Anatomic Changes Resulting from the Parasitism of Tomato by *Orobanche ramosa*

Barbara White Pennypacker, Paul E. Nelson, and Stephen Wilhelm

Research associate and professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park 16802; and professor, Department of Plant Pathology, University of California, Berkeley 94720.
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ABSTRACT

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Histologic studies were conducted on roots of tomato (*Lycopersicon esculentum*) cultivar VF 145 parasitized by *Orobanche ramosa*. The point of attachment of the parasite to the host consisted of a mass of undifferentiated, polymorphic *Orobanche* parenchymal cells extending from the tomato epidermis to the xylem tissue. The polymorphic cells contacted the host xylem cells, occasionally penetrated them, and then differentiated into parasite xylem vessel elements. These newly differentiated parasite xylem vessels connected the host xylem to the main vascular system of the parasite. Other polymorphic cells became tightly appressed to phloem sieve cells of the host. These undifferentiated cells probably absorbed nutrients from the sieve cells via the sieve areas and transported the nutrients back to the parasite. *O. ramosa* established both a xylem and a phloem connection with its host.

Orobanche ramosa L., commonly known as hemp or branched broomrape, is an achlorophyllous angiosperm parasite of the root systems of various solanaceous and leguminous plants (9,16,17,21). It has worldwide distribution and was first found in the United States in Kentucky (17) in the late 1800s. The parasite since has spread to California, where it infests several thousand acres of land in tomato production and causes severe damage (4).

O. ramosa annually produces an aboveground flower stalk that releases thousands of minute seeds (1,17). The seeds germinate only in the presence of a host crop, and they survive up to 13 yr in soil (7). Many investigators have tried to determine the mechanism of seed germination (12–14). Nash and Wilhelm (14) reported that the presence of gibberellic acid stimulates germination of *O. ramosa* seed and suggests that host roots may secrete gibberellins into the rhizosphere.

Attempts to control *O. ramosa* are complicated by the failure of the parasite to germinate in the absence of a host. In the absence of susceptible plants, it is difficult to detect the pathogen in infested soil. Ashworth (3) recently reported a technique to quantify the number of broomrape seeds in the soil; this method, coupled with field surveys, may provide a reliable index of the degree to which a field is infested. Fumigating with methyl bromide and then immediately covering the field with a polyvinyl tarpaulin has successfully controlled this parasite (21).

Broomrape attaches itself to the host root system by means of suckers extending into the host cortex, as Guettard (8) showed in 1746 in his work with glouteron (burdock, *Arctium lappa* L.). He indicated that connecting suckers were open on the distal end, allowing nutrients to flow from the host to the parasite. The mechanism by which the nutrient transfer occurred was not determined. Guettard's work on broomrape demonstrated clear understanding of the parasitic process and contributed to the modern concept of parasitism.

This study was initiated to determine the mechanism by which broomrape parasitizes tomato plants and the anatomic changes that occur in tomato roots parasitized by *O. ramosa*.

MATERIALS AND METHODS

Specimens of *O. ramosa* on tomato (*Lycopersicon esculentum* Mill.) roots were collected from infected plants growing in fields near Union City and Clarksburg, CA, during August 1975. In addition, *O. ramosa* specimens were obtained from the roots of infected tomato plants, cultivar VF 145, grown in root observation boxes and from similar plants grown in 15-cm clay pots during November 1975 and June 1976, respectively.

After careful removal from soil, the tomato specimens were fixed in formalin/acetic acid/alcohol (FAA) (11) and shipped to Pennsylvania. A diagram was made of each specimen and the specimens were given identifying numbers before being dehydrated in a tertiary butyl alcohol series, infiltrated, and embedded in Paraplast (Scientific Products, 2410 McGraw Rd., Obetz, OH 43207) (11). The specimens were oriented during embedding to allow both longitudinal and cross sections of the parasite tissue. The embedded specimens were softened for approximately 5 hr in a solution of 90 ml of 1% sodium lauryl sulfate (Dreft) and 10 ml of glycerin before sectioning at 10 μm on a Leitz rotary microtome (2). The sections were mounted serially on chemically cleaned slides and stained with either Johansen's Quadruple Stain (11) or Pianeze III B-chlorozol black E stain (20). Selected sections were photographed on Kodak Plus X Pan film with a Leitz Aristophot camera with a 4 × 5 in. Graflex back.

Histochemical tests were performed on the sectioned material for pectin (iron-absorption method), starch (IKI), gums (phloroglucinol and orcinol), and suberin (Sudan IV) (10,15). Sections also were examined under polarized light to detect possible cellulose breakdown.

RESULTS

The parasite. The initial infective propagule of *O. ramosa* is a microscopic seed (Fig. 1). The seed germinates in the presence of host tissue, and ultimately will invade the roots of the host plant. After successful establishment, the parasite produces a tubercle external to the host tissue. The tubercle has many rootlike projections (Fig. 2), each consisting of an epidermis, cortex, and vascular system (Fig. 3). The vascular system of *O. ramosa* is composed of short, irregular, rectangular xylem vessel elements (Fig. 3) that have both helical and reticulate secondary thickenings and scalariform perforation plates. Phloem tissue consists of sieve tube members (Fig. 4). The vascular system usually is surrounded by layers of cortical parenchymal cells containing starch and an epidermis.

In addition to the well-organized vascular tissue of *O. ramosa*, a similar although haphazardly arranged vascular system is produced outside the central vascular cylinder (Fig. 5). The randomly arranged system apparently arises from the differ-

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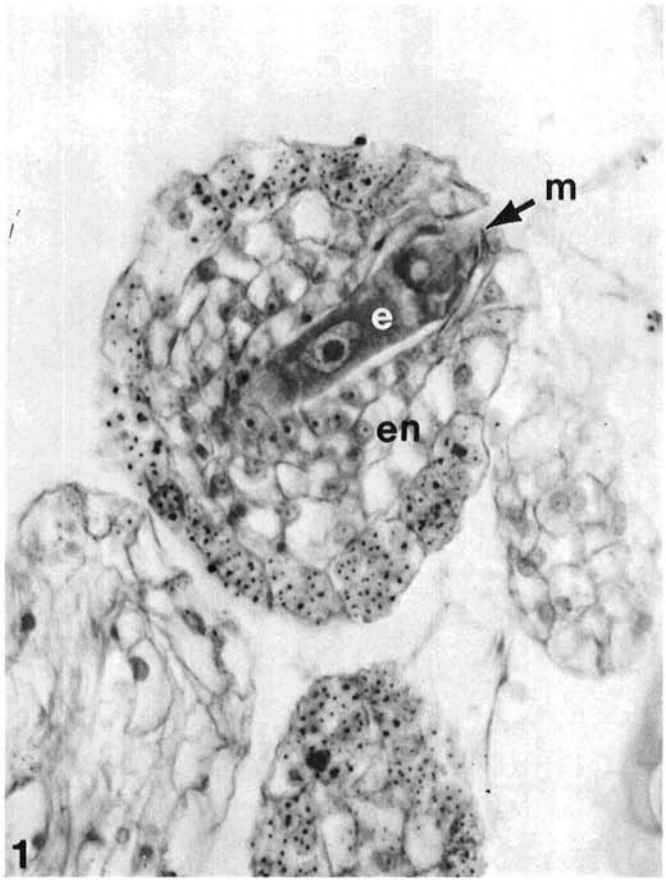
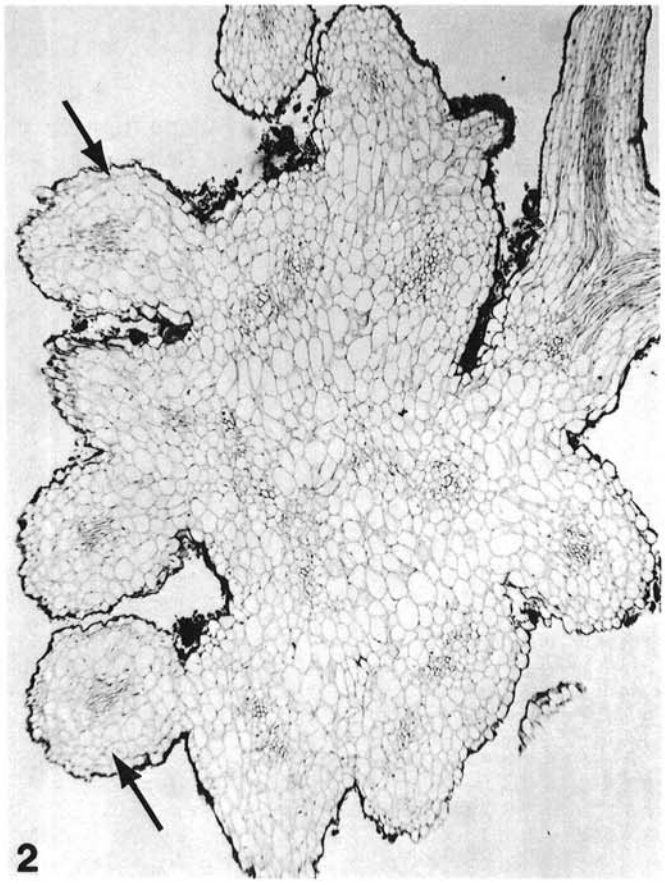
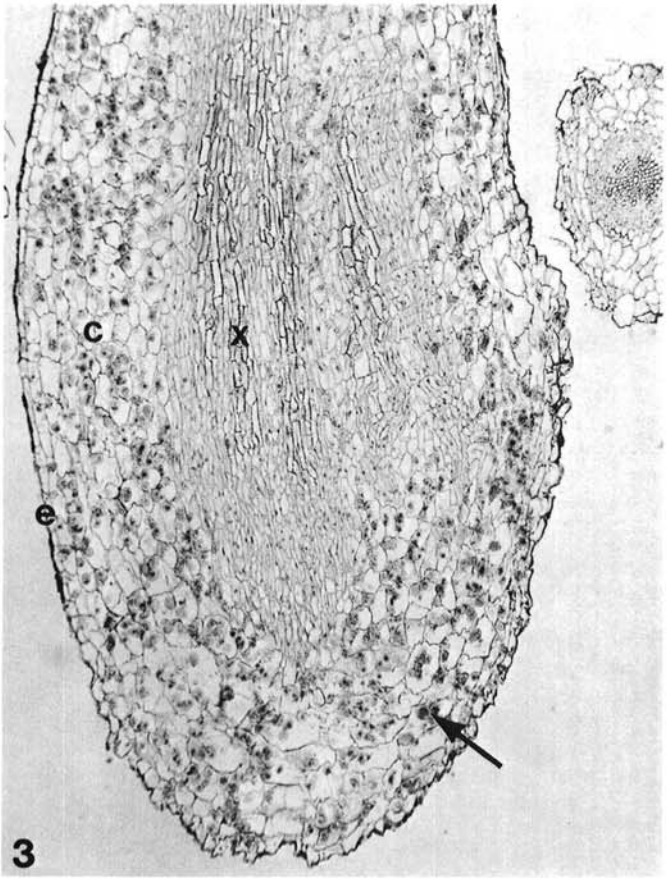
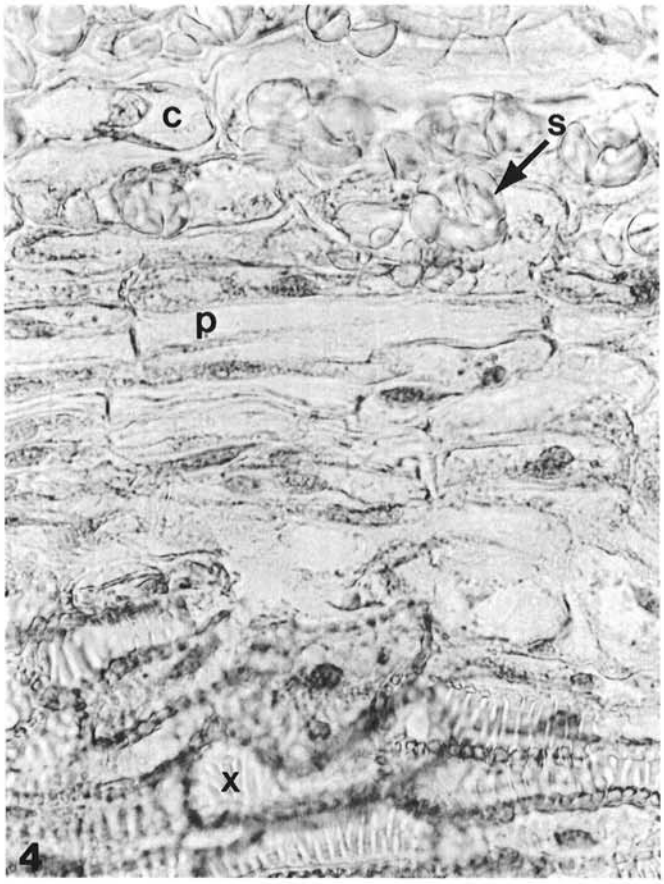
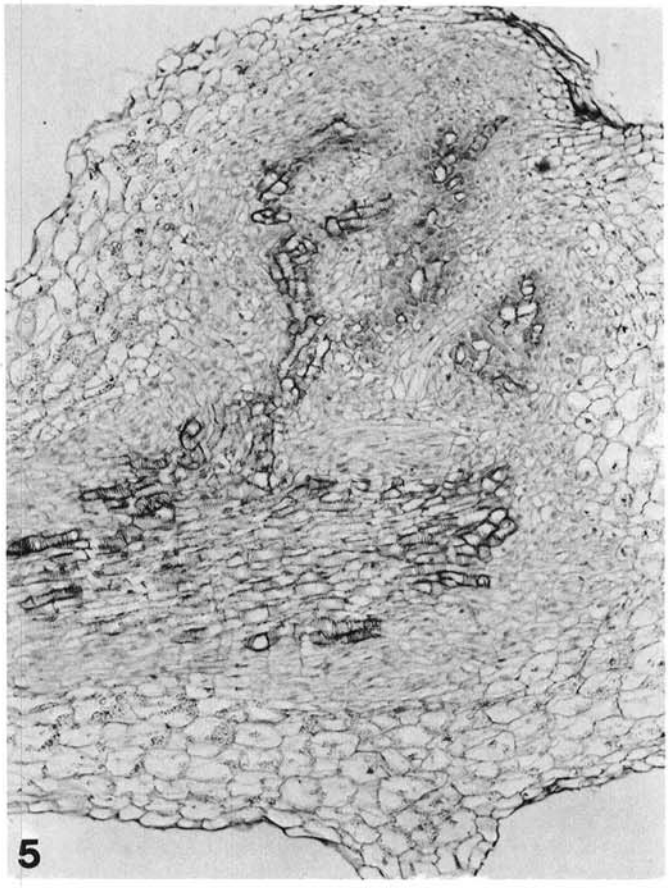


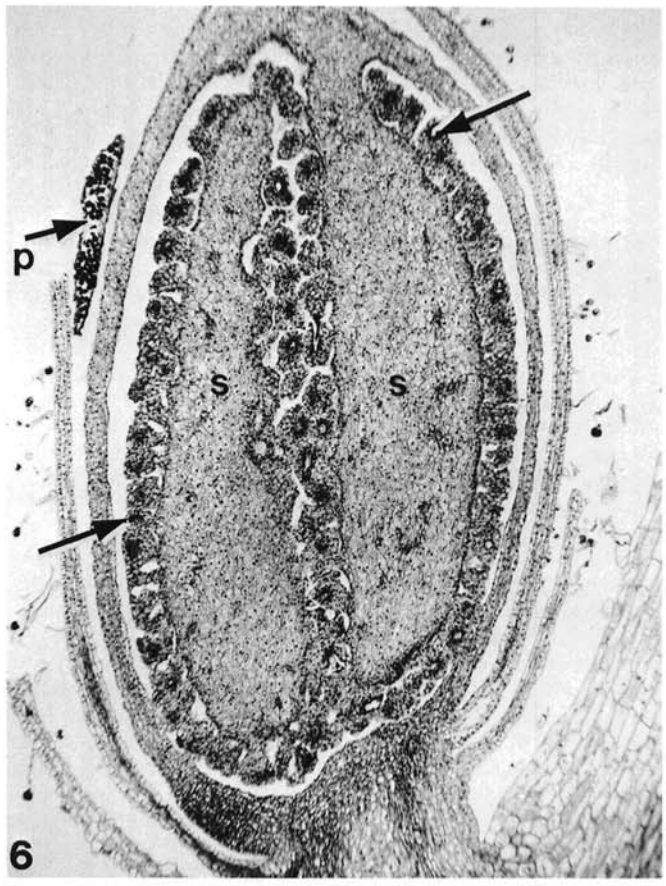


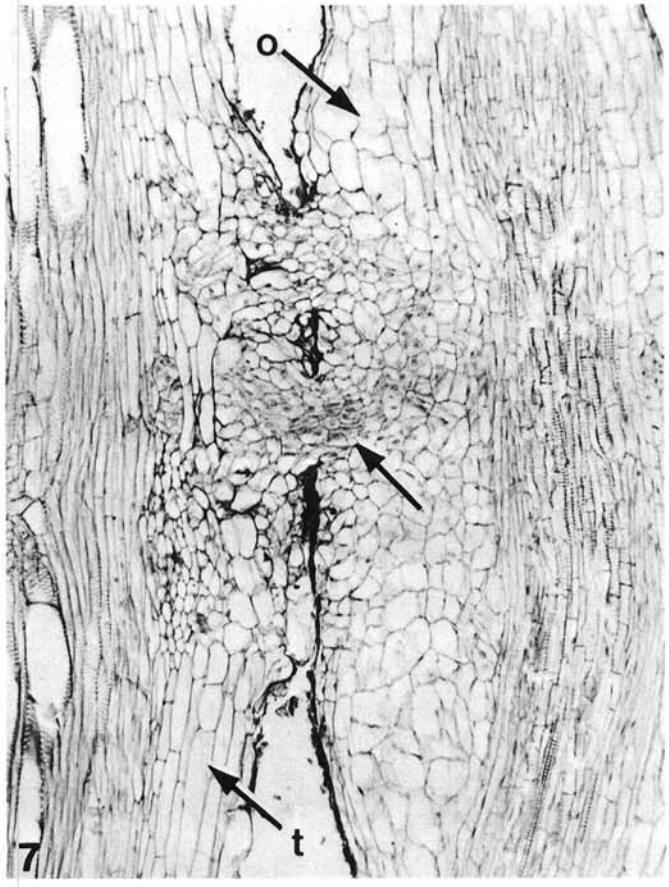


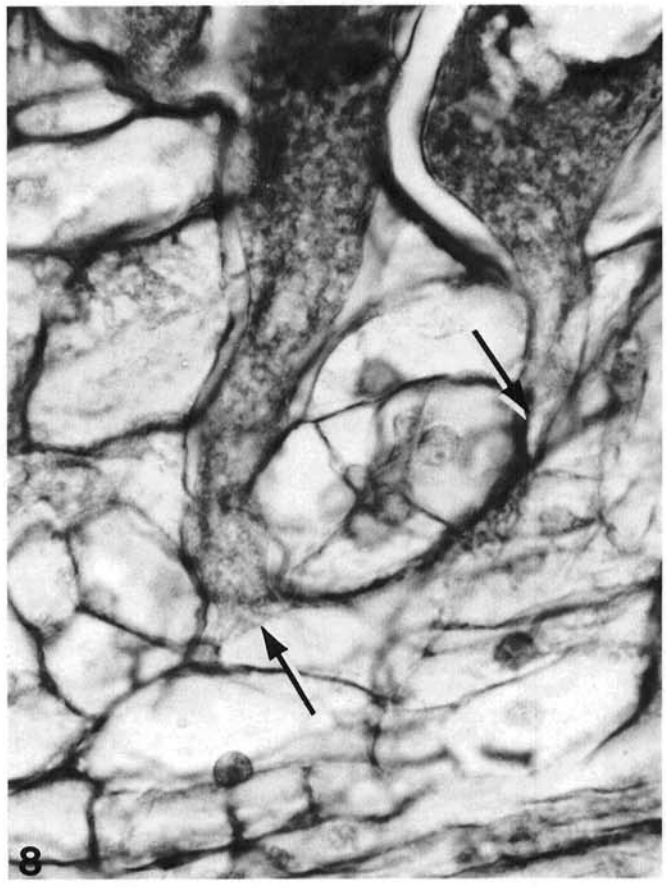


Figs. 1–4. Sections through *Orobanche ramosa* plants of varying ages. **1,** Cross section through an *O. ramosa* seed showing the embryo (**e**), endosperm (**en**), and micropyle (**m**). (×337) **2,** Cross section through a young *O. ramosa* turbercle. Note the rootlike projections (arrows). (×47) **3,** Longitudinal section of an *O. ramosa* "root" showing xylem (**x**), cortex (**c**), and epidermis (**e**). Note the starch grains (arrow) and the irregular shape of the xylem vessel elements. (×51) **4,** Cross section through an *O. ramosa* plant stained with lacmoid, a stain specific for callose, showing the cortex (**c**), phloem (**p**), xylem (**x**), and starch grains (**s**). (×430).









Figs. 5–8. Sections through *Orobanche* tissue and through tomato roots invaded by the parasite. **5,** Section through an older tubercle of *O. ramosa*. Vascular tissue is disorganized. (×74) **6,** Longitudinal section through the ovary of an *O. ramosa* flower. Note the numerous ovules (arrows), the pollen (**p**), and the starch (**s**). (×38) **7,** Longitudinal section of a tomato root (**t**) and *O. ramosa* (**o**) shows the initial stages of penetration by the parasite. Note the presence of granular elongated *Orobanche* cells (arrow). (×86) **8,** Cross section of a parasitized tomato root. Note the granular polymorphic cells and their apparent lack of a distinct cell wall (arrows). (×807).

entiation and division of cortical parenchyma cells. Such differentiation seems to be initiated in response to the presence of host tissue and initially consists of elongated, granular cells. These cells eventually develop the secondary thickenings of xylem vessel elements and probably function as such.

The tubercle of the parasite eventually initiates a floral meristem that produces a flower stalk with ovaries and ovules (Fig. 6). Large amounts of starch are stored in these reproductive tissues.

The host-parasite system. Attempts to isolate the initial infection of a tomato root by *O. ramosa* in plants grown in root observation boxes were unsuccessful, and the only specimens available came from field-grown tomato roots. The amount of time between infection and sampling of these roots was impossible to determine. Consequently, we were able to observe only one case of relatively early although secondary parasite penetration. In this specimen, the elongated, granular *Orobanche* parenchyma mentioned above were present between the host cells (Fig. 7), and no extensive host cell destruction was evident.

Once in the host cortex, the granular *Orobanche* cells continue to divide and occupy the host cortex adjacent to the point of entry. As colonization of the host progresses, the parasite forms many granular polymorphic cells. These cells usually are elongated and appear to lack a distinct cell wall (Fig. 8). The polymorphic cells grow laterally in the host tissue until they contact host xylem vessel elements (Fig. 9). Such contact appears to initiate further parasite cell differentiation and many of the polymorphic cells develop the secondary thickenings characteristic of xylem vessel elements (Fig. 10). The initial polymorphic cells of *Orobanche* may penetrate the xylem vessel elements of the tomato prior to differentiation into parasite xylem cells (Fig. 11). The differentiation process proceeds from the host xylem backward to the *Orobanche* tubercle and results in the formation of an *Orobanche* xylem vessel that extends from the host xylem tissue to the parasite main body (Fig. 12). The formation of xylem vessel elements to connect the parasite xylem vessel from tomato to the main vascular system of the parasite tubercle accounts for the apparently haphazard arrangement of vascular tissue in the tubercle. As a result of differentiation of polymorphic cells into xylem vessel elements, a direct connection is established between the tomato xylem elements and the vascular system of *O. ramosa*.

No such connection is apparent between tomato phloem tissue and *Orobanche* phloem cells. Phloem sieve cells are present in the *Orobanche* tubercle, but no phloem cells of the parasite have been identified in the tomato tissue. The quantity of starch present in *O. ramosa* tissue apparently indicates existence of some type of phloem connection between the two plants, since the obligate parasite *O. ramosa* cannot produce the sugars necessary for starch formation.

In the area where the parasite joins the host tissue are numerous polymorphic *Orobanche* cells that grow longitudinally as well as laterally. Host phloem sieve tubes are completely absent in this area of host/parasite contact, although they are present above and below the area. Polymorphic parasite cells are tightly appressed to the tomato sieve cells that are directly adjacent to the area of intensive host/parasite contact (Fig. 13). These cells may be able to obtain nutrients from the tomato sieve cells by absorption via the sieve areas. We did not observe the penetration of a host phloem cell by the polymorphic parasite cell, although this penetration often occurred in host xylem cells.

In the case of the xylem connection, the parasite initiates the formation of xylem vessel elements from many sides of the host xylem during the later stages of infection (Fig. 14); however, these vascular connections between host and parasite are restricted to the outer edge of the host xylem until late in pathogenesis when deep vascular penetration occurs (Fig. 15).

Penetration of tomato tissue by *O. ramosa* is not accompanied by crushing of host cells. Histologic evidence indicates that the polymorphic cells of the parasite cause selective disintegration of host cells, and then the parasite cells occupy the resulting space (Figs. 16 and 17). This mechanism of penetration and infection results in a close association between host cells and parasite cells, often making them difficult to distinguish.

In contrast to the parasite's effect on host cortical tissue, disintegration of the host xylem vessel elements is not extensive, but some vessel elements may partially disintegrate during penetration by the polymorphic cells (Fig. 18). In addition, the host xylem vessel elements are frequently occluded by tyloses that usually are located in the area of the host/parasite connection.

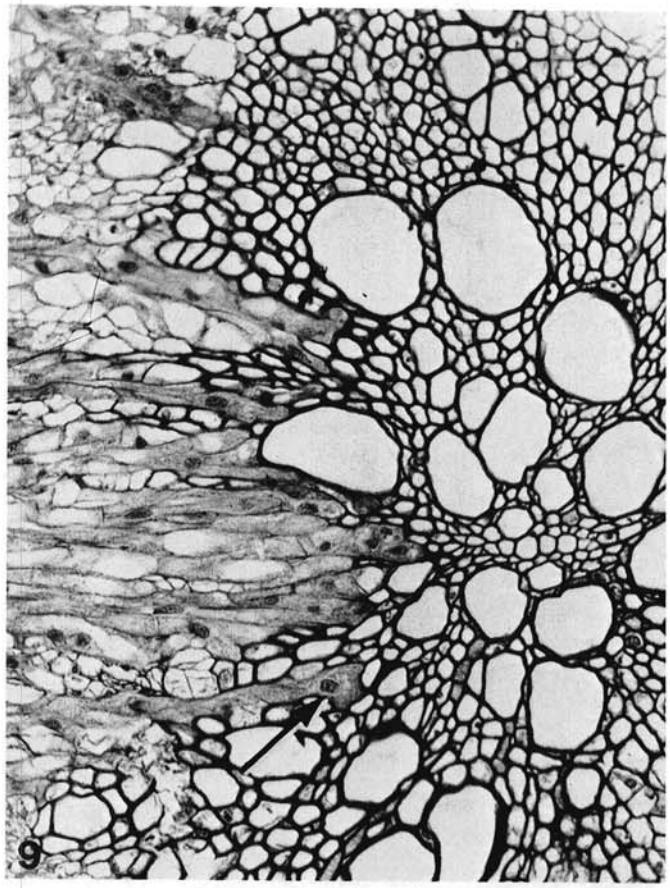
Histochemical tests for pectic materials, gums and gels, starch, cellulose, suberin, and callose were performed on selected material from the area of the host/parasite connection. Starch, present in large quantities in the parasite cortical cells, generally was absent in the tomato roots. In one case, very small starch grains were present in the tomato cortex, but they were associated with the initial thrust of the polymorphic cells into the host cortex. Large quantities of pectic materials were observed around the polymorphic cells in the tomato tissue. Strong positive reactions for pectic substances also were obtained in all *O. ramosa* tissue except the xylem, and in the cortex, phloem, and vascular cambium of the tomato. Tests for gums and gels were negative, except in one case in which a vascular plug in the *Orobanche* xylem was positive for gum. Reactions for suberin were occasionally positive in the polymorphic cells in the tomato tissue; these results were not consistent, however. Callose was detected in the sieve plates and sieve areas of both tomato and *O. ramosa* phloem tissue. Polarized light studies indicated that cellulose was present and intact in the xylem of both plants.

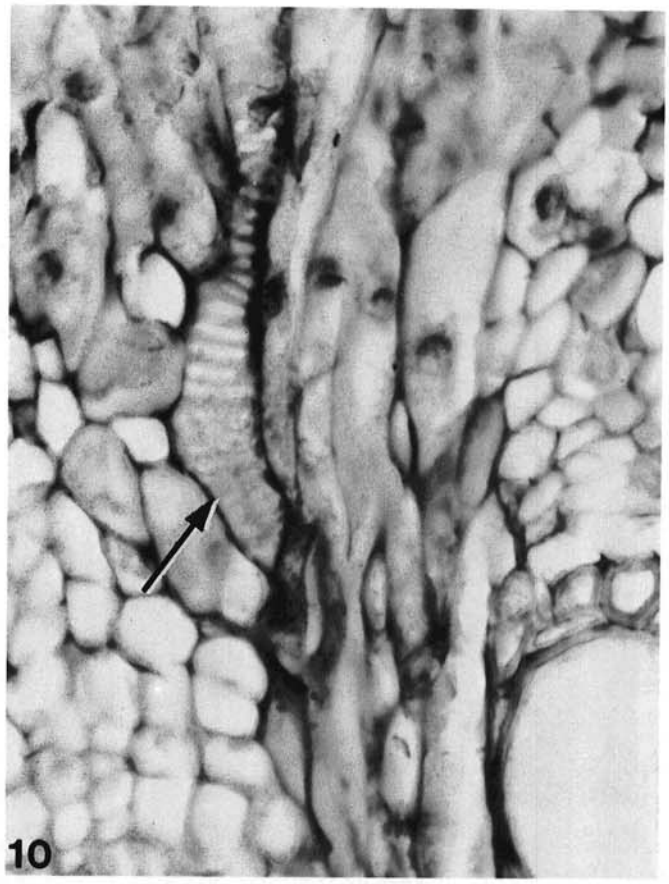
DISCUSSION

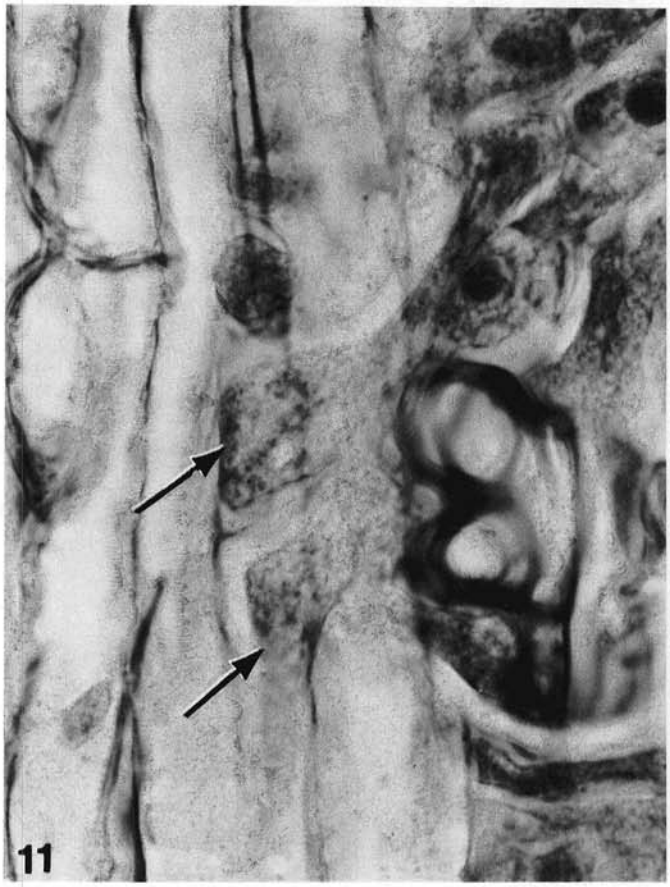
Our results show that *O. ramosa* establishes a connection with the xylem tissue of the tomato root. To form this vital link, the parasite relies on the activity of specialized cortical parenchymal cells. These cells, termed polymorphic because of their apparent lack of a definite cell wall in the initial stages, are capable of penetrating host tissue and growing through it until they contact host xylem cells. Dörr and Kollmann (5) reported the presence of similar cells in hemp parasitized by *O. ramosa*; they referred to them as haustorial cells. These investigators also were able to distinguish between the very similar host and parasite cells by means of the electron microscope. At first, we encountered difficulty in such differentiation but with practice were able to separate the two kinds of cells because of a difference in the granulation of the cytoplasm. It is possible that we misidentified some *Orobanche* cells that lacked the pronounced granulation.

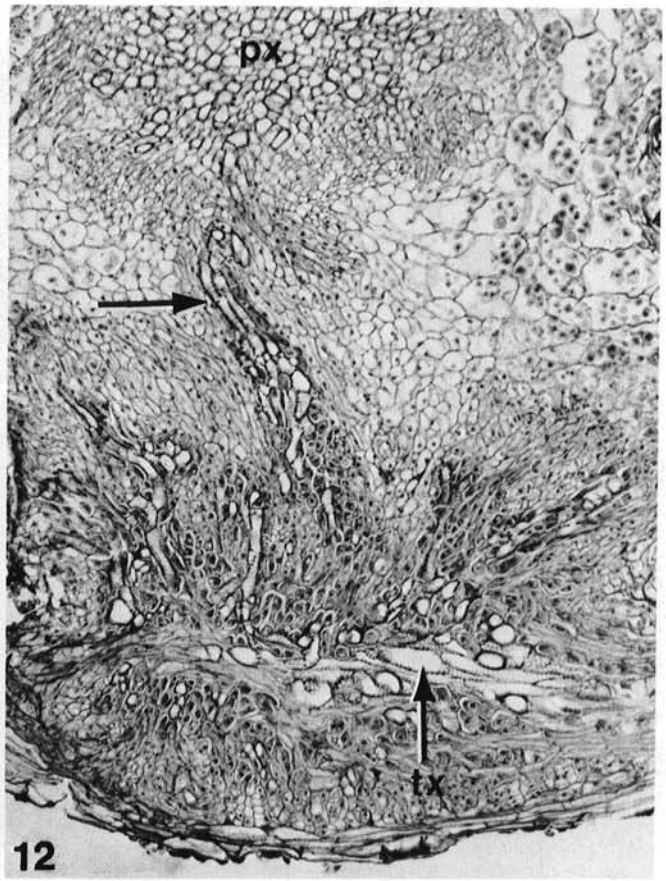
Initially, the polymorphic cells of the parasite were identical, but histologic evidence from more advanced infections indicated that some would become xylem vessel elements and others would be involved in nutrient transport. Contact alone with host xylem tissue may have initiated a parasite response that caused the polymorphic cell to differentiate into a xylem vessel element. It is also conceivable that a substance transported by the host xylem produced the stimulus for differentiation. The result was a complete parasite xylem vessel leading from the host vascular tissue to the main parasite vascular system.

Some polymorphic cells apparently failed to differentiate into specialized cells but rather appeared to become associated with the host phloem tissue. These cells and those that differentiated into xylem vessel elements often seemed to lack a cell wall. Dörr and Kollmann (6), working with *O. ramosa* in hemp, showed that a very thin cell wall was visible in cells viewed with an electron microscope. They further described a well-differentiated parasite phloem system in the host tissue, which was composed of three types of parasite cells: the contact cells that touch the host sieve cells and were undifferentiated, the transition sieve cells that lack nuclei and the true sieve tube members. We identified polymorphic cells that seemed to correspond to the contact cells of Dörr and Kollmann (6). These cells were undifferentiated, tightly appressed to the host sieve member and often extended longitudinally along those sieve cells. We were not able to distinguish the other cell types described above and concur with the authors' observation that these anatomic features are not visible under the light microscope. Although we could not identify the cells connecting the host phloem cells and the main body of the parasite, we conclude that the close association between the polymorphic cells and the host

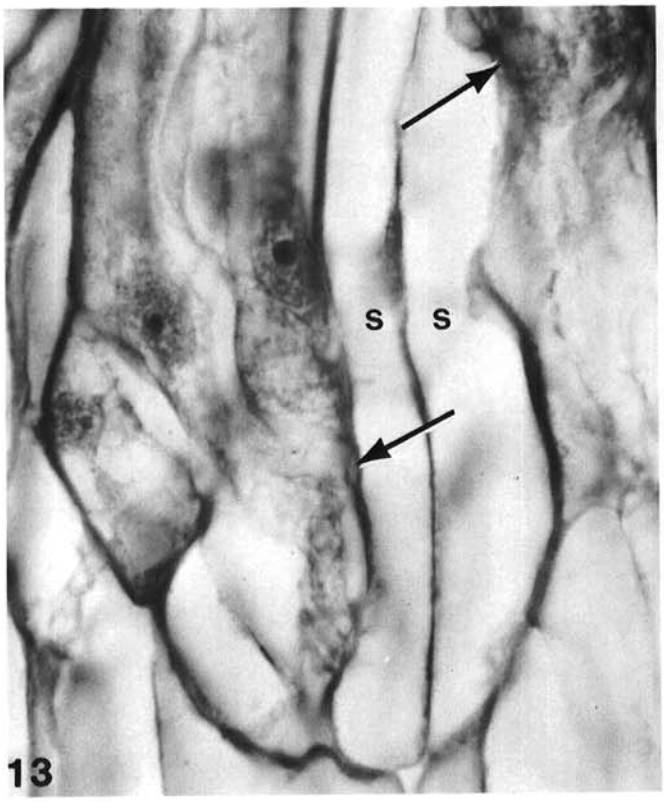


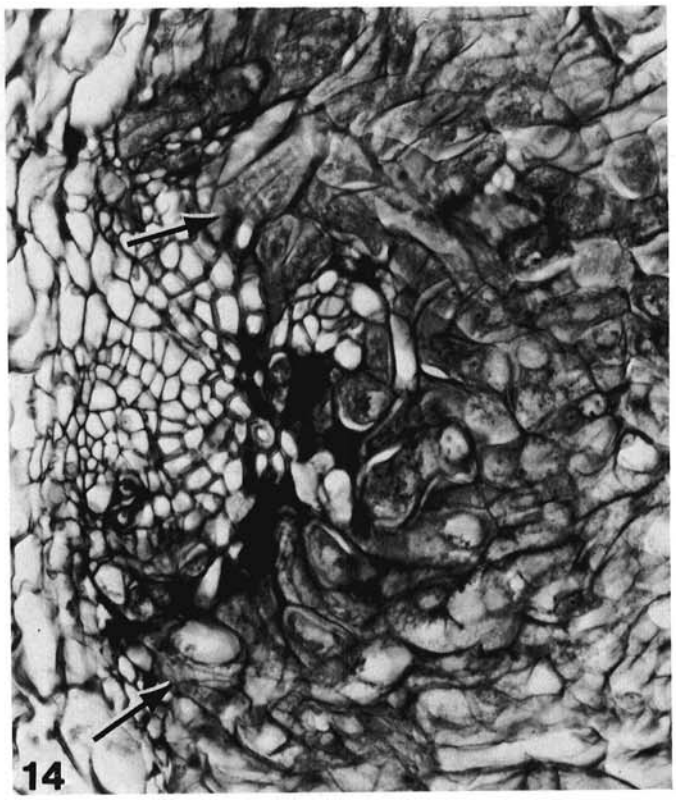


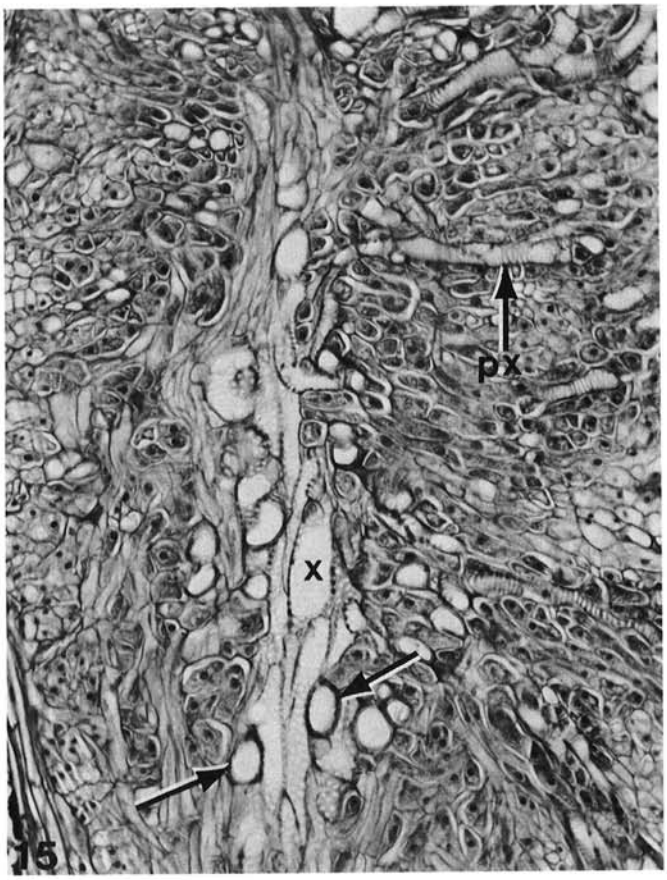


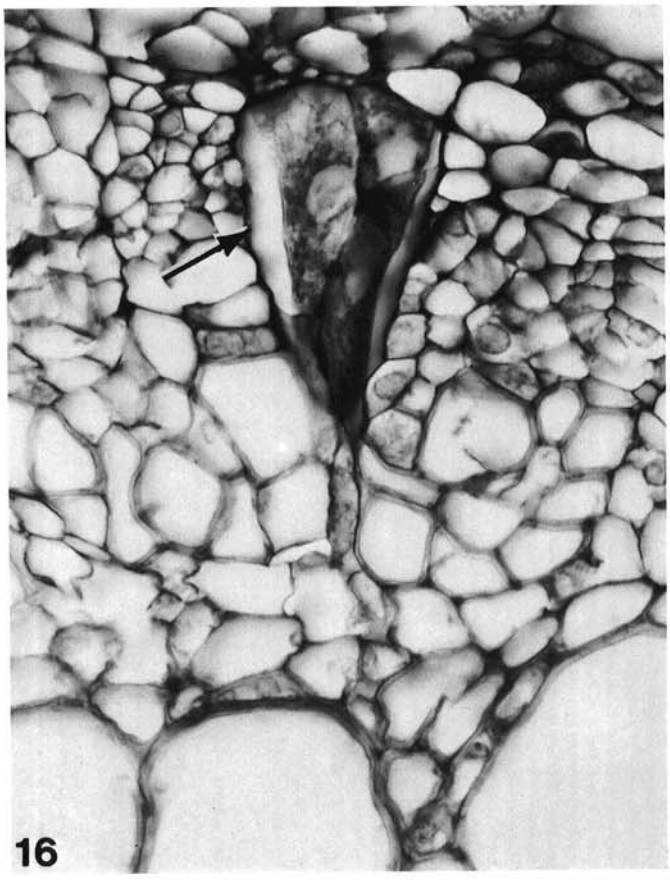


Figs. 9–12. Sections through tomato roots parasitized by *Orobanche ramosa*. **9,** Cross section through a tomato root shows the polymorphic cells (arrow) of the parasite. Note their apparent affinity for the host xylem tissue. (×180) **10,** Cross section of tomato root shows the differentiation of the polymorphic cells of the parasite into xylem vessel elements (arrow). (×420) **11,** Longitudinal section of a tomato root. Note the polymorphic cells, apparently lacking cell walls, that have penetrated the host xylem vessel elements (arrows). (×980) **12,** Longitudinal section through a tomato root parasitized by *O. ramosa*. The parasite xylem vessels (arrows) have formed between the tomato xylem (**tx**) and the parasite xylem (**px**). (×82).

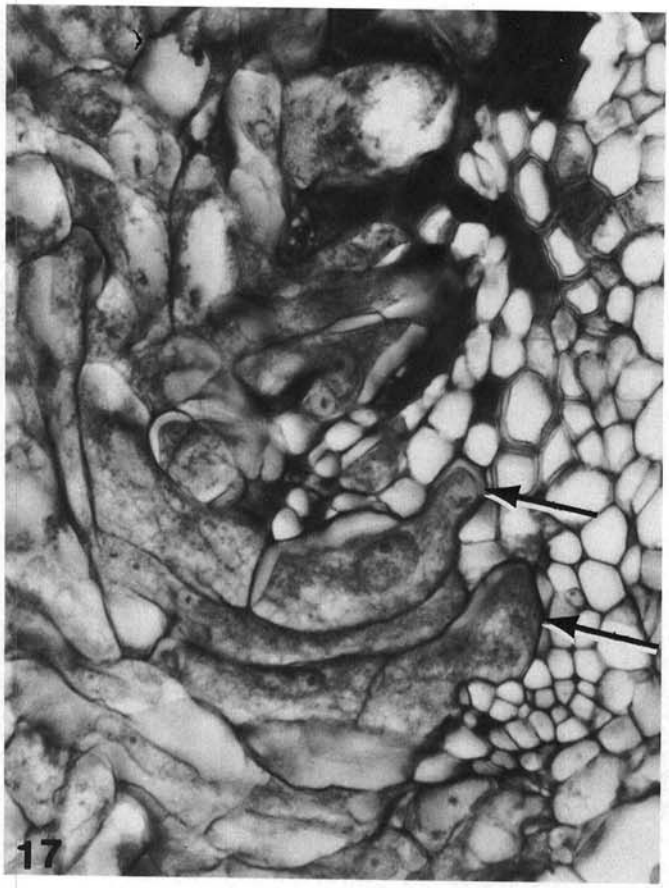


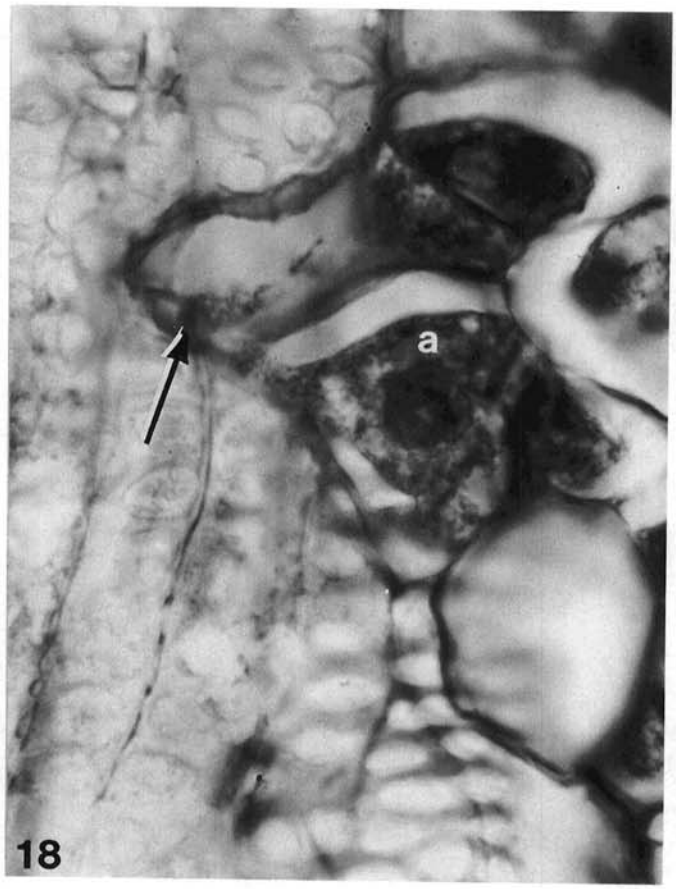






Figs. 13–16. Sections through tomato roots parasitized by *Orobanche ramosa*. **13,** Longitudinal section through a tomato root shows the polymorphic cells of the parasite (arrows) tightly appressed to host sieve cells (**s**). (×116) **14,** Cross section through a tomato root heavily parasitized by *O. ramosa*. The polymorphic cells (arrows) of the parasite have penetrated the host xylem tissue from several directions. (×360) **15,** Longitudinal section through a tomato root during the later stages of pathogenesis. Penetration of the parasite is deep and few host xylem vessels are not attacked (**x**). The dark-staining cross sections of xylem vessel elements (arrows) probably represent areas of connection between the host and parasite xylem where the parasite penetrated from above or below the plane of the photograph. Also note the formation of parasite xylem vessels (**px**). (×160) **16,** Cross section of a tomato root infected with *O. ramosa*. Note the cavity formed around the polymorphic parasite cell (arrow) and the lack of any distorted host cells, indicating breakdown of the host cells. (×608).





Figs. 17 and **18.** Sections through tomato roots invaded by *O. ramosa*. **17,** Cross section of a tomato root invaded by polymorphic cells (arrows) of *O. ramosa*. Note the absence of crushed cells around the polymorphic cells. (×592) **18,** Longitudinal section through a tomato root showing the apparent breakdown of a tomato xylem vessel element (arrow) and the penetration of the *O. ramosa* polymorphic cell (**a**). (×868).

sieve cells provides circumstantial evidence supporting the existence of a host/parasite phloem connection.

We did not observe mechanical damage to any host cells. On the contrary, there was evidence of enzymatic degradation of host cells in contact with *O. ramosa* cells. When the parasite cell contacts the host cell, the latter appears to disintegrate and the parasite, which grows very rapidly, then occupies the resulting space. Electron-microscopic studies (5) support the idea of enzymatic breakdown and show a loosening of the middle lamella between host cells. Large cavities form and are occupied by the parasite. Whether the parasite invades host cells and destroys them or whether it occupies spaces formed by the loosening of the middle lamella is open to question. As previously stated, we found no cell crushing or other mechanical damage that would be expected if large spaces were created between cells and then occupied by the parasite. It is possible that a combination of the two mechanisms is operating, resulting in the extremely close association between host and parasite.

Tyloses frequently were observed in the xylem vessel elements of tomato roots in the area of parasite activity. Tyloses were not observed in healthy tomato xylem vessels. These structures could represent an attempt by the tomato to wall off the parasitic invasion.

Histochemical tests substantiated previous reports (19) that the *Orobanche* plant contains large reserves of starch; no starch was found in the host root tissue. Whitney (18) reported that sucrose from faba bean is transported to *O. crenata*, where it is immediately hydrolyzed into fructose and glucose, thus maintaining a parasite-favorable concentration gradient. A similar transfer may be operating in the *O. ramosa*/tomato system with the carbohydrate from the tomato being transformed into starch in the parasite.

A buildup of pectic materials was noted along the perimeter of the long polymorphic cells. This material may represent the remains of cells broken down during the advance of the polymorphic cells into the xylem tissue. It is also possible that these polymorphic cells excrete or otherwise produce large quantities of pectic material along their perimeters. Dörr and Kollmann (5) reported a loosening of the middle lamella between cells adjacent to parasite cells, but they did not determine whether the pectic substances composing the middle lamella were destroyed. Our evidence suggests that these materials were not destroyed.

No other changes in cell constituents were detected in the tomato root tissue. The lack of observable chemical change in the tomato root may indicate that the parasite does not attack the tissues of the host beyond those that are necessary to establish a dynamic relationship with the host xylem and phloem. Apparently *O. ramosa* obtains needed metabolites from the host vascular system rather than from a combination of host vascular translocates and nutrients gained by the destruction of host tissue.

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