Gall Formation on *Cirsium arvense* by *Ditylenchus dipsaci*[1]

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Abstract: Ditylenchus dipsaci was found to cause gall formation on the stems of *Cirsium arvense*. The galls were characterized by extensive hypertrophy and hyperplasia, differentiation of nutritive tissue, nuclear modification, and a central cavity containing nematodes. These findings emphasize the importance of host response in investigations of host-parasite interactions and suggest that *D. dipsaci* may be evolving a host race by reproductive isolation within the confines of a plant gall. *Key Words:* host race, stem and bulb nematode, histopathology.

Plant-parasitic nematodes induce a wide variety of host responses (2, 27). Some nematodes simply cause tissue necrosis as they feed, while others induce nurse cells or syncytia. Nematodes of the genera *Anguina* and *Nothanguina* cause complex galls composed of various layers of modified cells. Categorizing nematodes in terms of the host response or modifications induced is often difficult, however, since some species cause different responses in different hosts. For example, Dropkin (2) reported that *Meloidogyne incognita* induced large galls on okra but small or no galls on certain varieties of soybeans and grasses.

Ditylenchus dipsaci (Kühn) Filipjev is an obligate parasite of numerous higher plants, including *Cirsium arvense* (L.) Scop. (Canada thistle), on which it causes stem swellings (3, 21). However, there exists some confusion as to whether this host response should be considered a gall. This paper discusses morphological changes and damage to infected stems of *C. arvense*.

MATERIALS AND METHODS

Larvae of *D. dipsaci* were obtained from infected *C. arvense* collected in a pasture near Regina, Saskatchewan. The nematodes were extracted by placing pieces of stem overnight in sterile distilled water and supplying continuous aeration. Larvae that egressed from the tissues were concentrated in distilled water, and used immediately for inoculations.

Root fragments of healthy *C. arvense* were washed in water and placed in 12.5-cm-diam plastic pots containing vermiculite. Every second day, all pots received an excess of modified Hoagland's solution (10) containing nitrogen at 10.5 μg/g supplied as ammonium nitrate (NH_4NO_3). On alternate days the pots received sufficient distilled water to reach saturation of the vermiculite medium. Pots were maintained in the greenhouse under the following conditions: temperature, 22±5 C; day length, 14 h; light intensity, minimum 8,600 lux; relative humidity, 30±10%.

When the first shoots of *C. arvense* emerged, twelve pots were paired and six

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were inoculated each with about 10,000 nematodes in 1 ml of sterile distilled water. The suspension was dispersed onto the apical meristems of shoots that had emerged and onto the surface of the moist vermiculite.

Infected and healthy tissues were fixed in formalin acetic-acid-alcohol (F.A.A.) (13) at regular intervals 30–60 days after inoculation. Because of the growth habit of *C. arvense* and its ability to regenerate from root-borne stem buds, infected plants at various stages of development were available for study. Tissues from a total of 21 plants were sectioned.

Fixed tissues were washed overnight in water, dehydrated in a tertiary alcohol series, and embedded in paraffin (12, 13). All tissues were sectioned at 8 μm. Most sections were stained with safranin and fast green (8), but at least one of the series was stained with haematoxylin, safranin, and fast green (12), and one with periodic acid-Schiff's and counterstained with fast green (12).

RESULTS

Swellings were induced by *D. dipsaci* at or near the apical meristem of shoots collected from the field and shoots inoculated in the laboratory. The swellings were usually elliptical, with a diameter twice that of normal stems (Fig. 1). Leaves near the swellings became chlorotic as the stems increased in size. Stems at early stages of infection also were green, but turned brown once the swelling reached maximum size. Infected plants averaged about 15 cm in height before growth ceased.

Dissections of maturing infected stems 60 days after inoculation revealed a cavity filled with numerous *D. dipsaci* at various stages of development (Fig. 2). Infected stems were characterized also by irregularly shaped masses of cells extending into the cavity (Fig. 2). Deformation and swelling of the shoot apex was most evident in longitudinal sections (Fig. 3). Nematodes were concentrated around the edges of the cavity in such sections more than around the cell

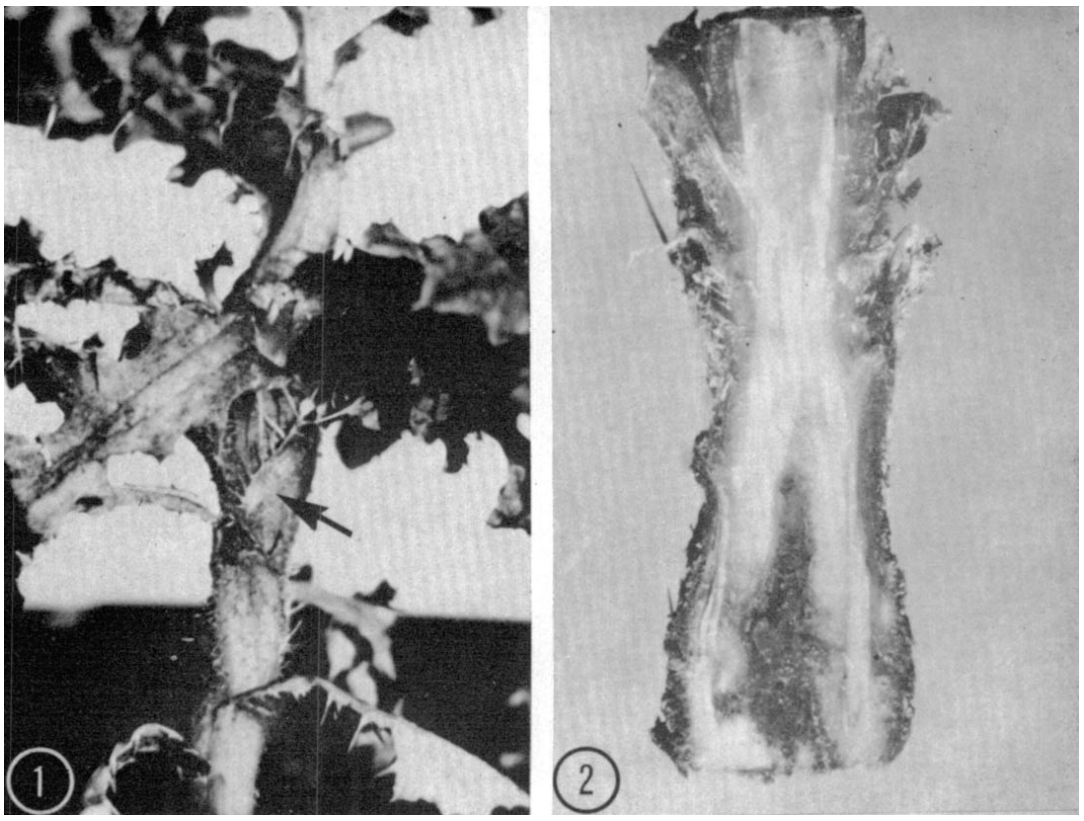


FIG. 1-2. *1)* Mature swelling on the stem of *C. arvense* (arrow). ×2.5. *2)* Dissection of mature stem of *C. arvense*. Note the swelling and central cavity, bordered by irregularly shaped masses of cells. ×6.5.

masses extending into the cavity. Most nematodes were associated with the pith, inside the area delineated by vascular bundles. Swellings found in the field were identical in morphology to swellings obtained in the greenhouse.

Infected tissues fixed when the shoots were about 4 mm above the surface (30 days after inoculation) showed nematodes feeding in cortical parenchyma tissues (Fig. 4). The area where nematodes penetrated the epidermis was found in several sections, and evidence of their feeding was visible as they moved towards the base of the meristematic region. Feeding in cortical parenchyma tissues at this stage caused separation of cells, resulting in the enlargement of intercellular spaces. When nematodes fed intensively in cortex tissues, they induced extensive cell separation, some necrosis, and hypertrophy (Fig. 5). However, no hyperplasia was observed in infected cortex. Nematodes in this region were found both intra- and intercellularly.

As the shoots matured, the concentrations of nematodes were largest beneath the apical meristem. Extensive cell separation, some hypertrophy, and the first indications of cellular dedifferentiation (Fig. 6) occurred soon after feeding began amongst cells below the apical meristem. Clusters of cytoplasmically dense cells which stained purple with safranin fast green occurred amongst cells of the pith around the surface of the cavity near nematode feeding sites (Fig. 7). The cells were hyperplastic and had enlarged nuclei. Some of these cells were also multinucleate. Cytoplasmically rich pith parenchyma cells developed also amongst vascular tissues as the swellings grew (Figs. 8, 9). Nematodes feeding on vascular bundles often caused direct tissue damage (Figs. 8 and 9).

The extent of nematode-induced proliferation and damage is most evident when normal (Fig. 10) and infected (Fig. 11) stems of comparable age (60 days) are compared. Cells of the cortex and pith in normal stems (Fig. 10) are of similar size and shape and are arranged in an orderly fashion around the vascular bundles. In infected stems (Fig. 11), pith cells are of irregular size and shape and exhibit conditions of hyperplasia and hypertrophy. Cells of the cortex are also of irregular size

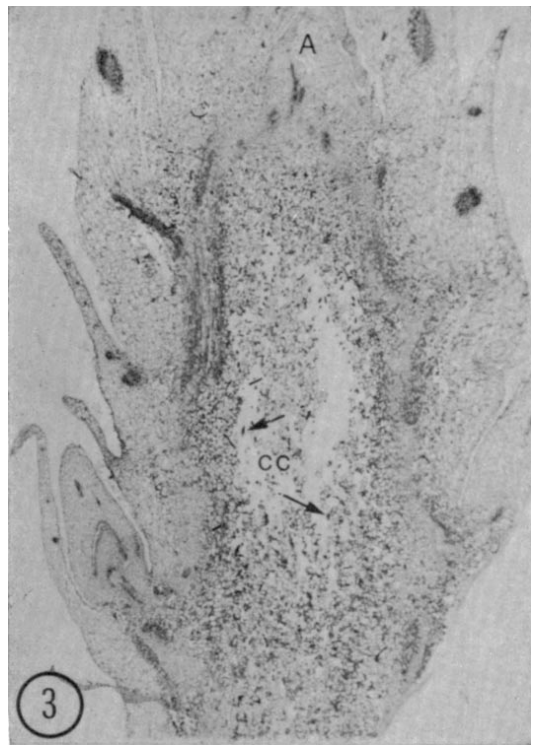


FIG. 3. Longitudinal section of stem taken below the apex (A) inhabited by *D. dipsaci*. Note the central cavity (cc) and nematodes (arrows). ×10.

and shape. Cell division in both the fascicular and interfascicular cambium continues as the tissues mature (Fig. 11). Clusters of cytoplasmically dense cells were found in the pith near feeding nematodes until the tissues became necrotic and the plants died.

DISCUSSION

Ditylenchus dipsaci causes a variety of host responses on leaves and stems of various plants and the response appears to vary with the host attacked. Webster (27) refers to *D. dipsaci* as causing tissue breakdown and necrosis, while other workers (2, 15) refer to the swellings as galls, without giving reasons. Goodey (6) distinguished between the localized "gall-like" lesions caused by *D. dipsaci* and "true-galls" incited by *Anguina* species. We believe that our morphological studies of infected *C. arvense* stems provide evidence that the population of *D. dipsaci* from Southern Saskatchewan forms galls.

Mani (16) describes nematode galls of aerial parts as having pronounced hypertrophy and hyperplasia, a central cavity

containing nematodes, and a zone of cells with abundant cytoplasm that line the cavity. According to Viglierchio (24) nematode galls are simply pathological swellings or enlargements of a plant part, irrespective of histological organization or structure. A common characteristic of most galls, however, whether formed by nematodes, mites, or insects, is the presence of modified cells arranged in layers which encircle the feeding site. Some nematode-induced galls, like most insect galls, are characterized by a relatively constant size and shape and the presence of definitive tissues (5). Less highly developed nematode-induced galls may lack definitive tissues and a constant size and shape, but they still have the layer of characteristic cells near feeding sites (26). These cells are called nutritive cells and are induced and eaten by all gall formers (16). Nutritive cells are cytoplasmically rich, contain fragmented vacuoles, exhibit nuclear and nucleolar hypertrophy, are rich in mitochondria, and contain high concentrations of lipids, hydrolytic enzymes, and amino acids (17, 19). Cells of this type appear to be present in *C. arvense* swellings infected by *D. dipsaci*. Godfrey (4) and Krusberg (14) were probably referring to the same cells when they reported hyperplastic strands of irregularly attenuated small cells with granular cytoplasm lining the cavities of swellings induced by *D. dipsaci*. The *D. dipsaci* we observed in the stems of *C. arvense* caused cell hypertrophy and separation at early stages of invasion in cortical parenchyma, whereas nutritive cells were not induced until feeding began on cells near the apical meristem. Maturing swellings were characterized by cell hypertrophy, hyperplasia, a poorly defined central cavity, and the proliferation of nutritive cells amongst pith parenchyma and vascular bundles (Figs. 2, 9, 11).

Most gall formers attack and modify meristematic or actively growing tissues (18), and the same was observed in our plants inhabited by *D. dipsaci*. Goodey (6) and Krusberg (14) observed that younger or meristematic cells were more susceptible to modification by nematodes than were older cells, and also that physiologically older tissues, such as cortical parenchyma, exhibited only cell separation rather than a galling response. Those observations are consistent with ours, for we found the first indications of nutritive cells and tissue swelling just beneath the apical meristems and near the cambium.

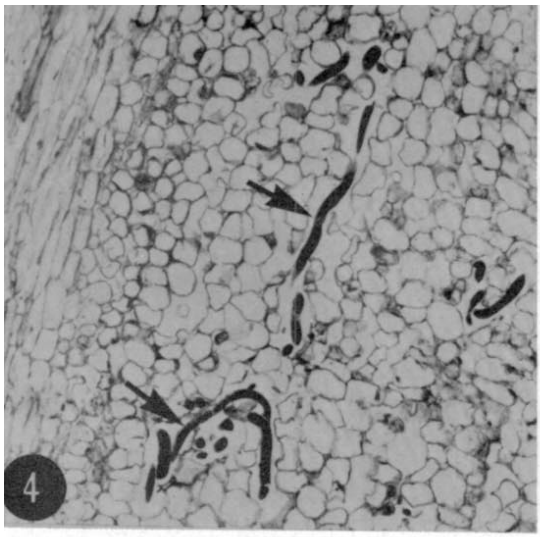
Not all *D. dipsaci* populations are capable of gall formation. Webster (27), for example, does not consider his populations of *D. dipsaci* infecting lucerne as gall formers since he observed only loose hypertrophied cells. Other workers give little morphological information on swellings induced by *D. dipsaci* that they describe (2, 4, 6, 24).

Sturham (23) suggests that biological races have occurred by either geographic isolation or ecological or physiological isolation within a host plant. Steiner (22) suggested that a nematode population could become more specialized and highly host-specific as the number of generations on a host increased. A nematode population could presumably become isolated if reproduction occurred mainly within galls of certain host species, and we suggest that that has occurred with *D. dipsaci* on *C. arvense*. Once a population of *D. dipsaci* has evolved the ability to induce galls, it would likely become host-specific and organ-specific, as have species of *Anguina* (5, 7) and most other gall-forming animals (16).

Perennial crops or crops growing in continuous monoculture are well suited to the establishment of a host race, and this selection could be maintained with limited heterogeneity (9). *C. arvense* is a perennial, and infestations of this weed are characteristically dense and relatively permanent (20). The ability of *D. dipsaci* to initiate galls on this plant and thereby become isolated within its host (25), leads us to postulate that it may be evolving a host-specific race.

D. dipsaci is a notorious pest of many plants, known to cause tissue breakdown, swelling, necrosis, and localized deformations of stem and leaf tissues. All *C. arvense* that we examined were retarded in growth, although the first visible damage was chlorosis near the gall. As the galls matured, the stems became twisted and death often occurred when the plants were about 15 cm in height. Hypertrophy of cortex and pith cells and damage to vascular bundles were also observed.

We suggest that the presence of nutritive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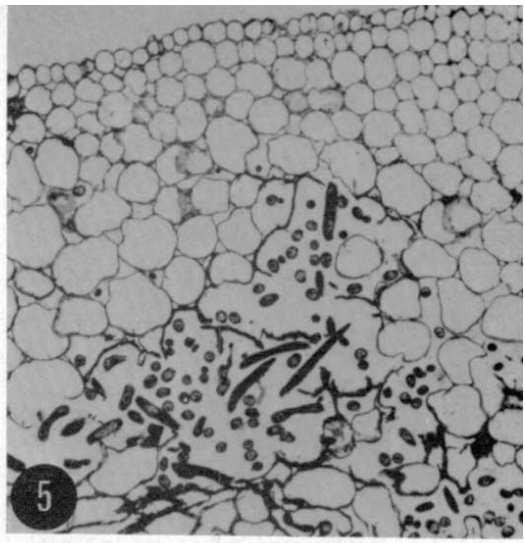
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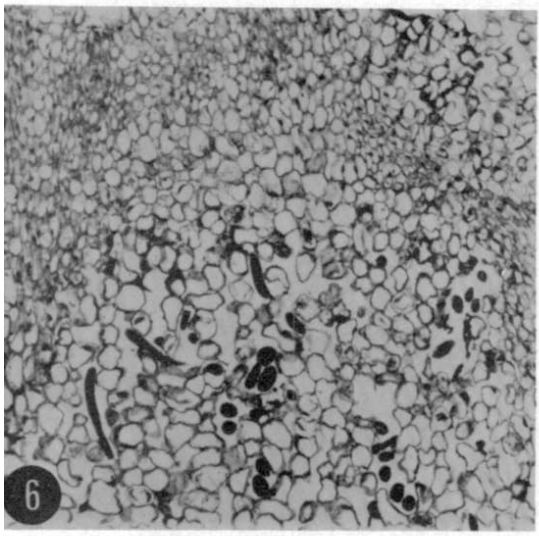
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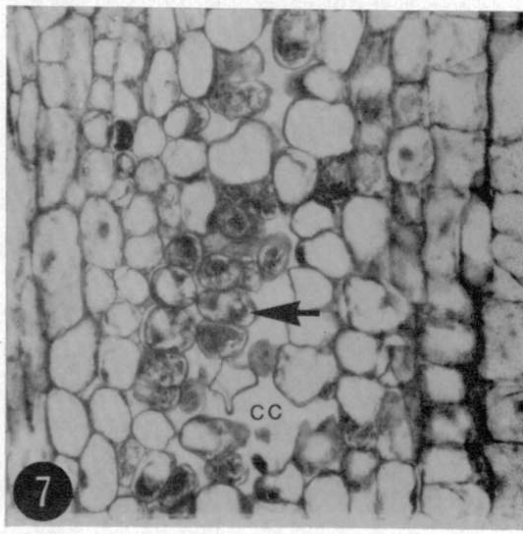
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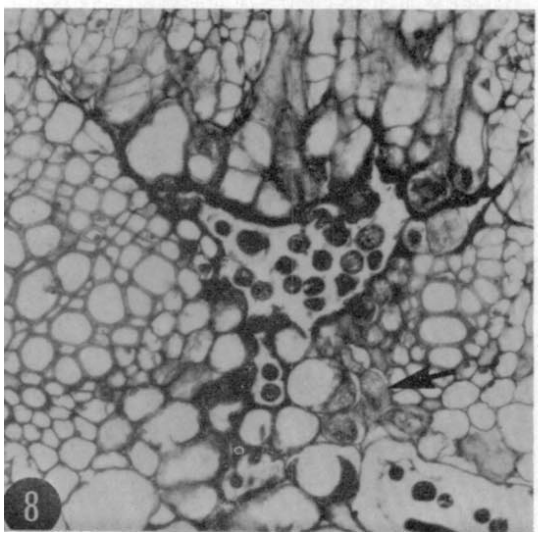
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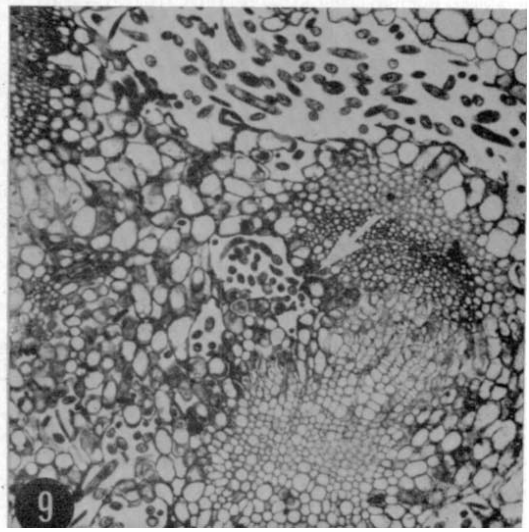
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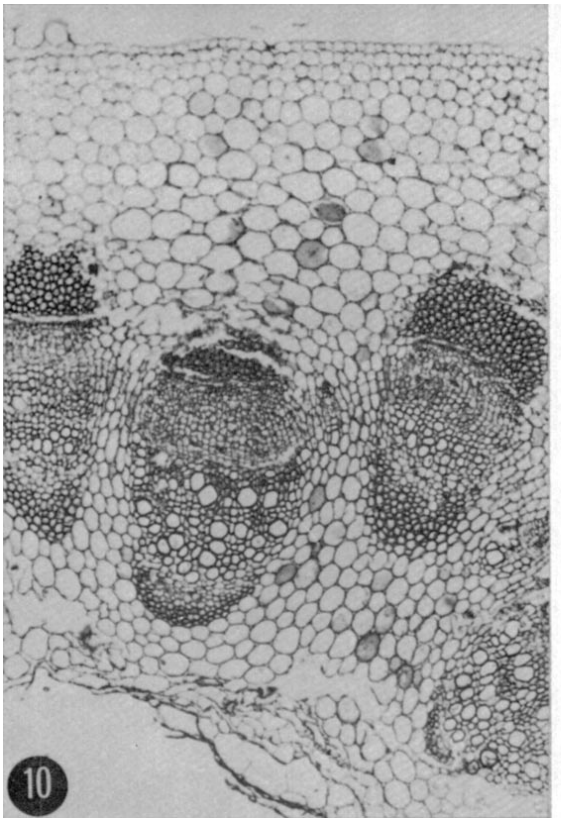

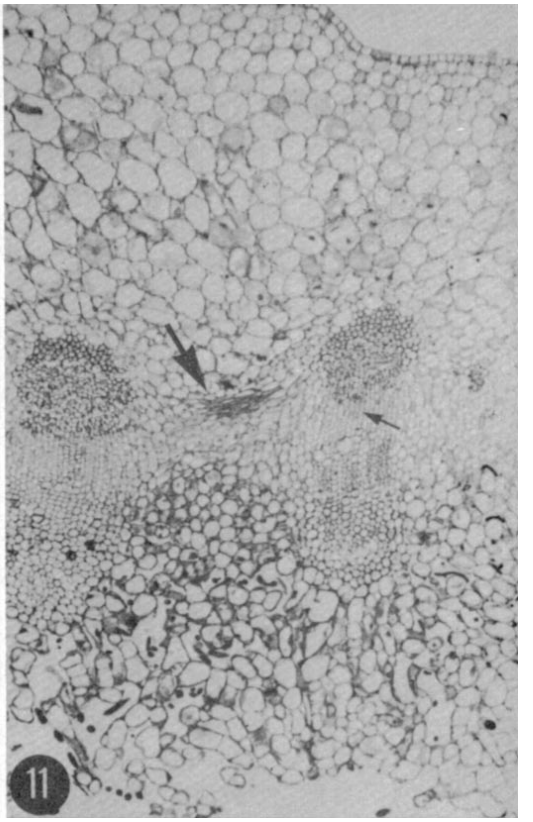

FIG. 10-11. *10)* Cross-section of normal *C. arvense* stem. ×75. *11)* Cross-section of infected *C. arvense* stem at maturity, showing hypertrophy and hyperplasia in the pith parenchyma. Note the hypertrophy in both the fascicular (small arrow) and interfascicular cambium (large arrow). ×75.

cells in the *D. dipsaci* gall provides evidence of damage besides the more obvious cell separation and disruption of vascular bundles. It has been shown with radioactive tracers (11) that nutritive cells act as powerful physiological sinks attracting assimilates to the gall and gall formers. Nutritive cells and syncytia in nematode galls probably serve in a similar manner (1, 26) and it can be postulated that the volume of the nutritive layer is an indication of the extent of the sink effect.

Although the amount of nutritive tissue in stem galls of *C. arvense* induced by *D. dipsaci* is less than in advanced nematode galls such as those induced by *Anguina* species, we suggest that they are acting as physiological sinks. Browning of leaves near the gall may be due to this effect. The incurred drain may be minor compared with damage inflicted in vascular bundles, yet it must be considered in assessing damage.

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FIG. 4-9. *4)* Longitudinal section of infected shoot showing nematodes (arrows) feeding on cortical parenchyma cells. ×80. *5)* Cross-section of infected stem showing nematodes and cell hypertrophy, collapsed cells and increased intercellular spaces in the cortical parenchyma. ×80. *6)* Longitudinal section below the apical meristem showing the first cellular differentiation of cytoplasmically dense cells. ×75. *7)* Longitudinal section of infected stem showing cytoplasmically dense cells (arrow) found lining the central cavity (cc). Note the lack of vacuoles, nuclear modifications and evidence of hyperplasia. ×195. *8)* Cross-section of infected stem showing nematode damage to vascular bundle and adjoining tissues. Cytoplasmically dense cells (arrow) have also differentiated amongst vascular tissues, pith, and cortical parenchyma. ×195. *9)* Cross-section of mature stem showing vascular tissues damage by nematode feeding (arrow). ×75.

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