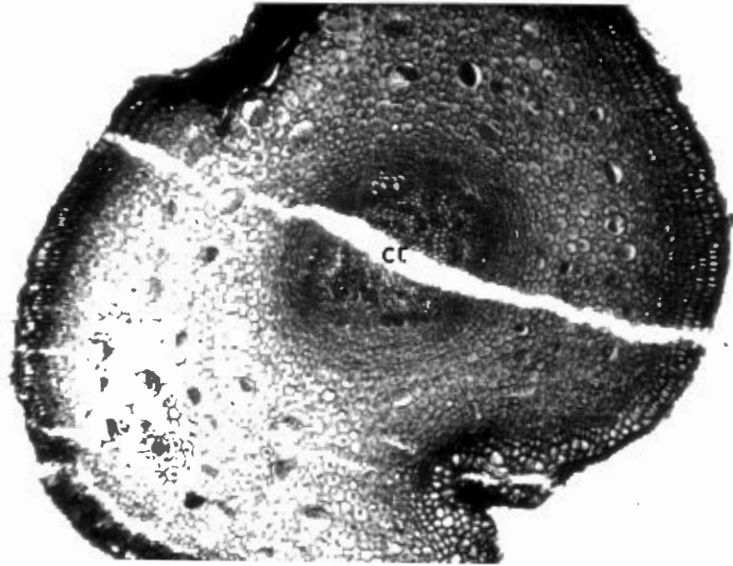


Fig. 16

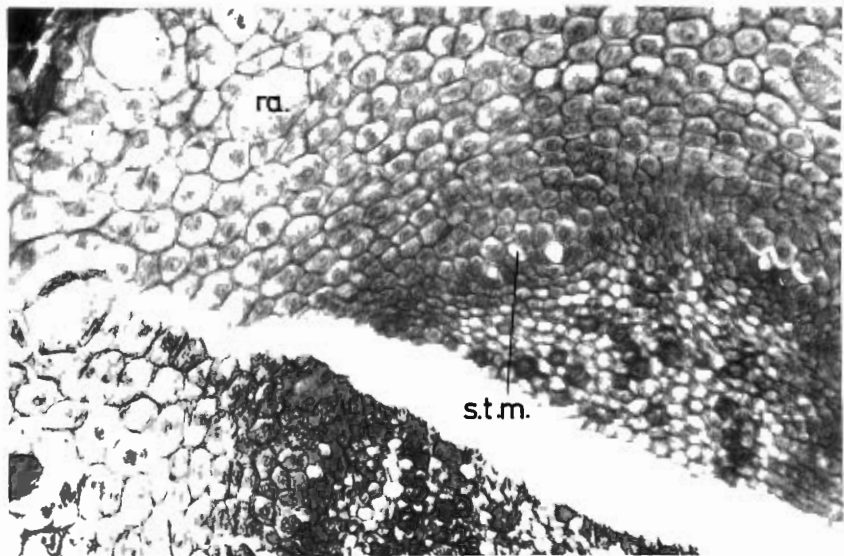


Fig. 17

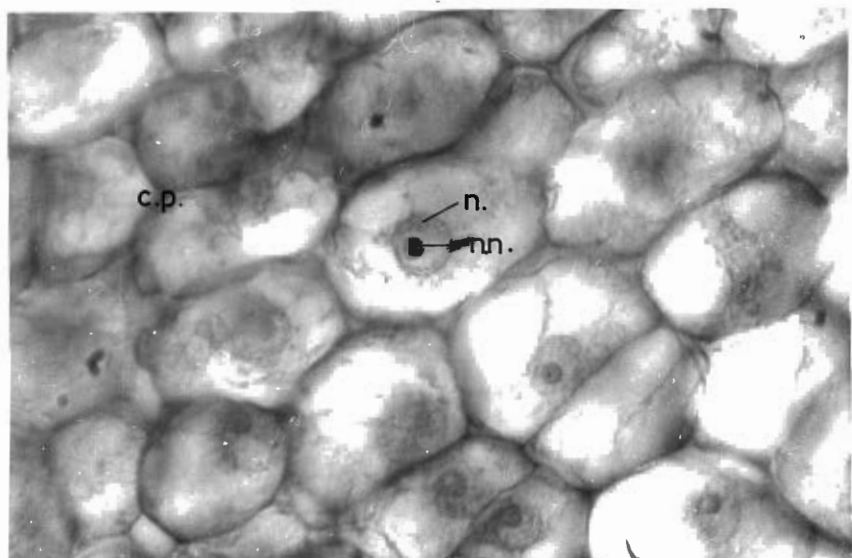


Fig. 18: 1202 C. Cross-section of root infected with *M. hapla*, 10 mm from root tip, showing epidermis (ep.); point of infection (p.i.); necrosis (nec.) and cortex parenchyma (c.p.). X 165.

Fig. 19: Steen. Cross-section of uninfected root, 1 000µm from root tip, showing protoxylem element (px.). X 1 070.

Fig. 20: 1202 C. Cross-section of uninfected root, 10 mm from root tip, showing protoxylem (px.); metaxylem (mx.); xylem parenchyma (xp.); nucleus (n.) and tannin (tan.). X 960.

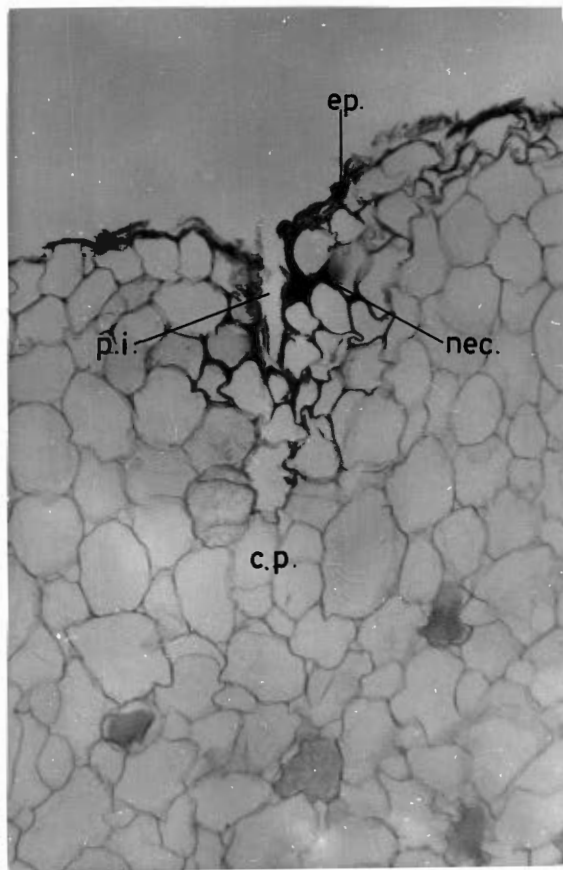


Fig. 18

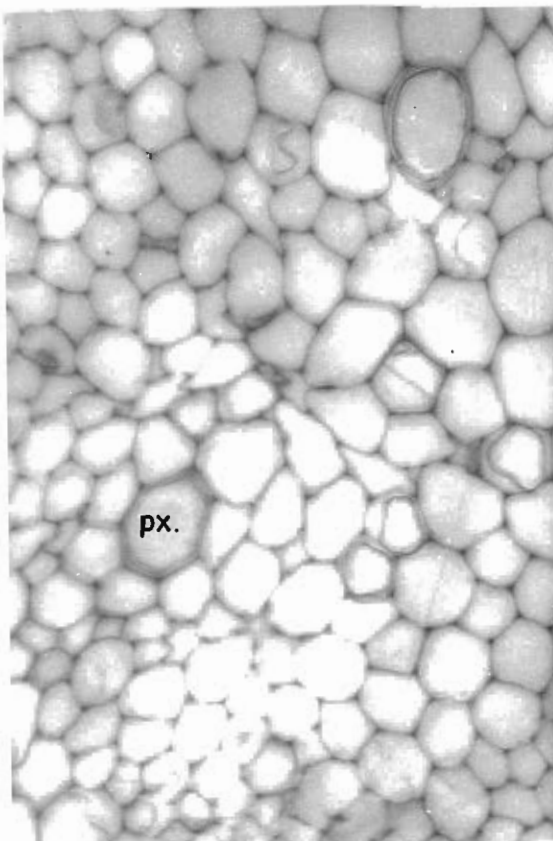


Fig. 19

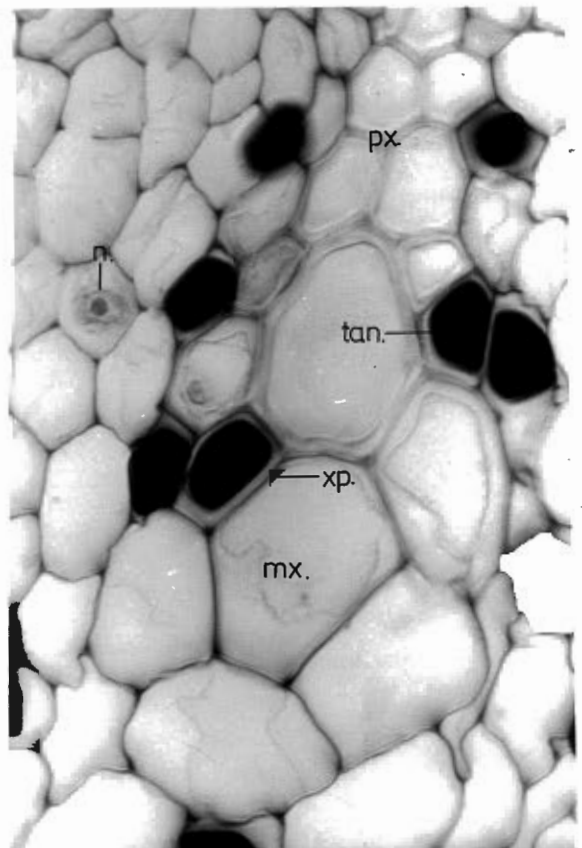


Fig. 20

Fig. 21: Dogridge. Cross-section of uninfected tetrach root, 38 mm from the root tip, showing cortex parenchyma (c.p.); endodermis (end.); pericycle (perc.); phloem (phl.); xylem (x); pith (pth.) and tannin (tan.). X 110

Fig. 22: 1202 C. Cross-section of uninfected pentarch root, 40 mm from root tip, showing cortex parenchyma (c.p.); endodermis (end.); pericycle (perc.); phloem (phl.); pith (pth.); xylem (x); cambium (cam.) and tannin (tan.). X 100.

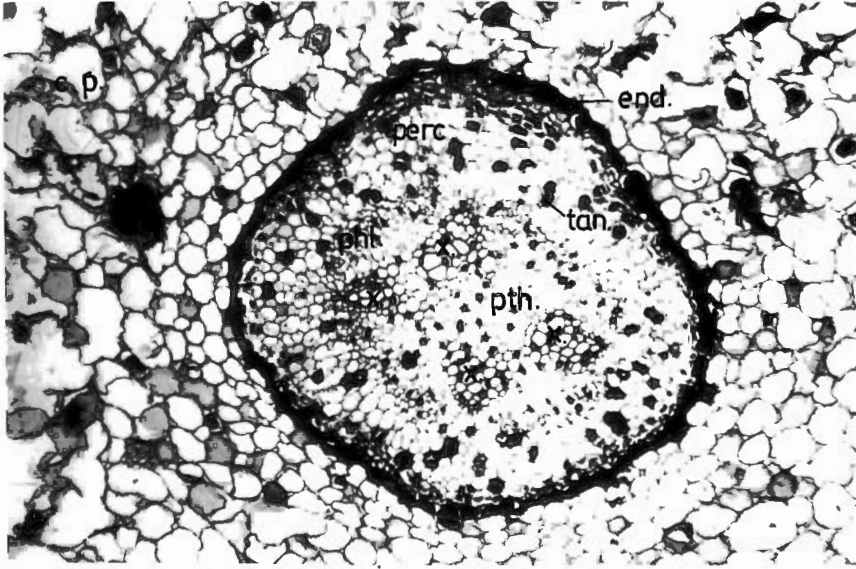


Fig. 21

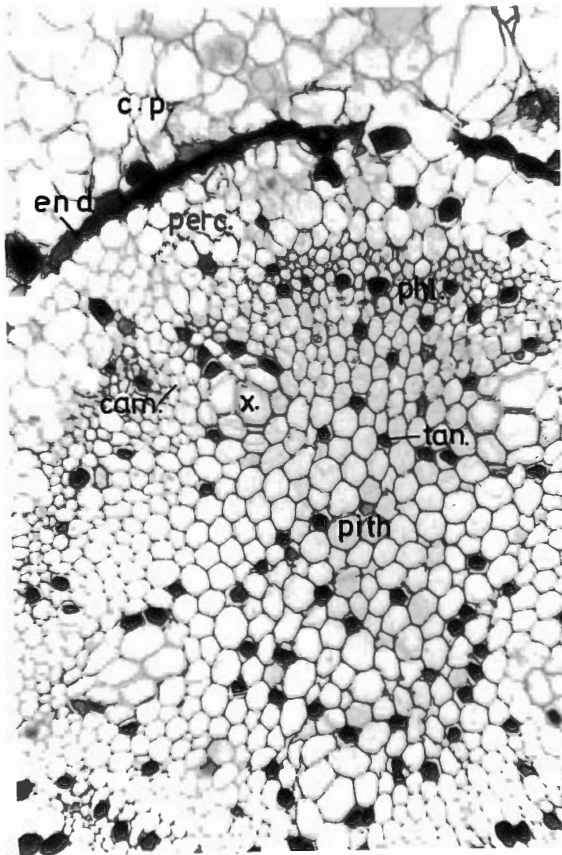


Fig. 22

Fig. 23: Dogridge. Cross-section of uninfected root, 30 mm from root tip, showing endodermis (end.); cortex parenchyma (c.p.); metaxylem (mx.); pericycle (perc.); primary phloem (p. phl.); vascular cambium (cam.); protoxylem (px.); pith (pth.); cambium (cam.). X 150.

Fig. 24: Enlargement of part of xylem in Fig. 23. Figure 1 in Figs. 23 and 24 corresponds. Xylem parenchyma (x.p.); metaxylem (mx.); protoxylem (px.). X 625.

Fig. 25: Dogridge. Enlargement of part of vascular cambium (cam.) on left hand side of Fig. 23. Figure 2 in Figs. 23 and 25 corresponds. Metaxylem (mx.); protoxylem (px.); primary phloem (p. phl.). X 625.

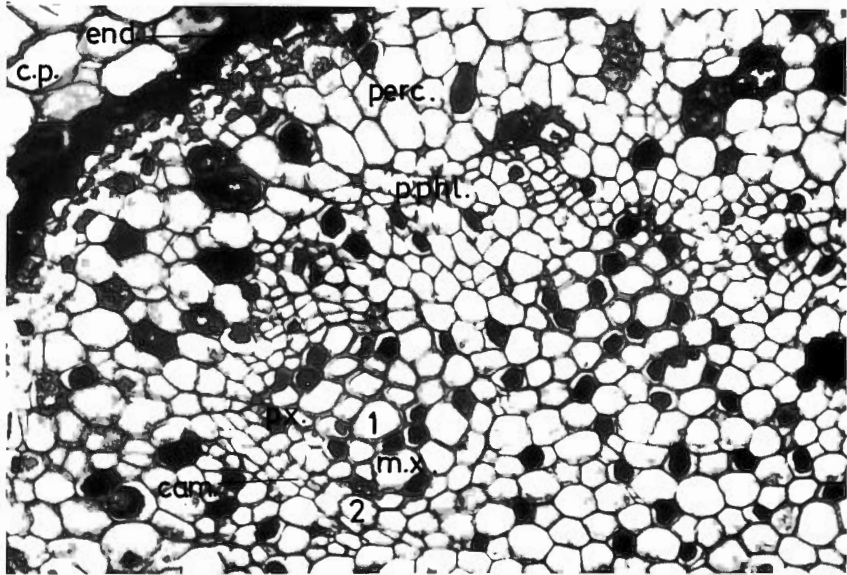


Fig. 23

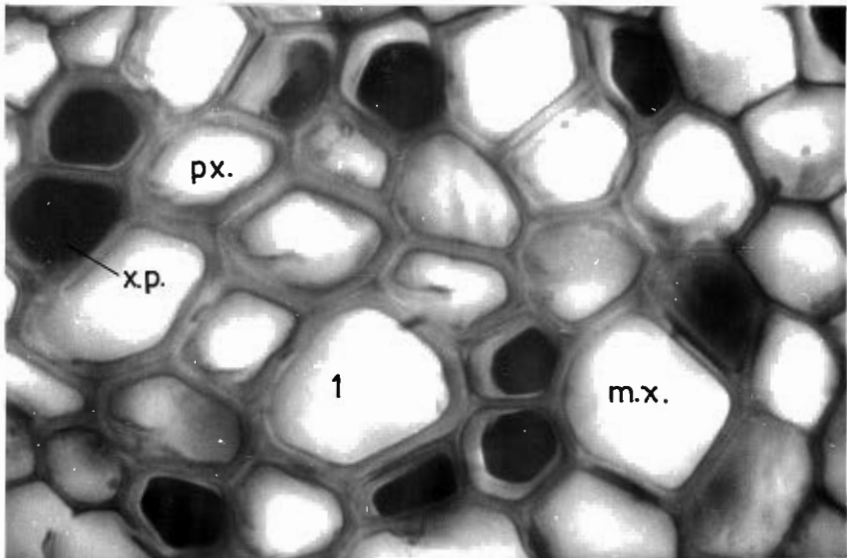


Fig. 24

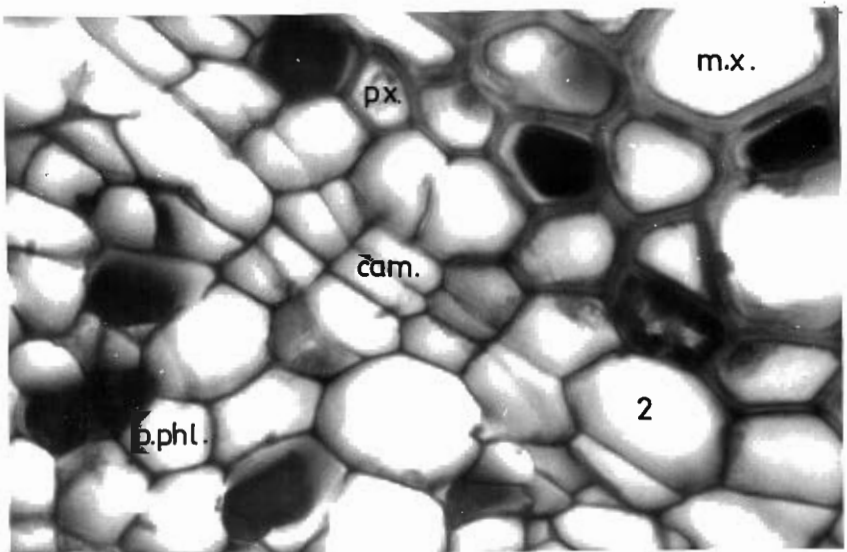


Fig. 25

Fig. 26: 99 R. Cross-section 2 mm from root tip, five days after inoculation with *M. hapla*, showing cortex parenchyma (c.p.); endodermis (end.); pericycle (perc.); first indication of syncytium near xylem poles (x. pl.) being affected; syncytium (g.c.). X 240.

Fig. 27: 99 R. Cross-section 12 mm from root tip, five days after inoculation with *M. hapla*, showing cortex parenchyma cells (c.p.); endodermis (end.); damaged pericycle (perc.); syncytia (g.c.) near xylem elements (x.); pith (pth) cells containing tannin (tan.). X 325.

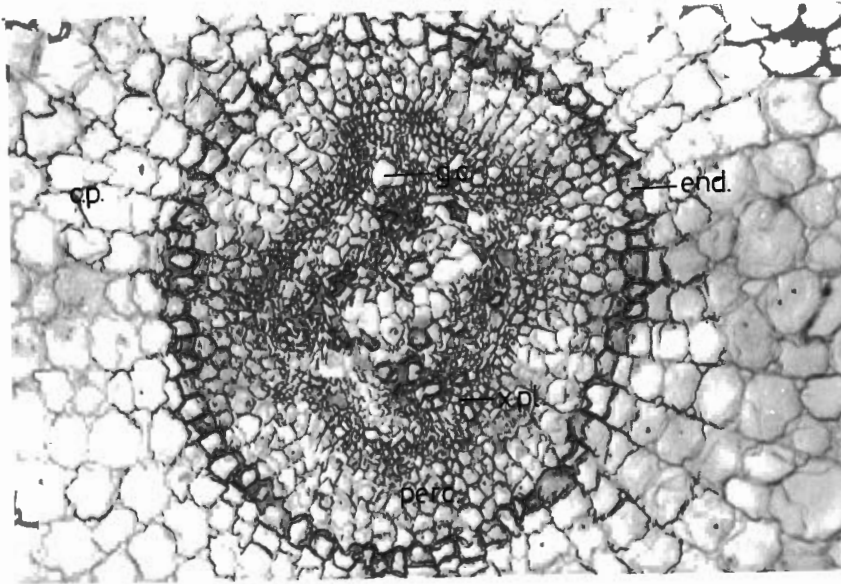


Fig. 26

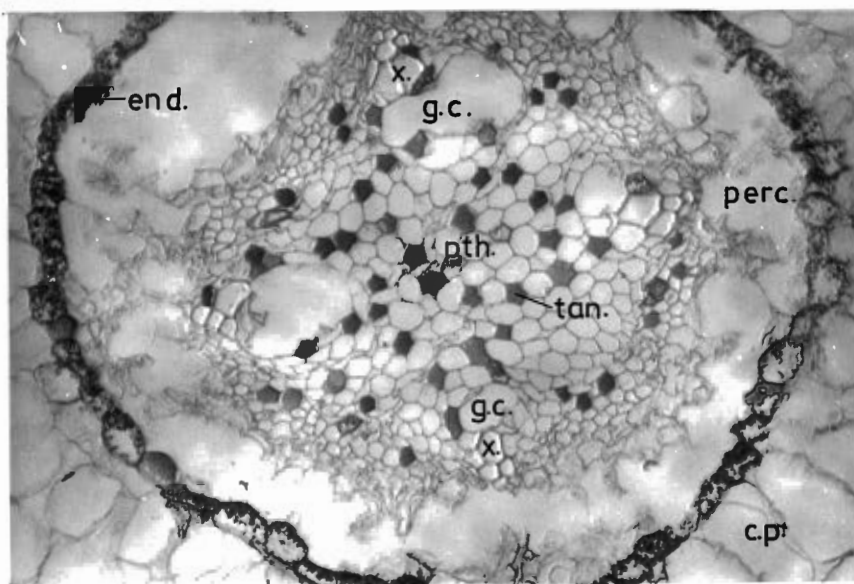


Fig. 27

Fig. 28: Jacquez. Cross-section of central cylinder of young root, 50 mm from root tip, 10 days after inoculation with *M. hapla*, showing:

- (a) cortex parenchyma (c.p.)
- (b) endodermis (end.) forming a bulge opposite proliferated pericycle (p. perc.). On the bulge typical endodermal cells (end.) are absent
- (c) part of a nematode (nem.) adjacent to a syncytium (g.c.)
- (d) primary phloem (phl.); xylem pole elements (x. pl.); granulated protoplasm (p.). X 30.

Fig. 29: Jacquez. Cross-section of root, 50 mm from root tip showing:

- (a) part of nematode (nem.) near the endodermis (end.).
As in Fig. 28 the endodermis forms a bulge opposite the nematode's feeding site and the pericycle cells (perc.) are proliferated
- (b) cortex parenchyma cells (c.p.); typical endodermal cells (end.) are absent. X 30.

Fig. 30: Jacquez. An enlargement of Fig. 29.

Note: No indication of necrosis near nematode and many tannin-containing cells (tan.); nematode (nem.). X 90.

Fig. 28

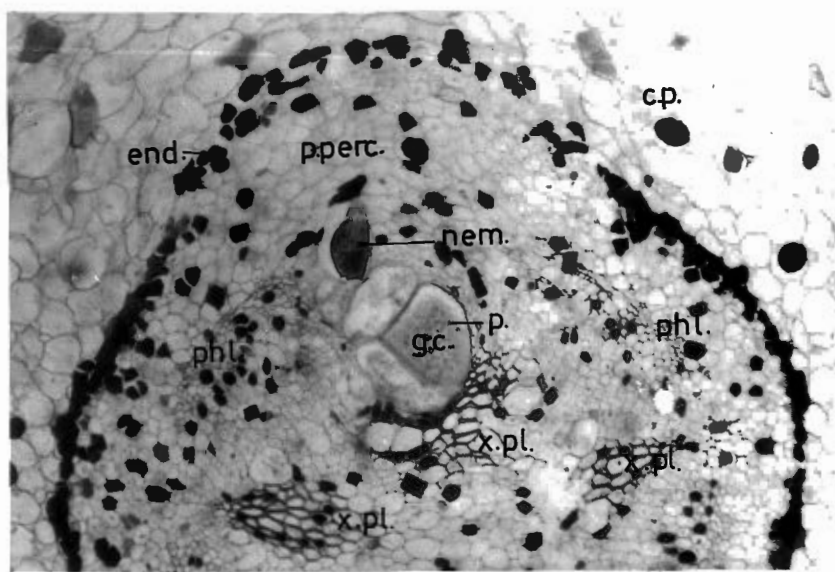


Fig. 29

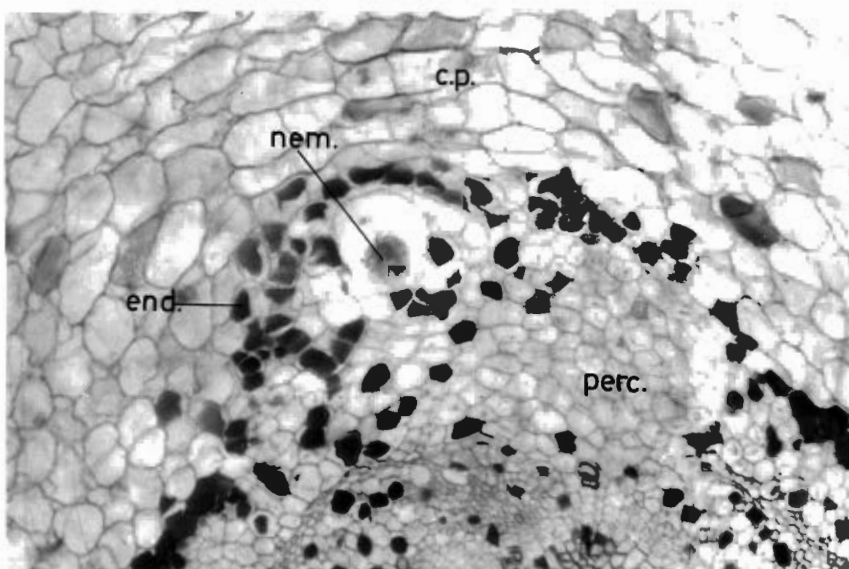
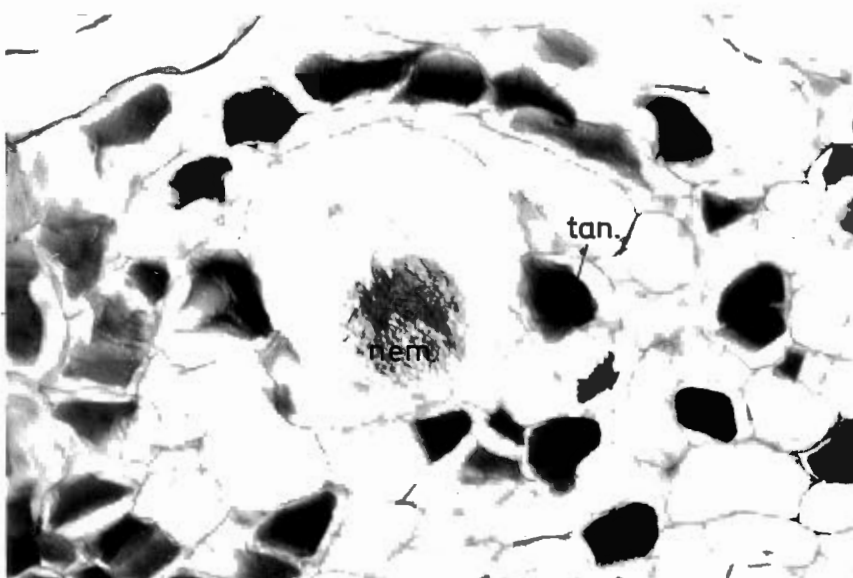


Fig. 30



Figs. 31-35: Jacquez. Cross-section of part of central cylinder, showing syncytia, 50 mm from root tip, ten days after inoculation with *M. hapla*. X 670.

Fig. 31: Three adjacent syncytia (g.c.) of which one is multinucleate.

Note:

- (a) enlarged nuclei (n.) and nucleoli (n.n.)
- (b) granulated cytoplasm (p.)
- (c) partially dissolved cell-walls (d.c.w.) (*lower left*) can be seen, while on the right hand side the walls of the syncytia (g.c.w.) appear very thick. X 670.

Fig. 32: Two adjacent syncytia (g.c.), one with a lobed nucleus (l.n.).

Both cells contain granulated cytoplasm (p.); syncytium-wall (g.c.w.). X 670.

Fig. 33: Three adjacent syncytia (g.c.). Note: The walls of some of the cells bordering the syncytia are partially dissolved (d.c.w.). Nuclei (n.) in the bordering cells are ellipsoid and much smaller than the one in the syncytia; nucleus (n.); nucleoli (n.n.); syncytium-wall (g.c.w.); tannin (tan.). X 670.

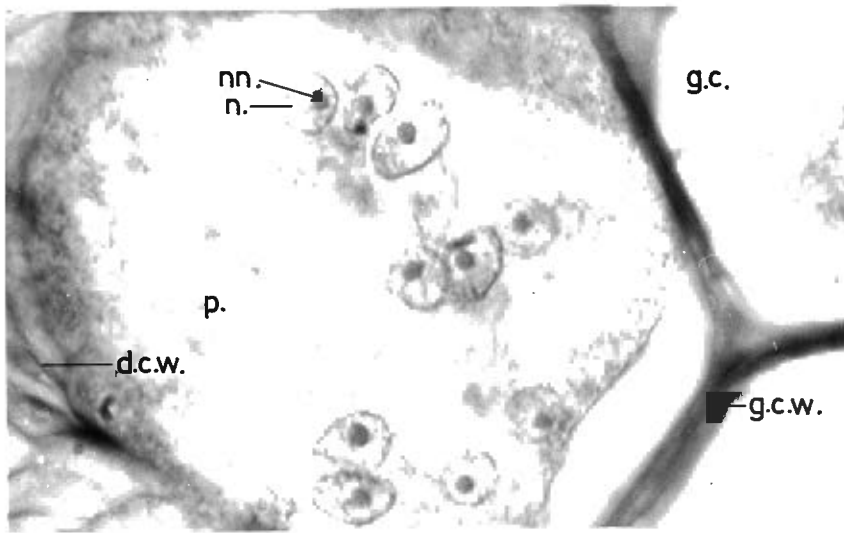


Fig. 31

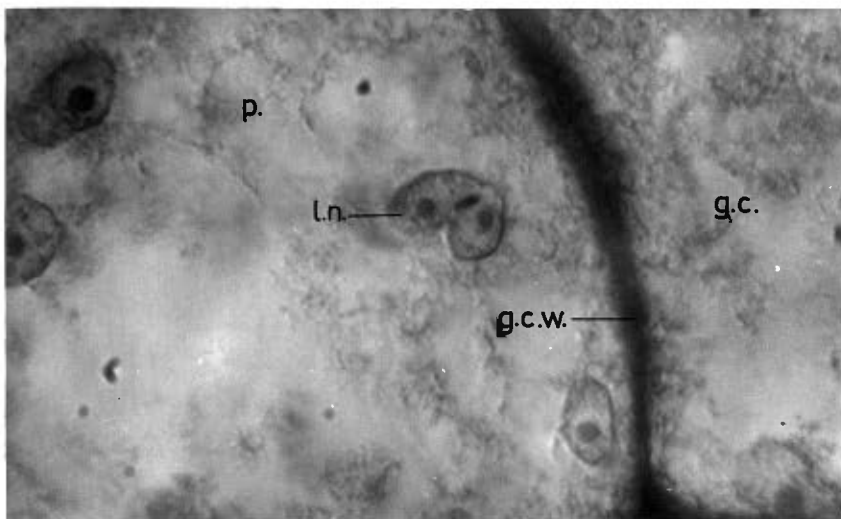


Fig. 32

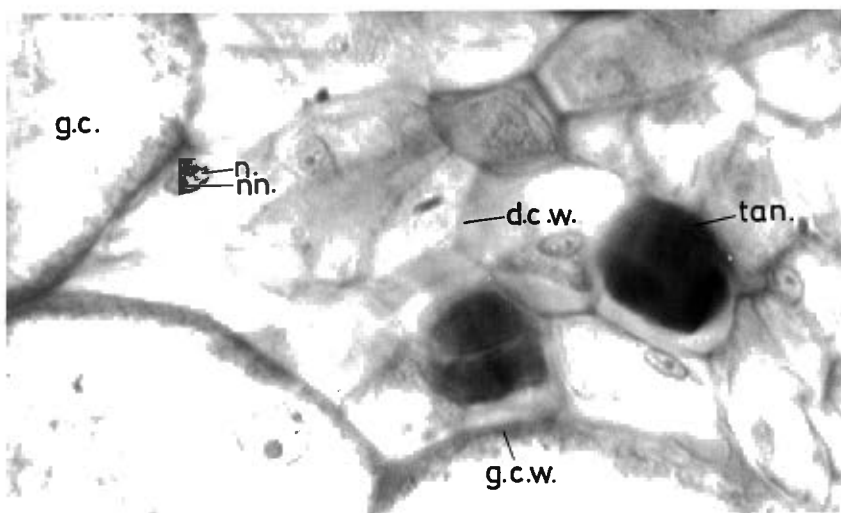


Fig. 33

Fig. 34: (a) four multinucleate syncytia (g.c.) adjacent to primary xylem (x.) bundle
(b) cell-wall (c.w.) of syncytia appear much thicker than those of adjacent cells. X 275.

Fig. 35: Multinucleate syncytia (g.c.) adjacent to deformed primary phloem cells, with enlarged nuclei (n.) in syncytia and partially dissolved cell-walls(d.c.w.) of adjacent cells; syncytium-wall (g.c.w.). X 670.

Fig. 36: Jacquez. Cross-section of central cylinder of root, 50 mm from root tip, showing:
(a) syncytium (g.c.); (b) xylem (x.) elements and
(c) granulated cytoplasm (p.) in syncytium (g.c.)
Figure 1 in figures 36 and 37 corresponds. X 275.

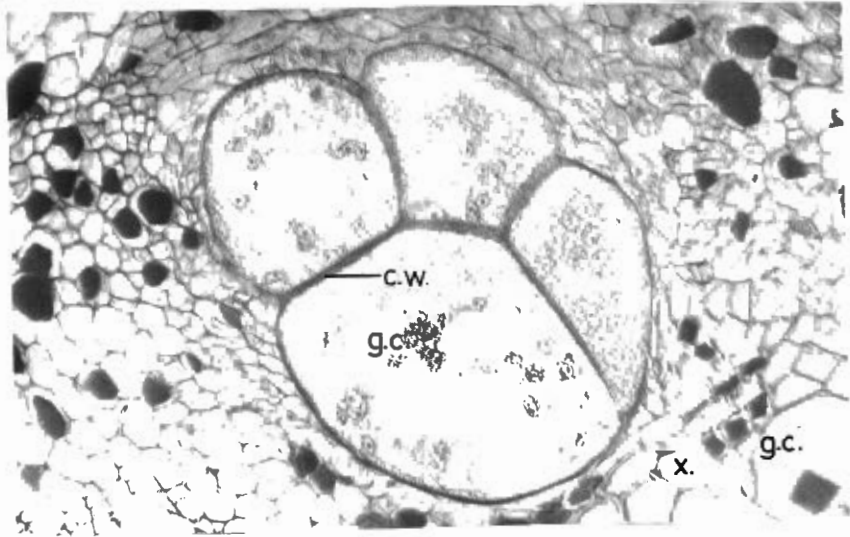


Fig. 34

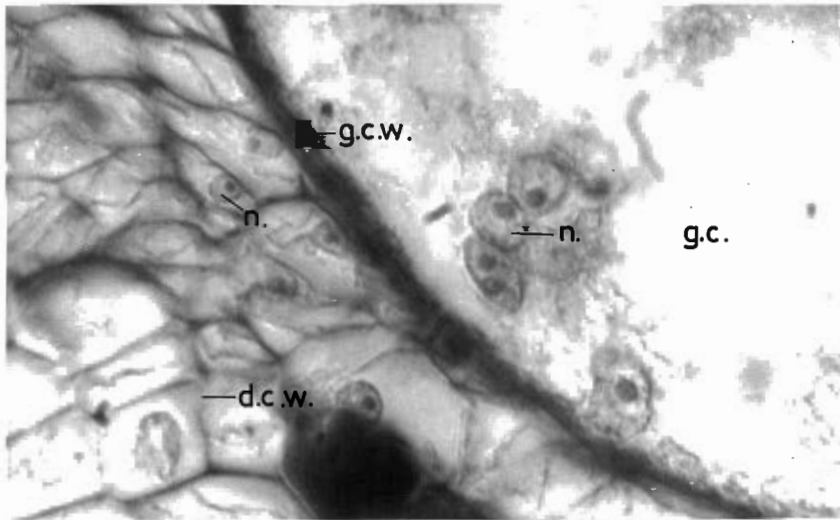


Fig. 35

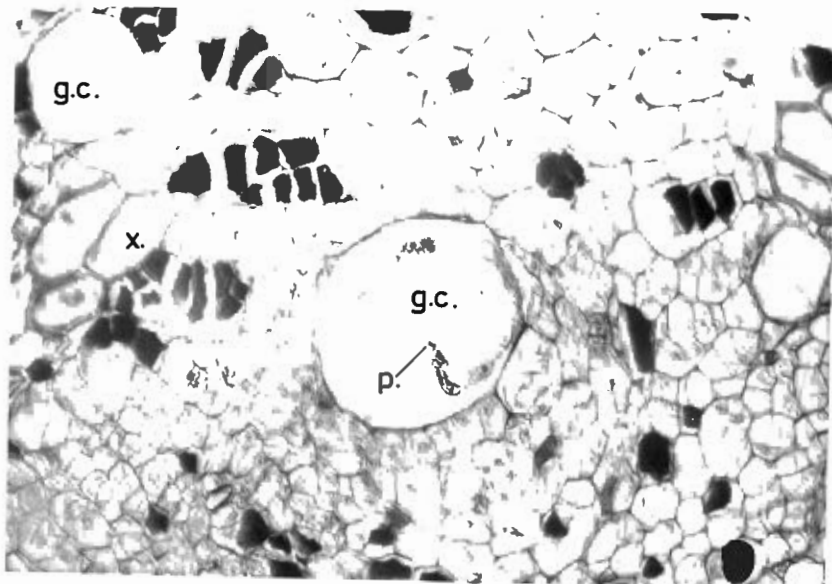


Fig. 36

Fig. 37: Jacquez. Enlargement of Fig. 36. Figure 1 in Figs. 36

and 37 corresponds. Note:

- (a) enlarged, ellipsoid nuclei (n.) with spherical nucleoli (n.n.)
- (b) granulated cytoplasm (p.)
- (c) cell-wall of syncytium (g.c.w.)
- (d) cell-walls of adjacent cells apparently partially dissolved (d.c.w.); syncytium (g.c.); tannin (tan.) X 670.

Fig. 38: Jacquez, Cross-section of central cylinder, 10 days after inoculation with *M. hapla*, and 50 mm from root tip.

Note:

- (a) syncytium (g.c.) contains granulated cytoplasm (p.)
 - (b) xylem elements (x.) adjacent to syncytium (g.c.)
 - (c) xylem parenchyma (x.p.) containing tannin (tan.).
- X 340.

Fig. 39: Jacquez. Cross-section, 70 mm from root tip, showing female nematode in pericycle (perc.). Note:

- (a) absence of necrosis near nematode (nem.)
 - (b) cells containing tannin (tan.)
 - (c) syncytium (g.c.) visible in left hand corner.
- X 340.

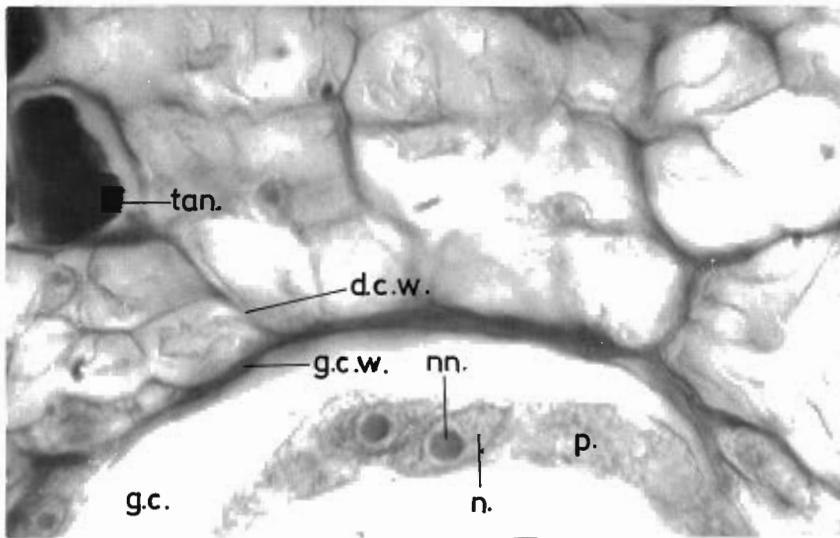


Fig. 37

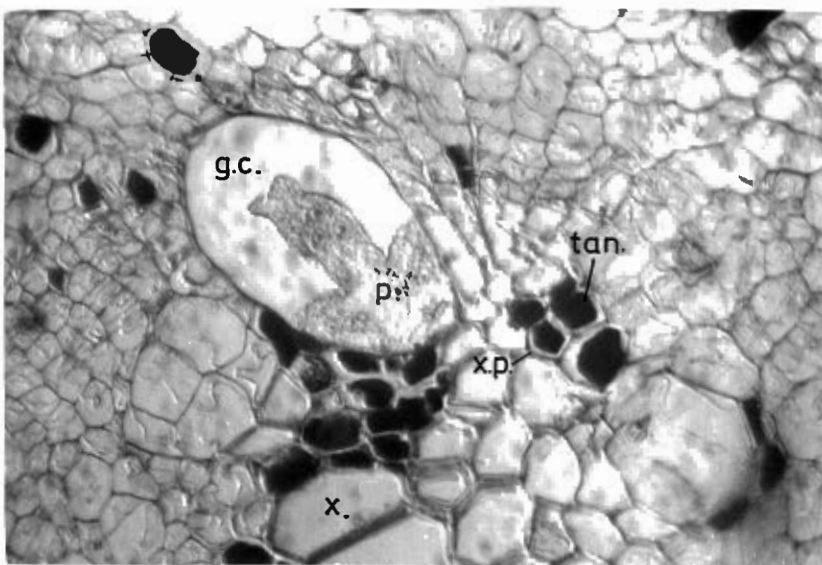


Fig. 38

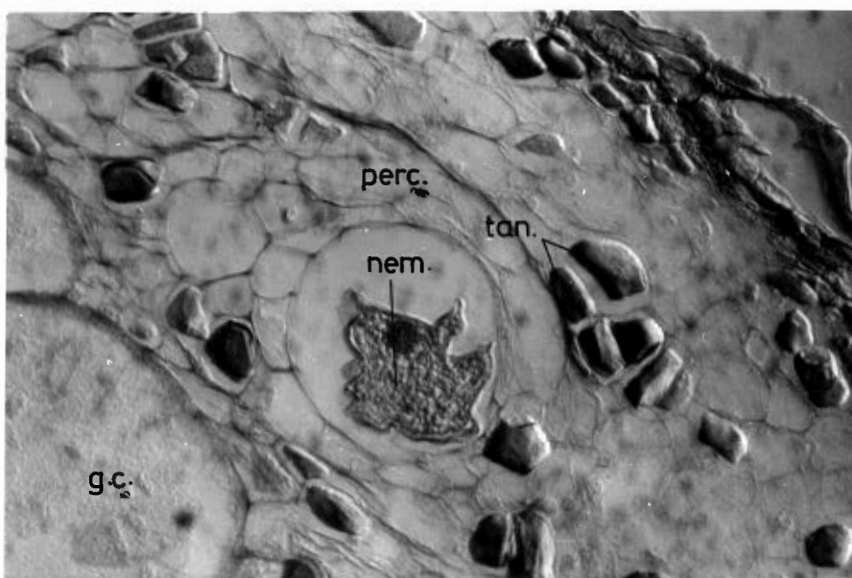


Fig. 39

Fig. 40: Jacquez. Cross-section, 50 mm from root tip, 25 days after inoculation with *M. hapla*, showing:

- (a) head of nematode (n.h.) with median bulb (m.b.)
opposite xylem pole
- (b) three multinucleate syncytia (g.c.) of which the
nuclei (n.) have partly disintegrated
- (c) dissolved wall of syncytium opposite lip region (l. rn.)
of nematode (d.c.w.). X 215.

Fig. 41: Jacquez. Cross-section, 10 mm from root tip, 30 days after inoculation with *M. hapla*, showing syncytia (g.c.) adjacent to primary phloem (p. phl.) tissue.

- Note:
- (a) difference in appearance of cell-walls of
syncytium (g.c.) and those of adjacent cells
 - (b) dark coloured cytoplasm appressed to the cell-walls
 - (c) head of nematode (n.h.) wedged in between syncytia
(g.c.). X 215.

Fig. 42: Jacquez. Cross-section of root, 70 mm from root tip, 20 days after inoculation with *M. hapla*, showing:

- (a) lateral root (l.r.) initiated opposite xylem pole (x. pl.),
- (b) tracheary elements (tra.) of lateral root (l.r.)
- (c) tannin (tan.)
- (d) syncytium (g.c.). X 300.

Fig. 40

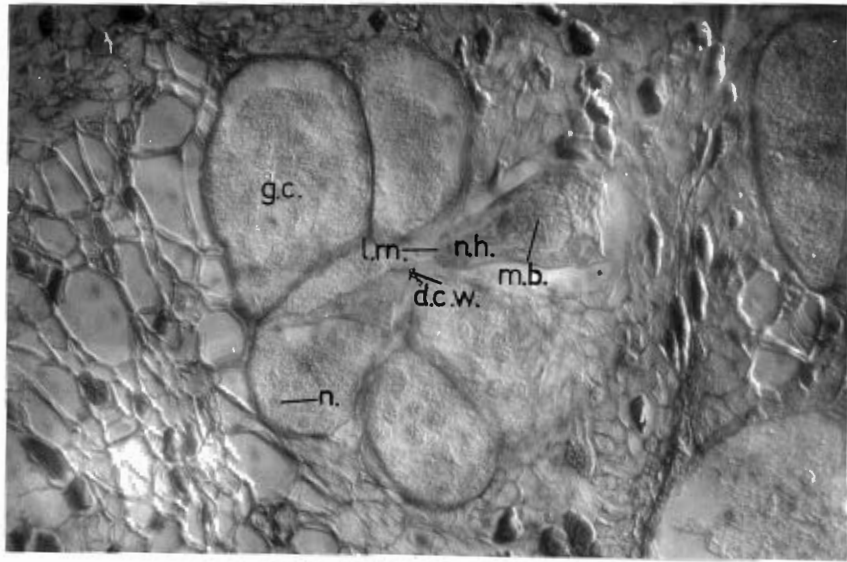


Fig. 41

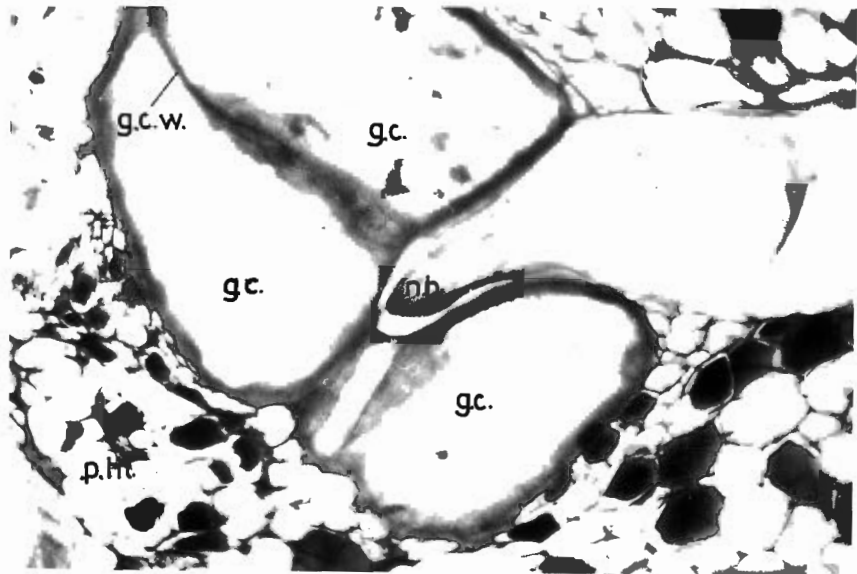


Fig. 42

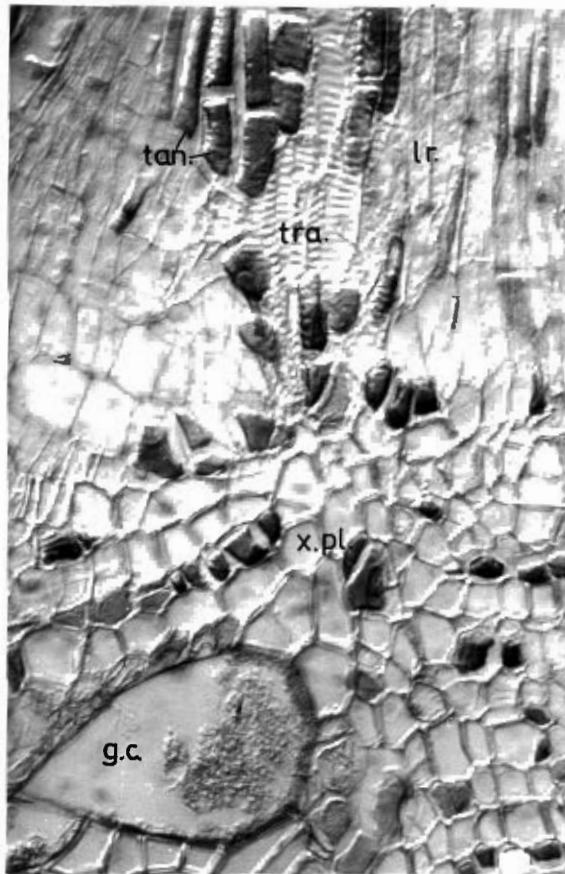


Fig. 43: Jacquez. Cross-section, 70 mm from root tip, showing an almost square syncytium (g.c.) adjacent to xylem elements (x). Note:

- (a) syncytium (g.c.) is void of nuclei and granulated cytoplasm (p.) is present
- (b) cells which are in contact with syncytia are not crushed
- (c) cell-walls of syncytia (g.c.) in lower right hand corner of this Figure appear to be partly dissolved (d.c.w.)
- (d) there is very little difference in thickness between the cell-wall of the syncytium and those of the adjacent cells. X 280.

Figs. 44-45. Jacquez. Cross-section, 10 mm from root tip, 30 days after inoculation with *M. hapla*, showing:

- (a) abnormal xylem (ab. x.) adjacent to syncytia (g.c.)
- (b) abnormal vascular cambium (ab. cam.) encircling the abnormal xylem. X 270.

Fig. 43

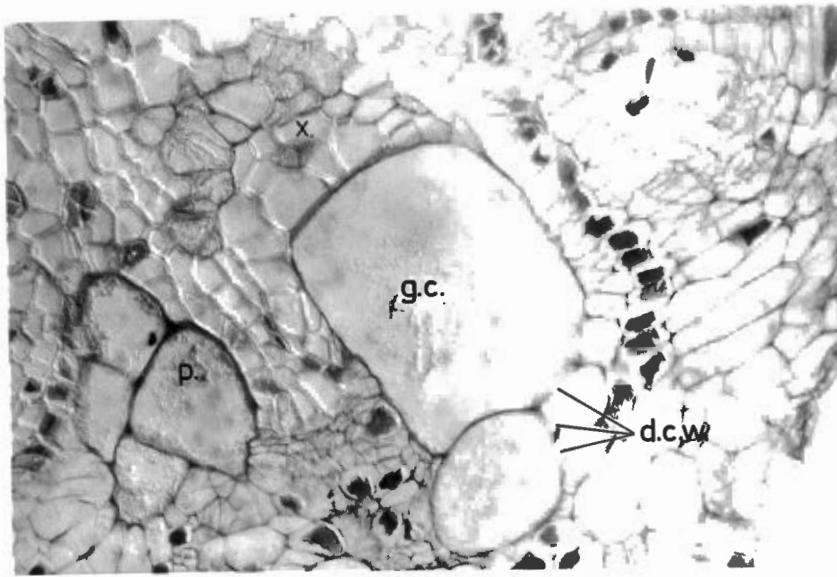


Fig. 44

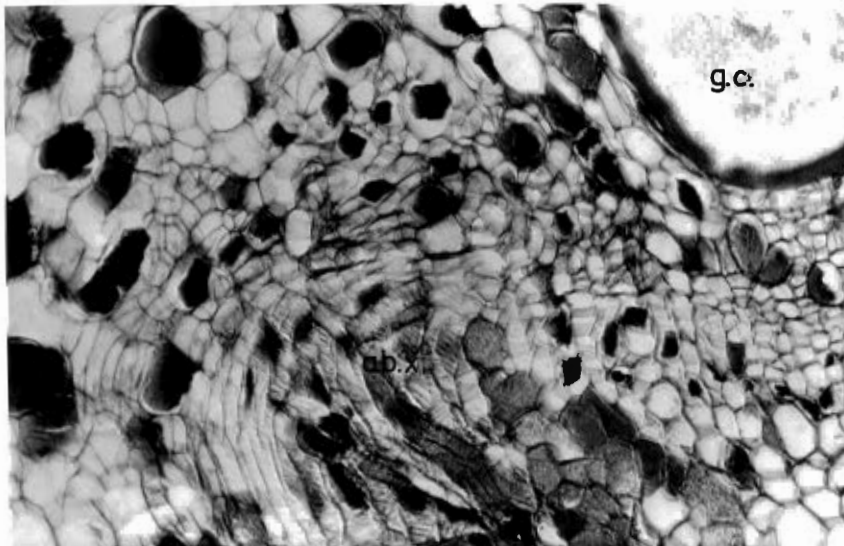


Fig. 45

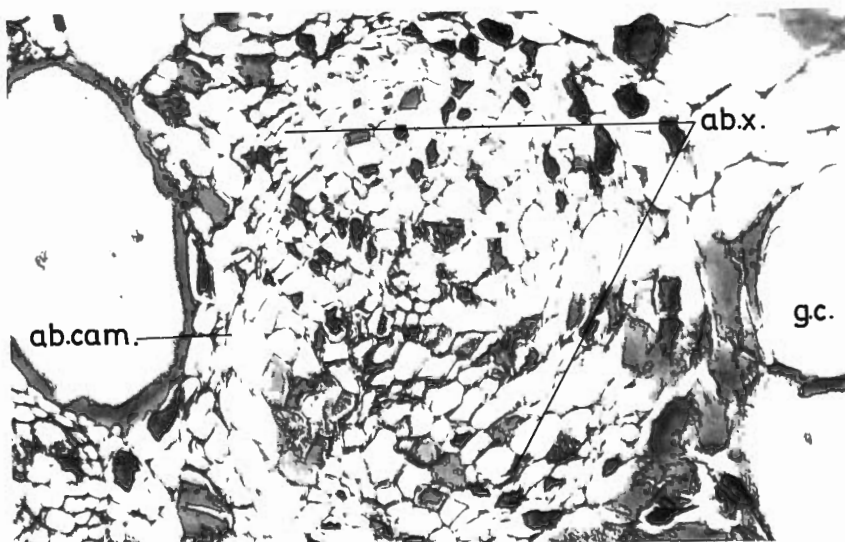


Fig. 46: Jacquez. . . Cross-section, 70 mm from root tip showing: cortex parenchyma cells (c.p.); endodermis (end.); pericycle (perc.); primary phloem (p. phl.); syncytium (g.c.) formed in the cambial region; xylem elements (x.). X 250.

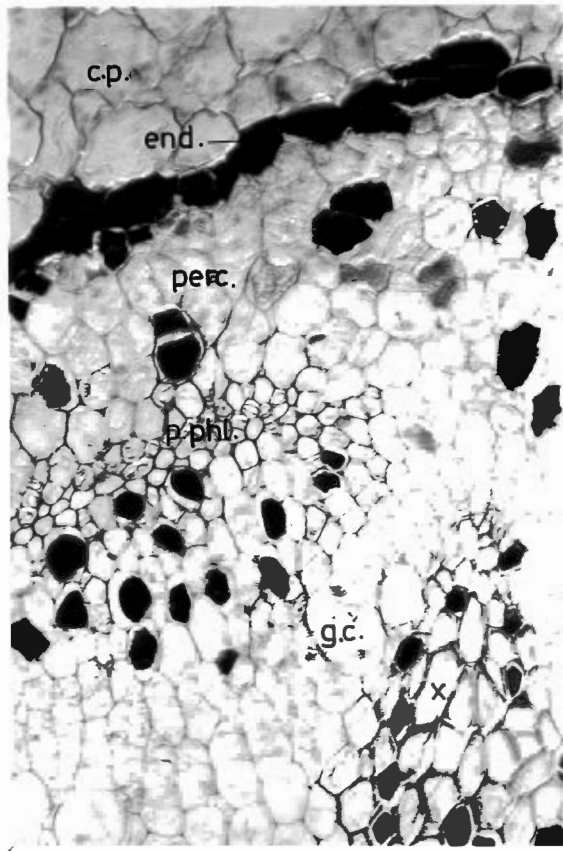


Fig. 46

Figs. 47-49: Jacquez. Cross-sections, 10 mm from root tip, 30 days after inoculation with *M. hapla*. Sections shown in Figs. 47 and 49 are 750µm apart, showing exodermis (ex.); gelatinous egg sac (g.e.s.); abnormal xylem (ab. x.); pericycle (perc.); syncytium (g.c.); nematode eggs (e.); female nematode (n.f.); nematode head (n.h.) and endodermis (end.).

Note:

(a) large number of syncytia (g.c.) in all three

Figures

(b) egg masses (e.) are outside the stele

(c) absence of root galls or knots

(d) egg masses (e.) and gelatinous egg sac (g.e.s.).

X 40.

Fig. 47

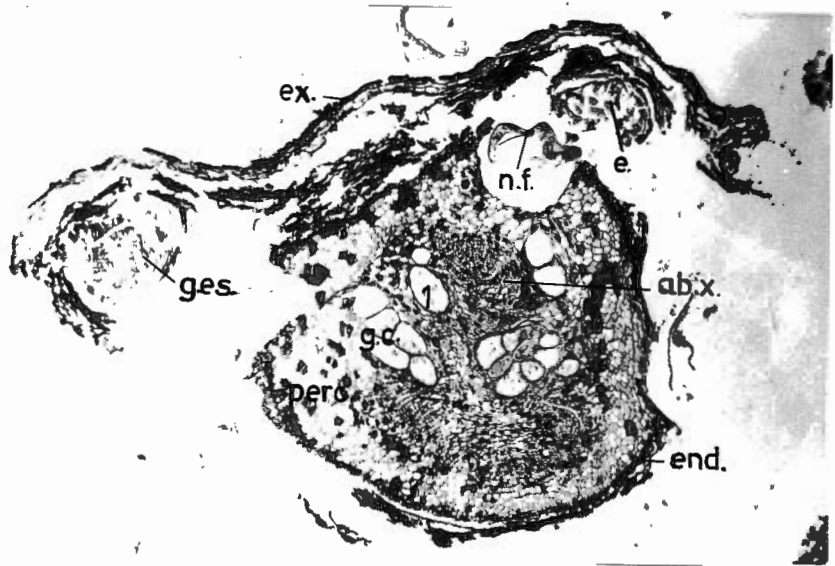


Fig. 48



Fig. 49

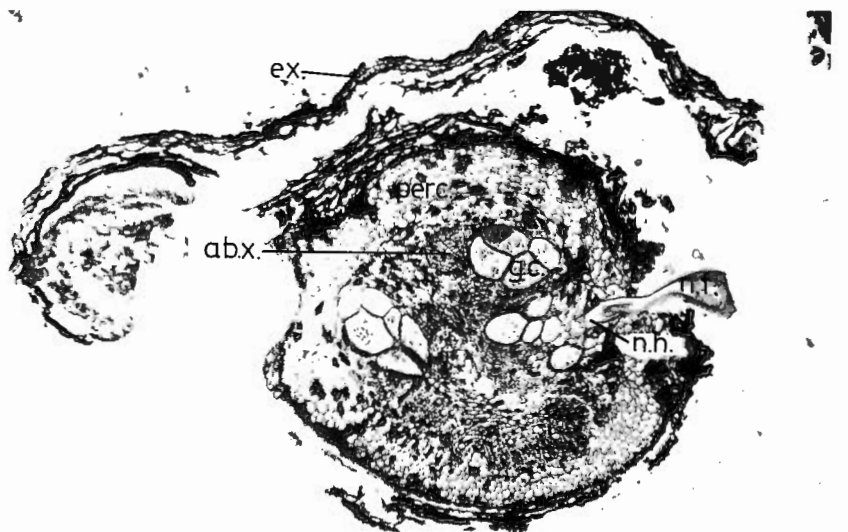


Fig. 50: Jacquez. Cross-section, 70 mm from root tip, 20 days after inoculation with *M. hapla*.

Note:

- (a) long neck of female nematode (n.f.) feeding next to syncytium (g.c.)
- (b) absence of eggs. X 110.

Fig. 51: Enlargement of female (n.f.) in Fig. 50.

Note:

Large space round female's head (n.h.) with no indication of mechanical breakdown of cell-walls; median bulb (m.b.) clearly visible. X 295.

Fig. 50

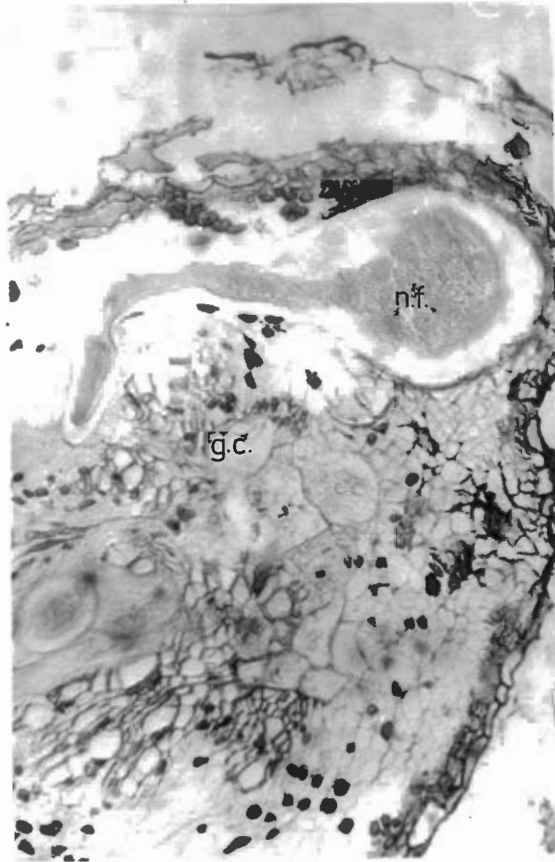
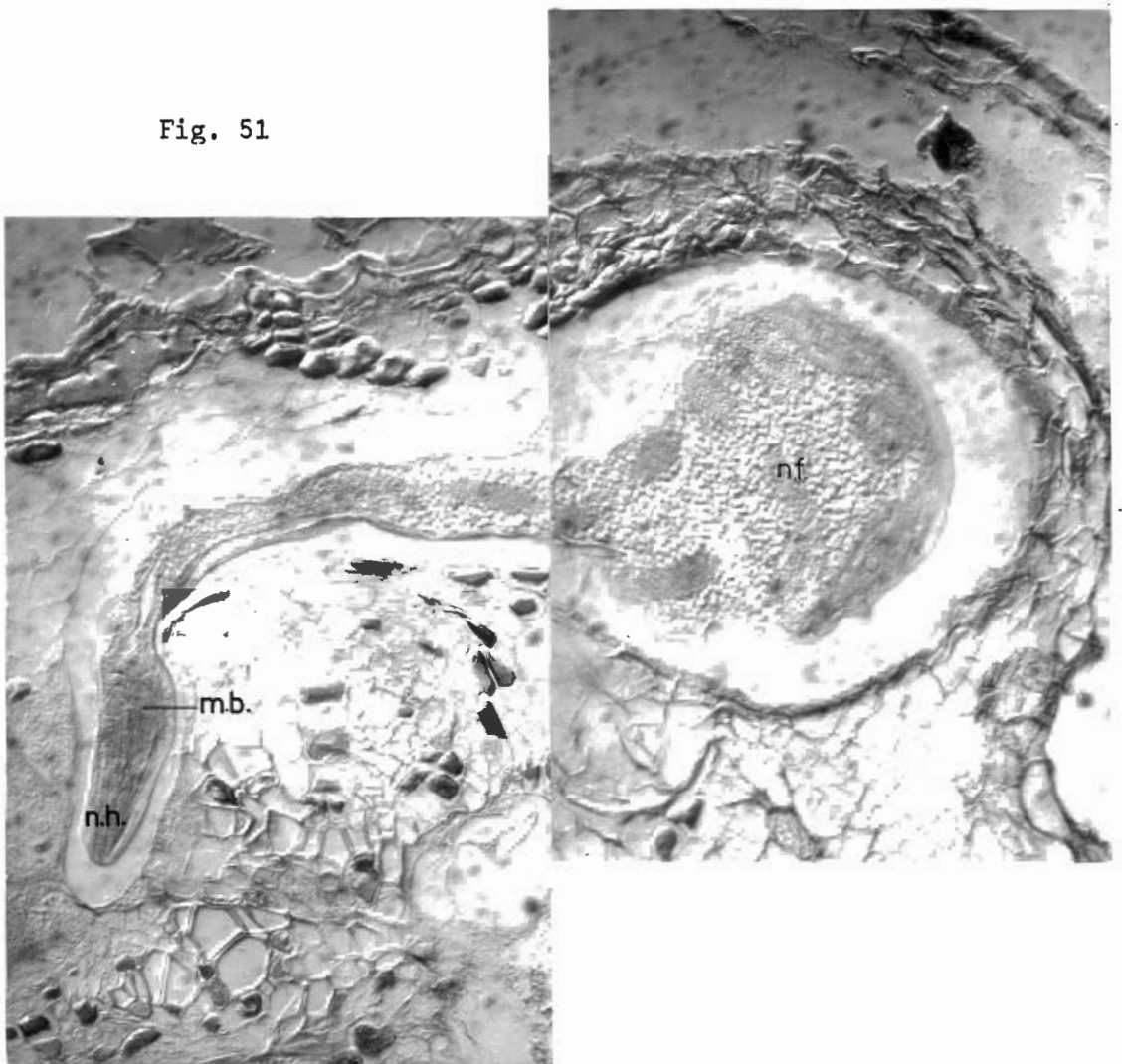


Fig. 51



- Fig. 52: White French. Tangential longitudinal section, very close to surface of root, showing position of seven egg masses (e.) and cells of periderm (perd.); pericycle (perc.). X 35.
- Fig. 53: White French. Tangential longitudinal section, very close to surface of root, showing egg masses (e.) and the remains of female nematodes (n.f.). Note hardly any necrosis of cells adjacent to nematodes' nests; pericycle (perc.); Figure 1 in Figs. 53 and 55 corresponds. X 90.
- Fig. 54: White french. Tangential longitudinal section, very close to surface of root, showing cross-section of an egg mass; distribution of eggs (e.) embedded in gelatinous material (j); pericycle (perc.). Note hardly any necrosis of cells at border of nematode nest (b.n.n.). X 380.

Fig. 52

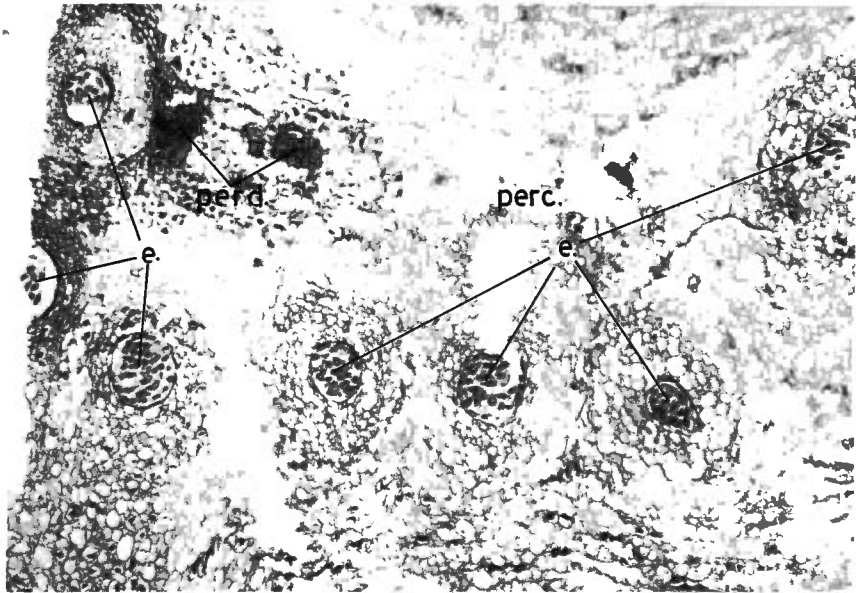


Fig. 53

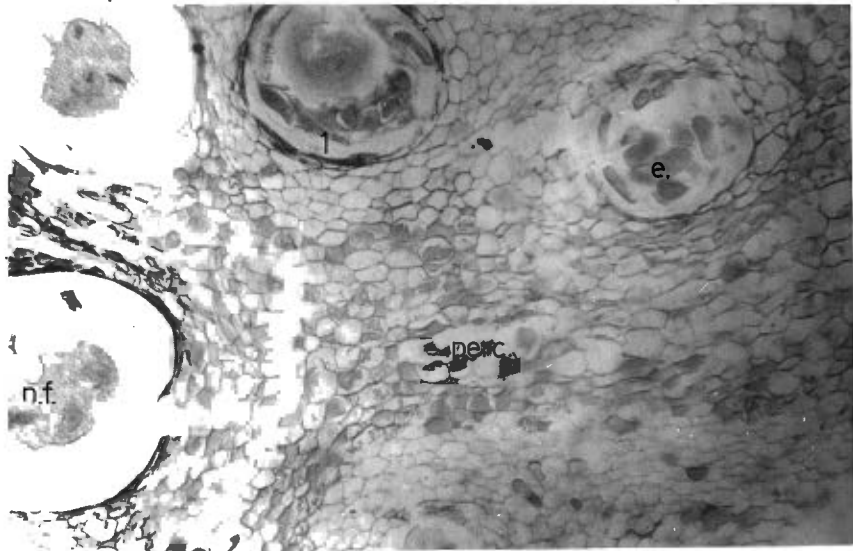


Fig. 54

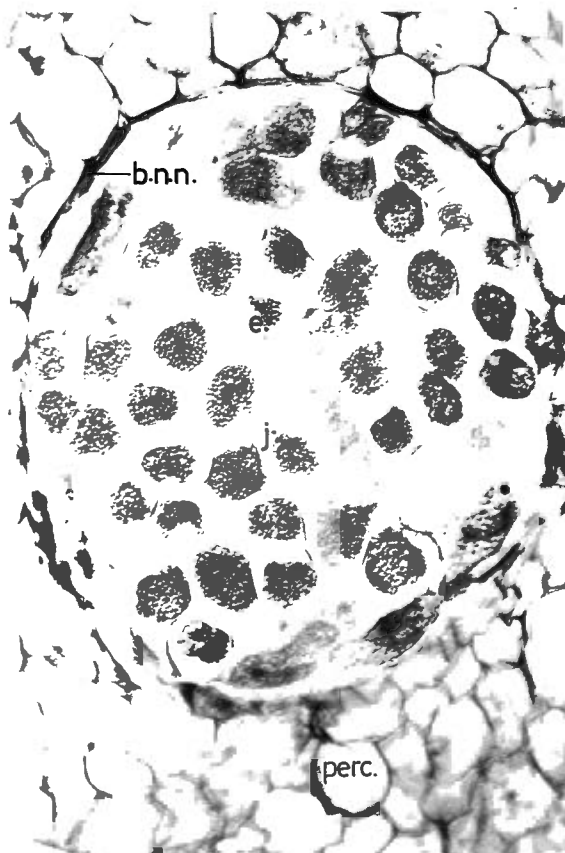


Fig. 55: White French. Tangential longitudinal section, through pericycle of root and through nematode egg mass, but further from surface than that shown in Fig. 54.

Figure 1 in Figs. 53 and 55 corresponds.

Note:

Nematode eggs (e.) and vulva opening (vul.) of nematode and very slight necrosis at border of nematode nest (b.n.n.). Nematode nest is surrounded by pericycle (perc.) cells. X 380.

Fig. 56. White French. Tangential longitudinal section, through pericycle of root, showing nematode nests (n. nt.) and remains of nematodes (nem.). Nematode nests are about 14 cells apart. Note very slight necrosis of plant cells bordering nematode nest; pericycle (perc.).

X 140.

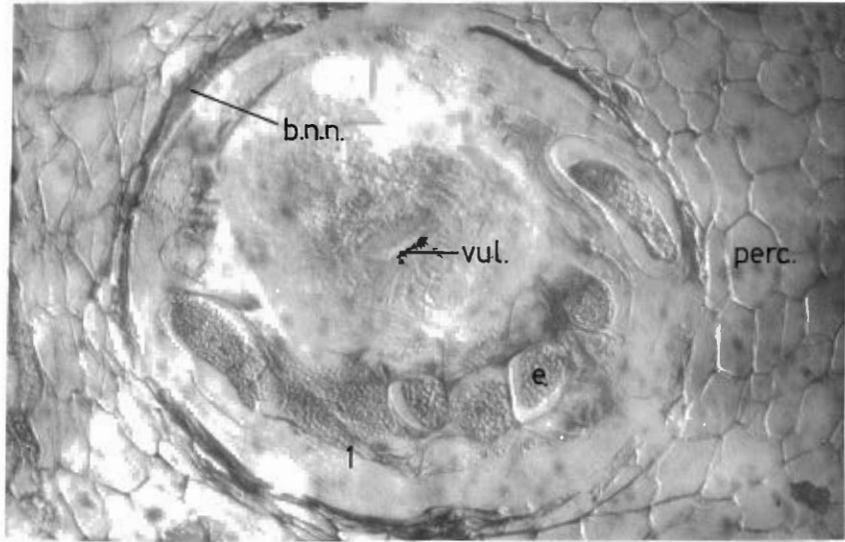


Fig. 55

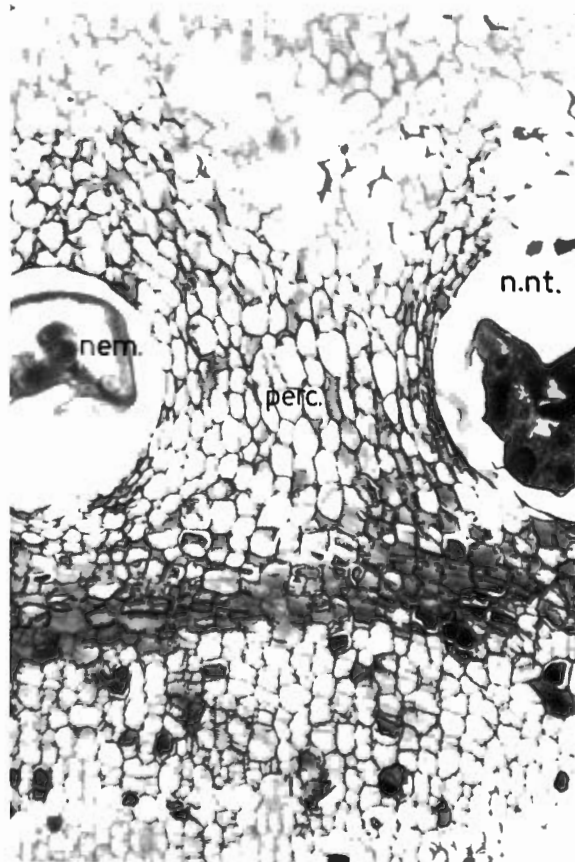


Fig. 56

Fig. 57: Salt Creek. Tangential longitudinal section,
through pericycle of root, showing nematode nests
(o.n.n.); pericycle (perc.). X 70.

Figs. 58-60: Jacquez. Nematode larvae (l.) in root,
24 hours after roots have been exposed to nematode
infection. Note different positions of larvae.
Fig. 58 illustrates anterior (ant.) and posterior (post.)
parts of larvae. X 115.

Fig. 57

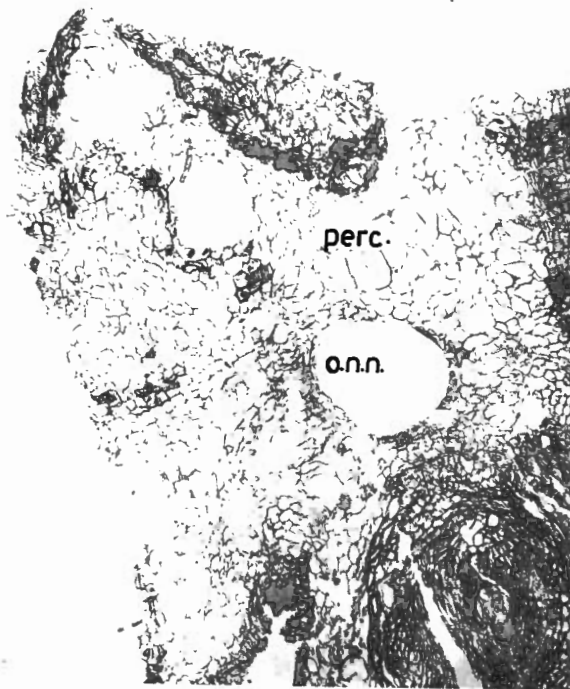


Fig. 58

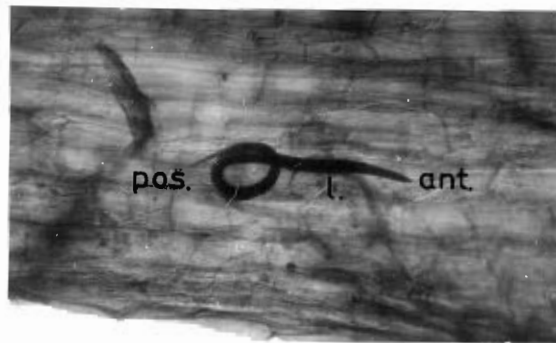


Fig. 59



Fig. 60



Fig. 61; Jacquez. Tangential longitudinal section of root, showing nematode path (n.p.) with slight necrosis (nec.) of cells in xylem ray. A large number of cells contain tannin (tan.). X 210.

Fig. 62: White French. Radial tangential section, showing young nematode larva (nem.) near xylem. Many cells contain tannin (tan.); tracheary element (tra.). X 210.

Fig. 61

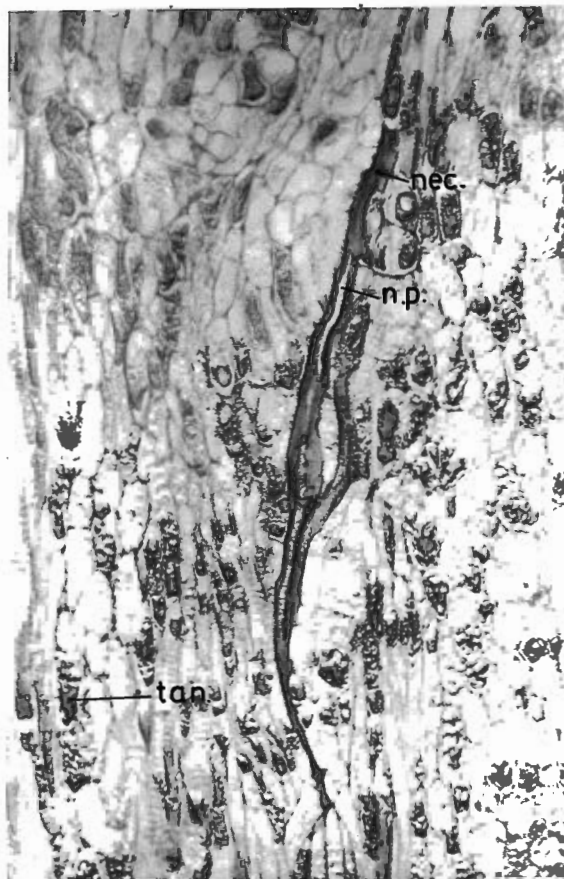


Fig. 62

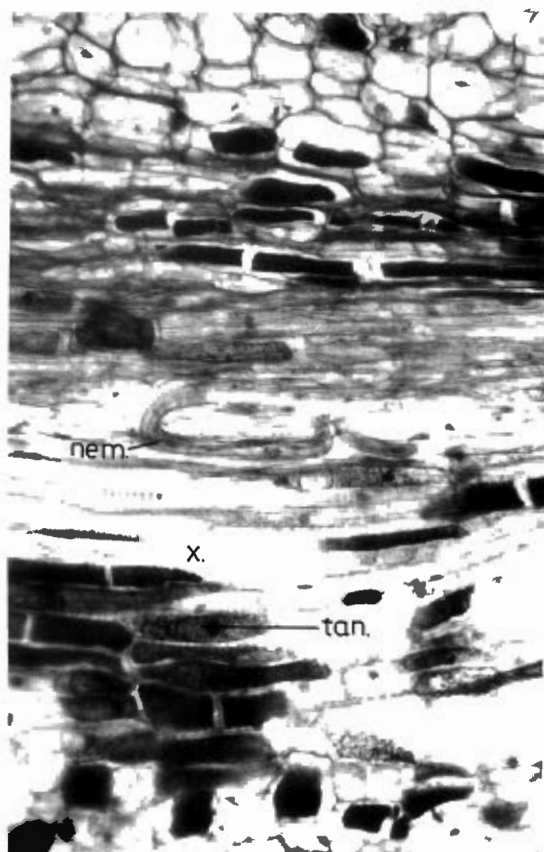


Fig. 63: White French. Deep tangential section, showing nematode larva (nem.) at the "spike" stage of development near xylem (x.); tannin (tan.); posterior (post.) and anterior (ant.) parts of larva nematode. X 270.

Fig. 64: 1202 C. Cross-section of one-year-old root, showing anterior part of nematode (nem.) in vascular ray (par. r.). Note phloem (phl.) on both sides of nematode, and large number of parenchyma cells of phloem and pericycle containing tannin (tan.); anterior part of nematode (ant.); xylem (x.). X 270.

Fig. 63

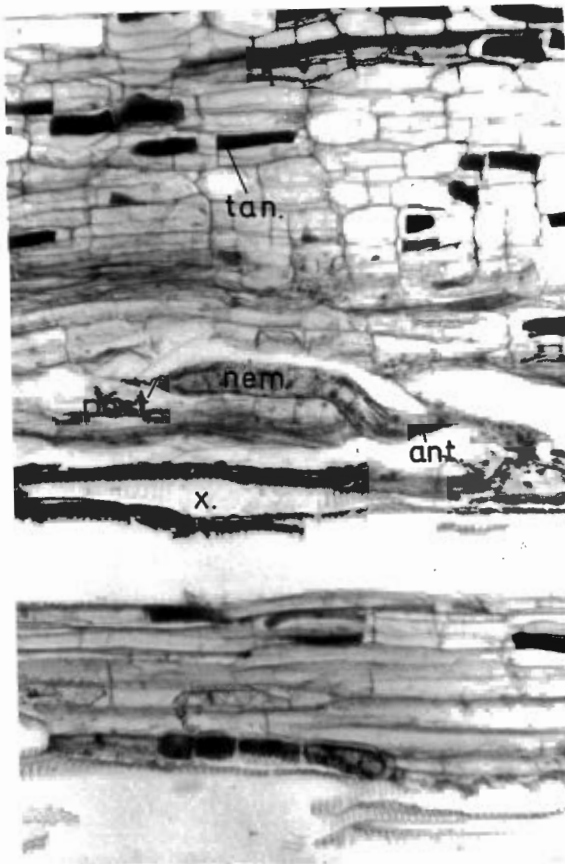


Fig. 64

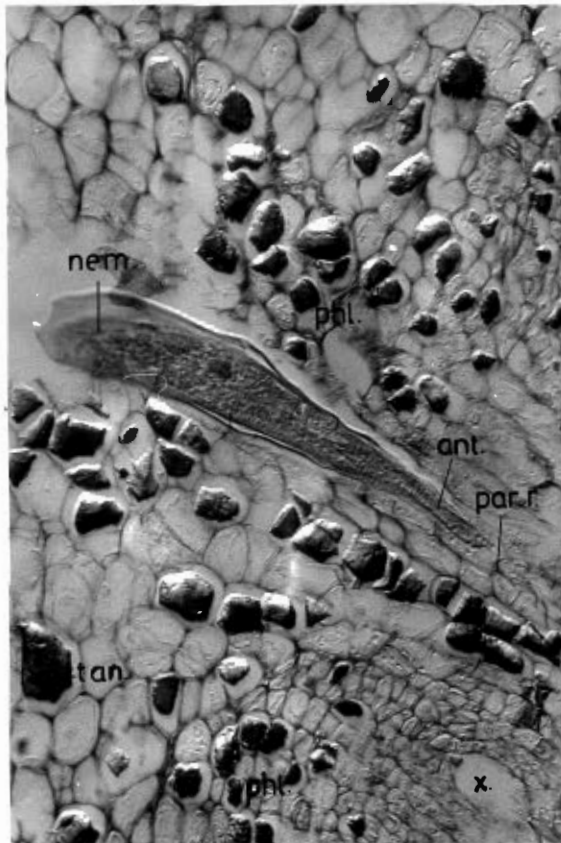


Fig. 65: White French. Radial longitudinal section of root, showing female nematode (nem.) in advanced stage of development with only part of tail tip (t.t.) left; anterior (ant.); posterior (post.); tannin (tan.). X 190.

Fig. 66: White French. Radial longitudinal section of root, showing two female nematodes (n.f.) and syncytia (g.c.). Note vulva (vul.) of one female clearly visible; nematode head (n.h.); necrosis (nec.); infected area (i.a.). X 190.

Fig. 67: White French. Radial longitudinal section of root, showing posterior end of female nematode (n.f.) and her eggs (e.). Note nuclei (n.) visible in eggs. X 145.

Fig. 68: Jacquez. Tangential longitudinal section of root, showing female nematode (n.f.) and syncytia (g.c.); nematode head (n.h.); necrosis (nec.). X 145.

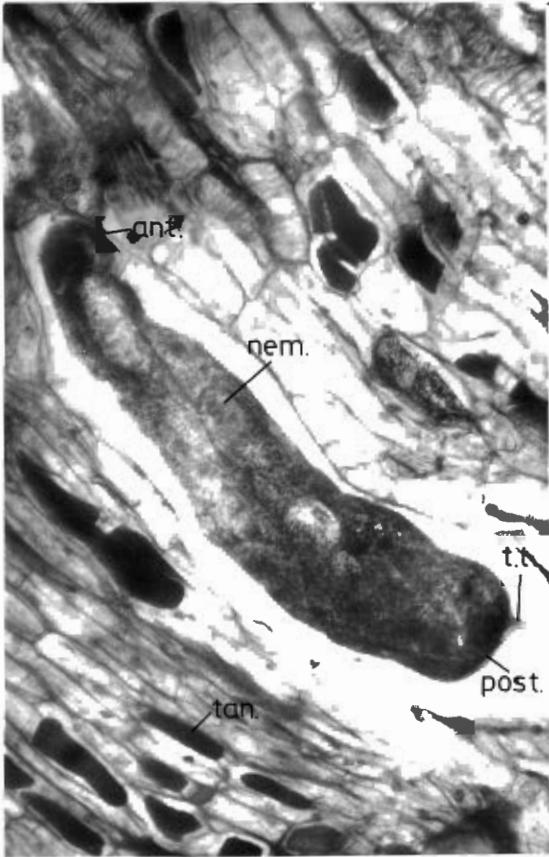


Fig. 65

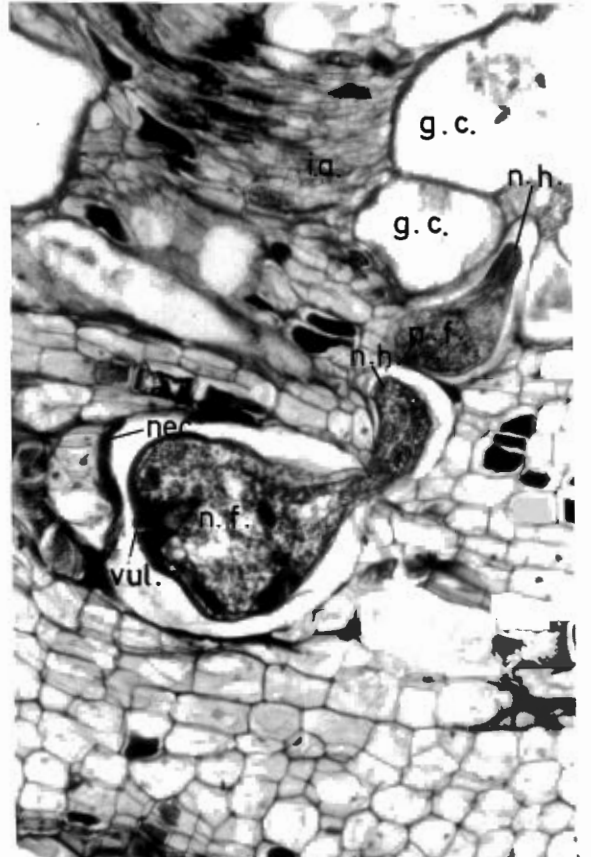


Fig. 66



Fig. 67

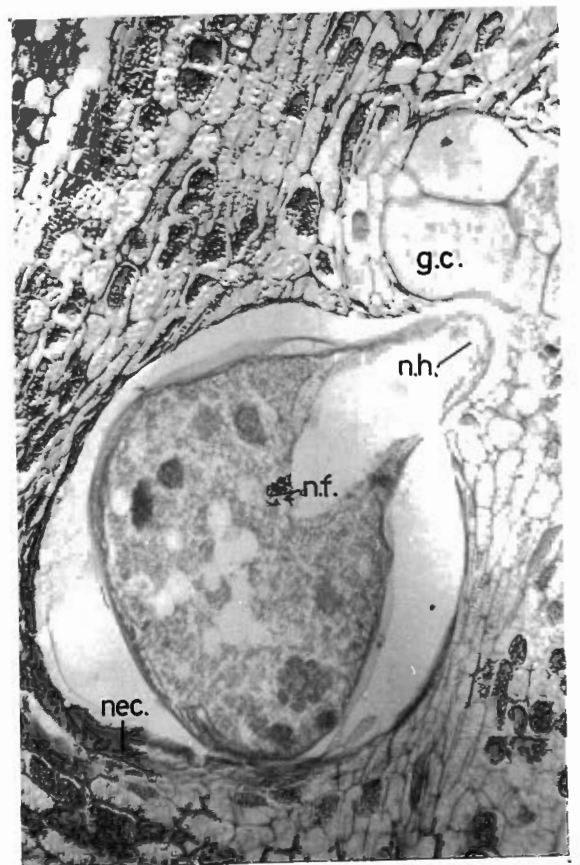


Fig. 68

Fig. 69: White French. Radial longitudinal section of root, showing posterior part of female nematode (n.f.) with growth zone of ovary (g.z.o.) and slight necrosis (nec.) of cells adjacent to nematode's nest (n. nt.). X 220.

Fig. 70: 1202 C. Tangential longitudinal section of root, with part of female nematode's (n.f.) body, exposing two clearly visible eggs (e.). Note necrosis (nec.) of cells bordering nematode's nest (n. nt.); tannin (tan.). X 220.

Fig. 71: Jacquez. Tangential longitudinal section of root, showing part of female nematode's (n.f.) body. Note slight necrosis (nec.) of cells adjacent to nematode's nest (n. nt.). X 220.

Fig. 69

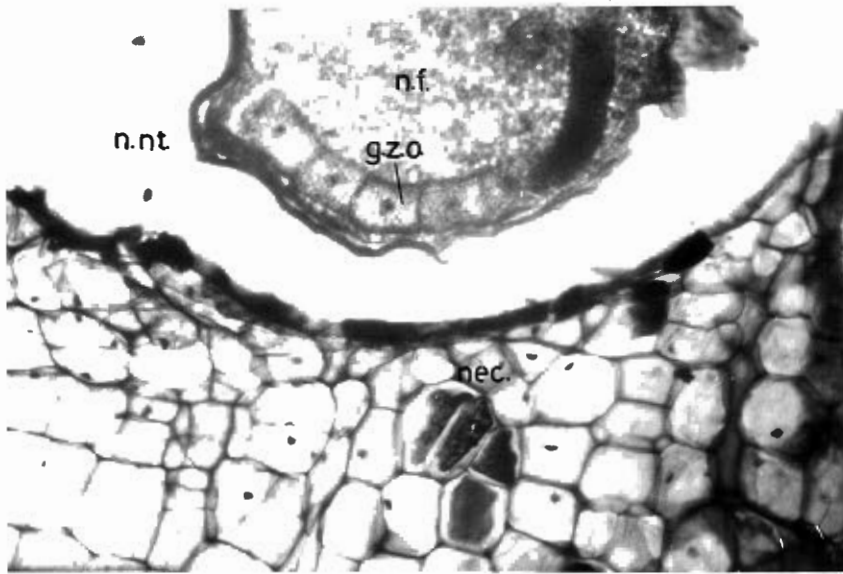


Fig. 70

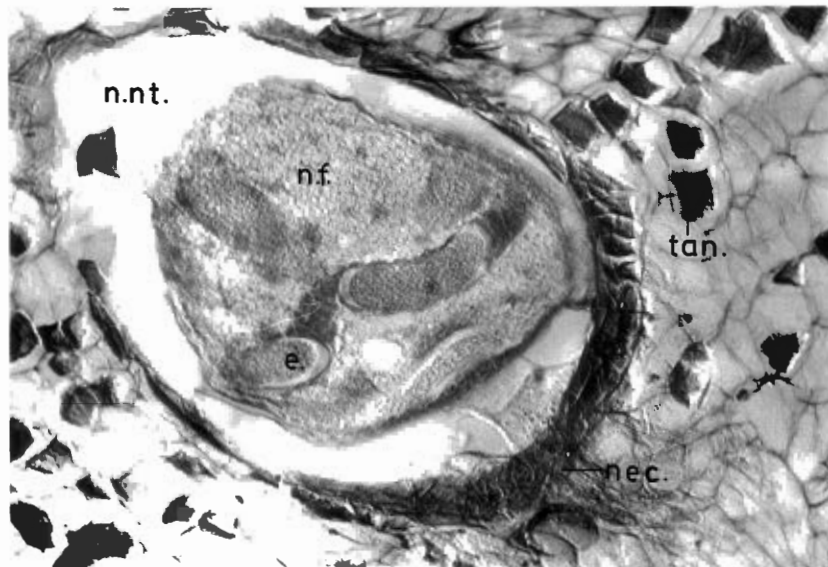


Fig. 71

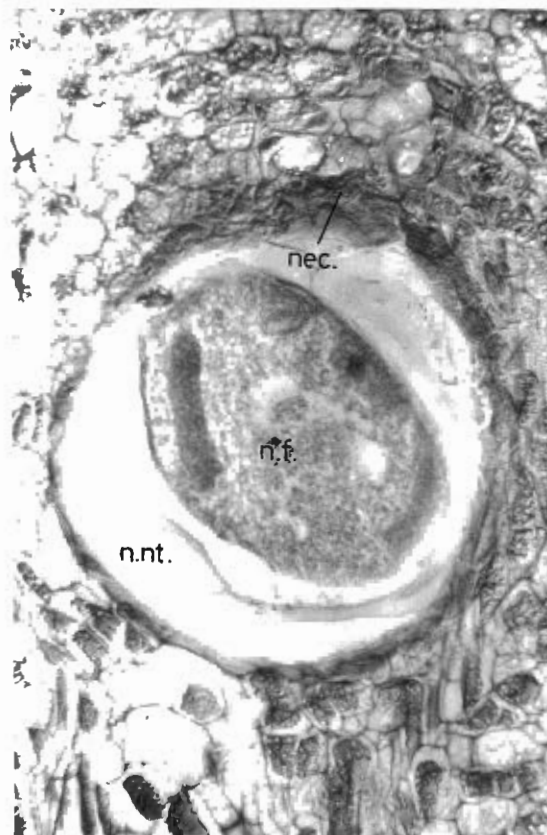


Fig. 72: White French. Radial longitudinal section of root, photographed at low magnification to show:

- (a) female nematodes (n.f.) with their egg masses (e.)
- (b) large number of syncytia (g.c.) in secondary xylem
- (c) unspoiled pith (pth.); wound periderm (w.p.)
- (d) root is attacked from both sides
- (e) periderm (perd.). X 30.

Fig. 73: White French. Tangential longitudinal section of one-year-old root, showing syncytia (g.c.) with abnormal xylem (ab. x.) in their neighbourhood; wedge-shaped parenchyma tissue in xylem ray (par. r.). X 90.

Fig. 74: 1202 C. Cross-section of one-year-old root, showing exceptionally long syncytia (g.c.) at base of lateral root (l.r.); xylem (x.); pith (pth.) abnormal parenchyma of lateral root (ab. p.). X 90.

Fig. 72

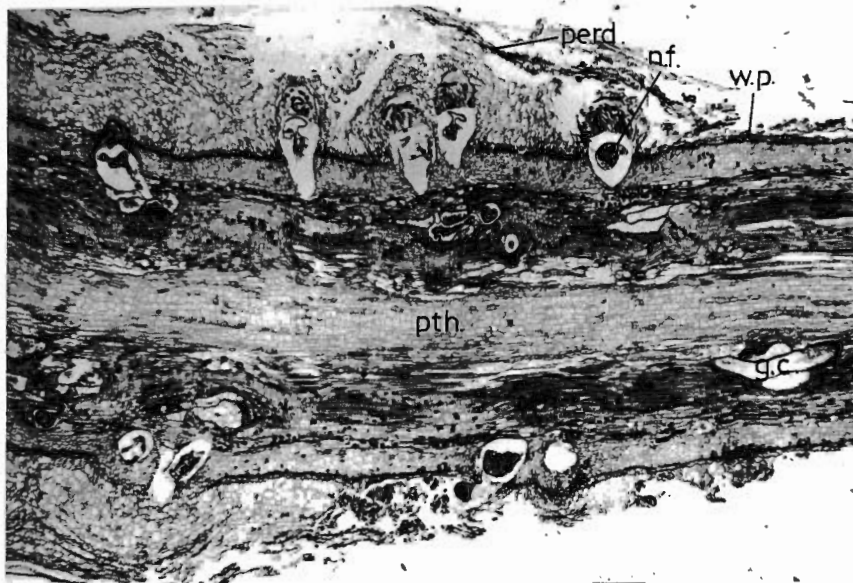


Fig. 73

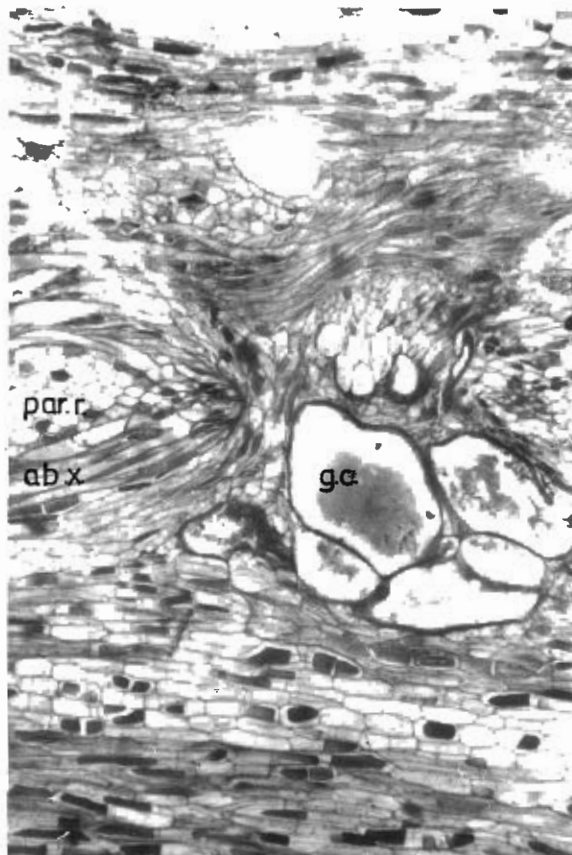
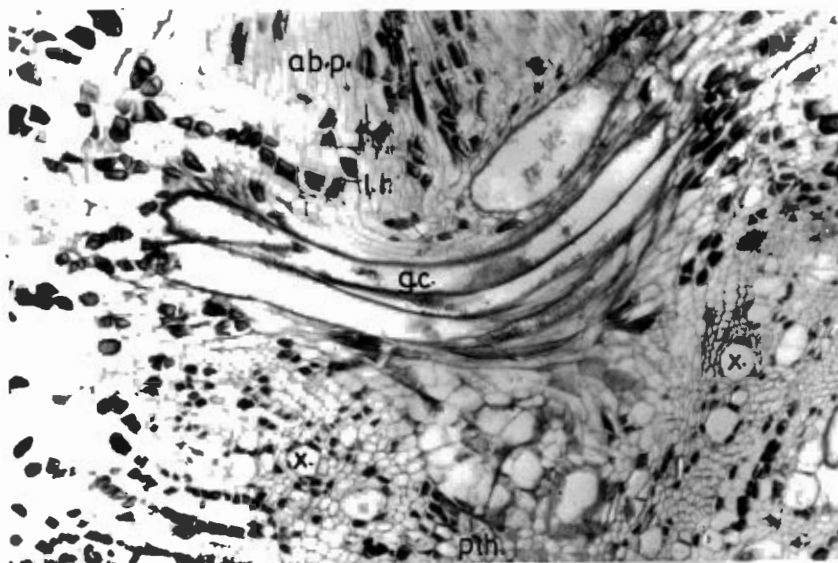


Fig. 74



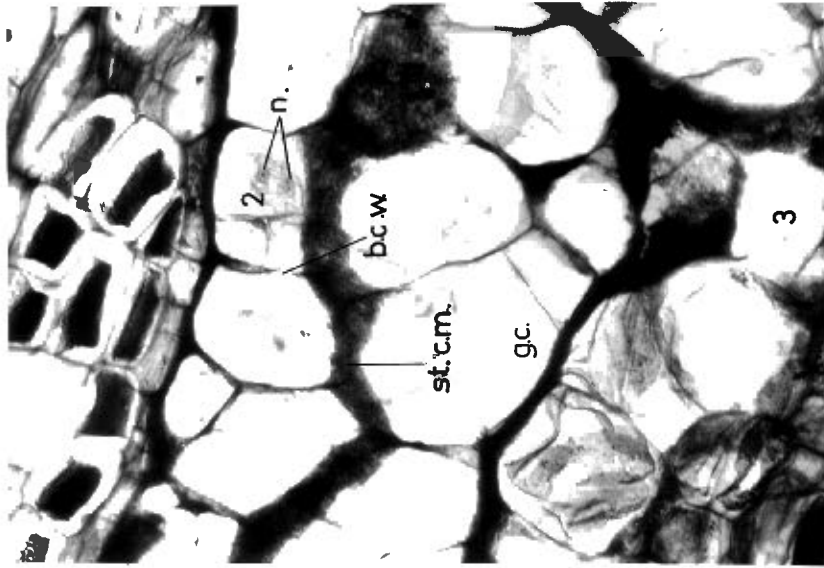


Fig. 77

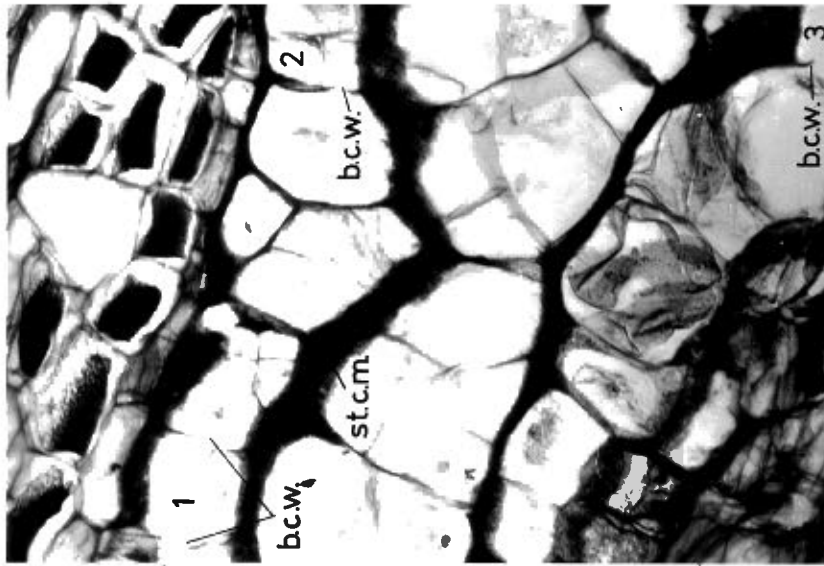


Fig. 76

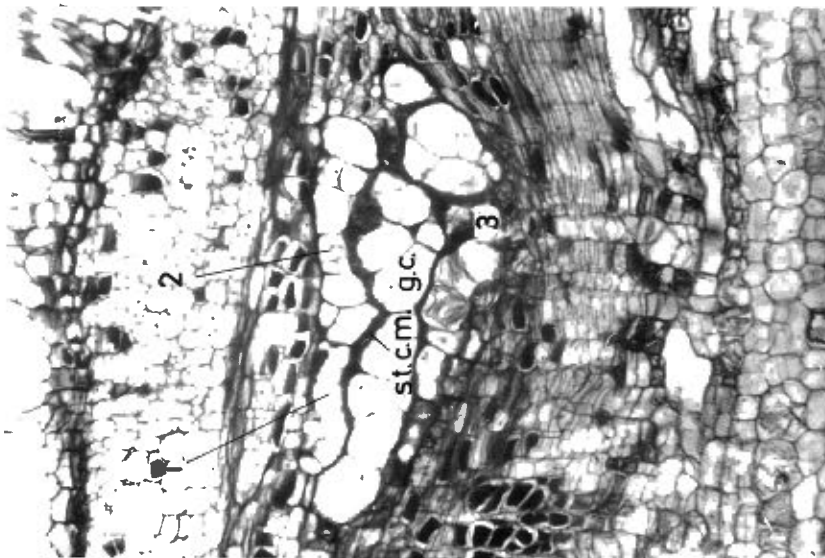


Fig. 75

Figs. 75-80: White French. Radial longitudinal section of one-year-old root, showing syncytia (g.c.) being formed by dissolution and breaking down of cell-walls (b.c.w.). Note some walls of syncytia (g.c.) appear very thick and other very thin. The thickening is partly caused by stained cytoplasm (st.c.m.) appressed to the cell-walls.

Figures 1, 2 and 3 in Figs. 75, 76, 77, 78, 79 and 80 correspond. Enlarged nuclei (n.) are visible in Figs. 77 and 79.

Enlargements: Fig. 75, X 90; Fig. 76, X 270; Fig. 78, X 270; Fig. 78, X 1 375; Fig. 79, X 1 375; Fig. 80, X 1 375.

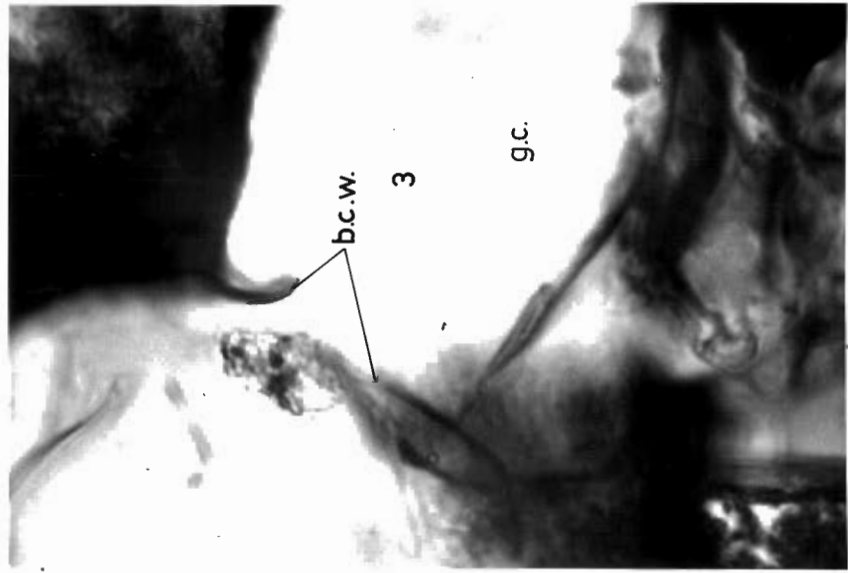


Fig. 80

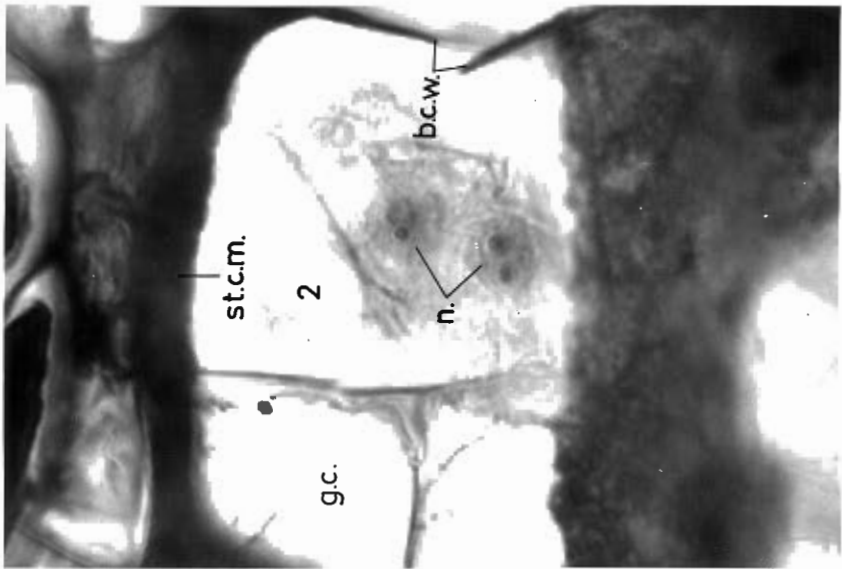


Fig. 79

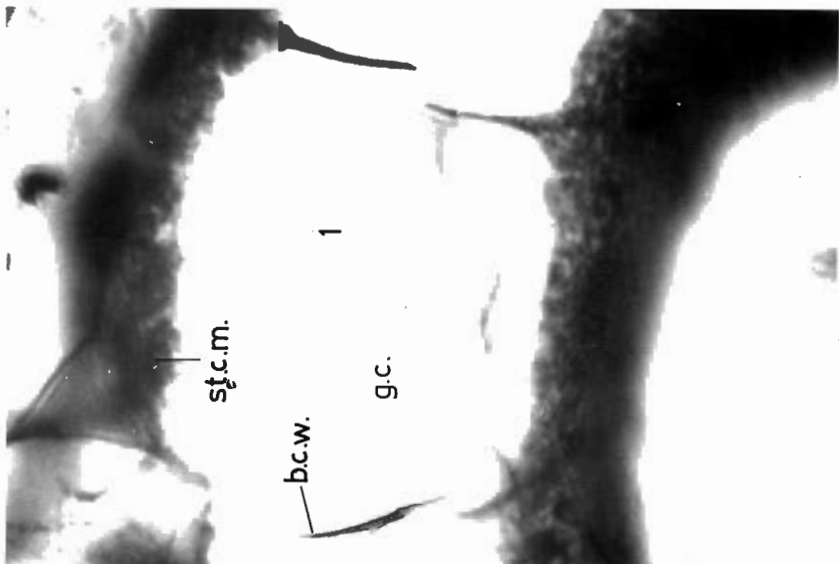


Fig. 78

Fig. 81: White French. Radial longitudinal section,
showing broken cell-wall (b.c.w.) between syncytia (g.c.).
X 230.

Fig. 82: An enlargement of broken cell-wall as seen in Fig. 81.
Figure 1 in these two Figures corresponds; broken
cell-wall (b.c.w.); syncytium (g.c.). X 1 060.

Fig. 81

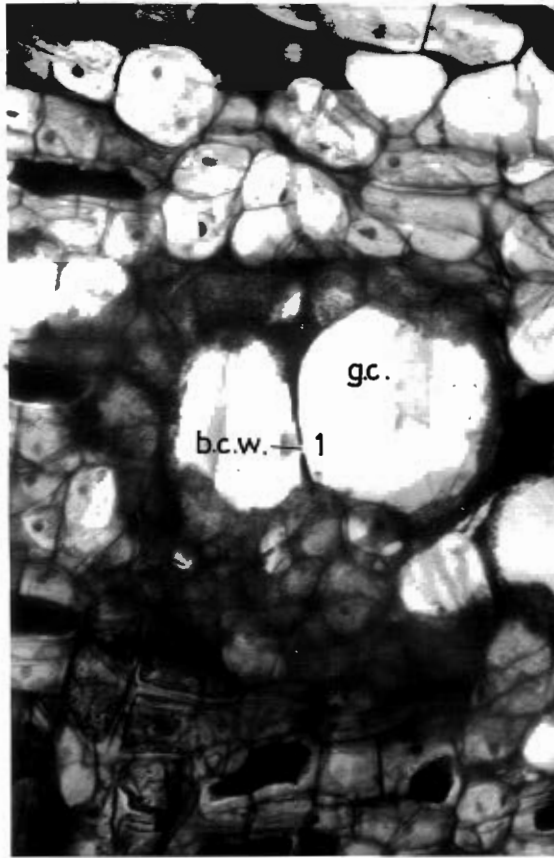


Fig. 82

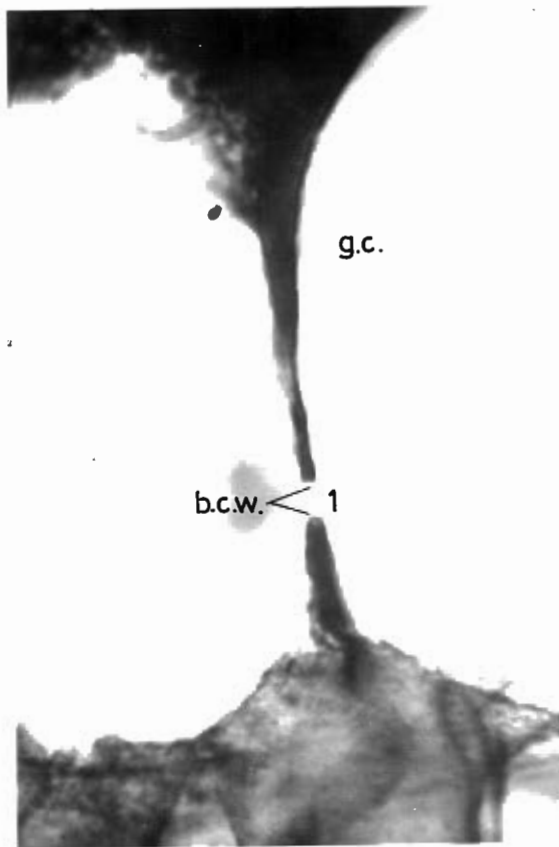


Fig. 83: White French. Radial longitudinal section, showing:

- (a) xylem vessels (x.)
- (b) syncytium (g.c.) with partially dissolved cell-wall (d.c.w.) and stained cell material (st.c.m.)
- (c) tannin (tan.) in parenchyma cells.

X 260.

Fig. 84: White French. Enlargement of part of Fig. 83.
Figure 1 in Figs. 83 and 84 corresponds.
Partially dissolved cell-walls (d.c.w.);
nucleus (n.); syncytium (g.c.). X 1 140.

Fig. 83

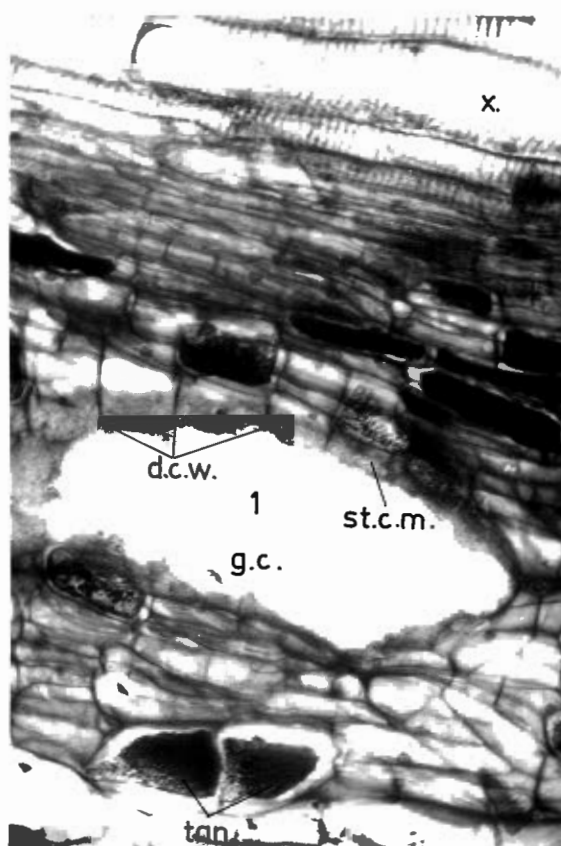


Fig. 84

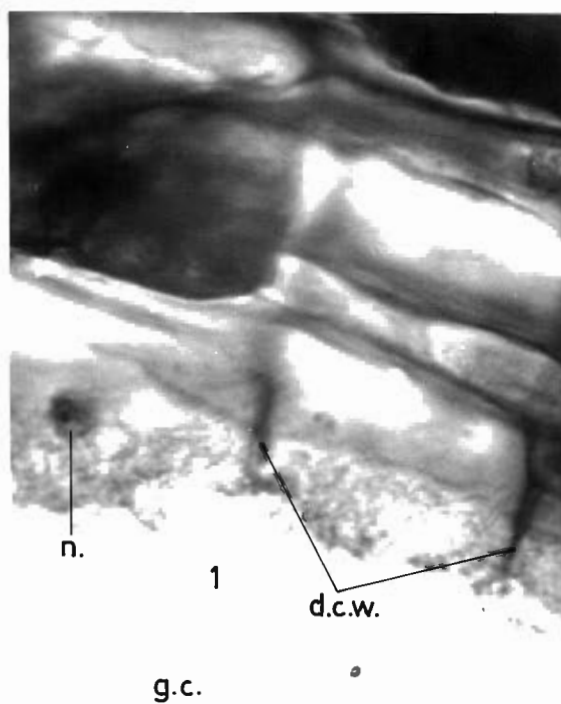


Fig. 85: White French. Radial longitudinal section, of a one-year-old root, showing:

- (a) head of nematode (n.h.) (*upper left*), wedged in between thickened cell-walls (c.w.)
- (b) partially dissolved cell-walls (d.c.w.) bordering the thick syncytium-wall (g.c.w.); syncytium (g.c.). X 260.

Fig. 86: White French. Radial longitudinal section, of a one-year-old root.

Note:

- (a) cell-walls (c.w.) (*top*) appear to be normal while those between the syncytia (g.c.) are partially dissolved (d.c.w.)
- (b) some cells (*bottom*) have two nuclei (n.).

X 260.

Fig. 87: White French. Radial longitudinal section, of a one-year-old root, showing syncytium (g.c.) partially vacuolated (vac.); cell contents pressed against syncytium-wall (g.c.w.); tannin (tan.). X 260.

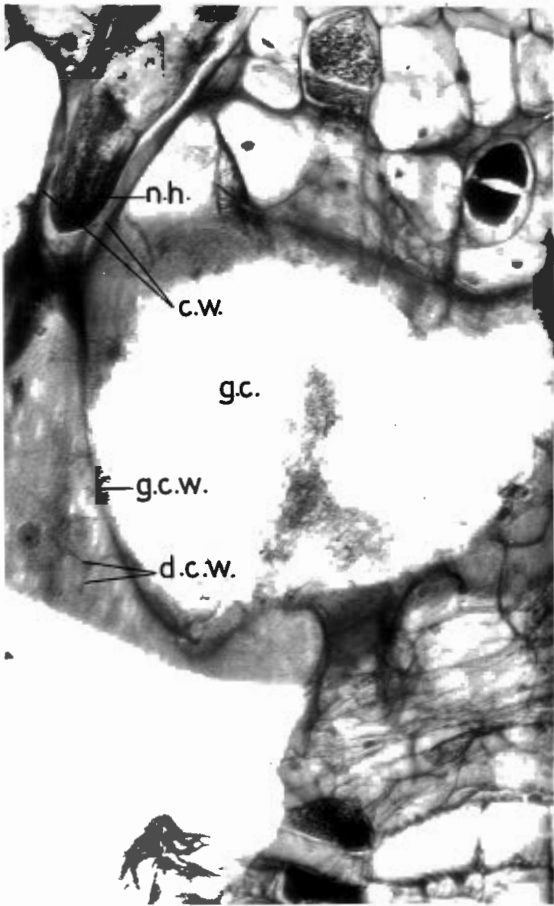


Fig. 85

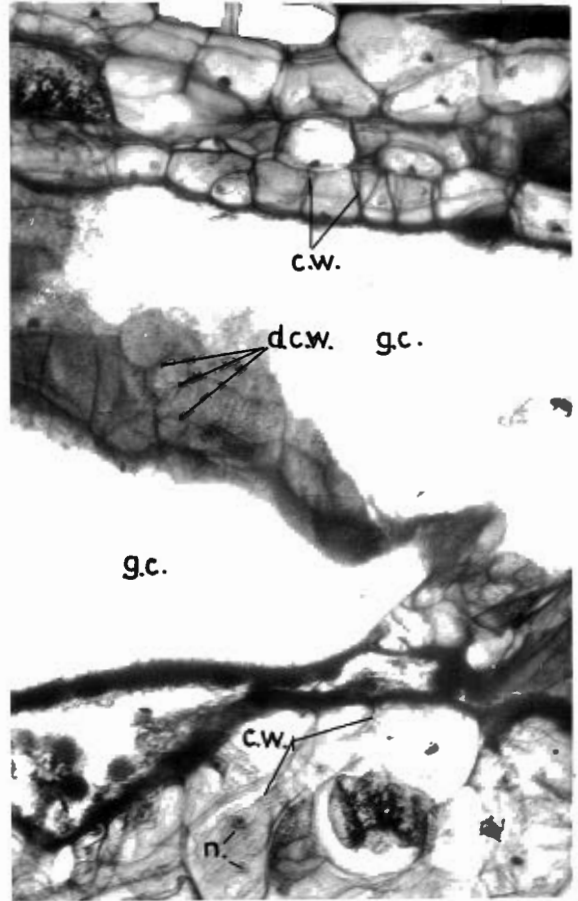


Fig. 86

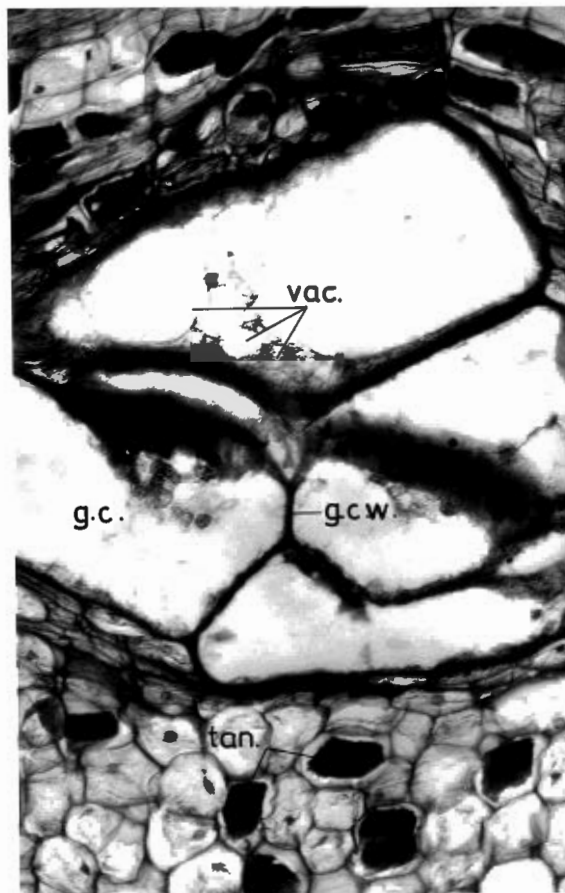


Fig. 87

Fig. 88: White French: Radial longitudinal section, of a one-year-old root, showing:

- (a) cytoplasm (p.) and partially disintegrated nuclei (n.) in a syncytium (g.c.)
- (b) stained cell material (st.c.m.) appressed to the cell-wall (c.w.) of the syncytium (g.c.)
- (c) opening (op.) between the two syncytia (g.c.); tannin (tan.). X 260.

Fig. 89. White French. Cross-section of a one-year-old root, stained with anilin chloride.

Note:

- (a) syncytium (g.c.) with cell-wall (c.w.) of uneven thickness in primary xylem
- (b) granulated cell material (p.) appressed to cell-walls and filling one syncytium. X 240.

Fig. 88

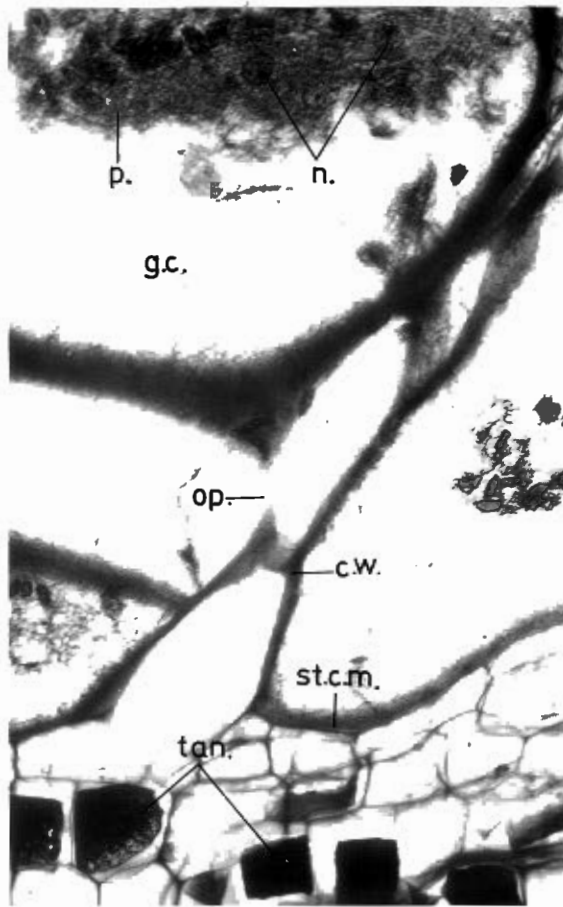
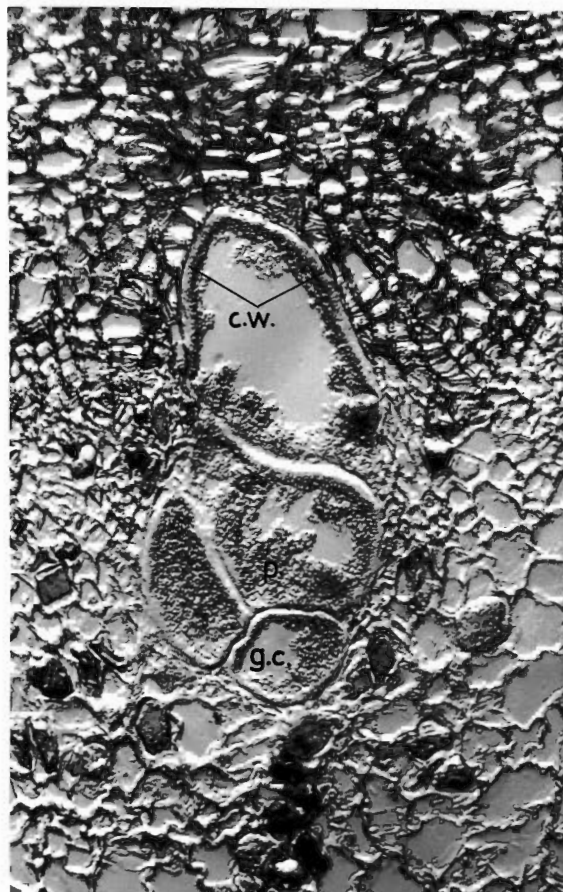


Fig. 89



Figs. 90-93: White French. Series of photomicrographs of a radial longitudinal section, of a one-year-old root, showing: Periderm (perd.); proliferated pericycle (p. perc.); nematode eggs (e.); nematode nest (n. nt.); syncytia (g.c.); tannin (tan.) in secondary phloem parenchyma cells; xylem elements (x.); pith (pth.); distortion of cells in vicinity of syncytia.

X 90.

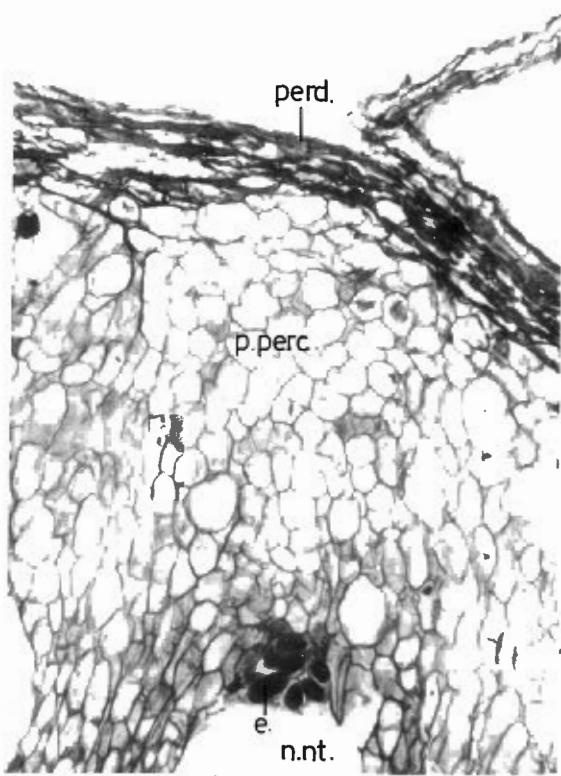


Fig. 90

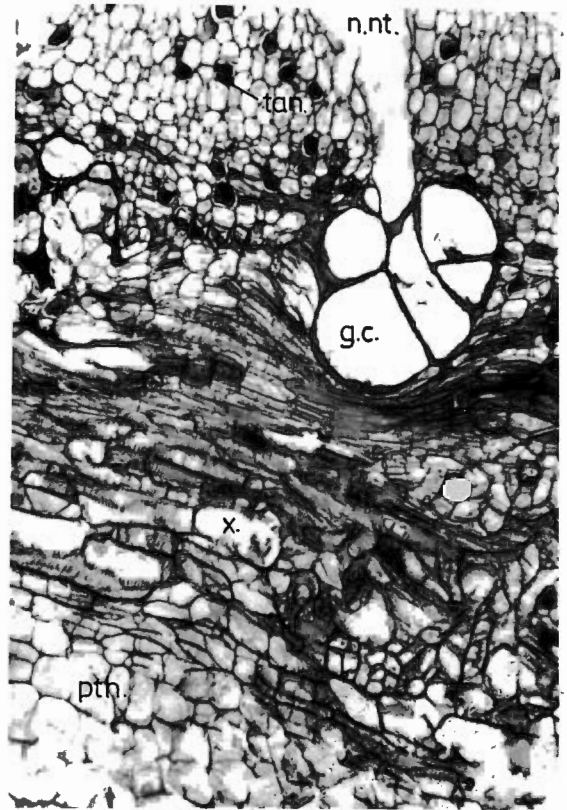


Fig. 92

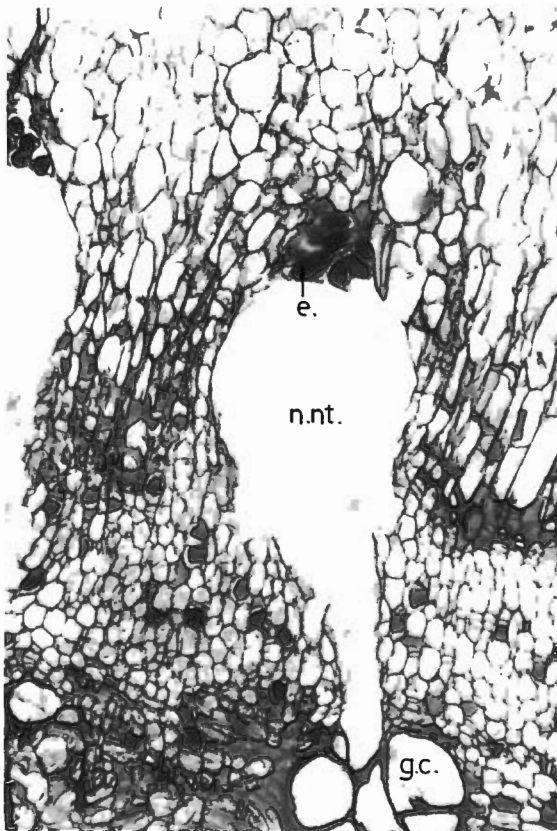


Fig. 91

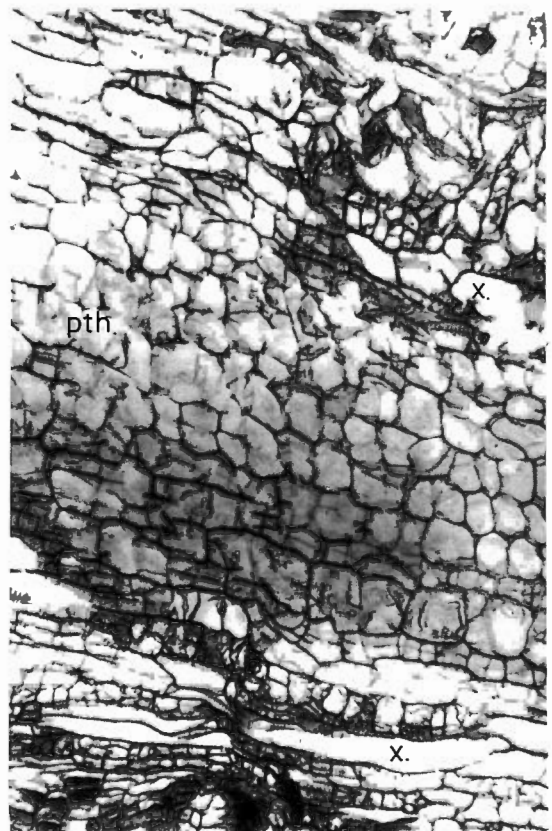


Fig. 93

Fig. 94: White French. Radial longitudinal section of root, showing group of syncytia (g.c.) near xylem (x.) elements; granulated cytoplasm (p.). X 260.

Fig. 95: White French. Radial longitudinal section of root, showing part of syncytia (g.c.) and stained cytoplasm (p.). Note semi-transparent (tp.) areas in obliquely cut cell-wall. X 260.

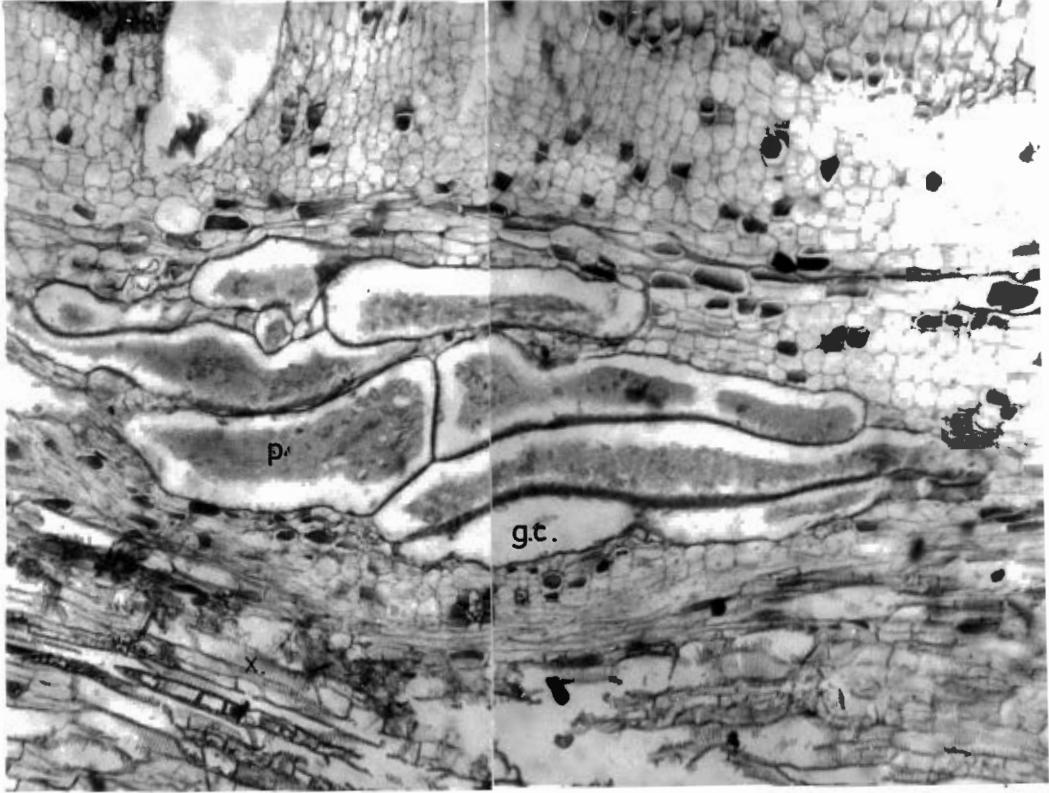


Fig. 94

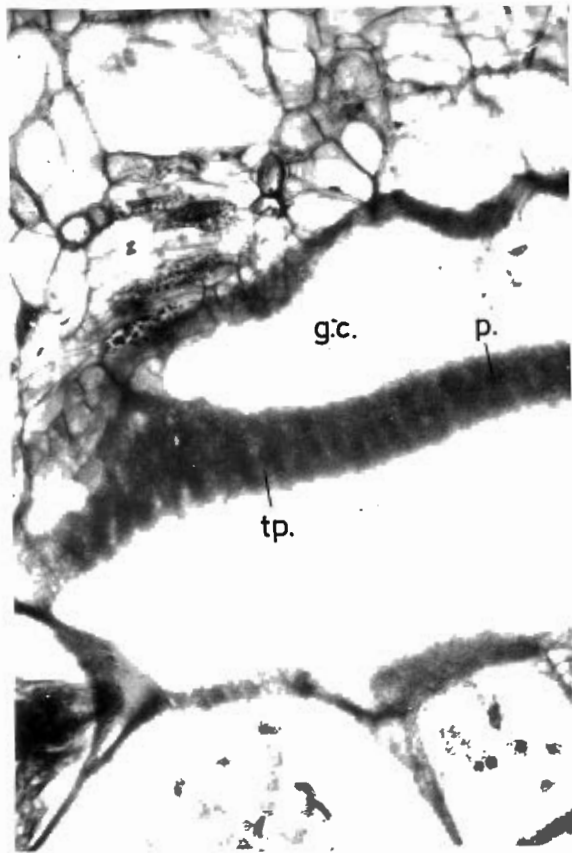


Fig. 95

Figs. 96-98: Steen. Cross-section of one-year-old root, shows penetration into root by *M. hapla* and necrosis caused by nematodes.

Sections illustrated in Figs. 96 and 97 are 1,8 mm apart and sections illustrated in Figs. 97 and 98 are 20 mm apart. X 45.

Fig. 96: Note proliferation of pericycle (p. perc.); syncytia (g.c.), one with necrosis (nec.) of adjacent cells.
X 45.

Fig. 97: Note gelatinous matrix (g.m.); nematodes (nem.) perished between outer periderm (perd.) and a second periderm (w.p.). X 45.

Fig. 98: Mass of necrotic cells (nec.) surrounded by wound periderm (w.p.) in pericycle. Necrosis (nec.) is also present in primary xylem (pr. x.); xylem (x.); phloem.). X 45.

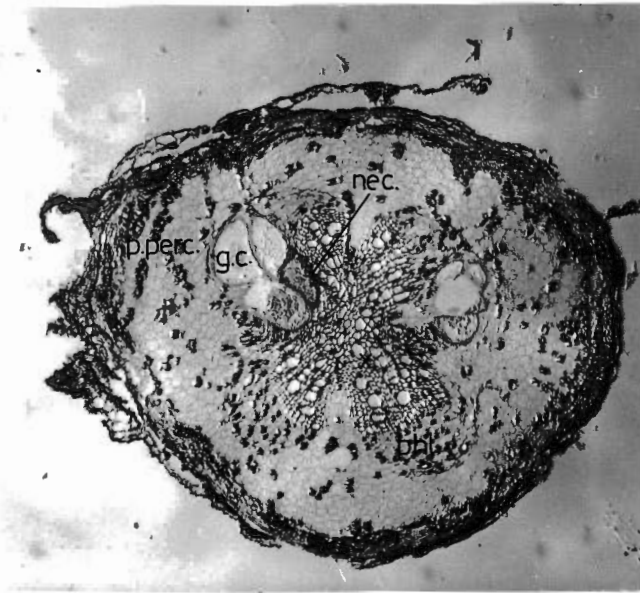


Fig. 96

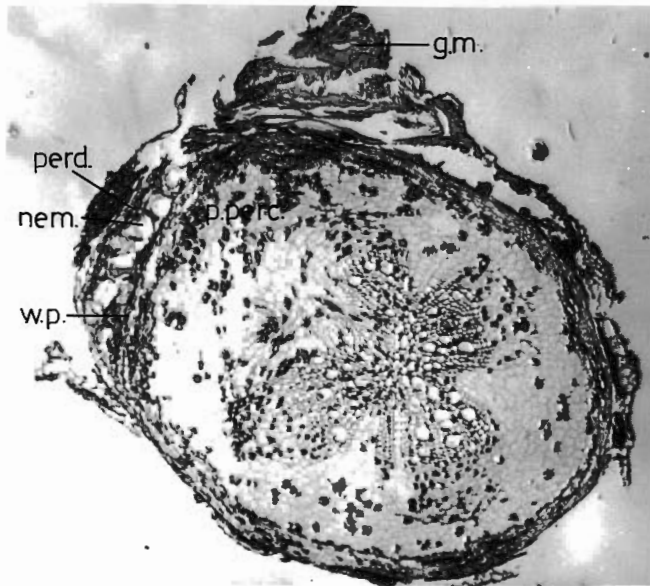


Fig. 97

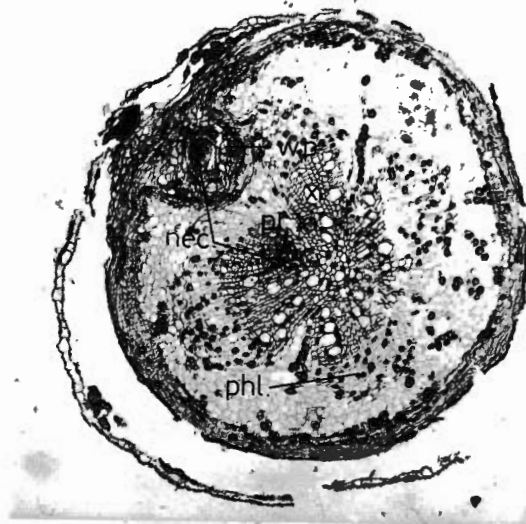


Fig. 98.

Fig. 99: Steen. Cross-section of one-year-old root, showing three nematode females (nem.) enclosed between outer periderm (perd.) and wound periderm (w.p.) and thus being prevented from penetrating root any further. X. 260.

Fig. 100: Steen. Cross-section of one-year-old root, showing a discontinuous band of wound periderm (w.p.) and nematode female invasion through the discontinuity of the wound periderm, to penetrate deep into the phloem (phl.); infected area (i.a.); gelatinous matrix (g.m.); syncytia (g.c.); xylem (x.); necrosis (nec.). X 45.

Fig. 101: Steen. Cross-section of one-year-old root, showing xylem elements (x.); xylem ray (par. r.); nematode path (n.p.); syncytia (g.c.); necrosis (nec.). X 260.

Fig. 99

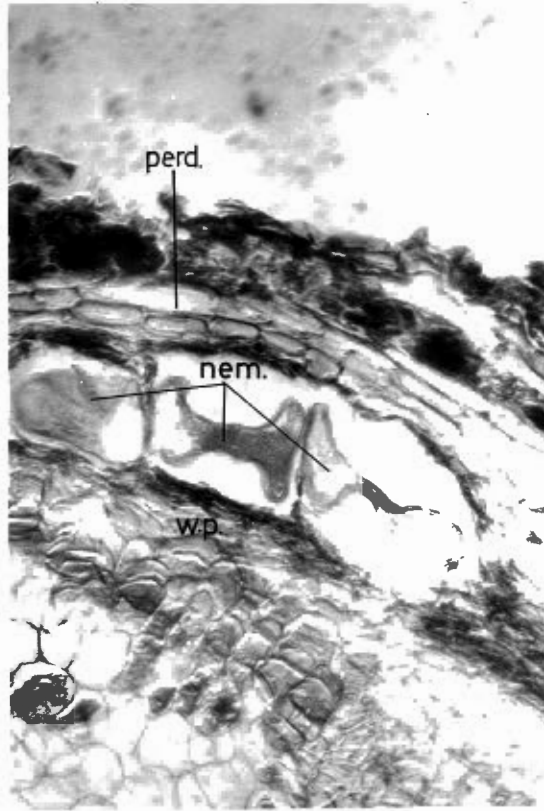


Fig. 100

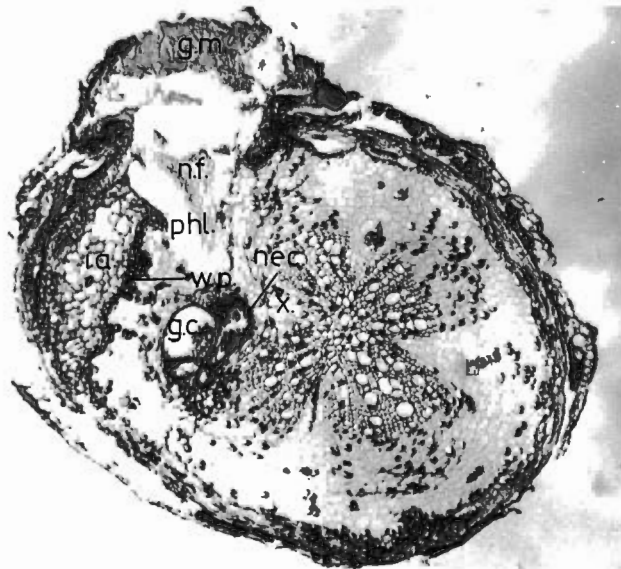


Fig. 101

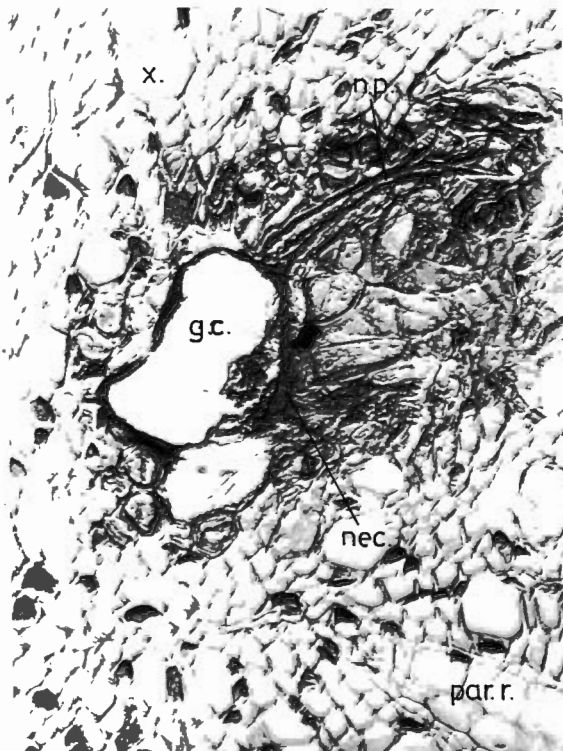


Fig. 102: Jacquez. Cross-section, showing nematode nests (n. nt.) outside wound periderm (*top right*) and wound periderm (w.p.) (*left*) interrupted by large nematode nest; phloem (phl.); xylem (x.); proliferated pericycle (p. perc.). X 55.

Fig. 103: Jacquez. Cross-section, showing gelatinous matrix (g.m.); remains of female nematode (nem.); wound periderm (w.p.); partly formed syncytia (g.c.); proliferated pericycle (p. perc.). X 100.

Fig. 102

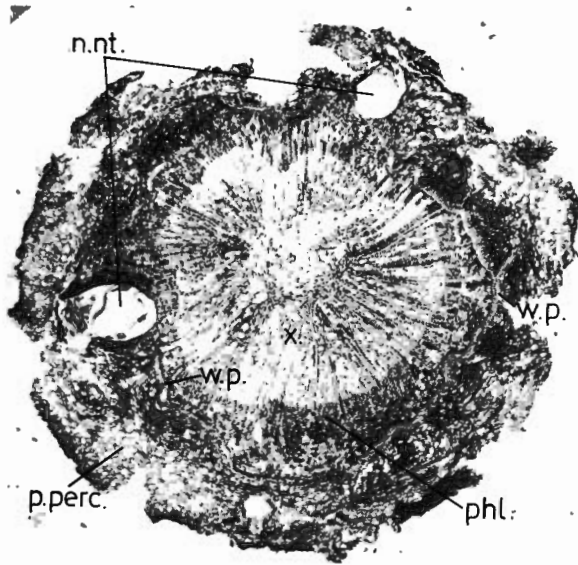
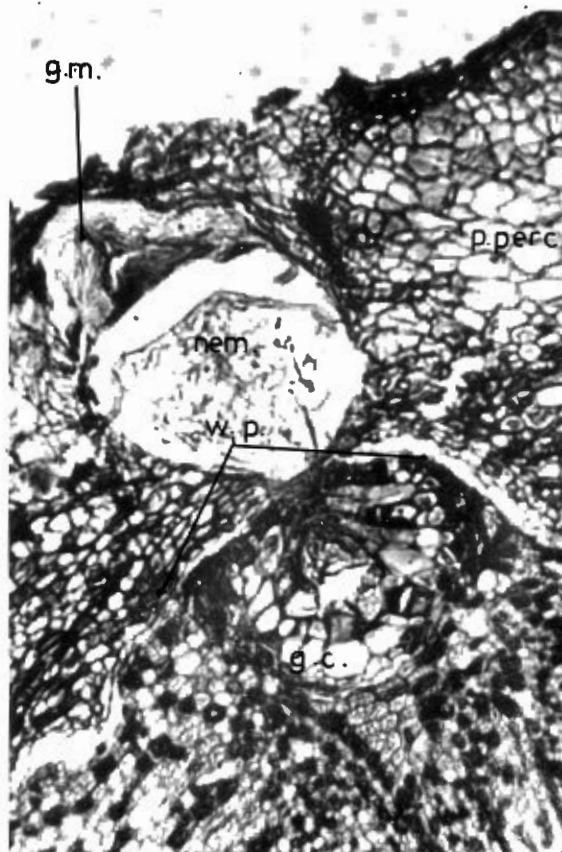


Fig. 103



Figs. 104-111: Dogridge, Salt Creek and 99 R.

Cross-sections of two-year-old roots, infected with *M. hapla* at end of first year.

Figs. 104-105: Dogridge. Cross-sections, illustrated in Figs. 104 and 105 are 750µm apart. These Figures show wound periderm (w.p.) fluctuating at different distances from the periderm (perd.). In Fig. 104 two wound periderms are clearly visible in some places as well as a slight necrosis (nec.) in the xylem (x.); proliferated pericycle (p. perc.); phloem (phl.). X 30.

Fig. 106: Salt Creek. Two wound periderms (w.p.); partly obliterated vessels (o.x.) in the secondary xylem; necrosis (nec.) of cells bordering two vessels (x.) is visible; periderm (perd.); phloem (phl.). X 65.

Fig. 107: 99 R. A second periderm (s.p.) almost parallel to the outer periderm (perd.) is visible, as well as necrosis (nec.) of cells in the secondary xylem (x.); tyloses (ty.). X 30.

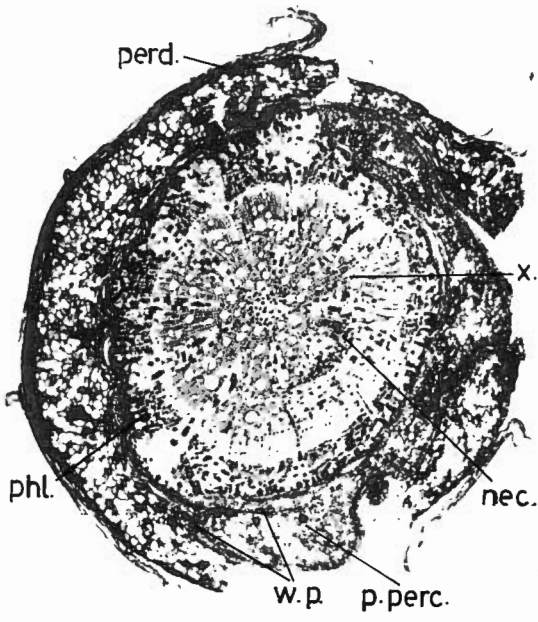


Fig. 104

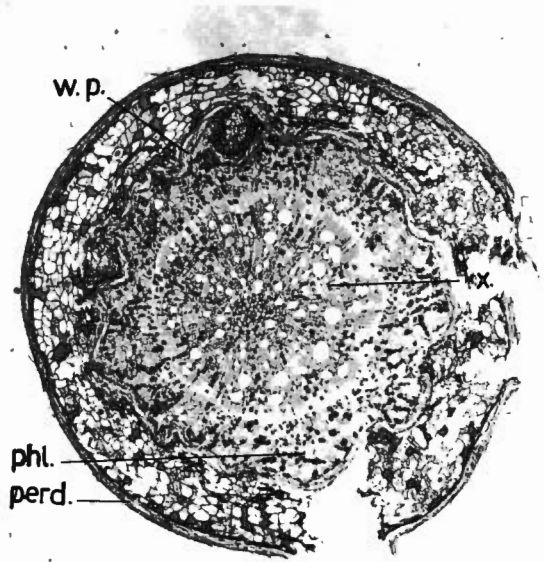


Fig. 105

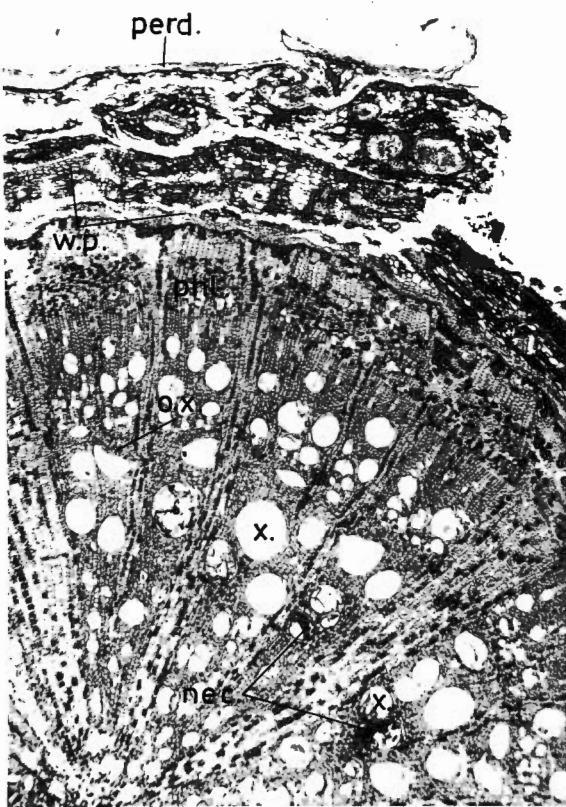


Fig. 106

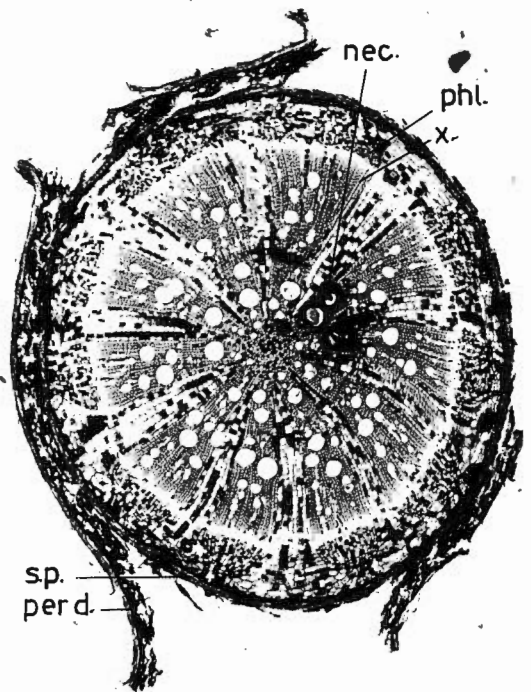


Fig. 107

Fig. 108: 99 R. Cross-section, 300 μ m from section shown in Fig. 107. Note more severe necrosis (nec.) in secondary xylem (x.) than shown in Fig. 104; phloem (phl.); periderm (perd.); second periderm (s.p.). X 30.

Fig. 109: 99 R. The beginning of necrosis (nec.) of xylem (x.) tissues. Some vessels contain gums (gu.); tyloses (ty.); xylem ray (par. r.); pith (pth.). X 85.

Fig. 110: 99 R. A very advanced form of necrosis (nec.) in secondary xylem (x.); wound periderm (w.p.) formed far from surface of roots.

Note: Dome-shaped lesion "slit" on outer margin of first year annual ring. In second annual ring outside lesion a mass of almost undifferentiated xylem (un.x.) tissue with few tracheary elements occurs; periderm (perd.); phloem (phl.); necrosis (nec.); proliferated pericycle (p. perc.) between two periderms (*right*). X 30.

Fig. 111: 99 R. Close-up view of lesion similar to the one illustrated in Fig. 110. Many cells in xylem (x.) are filled with gums (gu.); abnormal amount of parenchyma (ab. p.) in second annual ring. X 85.

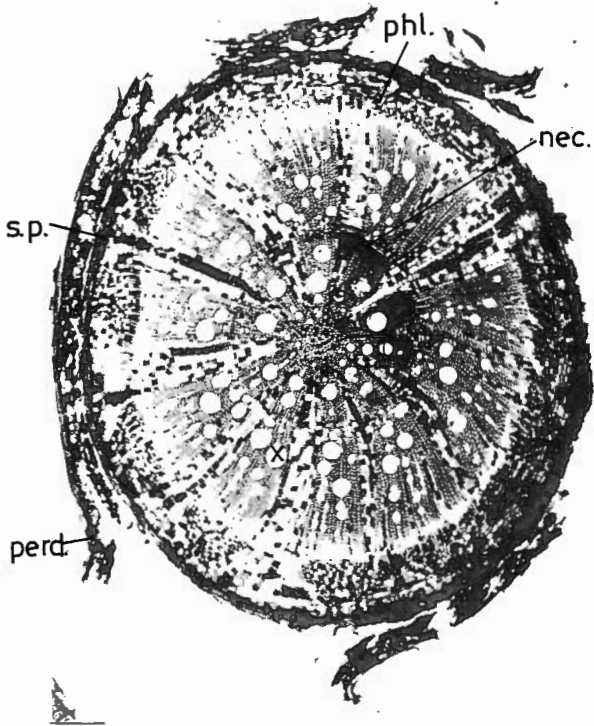


Fig. 108

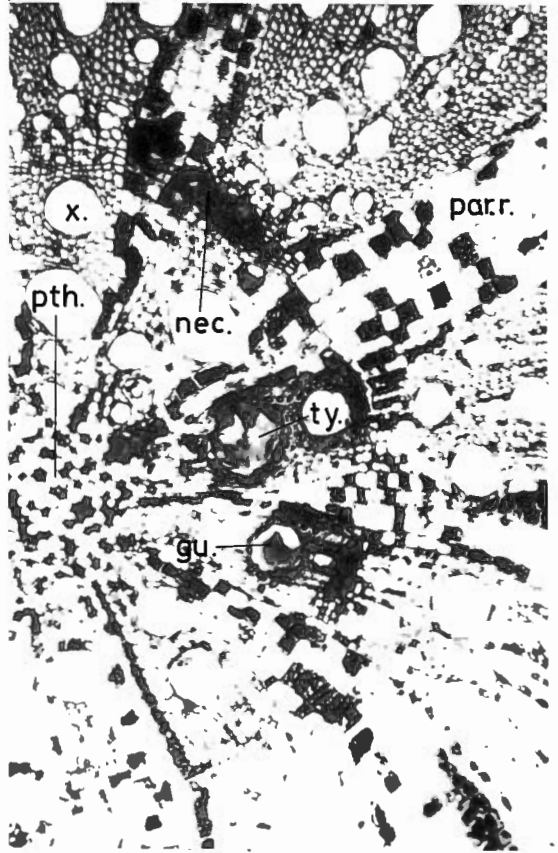


Fig. 109

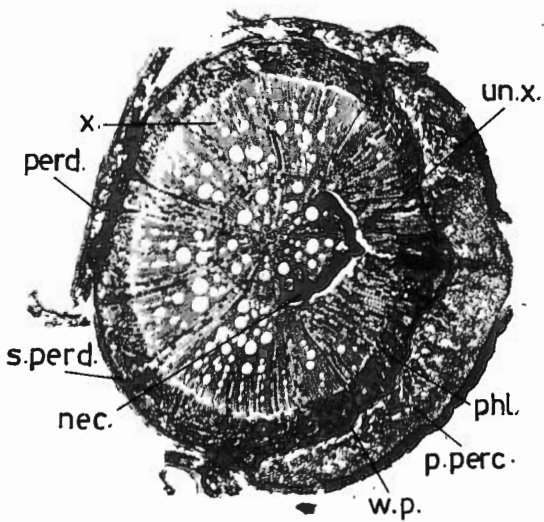


Fig. 110

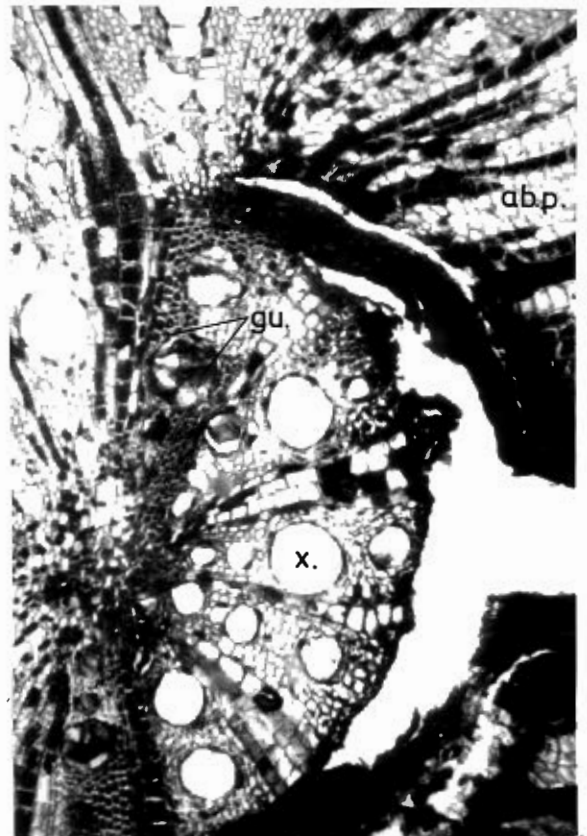


Fig. 111

Figs. 112-114: 1202 C. A series of photomicrographs of a cross-section of a one-year-old root.

Fig. 112: Proliferated pericycle (p. perc.); syncytium (g.c.).
X 340.

Fig. 113: Syncytia (g.c.) and thin-walled infected parenchyma cells (i. par.) seen between two groups of xylem elements (x.) i.e. in the xylem rays. X 340.

Fig. 114: Syncytia (g.c.) with neighbouring infected parenchyma cells (i. par.) between two groups of xylem elements (x.) in xylem ray; pith (pth.). X 340.

Fig. 115: 1202 C. Enlargement of central part of Fig. 114. Figure 1 in Figs. 114 and 115 corresponds; syncytium (g.c.); abnormal xylem (ab. x.). X 1 020.

Fig. 116: 1202 C. Enlargement of part of syncytium (g.c.) seen in Fig. 115. U-shaped wall thickening with an appressed layer of cell contents. Note adjacent abnormal tracheary elements (ab. x.).
X 1 450.

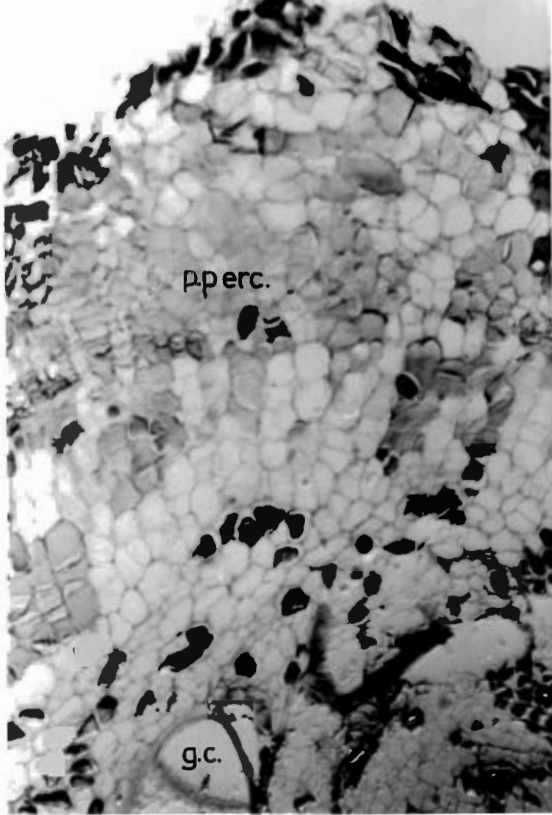


Fig. 112

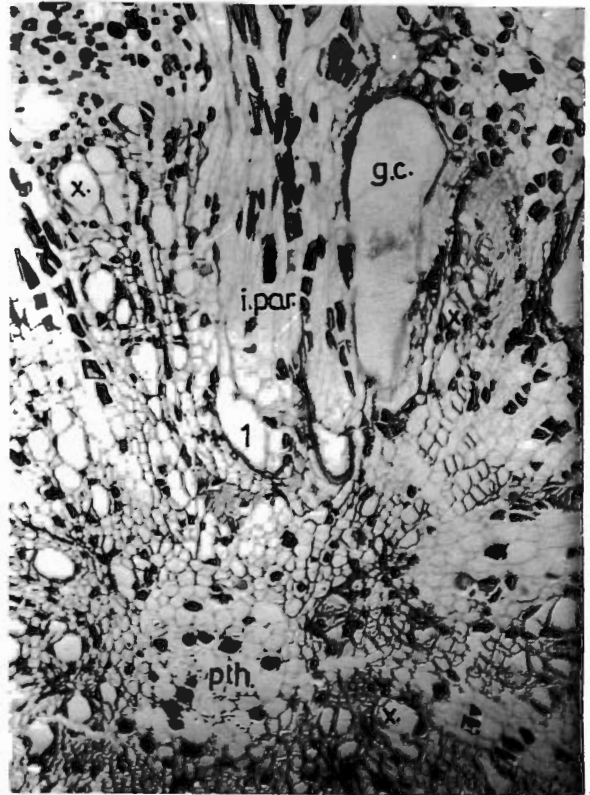


Fig. 114

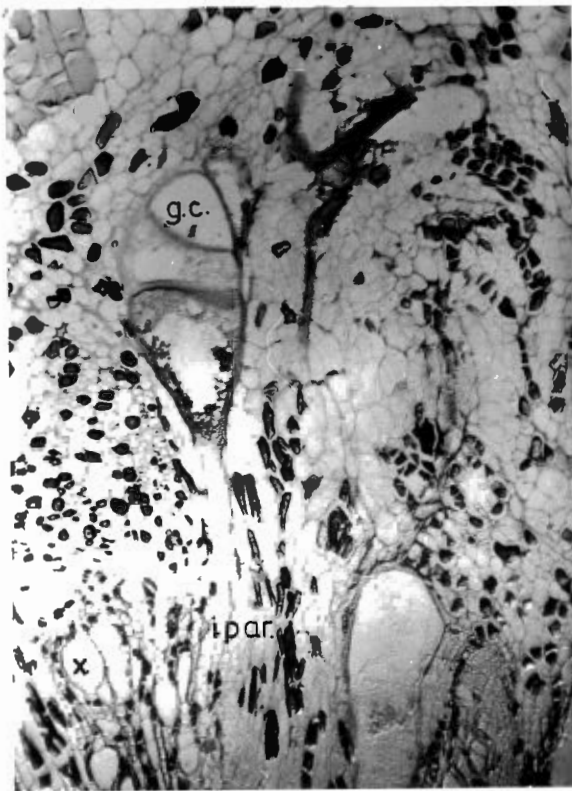


Fig. 113

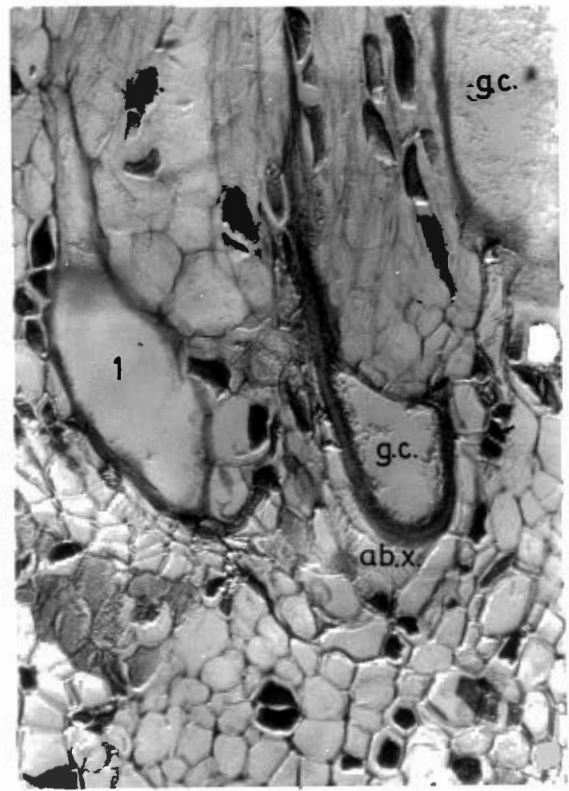


Fig. 115

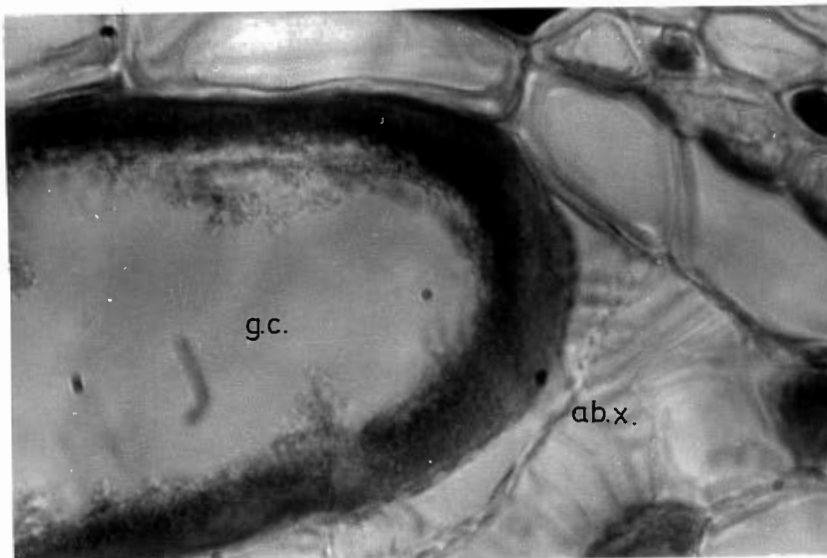


Fig. 116

Figs. 117-120: Jacquez. A series of photomicrographs of a cross-section through one-year-old root.
X 145.

Fig. 117: Proliferated pericycle (p. perc.) abnormally shaped phloem ray cells (phl. r.).

Fig. 118: Semi-tangentially stretched cells of phloem ray (phl. r.). Elongated syncytium (g.c.) stretching from young secondary phloem (y.s.phl.) across vascular cambium (cam.) into secondary xylem (s.x.). Wound periderm (w.p.) in older secondary phloem (o.s.phl.), cutting across widened phloem ray (phl. r.); infected parenchyma (i. par.).

Fig. 119: End of elongated syncytium (g.c.). Note abnormal parenchyma (i. par.) in xylem ray and destruction of primary xylem (pr. x.); xylem (x.).

Fig. 120: Pith with four groups of abnormal cells in infected area (i.a.) replacing primary xylem bundles (pr. x.). Necrosis (nec.) is clearly visible (*bottom left*).

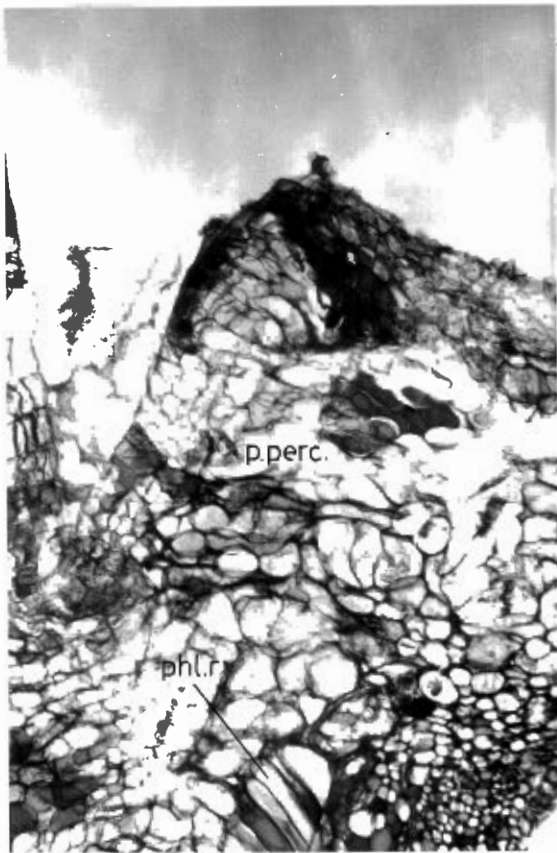


Fig. 117

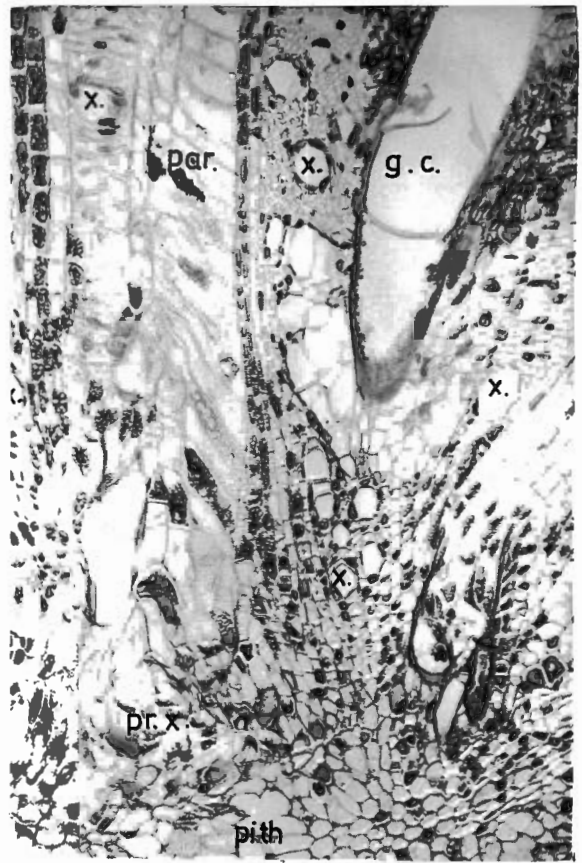


Fig. 119

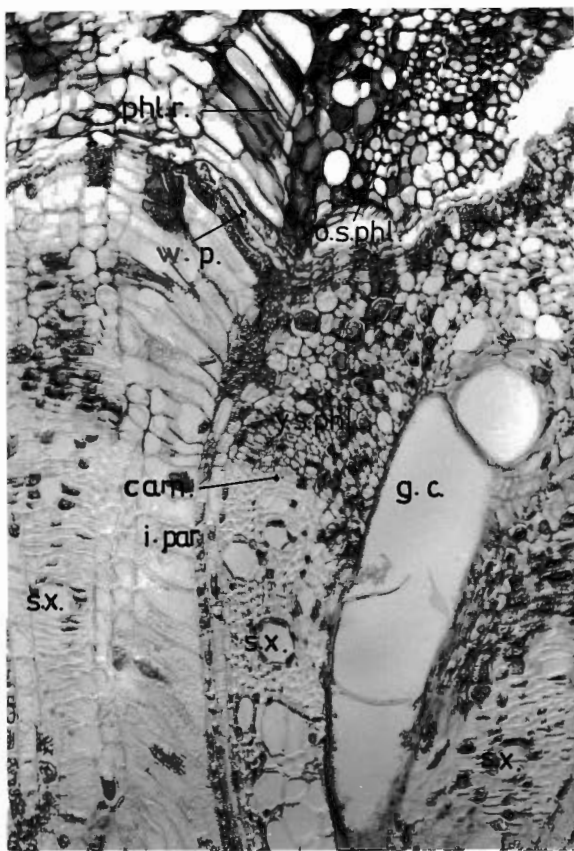


Fig. 118

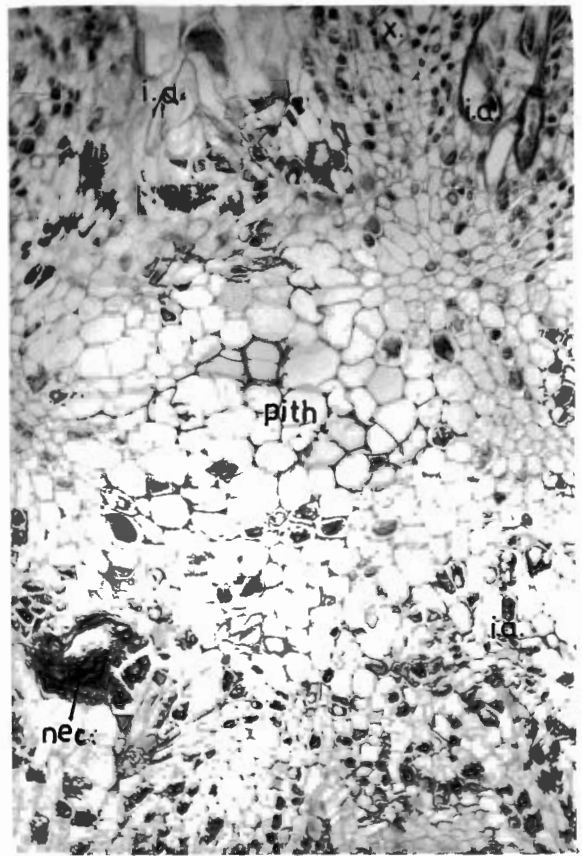


Fig. 120

Figs. 121-122: Jacquez. A series of photomicrographs of a cross-section, through a one-year-old root.
X 145.

Fig. 121: Proliferation of pericycle (p. perc.); wound periderm (w.p.) formed in old secondary phloem tissue; semi-tangentially stretched cells in vascular ray (par. r.).

Fig. 122: Cambium cells (cam.) (*middle left*) are normal but on the right they are almost absent; cells of vascular ray are abnormally stretched (i. par.); xylem (x.); old secondary phloem (o.s.phl.); young secondary phloem (y.s.phl.); wound periderm (w.p.).

Fig. 123: Jacquez. Wedge-shaped syncytium (g.c.) next to three other syncytia. Note normal vascular cambium (cam.) (*middle right*) and abnormal tissue left of the syncytium; cytoplasm (p.) is visible in syncytium; wound periderm (w.p.); phloem (phl.). X 145.



Fig. 121

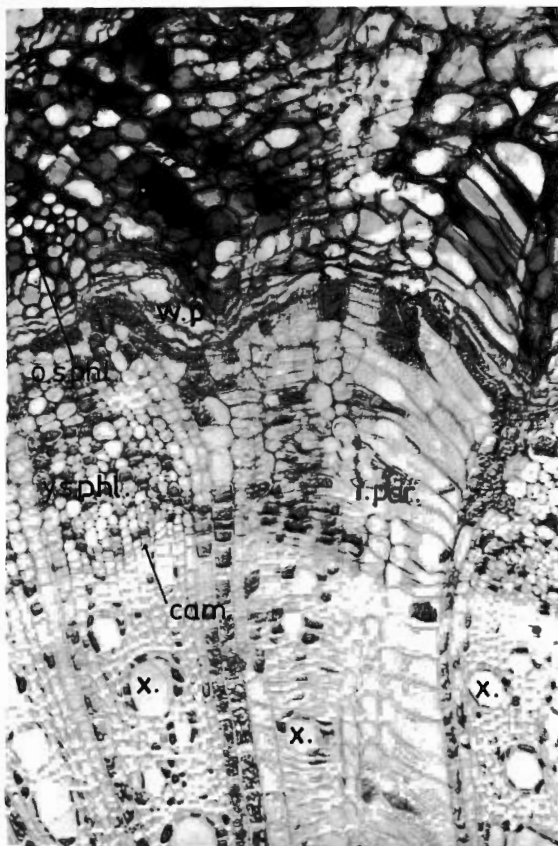


Fig. 122

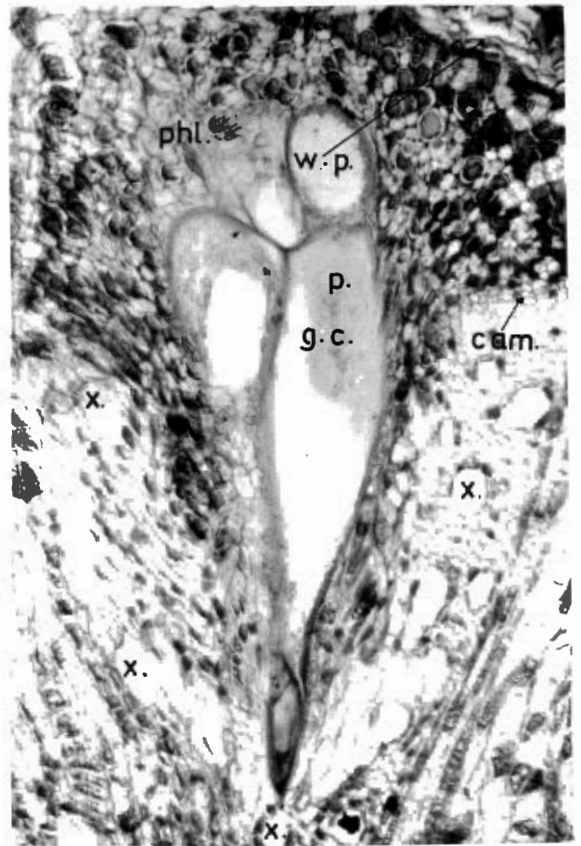


Fig. 123

Fig. 124: Jacquez. Part of tangential longitudinal section, of infected root showing obliteration (ob.) of vascular ray cells. Note first indications of probable cell-wall dissolution (c.w.d.). X 145.

Fig. 125: Jacquez. Cross-section of two-year-old root, showing intrusion (it.) of new cell growth into an old syncytium (g.c.); obliteration (ob.) of cells. X 220.

Fig. 124.



Fig. 125

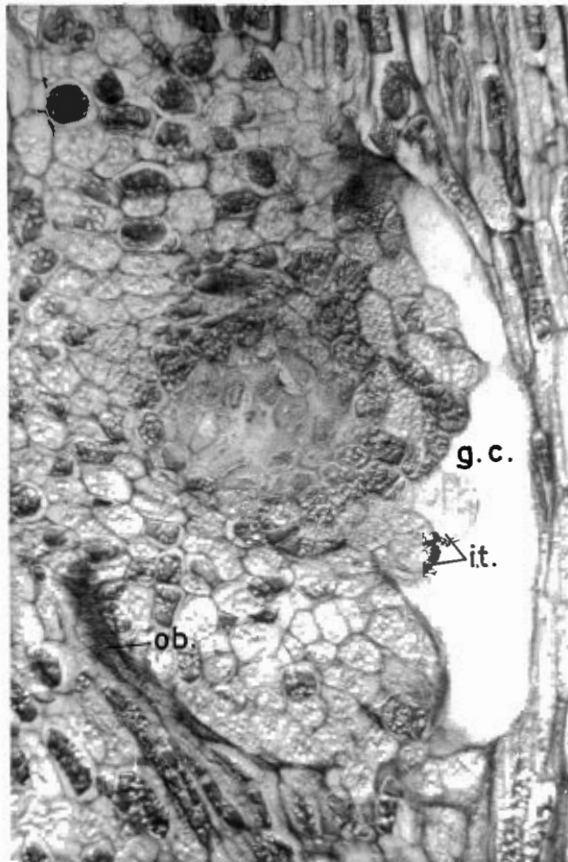


Fig. 126: Jacquez. Cross-section, showing proliferation of pericycle (p. perc.); wound periderm (w.p.) formed in secondary phloem (phl.); pith (pth.); necrosis (nec.); xylem (x.); cambium (cam.).
X 50.

Fig. 127: Jacquez. Cross-section, showing wound periderm (w.p.) formed in secondary phloem. Younger secondary phloem (y.s.phl.) and phloem ray (phl. r.) between wound periderm (w.p.) and vascular cambium (cam.) appear normal, and are rich in starch embedded in tannin (tan.); old secondary phloem (o.s.phl.).
X 145.

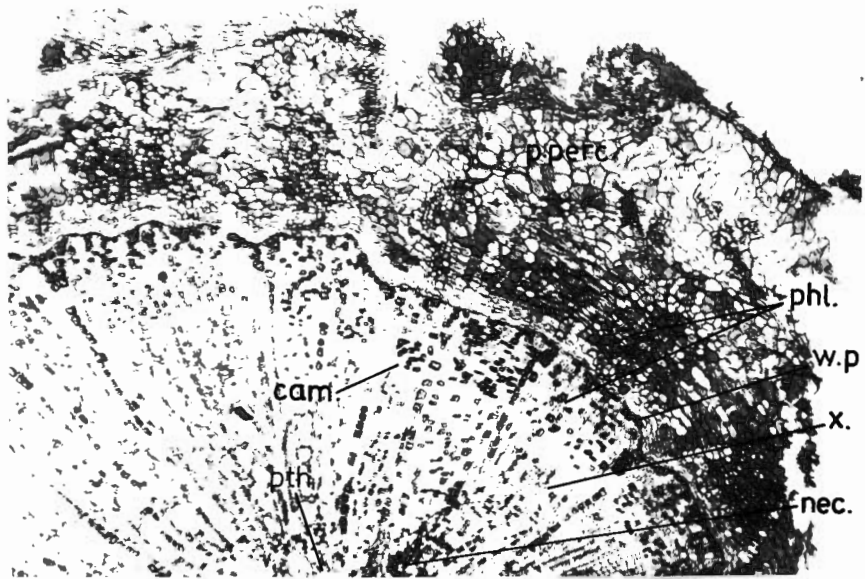


Fig. 126

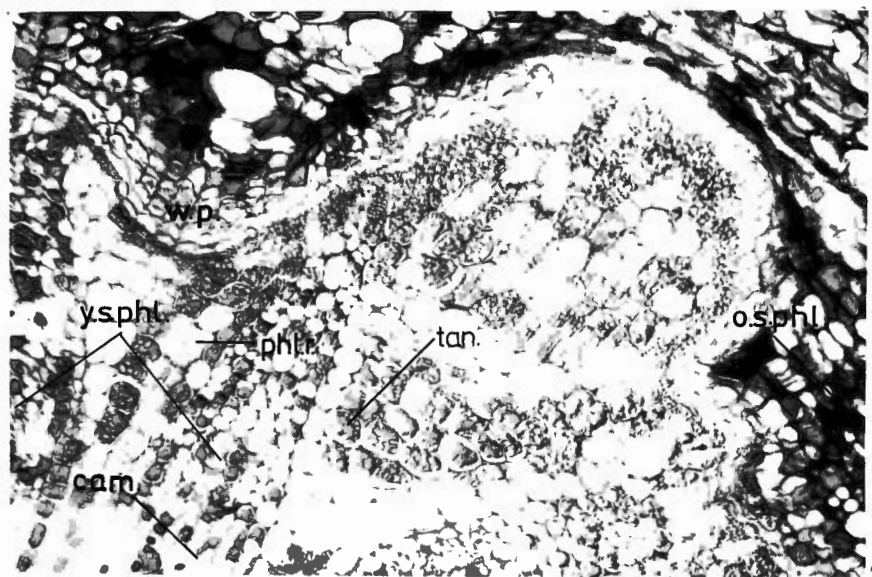


Fig. 127

Fig. 128: Jacquez. Cross-section, showing one-year-old root, with intersecting wound periderm (w.p.) layers. These wound periderms cut off the older secondary phloem (o.s.phl.) from the younger (y.s.phl.); dilated vascular ray (par. r.) near wound periderm; vascular cambium (cam.). X 145.

Figs. 129-130: Jacquez. Two photomicrographs of a cross-section, of one-year-old root showing a nematode nest (n. nt.) and adjacent tissues. X 160.

Fig. 129: Nematode eggs (e.). Wound periderm (w.p.) on the left of nematode nest (n. nt.) is clearly visible, but on the right it is absent; younger secondary phloem (y.s. phl.); infected area (i.a.).

Fig. 130: (a) nematode nest (n. nt.)
(b) normal vascular cambium (cam.) in left half of Figure; to the far right they are either abnormal or absent. Where vascular cambium cells are normal the adjacent xylem and phloem cells are also normal
(c) vascular ray (par. r.); xylem (x.); infected parenchyma cells (i. par.); phloem (phl.); wound periderm (w.p.).

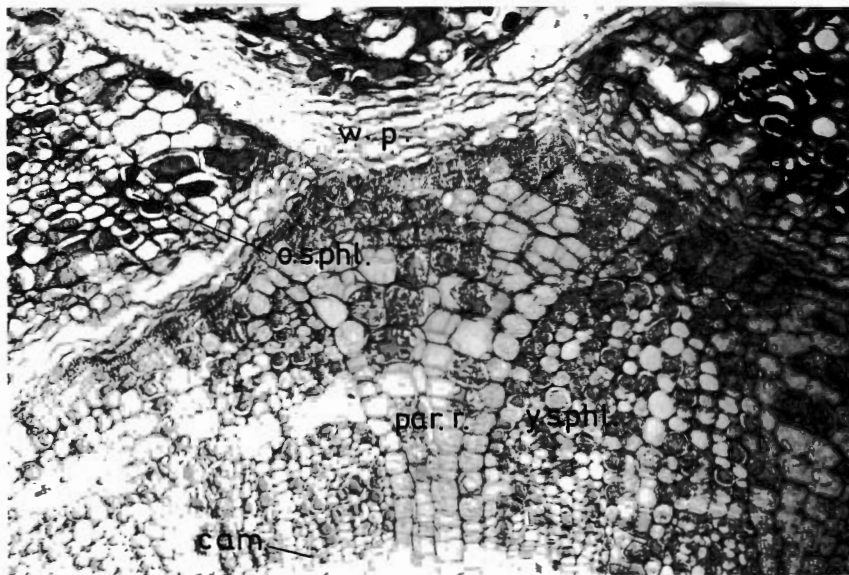


Fig. 128

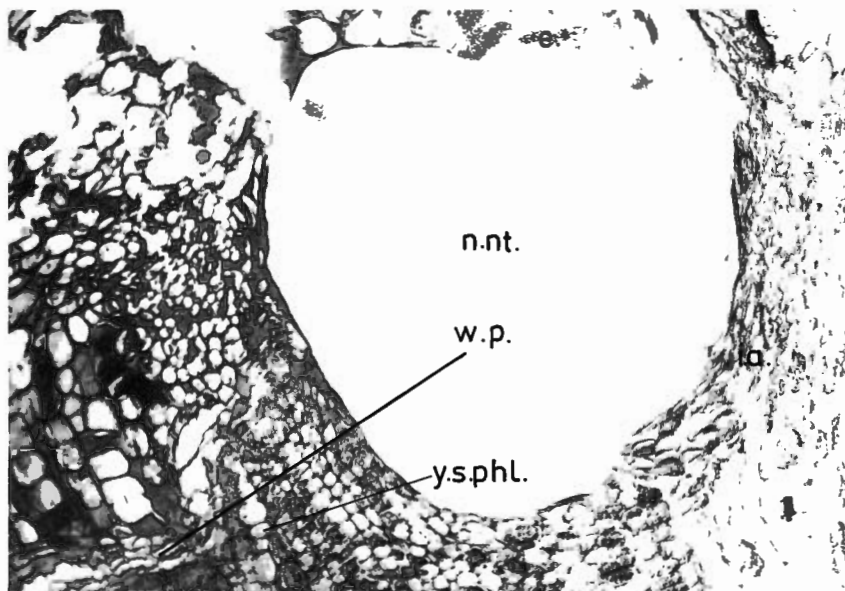


Fig. 129

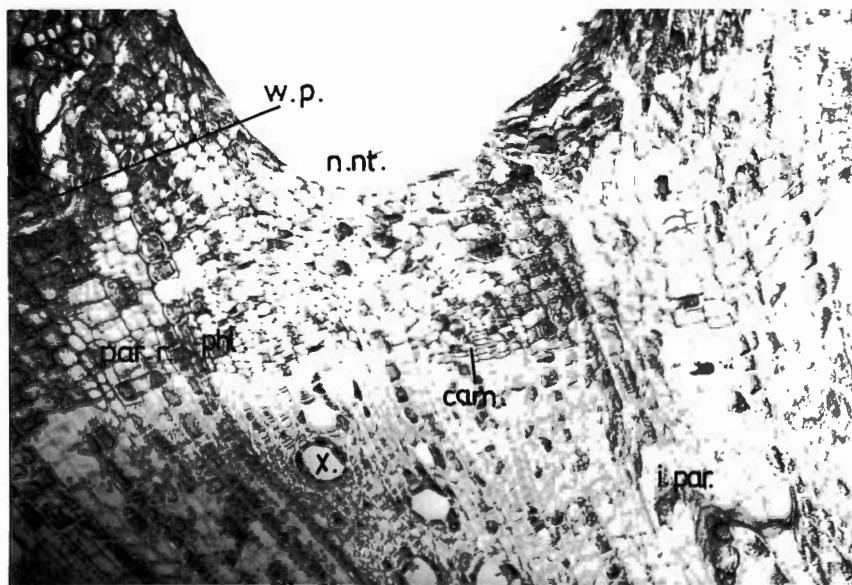


Fig. 130

Fig. 131: Steen. Cross-section of one-year-old root, showing nematode nest (n. nt.) with periderm (perd.) on the outside and wound periderm (w.p.) on the inside; parenchyma cells (par.) of the pericycle with starch (sta.); phloem (phl.); vascular cambium (cam.); secondary xylem (s.x.). X 115.

Fig. 132. Steen. An enlargement of part of Fig. 131. Figure 1 in Figs. 131 and 132 corresponds. Cells in infected area are almost void of starch while those on the centripetal side of the wound periderm (w.p.) are filled with starch (sta.); periderm (perd.); parenchyma (par.); nematode nest (n. nt.). X 290.

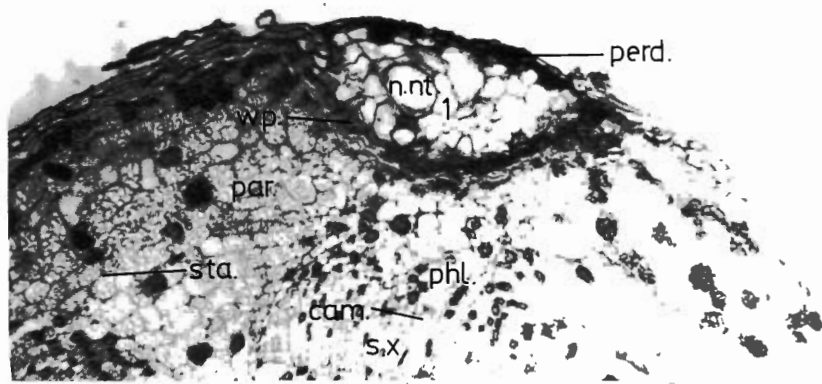


Fig. 131

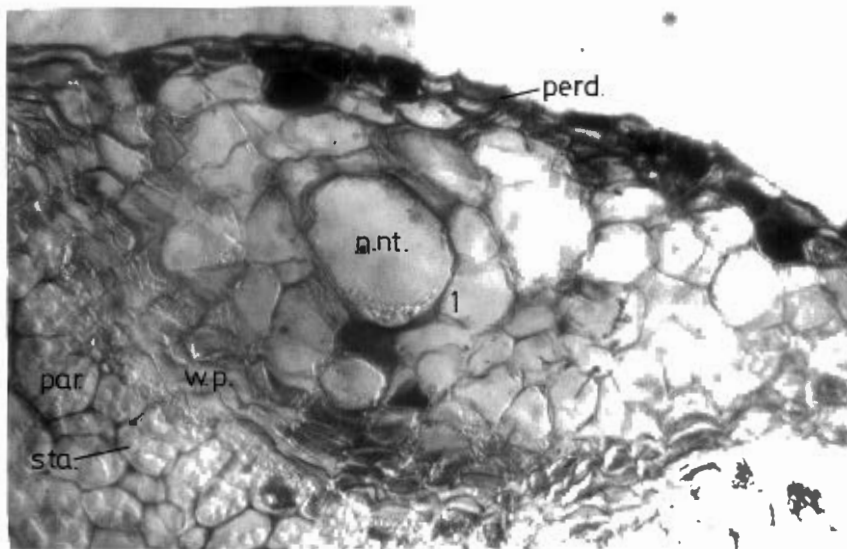


Fig. 132

Figs. 133-135: Steen. A series of photomicrographs of a cross-section, of a one-year-old root, attacked by *M. hapla*.

Fig. 133: Wound periderm (w.p.) protecting part of phloem (phl.) and xylem (x.) against nematode attacks. Lateral root (l.r.), however, is badly damaged; infected area (i.a.). X 125.

Fig. 134: Necrosis (nec.) in vascular ray (par. r.) and in primary xylem (pr. x.).

Note:

- (a) necrosis has caused a serious lesion or slit (sl.)
- (b) secondary xylem vessels (s.x.) on either side of necrosis, and pith cells (pth.) are apparently normal. X 330.

Fig. 135: Cross-section of one-year-old root, attacked by *M. hapla*, showing serious necrosis of primary xylem bundles (pr. x.); pith (pth.) cells apparently normal; xylem ray (par. r.); necrosis (nec.). X 130.

Fig. 133

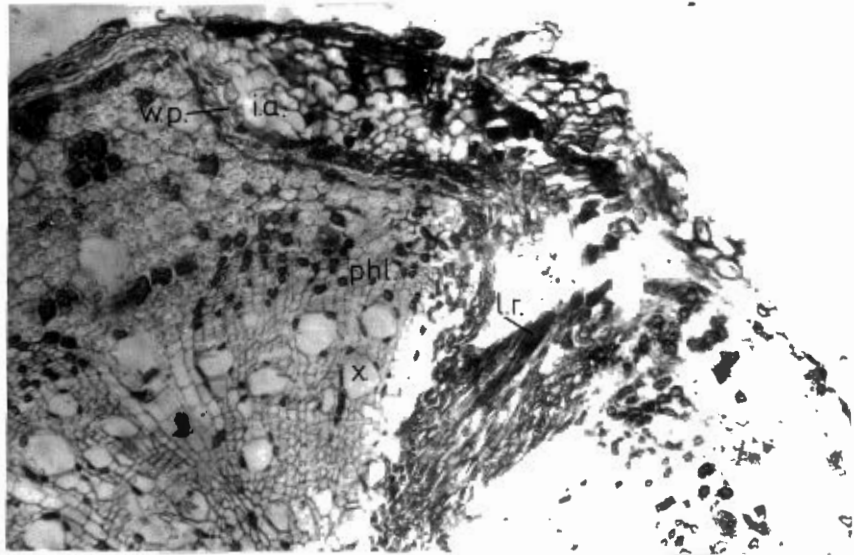


Fig. 134

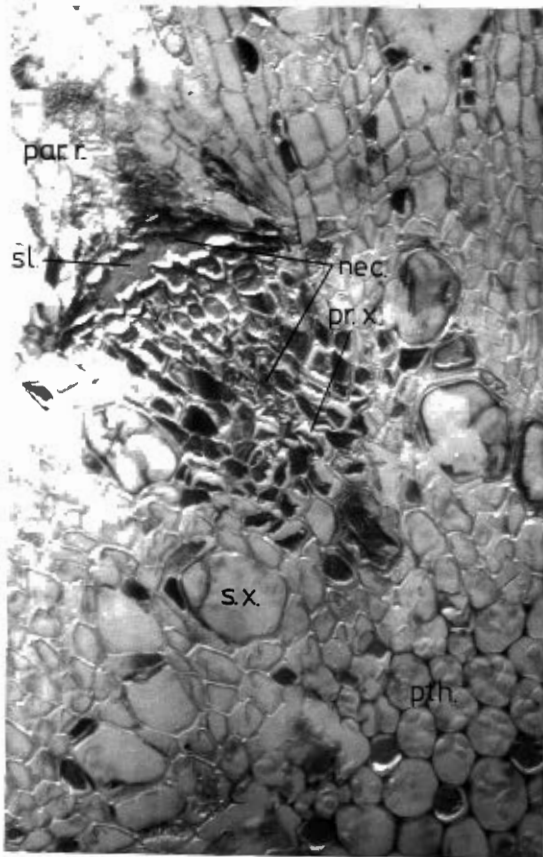
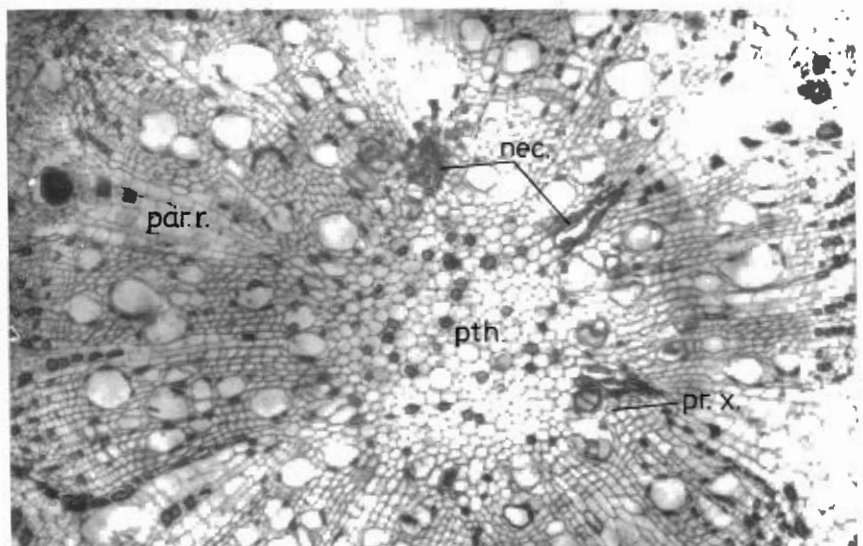


Fig. 135



Figs. 136-138: Steen. Series of photomicrographs of a cross-section of a one-year-old root, infected by *M. hapla*. Discoloured elements of a lateral root (l.r.) visible in all three Figures. X 250.

Fig. 136: Wound periderm (w.p.) (*upper left*); obliteration (ob.) in phloem (*upper left*); necrosis (nec.); lateral root (l.r.).

Fig. 137: Necrosis (nec.) of tracheary elements (tra.) in base of lateral root (l.r.). Note xylem (x.) elements of main root on either side of lateral root.

Fig. 138: Necrosis (nec.) of primary xylem tissue of main root below base of lateral root; pith (pth.) cells, apparently normal, contain starch (sta.) and tannin (tan.); primary xylem (pr. x.).

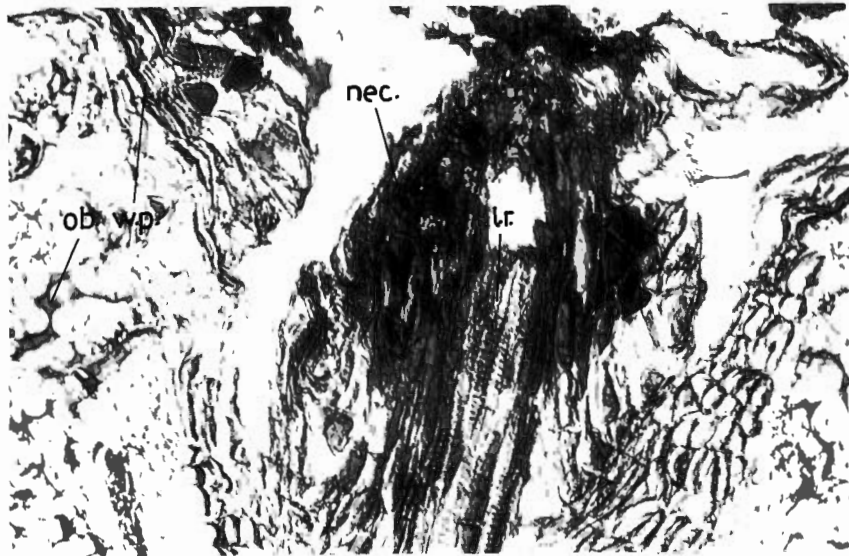


Fig. 136

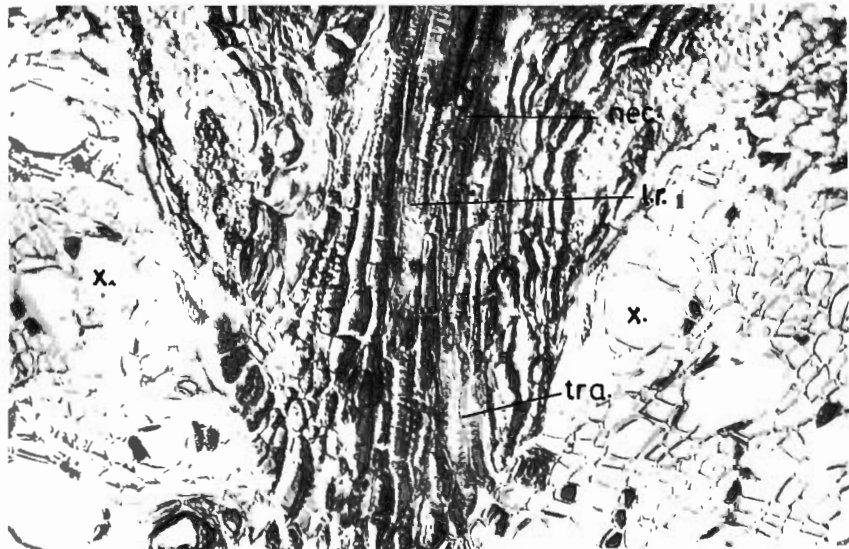


Fig. 137

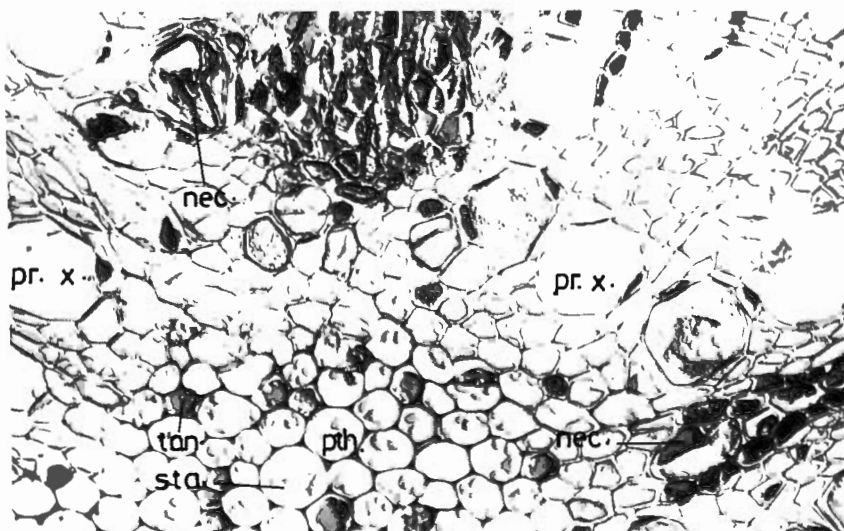


Fig. 138

Figs. 139-141: 99 R. A series of photomicrographs of a cross-section of an infected one-year-old root, showing necrosis (nec.) of lateral root (l.r.). Necrosis ends at base of lateral root. Secondary xylem (s.x.) of main root is visible on both sides of lateral root; pericycle (perc.); tannin (tan.). X 250.

Fig. 142: 99 R. An enlargement of part of Fig. 141. Figure 1 in Figs. 141 and 142 corresponds. Note nuclei (n.) and tannin (tan.) in xylem parenchyma cells; tracheary elements (tra.). X 800.

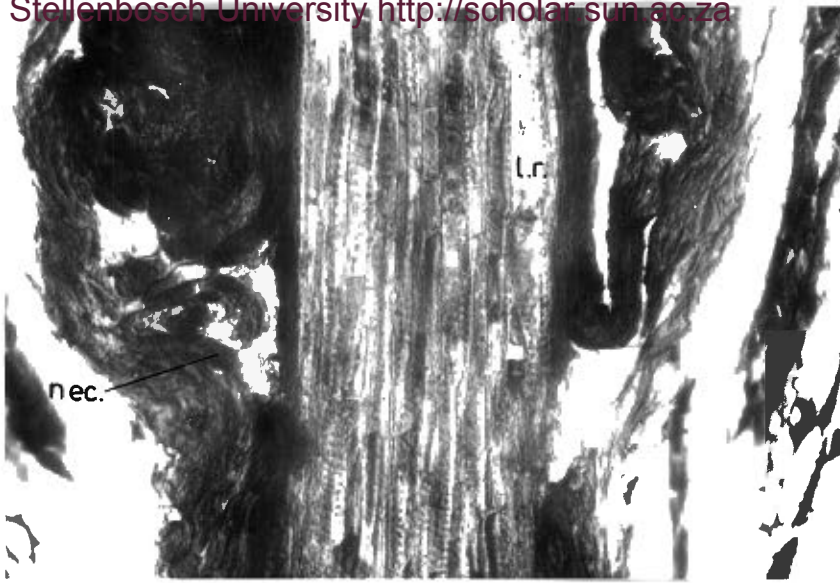


Fig. 139

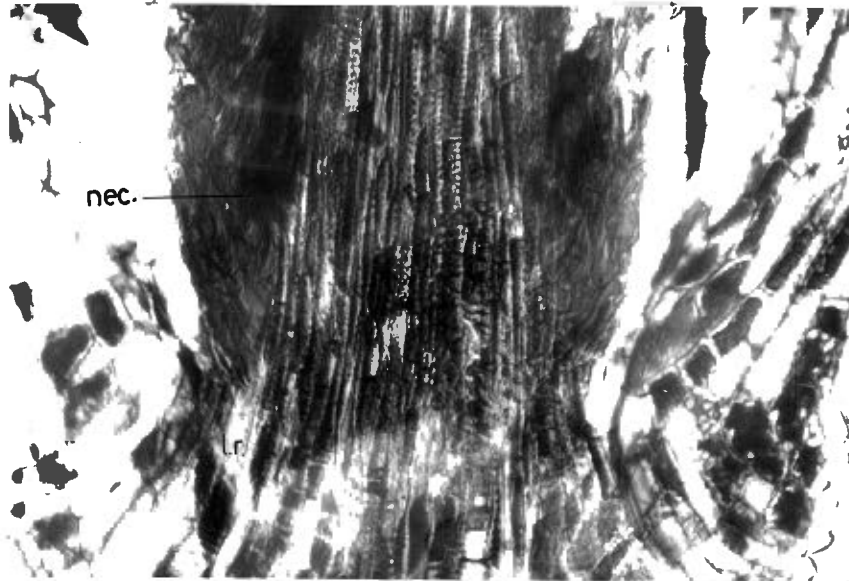


Fig. 140

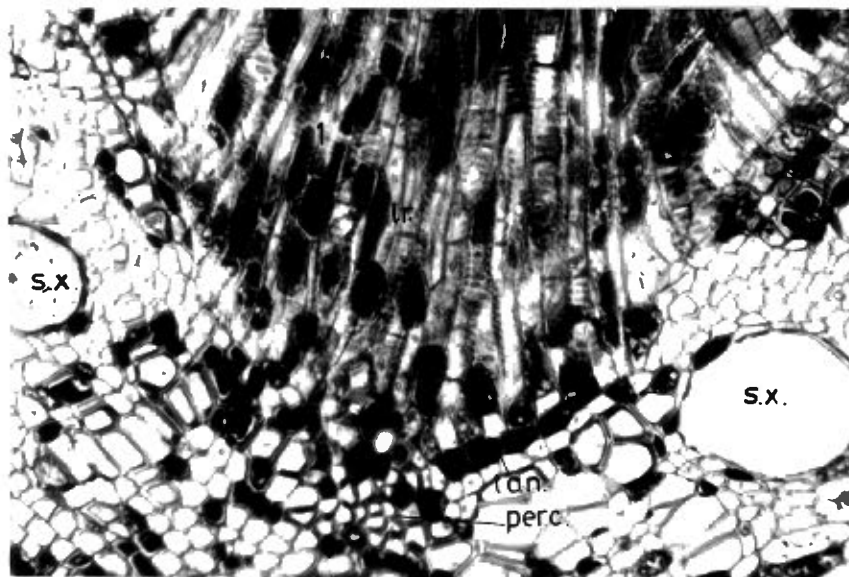


Fig. 141

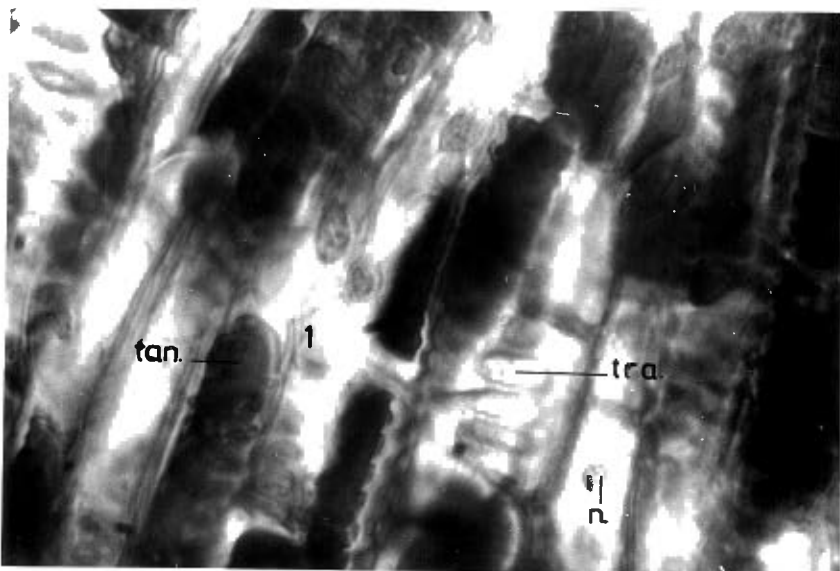


Fig. 142

Fig. 143: 99 R. Cross-section of two-year-old root, infected with *M. hapla* at beginning of second year, showing dead and deformed female nematodes (d.n.f.) enclosed between the first and second periderms (s.p.). Note phloem bundle (phl.), dilated phloem ray (phl. r.) and xylem rays (par. r.) are apparently normal; first periderm (perd.); xylem (x.). X 90.

Fig. 144: 99 R. Enlargement of a cross-section of two-year-old root, infected as in Fig. 143, showing remains of nematodes (d.n.f.) enclosed between outer periderm (perd.) and second periderm (s.p.); pericycle (perc.). X 235.

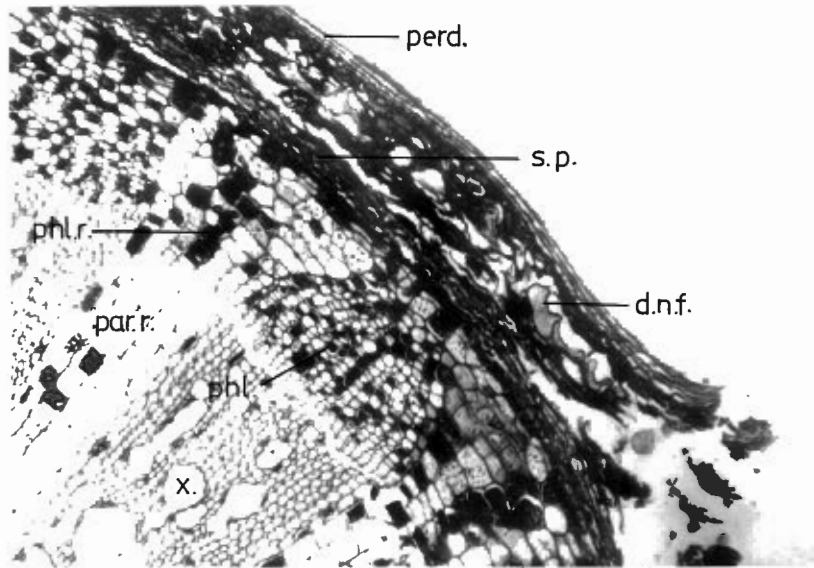


Fig. 143

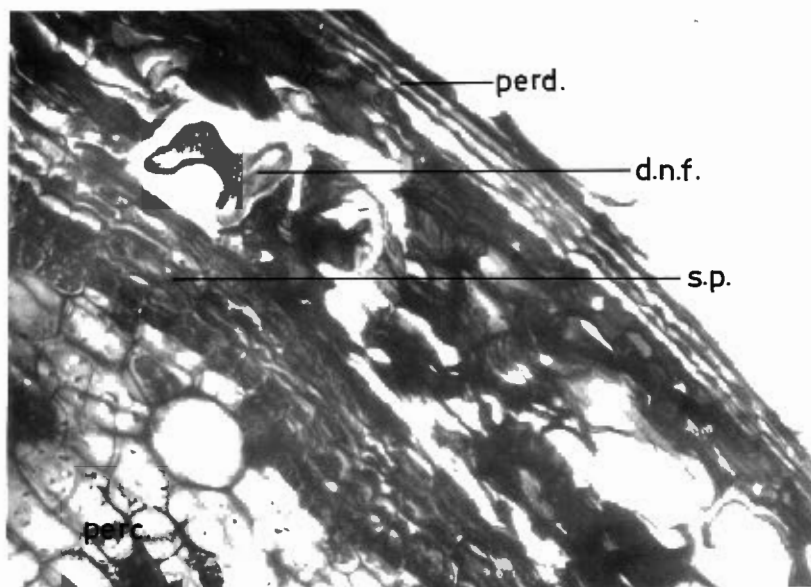


Fig. 144

Fig. 145: 99 R. Cross-section of two-year-old root, infected with *M. hapla* as in Fig. 143, showing periderm (perd.); proliferated pericycle (p. perc.) cut off by wound periderm (w.p.); muriform phloem parenchyma tissue (m.t.); vascular cambium (cam.); phloem (phl.).
X 90.

Fig. 146: 99 R. Cross-section showing an enlargement of muriform parenchyma tissue (m.t.), which is the youngest secondary phloem, formed by vascular cambium (cam.). Nuclei (n.) just visible in vascular cambium and phloem parenchyma; wound periderm (w.p.). X 250.

Fig. 147: 99 R. Enlargement of muriform tissue shown in Fig. 146. Figure 1 in Figs. 146 and 147 corresponds. Note nuclei (n.) and nucleoli (n.n.) are clearly visible; tannin (tan.). X 770.

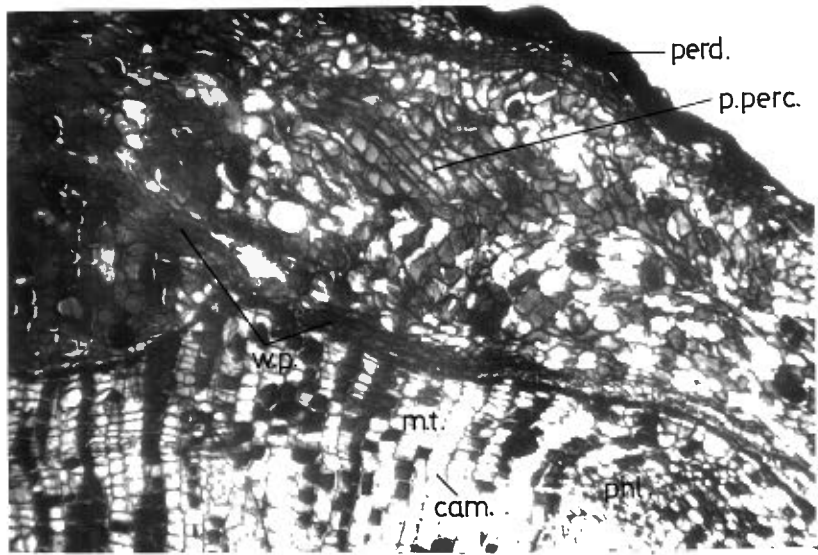


Fig. 145

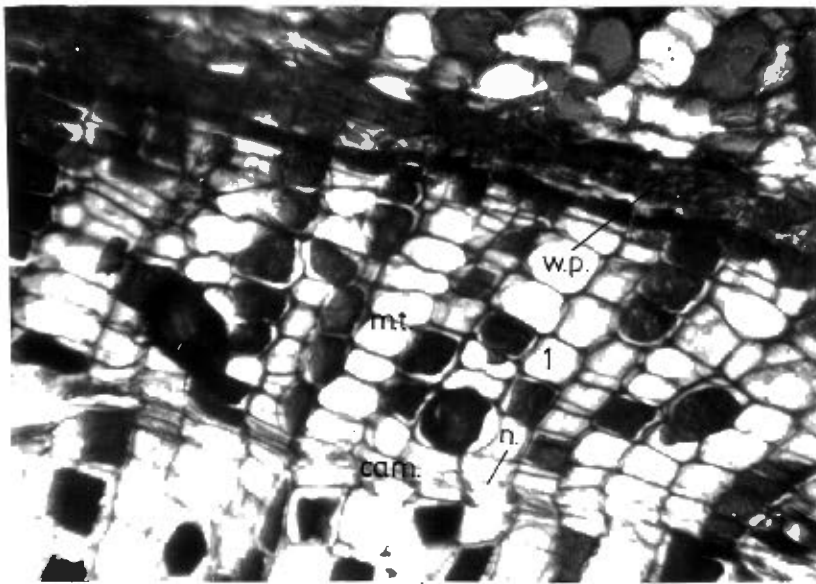


Fig. 146

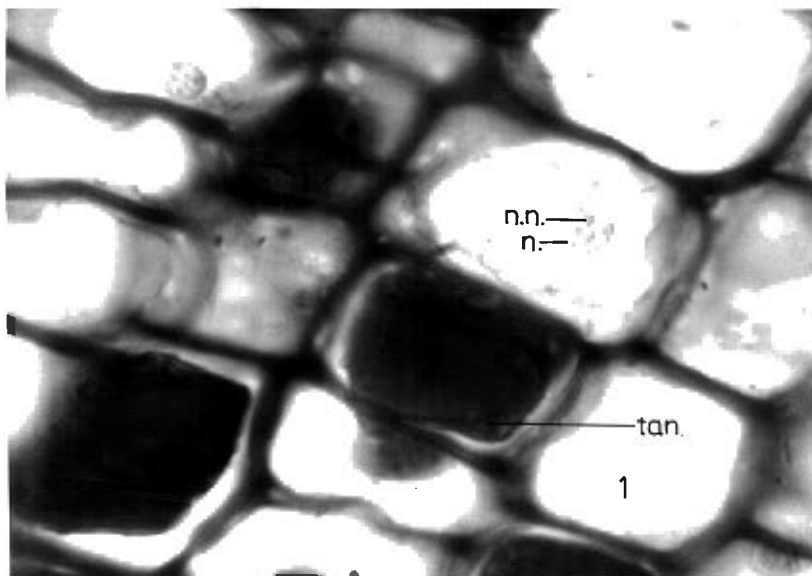


Fig. 147

Figs. 148-150: 99 R: Photomicrograph of a cross-section, showing influence of *M. hapla* infection on vascular cambium (cam.) of a two-year-old root, exposed to infection at the beginning of the second year.

Fig. 148: Secondary phloem (s. phl.) with tannin (tan.) containing phloem parenchyma. Compressed vascular cambium (cam.); secondary xylem (s.x.). X 290.

Fig. 149: Enlargement of compressed vascular cambium (cam.) cells. Figure 1 in Figs. 148 and 149 corresponds; secondary xylem (s.x.); nuclei (n.). X 1 360.

Fig. 150: A further enlargement of compressed vascular cambium cell (cam.) with distorted radial cell-wall (r.w.). Note nuclei (n.) and nucleoli (n.n.) in cambium (cam.); tannin (tan.). X 2 430.

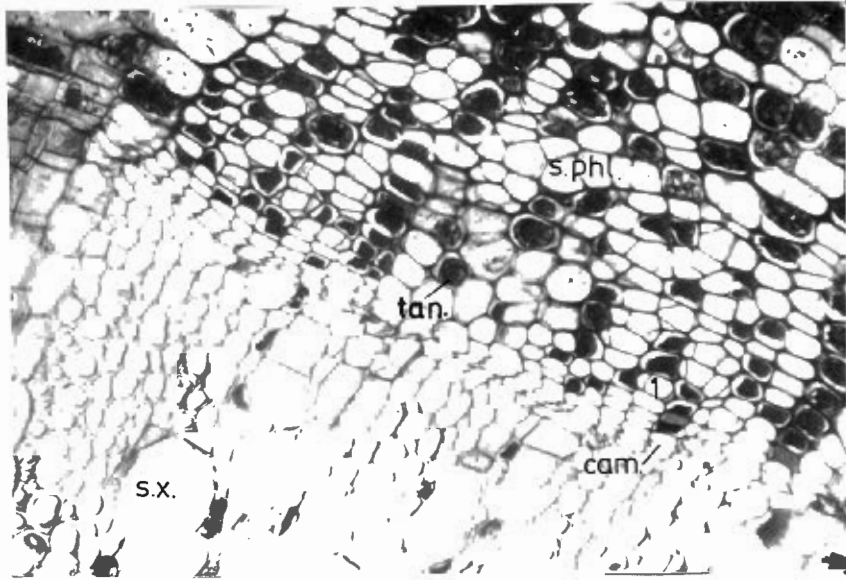


Fig. 148

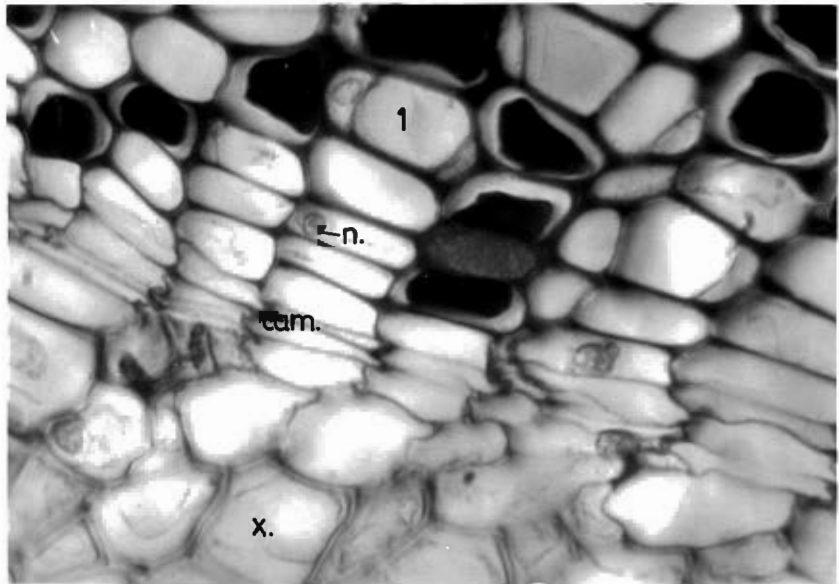


Fig. 149

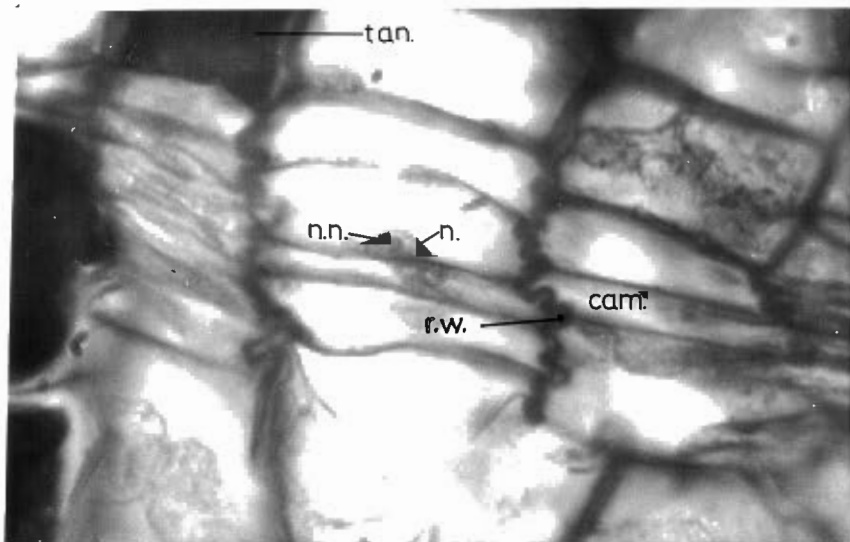


Fig. 150

Figs. 151-154: 99 R. Two-year-old root infected with *M. hapla* at beginning of second year. Cross-sections through a lesion or slit, in the summer wood of the first annual ring and spring wood of the second annual ring. The sections shown in Figs. 151 and 154 are almost 100µm apart.

Section shown in Fig. 154 is cut through a slit (sl.). Tyloses (ty.) and gums (gu.) occur in the vessels; xylem ray (par. r.); xylem (x.); perforation plate (pf.) in tangentially elongated vessel; tannin (tan.); necrosis (nec.). X 290.

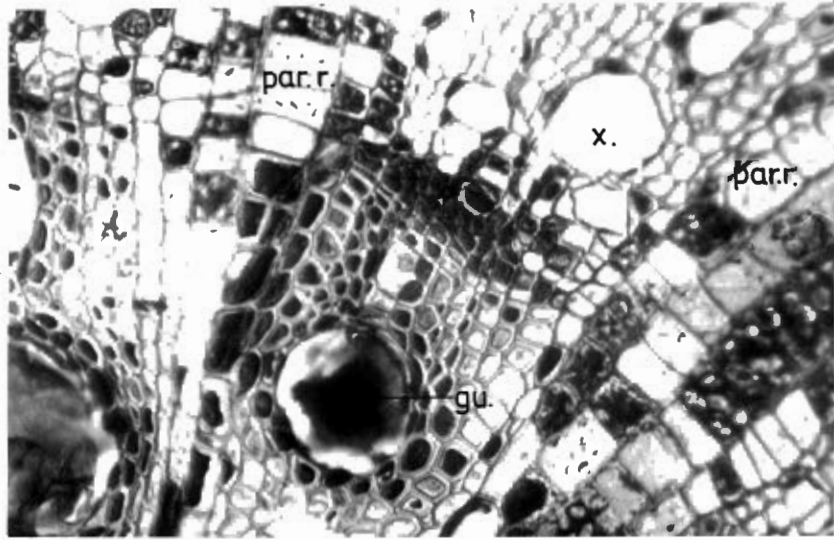


Fig. 151

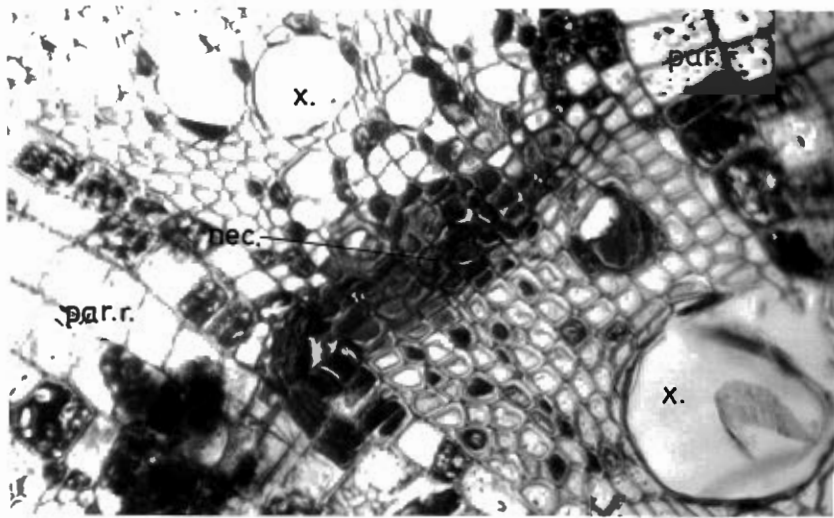


Fig. 152

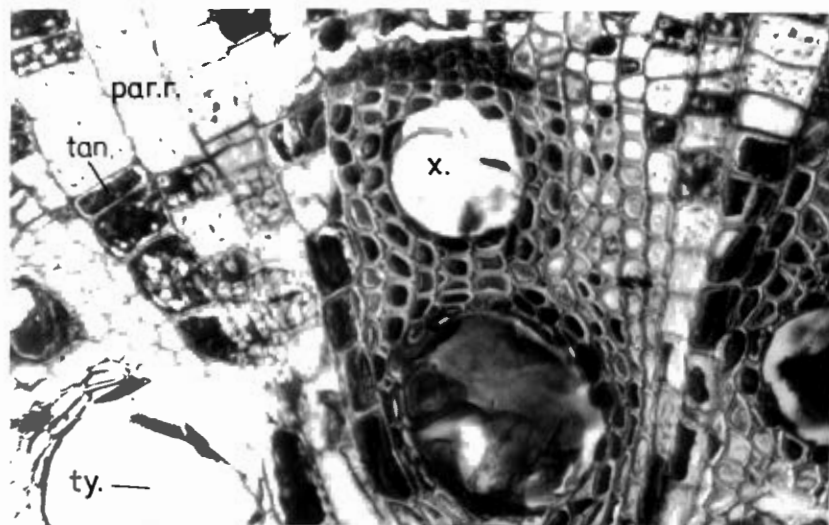


Fig. 153

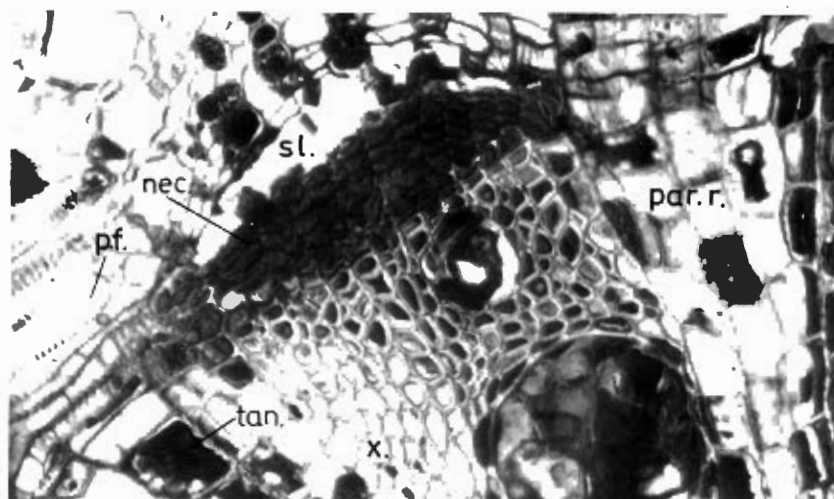


Fig. 154

Fig. 155: 99 R. Cross-section showing two-year-old root, infected with *M. hapla* at beginning of the second year.

Note:

- (a) pith (pth.) cells apparently normal
- (b) primary xylem (pr. x.) cells are slightly necrotic
- (c) secondary xylem (s.x.) vessels apparently normal; vascular ray (par. r.). X 290.

Fig. 156: 99 R. Cross-section of two-year-old root, infected with *M. hapla* as in Fig. 155, showing three vessels occluded with tyloses (ty.); vascular ray (par. r.); pith (pth.); xylem (x.). X 290.

Fig. 157: Salt Creek. Cross-section of two-year-old root, infected with *M. hapla* as in Fig. 155, showing:

- (a) the summer wood of the first annual ring pressed into a vessel causing partial occlusion (p.o.)
- (b) vessel (*lower right*) filled with tyloses (ty.)
- (c) vascular ray (par. r.) (*lower left*) apparently normal; secondary xylem (s.x.). X 360.

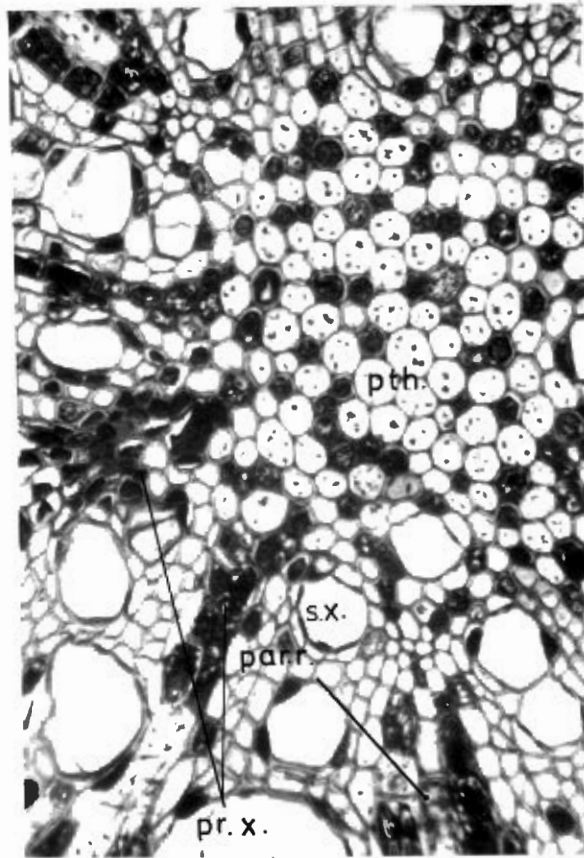


Fig. 155.

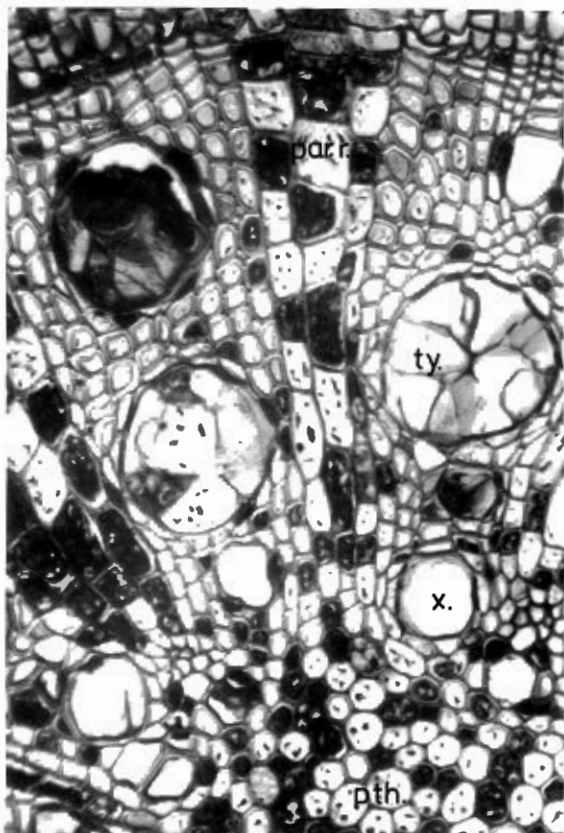


Fig. 156

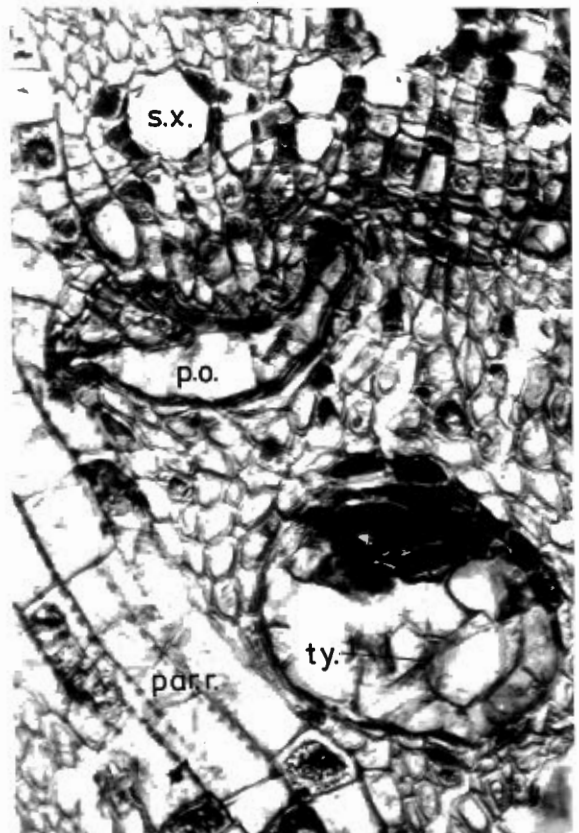


Fig. 157

Fig. 158: 1202 C. Radial longitudinal section of two-year-old root, infected at the beginning of the second year with *M. hapla*, showing large number of tyloses (ty.) in vessel. Tannin (tan.) in xylem parenchyma. X 530.

Fig. 159: 1202 C. Enlargement of a radial longitudinal section, through a xylem vessel (x.) of a two-year-old root, infected as in Fig. 158, showing tyloses (ty.) in vessel (x.); pith membrane (p.m.) and xylem parenchyma (par.) cell. X 760.

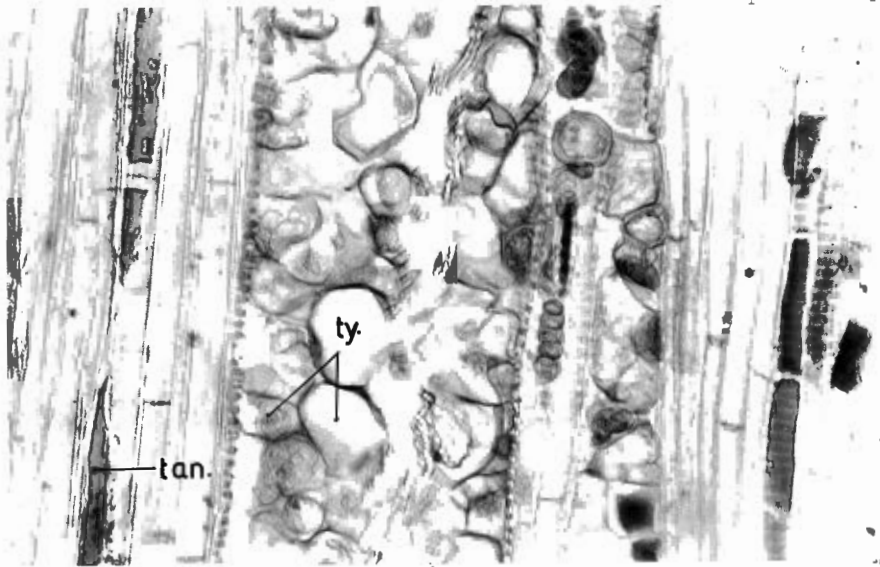


Fig. 158

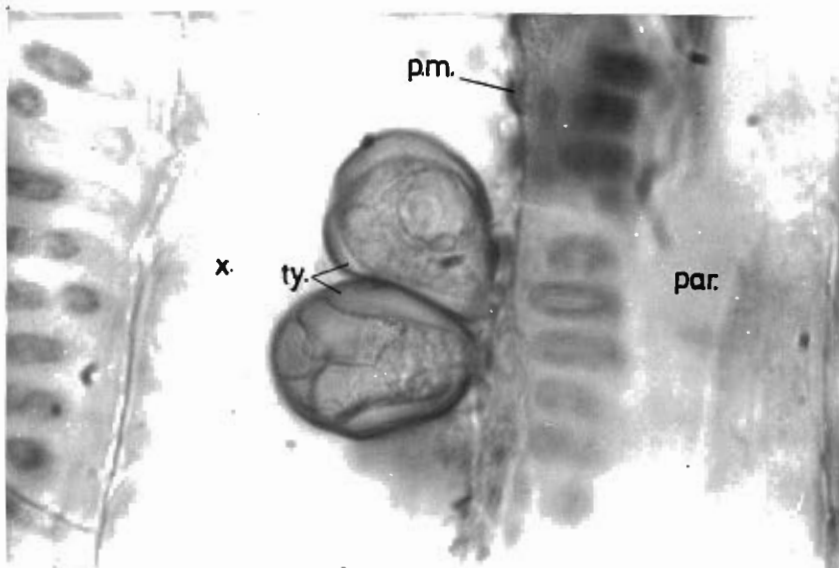


Fig. 159

Figs. 160-161: 99 R. Two cross-sections through a lesion of two-year-old root, infected with *M. hapla*, at the beginning of the second year, showing:

- (a) abnormal tangentially elongated xylem vessels in the vascular ray (par. r.); scalariform tracheid (sc. t.) (Fig. 160); tyloses (ty.) (Fig. 161); perforation plates (p.f.) (Fig. 161)
- (b) tearing of xylem (x.) tissue between first and second annual ring (Fig. 161). X 290.

Fig. 162: Dogridge. Cross-section of two-year-old root, infected with *M. hapla* as in Figs. 160-161, showing:
Abnormal xylem (ab. x.) and phloem (ab. phl.) elements. tangentially elongated opposite necrotic mass (nec.); phloem (phl.); wound periderm (w.p.); muriform parenchyma tissue (m.t.) in phloem ray; vascular ray (par. r.); xylem (x.). X 290.

Fig. 163. Dogridge. An enlargement of part of Fig. 162. Figure 1 in Figs. 162 and 163 corresponds.
Abnormal xylem elements (ab. x.) running tangentially opposite necrotic mass (nec.); xylem (x.); vascular ray (par. r.); tannin (tan.) in parenchyma cells.
X 290.

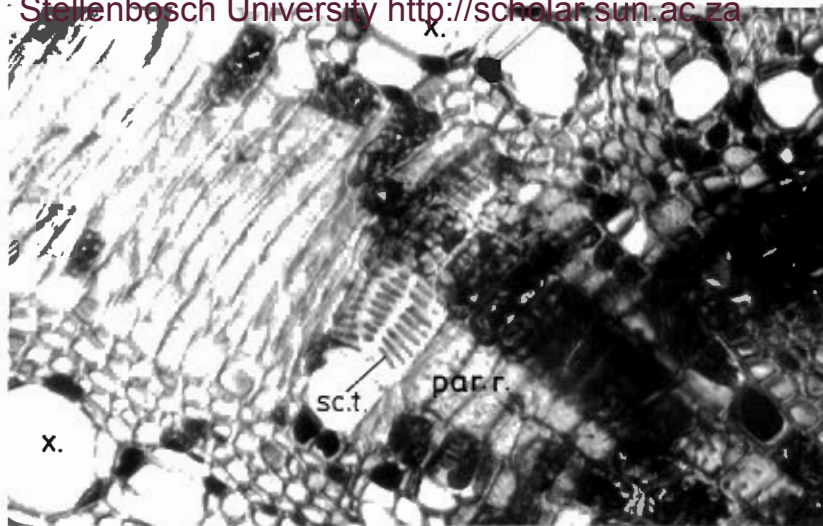


Fig. 160

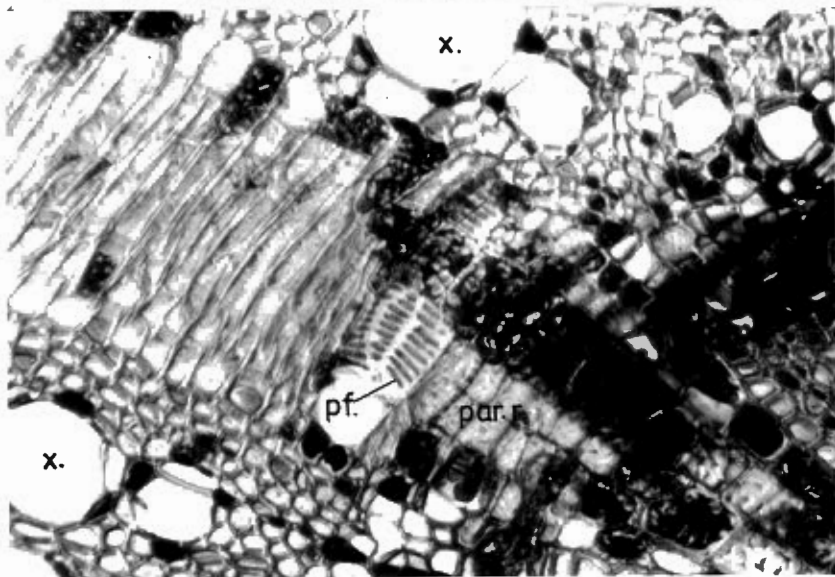


Fig. 161

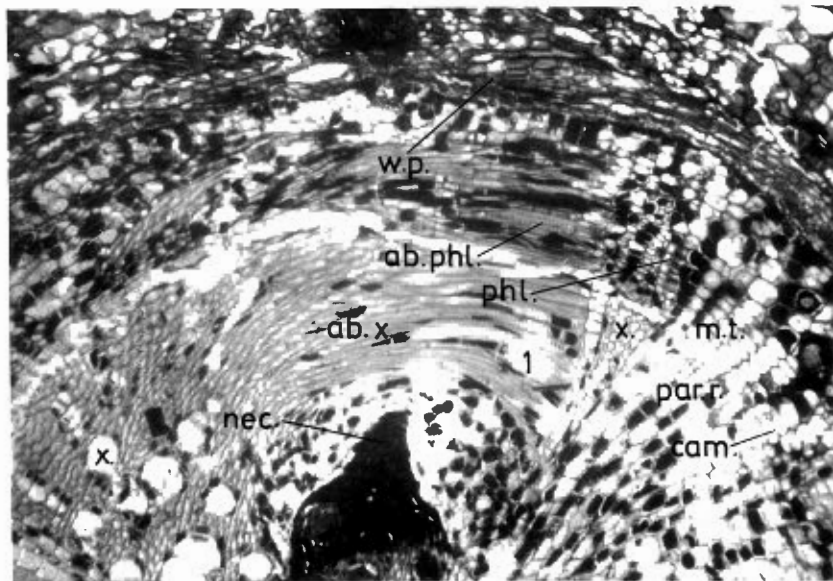


Fig. 162

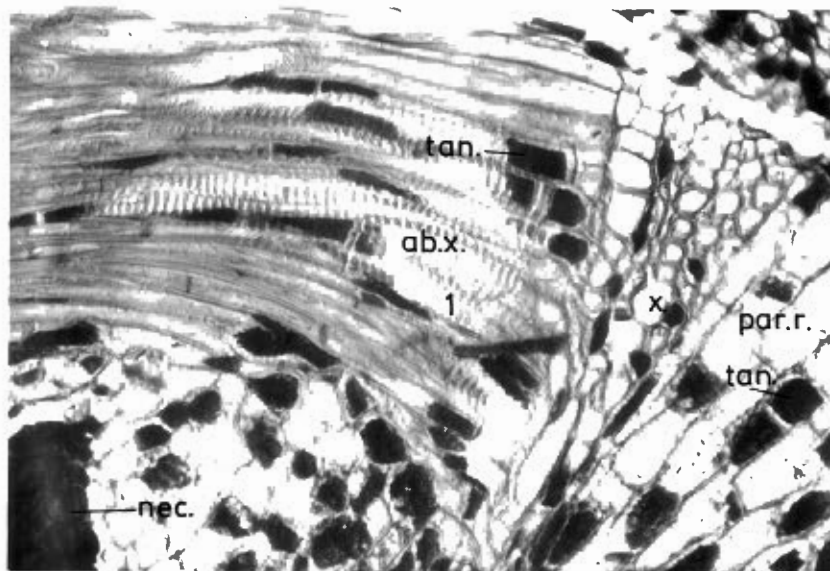


Fig. 163

Fig. 164: Dogridge. Cross-section of two-year-old root, infected as in Figs. 160-161. Close-up view of necrosis as seen in Fig. 162. In the immediate vicinity of the necrosis (nec.) some of the vessels are also occluded with coloured tyloses (ty.), but further away they are apparently normal; vascular ray (par. r.); xylem (x.). X 290.

Fig. 165: Dogridge. Cross-section of two-year-old root, infected as in Figs. 160-161. Close-up view of xylem on both sides of necrotic mass (nec.) and torn tissue in the secondary xylem (x.).

Note:

- (a) the upper part of the photomicrograph points towards the vascular cambial region, and shows a large mass of undifferentiated xylem parenchyma tissue (ab. p.) in the second annual ring, but no tracheary elements
- (b) the lower part of the photomicrograph shows the first annual ring which is apparently normal; tyloses (ty.). X 290.

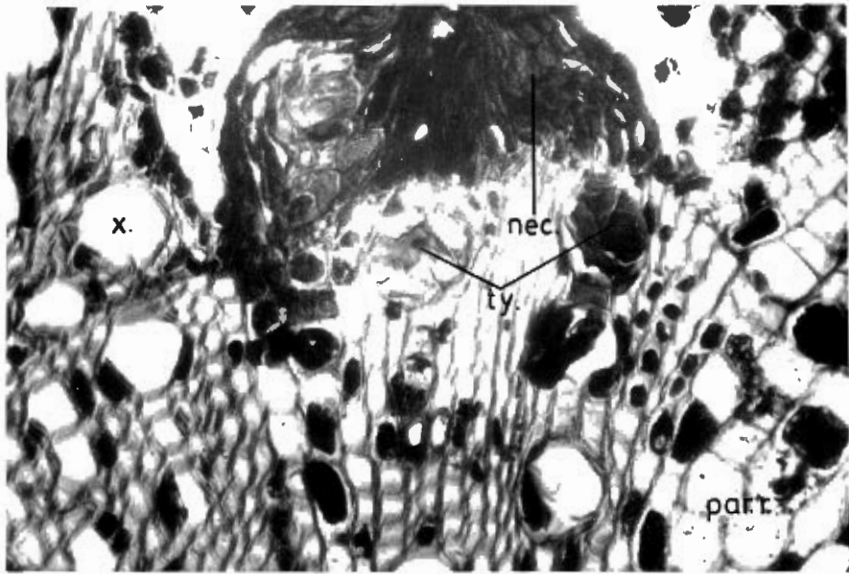


Fig. 164



Fig. 165

FIGURE INDEX

(Numbers indicate pages bearing photomicrographs of cultivars concerned)

1. Dogridge 92, 96, 98, 157, 193, 195.
2. Jacquez 82, 86, 88, 102, 104, 106, 108, 110, 112,
114, 116, 118, 124, 126, 130, 132, 155, 163,
165, 167, 169, 171.
3. Salt Creek 124, 157, 189.
4. Steen 90, 94, 151, 153, 174, 175, 177.
5. White French 82, 84, 86, 120, 122, 126, 128, 130, 132,
134, 137, 139, 141, 143, 145, 147, 149.
6. 99 R 88, 90, 100, 157, 159, 179, 181, 183, 185,
187, 189, 193.
7. 1202 C 94, 96, 128, 132, 134, 161, 191.

= = = = =

LITERATURE CITED

- Abesadze, K.J., Makarevskaja, E.E., Zchakaja, K.E., 1930. Über die verschiedenen Grade der Widerstandfähigkeit gegen Reblaus allgemein verbreiteter georgischer Rebsorten, bedingt durch die Unterschiede in der anatomischen Struktur ihrer Wurzeln. Sci. papers of the applied Sect. of the Tiflis Bot. Gdns. 8: 147-154.
- Akai, S., 1959. Histology in defense plants, p.391-434. In Horsfold & Dimond. Plant Pathology. New York and London: Academic Press.
- Alexandrov, W.G., 1926. Von den Eigenheiten in der Lage der Kristalle und Eiweiss enthaltenden Zellen in den Wurzeln und Stengeln der Weinrebe. Bot. Arch. 14: 461-466.
- Allen, M.W., 1952a. Observations on the genus *Meloidogyne* Goeldi 1887. Proc. helminth. Soc. Wash. 19: 44-51.
- Allen, M.W., 1952b. Root-knot and root lesion nematodes. California Agriculture 6(5): 8.
- Anders, F., 1955. Zur biologischen Charakterisierung der galleninduzierenden substanz aus dem Speicheldrusen sekret der Reblaus (*Viteus (Phylloxera) vitifolii* Shimer). Verh. dt. zool. Ges. Erlangen. 421-428.
- Anders, F., 1957a. Über gallenerregenden Agenzien der Reblaus (*Viteus (Phylloxera) vitifolii* Shimer). Vitis 1: 121-124.
- Anders, F., 1957b. Neure Auffassungen über die Reblaus-Resistenz. Vitis 1: 142-152.
- Anders, F., 1958. Das galleninduzierende Prinzip der Reblaus (*Viteus vitifolii* Shimer). Verh. dt. zool. Ges., Frankfurt: 355-363.
- Anders, F., 1960a. Untersuchungen über das cecidogene Prinzip der Reblaus (*Viteus vitifolii* Shimer), I. Biol. Zbl. 79: 47-58.
- Anders, F., 1960b. Untersuchungen über das cecidogene Prinzip der Reblaus (*Viteus vitifolii* Shimer), II. Biol. Zbl. 79: 679-700.
- Anders, F., 1961. Untersuchungen über das cecidogene Prinzip der Reblaus (*Vitis vitifolii* Shimer), III. Biol. Zbl. 80: 199-233.
- Baranov, P.A., 1946. Strojenje vinogradnoj lozi. Ampel. SSSR. I: 217-346.
- Barrons, K.C., 1939. Studies of the nature of root-knot resistance. Journ. Agric. Res. 58: 263-271.

- Baxter, R.I. & Blake, C.D., 1968. *Pratylenchus thornei* - A cause of root necrosis in wheat. *Nematology* 14: 351-361.
- Becker, H., 1957. Untersuchungen über den Befall von Unterlagreben durch die Reblaus. *Verhandl. d. IV. Int. Pflsch. Kongr. Hmb.* 1: 783-785.
- Beckman, C.H., 1966. Cell irritability of localization of vascular infections in plants. *Phytopathology* 56(7): 821-824.
- Bergman, B.H.H., 1958. Het beitecystenaaltje en zijn bestrijding. V. Enige microscopische waarnemingen betreffende de ontwikkeling van larven *Heterodera schachtii* in de wortels van vatbare en resistente planten. *Mededel. Inst. Rationele Suikerprod.* 28: 151-169.
- Bessey, E.A., 1911. Root-knot and its control. *Bull. U.S. Bur. Plant Ind.* No. 217: 89 pp.
- Birchfield, W., 1962. Host-parasite relations of *Rotylenchus reniformis* on *Gossypium hirsutum*. *Phytopathology* 52: 862-865.
- Birchfield, W., 1964. Histopathology of nematode-induced galls of *Echinochloa coluum*. *Phytopathology* 54: 888 (Abstr.).
- Birchfield, W. & Jones, J.E., 1966. A new cotton variety with root-knot nematode and *Fusarium* wilt resistance. *Phytopathology* 56(8): 871. (Abstr.).
- Bird, A.F., 1960. Additional notes on the attractiveness of roots to plant parasitic nematodes. *Nematologica* 5: 217.
- Bird, A.F., 1961. The ultrastructure and histo-chemistry of nematode induced giant cells. *J. Biophys. Biochem. Cytol.* 11: 701-715.
- Bird, A.F., 1962a. The inducement of giant cells by *Meloidogyne javanica*. *Nematologica* 8: 1-10.
- Bird, A.F., 1962b. Orientation of the larvae of *M. javanica* relative to roots. *Nematologica* 8: 275-285.
- Bird, A.F. & Millerd, A., 1962. Respiration studies on *Meloidogyne*-induced galls in tomato roots. *Nematologica* 8: 261-266.
- Blake, C.D., 1966. The histological changes in banana roots caused by *Radophobus similis* and *Helicotylenchus multicinctus*. *Nematologica* 12: 129-137.
- Blair, G.P. & Darling, H.M., 1968. Red ring disease of the coconut palm, inoculation studies and histopathology. *Nematologica* 14: 395-403.
- Bloch, R., 1941. Wound healing in higher plants. *Botanical Review* 7: 110.

- Boubals, D., 1954. Les nematodes parasites de la vigne. *Progrès agric. vitic.* 141: 173-182; 204-228.
- Bowman, P. & Bloom, J.R., 1966. Breaking the resistance of tomato varieties to *Fusarium* wilt by *Meloidogyne incognita* (Abstr.). *Phytopathology* 56(8): 871.
- Breider, H. & Husfeld, B., 1938. Die Schädigung der Rebe durch die radicole Form der Reblaus (*Phylloxera vastatrix*). *Gartenbauwissenschaft* 12: 41-69.
- Britz, C.J., 1968. 'n Anatomiese studie van *Vitis* wortels gesond en beskadig deur filloksera. Ongep. M.Sc.-thesis, Universiteit van Stellenbosch.
- Brodie, B.B. & Cooper, W.E., 1964a. Pathogenicity of certain parasitic nematodes on upland cotton seedlings. *Phytopathology* 54: 1019-1022.
- Brodie, B.B. & Cooper, W.E., 1964b. Relation of parasitic nematodes to postemergence damping-off of cotton. *Phytopathology* 54: 1023-1027.
- Bruhn, C. & Koch, W., 1962. Beobachtungen über den Befall mittelamerikanischer Dioscoreenarten durch *Meloidogyne incognita* (Kofoid & White). *Nematologica* 8(4): 307-312.
- Burdett, J.F., Bird, A.F. & Fischer, J.M., 1963. The growth of *Meloidogyne* in *Prunus persica*. *Nematologica* 9: 542-546.
- Caveness, F.E. & Panzer, J.D., 1960. Nemic galvanotaxis. *Proc. helminth. Soc. Wash.* 35: 241-250.
- Christie, J.R., 1936. The development of root-knot galls. *Phytopathology* 26(1): 1-22.
- Christie, J.R., 1949. Host-parasite relationships of the root-knot nematodes, *Meloidogyne* spp. III. The nature of resistance in plants to root-knot. *Proc. helminth. Soc. Wash.* 16: 104-108.
- Cohn, E., 1964a. Host response to the penetration of the citrus nematode, *Tylenchulus semipenetrans* Cobb. *Nematologica* 10: 77-78.
- Cohn, E., 1964b. Penetration of the citrus nematode in relation to root development. *Nematologica* 10: 594-600.
- Cohn, E., 1965. On the feeding and histopathology of the citrus nematode. *Nematologica* 11: 47-54.
- Coombe, B.G., 1963. Phylloxera and its relation to South Australian viticulture. *Tech. Bull. Dep. Agric. S. Aust.* 31.

- Cornu, M., 1878. Études sur la *Phylloxera vastatrix*. Mémoires de l'Académie des Sciences, Paris XXVI(1): 146-150.
- Crittenden, H.W., 1962. Effect of *Meloidogyne incognita acrita* in nodulating and non-nodulating strains of soybean. *Phytopathology* 52: 163. (Abstr.).
- Daulton, R.A.C. & Nusbaum, C.F., 1961. The effect of soil temperature on the survival of the root-knot nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica* 6: 280-294.
- Davide, R.G. & Triantaphyllou, A.C., 1965. Influence of foliar applications of maleic hydrazide on various host plants upon sex differentiation in *Meloidogyne javanica* and *M. incognita*. *Nematologica* 11: 35. (Abstr.).
- Davis, R.A. & Jenkins, W.R., 1960. Histopathology of gardenia (*Gardenia jasminoides* Veitchi), infected with three species of *Meloidogyne*. *Nematologica* 5: 228-230.
- Dean, J.L. & Struble, F.B., 1953. Resistance and susceptibility to root-knot nematodes in tomato and sweet-potato. *Phytopathology* 43: 290. (Abstr.).
- Despeissis, A., 1895. Phylloxera of the vine. J. Dept. Agric. Res. South Wales.
- Dropkin, V.H., 1959. Varietal response of soybeans to *Meloidogyne* a bioassay system for separating races of root-knot nematodes. *Phytopathology* 49: 18-23.
- Dropkin, V.H. & Nelson, P.E., 1960. The histopathology of root-knot nematode infections in soybeans. *Phytopathology* 50: 442-447.
- Dropkin, V.H. & Boone, W.R., 1966. Analysis of host-parasite relationships of root-knot nematodes by single-larva inoculations of excised tomato roots. *Nematologica* 12: 225-236.
- Dropkin, V.H. & Webb, R.E., 1967. Resistance of Axenic tomato seedlings to *Meloidogyne incognita acrita* and *M. hapla*. *Phytopathology* 57: 584-587.
- Dropkin, V.H., 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. *Phytopathology* 59: 1632-1637.
- DuCharme, E.P., 1959. Morphogenesis and histopathology of lesions induced on citrus roots by *Radopholus similis*. *Phytopathology* 49: 388-395.
- Eames, A.J. & MacDaniels, L.H., 1947. An introduction to plant anatomy. (2nd ed.). New York: McGraw-Hill.

- Endo, B.Y., 1964. Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. *Phytopathology* 54: 79-88.
- Endo, B.Y., 1965. Histological responses of resistant and susceptible soybean varieties and back-cross progeny to entry and development of *Heterodera glycines*. *Phytopathology* 55(4): 375-381.
- Esau, K., 1933. Pathologic changes in the anatomy of leaves of sugar beet, *Beta vulgaris* L., affected by curly top. *Phytopathology* 23: 679-712.
- Esau, K., 1948a. Phloem structure in the grape vine, and its seasonal changes. *Hilgardia* 18(9): 217-296.
- Esau, K., 1948b. Anatomic effects of the viruses of Pierce's disease and phony peach. *Hilgardia* 18(12): 423-461.
- Eversmeyer, H.E. & Dickerson, O.J., 1966. Histopathology of root-knot nematode infected peony roots. *Phytopathology* 56: 816-820.
- Fairbrother, R.W., 1943. A textbook of bacteriology. London: William Heinemann, Medical Books, Ltd.
- Feder, W.A. & Feldmesser, J., 1961. The spreading decline complex: the separate and combined effects of *Fusarium* spp. and *Radopholus similis* on growth of Duncan grapefruit seedlings in the greenhouse. *Phytopathology* 51: 724-726.
- Flerov, A.F. & Kovalenko, E., 1952. Anatomiseskije izmenijaije tkanjah tserenkov vinogradnoj lozi pod vlijanjiem alfanaftalikusnoj kisloti. *Dan. S.S.S.R.* 85: 433-436.
- Foëx, G., 1877. Deuxième note relative aux efforts produit par le phylloxera sur les racines de divers cãpages americans et indigenẽers. *Rev. de Viticulture* 84: 115-127.
- Frazier, W.A. & Dennett, R.K., 1949. Isolation of *Lycopersicon esculentum* type tomato lines essentially homozygous resistant to root-knot. *Proc. Am. Soc. Hort. Si.* 54: 225-236.
- Galet, P., 1956. Cãpages et Vignobles de France. 1. Montpellier: Paul Dehan.
- Gardner, M.W., 1925. Hyperplastic crushing of the tracheal tubes in mosaic tomato stems. *Phytopathology* 15: 759-761.
- Giamalva, M.J., Martin, W.J. & Hernandez, T.P., 1963. Sweet-potato varietal reaction to species and races of root-knot nematodes. *Phytopathology* 53: 1187-1189.

- Gilbert, J.C. & McGuire, D.C., 1952. Root-knot resistance in commercial type tomatoes in Hawaii. Proc. Am. Soc. Hort. Sci. 60: 401-411.
- Godfrey, G.H. & Oliveira, J.M., 1932. The development of the root-knot nematode in relation to root tissues of pineapple and cowpea. Phytopathology 22(4): 325-348.
- Goodey, T.B., 1948. The galls caused by *Anguillulina balsamophila* (Thorne) Goodey on the leaves of *Wyethia amplexicaulis* Nutt. and *Beilsamorhiza sagittata* Nutt. J. Helminthol. 22: 109-116.
- Guillon, J.M., 1905. Etude générale de la Vigne. Paris: Masson et Cie.
- Hegedüs, A., Kozma, P., Németh, M., 1966. A szőlő. Budapest: Akad. Kiadó.
- Hemmi, T., 1916. On a new canker disease of *Prunus yedoensis*, *P. mume* and other species caused by *Valsa japonica* Miyabe et Hemmi sp. n. J. Coll. Agr. Tōhoku Imp. Univ. 7: 257-319.
- Henke, O., 1958. Untersuchungen über die biochemischen Grundlagen der Reblausresistenz der Reben. Phytopathology Z. 32: 149-164.
- Hofmann, E.L., 1957. Die histologie der Nodositäten verschiedener Rebensorten bei Reblausbefall. Vitis 1: 125-141.
- Horsfall, J.G. & Dimond, A.E., 1959. Plant Pathology Vol. I. The diseased plant. New York & London: Academic Press.
- Huang, C.S., 1966. Host-parasite relationships of the root-knot nematode in edible ginger. Phytopathology 56: 755-759.
- Huang, C.S. & Maggenti, A.R., 1969a. Mitotic aberrations and nuclear changes of developing giant cells in *Vicia faba* caused by root-knot nematode, *Meloidogyne javanica*. Phytopathology 59: 447-455.
- Huang, C.S. & Maggenti, A.R., 1969b. Wall modifications in developing *Cucumis sativus*. Induced by root-knot nematode, *Meloidogyne javanica*. Phytopathology 59: 931-937.
- Jacob, H.E., 1947. Grape growing in California. Calif. Agric. Ext. Serv. Circ. No. 116.
- Jones, F.G.W., 1957. Resistance breaking biotypes of the potato root eelworm (*Heterodera rostochiensis* Woll.). Nematologica 2: 185-192.
- Jones, F.G.W., 1960. Some observations and reflections on host finding by plant nematodes. Meded. Landb. Hooges. Gent. 25: 1009-1024.

- Kirkpatrick, J.D., Mai, W.F. & Fischer, E.G., 1964. Effect of phosphorus and potassium nutrition of sour cherry on the soil population levels of five plant-parasitic nematodes. *Phytopathology* 54: 706-712.
- Kövessi, F., 1903. *Récherches biologiques sur l'autement de la Vigne*. *Rev. Gen. Bot.* 13: 1-23.
- Kroemer, K., 1923. *Organographie, Anatomie und Physiologie der Rebe*. In: Babo und Mach, *Handb. d. Weinbaues* 1: 256-316. Berlin: Paul Parey.
- Krusberg, L.R. & Nielsen, L.W., 1958. Pathogenesis of root-knot nematodes to the Porto Rico variety of sweet-potato. *Phytopathology* 48: 30-39.
- Krusberg, L.H., 1961. *Studies on the culturing and parasitism of plant*
- Krusberg, L.R. 1967. Influence of host physiology on nematode populations. *Phytopathology* 57: 653-655.
-
- Lavergne, G., 1901. *La Anguilula en Sud-América*. *Revista Chilena de Historia Natural. Valparaiso* 5(4): 85-91.
- Liao, S.C. & Dunlap, A.A., 1950. Arrested invasion of *Lycopersicum peruvianum* roots by the root-knot nematode. *Phytopathology* 40: 216-218.
- Lider, L.A., 1959. Nematode resistant root-stocks for California vineyards. *Division of Agric. Sc. Univ. Calif.*
- Linford, M.B., 1939. Attractiveness of roots and excised shoot tissues to certain nematodes. *Proc. helminth. Soc. Wash.* 6: 11-18.
- Littrell, R.H., 1966. Cellular responses of *Hibiscus esculentus* to *Meloidogyne incognita acrita*. *Phytopathology* 56(5): 540-544.
- Loewenberg, J.R., Sullivan, T. & Schuster, M.L., 1960. Gall induction by *Meloidogyne incognita* by surface feeding and factors affecting the behaviour pattern of the second stage larvae. *Phytopathology* 50: 322-323.
- Lownsbery, B.F. & Viglierchio, D.R., 1960. Mechanism of accumulation of *Meloidogyne incognita acrita* around tomato seedlings. *Phytopathology* 50: 178-179.
- Lucas, G.B., Sasser, J.N. & Kelman, A., 1955. The relationship of root-knot nematodes to Granville wilt resistance in tobacco. *Phytopathology* 45: 537-540.
- Mangin, L., 1895. *Sur les présence de thylles gommeuses dans la vigne*. *Acad. Sci. Paris, Compt. Rend.* 119: 514-516.

- Mankau, G.R., 1956. Studies on the host parasitic relationships of *Heterodera trifolii* (Goffart) Oostenbrink, 1951. Ph.D. thesis, Univ. Illinois. (L.C. Card No. Mic. 18 172). Univ. Microfilms, Ann. Arbor. Michigan. 107 p.
- Manzoni, G., 1952. Considerazioni su differenze anatomiche in radici di barbatelli di *Vitis vinifera*, *V. riparia*, *V. rupestris*, *V. berlandieri*. Annali della sperimentazione agraria nuova serie 7(S): 299-337.
- Martin, W.J., 1954. Parasitic races of *Meloidogyne incognita* and *M. incognita* var. *acrita*. Plant. Dis. Repr., Suppl. No. 227: 86-89.
- Martin, W.J., Newsom, L.D. & Jones, J.E., 1956. Relationship of nematodes to the development of *Fusarium* wilt in cotton. Phytopathology 46: 285-289.
- McClure, M.A. & Viglierchio, D.R., 1966. The influence of host nutrition and intensity of infection on the sex ratio and development of *Meloidogyne incognita* in sterile agar cultures of excised cucumber roots. Nematologica 12: 248-258.
- McGuire, J.M., Walters, H.J. & Slack, D.A., 1958. The relationship of root-knot nematodes to the development of *Fusarium* wilt in alfalfa. Phytopathology 48: 344. (Abstr.).
- Metcalfe, C.R. & Chalk, L., 1950. Anatomy of the Dicotyledons. Oxford: University Press.
- Miles, L.E., 1939. Some tests of varietal susceptibility to a combination of root-knot nematode and cotton wilt. Phytopathology 29: 974-978.
- Millardet, A., 1897. Alterations phylloxeriques sur les racines. Rev. de Viticulture 8.
- Mizogami, T., 1947. Anatomical research on resistance of sweet-potato to the root-knot nematode. Proc. Meeting Agric. Res. Workers in Kyushu Vol. 1: 19-20. (Translation by Shoji Kayashima at North Carolina State College, March, 1958).
- Morgan, G.T. & McAllen, J.W., 1962. Hydrolytic enzymes in plant-parasitic nematodes. Nematologica 8: 209-215.
- Morris, L.L. & Mann, L.K., 1955. Wound healing, keeping quality and compositional changes during curing and storage of sweet-potatoes. Hilgardia 24: 143-183.
- Mountain, W.B. & Patrick, Z.A., 1959. The peach replant problem in Ontario. 7. The pathogenicity of *Pratylenchus penetrans* (Cobb) Filipjev and Stek., 1941. Can. J. Bot. 37: 459-470.

- Neal, J.C., 1889. The root-knot disease of the peach, orange and other plants in Florida, due to the work of *Anguillula*. U.S. Dept. Agric. Div. Ent. Bull. 20.
- Nemec, B., 1910. Das Problem der Befruchtungsvorgänge und andere zytologische Fragen. Teil 4. Vielkernige Riesenzellen in *Heterodera* gallen. Berlin: Gebr. Borntraeger, pp. 151-173.
- Niklowitz, W., 1955. Histologische Studien an Reblausgallen und Reblaus Abwehrnekrosen. *Phytopathology Z.* 24: 299-340.
- Nusbaum, C.J., 1958. The response of root-knot infected tobacco plants to foliar applications of maleic hydrazide. *Phytopathology* 48: 344. (Abstr.).
- Owens, R.G. & Specht, H.N., 1964. Root-knot histogenesis. *Contrib. Boyce Thompson Inst.* 22: 471-489.
- Parniewski, D., 1962. Untersuchungen über Wurzelbau und die Reblausresistenz von Unterlagsreben. (Investigations on root structure and resistance to *Phylloxera vitifolii* in vine rootstocks). Diss. Hochsch. Hohenheim.
- Peacock, H.A., 1966. Elementary microtechnique. London: Edward Arnold (Publishers) Ltd.
- Perold, A.I. & Tribolet, I., 1912. American stocks for Cape vineyards. *Union of S.A. Agric. Journ.* Vol. IV: 99-109; 225-257.
- Perold, A.I. & Wagner, W., 1914. Ondersoek in verband met geënte wynstokke in die Kaapprovinsie. *Unie van S. Afr. Dept. v. Landb. Bulletin No.* 49.
- Perold, A.I., 1926. *Handboek oor Wynbou*. Stellenbosch: Pro Ecclesia Drukkery.
- Petri, L., 1909. Über die Wurzelfäule phylloxerierter Weinstocke. *Z. Pflkrankh.* 19: 18-48.
- Piegat, M. & Wilski, A., 1963. Changes observed in cell nuclei in roots susceptible and resistant potato after their invasion by potato root eelworm (*Heterodera rostochiensis* Woll.) larvae. *Nematologica* 9: 576-580.
- Pitcher, R.S., Patrick, Z.A. & Mountain, W.B., 1960. Studies on host-parasite relations of *Pratylenchus penetrans* (Cobb) to apple seedlings. 1. Pathogenicity under sterile conditions. *Nematologica* 5: 309-314.
- Pongrácz, D.P., 1969. Vergelykende anatomiese studies van een- en driejarige wingerdwortels (*Vitis* spp.). M.Sc. Landbou-skripsie, Universiteit van Stellenbosch.

- Powell, N.T. & Nusbaum, C.J., 1960. The black shank root-knot complex in flue-cured tobacco. *Phytopathology* 50: 899-906.
- Powell, N.T., 1963. The role of plant parasitic nematodes in fungus diseases. *Phytopathology* 53: 1.
- Powers, H.R. Jr., 1954. The mechanism of wilting in tobacco plants affected by black shank. *Phytopathology* 44: 513-521.
- Raski, D.J. & Lider, L., 1959. Nematodes in grape production. *California Agriculture* 13(9): 13-15.
- Raski, D.J. & Hewitt, W.B., 1960. Experiments with *X. index* as a vector of fanleaf virus of grapevines. *Nematologica* 5: 166-170.
- Raski, D.J., Hart, W.H. & Kasimatis, A.N., 1965. Nematodes and their control in vineyards. *California Agriculture Exp. Sta. Ext. Service Circular* 633: 1-23.
- Rathay, E., 1896. Ueber das Auftreten von Gummi in der Rebe and ueber "Gommose bacillaire". Separate from Jahresber. und Programm der K.K. Oenolog. u. Pomolog. Lehranstalt in Klosterburg. 84 p. Wien.
- Ravaz, L., 1897. Contribution a l'étude de la résistance phylloxérique. *Revue de Viticulture* 12(63): 164-166.
- Riffle, J.W. & Kuntz, J.E., 1967. Pathogenicity and host range of *Meloidogyne ovalis*. *Phytopathology* 57: 104-107.
- Riggs, R.D. & Winstead, N.N., 1959. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. *Phytopathology* 49: 716-724.
- Rodrigues, A., 1942. Raizes aéreas na *Vitis vinifera* L. *Agr. Lusit.* 4: 5-30.
- Rohde, R.A., 1965. The nature of resistance in plants to nematodes. *Phytopathology* 55: 1159-1162.
- Ross, J.P., 1958. Host parasite relationships of the soybean cyst nematode in resistant soybean roots. *Phytopathology* 48: 578-579.
- Rovira, A.D., 1956a. Plant root excretions in relation to the rhizosphere effect. I. The nature of root exudate from oats and peas. *Plant and Soil* 7(2): 178-194.
- Rovira, A.D., 1956b. Plant root excretions in relation to the rhizosphere effect. II. A study of the properties of root exudate and its effects on the growth of micro-organisms isolated from the rhizosphere and control soil. *Plant and Soil* 7(2): 195-208.

- Sass, J.E., 1961. Botanical microtechnique. Iowa, Iowa State College Press.
- Sauer, M.R., 1962. Distribution of plant parasitic nematodes in irrigated vineyards at Merbein and Robinvale. Austr. J. exp. Agric. Anim. Husb. 2: 8-11.
- Schäfer, R., 1966. Nematoden in weinbau Chiles. Weinberg und Keller 13: 452-458.
- Schanderl, H., 1957. Über das Vorkommen und die Bildungsweise der Gerbstoffe und der Anthozyane in den Rebengewachsen. Mitt. Klosterneuburg 7 A/5: 229-275.
- Schindler, A.F., Stewart, R.N. & Semenik, P., 1961. A synergistic *Fusarium*-nematode interaction in carnations. Phytopathology 51: 143-146.
- Schneider, H. & Baines, R.C., 1964. *Tylenchus semipenetrans* parasitism and injury to orange tree roots. Phytopathology 54(10): 1202-1206.
- Schneider, H., 1968. Anatomy of greening-diseased sweet orange stocks. Phytopathology 58: 1155-1160.
- Seinhorst, J.W. & Sauer, M.R., 1956. Eelworm attacks on vines in the Murray valley irrigation area. J. Austr. Inst. Agric. Sci. 22: 296-299.
- Sembner, G., 1963. Anatomie der *Heterodera-Rostochiensis*-gallen in Tomatenwurzeln. Nematologica 9: 55-64.
- Sheldrake, A.R. & Northcote, D.H., 1968. The production of auxin by autolysing tissues. Planta 80: 227-236.
- Shibuya, M., 1952. Studies on the varietal resistance of sweet-potato to the root-knot nematode injury. Mem. Fac. Agr. Kagoshima Univ. 1: 1-22.
- Smith, A.L., 1954. Problems on breeding cotton for resistance to nematodes. Plant Disease Repr. Suppl. 227: 90-91.
- Smith, J.J. & Mai, W.F., 1965. Host-parasite relationships of *Allium cepa* and *Meloidogyne hapla*. Phytopathology 55(6): 693-697.
- Smith, L.M. & Stafford, E.M., 1955. Grape pests in California. Division of Agricultural Sciences Univ. of California.
- Smitn, P.C., 1967. Observations on nematodes in vineyards in the Western Cape Province, with special reference to the genera *Xiphinema* and *Longidorus*. M.Sc. Agric. Thesis, Univ. of Stell.
- Snyder, E., 1936. Susceptibility of grape root-stocks to root-knot nematode. Circular no. 405, U.S. Dept. of Agric., Washington.
- Staehlin, M., 1927. Kenntnis des einjährigen Rebholzes und dessen Reife. Separat Abdr. Landw. Jahrb. d. Schweiz: 337-386.

- Steiner, G., 1925. The problem of host selection and host specialization of certain plant-infecting nemas and its application in the study of nematode pests. *Phytopathology* 15(9): 499-534.
- Stewart, F.C., 1963. *Plant physiology*. New York & London: Academic Press.
- Struckmeyer, E.B., Beckman, C.H., Kuntz, J.E. & Riker, A.J., 1954. Plugging of vessels by tyloses and gums in wilting oaks. *Phytopathology* 44: 148-153.
- Szigeti-Gyula, A., 1905. Adatok néhány guakorlatilag fontosabb, szőlőfajta gyökereinek anatómiájához különös tekintettel a *Phylloxera biantalmára*. *Növ. Közl.* 4: 45-62.
- Taylor, A.L., Barker, H.D. & Owens, O.P., 1939. Preliminary report on cotton wilt-nematode experiments at Lamberton, North Carolina. *Phytopathology* 29: 752. (Abstr.).
- Thorne, G., 1961. *Principles of nematology*. New York, McGraw-Hill.
- Townshend, J.L., 1963a. The pathogenicity of *Pratylenchus penetrans* to celery. *Can. J. Plant Sci.* 43: 70-74.
- Townshend, J.L., 1963b. The pathogenicity of *Pratylenchus penetrans* to strawberry. *Can. J. Plant Sci.* 43: 75-78.
- Triantaphyllou, A.C., 1960. Sex determination in *M. incognita* Chitwood, 1949 and intersexuality in *M. javanica* (Treub, 1855) Chitwood, 1949. *Ann. Inst. Phytopathol. Benaki* 3: 12-31.
- Troll, J. & Rohde, R.A., 1966. Pathogenicity of *Pratylenchus penetrans* and *Tylenchorhynchus claytoni* on turfgrasses. *Phytopathology* 56(9): 995-998.
- Turner, L.M., 1935. Anatomy of aerial roots of *Vitis rotundifolia*. *Bot. Gaz.* 96: 367-371.
- Tyler, J., 1933. The root-knot nematode. *California Agric. Exp. Sta. Cir.* 330: 1-30.
- Van Fleet, D.S., 1961. Histochemistry and function of the endodermis. *The Botanical Review* 27(2): 166-220.
- Van Gundy, S.D. & Tsao, P.H., 1963. Infesting citrus seedlings with the citrus nematode, *Tylenchulus semi-penetrans*. *Phytopathology* 53: 228-229.
- Van Gundy, S.D. & Kirkpatrick, J.D., 1964. Nature of resistance in certain citrus rootstocks to citrus nematode. *Phytopathology* 54: 419-427.
- Van Niekerk, S.W. & Theron, C.J., 1927. Amerikaanse onderstokke. Ondersoek van geënte wingerde. *Unie van S. Afr. Dept. van Landbou, Bulletin No. 10.*

- Vaughan, J.G., 1955. Staining angiosperm shoot apices with Heidenhain's iron hematoxylin and aniline blue. *Stain Technol.* 30(2): 79-82.
- Viala, P., 1910. *Ampelographie* 1. Paris: Mao son et Cie.
- Vuillemin, P. & Legrain, E., 1894. Symboise de l'*Heterodera radicicola* avec plantes cultivées au Sahara. *Compt. Rend. Acad. Sci. (Paris)* 118: 549-551.
- Walters, H.J. & Slack, D.A., 1956. *Fusarium*-wilt plus nematodes ... hard on alfalfa. *Arkansas Farm Research* 5: 7.
- Watermeyer, T.L., 1908. *Vinifera* x American hybrid stocks. *Union of S. Afr. J. Agric.* XXXIII: 436-437.
- Webber, A.J. Jr. & Barker, K.R., 1967. Biology of pseudo root-knot nematode *Hypoperine ottersoni*. *Phytopathology* 57: 723-728.
- Weber, D., 1909. Adatok néhány gyakorlatilag fontosabb szőlőfajta gyökereinek anatómiai megkülönböztetéséhez. *Amp. Evk.* 3: 17-22.
- Weischer, B., 1960. Untersuchungen über das Auftreten pflanzenparasitärer Nematoden in Weinbergböden. *Nematologica* 5, Suppl. 11: 29-39.
- Wood, R.K.S., 1967. *Physiological plant pathology*. Oxford & Edinburgh: Blackwell Scientific Publications.

-----oo0oo-----