Mycoparasitic Relationships: Gonatobotrys simplex Parasitic on Alternaria tenuis

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This is the second of a continuing series of papers on the subject of mycoparasitic relationships.

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ABSTRACT

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Ultrastructural detail of the host-parasite interface of a biotrophic contact mycoparasitic relationship involving *Alternaria tenuis* and *Gonatobotrys simplex* is presented. Membrane-lined plasmodesmata traverse the wall between the two fungi and thus provide intimate cytoplasmic contact. The wall of the host, *A. tenuis,* at the contact interface is thickened; the wall of *G. simplex* either was not resolved by the preparative and micrographic techniques or it is absent. The contact cell of *G. simplex* is separated from the main hypha of *G. simplex* either by an occluded septal pore surrounded by Woronin bodies or by a septum with an open pore.

Mycoparasitism is the parasitism of one fungus on another. Both necrotrophic and biotrophic modes of mycoparasitism exist in nature. The latter type is known to occur in three distinct modes: (i) internal parasitism, exemplified by the chytrids that develop within cells of host fungi (15,16); (ii) haustorial parasitism in which haustoria are produced by the parasite within the host, e.g., *Piptocephalis virginiana* on various species of Mucorales (5); and (iii) contact parasitism, where no internal hyphae or haustoria are produced by the parasite. Barnett and Binder (2) recently reviewed the literature pertaining to mycoparasitism, and discussed in some detail the modes of parasitism by the five known species of biotrophic contact mycoparasites.

In 1963, Whaley and Barnett (23) described a biotrophic contact relationship involving *Gonatobotrys simplex* Corda, the parasite, and *Alternaria tenuis* Nees, the host. They reported that parasitic contact was established via special short-branch contact cells. The contact branches of the parasite varied from globose protrusions to curved or fingerlike appendages. The authors postulated that these contact branches served as specialized absorptive hyphae, perhaps acting as sites of enzyme synthesis to aid in absorbing nutrients from the host cell. Furthermore, they suggested that these contact hyphae brought about increased permeability of the host cell plasmalemma to nutrients and certain growth factors required for axenic growth, one of which they termed mycotrophein.

To understand more clearly how the contact hyphae of *G. simplex* affect the host cells during parasitism, this host-parasite relationship was studied ultrastructurally. The results illustrate a unique means of mycoparasitism; parasitism through plasmodesmata.

MATERIALS AND METHODS

Isolates of *G. simplex* (W. Va. Univ. isolate no. 1692) and *A. tenuis* (W. Va. Univ. isolate no. 1812) were obtained in both mixed and axenic cultures from H. L. Barnett (W. Va. Univ., Morgantown) and were maintained on either potato-dextrose agar or on a glucose-yeast extract agar (GYS) (23). Axenic cultures of *G. simplex* were maintained on the GYA medium supplemented with thiamine HCl (4 mg/liter), biotin (20 μg/liter), and with mycotrophein (6.2 mg/ml) prepared from *Arthrobotrys musiformis* Drechs. (W. Va. Univ. isolate no. 1297) as described previously (23).

Conidia of *G. simplex* and conidia and hyphal fragments of *A. tenuis* were scraped from their respective cultures and suspended in distilled water. The suspensions were poured over GYA-coated microscope slides as mixed culture suspensions. Conidia of *G. simplex* also were poured over GYA-coated slides which had been seeded 24 or 48 hr previously with *A. tenuis.*

The agar-slide cultures were prepared for electron microscopy (12) after 24 to 72 hr of mixed growth of the two fungi on the same slide at 22 C. Fixation, dehydration, and embedding procedures were similar to those previously described (14). Hyphae of the two interacting fungi, selected and photographed by light microscopy in the polymerized embedment, were cut out and remounted so that transverse sections could be made of the hyphae. The sections were post-stained with aqueous 1% barium permanganate for 30 sec followed by a water rinse and a 0.05% citric acid destain treatment for

30 sec. Following another water rinse, the sections were stained further with saturated aqueous uranyl acetate for 2 min, then with lead citrate for 1 min, all at 21 C. In most instances, ribbons of serial thin-sections were collected on 1- × 2-mm single-slot Formvar-coated grids and examined in their entirety with a JEOL 100B electron microscope operating at 60 kV.

Slide cultures of the interacting fungi also were examined and photographed in vitro with Zeiss phase-contrast and Nomarski interference-contrast light microscope optics.

RESULTS

The primary sites of interest in this study were the host-parasite interfaces where the contact cells of the parasite

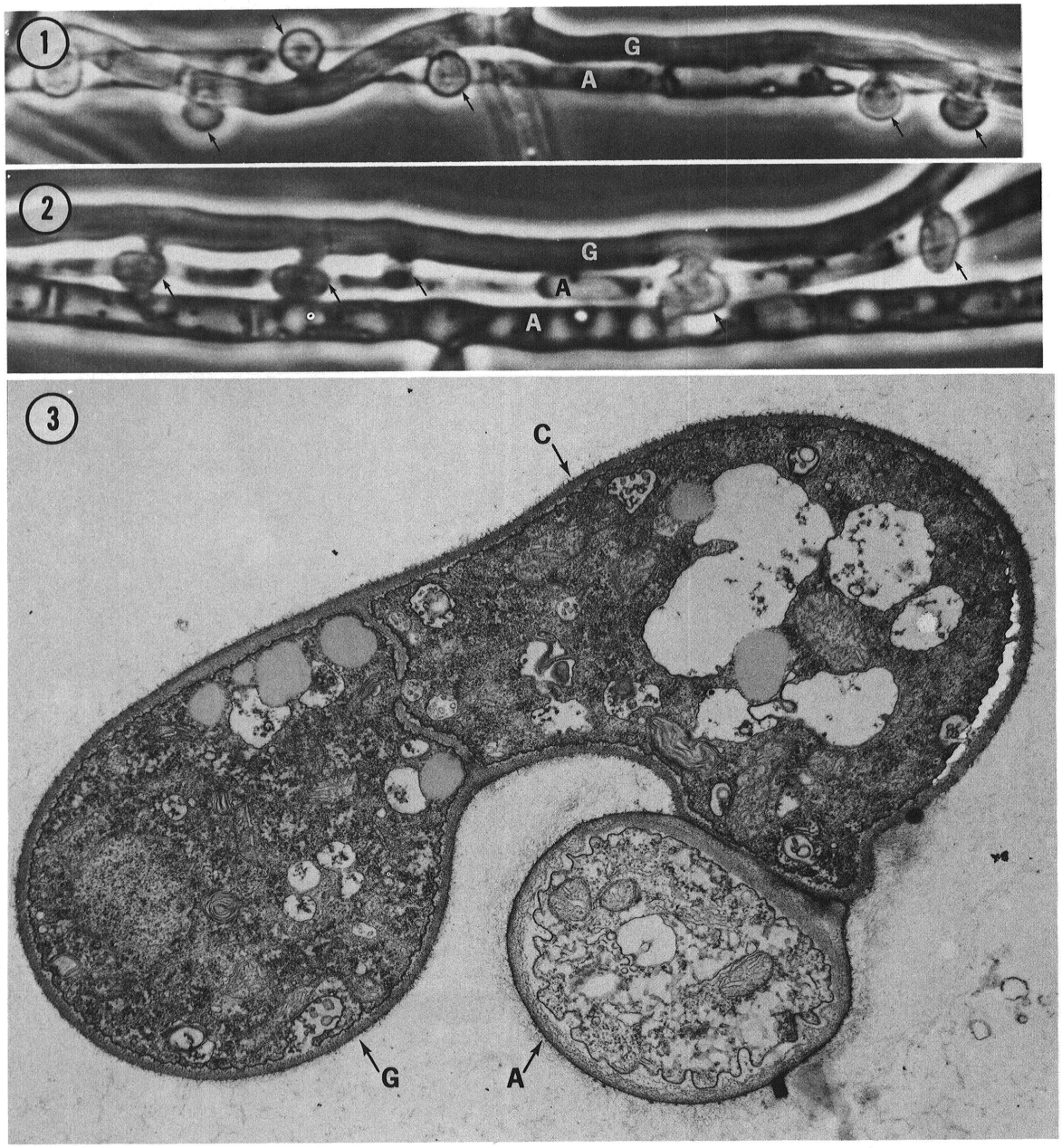

Fig. 1-3. 1) Phase-contrast light micrographs of *Gonatobotrys simplex* hyphae (G) growing parallel to host hyphae of *Alternaria tenuis* (A). Contact cells (arrows) of *G. simplex* curve around contact host hyphae. **2)** When two host hyphae grow intimately parallel to each other, the contact branch of *G. simplex* occasionally extends to contact both host hyphae as shown in the two left contact sites of Fig. 2. Both approximately × 1,800. **3)** Electron micrograph of a transverse section through hyphae of the parasitic fungus *Gonatobotrys simplex* (G) and the host fungus *Alternaria tenuis* (A) showing a curved contact cell (C), which is separated from its parent hypha by a septum. The contact site is not representative of a median section through this area. × 19,950.

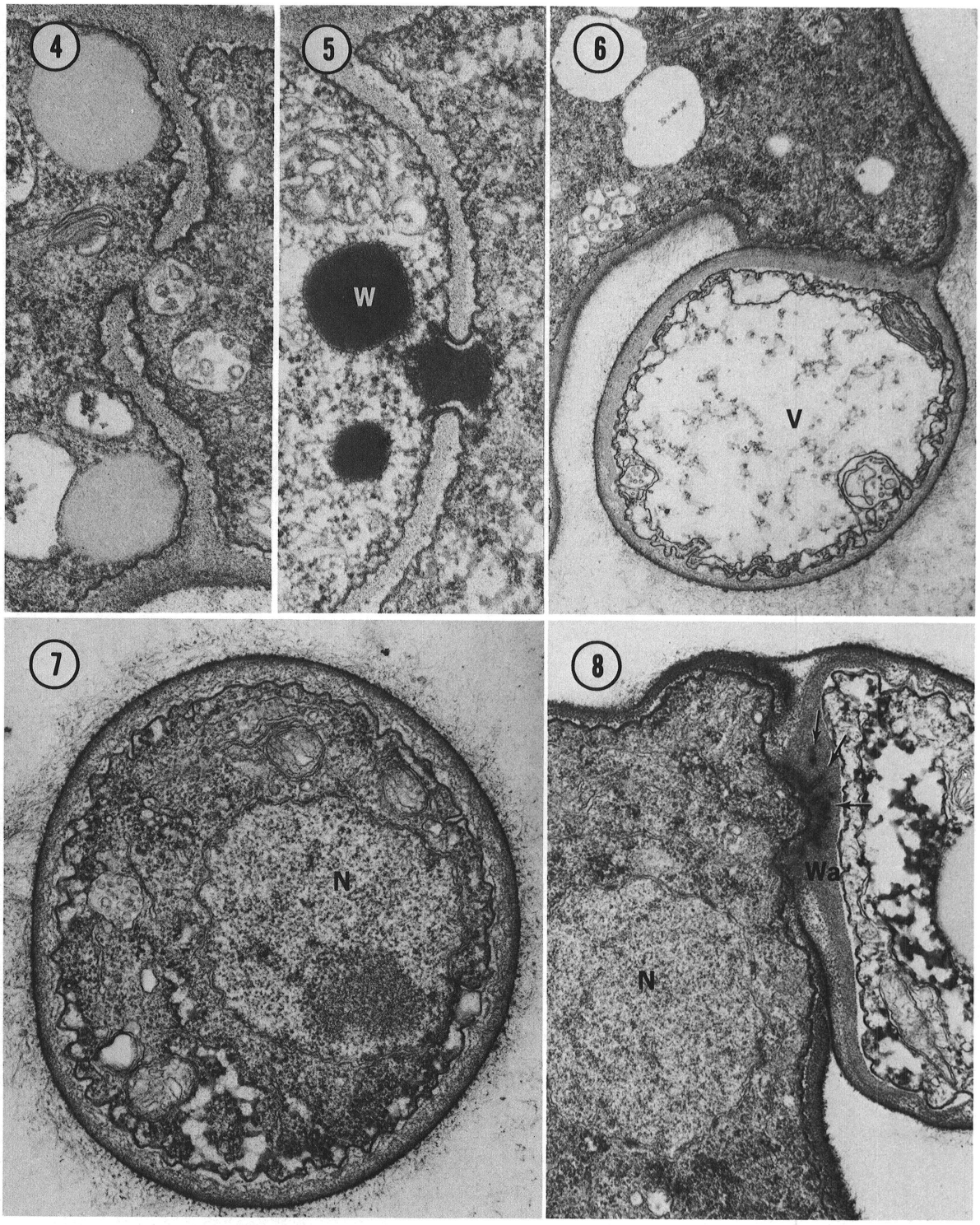

Fig. 4-8.. 4-5) Median sections through pores of septa that separate the contact cell (right) of *Gonatobotrys simplex*. Septal pore in Fig. 4 (same as Fig. 3) is unoccluded; that in Fig. 5 is occluded with an electron-opaque plug. Woronin bodies (W). × 48,000 and 48,800, respectively. **6)** Transverse section through a hypha of *Alternaria tenuis* in which a vacuole (V) occupies most of the cytoplasmic space. The figure represents a near-median section through the contact site. × 20,200. **7)** Transverse section through a hypha of *A. tenuis* some distance from a contact site. The cytoplasm contains a greater concentration of organelles, and fewer vacuoles, than seen in host cytoplasm adjacent to contact sites; e.g., Fig. 3, 6, and 9. Nucleus (N). × 34,800. **8)** Near-median section through the contact site of a contact cell (left) of *G. simplex* with a hypha of *A. tenuis* (right). Secondary material deposited as a wall apposition (Wa) to the inner surface of the original *A. tenuis* wall is illustrated. Segments of several plasmodesmata (arrows) are seen in the wall of the host. Nucleus (N). × 30,200.

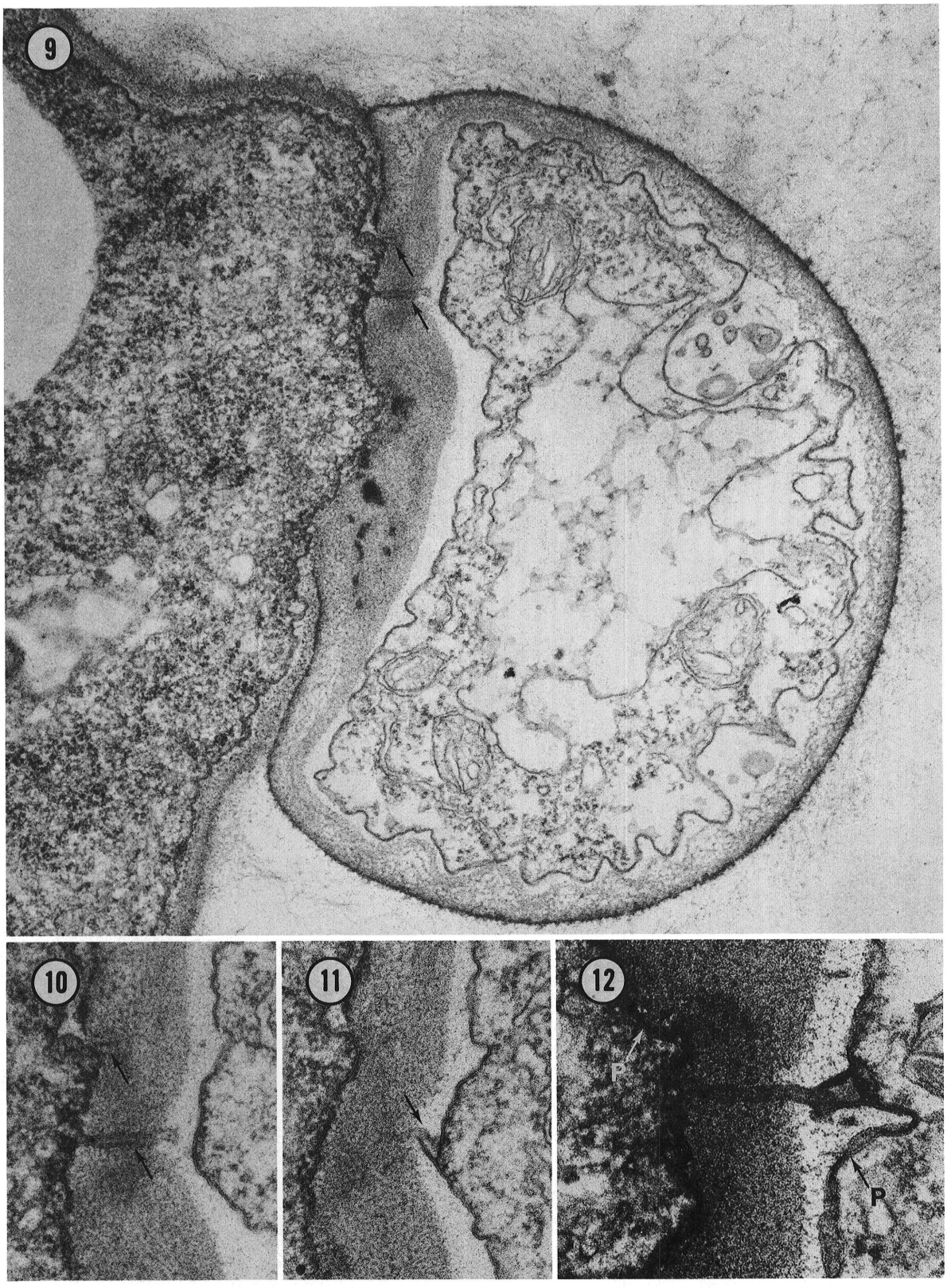

Fig. 9-12. 9) Near median section through the host-parasite interface of the contact cell of *Gonatobotrys simplex* (left) and a hypha of *Alternaria tenuis* (right). Segments of two membrane-bounded plasmodesmata (arrows) are seen. ×45,600. **10-12)** Median sections through plasmodesmata. Figures 10 and 11 are adjacent sections of the same plasmodesmata (arrows) in Fig. 9. The plasmodesmata membranes are continuous with the plasma membranes (P) of the contact cell (left) and of the host (right). × 78,600, × 78,600, and × 103,000, respectively.

adjoined the host cells. As observed and illustrated by Whaley and Barnett (23), contact between the two fungi occurs primarily in two ways: (i) where both the host and the parasite produced short hyphal branches and made contact or, alternately, where the host grew and contacted germinated spores of *G. simplex;* and, (ii) where only the parasite produced short branches and contacted *A. tenuis* hyphae (Fig. 1 and 2). The latter mode of contact was noted more frequently and was the subject of this study, primarily because of better orientation during sectioning. By sectioning transversely, cross-sections of both the parasite and host hyphae as well as a median view through the contact branch and interface could be obtained (Fig. 3).

Observations by both light and electron microscopy indicated that more than one contact branch could be produced by the same *G. simplex* cell (Fig. 1) and that the contact branch rarely grew straight to the host cell, but nearly always curved slightly around the host hypha to make contact (Fig. 1-3). The contact branch of *G. simplex* was separated from the main hypha by a septum that contained a simple pore (Fig. 3-5). The pore usually was plugged with an electron-opaque inclusion and surrounded by Woronin bodies (Fig. 5). Occasionally, the pore was not plugged and Woronin bodies were absent (Fig. 3 and 4).

The cytoplasm of *G. simplex* was relatively dense and contained organelles typical of younger, more active regions of ascomycete hyphae. In contrast, the cytoplasm of *A. tenuis* was less condensed in the hyphal regions where it was contacted by *G. simplex*. A vacuole typically filled most of the cytoplasm (Fig. 6). However, at points along *A. tenuis* hyphae, away from the contact sites, the cytoplasm was less vacuolate and more condensed (Fig. 7).

Hyphae of *A. tenuis* often were flattened (Fig. 3, 6, 8 and 9) at the sites of contact by *G. simplex*. In addition, the hyphal wall was thicker at these interface sites. Much of the additional wall material formed after the original hyphal wall was developed (Fig. 3, 8, and 9). The plasmalemma of *A. tenuis* appeared somewhat retracted from the wall, particularly in the interface region. It also was away from the wall elsewhere around the circumference of the hyphae, both in and away from regions of contact by *G. simplex*. Whether this was the result of "plasmolysis" due to the molarity of the solutions used during preparation for electron microscopy was not determined. Although the plasmalemma was retracted at various points from the wall, it was undulate, and tended to conform to the wavy inner layer of the wall.

The wall of *G. simplex* was seen clearly except where intimate contact was made with *A. tenuis* (Fig. 8, 9). Either it was very thin and not discernible from the wall of *A. tenuis* or it was absent altogether at the interface. Occasionally areas of *G. simplex* cytoplasm were seen protruding slightly into the wall of *A. tenuis;* however, large cytoplasmic bridges connecting the two cells were not seen. Instead, plasmodesmata bridged the cytoplasms of the host and parasite. They were bounded by a membrane, 26-31 nm in diameter, and apparently did not appear to be occluded (Fig. 9-12). The membranes of the plasmodesmata were continuous with the plasmalemma of both cells, but were not associated or connected to endoplasmic reticulum as reported for higher plant cells (20, 21, 24) or in some fungi (cf. 18). The plasmodesmata generally were sinuous as they traversed the wall of *A. tenuis* and they could be determined to bridge the cytoplasm of the two fungi only from serial sections. Occasionlly plasmodesmata traversed the wall directly (Fig. 9-12). Ribosomes or other recognizable cytoplasmic constituents could not be detected within them.

DISCUSSION

The hypothesis that biotrophic contact mycoparasites parasitize their respective host by simple contact and induce increased nutrient absorption by causing an increase in the permeability of the host plasmalemma has been proposed by Barnett and his co-workers (2, 3, 22, 23). The evidence for such a hypothesis has been based largely on the mode of parasitism observed by light microscopy and on nutritional studies of the various contact mycoparasites. From the present study, it is clear that more than simple contact and plasmalemma permeability changes were involved. Actual cytoplasmic continuity, via plasmodesmata, between *G. simplex* and *A. tenuis* was observed. Such structures could serve as direct avenues for nutrient and growth factor; e.g., mycotrophein, uptake by the parasite.

The discovery of plasmodesmata within the wall of *A. tenuis* between the two fungi poses intriguing questions. For example, when are they formed? The hyphal wall of *A. tenuis* was formed well in advance of contact by *G. simplex*. Thus, did these plasmodesmata "push" intrusively through a mature, polymerized wall of *A. tenuis*? Plasmodesmata, as known in higher plants, generally are believed to be formed from vesicles and endoplasmic reticulum entrapped in the developing cell wall following cytokinesis (21, 24). However, there is supportive evidence, reviewed by Robards (21), that they can be intrusive as well, and thus may be quite dynamic structures.

Plasmodesmata have been observed previously in only one other type of host-parasite relationship. Species of *Cuscuta* parasitizing various higher green plants have plasmodesmatal relationships (4, 8, 9, 17), through which the cytoplasm of the host and parasite possibly are connected. In addition, other channel-like areas have been reported to occur between the haustorial cells of *Puccinia graminis tritici* and its host (10). They have not been confirmed, however, by more recent investigators.

Do plasmodesmata exist in other biotrophic contact mycoparasite relationships? In all, five species of contact mycoparasites have been reported in the literature. The other four are: *Calcarisporium parasiticum* Barnett (3); *Stephanoma phaeospora* Butler and McCain (7, 19); *Gonatobotryum fuscum* Sacc. (22); and *Gonatorhodiella highlei* Smith (11). The ultrastructure of the host-parasite interfaces with these relationships have not been reported. However, recent work with *C. parasiticum* parasitism of *Physalospora obtusa* revealed that even larger pores exist (Hoch, *unpublished).* In this relationship a "buffer cell" (3) produced by the parasite contacts the host hyphal tips. The contact interface is digested away, leaving a large opening between the two fungi, much as one might expect to observe as a result of anastomosis.

The thickened wall of *A. tenuis* at the contact site was interpreted as a deposition of additional wall material and not a swelling or expansion of the existing wall. Firm evidence for this conclusion, such as secretory vesicles in the immediate area, was not presented, in part because earlier sequences of contact were not examined. However, the fibrillar nature of the thickened wall did not appear swollen or loosened, but, in fact, appeared more condensed. Wall appositions caused by fungal attack or mechanical injury are well known in higher plants to be additions to the wall mass (1, 6). They also are known to some extent in fungi (13). The wall apposition in *A. tenuis* was precipitated most likely as a response to contact or chemical stimuli from *G. simplex*.

The results of this study have supplemented our present knowledge of biotrophic contact mycoparasites with greater detail of the host-parasite interface. In addition, this is the first time that plasmodesmata or other such pores have been shown to be intimately involved in parasitism involving two taxonomically different fungi. With the knowledge that cytoplasmic continuity exists in at least two contact mycoparasites, they are worth further consideration as useful tools for transmitting and infecting fungi with mycoviruses.

LITERATURE CITED

1. AIST, J. R. 1976. Papillae and related wound plugs of plants. Annu. Rev. Phytopathol. 14:145-163.
2. BARNETT, H. L., and F. L. BINDER. 1973. The fungal host-parasite relationship. Annu. Rev. Phytopathol. 11:273-292.
3. BARNETT, H. L., and V. G. LILLY. 1958. Parasitism of Calcarisporium parasiticum on species of Physalospora and related fungi. W. Va. Agric. Exp. Stn. Tech. Bull. 420-T. 37 p.
4. BENNETT, C. W. 1944. Studies of dodder transmission of plant viruses. Phytopathology 34:905-932.
5. BERRY, C. R., and H. L. BARNETT. 1957. Mode of parasitism and host range of Piptocephalis virginiana. Mycologia 49:374-386.
6. BRACKER, C. E., and L. J. LITTLEFIELD. 1973. Structural concepts of host-pathogen interfaces. Pages 159-318 *in* R. J. W. Byrde and C. V. Cutting, eds. Fungal pathogenicity and the plants' response. Academic Press, New York. 499 p.
7. BUTLER, E. E., and A. H. MC CAIN. 1968. A new species of Stephanoma. Mycologia 60:955-959.
8. DÖRR. I. 1968. Plasmatische Verbindungen zwischen artfremden Zellen. Naturwissenschaften 55:396.
9. DÖRR, I. 1969. Feinstruktur intrazellular wachsender Cuscuta-Hyphen. Protoplasma 67:123-137.
10. EHRLICH, M. A., and H. G. EHRLICH. 1971. Fine structure of the host-parasite interfaces in mycoparasitism. Annu. Rev. Phytopathol. 9:155-184.
11. GAIN, R. E., and H. L. BARNETT. 1970. Parasitism and axenic growth of the mycoparasite Gonatorhodiella highlei. Mycologia 62:1122-1129.
12. HOCH, H. C. 1974. Preparation of fungal hyphae grown on agar-coated microscope slides for electron microscopy. Stain Technol. 49:318-320.
13. HOCH, H. C., and M. S. FULLER. 1976. Mycoparasitic relationships. I. Morphological features of interactions between Pythium acanthicum and several fungal hosts. Arch. Microbiol. 112:(In press).
14. HOCH, H. C., and J. E. MITCHELL. 1972. The ultrastructure of Aphanomyces euteiches during asexual spore formation. Phytopathology 62:149-160.
15. KARLING, J. S. 1942. Parasitism among the chytrids. Am. J. Bot. 29:24-35.
16. KARLING, J. S. 1960. Parasitism among chytrids. II. Chytridiomyces varrucosus sp. nov. and Phlyctochytrium synchytrii. Bull. Torrey Bot. Club 87:326-336.
17. KOLLMANN, R., and I. DORR. 1969. Strukturelle Grundlagen des zwischenzelligen Stoffaustausches. Ber. Dtsch. Bot. Ges. Gd. 82:415-425.
18. POWELL, M. J. 1974. Fine structure of plasmodesmata in a chytrid. Mycologia 66:606-614.
19. RAKVIDHYASASTRA, V., and E. E. BUTLER. 1973. Mycoparasitism by Stephanoma phaeospora. Mycologia 65:580-593.
20. ROBARDS, A. W. 1968. A new interpretation of plasmodesmatal ultrastructure. Planta 82:200-210.
21. ROBARDS, A. W. 1975. Plasmodesmata. Annu. Rev. Plant Physiol. 26:13-29.
22. SHIGO, A. L. 1960. Parasitism of Gonatobotryum fuscum on species of Ceratocystis. Mycologia 52:584-598.
23. WHALEY, J. W., and H. L. BARNETT. 1963. Parasitism and nutrition of Gonatobotrys simplex. Mycologia 55:199-210.
24. VOELLER, B. R., M. C. LEDBETTER, and K. R. PORTER. 1964. The Plant Cell: Aspects of its form and function. Pages 245-312 *in* J. Brachet and A. E. Mirsky, eds. The Cell: Biochemistry, Physiology, Morphology, Vol. VI, Academic Press, New York. 564 p.