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A STUDY OF ULTRASTRUCTURAL CHANGES IN TOLERANT AND SUSCEPTIBLE
LINES OF ALFALFA INDUCED BY STEM NEMATODE

(DITYLENCHUS DIPSACI KÜHN)

by

Doris C. N. Chang

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Science

(Ultrastructural Plant Cytology)

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1971

ACKNOWLEDGMENTS

I express my deepest appreciation to Dr. E. J. Gardner, Dean of the School of Graduate Studies at Utah State University, for providing me with the NEA Type IV Graduate Fellowship, without which I would have been unable to complete this degree, and to Dr. W. F. Campbell, my major professor, for his profound interest in this project and the effort and time he has devoted to it. I wish to thank the members of my committee: Drs. G. D. Griffin and H. P. Stanley are acknowledged for their participation, assistance and consultation. To Dr. D. R. McAllister for his constant encouragement and advice, I am grateful. I likewise thank Drs. J. O. Evans and O. S. Cannon for their encouragement and assistance.

A special thanks goes to Mr. W. W. Waite for providing the nematodes and the preparation of the inoculated samples.

Deep appreciation is extended to my family and friends in Taiwan, China, and those in the United States for constant moral support which has made my life more enjoyable during my graduate studies.

Doris C. N. Chang
Doris C. N. Chang

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ABSTRACT

A Study of Ultrastructural Changes in Tolerant and Susceptible
Lines of Alfalfa Induced by Stem Nematode
(Ditylenchus dipsaci Kühn)

by

Doris C. N. Chang, Doctor of Philosophy

Utah State University, 1971

Major Professor: Dr. W. F. Campbell
Department: Plant Science

Fine structural analyses of host tissue (alfalfa, Medicago sativa L.) response to infection by the stem nematode (Ditylenchus dipsaci Kühn) were conducted. Hypocotyl regions were taken on 1, 3 and 7 days after inoculation.

Electron micrographs of infected tissue indicated the types of damage were the same between Lahontan (tolerant line) and Ranger (susceptible line). Only the infection rate (in percent) and degree of damage were different between lines and among the different temperatures (15, 20 and 25 C). The higher the temperature, the more injury resulted. After 3 to 7 days of infection, the symptoms observed were swelling and broken endoplasmic reticulum (ER), distended and broken chloroplasts, loss of nuclear material and bulging and rupturing of nuclear envelopes. Heavily infected cell walls showed more osmophilic substances on one side. Infected cytoplasm contained more ER (both rough and smooth ER), ribosomes, vesicles and Golgi apparatus, suggesting increased metabolic activities.

Lobing nuclei were observed in all treatments. Lipid content varied with temperature in one-day-old seedlings. At 15 and 25 C, electron dense substances were commonly found along the tonoplast, intercellular spaces and on the cell wall. Also some enlarged ER were noted in the non-infected controls at these temperatures.

From the fine structural studies of host tissue it is not possible at this time to determine the nature of resistance of alfalfa lines to nematode infection. More studies at both the biochemical and electron microscopical levels are needed. Further, studies on the activities of the nematodes at the various temperatures during the infection periods would be primarily important.

(89 pages)

INTRODUCTION

Alfalfa (Medicago sativa L.) is by far the most important forage crop in Utah as well as in the United States as a whole. In the state of Utah and the United States, respectively, alfalfa is grown on 450,000 and 29,008,000 acres from which 1,282,000 and 72,476,000 tons of hay are harvested (USDA, 1967). Alfalfa contains about twice as much digestible protein as clover, and about four times as much as timothy-clover hay or corn silage. Moreover, the alfalfa leaf protein may have potential as a source of protein for humans (Pirie, 1966, 1969). This crop is high in mineral content and contains at least ten different vitamins. It is an especially important source of vitamin A. Furthermore, alfalfa encourages the formation of a well-structured and nitrogen enriched soil layer (Martin and Leonard, 1967).

Because alfalfa is a relatively long-lived perennial, once it is established, the farmer or rancher can realize great monetary return for his investment. Many fields of alfalfa, however, are weakened each year by the alfalfa stem nematode (Ditylenchus dipsaci Kühn) which is a microscopic, slender roundworm approximately 1/12-inch long and 1/900-inch wide. Conservative estimates place the loss to this organism in Utah alone at approximately \$1,500,000 annually (Griffin, personal communication). In the United States, this loss is about \$86 million annually (Anonymous, 1971). The loss in terms of dollars of all crops to nematodes in the United States is estimated at \$1.6 billion. This value plus the amount spent for nematode control approaches \$1.7 billion

annually. Once the alfalfa plants are weakened by the alfalfa stem nematodes, bacteria and other pest organisms take over the fields.

Because chemical control of the nematodes is not yet feasible (Griffin, 1967; Sherwood et al., 1967), the only practical means of control is through the breeding and developing of resistant varieties (Grundbacher and Stanford, 1962a; Sherwood et al., 1967). However, Grundbacher and Stanford (1962a) reported that resistance to the stem nematode decreased in Lahontan alfalfa (Medicago sativa L.) with increased temperatures. Griffin (1968) further reported that there was no relationship between host response and numbers of nematodes invading susceptible and resistant alfalfa. Infection occurred at temperatures between 5 and 30 C with maximum infection at 20 C. Ranger (a susceptible line) was susceptible at temperatures from 10 to 30 C, but the susceptibility of Lahontan (a tolerant line) increased slightly as temperature increased from 5 to 20 C and increased sharply at 25 and 30 C. The hypocotyl, cotyledonary petioles, and to a lesser extent the epicotyl were invaded first. It is assumed that changes occur at the ultrastructural level long before visible symptoms are evident. Definition of these changes may elucidate basic and subtle differences associated with tolerant and susceptible alfalfa lines. This study, at the fine structural level, sought to answer the following questions:

1. What are the differences in fine structure between Lahontan (tolerant) and Ranger (susceptible) alfalfa lines?
2. What changes are induced by stem nematode infection in these lines?

3. What are the effects of number of nematodes in relation to host response?
4. Are subtle, but detectable, differences in pectin content of the middle lamellae of host cell wall tissue evident before and/or after infection?
5. Are there subtle, but detectable, temperature effects between the two alfalfa lines?
6. What effect does temperature have on the expression of nematode induced host responses?
7. What detectable changes occur in Lahontan that may account for its altered tolerance to stem nematode attacks with increased temperature?

REVIEW OF LITERATURE

Host Tissue ResponsesStructural studies

It has been established that the first visible symptom of infection by the alfalfa stem nematode Ditylenchus dipsaci (Kühn) is the formation of a gall or a swelling of the stem (Grundbacher and Stanford, 1962a; Radewald and Hart, 1965). The hypocotyl, cotyledonary petioles, and to a lesser extent the epicotyl were invaded first (Griffin, 1968). Infected plants were dwarfed and misshapen, and underground storage structures rotted. Cells near the nematode enlarged and separated; cytoplasm withdrew, and cell walls collapsed, forming cavities in tissues. In aseptic infections with one or two nematodes, leaf palisade parenchyma of alfalfa (Medicago sativa L.) leaflets contained masses of cells with dense, granular cytoplasm (Krusberg, 1961). The conspicuous galling of young alfalfa stems, caused by cell hypertrophy, was visible within 24 hours after inoculation. As early as 12 hours after inoculation, cavities developed in the cotyledonary cortex, the epidermal cells had enlarged, and cell reaction to dyes had changed. Vascular damage was not conspicuous, but did occur in old infections. Necrosis was not common in the young alfalfa tissues. The outstanding feature of these infections was that only a few nematodes affect many cells.

Histopathological studies of D. dipsaci on alfalfa have indicated distinct varietal differences in young seedlings following infection

(Bingefors, 1961, 1962). In tolerant alfalfa, the nematodes penetrated and were found in cavities in the plant (Bingefors, 1961). Cell walls adjacent to such cavities indicated a strong affinity for safranin. The cells in surrounding tissues were intact, and the cellular structure around the cavities showed very little disruption. By contrast, in susceptible alfalfa there was a very pronounced swelling following infection (Bingefors, 1961). The cellular structures were disrupted in the vicinity of the nematodes. Moreover, the middle lamellae of each affected cell appeared to be dissolved, with a gradient of damage extending to the middle lamellae of adjacent and surrounding cells. Further, the cell walls did not exhibit an affinity for the safranin stain. Bingefors (1961, 1962) and Krusberg (1963a) further noted cell enlargement, a disappearance of chloroplasts and an increase in intercellular space in parenchyma tissue following infection. Krusberg (1963a) reported that effects often preceded direct contact of the nematodes with the cells, suggesting that salivary secretions might be diffusing in advance of the nematodes. Many phytoparasitic nematodes, e.g., Rotylenchulus, affect only those cells upon which they feed, or a limited number of cells in the immediate vicinity of the feeding site (Birchfield, 1962). D. dipsaci, however, causes changes in cells at great distances from itself (Dropkin, 1969).

All the above results are based exclusively on observations made with the light microscope and classical histological procedures. A few investigators are beginning to use the electron microscope to study the dynamic aspects of this subject. However, little data (Bird, 1961; Huang and Maggenti, 1969; Chang, Campbell and Griffin, 1970; Paulson and Webster, 1969, 1970) are available on nematode-induced changes in

ultrastructure of the host tissue as shown by electron microscopy. Bird (1961) studied nematode-induced giant cells of tomato root, and found that the giant cells contained many mitochondria, proplastids, Golgi bodies, and a dense endoplasmic reticulum; the cytoplasm resembled that in meristematic cells during interphase, and appeared to be in a metabolically-active condition. Huang and Maggenti (1969) reported that the giant cells became multinucleate as a result of karyokinesis without subsequent cytokinesis. Paulson and Webster (1970) took sequential samples of tomato roots and observed that giant cell development was characterized by changes in the cell walls, cytoplasm, and organelles. Electron dense cytoplasm and the decreasing size of the large central vacuole were first observed. Then some regions of the giant cell walls became excessively thickened, while other parts remained relatively unchanged. In old giant cells, projections of wall material extended far into the cytoplasm. Microtubules occurred adjacent to the walls in all stages of giant cell development. As the giant cell developed, the nuclei became swollen and irregularly shaped; nucleoli became enlarged, and clumps of chromatin accumulated near the distinct nuclear membrane. Plastids did not change significantly, whereas mitochondria appeared to form vesicles, and the number of dictyosomes increased during the later stages of giant cells. In still later stages of giant cell development, smooth and rough endoplasmic reticulum were extensively produced in the form of vesicles and cisternae. Other histopathological studies of host tissues have been conducted either on nematodes other than D. dipsaci or on other host tissues (Bird, 1962; Blake, 1962a, 1962b, 1966; Christie, 1936; Dijkstra, 1957; Endo, 1964, 1965; Hussey and Krusberg,

1968; Muse, 1969; Piegat and Wilski, 1965; Siddiqui and Taylor, 1969, 1970; Van Gundy and Kirkpatrick, 1964).

Biochemical studies

Considerable research has been done on biochemical changes that occur in host tissues (alfalfa, Medicago sativa L.) following infection with the stem nematode Ditylenchus dipsaci (Cole and Krusberg, 1967; Cutler and Krusberg, 1968; Grundbacher and Stanford, 1962b; Howell and Krusberg, 1966; Krusberg, 1960a, 1961, 1963a, 1963b, 1964, 1967; Krusberg and Blichenstaff, 1964; Sherwood and Huisingsh, 1970; Viglierchio, 1971).

Plant parasitic nematodes have enzymes that degrade modified cellulose. These enzymes are absent or present only in low concentrations in microbivorous forms (Dropkin, 1963; Krusberg, 1960b; Myers, 1965). The alfalfa stem nematodes are thought to secrete pectinases, which dissolve the middle lamellae between cells, and other irritants, which stimulate the formation of the actual galls. It has been demonstrated that the homogenates of D. dipsaci contained polygalacturonase (Krusberg, 1964, 1967; Riedel and Mai, 1971) and a pectin-transeliminase (Krusberg, 1964, 1967). Extracts of a population of D. dipsaci from alfalfa (Krusberg, 1960b) contained pectinmethylesterase. Goffart and Heiling (1962) claimed a pectinase was secreted into water and oatmeal agar by D. dipsaci. Sherwood and Huisingsh (1970) found pectic enzymes in Atlantic alfalfa buds infected by D. dipsaci. Actual evidence that pectinases are effective in destruction of the middle lamella, however, is not yet conclusive. Krusberg (1963a) could not macerate alfalfa tissue with extracts of D. dipsaci nor could he find histochemical

evidence for pectin removal. Krusberg (1967) concluded that widespread degradation of pectic compounds in the middle lamellae of alfalfa plants by nematode pectinase was not a factor in pathogenesis. Also, these enzymes occurred in phytonematodes which did not cause cell separation in their hosts.

The evidence for nematode-induced changes in plant growth regulatory substances is fairly strong (Cutler and Krusberg, 1968; Viglierchio and Yu, 1965). Yet, it has not been possible to distinguish between the introduction of a growth regulator by the nematode, and the induction of changes in levels or gradients of endogenous growth regulators of the host, or inhibition of enzymes that degrade growth regulators (Dropkin, 1969).

Recent work on decapitated pea epicotyls indicated that IAA played a central role in the control of cellulase synthesis (Fan and Machlachlan, 1967). The authors postulated that IAA depressed that part of the genome which synthesized RNA for cellulase, and therefore acted on transcription. The cellulase presumably moves to the cell walls where it is rapidly inactivated. The enzyme may change wall plasticity and may influence cellulose deposition by providing sites for attachment of new cellulose. Many of the effects of nematodes are parallel to those reported in the work on IAA. Hyperplasia, hypertrophy, and the breakdown of cell walls all developed in decapitated pea epicotyls treated with endogenous IAA. The pattern of cell wall lysis in D. dipsaci infections of stems does not seem to result from passive responses to enzymes emanating from the nematodes, but rather suggests an active host participation in response to some controlling force from the parasite (Dropkin, 1969).

Many other biochemical studies of host tissues infected by nematodes have been done (Acedo and Rohde, 1970; Bird, 1961; Colotelo and Ward, 1961; Johnson and Viglierchio, 1969; Muse et al., 1970; Orion and Minz, 1968; Wilski and Giebel, 1966; Veech and Endo, 1969).

Mechanism of galling

The physiology of plant gall or tumor formation has intrigued investigators and has been studied by them for years. Yet no definite pattern has been revealed to explain the observed growth changes.

Sayre (quoted by Mountain, 1960) suggested a mechanism involving IAA for gall induction by root-knot nematodes. After penetration into root tissues, larvae supposedly secreted proteolytic enzymes which hydrolyzed IAA protein complexes releasing IAA to stimulate gall formation. Sayre further suggested that nematode proteases hydrolyzed plant proteins, releasing tryptophan which was transformed by plant enzymes to IAA. Leopold (1960) reported that auxins promoted hydrolysis of plant cell proteins.

Krusberg (1961) found free tryptophan in alfalfa shoot galls caused by D. dipsaci but none in healthy tissues and postulated that it might be involved indirectly in gall induction. Cutler and Krusberg (1968) reported a plant-growth regulator with indole-acetic methylester (IAM) characteristics in the extracts of D. dipsaci. They estimated it to be present at a rate of 1 µg/100 mg dry wt of nematodes. Viglierchio and Yu (1965) noted auxin inactivators in Ditylenchus and observed a reduction of auxins in alfalfa-shoot tips infected with D. dipsaci. In the case of nematodes there is no convincing evidence for the origin of auxins, whether in the reacting host cells or in the parasites

themselves. There have been, however, speculations that the host-cell auxin level is augmented by a nematode injection fluid secreted through the stylet, which releases plant-bound auxin. Also, it has been suggested that the auxin present within nematodes occurs by the intake and accumulation of that normally occurring in the plant cell (Mountain, 1960). Recent experiments incorporating radioactively labelled IAA in culture media, however, have indicated that D. dipsaci and D. trififormis were unable to take up significant amounts of the label (Cutler and Krusberg, 1968). It is evident, therefore, that not all auxin-containing nematodes would be able to obtain their free auxins from the host cells.

Krusberg (1963a) stated that mere hydrolyzed products of proteins would not seem sufficient to stimulate gall formation, since all plant nematodes probably secrete proteases into plant cells, but relatively few species stimulate galling.

It is tempting to explain the extensive cell lysis observed around D. dipsaci by suggesting that the nematodes emit hydrolytic enzymes into plant tissues. Pectinases are the enzymes most frequently proposed (Goffart and Heiling, 1962; Krusberg, 1964, 1967; Sherwood and Huisingsh, 1970). No strong consistent evidence, however, has yet been found to support this claim (Krusberg, 1963; Dropkin, 1969).

Huang and Maggenti (1969) noted that giant cell nuclei in the roots of Vicia faba infected with Meloidogyne javanica were derived from repeated mitoses of the original diploid cells without subsequent cytokinesis. Owens and Novotny (1960) and Littrell (1966) reported that both cell-wall dissolution and mitoses were responsible for giant cell formation.

Nature of resistance

Resistance is a characteristic of the host plant. However, various environmental factors such as temperature, soil type, host nutrition, age of the plant and previous cropping history may alter the expression of resistance (Rohde, 1960).

Temperature effects on the rate of penetration and reproduction of nematode in the host have been found in Ditylenchus (Griffin, 1968; Grundbacher and Stanford, 1962a; Hanna and Hawn, 1965), Meloidogyne (Tyler, 1933), Pratylenchus (Mountain, 1957), and Trichodorus (Rohde and Jenkins, 1957).

Several authors have noted that soil type has an effect on pathogenicity of plant-parasitic nematodes. Bessey (1911) transplanted plants infected with root-knot nematodes into pots containing susceptible hosts. In heavy clay soils, susceptible plants were not attacked.

Potassium has been found to be associated with the resistance to root-knot nematodes (Bessey, 1911; Otief, 1953; Crittenden, 1954). In several plant diseases, in which enzymes have been implicated, increased calcium (Ca++) content of plant tissue was correlated with increased resistance (Alghisi and Ferraza, 1967; Bateman and Lumsden, 1965; Bateman and Millar, 1966; Corden, 1965; Thomas, 1966). Probably Ca++ forms enzyme-resistant complexes with polygalacturonates in the middle lamellae.

Older plants seem to be more resistant to nematode attacks than young seedlings, such as tea plants to Meloidogyne infection (Loos, 1953).

Previous cropping may be a factor in selecting out more pathogenic strains of nematode species. In Australia, tomato varieties resistant to M. javanica break down after five years of continuous cropping in the same fields (Giles and Hutton, 1958).

According to Rohde (1960), the mechanisms of resistance to nematodes may be summarized as follows:

1. Root secretions as a mechanism of resistance (Winslow, 1955)
2. Resistance to penetration (Sasser, 1954)
3. Internal factors involved in resistance (Seinhorst, 1956a; Bingefors, 1957; Dijkstra, 1957)
4. Resistance related to a change in the sex ratio (Cobb, Steiner and Christie, 1927; Williams, 1956)
5. Production of resistant factors in plants (Riggs and Winstead, 1958; Swink and Finkner, 1956)

Studying the resistance in clover and alfalfa to the stem nematode, Ditylenchus dipsaci, Bingefors (1957) suggested the possibility that there were slight differences in the chemical composition of the middle lamellae in resistant red clover varieties, and a few major genes were mainly responsible for the resistance.

The resistance of Lahontan to D. dipsaci was expressed as reduced swelling of the host tissue and reproduction of the nematode, but not as reduced penetration. This has been agreed upon among several investigators such as Bingefors (1957), Griffin (1968), Sherwood and Huis Singh (1970), and Wynne and Busbice (1968).

Sherwood and Huis Singh (1970) studied calcium nutrition and resistance of alfalfa to D. dipsaci and found that divalent/monovalent cation content of tissues modifies the expression of resistance by

alfalfa buds. Therefore, they suggested that the differences in resistance of varieties might be related to differences in their ability to accumulate cations. The basic nature of resistance, however, remains unknown.

Alfalfa Stem Nematode--*Ditylenchus dipsaci* Kühn

The first record of *Ditylenchus dipsaci* is that of Schwertz in 1855 (Thorne, 1961), who recorded certain diseased conditions of rye, oats, clover, and other crops but did not observe the nematodes. Kühn (1857) discovered *D. dipsaci* (*Anguilla dipsaci*) in teasel, *Dipsacus fullonum* L., and recognized those in rye as being similar in form. In 1881 Kühn first recognized alfalfa as a host.

The bulb and stem nematode, *D. dipsaci*, is a complex group of populations that attack several hundred different plants varying from fuller's teasel, alfalfa, clover, and weeds to the bulbs of tulips, narcissi, hyacinths, onions, and garlic (Thorne, 1961). This nematode is very widely distributed, but it is more serious as a pest in cooler and moister climates (Winslow, 1960).

The genus *Ditylenchus* was established by Filipjev in 1934. With the erection of this genus, a large number of species previously included in *Tylenchus* or *Anguillulina* were transferred to *Ditylenchus*. *Ditylenchus dipsaci* (Kühn, 1857) was designated as the type species by Filipjev. At that time the genus comprised 14 species. In 1949, Thorne amended the generic diagnosis. At present the genus includes more than 30 species.

One of the outstanding features of *D. dipsaci* is the remarkable race specialization, there being a dozen or more known races or biotypes

morphologically indistinguishable but each having definite host preferences (Winslow, 1960). Another feature is that anabiosis developed to a high degree in D. dipsaci. Fielding (1951) reported that these nematodes were 100 percent alive after 20 years dormancy in Hypochoeris radicata. The anabiosis of D. dipsaci is probably confined to the fourth stage larvae or preadult. We have no exact information on the physiology of dormant nematodes but it is generally presumed that metabolism proceeds very slowly, the fat stored in the body being gradually used up (Winslow, 1960).

The nematode is disseminated by unclean seeds, bulbs, cuttings, machinery, and irrigation water (Thorne, 1961).

Control of the nematode can be done by: (a) crop rotation (sugar beets, grain, corn, potatoes, tomatoes and other common crops are suitable) and (b) resistant varieties (in alfalfa, Resistador, Lahontan, Talent and Kayseri).

Structural studies

Filipjev (1934) and Thorne (1949) were prominent early researchers who studied this genus of nematode. The description made by Thorne in 1949 is still the most widely used reference for this nematode.

Recently Evans and Fisher (1970) studied the excretory systems of three Ditylenchus spp. by light microscopy. Yuen (1967, 1968) is the only one who has studied D. dipsaci by electron microscopy. Other works that have been conducted on the ultrastructure of nematodes are on Rhabdits (Beams and Sekhon, 1967), Ascaris (Bird and Deutsch, 1957), Meloidogyne (Bird and Rogers, 1965), Trichodorus (Hirumi et al.,

1968), Heterodera (Wisse and Daems, 1968), Anguina (Wu, 1968), and others (Johnson, Van Gundy and Thomson, 1970a, 1970b).

Biochemical studies

The majority of biochemical work concerns hydrolytic enzymes present in nematode homogenates. Myuge (1957), Tracey (1958), Krusberg (1960b, 1964), Muse et al. (1970), Riedel and Mai (1971) found that the hydrolytic enzymes present in the D. dipsaci homogenates were amylase, cellulase, chitinase, glucosidases, pectinases and protease. Krusberg (1960b), Hussey and Krusberg (1971) and Dickson, Huisinigh and Sasser (1971) detected other enzymes such as hexokinase, glucosephosphate isomerase, phosphoglyceromutase, enolase, lactic dehydrogenase, malate dehydrogenase, acid and alkaline phosphatase, esterase, isocitric dehydrogenase, oxalosuccinic decarboxylase and fumarase in D. dipsaci.

Eriksson and Granberg (1969) have attempted to study the D. dipsaci races by using electrophoresis in acrylamide gel. Siddigui and Viglierchio (1970) have studied the effects of gamma irradiation on the motility, infection, reproduction and morphology of D. dipsaci.

MATERIALS AND METHODS

Preparation of Nematodes

The method for nematode sample preparation described by Thorne (1961, p. 41-52) was followed:

Nematodes in alfalfa stems were collected from a field located in Cache Valley, Utah, and were stored at 5 C overnight. Leaves were removed, and stems were cut into 1/8 or 1/4 inch long sections. The pieces of stems were placed in distilled water in a Baermann-funnel on which a David Bradley milk filter disc 6 inches in diameter was placed on a 4 mesh wire. The nematodes moved from the alfalfa tissue into the water, through the filter and settled in the bottom stem of the funnel. After an hour, the distilled water in the bottom of the Baermann-funnel, which contained the nematodes, was drained off. This suspension was stored at 4 C for 3 to 4 hours to precipitate the nematodes. The number of nematodes was then determined microscopically by placing a drop of suspension on a watch glass with a sub-divided bottom. Dilutions were made until each drop contained about 10 nematodes. When stored at 5 C, the nematodes remained viable for at least 7 days.

Preparation of Alfalfa Seedlings

Seeds of alfalfa (Medicago sativa L.) cvs Ranger (a susceptible line) and Lahontan (a tolerant line) were scarified with 100 grit silicon carbide water sandpaper and germinated for 36 hours under

room temperature in petri dishes lined with filter paper and moistened with distilled water.

Inoculation and Plantings

The germinated alfalfa seeds were placed in a 10 x 14 inch metal container (100 seeds/container) of sterilized Provo sand, with a soil moisture of approximately 100% field capacity. One cc of nematode suspension containing either 20 or 100 nematodes was pipetted directly over the germinating seed. Seeds were covered with 0.25 inches of soil. The containers were placed in growth chambers at temperatures of 15, 20 and 25 C. Sixteen hours of light at about 3,000 ft-c were maintained.

Fixation

General

Samples were harvested at 1-, 3- and 7-day intervals. Hypocotyl sections (1 mm) of infected alfalfa seedlings showing visible symptoms and similar regions in non-infected controls were harvested and fixed by two methods:

(1) Karnovsky's fixative in cacodylate buffer pH 7.2 (1965) for 4 hours at room temperature, rinsed with cacodylate buffer and kept overnight in a refrigerator. Fixed secondarily with 2% osmium tetroxide in cacodylate buffer for 1 hour and washed with two changes of cacodylate buffer at 15 minutes each.

(2) One part of 5% glutaraldehyde, 1 part of 5% osmium tetroxide together with 2 parts of 0.2 M phosphate buffer, pH 7.2 (Trump and Bulger, 1966) for 1 hour on ice, and washed with phosphate buffer.

All samples were dehydrated with an ethanol-propylene oxide series for 15 minutes each and embedded in Epon 812 (Pease, 1964) with six parts of solution A and four parts of solution B. Polymerization was carried out by placing the specimens in Beem capsules and incubating at 45 and 60 C for 24 hours each.

Staining pectin of the cell wall

Hypocotyl sections of alfalfa seedlings were harvested and fixed in Karnovsky's fixative for 4 hours. Sections were fixed secondarily with 2% osmium tetroxide for 1 hour, rinsed with cacodylate buffer, and dehydrated in 35% ethanol as usual. The method for staining pectins for electron microscopy was followed (Albersheim and Killias, 1963). The samples were washed twice in 60% ethanol for 10 minutes each, and then put into 60% ethanol alkaline hydroxylamine solution for 1 hour at 4 C. They were then washed in 0.1 M HCl for 15 minutes, put into 2% FeCl_3 for 1 hour, then dehydrated with acetone-propylene oxide series and embedded in Epon 812.

Sectioning, Staining and Examining

1 μ sections for light microscopy

After sufficient polymerization and cooling of the Epon, the blocks were properly trimmed to the tissue. For orientation purposes 1 μ -thick sections were cut with a glass knife on a Sorvall MT-2 ultramicrotome.

Slides previously cleaned and stored in 60-95% ethanol with a few drops of 0.1 N HCl were air-dried and a drop of distilled water was added. A camel's hair brush was wetted and used to take the section

from the water trough to the slide. The slide was placed on a hot plate (40 C). After 15 minutes, Richardson's stain (Richardson, Jarett and Finke, 1960) was applied on the section and the slide was covered with a petri dish on the hot plate. After 1 or 2 minutes, excess stain was rinsed from the slide with a stream of distilled water. The slide was dried again on the hot plate and then examined with a light microscope.

Thin sections for electron microscopy

Blocks were trimmed to approximately 0.5 to 1.0 mm on a side. Sections were cut with a glass or diamond knife on a Sorvall MT-2 ultramicrotome. Only light gold or silver sections were placed on acetone-cleaned 3 mm 200 mesh copper grids. Sections were stained with saturated aqueous uranyl acetate at 60 C for 12 minutes (Watson, 1958) followed by lead citrate at room temperature for 3-5 minutes (Reynolds, 1963). Sections were examined with a Zeiss EM-9A and significant observations recorded photographically.

RESULTS AND DISCUSSION

Morphology

Seedling growth was directly influenced by temperature. No visible symptoms of nematode infection, however, were observed at any temperature in either Ranger or Lahontan 1 day after inoculation. Three days after planting, swollen hypocotyls could be seen at 20 C and 25 C in both cultivars. Visible symptoms were observed in seven-day-old seedlings at all temperatures (Figures 1, 2, and 3). The degree of swelling, however, was higher in Ranger (susceptible line) than in Lahontan (tolerant line) at all ages. These observations confirm those of Griffin (1968).

Light Microscopy

Figure 4 shows the cross section of control alfalfa hypocotyl. Figure 5 indicates that the nematode infection is only in the cortical region. Light microscopy of the infected areas in both Ranger and Lahontan substantiated the histopathological studies conducted earlier by Bingefors (1961, 1962) and Krusberg (1961).

Electron Microscopy

Nematode penetration of both Ranger and Lahontan occurs at temperatures between 5 and 30 C with maximum infection at 20 C (Griffin, 1968). Thus, 20 C was the temperature chosen for the most detailed study. The infection rate was lower at 15 C than at 20 C in Ranger.

Also the susceptibility of Lahontan increased sharply at 25 C. Thus, they were chosen as the other two temperatures for the purpose of comparing the temperature effects.

20 C

The swelling of hypocotyls in susceptible Ranger was usually visible after 3 to 7 days of inoculation. This is somewhat longer than the 24 hours reported by Krusberg (1961), and may indicate a differential cultivar response.

Control plants in both Ranger and Lahontan exhibited normal cell wall and cytoplasm. The cytoplasm contained a nucleus, mitochondria, ER, ribosomes, Golgi apparatuses, and vacuoles (Figures 6 and 22). Plasmadesmata were common on the cell walls (Figures 9 and 22). Control plants also contained normally developing chloroplasts bearing stroma, thylakoid units of the grana, and starch grains. Those chloroplasts devoid of starch grains were lens-shaped (Figures 9, 12 and 22), and those containing starch grains usually became oval or nearly round in shape (Figures 9, 11, 23 and 24).

Crystalline and paracrystalline arrays of electron dense granules were observed in the chloroplasts of control and infected plants at all ages and all treatments in both Ranger and Lahontan. This complex has been referred to as phytoferritin (Hyde et al., 1963; Jacobson, Swift and Bogorad, 1963; Robards and Robinson, 1968; Robards and Hympherson, 1967). These workers suggested that the phytoferritin was an iron-protein complex that allowed the plant to store iron in a non-toxic form. This theory is in accord with the phytoferritin that is most commonly found in differentiating plastids and often in meristematic cells and may provide iron for further development in

young cells, including the elaboration of photosynthetic material (Hyde et al., 1963; Robards and Hympherson, 1967). Robards and Robinson (1968) found that phytoferritin was completely unaffected by the treatments of deoxyribonuclease or ribonuclease, thus excluding the possibility that the inclusions were viral particles.

On the first day after inoculation with *D. dipsaci*, infected Ranger and Lahontan exhibited more lipid bodies per cell than did the controls (Figures 10, 11, 23 and 24). Sheetz and Crittenden (1966) histochemically demonstrated a greater abundance of lipids in the giant cells of rootknot-susceptible soybean than in rootknot-resistant plants. The number of lipid bodies, however, was less in Lahontan than in Ranger. No other changes were noted in the fine structure at this age.

Three days after planting, however, the chloroplasts in infected cells of Ranger were swollen and the outer chloroplast membrane was ruptured. Swelling of the thylakoid units was also noted (Figure 13). Moreover, in a few cases, the nucleus was swollen and the outer nuclear membrane was ruptured. Leakage of nuclear content was also observed (Figure 15). The permeability of the membranes had obviously been altered by the infection of nematodes, probably through enzymatic reactions. Moreover, compared to the controls, the cytoplasm of the infected cells became more dense with an abundance of ribosomes and numerous cisternae of smooth ER (Figures 16 and 17) and may indicate an injury response. Further, one side of the cell wall became more osmiophilic (Figure 17), suggesting chemical changes of the cell wall have been induced by nematode infection. In Ranger, the host tissue at the infection site, i.e., next to the nematode, showed a complete

loss of cytoplasm and an osmiophilic cell wall at the side adjacent to the nematode (Figure 18). These suggest that at the infection site, the injury responses are caused by mechanical suction and the enzyme secretion of the nematode.

With few exceptions, three days after planting Lahontan contained normally developing chloroplast, cytoplasm and nucleus even in the infected cells (Figures 25, 26 and 27). Lahontan alfalfa has been regarded as tolerant to nematode infection; therefore, less damage responses were expected. In a very few cases, however, when the cell was heavily infected, the infection responses at the fine structural level were the same as those in Ranger, i.e., ER became swollen in sausage-like chains, numerous vesicles formed, chloroplasts swelled, and the outer membranes of the chloroplasts ruptured. Swelling of thylakoid units of the grana was also noted (Figure 28). One important feature about Lahontan, even at the infection site, was that the cell wall remained unchanged (Figure 28).

In Ranger, seven days after infection, partial to complete disruption of chloroplasts was evident (Figure 19). Abundant "vesicles," which may have formed from ER, were found in the cytoplasm and the cell wall broke down causing a mixing of cytoplasm of adjacent cells (Figure 20). In Lahontan, seven days after infection, most chloroplasts were normal (Figures 30 and 31). Heavily infected areas, however, showed symptoms, such as leaking of nuclear content, swollen ER (Figure 32), and broken chloroplasts (Figures 32 and 33), as were observed in Ranger. But most cell walls still remained normal (Figure 32).

At all temperatures and all treatments, especially in one-day-old seedlings, large amounts of electron dense masses were present in both

cytoplasm and vacuoles (Figures 34 and 35). They varied in size and shape and have been identified as protein bodies (Bagley et al., 1963; Horner and Arnott, 1965). As the seed germinated, the protein bodies swelled and developed cavities within. Later these swollen bodies broke up into fragments which were digested and disappeared, and in seven-day-old seedlings very few protein bodies were visible. Lipid and protein bodies are the two major reserve foods stored in alfalfa seeds. Both were digested and disappeared gradually with increased age of the seedlings.

Myelin figures were found in some treatments (Figure 35) and apparently have no relation with the temperature treatment and nematode infection. Curgy (1968) suggested that myelin figures were seen only after aldehyde fixation or aldehyde/OsO₄ double fixation and indicated that fixation with OsO₄, with KMnO₄ or with a combined glutaraldehyde-OsO₄ mixture did not reveal such structures.

At 20 C, some chloroplasts were observed containing numerous small vesicles along the periphery of the chloroplasts in control and treated of both cultivars (Figure 35). Laetsch (1968) observed tubules of the peripheral reticulum on the chloroplasts in dicotyledons which possess the C₄-dicarboxylic acid pathway of photosynthetic CO₂ fixation. According to J. H. Hillard (personal communication, 1971), the presence (C₄) or absence (C₃) of parenchymatous bundle sheath on a leaf cross-section is the most reliable structural criterion for C₃ and C₄ plants. On this basis, then, alfalfa should be classified as a C₃ plant. However, tubules of peripheral reticulum were observed in the alfalfa chloroplasts. Thus, peripheral reticulum or vesicles may have no relation with type of photosynthetic pathways.

All of the above treated plants were inoculated with 20 nematodes per seedling. For a comparison of the host response and numbers of nematode each seedling was inoculated with 100 nematodes. Fine structural studies agreed with the statement made by Griffin (1968), i.e., the response of host tissue inoculated with 100 nematodes per plant did not show any more severity of infection than those inoculated with only 20 nematodes (Figures 37, 38 and 39). Griffin (1968) speculated that this response is due to the ability of D. dipsaci to invade resistant as well as susceptible alfalfa plants. He also found that the nematode population gradually declined in tolerant alfalfa, which agrees with the findings of Bingefors (1951) and Grumbacher and Stanford (1962a). In Ranger, the cytoplasm at the infected site was plasmolyzed (Figure 37). In Lahontan, slightly infected cells showed slight swelling of the ER only. In heavily infected cells, osmiophilic cell walls, broken and swollen ER, and numerous vesicles were observed (Figure 39).

The comparison of pectin content in the cell wall is shown in Figures 40 to 47. No obvious difference was noted in the pectin content of cell walls in treated or non-treated control plants at all ages in either Ranger or Lahontan. This finding agrees with the statement made by Krusberg (1963b, 1967) that there is no histochemical evidence from light microscopy for pectin removal, and the degradation of pectic compounds in the middle lamellae of alfalfa by nematode pectinase was not a factor in pathogenesis.

In summary, at 20 C, except for the lower degree of responses in Lahontan than in Ranger, almost no difference in structure has been observed between Lahontan and Ranger. The organelles showing damages

after infections were ER, chloroplasts, and nucleus. The lobing of nuclei was often observed in both treated and controls; therefore, it is not due to nematode infection and fits the interpretation made by Öpik (1966) that nuclear lobing is an indication of intense nuclear activity. Since lipid content is also found to be associated with nematode resistance in soybean (Sheetz and Crittenden, 1966), more investigations on lipids should be done. Studies on protein bodies at the same time also should not be neglected, for both protein and lipid are important components of the membranes (Davson and Danielli, 1952; Robertson, 1959). In light microscopic studies, Bingefors (1961, 1962) stated that the middle lamellae of each affected cell appeared to be dissolved. These fine structural studies, however, show that up to seven days after infection, the middle lamellae were not dissolved, and one side of the cell wall becoming more osmiophilic was the only response that was observed (Figures 13, 17, 18 and 39). Observations longer than seven days after inoculation should be carried out. In Lahontan, even in the infection sites, most cell walls remain unchanged. It has been speculated by Bingefors (1961, 1962) and Seinhorst (1956b) that differences in the chemical composition of the middle lamellae might account for resistance. Thus, these differences should be investigated in the future.

15 C

The basic fine structure of alfalfa hypocotyl tissue in plants grown at 15 C was similar to those grown at 20 C. The differences noted, however, were as follows. The lipid content in one-day-old non-infected plants was higher than in infected plants of both Ranger and Lahontan. This was a reverse from that observed at 20 C, and may

represent a temperature effect. Chemical analysis should further elucidate this point. Also, electron dense substances were often found in a somewhat continuous manner along the tonoplast (Figure 48), or in a semi-continuous manner in intercellular spaces (Figure 49). Further, some of the electron dense substances became separate spots distributed along the cell wall (Figure 50). At 20 C, these electron dense substances were only observed in a very few cases, but in 15 C they occurred rather frequently. Further investigation into their chemical nature and function would be most interesting.

The degree of damage in both Ranger and Lahontan to stem nematode infection at 15 C was less than that observed at 20 C. Most chloroplasts and nuclei were normal. Chloroplasts were, however, occasionally observed with peripheral vesicles formed from invagination of the inner membrane (Figure 51). This agrees with the chloroplast structure observed by Laetsch (1968) and Klein and Pollock (1968). Swollen ER were observed in both Ranger and Lahontan at all ages (Figures 53 and 62). Since there is no osmometer on campus, whether the swollen ER is due to unfavorable osmolarity of the fixating solution or due to the cool temperature effect is not distinguishable. It is entirely possible that any abnormal environmental condition (e.g. 15 C is somewhat cold for the growth of alfalfa seedlings) is able to cause the swelling of ER.

Generally, none of the nematode infected plants showed any visible symptoms in one-day-old seedlings at 15 C. Light and electron micrographs likewise showed normal cell structure.

In three-day-old seedlings, most chloroplasts near the infection sites were free of damage (Figures 55, 56, 57 and 58). In both Ranger

and Lahontan, cell walls usually were normal (Figures 50, 55 and 57). Endoplasmic reticulum in the infected areas were slightly swollen (Figures 56 and 58), and some were broken into short pieces (Figure 56). Numerous vesicles were also present in the cytoplasm (Figure 56). Chloroplasts contained peripheral vesicles and phytoferritin complexes in both infected and non-infected plants of all ages (Figures 56, 57, 58 and 59), indicating that the nematode infection has no effect on the peripheral vesicles and phytoferritin of chloroplasts. Lobed nuclei were observed in seedlings of all treatments (Figure 57). At the infection sites, the chloroplasts showed the same responses in both cultivars as those grown at 20 C (Figures 13 and 59). The nuclei in cells at the infection sites, however, exhibited less damage than those from plants grown at 20 C (Figures 15 and 59). Endoplasmic reticulum in this area exhibited exactly the same symptoms as those observed at 20 C (Figures 28 and 59).

Seven days after inoculation, the infected nucleus contained less nuclear material than the control (Figures 60 and 61). Chloroplasts and ER showed similar symptoms previously observed at 20 C. Slight swelling of ER was observed in control plants at this temperature (Figure 62), indicating possible low temperature effect. The cytoplasm of infected plants usually contained more actively secreting Golgi apparatus and vesicles (Figure 63). Since infected plants showed visible galling, it is logical to find more Golgi apparatus and vesicles in infected cytoplasm because Golgi vesicles are thought to be participating in cell wall formation (Larson, 1965; Rosen et al., 1964).

In Ranger, the severity of nematode infection at 15 C was slightly lower than that at 20 C, while Lahontan was much lower than Ranger (infection rate was 20% and 92%, respectively).

At 15 C, fine structural studies showed no qualitative difference between Lahontan and Ranger as compared to those at 20 C. Lipid content was the only quantitative difference found between 15 and 20 C. Quantitative histochemical or chemical analysis would be more reliable for the measurement of lipid quantity than electron micrographs.

Since few cell walls showed any change upon nematode infection at this temperature, it, therefore, emphasizes the need for determining the differences of chemical composition in cell walls of Lahontan and Ranger, and how this relates to different temperatures of the host tissues.

When the cells were infected, however, the type of damage was the same in both lines and not much different from those observed at 20 C, indicating cell walls may play a very important role in the tolerance of nematode infection in alfalfa.

25 C

At 25 C, the tolerance of Lahontan to nematode infection was much lower than at 20 C, the infection rate being 70% and 20%, respectively. Ranger showed nearly 100% infection (Figure 2) at this temperature. This agrees with the work of Griffin (1968). The degree of damage was slightly lower in Lahontan than in Ranger. With few exceptions, most of the damage responses were similar between both lines. One striking feature of the high temperature effect was the exhibition of partial to an all-round heavily swollen nuclear envelope in one-day-old

nematode infected Ranger seedlings (Figures 65 and 66). This suggests that secretion is emitted by the invading nematodes. Together with the cellular symptoms observed in the areas far removed from the invading nematodes, it presents strong evidence that the damages are caused by the salivary secretions diffusing in advance of the nematodes (Krusberg, 1963a). Numerous plasmadesmata were present (Figures 66 and 67). These symptoms were not observed in infected Lahontan plants.

In one-day-old Ranger seedlings, swollen ER in sausage-like chains were observed in many cells of the treated samples and some cells of non-infected control (Figures 68 and 69), suggesting a possible high temperature effect. Configurations of the swollen ER were similar to those of nuclear envelopes (Figures 65, 66 and 69), indicating a close relation between these two organelles. Separate and continuous electron dense substances similar to those observed at 15 C were also present quite often on the cell walls and tonoplasts in all treatments (Figures 65, 66, 68 and 70). Their function and chemical nature remain unidentified at the present time. Zee and O'Brien (1970) found similar electron-dense adcrusting substance deposited in the regions of the wall that lay between the primary pit fields in cells of wheat. Whether or not only special areas of the cell have these substances is not known.

At 25 C, more Golgi apparatuses and Golgi vesicles were noted in all treatments (Figure 71). Morphologically, the hypocotyls of 25 C treated seedlings were much bigger than the controls and those treated at 15 or 20 C (Figures 2 and 3). Thus, it is logical to find more Golgi apparatuses, Golgi vesicles and more cell wall surfaces in the differentiating young seedlings. In one-day-old Lahontan seedlings,

swollen ER (Figure 76) was the only symptom that had been detected. Lipid content was higher in Lahontan than Ranger in one-day-old samples. At three temperatures, three different patterns of lipid contents have been observed. Whether these are due to the true temperature effect remains to be determined.

Three days after planting, infected tissues in both Ranger and Lahontan exhibited the same responses as those at 20 C, such as swollen chloroplasts (Figure 72). At this age, the appearance of the cell wall showed the same characteristics as those at other temperatures. No swollen nuclear envelopes were observed at this age. Whether or not the swollen nuclear envelopes recover from the damage or break into pieces such as swollen ER is not known.

Seven days after inoculation, round and partially broken or completely disrupted chloroplasts and swollen ER were noted (Figures 74, 75 and 76). Mitochondria were abnormal in infected Ranger (Figure 74). Vascular bundle cells were not affected by the nematode infection in any treatment. The size of these cells was much smaller than the cortical cells (Figure 78). They also contained nucleus, mitochondria, chloroplasts, ribosomes, ER, cell walls, Golgi apparatuses and vacuoles.

CONCLUSION

In conclusion, at a given temperature, no qualitative differences in the fine structure of Lahontan and Ranger have been observed. Quantitatively, the amount of lipid bodies, ER, ribosomes, Golgi apparatuses and Golgi vesicles showed differences between treated and control within and between temperatures. At all temperatures, ER, chloroplasts and nuclei were the organelles that revealed recognizable symptoms after nematode infection at 3 and 7 days. Some of the cell walls in the heavily infected areas also became more osmophilic.

Except for infection rates (in percent) and the varying degree of damage at 15, 20 and 25 C, the heavily infected tissues showed the same types of damages.

This fine structural study does not provide a satisfactory explanation as to why Lahontan is tolerant and Ranger is susceptible or why the infection rates change at different temperatures. It is possible that the changes in infection rates are due to changes in nematode invading abilities at different temperatures.

For further investigations, many approaches can be applied to evaluate the tolerance of plants to stem nematode attacks, such as studying the changes in chemical composition of cell wall with various temperatures, analyzing the lipid and protein content of host tissues, and--most important of all--examining the nematode activities and amount of enzyme secretion under different environmental conditions. Longer infection times (over seven days) also need to be conducted.

ABBREVIATIONS, LEGENDS AND FIGURES

Explanation of figures

Abbreviations are as follows:

C: Chloroplast	Ne: Nematode
CW: Cell wall	NE: Nuclear envelope
CY: Cytoplasm	Nu: Nucleolus
EDS: Electron dense substance	Ph: Phytoferritin
ER: (Rough) Endoplasmic reticulum	Pl: Plasmadesmma
G: Grana	Pr: Protein body
GA: Golgi apparatus	PV: Periferal vesicle
GV: Golgi vesicle	R: Ribosomes
IS: Intercellular space	S: Starch
L: Lipid	SER: Smooth endoplasmic reticulum
M: Mitochondrion	T: Tonoplast
MF: Myelin figure	V: Vesicle
N: Nucleus	Va: Vacuole

Unless specified, all the treated samples were inoculated with 20 nematodes per plant.

Figure 1. Morphology of seven-day-old control alfalfa seedlings.

Figure 2. Morphology of seven-day-old nematode infected alfalfa seedlings. Note the swollen hypocotyl region.

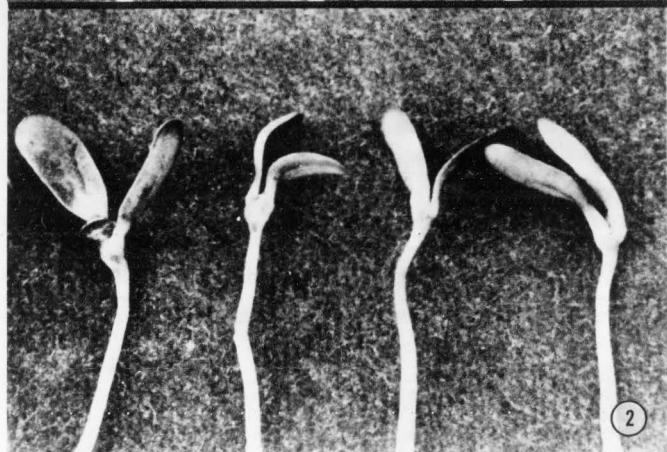
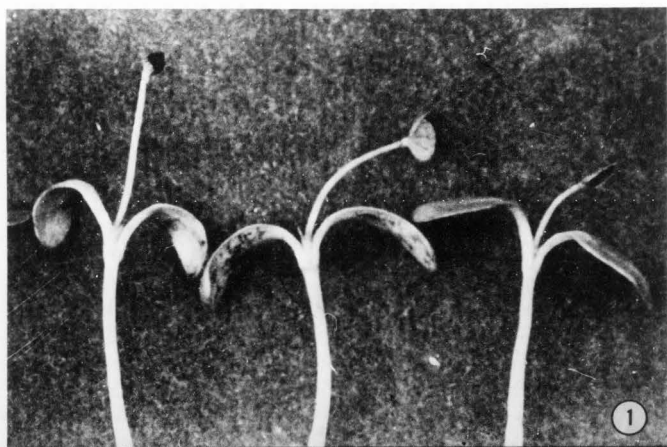


Figure 3. Morphology of seven-day-old alfalfa seedlings--(a) control and (b) infected plant. Note the swollen hypocotyl of the infected plant.

Figure 4. Cross section of hypocotyl region of control alfalfa seedling (1 μ).

Figure 5. Cross section of hypocotyl region of nematode infected alfalfa seedling (1 μ). Note the infection is only in the cortical region.

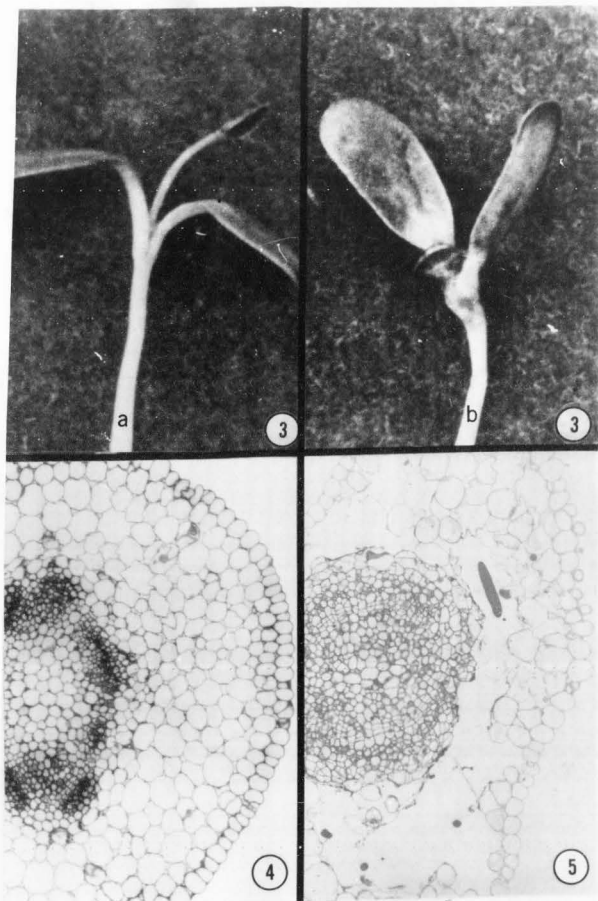


Figure 6. One-day-old Ranger alfalfa control plant, bearing normally developed cell wall, mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes, nucleus, vacuoles and chloroplasts. X 24,000

Figure 7. One-day-old nematode infected Ranger alfalfa plant. X 16,000

Figure 8. Phytoferritin in alfalfa chloroplast. X 52,400

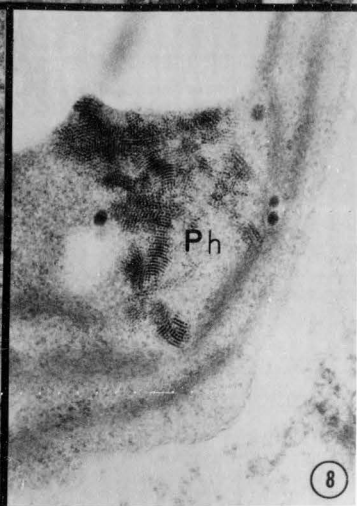
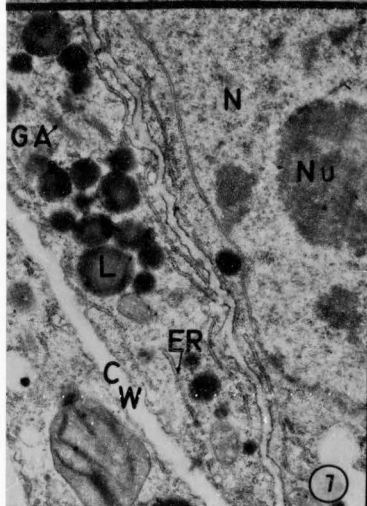
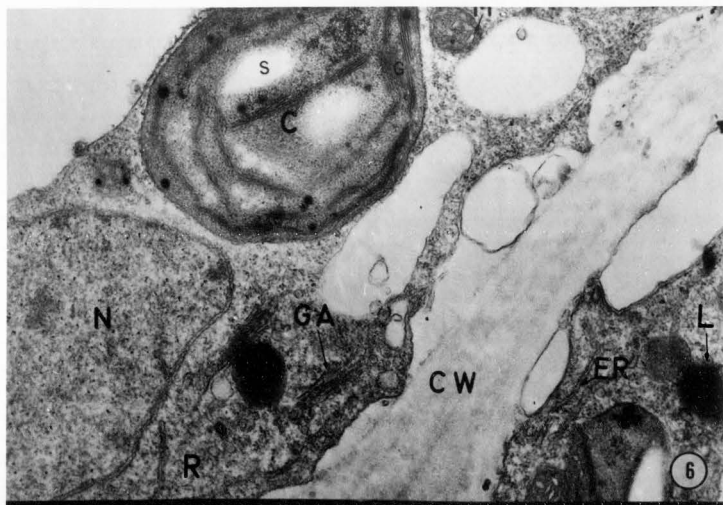


Figure 9. Starch grains and chloroplasts in one-day-old alfalfa control plant. X 24,000

Figure 10. Lipid content in one-day-old Ranger alfalfa control plant. X 9200

Figure 11. Lipid content in one-day-old Ranger alfalfa treated plant. X 9200

Figure 12. Typical shape of normal chloroplast devoid of starch grain. X 24,000

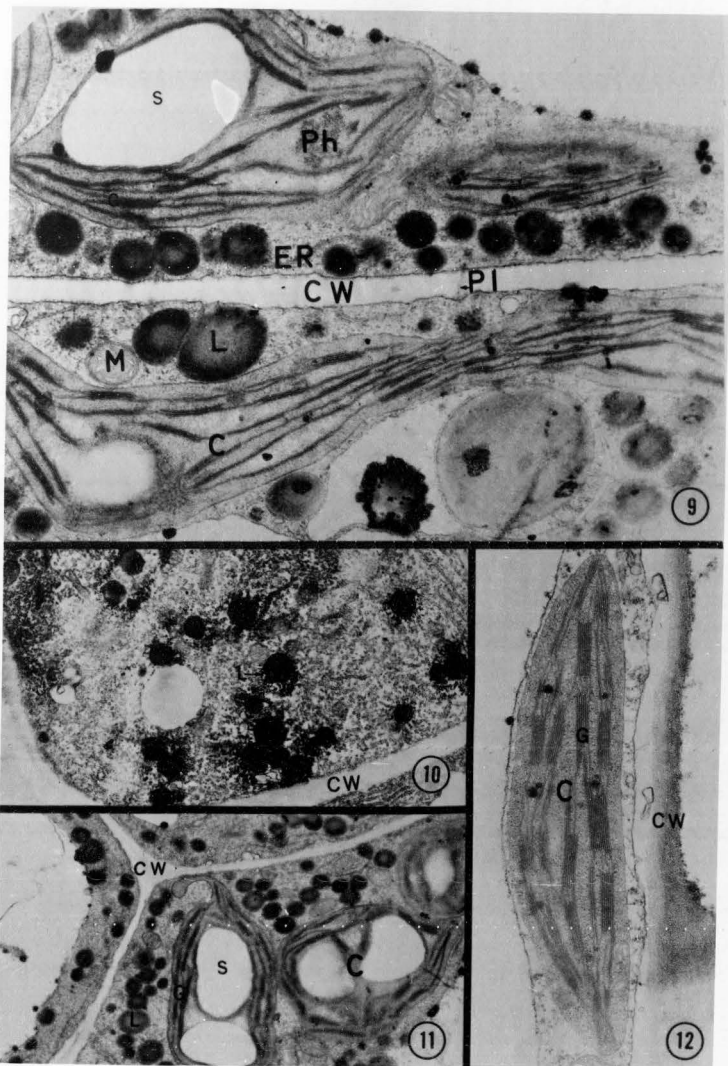


Figure 13. Three-day-old Ranger alfalfa infected plant. Note the osmiophilic staining of the side of the cell wall, the swollen chloroplasts, the rupture of the outer chloroplast membrane and the swollen thylakoid units (arrow). X 9200

Figure 14. Three-day-old Ranger alfalfa control plant with normal chloroplast, mitochondria, and nucleus. Note the lobing nucleus which is normal in alfalfa hypocotyl. X 13,800

Figure 15. Three-day-old Ranger alfalfa infected by nematode showing swelling and lobing nucleus, rupture of outer nuclear membrane (arrows), and the leakage of nuclear content. X 13,800

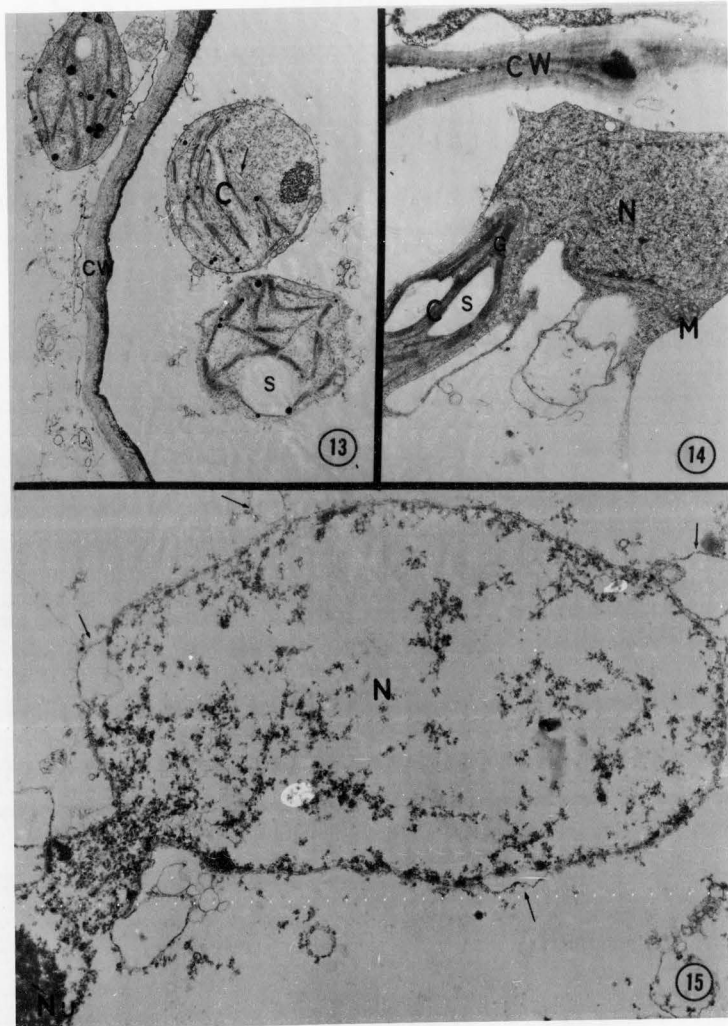
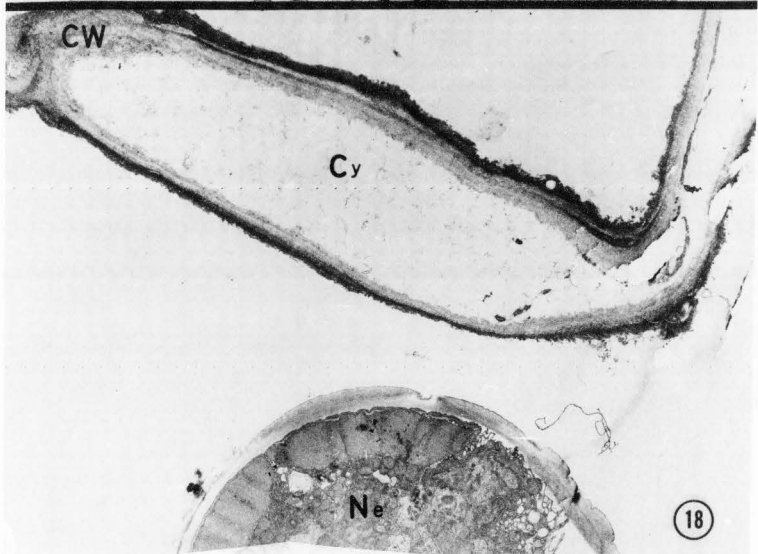
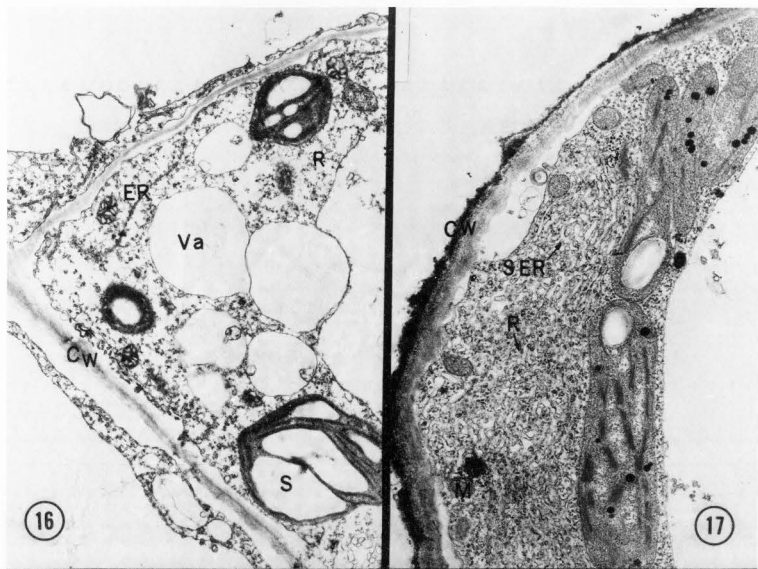


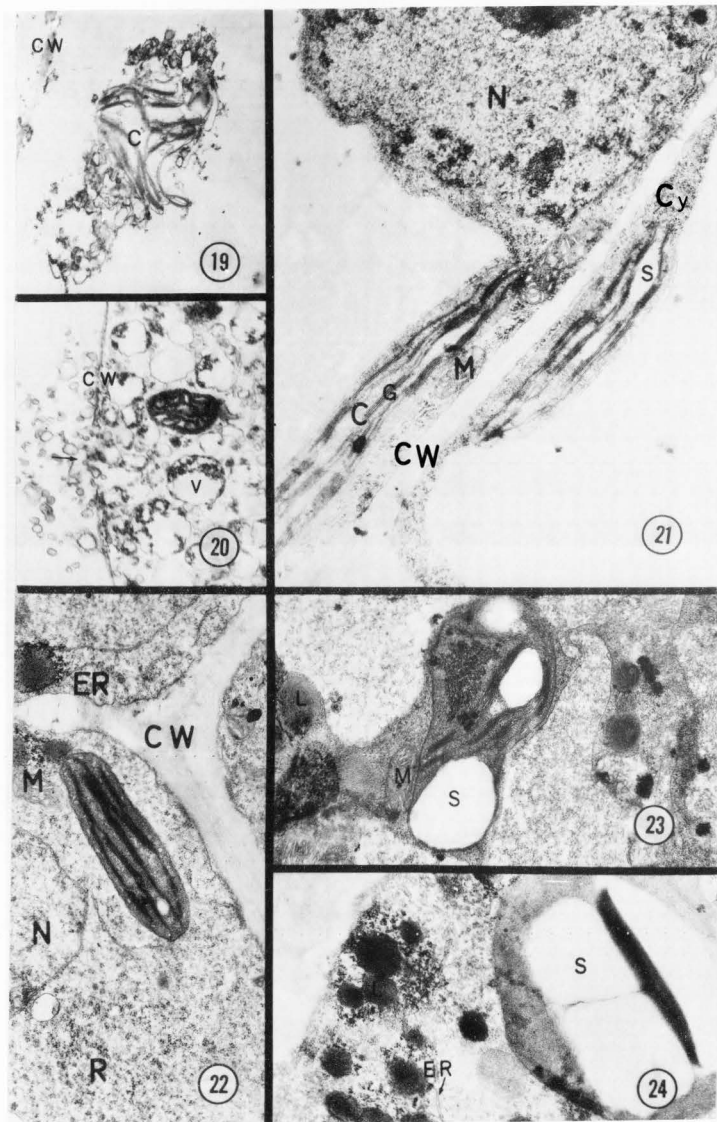
Figure 16. Three-day-old Ranger alfalfa control plant with normal cytoplasm. X 9200

Figure 17. Three-day-old Ranger alfalfa infected plant with dense cytoplasm and an abundance of ribosomes and numerous cisternae of smooth endoplasmic reticulum. X 9200

Figure 18. Three-day-old Ranger alfalfa infected tissue. Note the response of host tissue beside the nematode. X 5400



- Figure 19. Seven-day-old Ranger alfalfa infected plant showing the rupture of chloroplast. X 9200
- Figure 20. Seven-day-old Ranger alfalfa infected plant showing the broken cell wall and the mixing of the cytoplasm which contained numerous vesicles of two cells. X 16,000
- Figure 21. Seven-day-old Ranger alfalfa control plant with normal nucleus, mitochondria, cell walls, cytoplasm and chloroplasts. X 16,000
- Figure 22. One-day-old Lahontan alfalfa control plant with normal cell content. X 16,000
- Figure 23. Lipid content of one-day-old Lahontan alfalfa control plant. X 16,000
- Figure 24. Lipid content of one-day-old Lahontan alfalfa infected plant. X 16,000



- Figure 25. Three-day-old Lahontan alfalfa infected plant in most cases exhibited normally developed chloroplast. X 16,000
- Figure 26. Three-day-old Lahontan alfalfa infected plant with normally developed nucleus. X 16,000
- Figure 27. Three-day-old Lahontan alfalfa infected plant with normal cytoplasm. X 9200
- Figure 28. Three-day-old Lahontan alfalfa plant with heavily infected cytoplasm. Note the abnormal chloroplast, the sausage-like ER and the vesicles. X 9200
- Figure 29. Three-day-old Lahontan alfalfa control plant with normal cytoplasm. X 9200

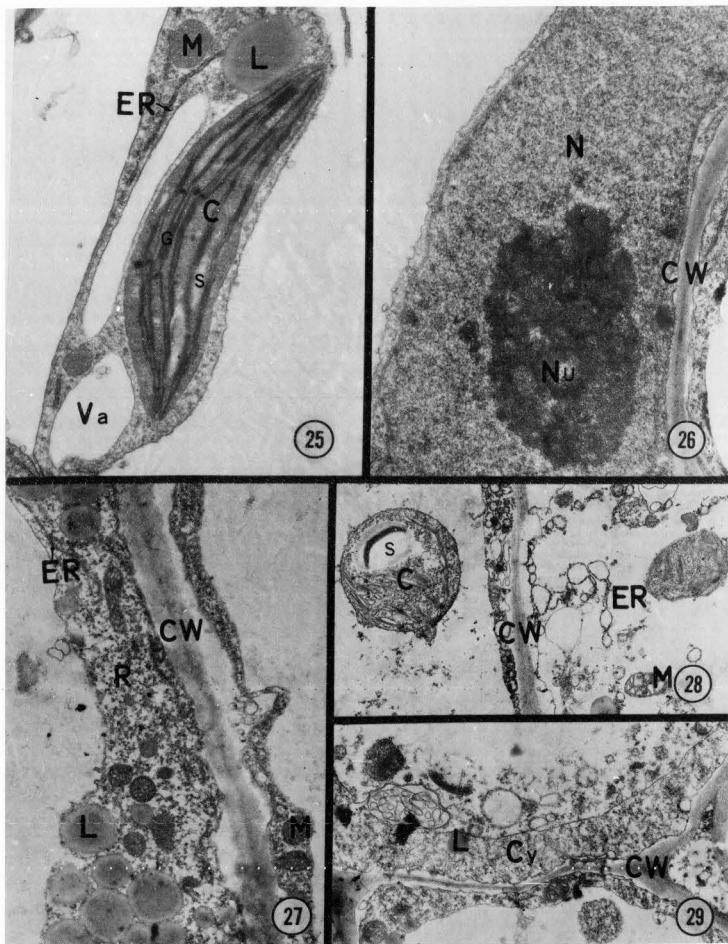


Figure 30. Seven-day-old Lahontan alfalfa infected plant. X 21,000

Figure 31. Seven-day-old Lahontan alfalfa infected plant. X 21,000

Figure 32. Seven-day-old heavily infected alfalfa plant (Lahontan) showing the abnormal nucleus and chloroplast. X 1400

Figure 33. Seven-day-old heavily infected alfalfa plant (Lahontan) showing the rupture of chloroplast membranes. X 9200

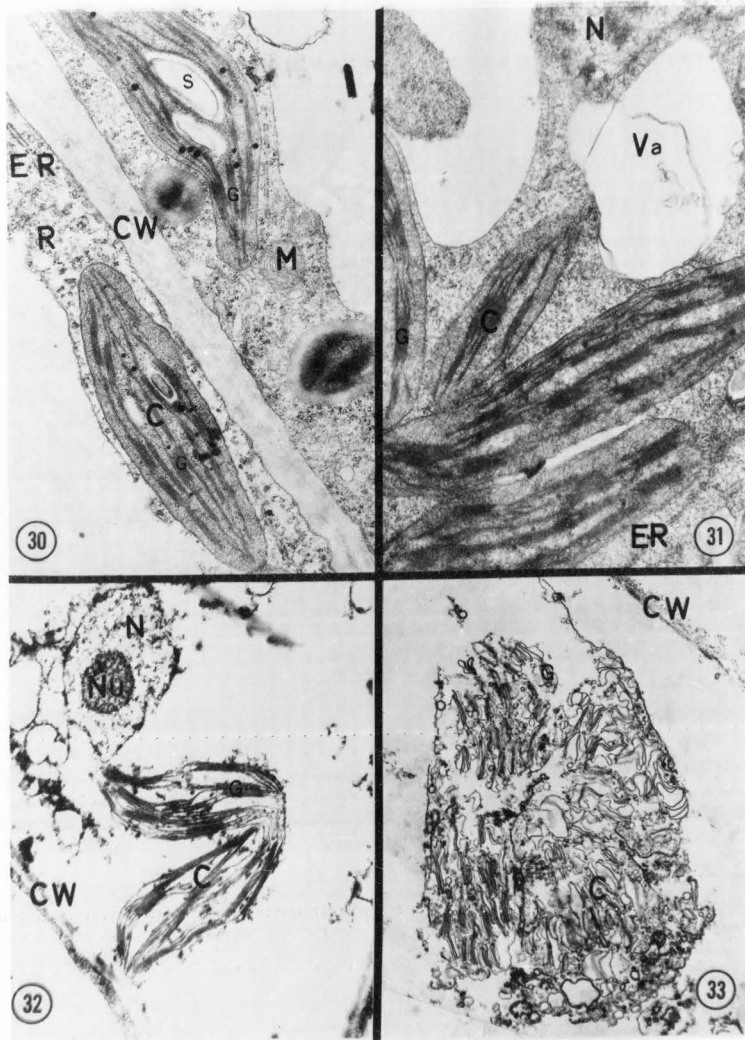


Figure 34. One-day-old alfalfa plants in all treatments contained numerous protein bodies of various sizes and shapes. X 9200

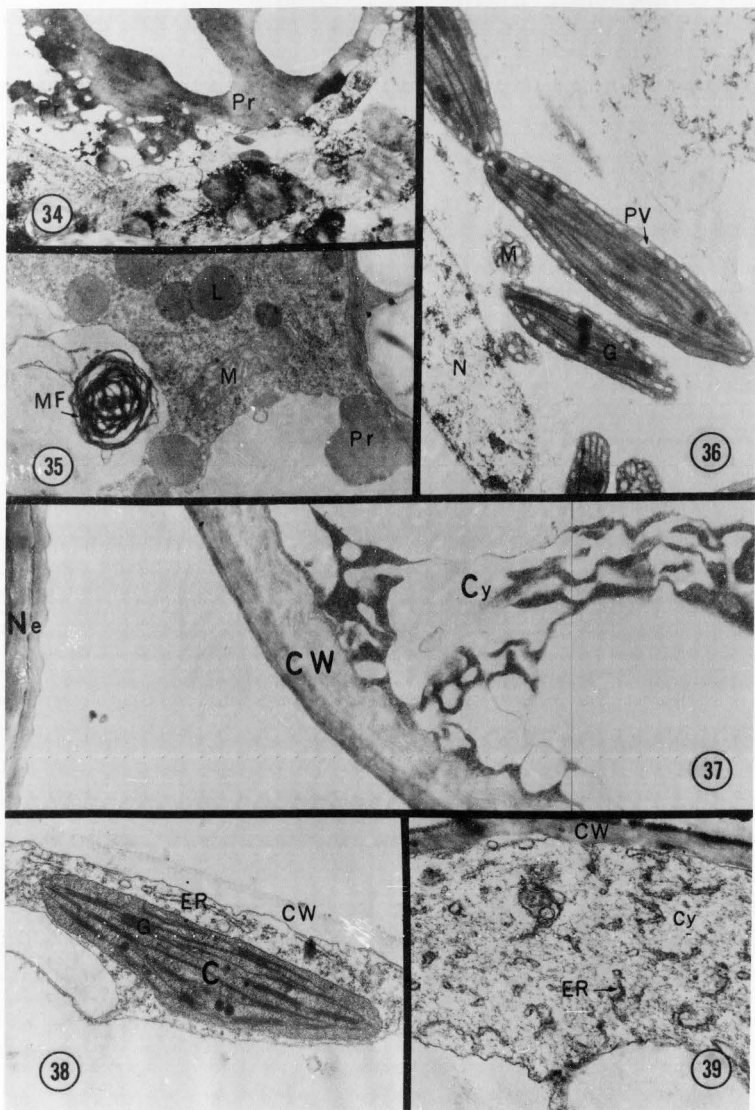
Figure 35. Myelin figure in alfalfa plant. X 16,000

Figure 36. Chloroplasts with peripheral vesicles at 20 C. X 16,000

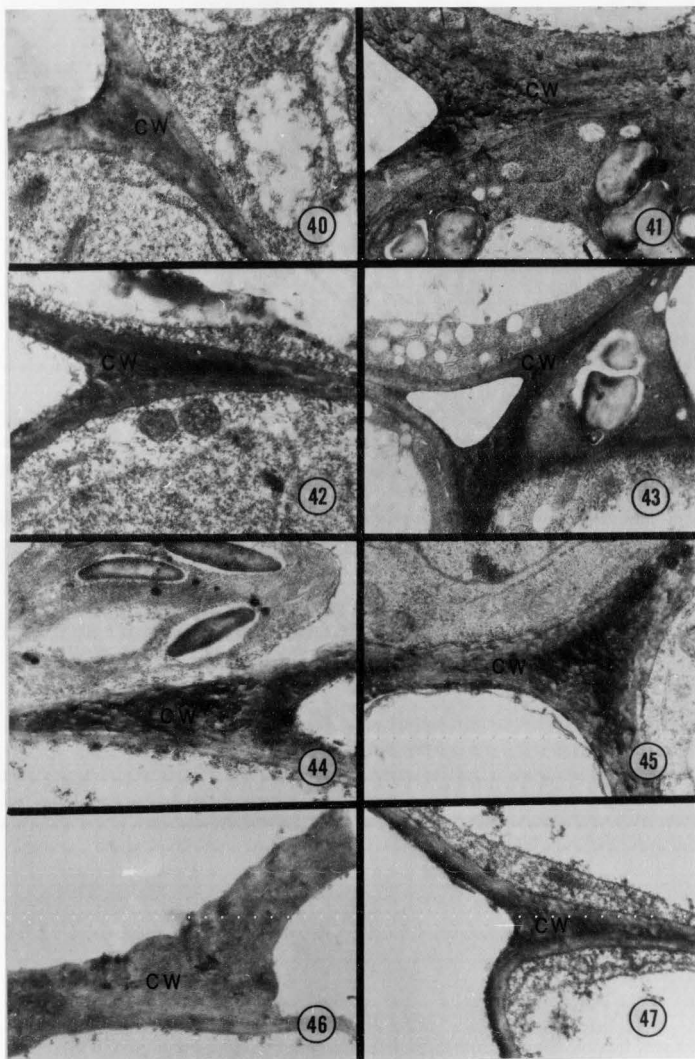
Figure 37. Seven-day-old Ranger alfalfa inoculated with 100 nematode per plant, showing the plasmolyzed cytoplasm. X 16,000

Figure 38. Seven-day-old Lahontan alfalfa inoculated with 100 nematode per plant, showing the slightly swelled ER. X 16,000

Figure 39. Seven-day-old Lahontan alfalfa inoculated with 100 nematode per plant, showing the osmiophilic cell wall and abnormal cytoplasm. X 16,000



- Figure 40. Pectin in the cell wall of one-day-old Ranger alfalfa control plant. X 16,000
- Figure 41. Pectin in the cell wall of one-day-old Ranger alfalfa infected plant. X 9200
- Figure 42. Pectin in the cell wall of one-day-old Lahontan alfalfa control plant. X 16,000
- Figure 43. Pectin in the cell wall of one-day-old Lahontan alfalfa infected plant. X 9200
- Figure 44. Pectin in the cell wall of seven-day-old Lahontan alfalfa control plant. X 16,000
- Figure 45. Pectin in the cell wall of seven-day-old Lahontan alfalfa infected plant. X 16,000
- Figure 46. Pectin in the cell wall of seven-day-old Ranger alfalfa control plant. X 16,000
- Figure 47. Pectin in the cell wall of seven-day-old Ranger alfalfa infected plant. X 16,000



- Figure 48. One-day-old alfalfa seedling at 15 C. Note the electron dense substances along the tonoplast. X 16,000
- Figure 49. Seven-day-old alfalfa seedling at 15 C. Note the electron dense substances in the intercellular space. X 16,000
- Figure 50. Seven-day-old infected Ranger alfalfa seedling at 15 C. Note the electron dense spots distributed along the cell wall and the normal nucleus and chloroplast. X 16,000
- Figure 51. Seven-day-old Ranger alfalfa seedling at 15 C. Peripheral vesicles in the chloroplast were formed by invagination of the inner membrane of chloroplast (arrow). X 36,000
- Figure 52. Seven-day-old Ranger alfalfa control plant at 15 C with normal nucleus, chloroplast, cell wall and cytoplasm. X 16,000
- Figure 53. One-day-old Lahontan alfalfa control plant at 15 C with swollen ER in the cytoplasm. X 9200

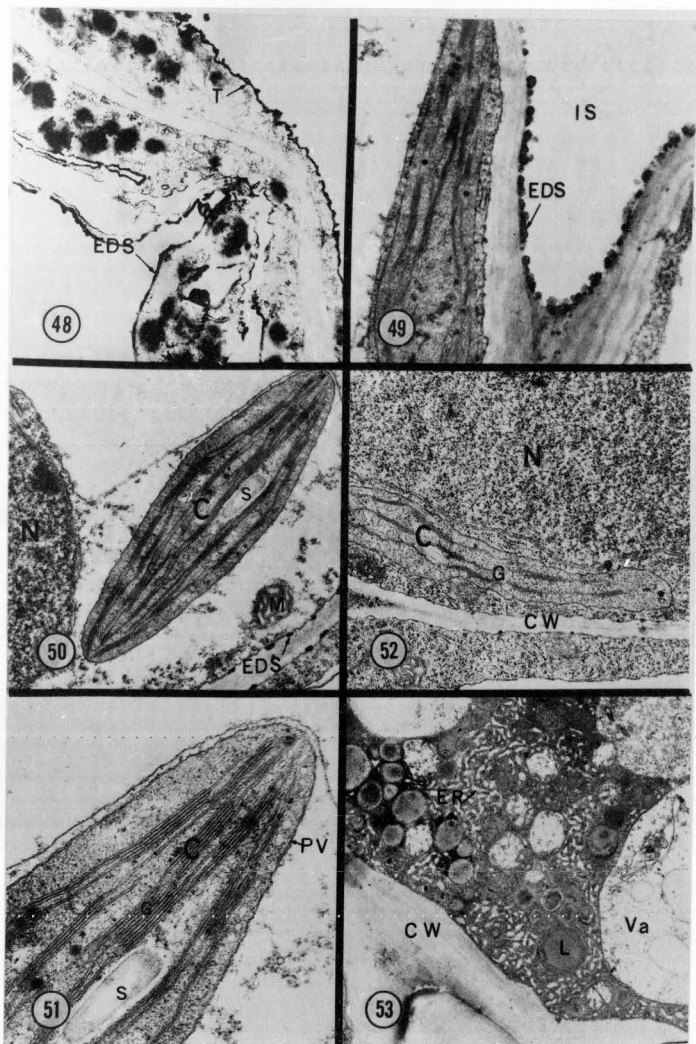
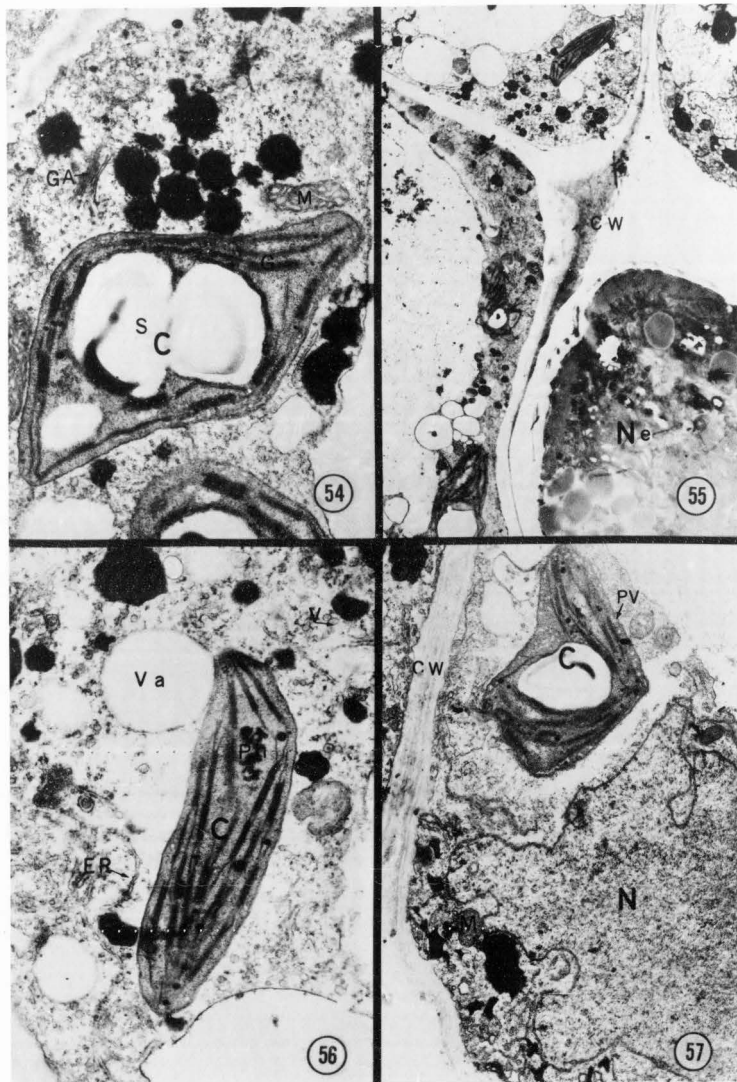


Figure 54. Three-day-old Lahontan alfalfa control plant at 15 C with normal cell contents. X 16,000

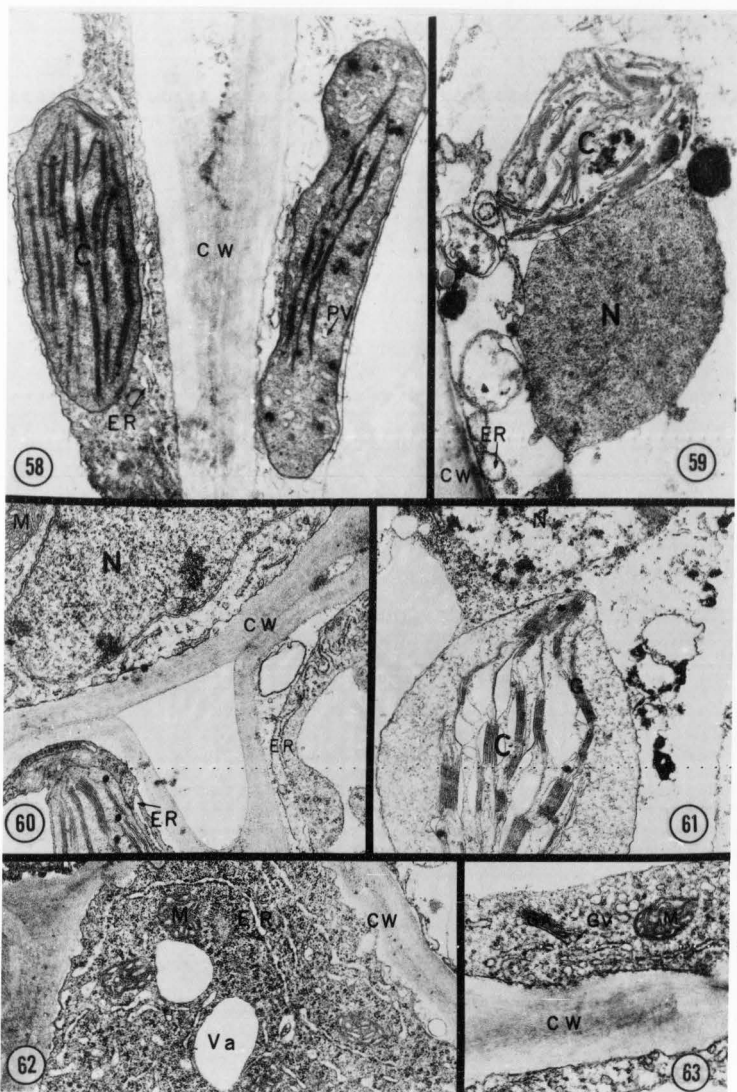
Figure 55. Three-day-old Lahontan alfalfa plant at 15 C showing nematode and host tissue. X 3600

Figure 56. Higher magnification of the host tissue adjacent to the invading nematode in Figure 55. Note the normal chloroplast with phytoferritin, the swollen and broken ER, and the numerous vesicles in the cytoplasm. X 16,000

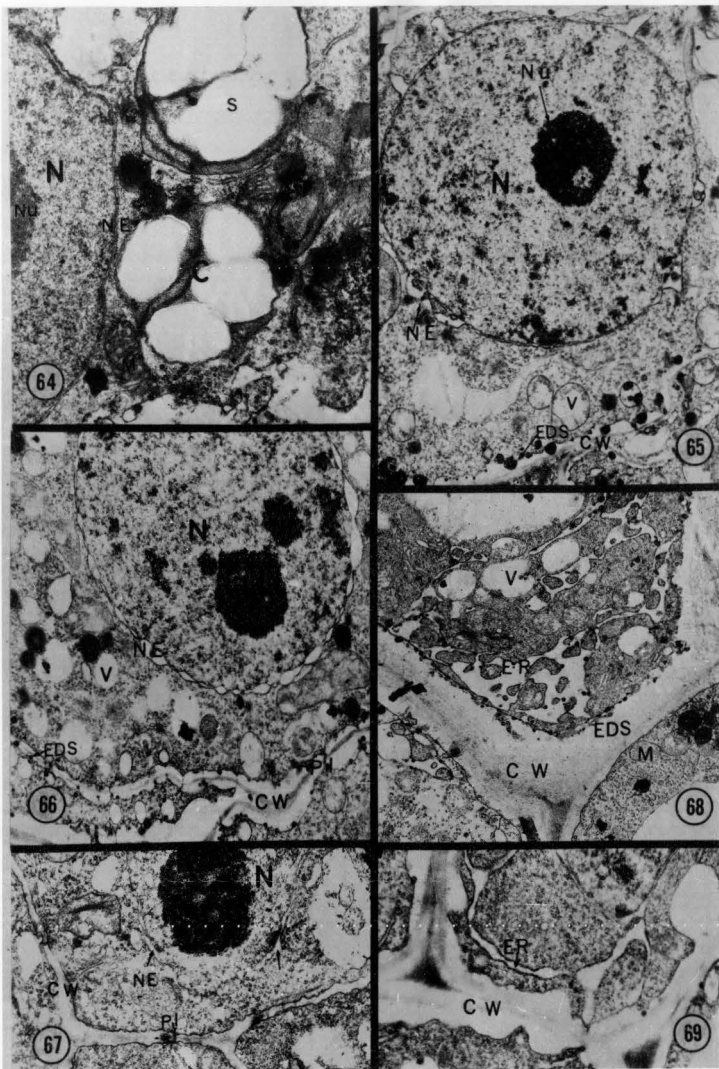
Figure 57. Three-day-old Lahontan alfalfa infected plant at 15 C with normal chloroplast and lobing nucleus in the cytoplasm. X 9200



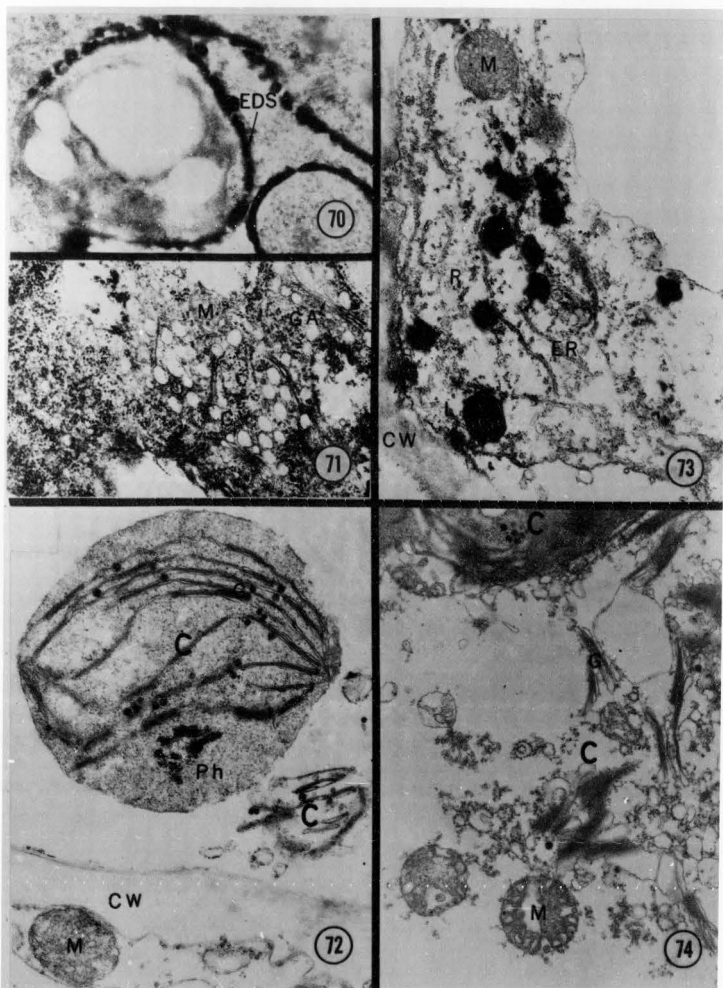
- Figure 58. Three-day-old Lahontan alfalfa infected plant at 15 C with two normal chloroplasts in adjacent cells. The chloroplast in one cell showed numerous vesicles in the stroma, whereas in the other one swollen ER were evident in the cytoplasm. X 16,000
- Figure 59. Three-day-old severely infected Lahontan alfalfa plant at 15 C with broken chloroplast, heavily swollen ER and a nucleus which still contained its nuclear material. X 16,000
- Figure 60. Seven-day-old Lahontan alfalfa control plant at 15 C with normal nucleus, cytoplasm, cell wall and chloroplast. Note the slightly swollen ER in the lower left corner. X 16,000
- Figure 61. Seven-day-old severely infected Lahontan alfalfa plant at 15 C with swollen chloroplast and ER and nucleus showing less nuclear material. X 16,000
- Figure 62. Seven-day-old Lahontan alfalfa control plant at 15 C with long dilated ER in the cytoplasm. X 16,000
- Figure 63. Seven-day-old Lahontan alfalfa infected plant at 15 C with active secreting Golgi apparatus and numerous Golgi vesicles. X 16,000



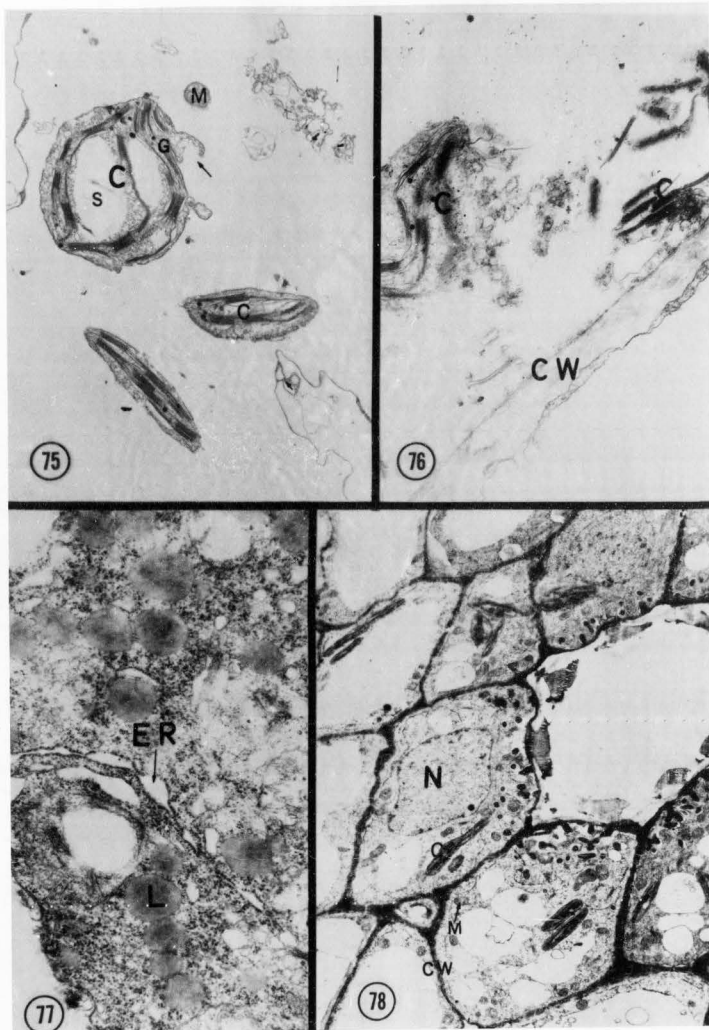
- Figure 64. One-day-old Ranger alfalfa control plant at 25 C with normal nucleus, chloroplast and cytoplasm. X 16,000
- Figure 65. One-day-old Ranger alfalfa infected plant at 25 C with partially swollen nuclear envelope and ~~wave-like cell wall~~ structure. Note the electron dense spots on the cell wall. X 9200
- Figure 66. One-day-old severely infected Ranger alfalfa plant at 25 C with all-round swollen nuclear envelope and electron dense substances. Note the numerous vacuoles in the cytoplasm and the plasmadesmata in the cell wall. X 9200
- Figure 67. Glutaraldehyde-OsO₄ combination fixation also showed a swollen nuclear envelope in one-day-old infected Ranger alfalfa plant at 25 C. X 16,000
- Figure 68. One-day-old Ranger alfalfa control plant at 25 C. The ER in some cells were heavily swollen. X 9200
- Figure 69. One-day-old Ranger alfalfa infected plant at 25 C showing swollen ER. X 16,000



- Figure 70. One-day-old alfalfa plant at 25 C with electron dense substances along the vacuoles. X 16,000
- Figure 71. One-day-old alfalfa plant at 25 C showing the general pattern of the actively secreting Golgi apparatus in the infected plant. X 24,000
- Figure 72. Three-day-old Ranger alfalfa control plant at 25 C with normal cytoplasm and cell wall. X 16,000
- Figure 73. Three-day-old severely infected Ranger alfalfa plant at 25 C with round and broken chloroplast and swollen ER. X 16,000
- Figure 74. Seven-day-old severely infected Ranger alfalfa plant at 25 C with round and completely disrupted chloroplasts. Note also the swollen mitochondria. X 16,000



- Figure 75. Seven-day-old infected Lahontan alfalfa plant at 25 C with round and outer membrane ruptured chloroplast (arrow). X 9200
- Figure 76. Seven-day-old heavily infected Lahontan alfalfa plant at 25 C with partially and completely broken chloroplasts. X 16,000
- Figure 77. One-day-old Lahontan alfalfa infected plant at 25 C with heavily swollen ER. X 24,000
- Figure 78. Vascular region of seven-day-old alfalfa plant at 25 C with normal cell contents. X 3600



LITERATURE CITED

- Acedo, J. R., and R. A. Rohde. 1970. Histochemical root pathology of Brassica oleracea capitata L. infected by Pratylenchus penetrans (Cobb) Filipjev and Schuurmans stekhoven (Nematoda: Tylenchidae). J. Nematol. 3:62-68.
- Albersheim, P., and U. Killias. 1963. Histochemical localization at the electron microscope level. Amer. J. Bot. 50:732-745.
- Alghisi, P., and G. Ferraza. 1967. Influenza esercitata dal sulla natura della sostanze pectiche presenti in piante di Radicchio e sulla resistenza di queste alla Sclerotinia minor. Riv. Patol. Veg. Pavia Ser. 4, 3:145-160.
- Anonymous. 1971. Estimated crop losses due to plant-parasitic nematodes in the United States. J. Nematol. (Suppl. Special Publ. No. 1).
- Bagley, B. W., J. H. Cherry, M. L. Rollins and A. M. Altschul. 1963. A study of protein bodies during germination of peanut (Archis hypogaea) seed. Amer. J. Bot. 50:523-532.
- Bateman, D. F., and R. D. Lumsden. 1965. Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of Rhizoctonia solani. Phytopathology 55:734-738.
- Bateman, D. F., and R. L. Millar. 1966. Pectic enzymes in tissue degradation. Ann. Rev. Phytopathol. 4:119-146.
- Beams, H. W., and S. S. Sekhon, 1967. Fine structure of the body wall and cells in the pseudocoelom of the nematode Rhibdits pellio. J. Ultrastruct. Res. 18:580-594.
- Bessey, E. A. 1911. Root-knot nematode and its control. Bull. U. S. Dept. Agric. Bur. Plant Ind. 217. 89 p.
- Bingefors, S. 1951. The nature of resistance to the stem nematode, Ditylenchus dipsaci (Kühn) Filipjev, in red clover Trifolium pratense L. Acta Agr. Scand. 1:180-189
- Bingefors, S. 1957. Studies on breeding red clover for resistance to stem nematodes. Pub. Inst. Plant Husb. R. Agric. Coll. Sweden, Uppsala. 123 p.
- Bingefors, S. 1961. Stem nematode in Lucerne in Sweden. II. Resistance in Lucerne against stem nematode. Knugl. Lant. Ann. 27:385-398.

- Bingefors, S. 1962. On resistance to stem nematode in Lucerne. *Nematologica* 7:17.
- Birchfield, W. 1962. Host-parasite relations of Rotylenchulus reniformis on Gossypium hirsutum. *Phytopathology* 52:862-865.
- Bird, A. F. 1961. The ultrastructure and histochemistry of a nematode-induced giant cell. *J. Biophys. Biochem. Cytol.* 11:701-715.
- Bird, A. F. 1962. The inducement of giant cells by Medoidogyne javanica. *Nematologica* 8:1-10.
- Bird, A. F., and K. Deutsch. 1957. The structure of the cuticle of Ascaris lumbricoides var. suis. *Parasitology* 47:319-328.
- Bird, A. F., and G. E. Rogers. 1965. Ultrastructure of the cuticle and its formation in Meloidogyne javanica. *Nematologica* 11: 224-230.
- Blake, C. D. 1962a. The etiology of tulip-root disease in susceptible and in resistant varieties of oats infested by the stem nematode, Ditylenchus dipsaci (Kühn) Filipjev. II. Histopathology of tulip-root and development of the nematode. *Ann. Appl. Biol.* 50:713-722.
- Blake, C. D. 1962b. Some observations on the orientation of Ditylenchus dipsaci and invasion of oat seedlings. *Nematologica* 8:177-192.
- Blake, C. D. 1966. The histological changes in banana roots caused by Radopholus similis and Helicotylenchus multicinctus. *Nematologica* 12:129-137.
- Chang, D. C. N., W. F. Campbell and G. D. Griffin. 1970. Ultrastructural changes in alfalfa induced by stem nematodes (Ditylenchus dipsaci Kühn). *Agron. Abstr.*, 62nd meeting, p. 7.
- Christie, J. R. 1936. The development of root-knot nematode galls. *Phytopathology* 26:1-22.
- Cobb, N. A., G. Steiner and J. R. Christie. 1927. Observations on giant cells in potato roots infected with Heterodera rostochiensis. *J. Helminthol.* 32:135-144.
- Cole, R. J., and L. R. Krusberg. 1967. Sterol composition of the nematodes Ditylenchus trifurmis and Ditylenchus dipsaci and host tissues. *Exptl. Parasitol.* 21:232-239.
- Colotelo, N., and E. W. B. Ward. 1961. β -glycosidase activity and cyanogenesis in the susceptibility of alfalfa to winter crown rot. *Nature (London)* 189(4760):242-243.

- Corden, M. E. 1965. Influence of calcium nutrition on Fusarium wilt of tomato and polygalacturonase activity. Phytopathology 55: 222-224.
- Crittenden, H. W. 1954. Factors associated with root-knot nematode resistance in soybeans. Phytopathology 44:388 (Abstr.).
- Culter, H. G., and L. R. Krusberg. 1968. Plant growth regulators in Ditylenchus dipsaci, Ditylenchus trififormis and host tissues. Plant and Cell Physiol. 9:479-497.
- Curgy, J. J. 1968. Influence du mode de fixation sur la possibilite d'observer des structures myélinigues dans les hépatocytes d'embryons poulet. J. Microscopie 7:63-80.
- Davson, H., and J. F. Danielli. 1952. The permeability of natural membranes. Cambridge Univ. Press, London.
- Dickson, D. W., D. Huisingh and J. N. Sasser. 1971. Dehydrogenases, acid and alkaline phosphatases and esterases for chemotaxonomy of selected Meloidogyne, Ditylenchus, Heterodera and Aphelenchus spp. J. Nematol. 3:1-16.
- Dijkstra, J. 1957. Symptom of susceptibility and resistance in seedlings of red clover attacked by the stem eelworm, Ditylenchus dipsaci (Kühn) Filipjev. Nematologica 2:228-236.
- Dropkin, V. H. 1963. Cellulase in phytoparasitic nematodes. Nematologica 9:444-454.
- Dropkin, V. H. 1969. Cellular responses of plants to nematode infections. Ann. Rev. Phytopathol. 7:101-122.
- 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 backcross progeny to entry and development of Heterodera glycines. Phytopathology 55:375-381.
- Eriksson, K. B., and J. Granberg. 1969. Studies of Ditylenchus dipsaci using electrophoresis in acrylamide gel. Nematologica 15:530-534.
- Evans, A. A. F., and J. M. Fisher. 1970. The excretory systems of three Ditylenchus spp. J. Nematol. 2:260-264.
- Fan, D. F., and G. A. Machlachlan. 1967. Massive synthesis of ribonucleic acid and cellulase in the pea epicotyl in response to indoleacetic acid, with and without concurrent cell division. Plant Physiol. 42: 1114-1122.

- Fielding, M. J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. Proc. Helm Soc. Wash. 18: 110-112.
- Filipjev, I. N. 1934. The classification of the free-living nematodes and their relation to the parasitic nematodes. Smithson. Misc. Coll. 89:1-63.
- Giles, J. E., and E. M. Hutton. 1958. Combining resistance to the root-knot nematode, Meloidogyne javanica (Treub) Chitwood, and fusarium wilt in hybrid tomatoes. Aust. J. Agric. Res. 9: 182-192.
- Goffart, H., and A. Heiling. 1962. Beobachtungen über die enzymatische Wirkung von Speicheldrüsensekreten pflanzenparasitärer Nematoden. Nematologica 7:173-176.
- Griffin, G. D. 1967. Evaluation of several techniques for screening alfalfa for resistance to Ditylenchus dipsaci. Plant Dis. Rep. 51:651-654.
- Griffin, G. D. 1968. The pathogenicity of Ditylenchus dipsaci to alfalfa and the relationship of temperature to plant infection and susceptibility. Phytopathology 58:929-932.
- Grundbacher, F. J., and E. H. Stanford. 1962a. Effect of temperature on resistance of alfalfa to the stem nematode (Ditylenchus dipsaci). Phytopathology 52:791-794.
- Grundbacher, F. J., and E. H. Stanford. 1962b. Genetic factors conditioning resistance in alfalfa to the stem nematode. Crop Sci. 2:211-217.
- Hanna, M. R., and E. J. Hawn. 1965. Seedling inoculation studies with the alfalfa stem nematode. Can. J. Plant Sci. 45:357-363.
- Hirumi, H., T. A. Chen, K. J. Lee and K. Maramorosch. 1968. Ultrastructure of the feeding apparatus of the nematode Trichodorus christiei. J. Ultrastruct. Res. 24:434-453.
- Horner, H. T., Jr., and H. J. Arnott. 1965. A histochemical and ultrastructural study of Yucca seed proteins. Amer. J. Bot. 50:523-532.
- Howell, R. K., and L. R. Krusberg. 1966. Changes in concentration of nitrogen and free and bound amino acids in alfalfa and pea infected by Ditylenchus dipsaci. Phytopathology 56:1170-1177.
- Huang, C. S., and A. R. Maggenti. 1969. Mitotic aberrations and nuclear changes of developing giant cells in Vicia faba caused by root-knot nematode, Meloidogyne javanica. Phytopathology 59:447-455.

- Hussey, R. S., and L. R. Krusberg. 1968. Histopathology of resistant reactions in Alaska pea seedlings to two populations of Ditylenchus dipsaci. Phytopathology 58:1305-1310.
- Hussey, R. S., and L. R. Krusberg. 1971. Disc-electrophoretic patterns of enzymes and soluble proteins of Ditylenchus dipsaci and D. trifurmis. J. Nematol. 3:79-84.
- Hyde, B. B., A. J. Hodge, A. Kahn and M. Birnstiel. 1963. Studies on phytoferritin. I. Identification and localization. J. Ultrastruct. Res. 9:248-258.
- Jacobson, A. B., H. Swift and L. Bogorad. 1963. Cytochemical studies concerning the occurrence and distribution of RNA in plastids of Zea mays. J. Cell Biol. 17:557-570.
- Johnson, R. N., and D. R. Viglierchio. 1969. A growth promoting substance occurring in an extract prepared from Heterodera schachtii larvae. Nematologica 15:159-160.
- Johnson, P. W., S. D. Van Gundy and W. W. Thomson. 1970a. Cuticle ultrastructure of Hemicycliophora arenaria, Aphelenchus avenae, Hirschmanniella gracilis and Hirschmanniella belli. J. Nematol. 2:42-58.
- Johnson, P. W., S. D. Van Gundy and W. W. Thomson. 1970b. Cuticle formation in Hemicycliophora arenaria, Aphelenchus avenae and Hirschmanniella gracilis. J. Nematol. 2:59-79.
- Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixation of higher osmolarity for use in electron microscopy. J. Cell Biol. 27:137A-138A.
- Klein, S., and B. M. Pollock. 1968. Cell fine structure of developing lima bean seeds related to seed desiccation. Amer. J. Bot. 55: 658-672.
- Krusberg, L. R. 1960a. Culturing, histopathology and biochemistry of Ditylenchus dipsaci and Aphelenchoides ritzena-bois on alfalfa tissues. Phytopathology 50:643 (Abstr.).
- Krusberg, L. R. 1960b. Hydrolytic and respiratory enzymes of species of Ditylenchus and pratylenchus. Phytopathology 50:9-22.
- Krusberg, L. R. 1961. Studies on the culturing and parasitism of plant parasitic nematodes in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfalfa tissues. Nematologica 6: 181-200.
- Krusberg, L. R. 1963a. Effect of galling by Ditylenchus dipsaci on pectins in alfalfa. Nematologica 9:341-346.

- Krusberg, L. R. 1963b. Host response to nematode infection. *Ann. Rev. Phytopathol.* 1:219-240.
- Krusberg, L. R. 1964. Investigation on Ditylenchus dipsaci polygalacturonase and its relation to parasitism of this nematode on alfalfa (Medicago sativa). *Nematologica* 10:72 (Abstr.).
- Krusberg, L. R. 1967. Pectinases in Ditylenchus dipsaci. *Nematologica* 13:443-451.
- Krusberg, L. R., and M. L. Blichenstaff. 1964. Influence of plant growth regulating substances on reproduction of Ditylenchus dipsaci, Pratylenchus penetrans and Pratylenchus zeae on alfalfa tissue culture. *Nematologica* 10:145-150.
- Kühn, J. 1857. Über das Vorkommen von Anguillula an erkrankten Blütenköpfen von Dipsacus fullonum L. *Zeitschr. wiss. Zool.* 9: 129-137.
- Larson, D. A. 1965. Fine structural changes in the cytoplasm of germinating pollen. *Amer. J. Bot.* 52:139-154.
- Laetsch, W. M. 1968. Chloroplast specialization in dicotyledons possessing the C-4-dicarboxylic acid pathway of photosynthetic CO₂ fixation. *Amer. J. Bot.* 55:875-883.
- Leopold, A. C. 1960. Auxins and plant growth. Univ. Calif. Press, Berkeley, Calif. 354 p.
- Littrell, R. H. 1966. Cellular responses of Hibiscus esculentus to Meloidogyne incognita acrita. *Phytopathology* 56:540-544.
- Loos, C. A. 1953. Meloidogyne brevicauda, n. sp., a cause of root-knot of mature tea in Ceylon. *Proc. Helm Soc. Wash.* 20:83-91.
- Martin, J. H., and W. H. Leonard. 1967. Principles of field crop production. 2nd edition. The MacMillan Co., New York.
- Mountain, W. B. 1957. Resistance as a function of tolerance levels, p. 1-7. In Cairns, E. J. (Ed.). Proc. of the S-19 workshop in phytonematology.
- Mountain, W. B. 1960. Mechanisms involved in plant-nematode relationships, p. 426-431. In Sasser, J. N., and W. R. Jenkins (Eds.). *Nematology, fundamentals and recent advances*. Univ. North Carolina Press, Chapel Hill, N. C.
- Muse, B. D. 1969. Histopathology of susceptible and resistant reaction in 'Wando' pea seedlings to two populations of Ditylenchus dipsaci. *J. Nematol.* 1:299 (Abstr.).

- Muse, B. D., L. D. Moore, R. R. Muse and A. S. Williams. 1970. Pectolytic and cellulolytic enzymes of two populations of Ditylenchus dipsaci on 'Wando' pea (Pisum sativum L.). J. Nematol. 2:118-124.
- Myers, R. F. 1965. Amylase, cellulase, invertase and pectinase in several free-living, mycophagus, and plant parasitic nematodes. Nematologica 11:441-448.
- Myuge, S. G. 1957. On the physiological specificity of the bulb nematode, Ditylenchus allii Beij. (In Russian). Zoologicheskii Zhurnal 36:620-622.
- Öpik, H. 1966. Changes in cell fine structure in the cotyledons of Phaseolus vulgaris L. during germination. J. Exp. Bot. 17: 427-439.
- Orion, D., and G. Minz. 1968. The influence of systematic plant growth substances on the galls induced by the root-knot nematode, Meloidogyne javanica. Phytopathology 58:384.
- Otiefka, B. A. 1953. Development of the root-knot nematode, Meloidogyne incognita as affected by potassium nutrition of the host. Phytopathology 43:171-174.
- Owens, R. G., and H. M. Novotny. 1960. Physiological and biochemical studies on nematode galls. Phytopathology 50:650 (Abstr.).
- Paulson, R. E., and J. M. Webster. 1969. Ultrastructural response of plant cells to gall-forming nematodes. J. Nematol. 1:22-23.
- Paulson, R. E., and J. M. Webster. 1970. Giant cell formation in tomato roots caused by Meloidogyne incognita and Meloidogyne hapla (Nematode) infection. A light and electron microscope study. Can. J. Bot. 48:271-276.
- Pease, D. C. 1964. Histological techniques for electron microscopy. 2nd edition. Academic Press, N. Y. 381 p.
- Pieglat, M., and A. Wilski. 1965. Cytological differences in root cells of susceptible and resistant potato varieties invaded by potato root eelworm (Heterodera rostochiensis Woll.) larvae. Nematologica 11:109-115.
- Pirie, N. W. 1966. Leaf protein as a human food. Science 152:1701.
- Pirie, N. W. 1969. Plants as sources of unconventional protein foods. XI Internal. Botanical Congress; All-Congress Symposium World Food Supply. Seattle, Wash., Aug. 28, 1969.
- Radewald, J. D., and W. H. Hart. 1965. The stem nematode disease of alfalfa. Calif. Plant Diseases #23, Univ. Calif. Agr. Ext. Ser. AXT-184.

- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212.
- Richardson, K. W., L. Jarett and E. H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* 35:313-330.
- Riedel, R. M., and W. F. Mai. 1971. A comparison of pectinases from Ditylenchus dipsaci and Allium cepa callus tissues. *J. Nematol.* 3:174-178.
- Riggs, R. D., and N. N. Winstead. 1958. Attempts to transfer root-knot resistance in tomato by grafting. *Phytopathology* 48: 344 (Abstr.).
- Robards, A. W., and P. G. Hympherson. 1967. Phytoferritin in plastids of the cambial zone of willow. *Planta* 76:169-178.
- Robards, A. W., and C. L. Robinson. 1968. Further studies on phytoferritin. *Planta* 82:179-188.
- Robertson, J. D. 1959. Ultrastructure of cell membranes and their derivatives. In Crook, E. M. (Ed.). Structure and function of subcellular components. Biochemical Soc. Symposium No. 16. Cambridge Univ. Press, London.
- Rohde, R. A. 1960. Mechanisms of resistance to plant-parasitic nematodes, p. 447-453. In Sasser, J. N., and W. R. Jenkins (Eds.). Nematology, fundamentals and recent advances. Univ. North Carolina Press, Chapel Hill, N. C.
- Rohde, R. A., and W. R. Jenkins. 1957. Effect of temperature on the life cycle of stubby-root nematodes. *Phytopathology* 47:29 (Abstr.).
- Rosen, W. G., S. G. Gawlik, W. V. Dashek and K. A. Siegesmund. 1964. Fine structures and cytochemistry of Lilium pollen tubes. *Amer. J. Bot.* 51:61-71.
- Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne spp.). *Bull. Univ. Md. Agric. Exp. Sta.* A-77. 31 p.
- Seinhorst, J. W. 1956a. Biologische rassen van het stengelaaltje Ditylenchus dipsaci (Künn) Filipjev en hun waardplanten. I. Reacties van vatbare en resistente planten op aantasing en verschillende vormen von resistente. *Tijdschr. PlZiekt.* 62: 179-188.
- Seinhorst, J. W. 1956b. Population studies on stem eelworms. *Nematologica* 1:159-164.

- Sheetz, R. W., and H. W. Crittenden. 1966. Histochemistry of soybean varieties resistant or susceptible to Meloidogyne incognita acrita. Phytopathology 56:586 (Abstr.).
- Sherwood, R. T., J. W. Dudley, T. H. Busbice and C. H. Hanson. 1967. Breeding alfalfa for resistance to the stem nematode, Ditylenchus dipsaci. Crop Sci. 7:382-384.
- Sherwood, R. T., and D. Huisinsh. 1970. Calcium nutrition and resistance of alfalfa to Ditylenchus dipsaci. J. Nematol. 2: 316-323.
- Siddiqui, I. A., and D. P. Taylor. 1969. Histopathogenesis of galls induced by Meloidogyne naasi in wheat and oat roots. J. Nematol. 1:304 (Abstr.).
- Siddiqui, I. A., and D. P. Taylor. 1970. Histopathogenesis of galls induced by Meloidogyne naasi in wheat roots. J. Nematol. 2: 239-247.
- Siddiqui, I. A., and D. R. Viglierchio. 1970. The effects of gamma irradiation on the motility, infectivity, reproduction and morphology of Ditylenchus dipsaci. Nematologica 16:459-469.
- Swink, J. F., and R. E. Finkner. 1956. Galactinol-weight relationships in breeding for resistance to the sugar beet nematode. J. Amer. Soc. Sugar Beet Tech. 9:70-73.
- Thomas, C. A. 1966. Calcium and water-insoluble pectic substances in safflower hypocotyls in relation to resistance to Phytophthora drechsleri. Phytopathology 56:985-986.
- Thorne, G. 1949. On the classification of the Tylenchida, new order (Nematoda: Phasmidia). Proc. Helminth. Soc. Wash. 16:37-73.
- Thorne, G. 1961. Principles of nematology. McGraw-Hill Book Co., Inc., N. Y. p. 131.
- Tracey, M. V. 1958. Cellulase and chitinase in plant nematodes. Nematologica 3:179-183.
- Trump, B. F., and R. E. Bulger. 1966. New ultrastructural characteristics of cells fixed in a glutaraldehyde-osmium tetroxide mixture. Lab. Invest. 15:368.
- Tyler, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:392-415.
- U.S.D.A. 1965. Losses in agriculture. Agr. Handbook 291.
- U.S.D.A. 1967. Agricultural statistics.

- Van Gundy, S. D., and J. D. Kirkpatrick. 1964. Nature of resistance in certain citrus rootstocks to citrus nematode. *Phytopathology* 54:419-427.
- Veech, J. A., and B. Y. Endo. 1969. The histochemical localization of several enzymes of soybean infected with the root-knot nematode Meloidogyne incognita acrita. *J. Nematol.* 1:265-274.
- Viglierchio, D. R. 1971. Nematodes and other pathogens in auxin-related plant-growth disorders. *Bot. Rev.* 37:1-21.
- Viglierchio, D. R., and P. K. Yu. 1965. Plant parasitic nematodes: A new mechanism for injury of hosts. *Science* 147:1301-1303.
- Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4:475-478.
- Williams, T. D. 1956. The resistance of potatoes to root eelworm. *Nematologica* 1:88-93.
- Wilski, A., and J. Giebel. 1966. β -glycosidase in Heterodera rostochiensis and its significance in resistance of potato to this nematode. *Nematologica* 12:219-224.
- Winslow, R. D. 1955. The hatching responses of some root eelworms of the genus Heterodera. *Ann. Appl. Biol.* 43:19-36.
- Winslow, R. D. 1960. Some aspects of the ecology of free-living and plant-parasitic nematodes, p. 341-415. In Sasser, J. N., and W. R. Jenkins (Eds.). *Nematology, fundamentals and recent advances*. Univ. North Carolina Press, Chapel Hill, N. C.
- Wisse, E., and W. Th. Daems. 1968. Electron microscopic observations on second stage larvae of the potato root eelworm Heterodera rostochiensis. *J. Ultrastruct. Res.* 24:210-231.
- Wu, L. Y. 1968. Morphological study of Anguina calamagrostis Wu, 1967. (Tylenchidae:Nematoda) with the electron microscope. *Can. J. Zool.* 46:467-468.
- Wynne, J. C., and T. H. Busbice. 1968. Effects of temperature and incubation period on the expression of resistance to stem nematode in alfalfa. *Crop Sci.* 8:179-183.
- Yuen, P. H. 1967. Electron microscopical studies on Ditylenchus dipsaci. I. Stomatal region. *Can. J. Zool.* 45:1019-1033.
- Yuen, P. H. 1968. Electron microscopical studies on Ditylenchus dipsaci. II. Oesophagus. *Nematologica* 14:385-394.
- Zee, S-Y., and T. P. O'Brien. 1970. Studies on the ontogeny of the pigment strand in the caryopsis of wheat. *Aust. J. Biol. Sci.* 23:1153-1171.

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