Entry of Ingested Plant Viruses into the Hemocoel of the Beetle Vector *Diabrotica undecimpunctata howardi*

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ABSTRACT

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Southern bean mosaic sobemovirus (SBMV) and the cowpea strain of tobacco mosaic tobamovirus (CP-TMV) are found in the hemocoel of some beetle vectors after they have fed on infected plants. The location in the alimentary canal through which ingested plant viruses enter the hemocoel of the spotted cucumber beetle *Diabrotica undecimpunctata howardi* was studied. Immunofluorescent and electron microscopy were used to detect the presence of ingested viruses in the gut lumen and the epithelial cells lining the midgut of the spotted cucumber beetle. Virus-specific immunofluorescent microscopy detected bean pod mottle virus and tobacco ringspot virus only in the lumen of the gut; these two viruses cannot be detected in the hemocoel. In beetles from which virus was recovered from the hemocoel, SBMV and CP-TMV were found both in the lumen of the gut and in the epithelial cells of the midgut. CP-TMV was observed by electron microscopy both in the epithelial cells of the midgut and in the hemocytes of the beetles that had acquired the virus. This suggests that plant viruses enter the hemocoel of the beetle through the peritrophic membrane-lined midgut but not through the cuticle-lined foregut or hindgut.

Additional keywords: Chrysomelidae, Coleoptera.

Four groups of plant viruses are transmitted by leaf-feeding beetles (4,7). These viruses are of worldwide importance (5). Specificity of transmission of plant viruses by leaf-feeding beetles is determined by the interaction of the virus with the host plant as well as by the interaction of the virus with the vector (7). Even though both beetle-transmissible and non-beetle-transmissible viruses are deposited on the leaves of host plants by the beetle in regurgitant during feeding, only beetle-transmissible viruses successfully infect the host plant (8). It has been shown that ribonuclease in beetle regurgitant prevents infection by non-beetle-transmissible viruses when these viruses are deposited in regurgitant on the leaf surface during feeding (9). The viruses in beetle regurgitant may originate from the digestive system or from the circulatory system of the beetle.

Freitag first recovered squash mosaic comovirus from the hemolymph of western striped cucumber beetles, *Acalymma trivittatum* (Mannerheim), and western spotted cucumber beetles, *Diabrotica undecimpunctata undecimpunctata* Mannerheim, following virus acquisition from infected tissue (3). Since then, other beetle-transmissible viruses, such as cowpea mosaic comovirus (19), the cowpea strain of southern bean mosaic sobemovirus (CP-SBMV) (16,20), and the type strain of southern bean mosaic sobemovirus (SBMV) (17), have been reported to occur in the hemolymph of the bean leaf beetle *Cerotoma trifurcata* (Forster), the spotted cucumber beetle *Diabrotica undecimpunctata howardi* Barber, and the striped cucumber beetle *Acalymma vittatum* (Fabricius). The hemolymph of the bean leaf beetle was shown to be a reservoir for CP-SBMV, and ingested CP-SBMV appeared very rapidly in the hemolymph of bean leaf beetles (16,20). On the basis of these reports, the generalization has been made that beetle-transmissible viruses are circulative in their beetle vectors (4,6,7).

Recently, however, we reported that some plant viruses that are efficiently transmitted by beetles are not circulative in their vectors (25). Specifically, bean pod mottle comovirus (BPMV) and the type strain of SBMV were not found in the hemolymph of viruliferous Mexican bean beetles, *Epilachna varivestis* Mulsant, and BPMV was not found in the hemolymph of viruliferous spotted cucumber beetles and bean leaf beetles. The relationship of plant viruses with their beetle vectors, therefore, can be noncirculative (those in which the viruses are restricted to the digestive system) or circulative (those in which the viruses enter the hemocoel of viruliferous beetles).

Some plant viruses that are not transmitted by beetles are also found in hemolymph after virus acquisition (17). This indicates that the interaction of these non-beetle-transmissible viruses with beetles is similar to the beetle-transmissible viruses in that some viruses not transmitted by beetles, such as the legume isolate of tobacco mosaic virus (CP-TMV), move from the digestive system into the hemocoel, while others, such as tobacco ringspot virus (TRSV), are restricted to the digestive system (25). These findings suggest that the gut acts as a selective barrier to movement of some plant viruses into the hemocoel and that virus movement into the hemocoel is not necessarily a prerequisite for beetle transmission of plant viruses.

The purpose of this research was to locate the site of plant virus passage from the digestive system of the beetle into the circulatory system with immunofluorescent and electron microscopy.

MATERIALS AND METHODS

Viruses. SBMV and CP-TMV, both of which are present in the hemocoel of spotted cucumber beetles after virus acquisition, and BPMV and TRSV, both of which are restricted to the digestive system of the spotted cucumber beetle after virus acquisition, were used in this study. SBMV and BPMV are beetle-transmissible viruses, while CP-TMV and TRSV are not. All of these viruses are stable, easily purified, mechanically transmissible, and cause systemic infections in bean (*Phaseolus vulgaris* L. 'Black Valentine'). All viruses were propagated in the greenhouse in Black Valentine bean except TRSV, which was propagated in cucumber (*Cucumis sativus* L. 'Boston Pickling'). The viruses were purified by the methods described by Gergerich et al (8).

Beetles. Adult spotted cucumber beetles were collected in the field from soybean, bean, and squash plants during the growing season and maintained for more than 1 wk on healthy Pinto bean plants before they were used. During the winter, adult spotted cucumber beetles were purchased from French Agricultural Research Inc., Lamberton, MN.

Virus acquisition from infected plants or from purified virus. Beetles were starved for 24 h and then individually given access to virus-infected leaves or to drops of purified virus (10–20 mg/ml) that had been mixed with an equal volume of 20% sucrose in 0.01 M phosphate buffer (BPMV in 0.1 M phosphate buffer), pH 7.2, in a petri dish at room temperature for 24 h. Only those beetles that had fed on virus-infected leaves or purified virus were used in this study. Each beetle was watched individually to ensure that it fed and to determine the beginning of the acquisition period. Beetles were labeled individually throughout the experiment after virus acquisition in order to facilitate and monitor sampling for immunofluorescent and electron microscopy.

Recovery of virus from the hemolymph. Beetles were held firmly, and one of the metathoracic legs was removed with a pair of forceps. The resulting drop of hemolymph (5–15 μl) was collected with a capillary tube, diluted 1:100–200 with 0.01 M phosphate buffer (BPMV with 0.1 M phosphate buffer), pH 7.2, and inoculated on Carborundum-dusted leaves of the appropriate local lesion host. The local lesion host used for virus detection of SBMV and BPMV was *P. vulgaris* L. 'Pinto'; for CP-TMV, *Chenopodium quinoa* Willd.; and for TRSV, *C. sativus*. Bean plants were inoculated when the primary leaves were fully expanded; *C. quinoa* was inoculated before blooming; and cucumber was inoculated at the cotyledon stage. The results of all hemolymph assays represent the combined data from at least two experiments in which 10–20 beetles were used in each treatment. The hemolymph from individual beetles was inoculated separately to one local lesion host plant. Hemolymph from five beetles that had not fed on virus was collected and inoculated separately as a control in each replicate.

TABLE 1. Recovery of viruses from hemolymph of spotted cucumber beetles 24 h after acquisition from infected leaves or from drops of purified virus

Acquisition source	Beetles with virus[a] in hemolymph (%)			
	SBMV	BPMV	CP-TMV	TRSV
Infected leaves[b]	19 (10/48)[c]	0 (0/26)	26 (10/38)	0 (0/70)
Purified virus[d]	38 (8/21)	0 (0/18)	41 (24/58)	0 (0/35)

[a]SBMV = southern bean mosaic sobemovirus; BPMV = bean pod mottle comovirus; CP-TMV = cowpea strain of tobacco mosaic tobamovirus; and TRSV = tobacco ringspot nepovirus.
[b]The acquisition tissue was systemically infected leaves of Black Valentine bean.
[c]The number of beetles having virus in the hemolymph per the number of beetles tested is in parentheses. Data are the pooled results of at least two experiments.
[d]Purified virus (10–20 mg/ml) was mixed with an equal volume of 20% sucrose in 0.01 M phosphate buffer (BPMV in 0.1 M phosphate buffer), pH 7.2.

For further sampling purposes, the beetles were divided into three groups on the basis of the results of infectivity tests: 1) those beetles that had acquired TRSV or BPMV (none of these beetles had virus in their hemolymph), 2) those beetles that had acquired SBMV or CP-TMV that did not have virus in their hemolymph, and 3) those beetles that had acquired SBMV or CP-TMV that did have virus in their hemolymph.

Beetle dissection. Beetles were immobilized in melted paraffin in a petri dish and immersed in fixative (2% paraformaldehyde for immunofluorescent microscopy and a modified Karnovsky's fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2, for transmission electron microscopy). A dissecting microscope was used for beetle dissection. The entire alimentary canal was removed and transferred into fresh fixative for at least 12 h before further processing.

Immunofluorescent microscopy. The alimentary canal was further cut into fore-, mid-, and hindgut. Different parts of the gut were embedded in embedding medium (O.C.T. compound, Lab-Tek Products, Naperville, IL) and quick-frozen. Cross sections 5–8 μm in thickness were cut with a cryostat microtome (model CTI, International Equipment Co., Needham Heights, MA) at −20 C and affixed to glass slides coated with poly-L-lysine (Sigma Chemical Co., St Louis, MO). The slides with adhering sections were immersed in 2% paraformaldehyde overnight at 4 C and then washed once in phosphate-buffered saline

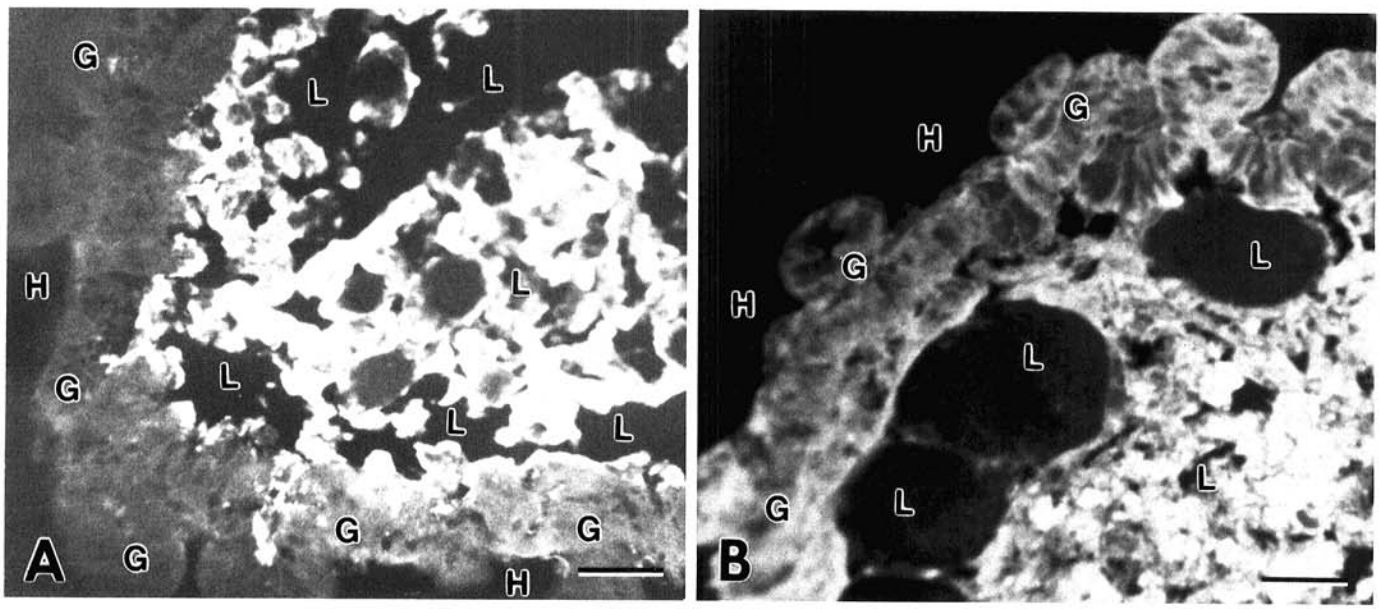

Fig. 1. Micrographs of indirect immunofluorescence-labeled cross sections of the midguts from spotted cucumber beetles. **A,** Midgut cross section from a beetle that had acquired bean pod mottle virus (BPMV) from infected leaves and that did not have virus present in the hemolymph. Fluorescence is restricted to the lumen (L) of the midgut tissue (G). H = hemocoel. Bar = 120 μm. Beetles that had acquired tobacco ringspot virus, southern bean mosaic virus (SBMV), and the cowpea strain of tobacco mosaic virus (CP-TMV) but that did not have virus present in the hemocoel gave results the same as those of BPMV-fed beetles. **B,** Midgut cross section from a beetle that had acquired CP-TMV from infected leaves and that had virus present in the hemolymph. Fluorescence is present both in the lumen (L) and in the tissue (G) of the midgut wall. H = hemocoel. Bar = 120 μm. Beetles that had acquired SBMV and that had virus in the hemolymph gave results the same as those of CP-TMV-fed beetles.

(PBS; 0.01 M phosphate, 0.85% NaCl, pH 7.0). The sections on slides were incubated with anti-virus rabbit immunoglobulin G (produced in our laboratory) diluted 1:100 in PBS for 2 h at 37 C or overnight at 4 C. After they were washed three times with PBS, the slides were incubated for 1 h at 37 C in fluorescein isothiocyanate-conjugated goat anti-rabbit serum (Sigma) diluted 1:50 in PBS. After two washings for 3 min each in PBS, the sections were mounted in mounting medium (glycerol-PBS, 1:1). The sections were examined under an epifluorescent microscope (model BHT, filter EY 455, Olympus Optical Instruments Co., Tokyo, Japan). The sections of gut from beetles treated with preimmune rabbit immunoglobulin G after virus acquisition feeding and sections of gut from beetles that fed on healthy leaves or on 10% sucrose were used as controls. Six alimentary canals were sectioned from each treatment. The experiment was repeated twice.

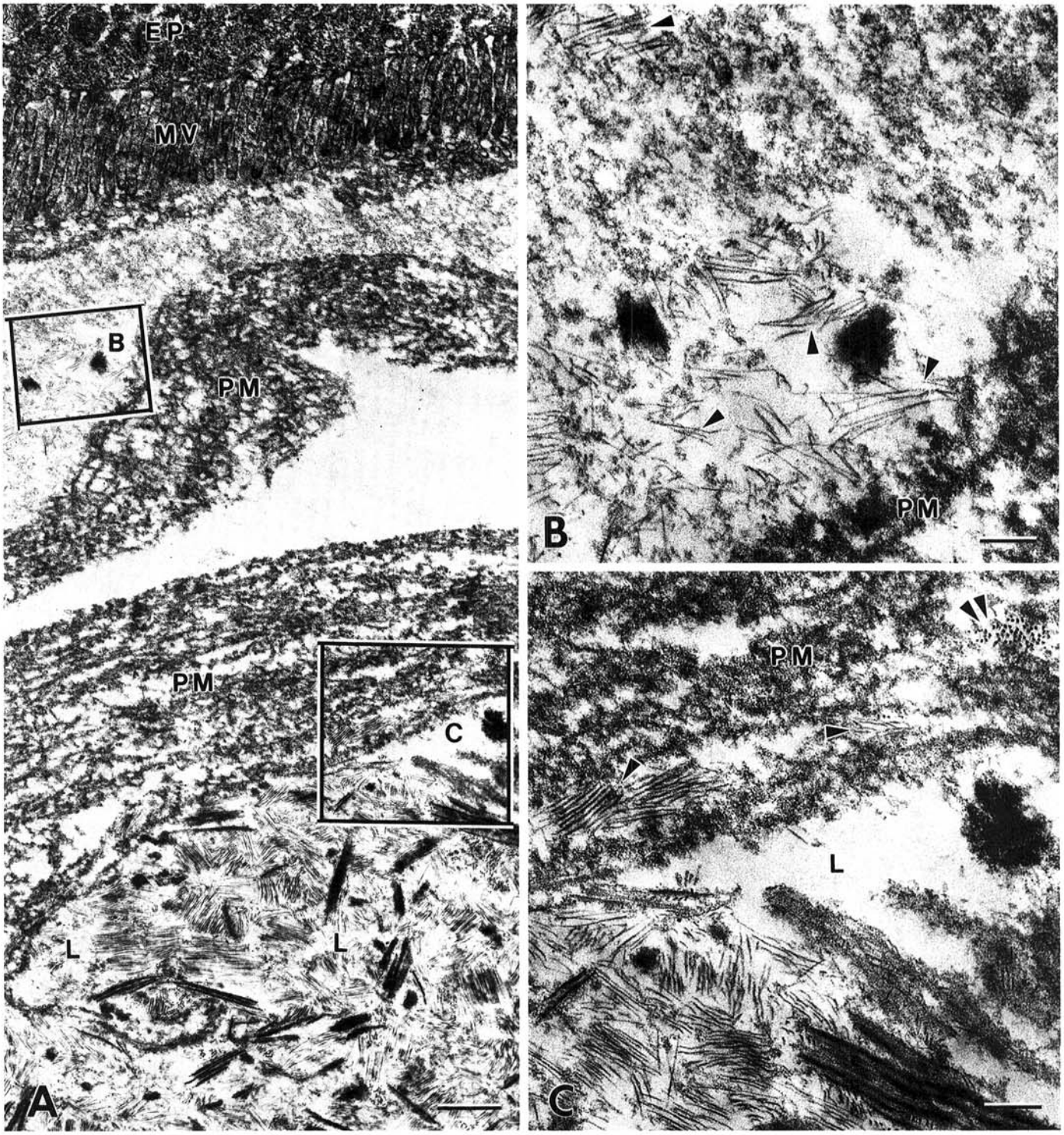

Fig. 2. Transmission electron micrographs of a cross section of the midgut from a spotted cucumber beetle that had acquired the purified cowpea strain of tobacco mosaic virus (CP-TMV) and that had virus present in the hemolymph. **A,** The majority of CP-TMV particles and crystals are confined to the lumen (L) of the midgut by the peritrophic membrane (PM); a few are embedded in the peritrophic membrane; some are in the space between the peritrophic membrane and the epithelial cells (EP). MV = microvilli. Bar = 500 nm. **B,** Higher magnification of the upper boxed area in Fig. 2A shows TMV particles and crystals (arrowheads). PM = peritrophic membrane. Bar = 167 nm. **C,** Higher magnification of the lower boxed area in Fig. 2A shows TMV crystals (arrowheads) embedded in the peritrophic membrane (PM). Some TMV particles are transversely sectioned (double arrowheads). L = gut lumen. Bar = 167 nm.

Electron microscopy. On the basis of the results of infectivity tests and immunofluorescent microscopy, the midguts of beetles from which CP-TMV had been recovered from the hemocoel and the midguts of beetles with no CP-TMV in the hemocoel were prepared for electron microscopic studies. In order to avoid confusion with some insect viruses or with viruslike particles that have been described in the epithelial cells of the spotted cucumber beetle (14), CP-TMV was chosen for electron microscopic studies, because this virus has a unique particle morphology that can be easily identified with the electron microscope. Seven to nine dissected midguts of beetles that had been stored in Karnovsky's fixative (up to 1 wk awaiting the results of infectivity assays) were washed twice with 0.05 M cacodylate buffer, pH 7.2, and then postfixed in 1% osmium tetroxide in the same buffer for 2 h. After two rinses of 2–3 min each in deionized water, the midguts were stained en bloc overnight in aqueous 0.5% uranyl acetate at 4 C and dehydrated through an ethanol series of 30, 50, 70, 80, 90 (10 min each), and 100% (three changes of 10 min each) and propylene oxide (two changes of 15 min each). The midguts were infiltrated under low vacuum with a mixture of 50% propylene oxide and 50% Spurr's medium for 4 h followed by 100% Spurr's medium for 24 h. The midguts were embedded in Spurr's medium and polymerized by overnight incubation in an oven at 70 C. Thin sections were double stained with uranyl acetate and lead citrate and examined under a JEOL-100 CX transmission electron microscope. Three to five beetles that had fed on healthy leaves or on 10% sucrose were used as controls. The experiment was repeated three times.

RESULTS

Infectivity tests. SBMV and CP-TMV were detected by infectivity assays in the hemolymph of spotted cucumber beetles that had acquired virus from infected leaves or from drops of purified virus. Fewer than 41% of the beetles contained either of these viruses in their hemolymph after feeding on infected leaves or from purified virus solutions. No infectious BPMV or TRSV was recovered from the hemolymph of spotted cucumber beetles (Table 1). None of the viruses was detected in the hemolymph of control beetles.

Immunofluorescent microscopy. Examination of immunofluorescence-labeled sections of the foregut and hindgut of spotted cucumber beetles revealed that 24 h after acquisition feeding, virus-specific fluorescence was confined to the lumen of both the foregut and hindgut (data not shown). These results were obtained with all viruses and treatments. In contrast, only a nonspecific bright yellow autofluorescence of gut tissue and food materials was observed in controls (data not shown). Under the epifluorescent microscope, the cuticle of the foregut and hindgut was distinct, since a clearly defined nonspecific bright yellow autofluorescence was produced by the cuticle in all beetles (data not shown).

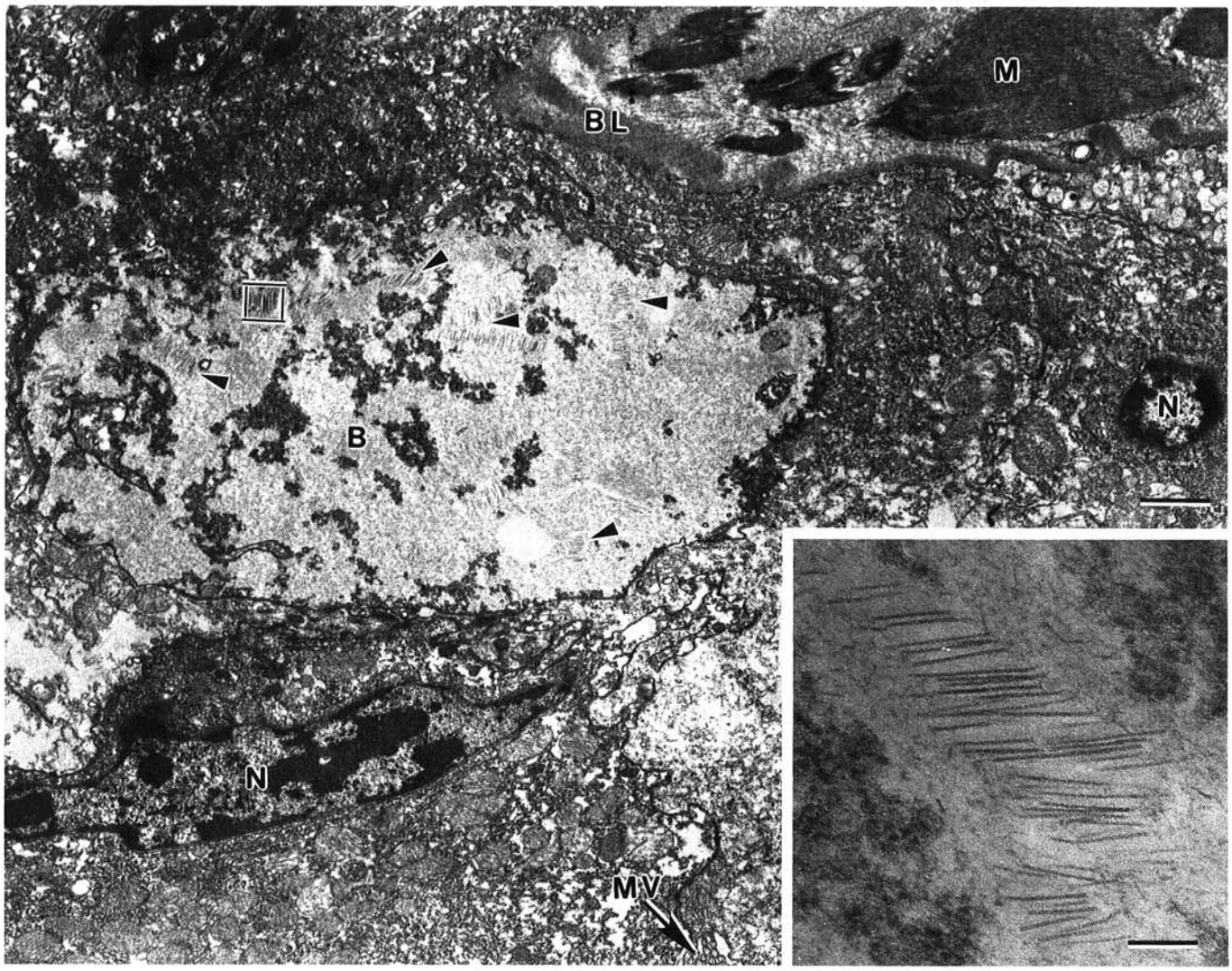

Fig. 3. Transmission electron micrograph of a cross section of the midgut from a spotted cucumber beetle that had acquired the purified cowpea strain of tobacco mosaic virus (CP-TMV) and that had virus present in the hemolymph. A large membrane-bound body (B) with many CP-TMV particles and crystals (arrowheads) is present in the cytoplasm of a midgut epithelial cell. BL = basal lamina; M = circular muscle; MV = microvilli; and N = nucleus. Bar = 1,000 nm. Inset, higher magnification of the boxed area showing TMV particles. Bar = 125 nm.

The results of immunofluorescent labeling of midgut transverse sections can be divided into three different categories corresponding to the results of infectivity tests for virus in the hemolymph of viruliferous beetles. The first category includes the beetles that had acquired BPMV or TRSV (these viruses do not move into the hemocoel of the spotted cucumber beetle). The second category includes beetles that had acquired SBMV or CP-TMV but from which no infectious virus was recovered from the hemocoel. In these two categories, virus-specific fluorescence was confined to the lumen of the midgut (Fig. 1A). In contrast, beetles in the third category that had SBMV or CP-TMV in their hemolymph had virus-specific fluorescence not only in the lumen of the midgut but also in the tissue of the midgut wall (Fig. 1B).

Electron microscopy. Electron microscopic examination of transverse sections of midguts of beetles from which virus was recovered from the hemocoel revealed the presence of numerous aggregates of CP-TMV particles in the gut lumen (Fig 2A). These tightly packed aggregates often exhibited paracrystalline arrays of various forms (Fig. 2). Highly concentrated virus aggregates were gradually diminished in distribution at the periphery of the lumen by one to three layers of the netlike structure that consisted of an electron-dense meshwork of fine filaments similar to the peritrophic membrane of other insects (1,18). This structure is assumed to be the peritrophic membrane of the spotted cucumber beetle and will be referred to as such hereafter. The concentration of virus aggregates appeared to be much higher in the middle of the lumen than in the ectoperitrophic space (the space between the peritrophic membrane and midgut epithelium). Virus particles in this space were sparse, but some of them were still in crystalline form (Fig. 2B).

Aggregates of CP-TMV were also present in the cytoplasm of the epithelial cells of the midgut (Fig. 3). Individually scattered CP-TMV particles in the cytoplasm were not observed in the epithelial cells in any of the specimens examined. Instead, many virus particles and aggregates of virus particles, some of which were in crystalline arrays, were observed in the membrane-bound bodies in the epithelial cells.

At the junction between the epithelial cells of the midgut and the hemocoel are the basal lamina, smooth muscle fibers, and nonepithelial hemocytes usually associated closely with the hemocoel side of the basal lamina (Fig. 4). Most hemocytes in the hemocoel attached to the basal lamina contained CP-TMV particles in the cytoplasm. These particles occurred in membrane-bound packets of various sizes and numbers and were usually in tightly packed aggregates (Fig. 4), suggesting that they were phagocytized by hemocytes. In fact, these membrane-bound packets containing CP-TMV aggregates are structurally indistinguishable from those phagocytized CP-TMV particles in beetle hemocytes formed after the injection of purified virus into the hemocoel (13). However, no virus particles were found in the muscle cells associated with the digestive system. We were unable to observe the process of passage of virus particles through the cell membrane and basal lamina of the midgut wall.

Midguts from more than 30 spotted cucumber beetles from four experiments in which infectious SBMV had been recovered from the hemocoels by infectivity tests were processed for electron

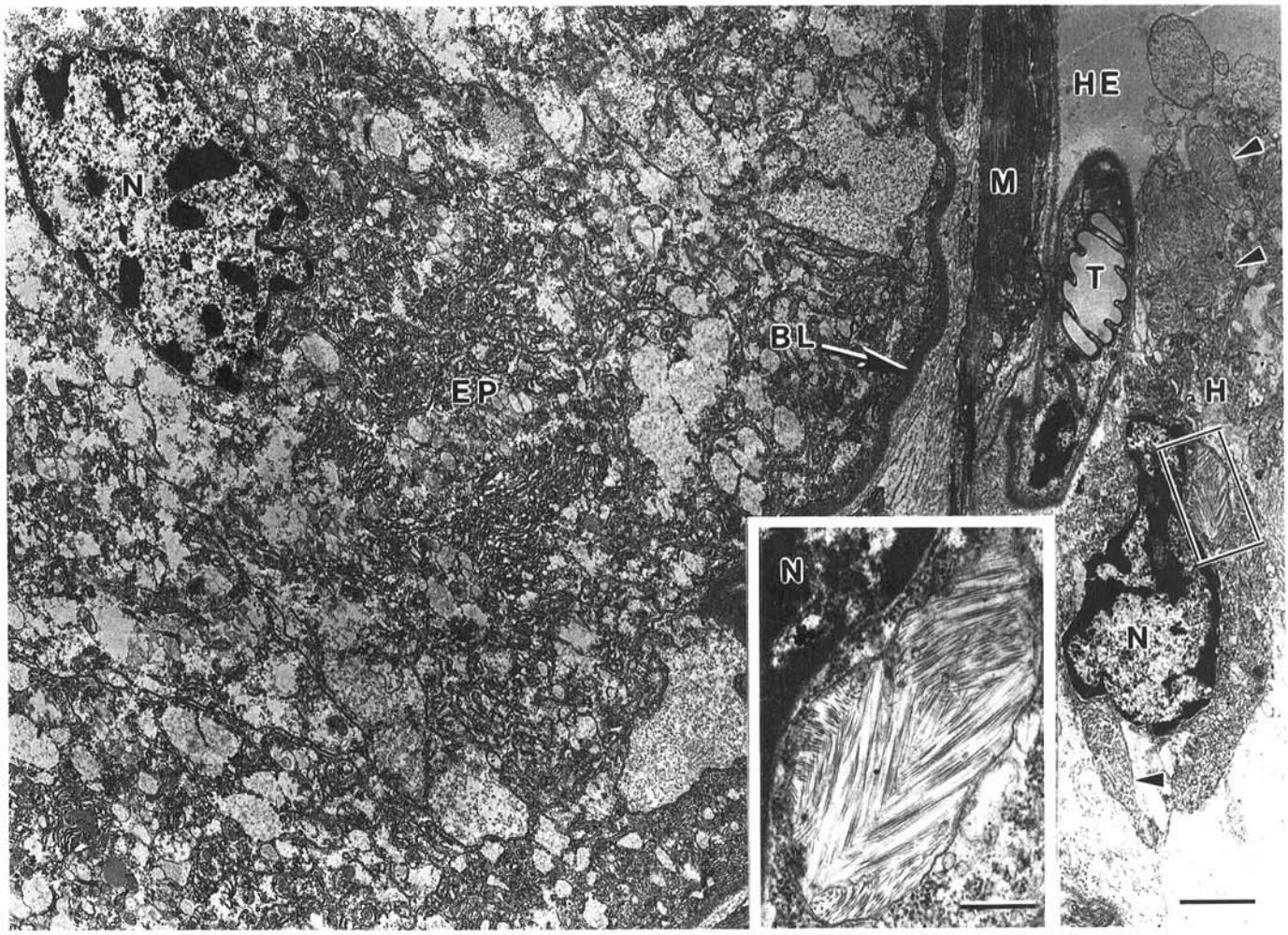

Fig. 4. Transmission electron micrograph of a cross section of the midgut from a spotted cucumber beetle that had acquired the purified cowpea strain of tobacco mosaic virus (CP-TMV) and that had virus present in the hemolymph. Numerous CP-TMV particles and crystals (arrowheads) appear to have been phagocytized by a hemocyte (H) located adjacent to basal lamina in the hemocoel. BL = basal lamina; EP = epithelial cell; HE = hemocoel; M = muscle; N = nucleus; and T = tracheole. Bar = 550 nm. Inset, higher magnification of the area to the left side of the unlabeled arrow showing TMV particles. Bar = 140 nm.

microscopic study. SBMV particles were found in the midgut lumen, associated with the peritrophic membrane, and in the ectoperitrophic space. However, we were unable to locate SBMV in the epithelial cells of the midgut (data not shown).

DISCUSSION

Using immunofluorescent microscopy, we identified the midgut as the portion of the gut where virus passage into the hemocoel occurs. Further efforts were focused on the midgut for examination by electron microscopy at the ultrastructural level. When spotted cucumber beetles were fed on virus-infected leaves or drops of purified virus, some viruses, such as BPMV and TRSV, were never recovered from the hemocoel (25). Other viruses, such as SBMV and CP-TMV, were recovered from the hemocoel of some of the beetles that had acquired virus. It is important to note that BPMV and SBMV are beetle-transmissible viruses while TRSV and CP-TMV are not, indicating that virus movement into the hemocoel is not involved in beetle vector specificity (25). Examination with fluorescent microscopy revealed that for individual beetles from which virus was not recovered from the hemocoel, virus was found only in the lumen of the fore-, mid-, and hindgut. However, for those individual beetles from which SBMV and CP-TMV were recovered from the hemocoel, virus was found both in the lumen of all three parts of the gut and in the epithelial layer of the midgut. Therefore, on the basis of immunofluorescent microscopy, it appears that plant viruses enter the hemocoel of some beetles only through the peritrophic membrane-lined midgut rather than through the cuticle-lined fore- or hindgut.

Morphologically, the alimentary canal of insects is divided into three primary regions according to their embryonic origin. Fore- and hindgut are formed from ectodermal invagination, whereas the midgut is of endodermal origin (22). This results in marked histological and structural differences among the regions of the gut. The fore- and hindguts are both lined with sclerotized, less permeable cuticle, whereas the midgut is lined with the more permeable structure called the peritrophic membrane. The peritrophic membrane directly covers ingested food in the midgut and is known to have many important functions, among which protection and selective permeability are the most important (15,18). The peritrophic membrane consists of a network of chitin in a protein-carbohydrate matrix (15,22). In Coleoptera, the peritrophic membrane is formed by secretion from the microvilli of midgut epithelial cells (15). However, the ultrastructure of the peritrophic membrane of beetles in the family Chrysomelidae has not been described. Three distinct layers of peritrophic membrane near the microvilli of epithelial cells (Fig. 2A) clearly illustrate that the peritrophic membrane of the spotted cucumber beetle is morphologically similar to that of other insects. In a study of the mosquito *Aedes taeniorhynchus* (Wiedemann) infected with mosquito iridescent virus, Stoltz and Summers (21) first demonstrated that the peritrophic membrane was a very effective physical barrier to the passage of the virus particles from the alimentary canal through the epithelium into the hemocoel. They were unable to find virus particles in the ectoperitrophic space. However, in some other cases, it has been demonstrated that insect viruses modify the peritrophic membrane to enter the epithelial cells (2). The work reported here showed that the peritrophic membrane of the spotted cucumber beetle is not effective in blocking the passage of CP-TMV. Virions of CP-TMV, some of which were in crystalline form, successfully passed through the peritrophic membrane and entered the ectoperitrophic space without any observed damage to the peritrophic membrane. The mechanism by which CP-TMV passes through the peritrophic membrane of beetles is still unknown.

The site through which ingested viruses enter the hemocoel of insect vectors of plant viruses is different for different virus-vector combinations. Barley yellow dwarf luteovirus enters the hemocoel of its aphid vector, *Rhopalosiphum padi* (Linnaeus), through the almost transparent hindgut (10,11). Tomato spotted wilt tospovirus moves into the hemocoel of its larval thrips vector, *Frankliniella occidentalis* (Pergande), through the midgut. However, when adult thrips acquire the virus, the midgut acts as a barrier to block virus movement into the hemocoel (24). In contrast, most insect viruses get into the hemocoel of their hosts through the peritrophic membrane-lined midgut instead of through the cuticle-lined fore- or hindgut (12,23).

Only some plant viruses are transported from the digestive system into the circulatory system of some beetle vectors (25). The work reported here shows that ingested plant viruses are transported from the digestive system into the circulatory system of the beetle only through the peritrophic membrane-lined midgut. SBMV, BPMV, and TRSV all have similar size and particle morphology. However, only SBMV can be recovered from the hemolymph of the bean leaf beetle and the spotted cucumber beetle, and none of these viruses moves into the hemocoel of the Mexican bean beetle (25). This suggests that a specific interaction occurs between the virus and some component(s) in the midgut and that this interaction is necessary for the passage of ingested viruses from the midgut to the hemocoel.

LITERATURE CITED

1. Brandt, C. R., Adang, M. J., and Spence, K. D. 1978. The peritrophic membrane: Ultrastructural analysis and function as a mechanical barrier to microbial infection in *Orgyia pseudotsugata*. J. Invertebr. Pathol. 32:12-24.
2. Derksen, A. C. G., and Granados, R. R. 1988. Alteration of a lepidopteran peritrophic membrane by baculoviruses and enhancement of viral infectivity. Virology 167:242-250.
3. Freitag, J. H. 1956. Beetle transmission, host range, and properties of squash mosaic virus. Phytopathology 46:73-81.
4. Fulton, J. P., Gergerich, R. C., and Scott, H. A. 1987. Beetle transmission of plant viruses. Annu. Rev. Phytopathol. 25:111-123.
5. Fulton, J. P., Scott, H. A., and Gamez, R. 1975. Beetle transmission of legume viruses. Pages 123-131 in: Tropical Diseases of Legumes. J. Bird and K. Maramorosch, eds. Academic Press, New York.
6. Fulton, J. P., Scott, H. A., and Gamez, R. 1980. Beetles. Pages 115-132 in: Vectors of Plant Pathogens. K. F. Harris and K. Maramorosch, eds. Academic Press, New York.
7. Gergerich, R. C., and Scott, H. A. 1991. Determinants in the specificity of virus transmission by leaf-feeding beetles. Pages 1-13 in: Advances in Disease Vector Research. Kerry F. Harris, ed. Springer-Verlag, New York.
8. Gergerich, R. C., Scott, H. A., and Fulton, J. P. 1983. Regurgitant as a determinant of specificity in the transmission of plant viruses by beetles. Phytopathology 73:936-938.
9. Gergerich, R. C., Scott, H. A., and Fulton, J. P. 1986. Evidence that ribonuclease in beetle regurgitant determines the transmission of plant viruses. J. Gen. Virol. 67:367-370.
10. Gildow, F. E. 1985. Transcellular transport of barley yellow dwarf virus into the hemocoel of the aphid vector, *Rhopalosiphum padi*. Phytopathology 75:292-297.
11. Gildow, F. E. 1993. Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. Phytopathology 83:270-277.
12. Granados, R. R., and Lawler, K. A. 1981. In vivo pathway of *Autographa californica* baculovirus invasion and infection. Virology 108:297-308.
13. Kim, K. S. 1981. Ultrastructure of bean leaf beetle (*Cerotoma trifurcata*) hemocytes and their phagocytic activities on tobacco mosaic virus, a non-beetle-transmitted virus. J. Ultrastruct. Res. 75:300-313.
14. Kim, K. S., and Kitajima, E. W. 1984. Nonoccluded baculovirus- and filamentous virus-like particles in the spotted cucumber beetle, *Diabrotica undecimpunctata* (Coleoptera: Chrysomelid). J. Invertebr. Pathol. 43:234-241.
15. Richards, A. G., and Richards, P. A. 1977. The peritrophic membranes of insects. Annu. Rev. Entomol. 22:219-240.
16. Robison, M. D., and Scott, H. A. 1986. Cowpea southern bean mosaic virus in bean leaf beetle hemolymph. (Abstr.) Phytopathology 76:1127.
17. Scott, H. A., and Fulton, J. P. 1978. Comparison of the relationships of southern bean mosaic virus and the cowpea strain of tobacco mosaic virus with the bean leaf beetle. Virology 84:207-209.
18. Sieber, K. P., Huber, M., Kaslow, D., Banks, S. M., Torii, M., Alkawa, M., and Miller, L. H. 1991. The peritrophic membrane as a barrier: Its penetration by *Plasmodium gallinaceum* and the effect of a monoclonal antibody to ookinetes. Exp. Parasitol. 72:145-156.
19. Slack, S. A., and Fulton, J. P. 1971. Some plant virus-beetle vector

relations. Virology 43:728-729.
20. Slack, S. A., and Scott, H. A. 1971. Hemolymph as a reservoir for the cowpea strain of southern bean mosaic virus in the bean leaf beetle. Phytopathology 61:538-540.
21. Stoltz, D. B., and Summers, M. D. 1971. Pathway of infection of mosquito iridescent virus. I. Preliminary observations on the ingested virus. J. Virol. 8:900-909.
22. Terra, W. R. 1990. Evolution of digestive systems of insects. Annu. Rev. Entomol. 35:181-200.
23. Tinsley, T. W. 1979. The potential of insect pathogenic viruses as pesticidal agents. Annu. Rev. Entomol. 24:63-87.
24. Ullman, D. E., Cho, J. J., Mau, R. F. L., Westcot, D. M., and Custer, D. M. 1992. A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. Phytopathology 82:1333-1342.
25. Wang, R. Y., Gergerich, R. C., and Kim, K. S. 1992. Noncirculative transmission of plant viruses by leaf-feeding beetles. Phytopathology 82:946-950.