# Histopathology of Meloidogyne chitwoodi (Golden et al.) on Russet Burbank Potato

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*Abstract:* Pathogenesis of *M. chitwoodi* associated with potato (*Solanum tuberosum* cv. Russet Burbank) followed a pattern characteristic of root-knot nematodes. Giant cells developed in the phloem tissues of roots, stolons, and tubers and appeared to arise by hypertrophy and karyokinesis rather than cellular fusion. Gall formation was a function of parasite density and developed by hypertrophy of cortical cells. Brownish lesions which are symptomatic of tuber infection resulted from lignification of cortical cell walls in contact with egg matrix. *Key words: M. Chitwoodi,* potato (*Solanum tuberosum*), histopathology, pathogenesis.

*Meloidogyne chitwoodi* (Golden et al.) was recently discovered in the Pacific Northwest and has been demonstrated to be an aggressive parasite of potato crops (6). A root-knot nematode disease of potato has been known in this region for many years (1) and has generally been attributed to *Meloidogyne hapla* (2). Some of these incidences probably were misidentified.

Host responses to infection, which are significantly different from those described for infection by *H. hapla* (Chitwood) (3), led to the discovery of *M. chitwoodi*. The purpose of this study was to explore and describe the biological basis of these observed differences.

## MATERIALS AND METHODS

The inoculum used in the controlled experiments of this study was obtained from infected potato tubers (*Solanum tuberosum* cv. Russet Burbank) harvested from naturally infested commercial fields. Egg masses were excised from the tuber tissues and incubated in distilled water on a culture shaker. A suspension of $L_2$ juveniles was used to inoculate tomato (*Lycopersicum esculentum* cv. Rutgers) seedlings growing in a sterile potting mixture. The population of nematodes which developed on the tomatoes was used to inocu-

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late wheat (*Triticum aestivum* cv. Fielder). Egg masses which developed on the wheat roots were used to inoculate the experimental potato plants.

The potato plants used in these experiments were grown from certified foundation grade seed tubers. The cut seed pieces were planted in large plastic tubs (approximately 5-gallon capacity) filled with steamed sandy silt loam soil. The plants were grown under controlled conditions of 12-h days and day/night temperatures of 21 and 15 C, respectively. These environmental conditions were previously determined to be satisfactory for the growth and development of the host and parasite.

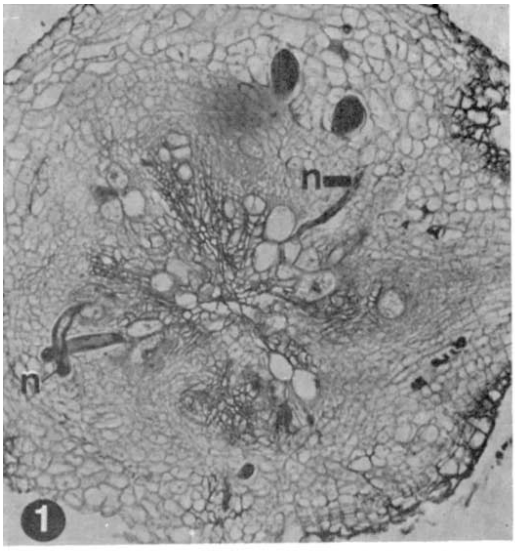
Infected roots, stolons, and tubers harvested at various intervals beginning 21 d after inoculation were thoroughly washed, fixed in formaldehyde-acetic-alcohol (100 parts distilled water, 20 parts 95% ethyl alcohol, 16 parts 38% formaldehyde, 2 parts glacial acetic acid), embedded in parawax, sectioned on a rotary microtome and stained. Except for the most delicate root tissues, all sections were cut at 25$\mu$m and stained in a 1% alcoholic solution of acid-fuchsin or saffranin and fast green, according to the schedules of Johansen (5), and observed under a microscope. Infected segments of roots, stolons, and tubers selected from several different plants were preserved and sectioned. Many of the tissue sections were discarded because they did not exhibit meaningful details, but 1,475 were studied intensively and were the basis of this report.

## RESULTS

Concentrations of juveniles were frequently observed surrounding the juncture of lateral roots (Fig. 1), near lesions caused by *Rhizoctonia solani* on stolons (Fig. 2), and near "eyes" on the tubers. No infections were observed to occur on the vertical stems, even in those portions growing below the soil line. Juveniles apparently penetrated through the epidermis or through wounds and natural openings and migrated inter- or intra-cellularly (Figs. 2 and 3, respectively) to the nearest phloem elements where feeding was initiated. This was evidenced by the orientation of intact nematodes in cross sections of host tissues (Figs. 1, 2, 3, 5, 6). The radial arrangement of nematodes around the vascular cylinder and their precise location within the phloem parenchyma suggested the involvement of chemical attractants. On one occasion preparasitic larvae placed near the cut end of a stolon, migrated directly through the stolon, and congregated around the vascular ring of the developing tuber.

The first evidence that parasitism had been initiated was manifested by an increase in protoplasmic density and granulation of cells proximate to the body of the larvae (Figs. 3, 4). These cells became progressively larger, multinucleated, and highly vacuolated (Fig. 5). The number of giant cells observed at each infection site varied, but there were never more than four (Fig. 6) when only one nematode was present. In those sections where the heads of the nematodes were clearly visible, they were always observed to occupy the intercellular spaces among the giant cells (Figs. 5, 6).

The roots of healthy potato plants have a diarch radial protostele with the two protoxylem points abutting the pericycle directly. The primary phloem is radially arranged with respect to the protoxylem points. In infected roots the anterior ends of the larvae were invariably embedded in the phloem and the giant cells were frequently bordered by xylem vessels (Fig. 7). This resulted in a morphological aberration of the vascular cylinder, but there was no evidence of increased cell number. Late second-stage and older nematodes were always within well defined cavities of the cortex (Figs. 2, 8, 10, 11), but there was no physical derangement of this tissue (Figs. 8, 11). The diameter of the cortical cells proximate to the developing females was greater than average, and when two or more resided at the same locus the roots became swollen (Fig. 9), thus producing the characteristic external symptoms of infection. The length of fully matured females is generally greater than the radius of adventitious roots; therefore, gravid females become erumpent and all egg masses are external to the epidermis. The erumpent posteriors of the females often are the only signs of infection. Roots, stolons, and tubers whose radii are greater than the length of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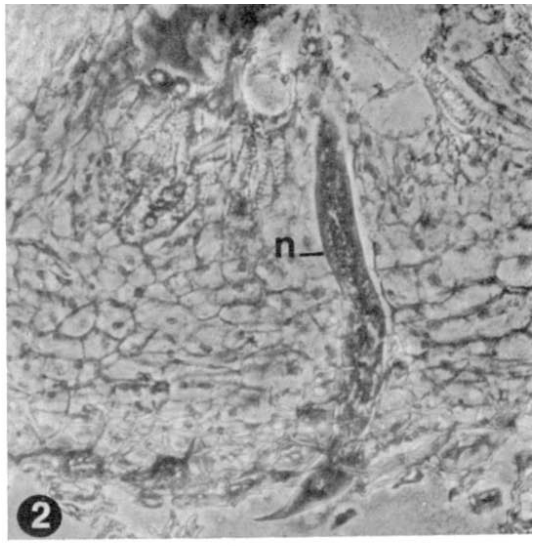
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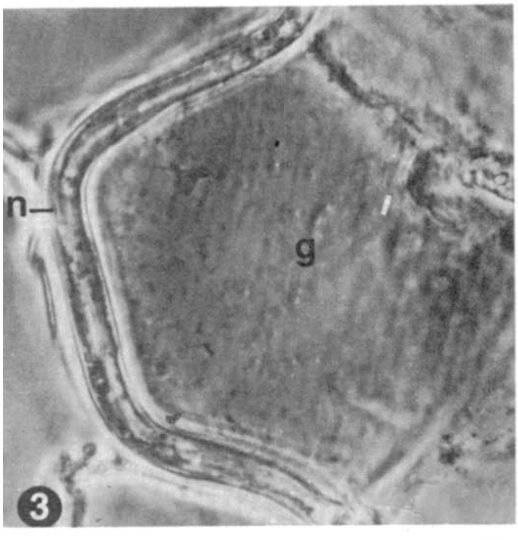
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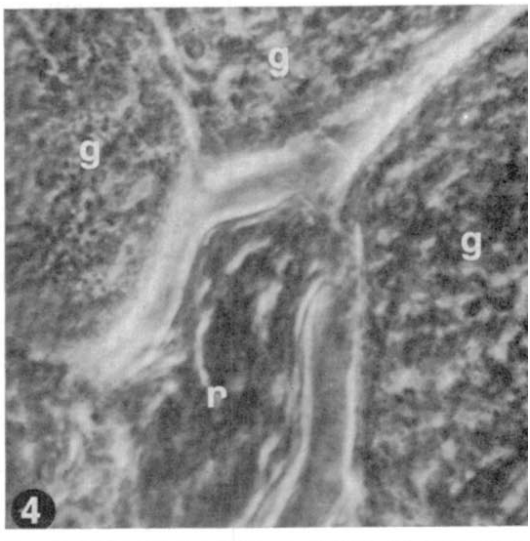
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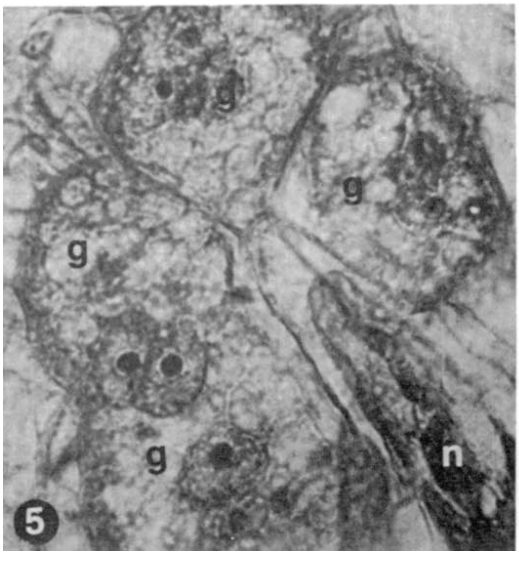
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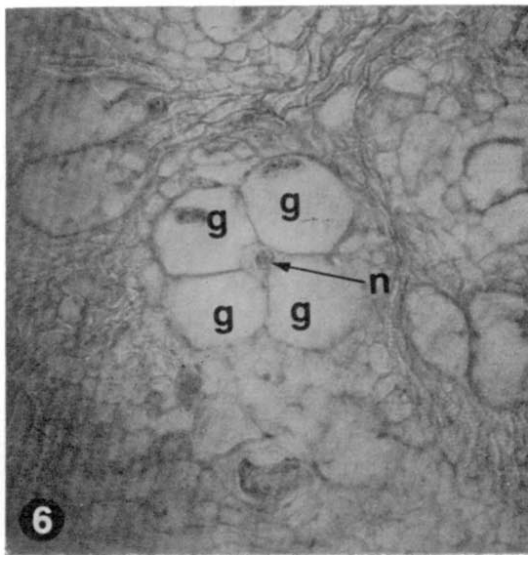
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mature females usually, but not always, display galls.

Infective juveniles frequently invaded stolons where they parasitized either primary or secondary phloem tissue. Consequently, lesions were found within the cortex, exterior to the vascular ring, and in the pith (Fig. 10). The pathology of stolon tissues was identical to that of root tissues except that egg masses were found in cavities within the cortex (Fig. 11). The walls of the cortical cells in contact with the egg masses were highly refractive and gave the appearance of being lignified (Fig. 12). In tubers, as in stolons, the lignified cortical cells formed what appeared to be a protective basket around the egg masses and developing juveniles. First-stage larvae were commonly observed in the eggs, and second-stage larvae were frequently observed among the eggs but, except in damaged tubers, never outside the protective basket.

## DISCUSSION

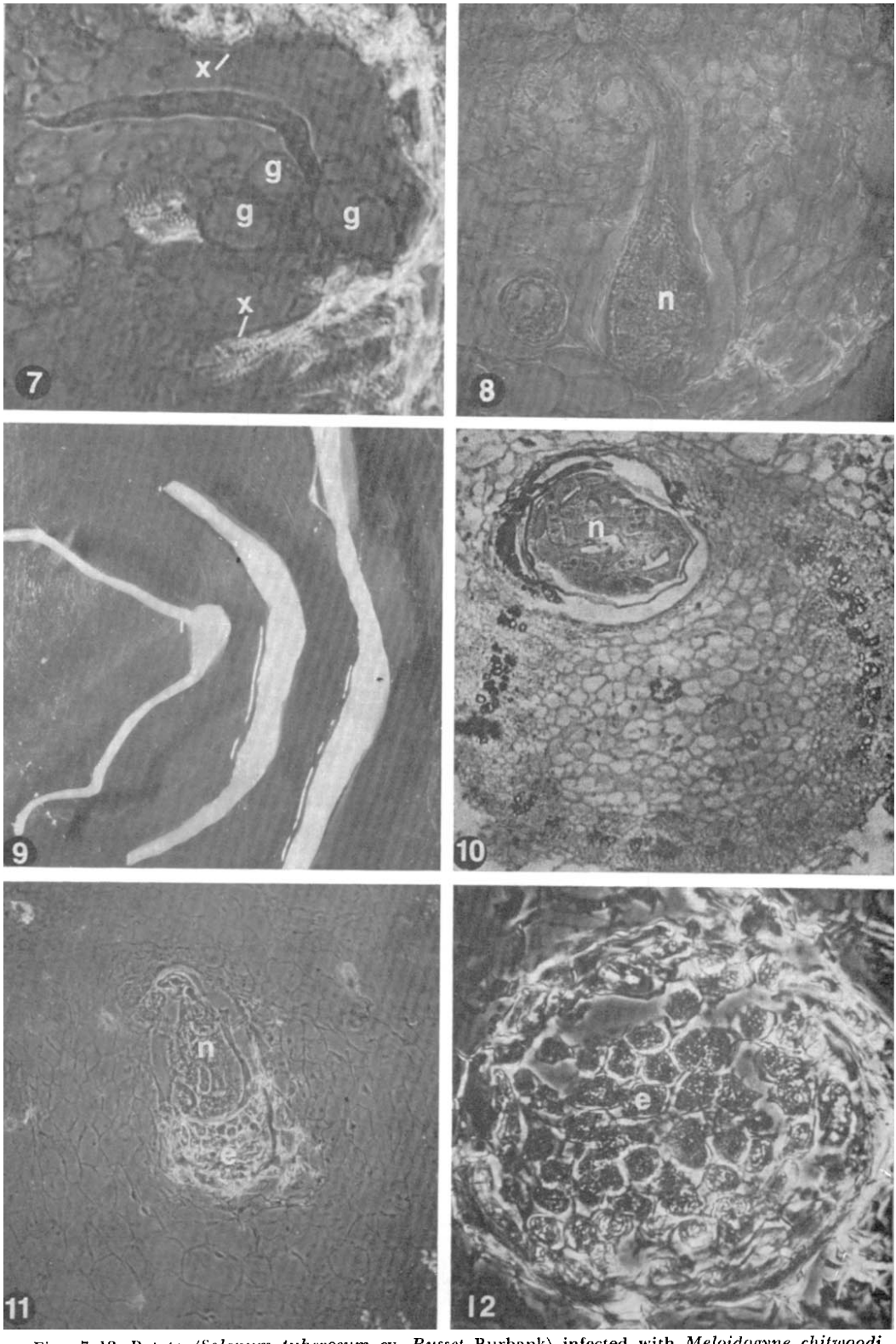
Root galling on Russet Burbank potato caused by *Meloidogyne hapla* (Chitwood) will occur within the temperature range of 20 to 30 C (3), but the optimum is approximately 25 C. Consequently, field surveys for root-knot in Idaho have customarily been initiated during late August or early September. The occurrence of severe root and tuber galling in two fields surveyed in mid-July 1978 was our first indication that a new biotype might be the responsible parasite. This survey was being conducted at the request of growers who had complained of their inability to achieve successful control by either soil fumigation or rotation with cereal grains. Soil samples from fields of both wheat and potatoes on these farms revealed that the population of Meloidogyne juveniles was higher in the wheat fields than in the potato fields. It was these observations which led to current studies of the root-knot problem on potatoes (6).

Symptoms of *Meloidogyne chitwoodi* infection in Russet Burbank potato are highly variable. Root infections are often difficult to detect without the aid of magnifying lenses, and tuber infections may or may not cause external galls to develop. Plants grown in soils wth a high population of *M. chitwoodi* invariably display irregular enlargement of roots, stolons, and tubers. Infections within the tubers of Russet Burbank are always manifested by the occurrence of brown lesions in the cortex and sometimes in the pith. Other cultivars have been observed to respond differently.

*Meloidogyne chitwoodi* is an aggressive parasite which often enters plant organs through wounds but appears quite capable of penetrating directly through the epidermis before it becomes suberized. After gaining entry into host tissues, the larvae may move through the cells but are more likely to move through the intercellular spaces along a direct pathway to the phloem where they feed within intercellular spaces of fundamental parenchyma. It is postulated that glandular secretions from the nematodes are absorbed by parenchymatous cells adjacent to them and that these substances induce metabolic changes which result in the development of coenocytic giant cells. These reactions are first manifested by an increase in the density and granulation of the cytoplasm. Progressive enlargement of nuclei, and especially nucleoli, is followed by multiplication of nuclei and cell enlargement. The synthesis of cytoplasm does not keep pace with cellular growth and the giant cell becomes highly vacuolated.

Cavities in the host tissues begin to form around the second-stage parasitic larvae and continue to enlarge until eggs are laid. This process is presumed to be due to lysis of adjacent cell walls because visible signs of

Figs. 1–6. Potato (*Solanum tuberosum* cv. Russet Burbank) roots infected with *Meloidogyne chitwoodi*. 1) Cross section of potato root with concentration of nematodes (n) at intersection of a lateral root. 2) Nematode (n) with tail protruding from Rhizoctonia (r) lesion on stolon. 3) Section of tuber with nematode (n) in the intercellular space between parenchyma cells; cytoplasm in cell (g) is becoming granulated. 4) Highly granulated cytoplasm in cells (g) surrounding nematode head (n). 5) Nematode (n) feeding in intercellular space of giant cells (g). 6) Cross sections of 4 giant cells (g) with nematode (n) in the intercellular space.




Figs. 7–12. Potato (*Solanum tuberosum* cv. *Russet* Burbank) infected with *Meloidogyne chitwoodi*. 7) Cross section of potato root showing xylem vessels (x) developing around giant cells (g). 8) Obese female (n) within well-defined cavity of tuber cortex 9) Characteristic symptoms of infected potato roots. 10) Female (n) developing within pith (p) of potato stolon. 11) Cross section of mature female (n) with egg mass (e) in potato tuber. 12) Cross section of egg mass (e) showing refractive deposit on surrounding cell wall.

cellular derangement such as would result from external pressure were not observed except in the most delicate roots.

Galls observed on the tubers are formed by hypertrophy of cortical cells rather than by hyperplasia involving an increase in cell number. This conclusion is based on the observtion that those cortical cells which constituted the galls were of greater diameter than those in noninfected tubers, but there was no increase in the average number of cell layers in the cortex. When the population density within the plant organs was high, whole segments of roots, stolons, and tubers were swollen but distinctive galls were rarely delimited.

The uniformity of distribution and development of nematodes within stolons and tubers suggested that under the conditions of these experiments penetration occurred primarily through the stolons before tuberization was initiated and that development of the nematodes and tubers progressed simultaneously. Sections of very small tubers ( < 1-cm-d) displayed an arrangement of infection sites along the vascular traces radiating outward from the stem attachment. As the tubers enlarged, the lesions became uniformly spaced around the vascular cylinder, generally in the cortex but occasionally within the pith. The absence of second-stage juveniles within mature tubers indicated that they are incapable of penetrating the suberized periderm.

The walls of cortical cells abutting the matrix of the egg masses turn brown, presumably due to the oxidation of phenolic compounds. Since this discoloration is limited to host cells in direct contact with the egg matrix and since it appears to be initiated before eggs are deposited, the chemical reactions involved are presumed to be induced by substances in the matrix. These host cells form a protective basket which serves to maintain the integrity of the egg mass and juveniles which emerge from it. These baskets are not susceptible to dissolution by pectinase, and juveniles which emerge from the eggs appear incapable of escape from them until they are broken by an external force. That this mechanism serves to ensure the survival of the species was demonstrated by using extracted eggs from tubers stored at 1 C for more than 2 yr as a continual source of inoculum. The egg masses remained intact and the eggs remained viable throughout this period even though the tubers were dehydrated. Infested tubers left in the field over winter have also been found to contain intact egg masses and viable eggs.

## CONCLUSIONS

Preparasitic juveniles of *Meloidogyne chitwoodi* are attracted to the phloem tissues by substances present in the intercellular spaces. It is possible that they derive their nourishment exclusively from these cellular exudates. Metabolic changes within the host cells which include increased protoplasmic density, enlargement of nucleoli, mitotic division of nuclei, hypertrophy, dissolution of cell walls, and lignification of cortical cell walls are induced by secretions from *M. chitwoodi*.

Histological evidence supported in every observable detail conclusions of Huang and Maggenti (4) with respect to giant cell formation but differ with respect to cell wall breakdown.

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