Ultrastructure of Fungal Plant Virus Vectors *Polymyxa graminis* in Soilborne Wheat Mosaic Virus-Infected Wheat and *P. betae* in Beet Necrotic Yellow Vein Virus-Infected Sugar Beet

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ABSTRACT

Langenberg, W. G., and Giunchedi, L. 1982. Ultrastructure of fungal plant virus vectors *Polymyxa graminis* in soilborne wheat mosaic virus-infected wheat and *P. betae* in beet necrotic yellow vein virus-infected sugar beet. Phytopathology 72:1152-1158.

The association of *Polymyxa graminis* with the viruses it transmits is thought to be internal; zoospores of *Polymyxa* do not acquire soilborne wheat mosaic virus (SBWMV) in vitro and cystosori of *Polymyxa* in dry SBWMV-infected roots remain able to transmit SBWMV to wheat seedlings for a long period of time. The ultrastructure of various stages in the life cycles of *P. graminis* and *P. betae* were examined in SBWMV-infected wheat and beet necrotic yellow vein virus (BNYVV)-infected sugar beet roots, respectively. Although particles of both viruses were observed in close contact with the fungal vector in the host tissue, they were always outside the fungal structures. Owing to the density of the fungal cytoplasmic contents, virus particles were not seen within fungal plasmodia, zoosporangia, or cystosori.

The genus *Polymyxa* has been reported not only from temperate zones in North America, Europe, and Japan (see Barr [1]), but recently also from Africa (24). *Polymyxa* spp. are economically important obligate root parasites of wheat and sugar beet crops. *Polymyxa graminis* Led. is considered to be the vector of several soilborne viruses such as soilborne wheat mosaic virus (SBWMV) (3,10,18), oat mosaic virus (13), barley yellow mosaic virus (15), and wheat spindle streak mosaic virus (19). Another species, *Polymyxa betae* Keskin, is associated with beet necrotic yellow vein virus (BNYVV) (4,11,22). Except for a partial micrograph of a *P. betae* zoospore showing particles resembling BNYVV (22), no micrographs showing the association of *Polymyxa* spp. and the viruses they transmit has been published. Several investigators have reported observing BNYVV in *P. betae* in ultrathin sections of zoospores (23), or in the fungal thallus (21,25). However, without micrographs these studies are hard to evaluate. We have not seen the virus inside either *P. graminis* or *P. betae* at any stage of their life cycles and report here on the ultrastructural association of *Polymyxa* with SBWMV and BNYVV. Micrographs for this study have been accumulated by us during the last 5–13 yr of investigating *P. graminis* and SBWMV (W.G.L) and *P. betae* and BNYVV (W.G.L and L.G.).

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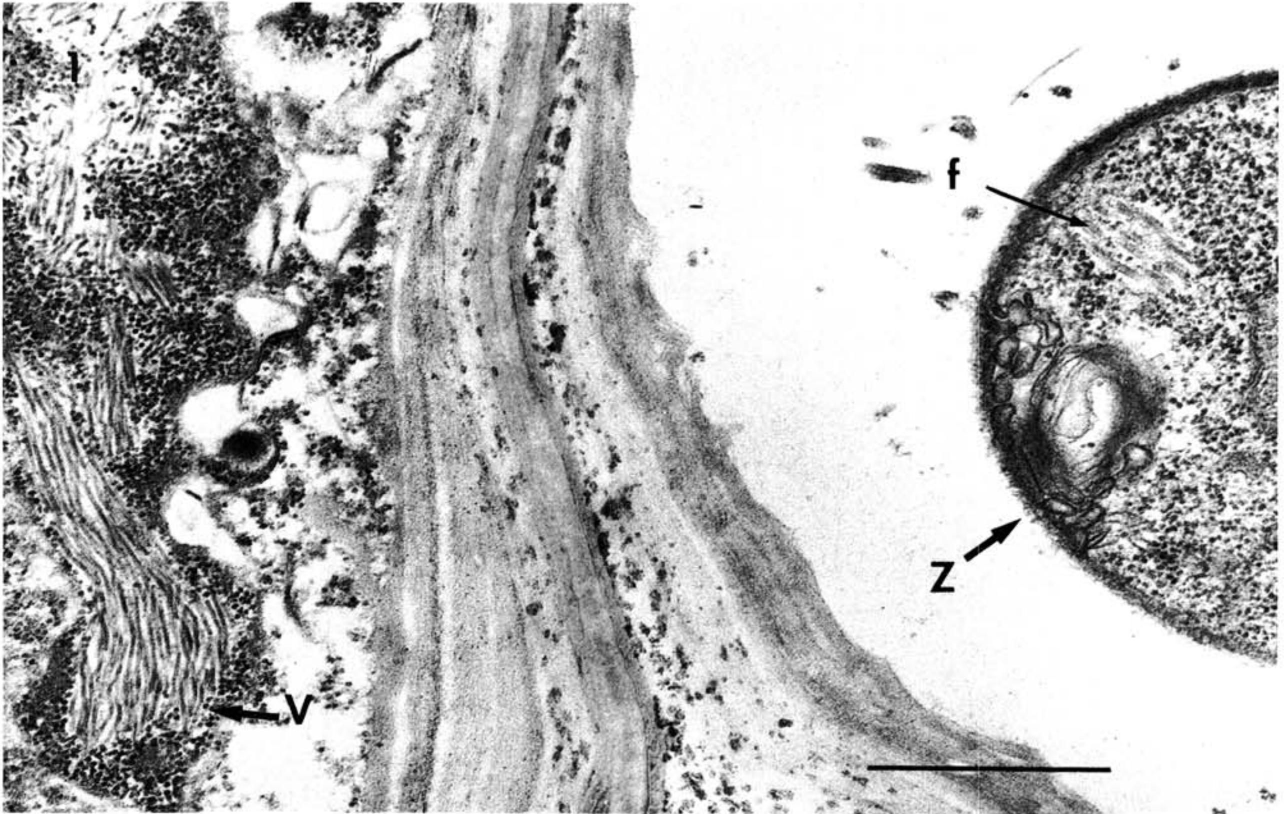
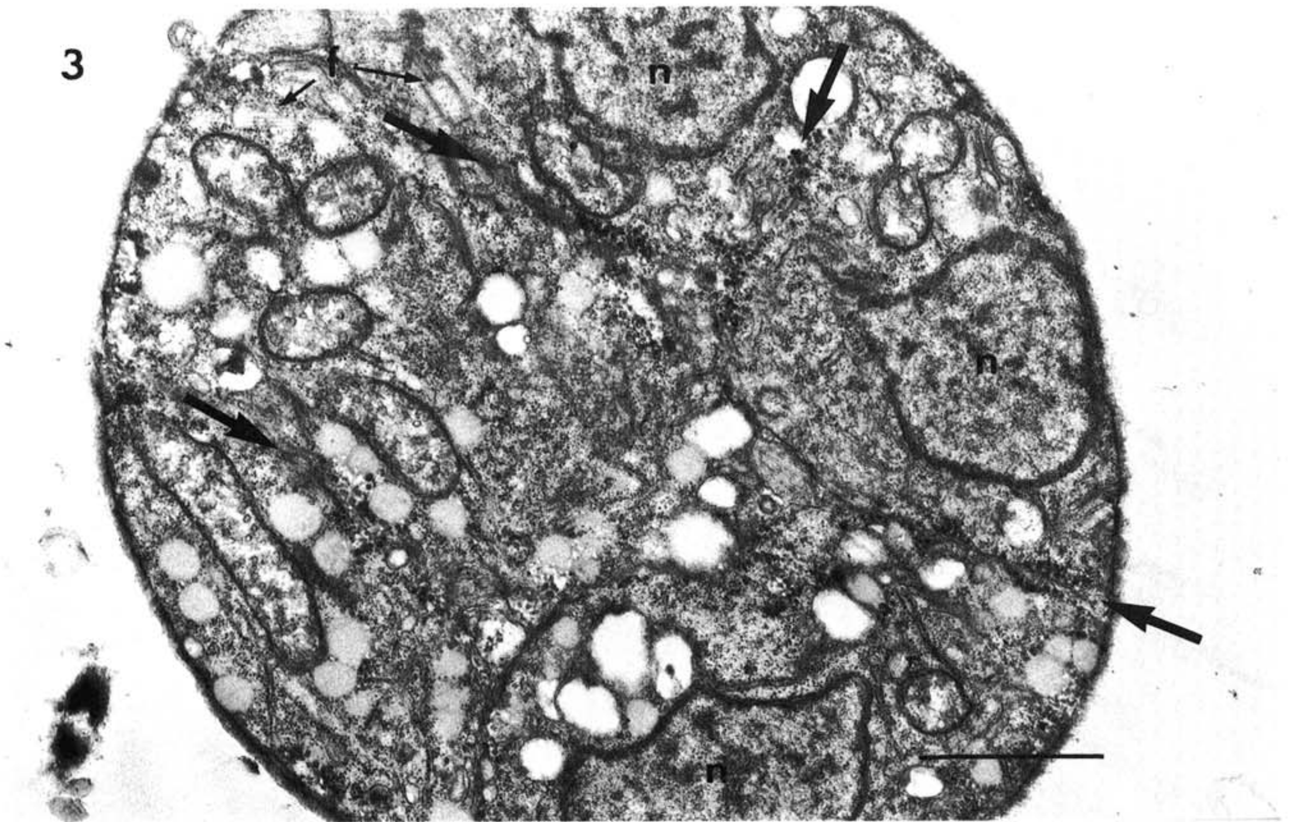
MATERIALS AND METHODS

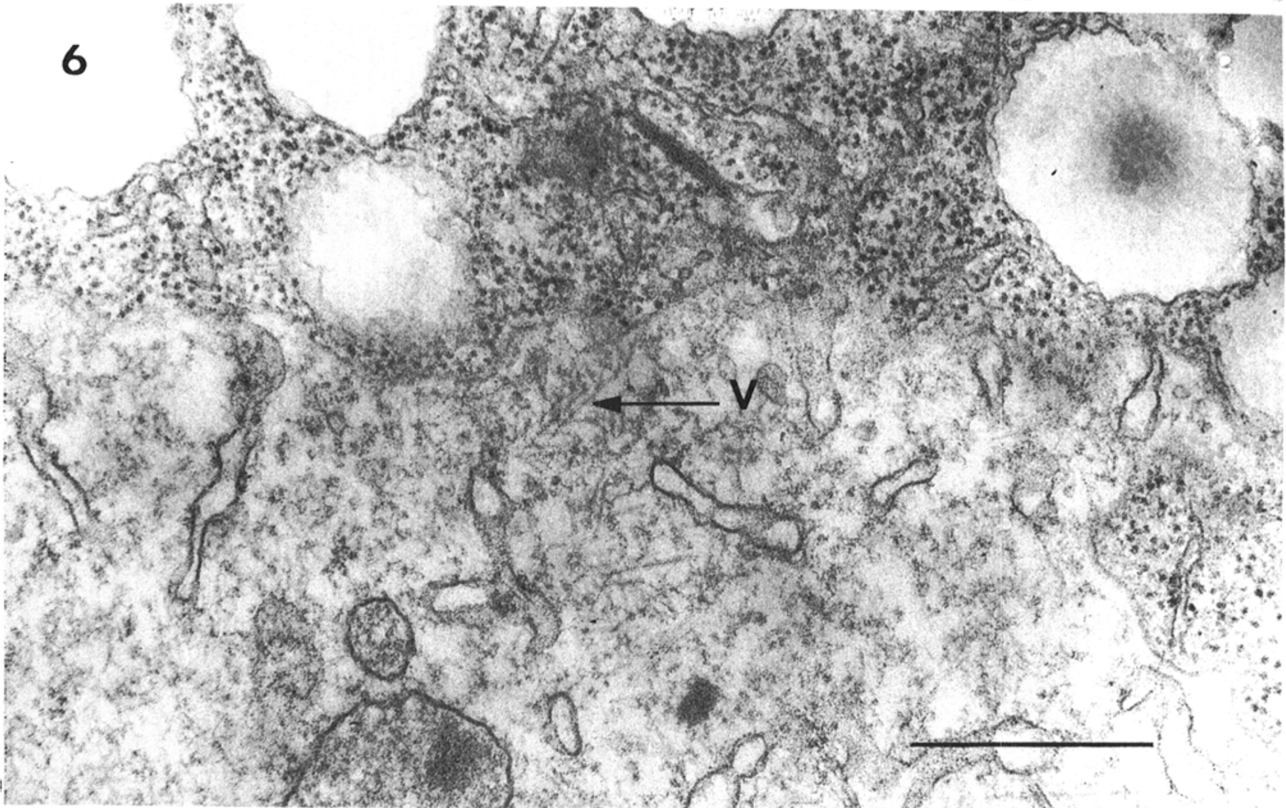
Culture of virus and fungus. *P. graminis* and SBWMV-infected wheat (*Triticum aestivum* L. 'Michigan Amber') were maintained in a growth chamber with a 9-hr day (100 μ Einsteins $m^{-2}\cdot sec^{-1}$) and temperature of 13 C in Lincoln, NE. Viruliferous fungus cultures were transferred regularly by rootwashings (18). Sugar beet (*Beta vulgaris* L. 'Monohil') or spinach (*Spinacia oleracea* L.) seed was sown directly in soil from fields known to be infested with *P. betae* and BNYVV and grown in a greenhouse in Bologna, Italy. After 1–2 mo, the plants were lifted, washed free of adhering soil, and examined for the presence of *P. betae* in the fine rootlets. Infected plants were transferred to pots filled with autoclaved river sand, drenched with one-tenth strength Hoagland's solution I (14), and several surface-sterilized seeds placed around it to germinate in place. Seeds were sterilized by a 10-min soaking in 1% (w/v) calcium hypochlorite in 0.1% Triton X-100, followed by three rinses



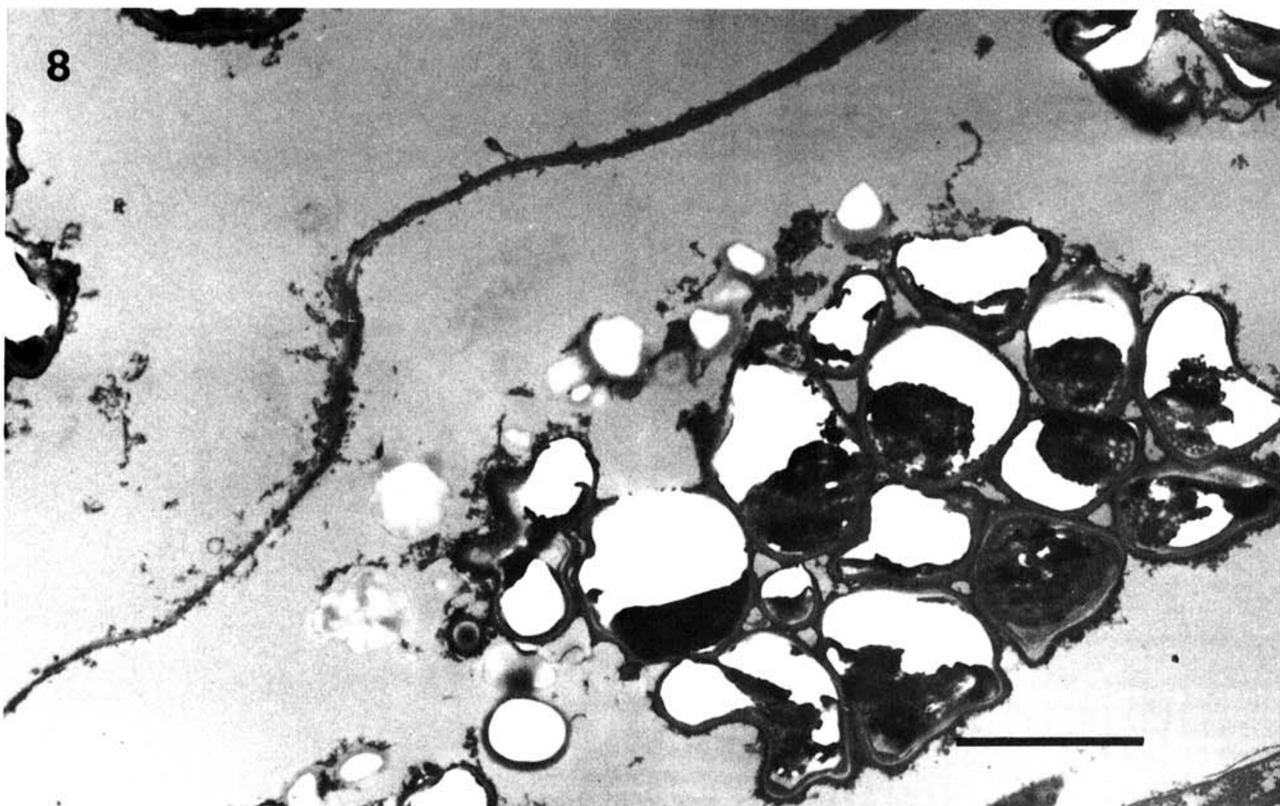
Figs. 1 and 2. 1, Zoospore (Z) of *Polymyxa graminis* in cells of soilborne wheat mosaic virus (SBWMV)-infected wheat root. f = flagellum. In the cell to the left aggregate of SBWMV (V). No virus could be distinguished from zoospore intracellular contents. Bar = 1,200 nm. **2,** Part of multichambered walled zoosporangium of *P. graminis* with mature zoospores. No SBWMV could be detected inside zoospores or in the extracellular space between zoospores. Bar = 2,000 nm.



Figs. 3 and 4. 3, Cross section through a plasmodium of *Polymyxa graminis* dividing into zoospores. Some cleavage planes are marked with heavy arrows. n = nucleus of zoospore, f = developing flagella. No soilborne wheat mosaic virus (SBWMV) could be identified. Bar = 1,000 nm. **4,** Plasmodium of *Polymyxa graminis* in a wheat root cell with SBWMV (V, arrows) attached to the outer membrane. No virus rods could be identified inside the plasmodium. m = mitochondrium. Bar = 500 nm.



Figs. 5 and 6. 5, Multinucleated (n) vacuolated plasmodium of *Polymyxa betae* in sugar beet seedling root cell. Root cell nucleus (N) and cell cytoplasm (c) surrounded by the plasmodium. Bar = 4,000 nm. **6,** Contact region between a vacuolated *P. betae* plasmodium dense with ribosomes (upper half of micrograph) and a sugar beet root cell cytoplasm low in ribosomes (lower half of micrograph) showing a characteristic chevron-shaped aggregate of BNYVV in the cell cytoplasm in contact with the plasmodium (V). BNYVV was seen in contact with a plasmodium in other infected cells but never inside plasmodia. Bar = 500 nm.



Figs. 7 and 8. *Polymyxa betae*: **7,** Section of Araldite-embedded, heavy-walled resting spores in a beet necrotic yellow vein virus (BNYVV)-infected sugar beet root cell. No BNYVV was seen in this or other sectioned cystosori. Bar = 2,000 nm. **8,** Poorly infiltrated *P. betae* resting spores sectioned after glutaraldehyde fixation in the absence of LiCl and embedment in Spurr's plastic. Cystospores of *P. graminis* were likewise poorly infiltrated under these conditions. Bar = 4 μm.

with sterile distilled water. The drenched pots were placed in transparent plastic bags, closed tightly, and incubated at 27 C with 16-hr daylength for 10–14 days. Part of the root system of plants infected by *P. betae* was assayed on *Chenopodium album* leaves for BNYVV infection. BNYVV induced local lesions and was positively identified in leaf dip preparations with homologous antiserum kindly supplied by T. Tamada (Japan).

Electron microscopy. Roots of plants doubly infected with *Polymyxa* and SBWMV or BNYVV were placed in 5% cold glutaraldehyde buffered with 0.075 M K_2HPO_4-citrate, pH 7.4, and during the last year of this investigation also containing 0.1 M LiCl (2). Earlier fixations were without LiCl. Tissue was fixed overnight at 5 C. Chlorotic local lesions of BNYVV on *C. album* leaves were excised, vacuum-infiltrated with 0.1 M KPO_4-citrate, pH 7.4, and placed at 5 C for 3–4 hr. The buffer was then replaced with cold buffered 2.5% glutaraldehyde for overnight fixation. The next day, root tissue was finely chopped with a razorblade, local lesions quartered and then postfixed in 0.1% OsO_4 at 0 C for 0.5 hr. Root tissue only was washed with eight changes of distilled water 15 min per change and then placed in 1% uranyl acetate in 70% alcohol or 70% acetone at 5 C overnight. After postfixation or uranyl acetate treatment, tissues were dehydrated in a cold acetone series and transferred to propylene oxide for subsequent epoxy plastic embedment. The following epoxy plastics were used: Spurr's plastic (20, normal formulation) Araldite 6005, Araldite 502, Araldite CY212 (Serva, West Germany), and Epon 812. Thin-sections were stained with uranyl acetate and lead citrate, or lead citrate only, and viewed either in an RCA-3G or Hitachi 12A, or a Zeiss EM 10A electron microscope.

RESULTS

Polymyxa graminis. All morphological steps of the growth cycle of *Polymyxa* were examined. Figure 1 shows part of a free-swimming *P. graminis* zoospore and SBWMV in a neighboring wheat root cell. Virus particles at high magnifications and in cross sections had a hollow central core and were clearly distinguishable from ribosomes. At no time was virus seen within free-swimming or in zoosporangia-enclosed zoospores (Fig. 2); ~300 *P. graminis* zoospores were examined. Straight rods were occasionally seen in the cellular space of the zoosporangium either before (Fig. 3) or after zoosporogenesis, but these could not be identified as SBWMV. Also, short viruslike hollow rods were seen and these were thought to be the initial stages of the flagellar rootlet. Flagellar initials (Fig. 3) appeared nearly simultaneously with the formation of cleavage planes. Figure 4 shows part of a *P. graminis* plasmodium in a SBWMV-infected wheat root cell. Virus particles were seen attached (mostly end-on) to the outer limiting membrane of the plasmodium, but not internally. It was not possible to discern individual virions clearly either as long or as short particles.

Polymyxa betae. In Fig. 5, part of a multinucleated plasmodium is shown in contact with host cell cytoplasm and the host's nucleus. Virus was not seen in any plasmodium (cross sections of 17 were examined), zoosporangium, zoospore, or the extracellular space between zoospores in zoosporangia. However, BNYVV, like SBWMV, occurred in contact with the exterior plasmodial wall (Fig. 6). Cytoplasm of *P. betae* plasmodia was heavily vacuolated and contained numerous ribosomes (Fig. 6, upper half) in contrast with cytoplasm of invaded host cells (Fig. 6, lower half). *P. betae* plasmodia were often very large and occupied nearly the entire cell.

Resting spores. Resting spores of either fungal species could not be distinguished at the ultrastructural level. Figure 7 shows the thick-walled resting spores of *P. betae* in a BNYVV-infected sugar beet root cell. Cross sections of over 435 resting spores of *P. betae* and several hundred of *P. graminis* were examined for virus particles and none were found. Resting spores were well fixed and well penetrated by Epon and all three Araldite plastics, but not by Spurr's plastic (Fig. 8). However, better penetration of resting spores with Spurr's plastic was achieved when roots infected by *P. betae* were fixed in the presence of LiCl. Overall, penetration of *P. graminis* and *P. betae* resting spores by the fixative and embedding plastic depended on both the fixation schedule and epoxy plastic employed.

DISCUSSION

It was not our intention during this investigation to describe the various stages in the life cycles of *Polymyxa*. These have been adequately described (6–9,16,17). Plasmodia of both species at times were very large, easily surpassing the original cell contents in mass. It appears, therefore, that the intracellular plasmodium is capable of uptake of extracellular nutrients. We could not determine whether the host cytoplasm in cells harboring the fungus was alive at the moment of fixation. Compared to the plasmodium, cytoplasm of an infected host cell had very few intact ribosomes when contrasted to neighboring uninfected cells. Cytoplasms of cells infected by *P. betae* contained fairly large amounts of BNYVV. Both viruses, SBWMV and BNYVV, were in close contact with plasmodia, but were not observed inside fungal structures at any stage of the life cycle of *Polymyxa.* Our fixation and embedding procedure resulted in both viruses being aggregated in host cell cytoplasm. It is, therefore, not unreasonable to expect that if the fungal structures also contained virus that these would appear as aggregates albeit possibly as small aggregates. Considering the large number of plasmodia, zoosporangia, and resting spores examined, chances are minimal that we could have missed even small virus aggregates in *Polymyxa* during the years of this investigation.

We do not attach any importance to the manner in which SBWMV is attached to the outer membrane of *P. graminis* plasmodia since fixation artifacts cannot be excluded. Most virus particles were at right angles to the plasma membrane, however. The right-angle attachment of rod-shaped virus to cell constituents has been reported before (5,12). We agree with Barr's observations (1) that both *Polymyxa* species are very similar to one another and differentiated only by host specificity. The similarity is extended to the fact that in neither species were virus particles detected. Cystosori were poorly infiltrated by Spurr's plastic, but not by Araldite or Epon epoxy plastics. Fixation in the presence of LiCl improved penetration of cystosori by Spurr's plastic, but not every time. Since ultrathin sections of material embedded in Spurr's plastic-embedded material also did not stain as well as those in Epon or Araldite-embedded materials, we mostly used Araldite plastics in the latter stages of this investigation. No difference was noted between the three Araldites. LiCl did not appreciably affect the ultrastructural appearance of cell contents and although not necessary when Araldite plastics were used, was routinely included in fixatives in the latter stages of this investigation.

Other means, such as immunocytochemical electron microscopy or electrophoretic analysis of isolated stages in the life cycle of *Polymyxa,* may have to be employed to detect the presence of SBWMV or BNYVV internally in *Polymyxa.*

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