Histopathological and histochemical studies on grapevine roots damaged by *Xiphinema index*

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SUMMARY

Roots of grapevine, healthy and attacked by nematodes, were studied to determine histopathological and histochemical changes caused by *Xiphinema index*. First visible symptoms of nematode feeding were hypertrophy of cells and a drastic reduction of cell divisions. Continued feeding led to cessation of longitudinal root growth and swelling of the root tip. At feeding sites multinucleate giant cells and mononucleate hypertrophied cells developed. The walls of these cells showed numerous enlarged "pit fields". Thickening of cell walls and cell wall dissolution as found in syncytia induced by some Heteroderidae were not observed, but incomplete cell separation and irregular wall formation were found in giant cells.

Nematode transformed cells showed a higher activity of peroxidase, monophenolmonooxigenase, cytochrome oxidase, β-glucosidase and nonspecific esterase than corresponding cells from healthy roots. This, however, is more related to the amount of cytoplasm than to an increased metabolic rate. Peroxidase activity was highest in cell walls. A typical phenomenon in nematode attacked roots was the increased activity of unspecific esterase and β-glucosidase in the hypodermis and in cells of the vascular bundle. In general there were only slight differences in enzyme activity between nematode induced giant cells and actively growing cells from healthy roots.

RÉSUMÉ

Etude histopathologique et histochimique des racines de vigne attaquées par le nématode Xiphinema index

Cette étude concerne les modifications histologiques et biochimiques causées par le nématode *Xiphinema index* aux racines de vigne *(Vitis vinifera)*. Les premières réactions pathologiques perceptibles consistent en une hypertrophie des cellules situées au voisinage de l'extrémité du stylet et une très forte réduction des divisions cellulaires dans toute l'extrémité de la racine. Si la nutrition du nématode se prolonge, il se produit un arrêt complet de l'élongation de la racine et une croissance en largeur de son extrémité. Différentes modifications histologiques sont alors provoquées ; les plus remarquables sont des cellules géantes, plurinucléées et à plasma dense, des cellules uninucléées fortement hypertrophiées et des cellules vides, nécrosées, ces dernières ayant été probablement atteintes directement par le stylet. Dans les parois des cellules géantes et des cellules hypertrophiées voisines on remarque, après coloration au bleu de toluidine, des plages généralement elliptiques et transparentes qui représentent probablement des plages ponctuées agrandies. Aucune de ces cellules modifiées ne présente d'épaississements ni de dissolution de la paroi comme il en existe dans le cas des syncitia induits par les *Heterodera*. Par contre, il a été observé dans les cellules géantes des divisions incomplètes, au cours desquelles la paroi, de structure normale, fait saillie dans une cellule d'apparence intacte. Les cellules géantes comprennent jusqu'à seize noyaux; il est supposé que ce phénomène est exclusivement dû à des divisions cellulaires synchrones et répétées sans formation de paroi.

Différentes méthodes histochimiques ont permis d'analyser la répartition et l'activité de peroxydases, monophenolmonoxygenases, cytochromoxydases, β-glucosidases et estérases non spécifiques dans les tissus des racines saines et attaquées. Toutes les cellules modifiées montrent une activité enzymatique supérieure à celle des cellules correspondantes normales, à l'exception toutefois des peroxydases localisées préférentiellement dans les parois cellulaires. Ce phénomène serait en relation avec l'augmentation de la masse cytoplasmique plutôt qu'avec un accroissement du métabolisme ; ainsi la teneur des cellules géantes en monophénolmonoxygénases et en cytochromoxydases n'est pas sensiblement augmentée et les cellules à plasma dense ont une teneur élevée en β-glucosidases et en estérases non spécifiques ; dans les cellules géantes et les cellules hypertrophiées ces deux enzymes sont principalement localisées dans des lysosomes. Il est caractéristique des racines attaquées qu'on y observe une

augmentation d'activité de ces deux enzymes dans l'hypoderme et les faisceaux cribro-vasculaires ; cette observation est également valable pour les peroxydases, surtout liées à la paroi cellulaire, et les estérases non spécifiques. Ces deux dernières enzymes sont surtout abondantes dans les parois cellulaires bordant les « vides intercellulaires » causés par le nématode. Les analyses ont montré qu'en ce qui concerne les enzymes citées, les cellules géantes n'étaient guère différentes des cellules en croissance active situées dans la zone d'élongation des racines saines. Il est suggéré que cette observation pourrait être également valable pour d'autres enzymes ou métabolites.

Recently Weischer and Wyss (1976) studied the feeding behaviour and pathogenicity of *Xiphinema index* on grapes. They described some changes in root tissue caused by the nematodes, the most conspicuous being the formation of multinucleate giant cells. Since the development and significance of the nematode induced alterations were little understood, further histopathological studies and some histochemical analyses were made.

Materials and methods

Healthy and nematode attacked feeder roots of grapevine (*Vitis vinifera* cv. Müller-Thurgau) from pots and from agar cultures were used for the experiments. For histological studies part of roots fed upon by nematodes and corresponding parts of healthy roots were processed as described by Wyss (1975*a*). For histochemical analyses the roots were fixed for 16 h at 4° C in buffered formalin (4%, pH 7.0) and washed for 3-4 h in distilled water. For the detection of cytochrome oxidase unfixed tissue was used. The roots were sectioned on a freezing microtome and the sections (10-25 µm thick) transfered to cold distilled water or buffer solution. They were then incubated in the respective media needed to detect the different enzymes. All methods used in our experiments were described in detail by Lojda, Gossrau and Schiebler (1976). Peroxidase activity was determined by the diaminobenzidine method modified after Graham and Karkorski (1966). Peroxisomes were demonstrated by the same compound following the method of Novikoff and Goldfischer (1969). For the monophenolmonooxigenase (tyrosinase), DL-β-3,4-dihydroxiphenylalanine was used as substrate in a method modified after Becker, Praver & Thatcher (1935). To demonstrate cytochrome oxidase, oxidative coupling between N-phenyl-p-phenylendiamine and 1-hydroxy-2-naphthoic acid was applied. The method described by Seligman *et al.* (1954) for the detection of β-glucuronidase was modified for β-glucosidase by using sodium-6-bromo-2-naphthyl-β-D-glucopyronosid as substrate. Unspecific esterase activity was demonstrated by simultaneous azocoupling between 1-naphthylacetate and fast blue B in a method modified after Gomori (1952).

Fig. 1. a : Longitudinal section through a healthy grapevine root showing the regular structure of the tissue.

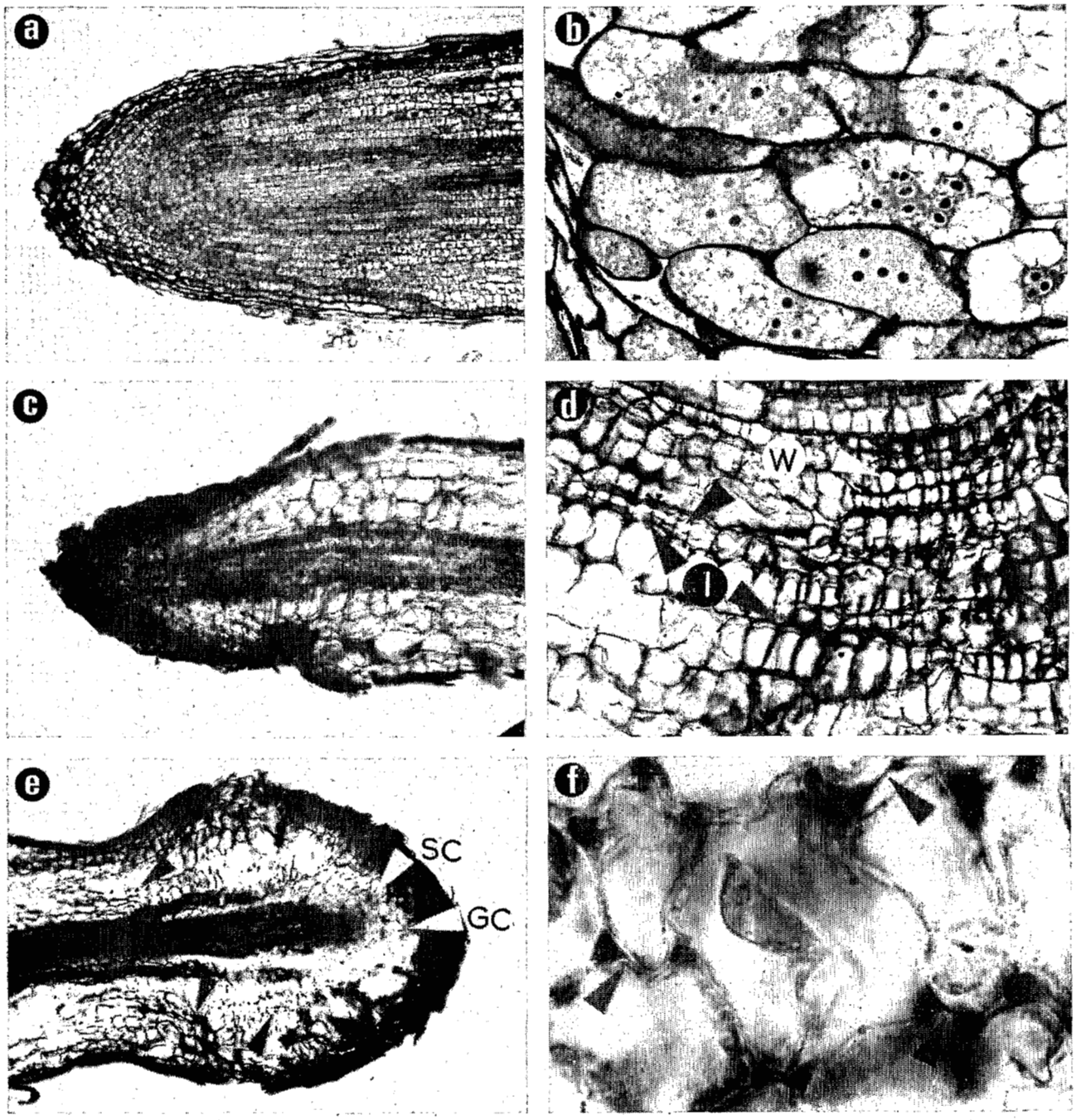
b : *X. index*-induced multinucleate giant cells in different stages of development as indicated by the degree of vacuolisation. The enlarged nuclei contain one or more greatly enlarged and deeply stained nucleoli.

c : Longitudinal section through a grapevine root attacked by *X. index* some distance behind the apical meristem. No plasm-rich giant cells are formed but cortical cells near feeding sites are greatly enlarged and partly separated by intercellular spaces. The apical meristem still functions. The differentiation of the stele proceeds to the root tip. The dark stained cells indicate monophenolmonooxigenase (tyrosinase) activity.

d : Cortex of a grapevine root fed upon by *X. index* showing a network of intercellular cavities (I) and rows of small round "windows" (W) as described in the text (p. 220). The dark staining indicates high activity of cell wall-bound peroxidase along the intercellular spaces.

e : Swollen root tip showing peroxidase activity localized mainly in the rhizodermis and the vascular bundle. As a response to nematode attack, intercellular spaces are formed showing cell wall-bound enzyme activity in the surrounding cell walls (black arrows). A detail is given in Fig. 1 f. Weak peroxidase activity is seen in the cytoplasm of cells at the very beginning of the stele (SC). Nearly no activity is found in the developing giant cells (GC).

f : Slightly hypertrophied cortical cells close to a feeding site. The dark stained triangles represent recently formed intercellular spaces showing high peroxidase activity. The arrows indicate progressing dissolution of the middle lamellae.


a
b
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After chemical treatment or staining the sections were embedded in "Zeiss W15" or glycerine-gelatine on glass slides for microscopic examination.

Results

Histological alterations

Feeding by *Xiphinema index* on young rootlets leads to considerable changes in shape and histological structure. First alterations became visible 6-8 hours after feeding began. In addition to the beginning of hypertrophy of cells, the number of cell divisions in damaged roots was drastically reduced to 1/100 of that found in comparable sections from healthy roots. Another early symptom was the frequent occurrence of thin straight walls in tangential direction. The shape of the root started to change 10-12 hours after the nematodes began to feed. The type of deformation varied according to the location and duration of feeding and the diameter of the root. Feeding near the root tip resulted in retarded growth, apical swelling and browning of the cortex. As a consequence of nematode attack various alterations in cell structure occurred. The following types of nematode transformed cells (NTC) could be distinguished :

a) multinucleate giant cells with small vacuoles (GC) ;
b) hypertrophied mononucleate parenchymatous cells (HC) and
c) necrotic cells (NC).

Underneath the necrotic cells, which had been fed upon by the nematodes, groups of multinucleate giant cells were formed (Fig. 1b). However, they do not always occur, particularly when the nematodes fed some distance from the root tip. In such cases parenchymatous cortical cells increased in size (Fig. 1c), necroses and browning of epidermal, subepidermal and cortical cells occurred and intercellular spaces developed (Fig. 1d, e). Root tips having a diameter less than 1 mm can be completely filled by the three types of NTC. In larger roots these cells were confined to one side or in the case of older roots to separate groups corresponding to the feeding sites. The well organized structure of a healthy root (Fig. 1a) was replaced by disorganized complexes of different NTC surrounded by irregular cortex tissue showing browning of the cell walls. In this tissue intercellular cavities are formed mainly in tangential walls. They gradually increase in size and finally form a network of cavities (Fig. 1d). When feeding is not continued the root tip can remain intact and eventually resume its longitudinal growth.

In callus tissue developing from cuts, the nematodes can induce the formation of slightly enlarged cells containing up to two enlarged nuclei and more cytoplasm than adjacent normal callus cells but not the type of multinucleate giant cells as described from root tips.

In the walls of giant cells stained with toluidine blue numerous faint blue fields of mostly elliptical shape were seen (Fig. 2a). In the early stage of giant cell formation they resemble the pit fields found in the walls of giant cells induced by *Meloidogyne incognita* in roots of *Impatiens balsamina* (Jones & Dropkin, 1976). Whether there are perforations of the wall representing plasmodesmata in these faintly stained fields could not be resolved by light microscopy. With expansion of the wall the "pit fields" enlarged mainly in the direction of cell elongation giving them a pronounced ellipsoid shape. There was always a distinct though very thin wall left. Such fields were not observed in the non-transformed parenchyma but a few were found in vascular parenchyma cells.

Cell wall dissolution, as known from syncytia induced by Heteroderidae, did not occur in grape roots. But sometimes incomplete cell separation occurred in giant cells with cell walls extending into the cell like a septum (Fig. 2b). Similar phenomena in fig roots attacked by *X. index* were interpreted by Wyss (1978) as due to cell wall dissolution. In grape roots such septa looked exactly like the real cell wall of giant cells having the same pattern of "pit field" distribution, thickness and staining behaviour. In grape roots cell wall breakdown only occurred in dead cells in the periphery of giant cell complexes. In these cases the edge had a fibrous, indistinct appearance,

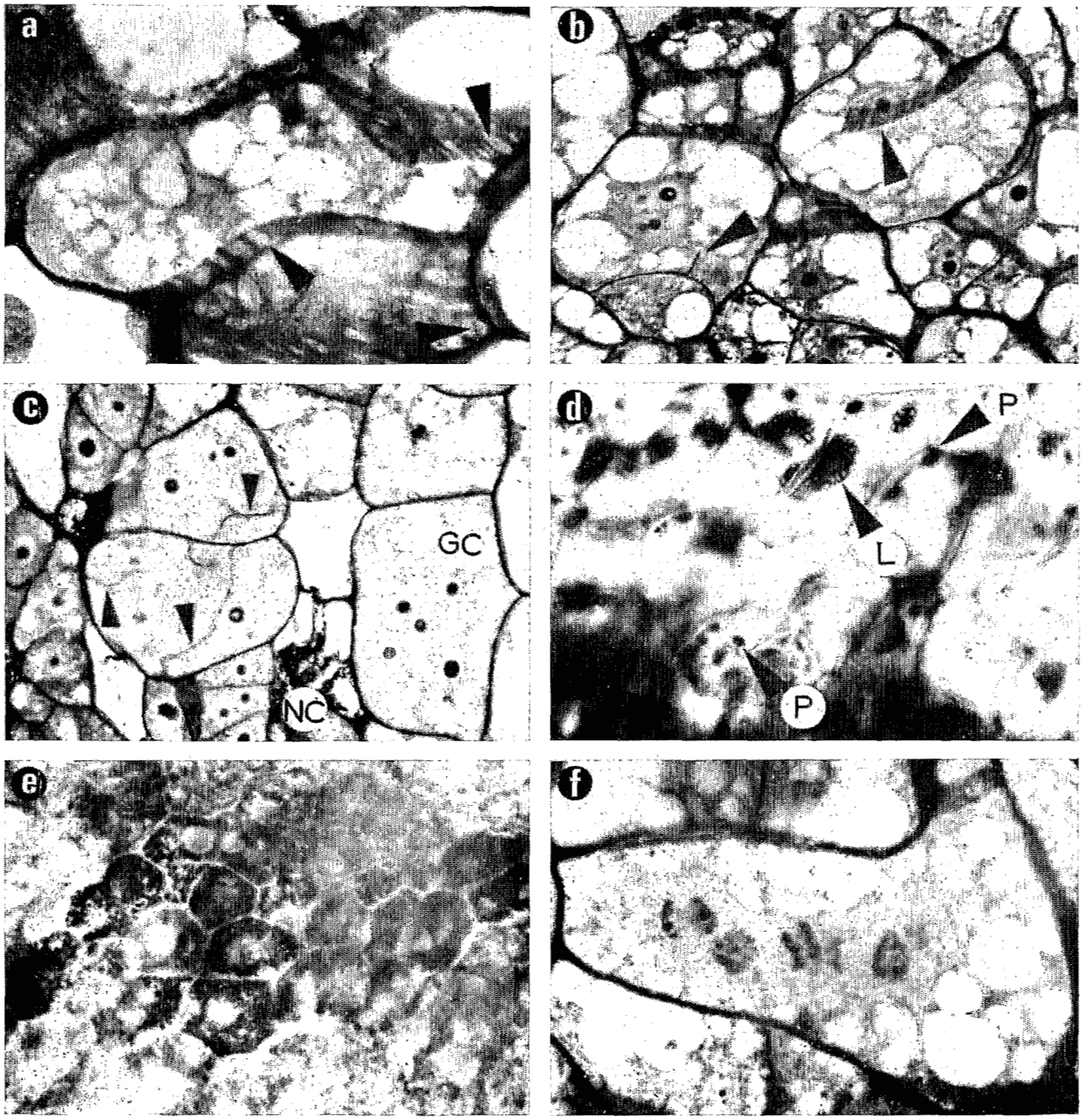

Fig. 2. a : Enlarged elliptical "pit-fields" (arrows) in the walls of giant cells.
b : Multinucleate giant cells with fragmentary cell walls (arrows).
c : Feeding site showing necrotic cells (NC) destroyed by nematode feeding, « normal» multinucleate giant cells (GC) and giant cells with irregular wall formation (arrows).
d : Histochemical localisation of sites of monophenolmonooxigenase activity in hypodermis and underlaying cells. Activity is seen in plastids (P) and leucoplasts (L) surrounding the nuclei. Leucoplasts contain two or more white starch granules.
e : Section through a root tip fed upon by *X. index* showing β-glucosidase activity in cytoplasm-rich cells out of the swollen part of the root tip. The dark granules are assumed to be lysosomes.
f : Giant cell undergoing five synchronous mitoses.

whereas the edge of a septum always was smooth and distinct.

Other giant cells showed irregular cell wall formation. Fig. 2c shows such cells together with necrotic cells destroyed by the nematodes and "normal" multinucleate giant cells. As mentioned above the number of cells in the giant cell complex undergoing mitoses was remarkably reduced. This seems to be in contradiction to the high number of nuclei per cell. Up to sixteen have been observed. Our studies revealed that in the early stage of giant cell formation the number of nuclei in a cell rapidly increases by synchronous mitoses (Fig. 2f) followed by an increase in cell size but not by cytokinesis. In the beginning the cytoplasm of such giant cells was dense and contained very small vacuoles only. The vacuoles increased in size, with the nuclei and the major part of the cytoplasm concentrated in clusters in the center of the cells (Fig. 1b).

Histochemical changes

Nothing has been published on the biochemical aspects of host-parasite relationships between grapevine and *Xiphinema index*. We therefore studied some enzyme systems involved in the physiology of this plant nematode interaction.

Peroxidase

Peroxidase activity in healthy grape root was most prominent in rhizodermis and hypodermis as well as in endodermis and some parts of the vascular parenchyma. Almost no activity was found in the cortex. The activity was mostly observed in and at the cell wall and rarely in the cytoplasm. Exceptions were the young cytoplasm-rich cells of the root tip. The peroxidase activity within the cytoplasm could be found at the very beginning of the stele and in outer cell layers of the root tip. Feeding by *Xiphinema index* greatly changed this pattern of peroxidase distribution. In addition to the described histological alterations, a general increase of enzyme activity in the rhizodermis and the vascular bundle occurred. A remarkable amount of activity was also found in the cortex around and posterior to giant cell complexes. The activity was always first detected in the tangential cell walls underneath the feeding sites, where the middle lamellae were dissolved and intercellular cavities formed (Fig. 1e, f). Enzyme activity spread with the extending intercellular spaces. In areas where the walls were still unseparated no activity could be demonstrated. Under the microscope the intercellular system appeared as a darkly stained network, whereas areas with undisturbed cell walls were unstained and appeared as round "windows" (Fig. 1d). In a later stage of development the outer layer of the giant cell complexes show high activity in the cell walls and also an increasing activity in the cytoplasm. However the central cells showed no enzyme activity. Peroxidase activity in cytoplasm was not localized in particular organelles.

Closely related to the increase in peroxidase activity was the observed incorporation into the cell walls of brown pigments which consisted of polymerized phenolic compounds.

Catalase activity was present in the cytoplasm of multinucleate giant cells to a larger extent than peroxidase. The nuclei were surrounded by small dark granules also present in the cytoplasm. These granules are peroxisomes containing catalase. The frequency of peroxisomes in giant cells was approximately the same as in the meristematic cells of the root tip.

Monophenolmonooxigenase

In healthy roots the histochemical reaction for monophenolmonooxigenase (tyrosinase, polyphenoloxidase, DOPA) showed high enzyme activity in rhizodermis and endodermis. The meristematic cells of the root tip had slightly less. Giant cells from attacked roots showed no distinctly increased enzyme activity. The staining resulting from the chemical reaction applied to show monophenolmonooxigenase was generally concentrated in granules around the nucleus and in leucoplasts distributed in the cytoplasm. In roots fed upon by nematodes, particularly at and around feeding sites, the number and size of such plastids increased in the hypodermis and the

adjacent two or three cell layers of the cortex. The staining became first visible in small granules around the nucleus. They then gradually increased in size and subsequently showed one to five small white starch granules. These enlarged and finally occupied the whole leucoplasts. Between the granules some enzyme activity was still visible (Fig. 2d).

Cytochrome oxidase

In young meristematic cells of healthy root tips cytochrome oxidase was visible as small dark spots scattered throughout the cytoplasm. The spots very probably are mitochondria. In the outer layers of the root tip and the youngest parts of the calyptra the cells showed an additional diffuse black-violet staining of the cytoplasm. High activity was also demonstrated in the vascular bundle particularly in pericycle and endodermis. The cytoplasm of giant cells in nematode-attacked roots had approximately the same amount of enzyme activity as the young plasm-rich cells of the root tip. High activity was found in the cortical parenchyma altered by nematode feeding in contrast to low activity in healthy cortex tissue. The cytochrome oxidase activity was particularly high in the beginning of the nematode-induced changes. Numerous dark spots, bigger and more densely packed than in healthy meristematic cells, marked the positions of enzyme activity in mitochondria. With progressive aging the enzyme activity decreased with the decreasing amount of cytoplasm.

β-glucosidase

In healthy roots a high β-glucosidase activity was found in the apical meristem and in the growing point of non emerged lateral roots. Activity was further demonstrated in cells of the vascular bundle and of the hypodermis. At feeding sites with multinucleate giant cell formation and galling of the surrounding tissue, high β-glucosidase activity was found in all cytoplasm-rich cells of these complexes. Figure 2e shows a group of intensively stained cells. The dark granules are assumed to be lysosomes known to contain large amounts of lytic enzymes, e.g. α- and β-galactosidase, β-glucosidase, phosphatase and nonspecific esterase. These enzymes are released when the cell wall is destroyed thus leading to the death of the cell. This probably happened to the brown cells in Figure 2e. Localisation and shape of this histochemically detected group allow us to interpret it as a feeding site.

In healthy tissue lysosomes were frequently encountered in cells of the vascular bundle where autolysis occurs in xylem elements and in the sclerenchyma. As a response to nematode attack the number of lysosomes in these cells and in the hypodermis was increased.

Nonspecific esterase

Nonspecific esterase activity proved to have a similar distribution in root tissue as peroxidase. Rhizodermis and the underlaying cell layer as well as the endodermis showed a higher enzyme activity than other parts of healthy roots. This activity was confined mainly to the cell walls. Only in the hypodermis, in some parenchymatous cells of the vascular bundle and particularly in the outer cell layers of the meristem, a higher amount of activity was present in the cytoplasm. At feeding sites the nematode induced giant cells gave a pronounced positive reaction in the cytoplasm. In contrast to the young plasm-rich cells in healthy roots whose cytoplasm was uniformely stained and contained clearly visible lysosomes, in the giant cells the stain was distinctly localized within cytoplasmic structures, mainly lysosomes. Activity was also found in granules (plastids ?) concentrated around the nucleus and along the tonoplast but also scattered throughout the cytoplasm.

Nonspecific esterase activity localized in lysosomes was often seen in the hypodermis and in cells of the vascular bundle. In the vicinity of feeding sites number and size of these bodies were increased as already described for β-glucosidase.

In the cortex altered by nematode feeding esterase activity was confined to those parts of the cell wall where cells had separated partially from each other forming intercellular spaces. In areas where the walls were still in contact no activity could be demonstrated.

Discussion

The histological changes observed in grape roots fed upon by *Xiphinema index* can be attributed to three different processes : strong reduction in meristematic activity ; galling by hypertrophy of cortical parenchyma and giant cell formation ; aging of the root tip by continued differentiation progressing to the very tip after cessation of longitudinal growth. Published results show that symptoms caused by root tip feeding nematodes vary from genus to genus and from species to species although there are some common phenomena. Feeding of *Longidorus africanus* causes hyperplasia of the cortical parenchyma (Cohn & Orion, 1970), while *L. elongatus* induces hypertrophy of undifferentiated cortical cells around the feeding sites (Wyss, 1975). In galls caused by *Hemicycliophora arenaria* in rough lemon the hyperplastic reaction of cortical cells is limited to a few cell layers close to the head of the nematode (Van Gundy & Rackham, 1961). *Dolichodorus heterocephalus* induces hypertrophy and hyperplasia of cortical and vascular tissues in tomato (Paracer, Waseem & Zuckerman, 1967).

The most obvious character of tip galls caused by *X. index* is the occurrence of multinucleate giant cells surrounding feeding sites. These cells resemble the syncytia induced by some sedentary tylenchids in also being densely packed with cytoplasm and having greatly enlarged and sometimes lobed nuclei. In all other aspects they are, however, completely different. There is no convincing evidence for cell wall breakdown and syncytia formation. The phenomenon of septa we observed may at first sight be attributed to cell wall dissolution but the appearence of such septate cells differs from all syncytia formations known so far. Syncytia as induced by some Heteroderidae result from cell wall dissolution occurring in a series of small and large holes, the remaining parts being thickened.

At the present time it can be stated that giant cells in grape roots induced by *X. index* need no wall breakdown to become multinucleate. The large number of nuclei results from repeated mitoses without cytokinesis.

In their paper Weischer and Wyss (1976) speculated that *Xiphinema*-induced giant cells might function as specialized nurse cells and because of their assumed modified and increased metabolic activity could be essential for the nematode to reproduce. Increase of enzyme activity has often been reported to occur in nematode infected tissue (Hussey & Krusberg, 1970 ; Veech & Endo, 1970). A large increase was found in the syncytium when compared with the surrounding cortical tissue. But all the transformed cells are richer in cytoplasm than the surrounding cortical tissue ; therefore, an intense staining does not necessarily indicate an abnormal high enzyme activity. It simply can reflect the greater quantity of cytoplasm. When estimating the enzyme activity of giant cells we therefore preferred to compare them with actively growing, cytoplasm-rich cells of healthy root tips. For all the enzymes located in the cytoplasm such as catalase, monophenolmonooxigenase, cytochrome oxidase, β-glucosidase and nonspecific esterase, no great difference from these root tip cells was found. Whether there are qualitative differences has not yet been investigated. It is known that as a consequence of infection the isozyme pattern can change (Hussey & Krusberg, 1970). In contrast to the above mentioned enzymes, peroxidase was mainly found attached to the cell wall. Also nonspecific esterase was demonstrated to be active at or in the cell wall mainly where intercellular spaces were formed. For a long period the function of cell walls in higher plants was regarded to be essential mainly as skeletal support. Now it is known that they are involved in some metabolic processes. They were found to contain a number of hydrolytic enzymes including peroxidase and phenoloxidase. These enzymes are often associated with the dynamic recycling functions of intracellular lysosomes. They can be released from the cell walls by pectic enzymes (Stephens & Wood, 1974) which must be expected to be very active at sites where intercellular spaces have just been formed. Nonspecific esterase is not yet reported to be present in the cell wall, but in lysosomes. In our studies the intensive browning of those parts of the cell wall bordering intercellular spaces may be due to the presence of these enzymes. Peroxidase also is reported to have

IAA-oxidase function thus possibly taking part in growth regulation (Shinshi & Noguchi, 1975).

β-D-glucosidase catalyses the hydrolysis of phenolic glucosides to their corresponding aglycones and glucose. Some of these aglycones are substrates of peroxidase and polyphenol-oxidase ; β-glucosidase was therefore assumed to be actively involved in necrosis formation in connection with the resistance of potatoes to *Globodera rostochiensis* (Giebel, 1974). The enzyme was thought to be injected by the nematodes into the plant cells. However, our own unpublished results strongly indicate that it is of plant origin. As also seen in grape roots studied here, β-glucosidase is most prominent in cytoplasm-rich active cells. The only indication for its suggested participation in necrosis is the observed higher activity in cells surrounding cells with browning walls. Our histochemical studies on giant cells induced by *X. index* in grape roots showed no remarkably higher activity of the enzymes studied than the actively growing cells from healthy root tips. This indicates that giant cells in these and other aspects do not differ much from active untransformed cells. Preliminary electron microscope studies support this view. The number and size of mitochondria and the number of plastids were not remarkably increased as compared to active, cytoplasm-rich cells from healthy root tips. Some giant cells were found showing irregular wall thickenings confined to small areas. This type of irregular deposits of wall material is a common phenomenon in parasitized plant tissue. We never observed the typical wall ingrowths described for transfer cells.

References

Becker, S. W., Praver, L. L. & Thatcher, H. (1935). Improved (paraffin section) method for dopa reaction : with considerations of dopa-positive cell, as studied by this method. *Arch. Derm. Syph.*, Berlin, 31 : 190.

Cohn, E. & Orion, D. (1970). The pathological effect of representative *Xiphinema* and *Longidorus* species on selected host plants. *Nematologica*, 16 : 423-428.

Giebel, J. (1974). Biochemical mechanisms of plant resistance to nematodes : A review. *J. Nematol.*, 6 : 175-184.

Gommers, F. J. & Dropkin, V. H. (1977). Quantitative histochemistry of nematode-induced transfer cells. *Phytopathology*, 67 : 869-873.

Gomori, G. (1952). *Microscopic histochemistry. Principles and practice.* Chicago, University Press.

Graham, R. C. & Karnovsky, M. J. (1966). The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney : ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.*, 14 : 291-302.

Hussey, R. S. & Krusberg, L. R. (1970). Histopathology of and oxidative enzyme patterns in Wando peas infected with two populations of *Ditylenchus dipsaci*. *Phytopathology*, 60 : 1818-1825.

Jones, M. G. K. & Dropkin, V. H. (1976). Scanning electron microscopy of nematode-induced giant transfer cells. *Cytobios*, 15 : 149-161.

Lojda, Z., Gossrau, R. & Schiebler, T. H. (1976). *Enzymhistochemische Methoden.* Berlin, Heidelberg, New York, Springer Verlag, 300 p.

Novikoff, A. B. & Goldfischer, S. (1969). Visualisation of peroxisomes (microbodies) and mitochondria with diaminobenzidine. *J. Histochem. Cytochem.*, 17 : 675-680.

Paracer, S. M., Waseem, M. & Zuckerman, B. M. (1967). The biology and pathogenicity of the awl nematode *Dolichodorus heterocephalus*. *Nematologica*, 13 : 517-524.

Seligman, A. M., Kwang-Chung Tsou, Rutenberg, S. H. & Cohen, R. B. (1954). Histochemical demonstration of β-D-glucuronidase with a synthetic substrate. *J. Histochem. Cytochem.*, 2 : 209-229.

Shinshi, H. & Noguchi, M. (1975). Relationships between peroxidase, IAA-oxidase and polyphenol oxidase. *Phytochemistry*, 14 : 1255-1258.

Stephens, G. J. & Wood, R. K. S. (1974). Release of enzymes from cell walls by an endopectate-transeliminase. *Nature, Lond.*, 251 : 358.

Van Gundy, S. D. & Rackham, R. L. (1961). Studies on the biology and pathogenicity of *Hemicycliophora arenaria*. *Phytopathology*, 51 : 393-397.

Veech, J. A. & Endo, B. Y. (1970). Comparative morphology and enzyme histochemistry in root knot resistant and susceptible soybeans. *Phytopathology*, 60 : 896-902.

Weischer, B. & Wyss, U. (1976). Feeding behaviour and pathogenicity of *Xiphinema index* on grapevine roots. *Nematologica*, 22 : 319-325.

Wyss, U. (1975 *a*). A routine method for investigations on the histology of feeding sites of plant-parasitic nematodes. *Nematologica*, 21 : 110-111.

Wyss, U. (1975 *b*). Feeding of *Trichodorus*, *Longidorus* and *Xiphinema*. In Lamberti, F., Taylor, C. E. & Seinhorst, J. W. (Eds) : *Nematode vectors of plant virusses*. NATO Advanced Study Institutes Series, Series A : *Life Sciences*, Vol. 2. London, New York, *Plenum Press*, 203-221.

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

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