# Aberrant Plastids in Barley Leaf Tissue Infected with Barley Stripe Mosaic Virus

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This research was a joint contribution of the Plant Science and Botany-Biology Departments at South Dakota State University and is published with the approval of the Director of the South Dakota State University Agricultural Experiment Station. Journal Series Paper No. 1434, South Dakota State University Experiment Station.

We are indebted to the Northern Grain Insect Research Laboratory for the use of the RCA EMU-3G electron microscope and darkroom facilities.

Accepted for publication 8 August 1977.

## ABSTRACT

MC MULLEN, C. R., W. S. GARDNER, and G. A. MYERS. 1978. Aberrant plastids in barley leaf tissue infected with barley stripe mosaic virus. Phytopathology 68: 317-325.

Barley leaf tissue systemically and mechanically infected with the ND18 strain of barley stripe mosaic virus (BSMV) contained numerous chloroplast aberrations including: swelling, deformation of membranes, cytoplasmic invaginations, peripheral vesicles, and BSMV particles attached to the limiting membrane. Several morphologically aberrant plastid types were located within single affected cells. Two defective types were characterized by the pellucid appearance of the stroma.

A variety of ultrastructural modifications have been found in chloroplasts of virus-infected plant tissues (6, 12, 21, 22). Both Gardner (8) using a moderately severe strain of barley stripe mosaic virus (BSMV) and Carroll (4) using the type strain (ATCC, No. 9) reported the occurrence of chloroplast swelling, deformation of membranes, cytoplasmic invaginations, and BSMV particles attached to the limiting membranes of barley (*Hordeum vulgare* L.) mesophyll plastids. Small peripheral vesicles have been observed in chloroplasts in cells infected with turnip yellow mosaic virus (TYMV) in several investigations (5, 10, 11, 12, 31). Similar structures were found by Moline (21) in physalis mottle virus-infected cells, Betto and co-workers (3) in TMV-infected tobacco leaves, Mohamed (20) in tobacco cells infected by tomato spotted wilt virus, Allen (1) working with wild cucumber mosaic virus, and Carroll (4) in BSMV-infected tissues. Hatta and Matthews (12) distinguished seven cytological stages of TYMV infection primarily on the development of peripheral vesicles in chloroplasts.

We report here on the occurrence of a heterogenous assortment of chloroplast abnormalities in the inoculated leaf and in systemic infections of ND18 BSMV transmitted either mechanically or through seed.

## MATERIALS AND METHODS

Mechanical inoculation was described earlier (18). Virus-infected barley seed was obtained from BSMV-inoculated (9) field-grown 'Larker' barley plants. Barley leaf tissues exhibiting primary acute, secondary acute, and chronic phases described by McKinney and Greeley (17) were prepared for light- and electron microscopy according to procedures described previously (18).

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## RESULTS

**Systemic infection, primary acute phase.**—Examination of thick-sections with the light microscope revealed portions of the leaf in which chloroplasts appeared normal and other segments in which the chloroplasts were distended and the cellular contents deranged. Electron microscopic studies showed that the area with normal-appearing chloroplasts contained considerable amounts of virus. Some chloroplasts had slight swellings or elongated protrusions of the envelope. The extensions encircled pockets of cytoplasm forming cytoplasmic invaginations. No virus particles were found in chloroplasts except in these cytoplasmic invaginations. A similar ameboid process which had enclosed a mitochondrion was observed in a healthy plant.

Electron microscopic studies of leaf tissue categorized as abnormal by light microscopy revealed several aberrations. Cells in uniformly chlorotic tissue frequently contained more than one type of plastid abnormality (Fig. 1). Two defective types were characterized by the pellucid appearance of the stroma caused in part by the paucity or absence of ribosomelike particles normally found in the stroma (Fig. 1). One of these chloroplast types (type 1) was irregular in shape and composed of twisted or convoluted membranes forming tubular networks (Fig. 1-B, 2-A). Elongated grana or anastomosed lamellae often were a prominent feature. The convoluted lamellar network was not apparent in the second type (Fig. 1-B, 2-B). However, compared to normal or control organelles, type 2 plastids were swollen and contained disarranged internal membranes. Both aberrant types rarely contained cytoplasmic invaginations or starch granules.

Chloroplasts containing an electron-dense stroma (type 3) often with cytoplasmic invaginations were the most common aberrant type observed in infected tissues (Fig. 1). The stroma was electron-dense, yet clear areas containing fibrils like those thought to contain DNA (14) frequently were discernible (Fig. 3-A). Distinct fibrils were not observed in plastids with an electron-lucent stroma or in plastids from control tissue. Dense-stroma chloroplasts often contained numerous small peripheral vesicles (Fig. 3). Some vesicles appeared to be double-membrane structures with the inner plastid membrane forming the outer vesicular membrane and the outer plastid membrane comprising the inner vesicular membrane (Fig. 3-A). However, further examination of the vesicles revealed that the inner plastid membrane only formed a depression in the stroma and did not contribute to the vesicular membranes (Fig. 3-B). Plastids frequently possessed peripheral vesicles arranged in rows or in sunken areas of the plastid with rosettes of vesicles. These vesicles were bounded by a single membrane with short necks in continuity with the outer plastid membrane. Virions commonly were observed attached to the outer membrane of the chloroplast. Upon rupture of the outer membrane, the plastid had an expanse of its envelope limited only by the inner membrane and was of irregular shape due to the sunken stroma (Fig. 3-C). Vesicle formation was not limited to mesophyll chloroplasts. Plastids of vascular parenchyma contained vesicles which appeared to be derived from membranes surrounding electron transparent areas in the stroma. Mesophyll chloroplasts contained vesicles derived from similar membranes.

**The inoculated leaf, secondary acute phase.**—Symptoms of the secondary acute phase appeared 1 or 2 days after the first appearance of primary acute symptoms or approximately 6 to 8 days postinoculation. Secondary acute reactions consisted of narrow chlorotic stripes which eventually became necrotic and involved the entire leaf blade, killing it.

Viewed with the light microscope, thick-sections of leaf tissue exhibiting chlorotic stripes revealed spherical rather than normal lenticular-shaped chloroplasts and some cells containing densely-stained shrunken cell contents. Electron microscopy confirmed these observations. Chlorotic leaf tissue contained chloroplasts which were similar to those categorized as type 3 in the primary acute phase of infection. No type 1 or 2 plastids were observed. Occasionally chloroplasts were found to have peripheral vesicles. The vesicles appeared to be connected to the outer plastid membrane and were bound by a single membrane. Rupture of the limiting membrane released the vesicles free into the cytoplasm or left them attached to small fragments of the membrane.

Examination of leaves with necrotic stripes revealed extensive portions with dense cellular contents (Fig. 4-A). That portion of the leaf which was chlorotic but not yet completely necrotic contained cells in various stages of dissolution (Fig. 4-B). Progressive chloroplast breakdown apparently was partially responsible for the increasing electron denseness of the cytoplasm, and the translucent elliptical structures in dense cytoplasm had the same staining characteristics and shape as starch granules found in intact chloroplasts (Fig. 4-B).

**Systemic infection from seed-borne virus, chronic phase.**—The symptoms expressed by plants infected from seed are those of the chronic phase. The emerging leaf contained numerous chlorotic flecks and stripes. As the plant matured, the chronic stripes became white and extended several centimeters up and down the leaf blade. The chlorotic flecks also became apigmented and were randomly scattered up and down the leaf.

Chloroplasts of the chronic tissue contained vesicles which appeared to be derived from a ramified tubular network (Fig. 5-A,B). Some plastids contained convoluted lamellae with small grana, consisting of only a few disks (Fig. 5-C). The very young plastids contained prolamellar bodies, consisting of disorganized tubules with few radiating lamellae (Fig. 5-D). No plastids comparable to types 1 and 2 were found.

## DISCUSSION

The relationship of the ND18 strain of BSMV to chloroplasts is consistent with that found in plant tissues infected with a moderately severe virus strain (8) or the type strain ATCC No. 69 (4). Extensions from chloroplasts, with or without the inclusion of cytoplasmic virions or organelles, are common in virus infections (6, 7, 15, 25, 27, 32). This phenomenon also occurs in healthy plants, but not so frequently (7).

Hatta and Matthews (12) published electron micrographs which depicted sunken areas formed by an invagination of both limiting plastid membranes and rosettes of double-membraned vesicles associated with the sunken part of the chloroplast membrane. Similar interpretations of TYMV-induced vesicles have been offered by Ushiyama and Matthews (31), Lafleche and Bové (see 1), and Hatta et al. (11). Sunken areas in chloroplasts of cells infected with ND18 BSMV (Fig. 3) are formed by the depression of the inner limiting membrane and the single membrane vesicles are attached to the outer plastid membrane by short necks. The hypothesis that the inner plastid membrane does not contribute to vesicular membranes in BSMV-infected cells is supported by our observation of peripheral sunken areas in chloroplasts limited by a single membrane, remaining after rupture of the outer membrane with attached vesicles (Fig. 3). This explanation of peripheral vesicle formation in BSMV-infected tissue agrees with that of Carroll (4). Single-membrane vesicles occasionally have been observed in cells infected with TYMV but were described as not having necks attached to the outer plastid membrane (12).

Matthews (16) reviewed the evidence supporting the hypothesis that small peripheral vesicles found in chloroplasts of diseased cells are intimately related to virus synthesis. The demonstration of definite virus/vesiculated-plastid relationship has been lacking; it has been found convincingly only in BSMV (4) and recently in TYMV (13). Close association of ND18 BSMV with chloroplasts in both barley and corn (19) supports Carroll's (4) suggestion that BSMV synthesis or assembly is linked to vesiculated chloroplasts. However, it is then difficult to explain the origin of massive amounts of virions in cells which appear to have nearly normal plastids. None of the plastids in such tissue had peripheral vesicles or virions closely associated with the outer

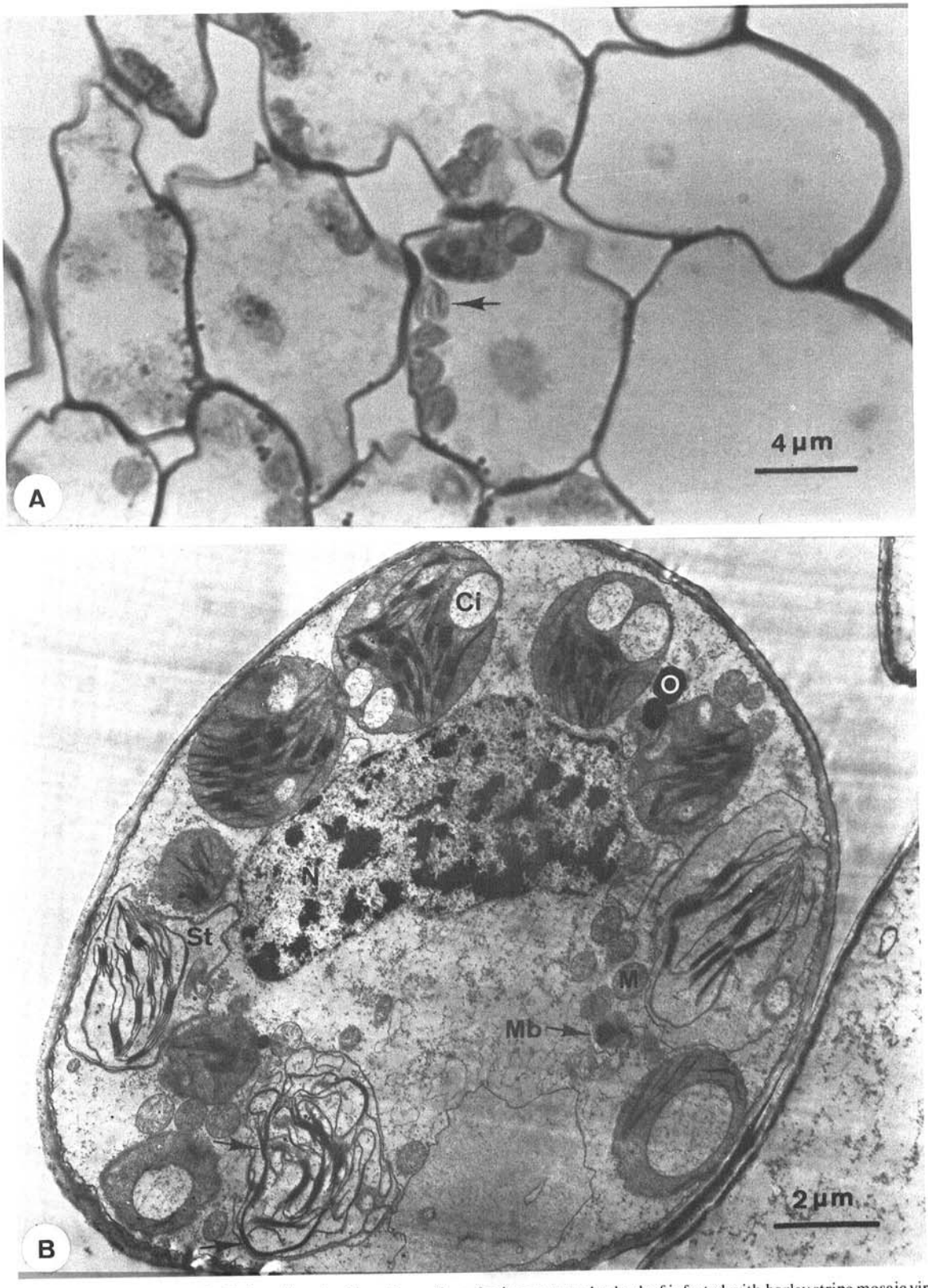


**Fig. 1-(A, B).** Cytology of cells in uniformly chlorotic portion of primary acute barley leaf infected with barley stripe mosaic virus. **A)** Light micrograph of mixed cell with pellucid-stroma chloroplast (arrow) and dense-stroma (type 3) chloroplasts. **B)** Electron micrograph of cell containing pellucid-stroma plastids with (type 1) and without (type 2, at St) convoluted lamellar network. Note elongated grana or anastomosed intergrana lamellae in type 1 chloroplasts (unlabeled arrows). Dense-stroma (type 3) plastids are above the nucleus. Legend: Ci = cytoplasmic invagination; M = mitochondrion; Mb = microbody; O = osmiophilic body; St = stroma.

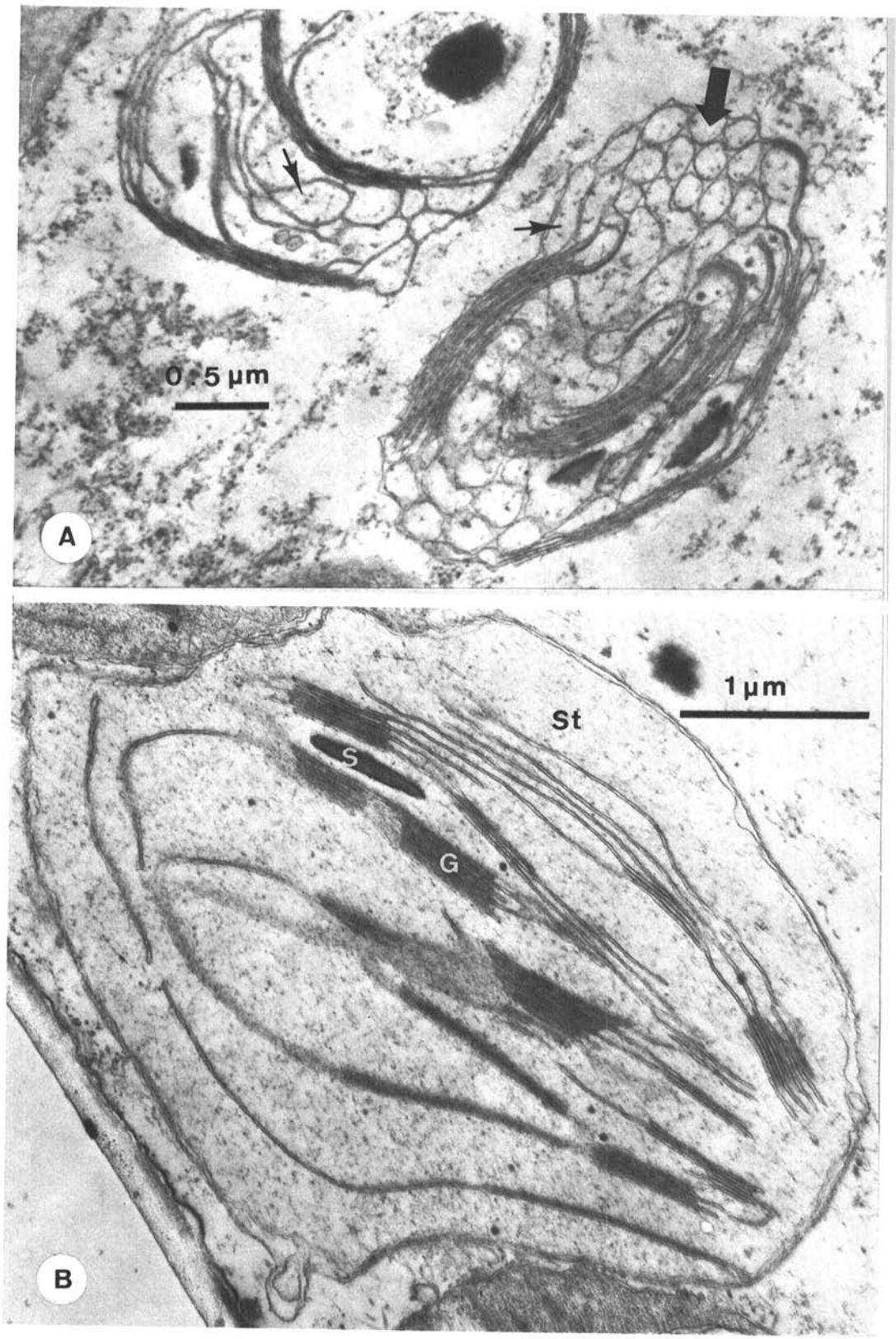


**Fig. 2-(A, B).** Pellucid-stroma plastids located in primary acute leaf tissue infected by barley stripe mosaic virus. **A)** Type 1 plastid composed of convoluted membranes forming tubular networks (small arrows) and vesicles (large arrows). **B)** Type 2 plastid characterized by the lack of distended lamellar membranes. Legend: G = grana lamellae; S = starch; St = stroma.

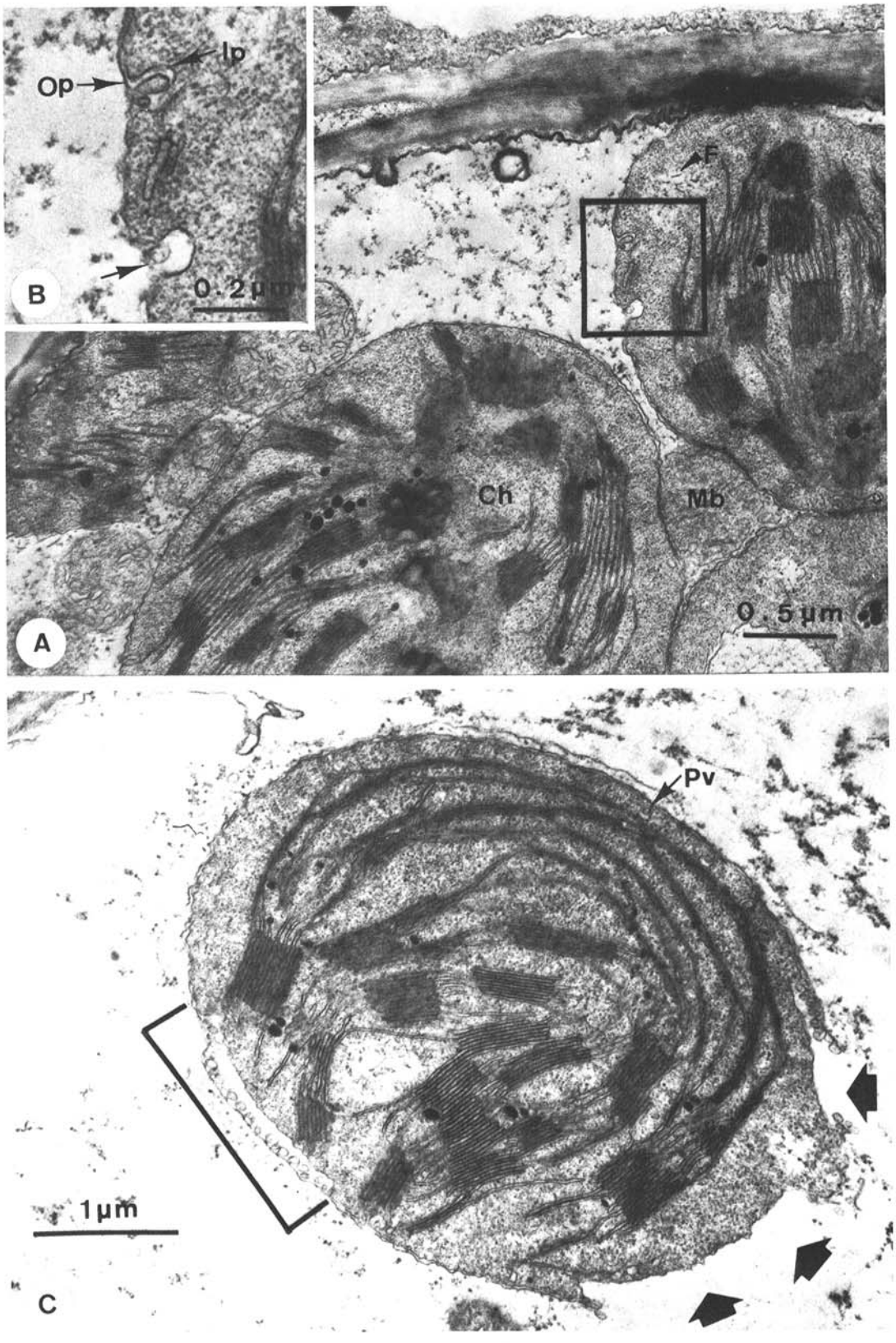


**Fig. 3.** Dense-stroma plastids (type 3) with peripheral vesicles from barley leaf tissue infected with barley stripe mosaic virus showing primary acute symptoms. **A)** Chloroplasts with peripheral vesicles (box) and presumptive DNA area (F). **B)** Enlargement of boxed-in area in Fig. 3-A. Inner plastid membrane forms invagination whereas the outer plastid membrane is attached to a vesicle. Unlabeled arrow indicates invagination or sunken area remaining after vesicle formation. **C)** Plastid with vesicles occurring singly and in rows. Broad arrows indicate area where outer membrane has ruptured leaving sunken stroma. Bracket indicates appearance of sunken area before outer membrane rupture. Legend: Ch = chloroplast; Ip = inner plastid membrane; F = fibrils; Mb = microbody; Op = outer plastid membrane; Pv = peripheral vesicle.

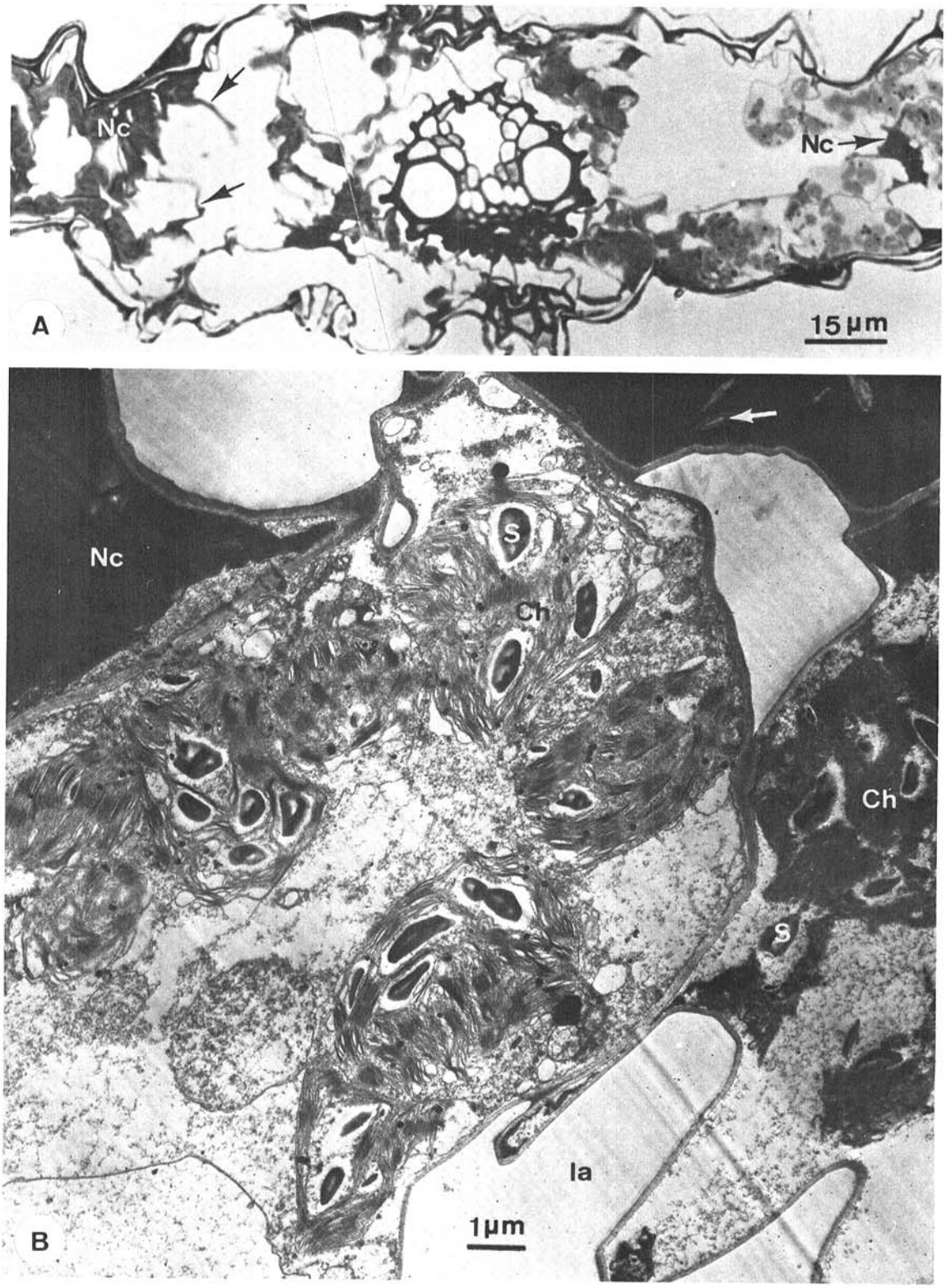


**Fig. 4-(A, B).** Cytology of a barley stripe mosaic virus-inoculated barley leaf with chlorotic and necrotic stripes (secondary acute). **A)** Cross section through leaf with chlorotic (right side of figure) and necrotic (left side of figure) stripes. Cellular contents were completely obscured and tissue collapsed (nonlabeled arrow) in necrotic areas. This figure is a montage of two photographs. **B)** Ultrastructure of same leaf in area with cells in various stages of dissolution. Cell in center is highly disrupted as compared to healthy. Chloroplasts are swollen, contain disorganized membrane systems and have numerous starch granules. Cell to right contains distended chloroplasts with electron-dense, obscured internal structure. The two upper cells are completely necrotic. Elliptical structures (arrow) observed in these cells appeared to be plastid starch granules. Legend: Ch = chloroplast; Ia = intercellular air space; Nc = necrotic cell; S = starch.

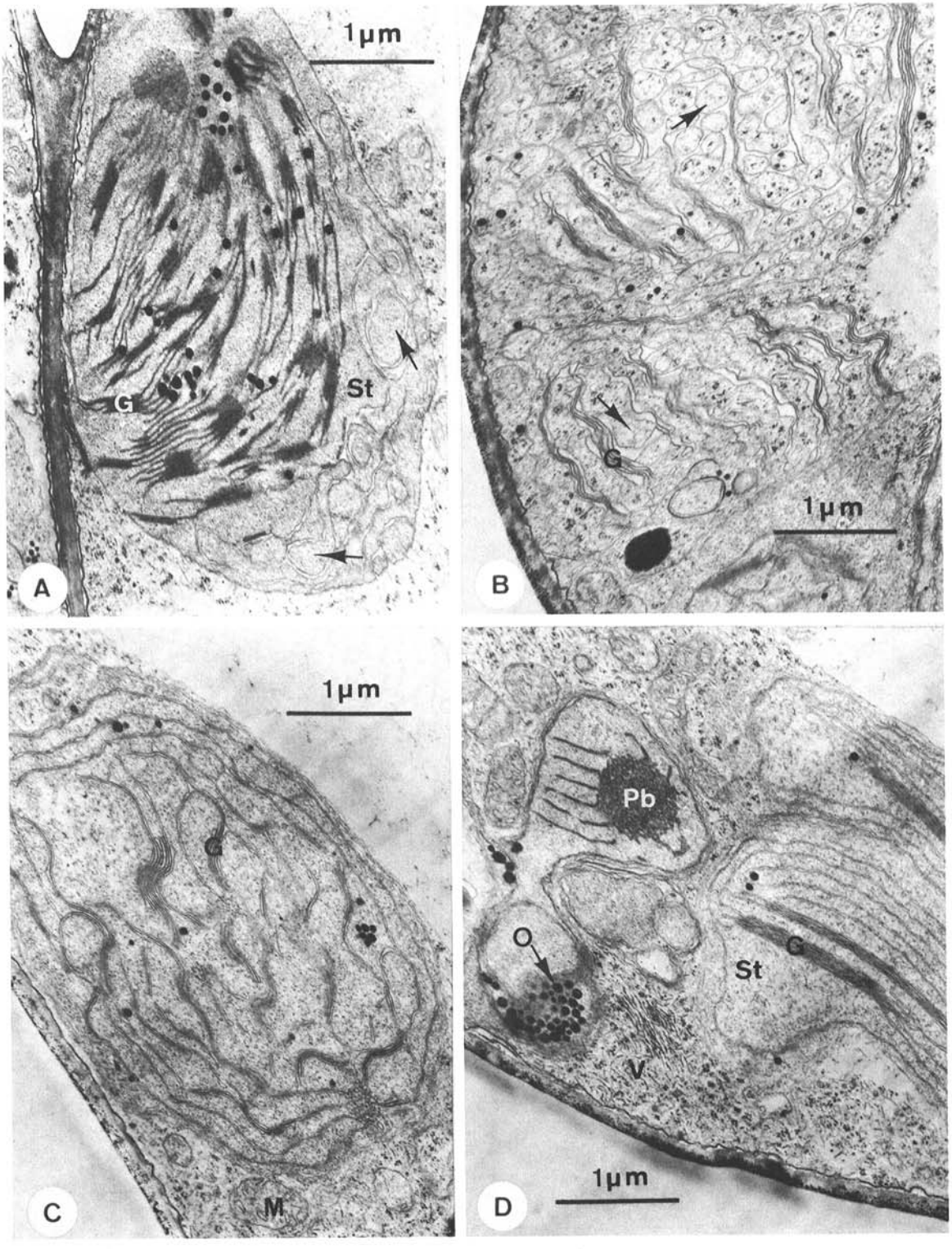


**Fig. 5.** Plastid aberrations found in mesophyll tissue of barley with chronic symptoms of barley stripe mosaic infection. **A)** Chloroplast with tubules and vesicles developing the stroma external to grana and intergrana lamellae systems. **B)** Plastids completely compartmentalized into vesicles (arrows) with narrow elongate wavy grana. **C)** Chloroplast with an unusual membrane system and small grana. **D)** Developing plastid with prolamellar body and osmiophilic bodies. Legend: G = grana; M = mitochondrion; O = osmiophilic body; St = stroma; V = virus.

membrane. It is interesting to note that this tissue contained nuclear virus (C. McMullen, *unpublished*). The nucleus as a possible site of BSMV synthesis or assembly was discussed by Gardner (8).

Our observation of "mixed cells" (cells with more than one plastid type) in BSMV-infected tissue is unique. Gardner (8) found BSMV induced changes in the chloroplasts of some cells and not others. However, no mixed cells were reported. It was suggested by Gardner that chlorplast response of BSMV infection might be based on genetic changes in the chloroplasts.

Some electron microscope data have been collected on mixed cells which are the result of cytoplasmic mutations [summarized by Redei and Plurad (24)]. It appears that only one other virus has been associated with mixed cells (2). Redei and Plurad (24) studied a nuclear mutator gene, which induced a variety of phenotypically different plastids, and suggested that many of these defective plastid types were the result of independent mutations in the plastid. These authors offered the following explanation for the existence of mixed cells in their study:

> "The differentiation of plastids under normal conditions in non-mutant cells is generally well synchronized. Thus if a single metabolic defect would cause the structural alterations one might expect identical or similar defects in all the plastids within single cells. This study clearly demonstrated a large variety of structural modifications within single cells suggesting that a portion of these is based on differences in the genetic constitution of the plastids concerned."

Arnott and co-workers (2) proposed three possible explanations for the occurrence of mixed cells in tomato leaf cells infected with TMV: differences in interaction between virus RNA and the plastome of individual plastids; factor(s) required for normal chloroplast development or maintenance becoming limiting as the result of virus synthesis; or the production of various phenotypes during a single developmental pattern progressing in an irregular sequence. In reference to the latter hypothesis, it seems just as likely that different kinds of plastids observed in a single cell could be intermediate steps in plastid degradation. However, we found mixed cells and aberrant plastid types 1 and 2 only in uniformly chlorotic portions of leaf tissue having symptoms characterized as the primary acute reaction. Neither mixed cells or aberrant plastid types 1 and 2 were observed in the other infection phases even though chloroplast degradation was evident.

It has been demonstrated that BSMV increases the frequency of triploid and aneuploid seeds in barley (26) and in corn that BSMV causes a genetic abnormality, designated "aberrant ratio" (AR) (23, 28, 29, 30). Pring (23) established recently that the ND18 strain of BSMV induces the AR phenomenon in corn. Our data presents the possibility that ND18 BSMV also may influence the phenotypes of plastids by interfering with the genetic condition of individual plastids or with chromosomal genes that code for structural or biochemical properties of the plastids. The latter possibility is of lesser credibility since, if chromosomal genes were involved, the 'plastidome' within a single cell should react the same.

We have found that certain cells, although containing large amounts of virus, contain chloroplasts with few ultrastructural abnormalities. These cells are found in the green portion of the systemically infected leaf. However, plastids in chlorotic regions of the leaf are severely altered. It appears that in leaf tissue exhibiting primary and secondary acute symptoms these degenerative chloroplast changes continue until plastid structure is completely unordered, the cytoplasm appears very electron-dense and the tissue becomes necrotic.

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