

Additional Evidence for Possible Autolytic Activity During Glutaraldehyde Fixation of Maize Dwarf Mosaic Virus-Infected Corn

Willem G. Langenberg and Helen F. Schroeder

Research Plant Pathologist, North Central Region, ARS, USDA; and Laboratory Technician, Department of Plant Pathology, University of Nebraska, Lincoln 68503, respectively.

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ABSTRACT

Fixation of corn leaf tissue infected with maize dwarf mosaic virus, strain B, was obtained after vacuum infiltration and 5 min fixation with a 0.1 M phosphate-citrate buffered 5% glutaraldehyde solution. Tissue was postfixed, without washing, in buffered 1% osmic acid for 15 min. Labile laminated aggregates and virus were preserved by this procedure but, as previously reported, not after conventional glutaraldehyde-osmic acid fixation. Preservation is attributed to prevention of enzymic degradation by brief fixation in glutaraldehyde.

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Additional key words: electron microscopy, ultrastructure.

In a previous publication (4), we reported that conventional glutaraldehyde fixation (3 h and 5%) did not preserve laminated aggregates (LA) in corn leaf tissue infected with maize dwarf mosaic virus (MDMV). The use of certain additives during fixation, or of fixatives considered detrimental to enzymic action, preserved laminated aggregates. We report here on further evidence that cellular lytic action should be considered as a causal factor in the disappearance of LA's from cells infected with MDMV.

Leaves of sweet corn (*Zea mays* L. 'Goldencross Bantam') systemically infected with MDMV-B, 10-14 days after manual inoculation, were cut in pieces about 1 × 1 mm and vacuum-infiltrated at room temp (25 C). After infiltration, fixation vessels were immediately placed in crushed ice for the duration of the fixation. Certain precautions were taken (4) to ensure that tissues used in the various fixation tests were comparable. The main fixation procedures were as follows: (i) 5% glutaraldehyde (GA, Biological Grade, Fisher Scientific Co., St. Louis, Mo.) in 0.1 M phosphate-citrate buffer, pH 7.2, for durations varying from 5 min to 3 h. Tissue was, or was not, washed in cold buffer before postfixation with cold buffered 0.5-2% osmic acid. Washing periods varied from no washing to four 15-min periods; (ii)

Phosphate-citrate buffered 5% GA containing 0.5-2% osmic acid for 5-15 min; (iii) 0.5-2% phosphate-citrate buffered osmic acid for 15 min.

All tissues were dehydrated with a cold acetone series, 15 min per change. At 100% acetone, tissue was brought to room temp and infiltrated with a graded series of a mixture of Spurr plastic (8) and Araldite 6005 (1:1, v/v), prepared as for Araldite 502 (6). Plastic was cured at 60 C for 18 h. Silver-to-gold sections were double-stained with uranyl acetate and lead citrate according to standard procedures (6) and viewed in a RCA-3G electron microscope.

Of all methods tested, corn leaf tissue was fixed best for 5 min in buffered GA followed by cold 1% osmic acid in the same buffer for 15 min, which was sufficient for the osmic acid to diffuse to the center of the tissue pieces. Diffusion of osmic acid could be followed visually by the movement of a black front from the edge of the tissue to the center. Dehydration with a cold acetone series was commenced at this point.

The result of this latter procedure is presented in Fig. 1. All cell constituents were well preserved in dense cytoplasm. Laminated aggregates were readily detected. Thin strands of cytoplasm traversing the central vacuole were preserved by this procedure (lower right Fig. 1), whereas with longer GA fixation periods such strands were rare or absent. Virus particles were also present.

Washing the tissue before osmic acid fixation was not

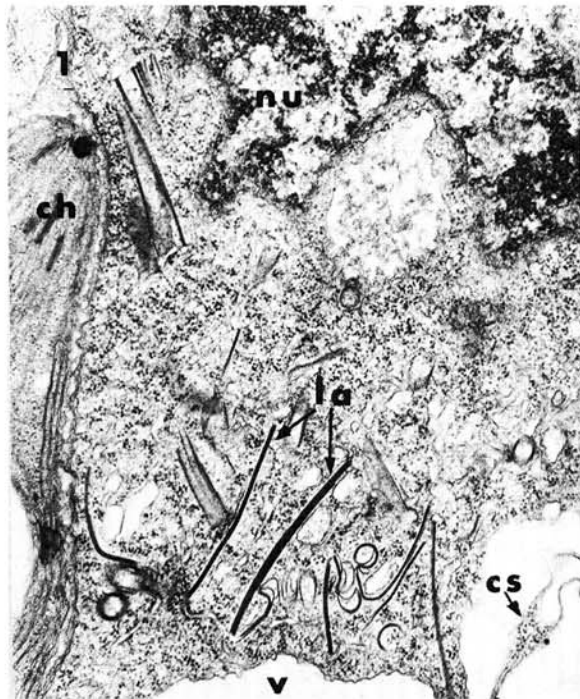


Fig. 1. Part of sheath cell of vascular bundle of corn infected with MDMV, fixed for 5 min in buffered glutaraldehyde and postfixed in 1% buffered osmic acid for 15 min. Central vacuole (V), cytoplasmic strand (CS), laminated aggregates (LA), chloroplast (CH), and nucleus (NU). 24,850 ×.

necessary. The osmic acid fixative was kept cold and replaced the glutaraldehyde in a cold vessel. Under these conditions, glutaraldehyde did not appreciably reduce osmic acid in the 15 min allowed for postfixation. Only expanded leaf tissue was used. It is unknown whether the same fixation results would be obtained with tissue where vacuum infiltration would not bring most cells into rapid contact with the fixative; e.g., root apex or meristematic tissue.

Tissue fixed for up to 3 h in glutaraldehyde only and dehydrated and embedded without washing or osmic acid postfixation showed a gradual reduction in density of cell contents with increasing time of fixation. However, differences existed between experiments; some would still show the existence of LA's in leached cytoplasm after 3 h, whereas in others the deterioration was apparent after 1.5 h GA fixation. Worst preservation, in terms of LA's, was generally present after 3 h GA fixation, followed by 1-2 h repeated changes of washing buffer, and a 1 h 1% osmic acid postfixation. The LA's of pokeweed mosaic virus, which is morphologically similar to MDMV, were partially degraded in *Chenopodium* leaves (3). However, the partial degradation of the LA's was not tied to the method of tissue preparation for electron microscopy.

The addition of 0.01 M 2-mercaptoethanol during 5 min of fixation in GA, with four 5-min washes in buffer to remove this reducing agent before 15 min of osmic acid postfixation, did not improve the appearance. Therefore investigation of its use was not pursued further. Brief washing (four times 5 min) in cold buffer before osmic acid postfixation also did not improve the results, and washing between GA and osmic acid fixation was entirely omitted. Simultaneous fixation in GA and osmic acid did not improve the preservation of tissue in general, although it did preserve the LA's.

Osmic acid alone also produced less desirable results. Laminated aggregates were preserved, however, as had been reported previously (4).

Times and percentages used in glutaraldehyde fixation of plant tissues differ widely. Reports vary from 1 h in 3% GA (10) to 48 h in 6.25% GA (7), with 3 h and 5% most commonly used. It is seldom stated why certain time periods, or percentages of GA, are preferred over others, and application seems to be entirely arbitrary. Customary washing practices to remove unreacted GA from tissue before osmic acid postfixation also vary widely, and often washing times are not given. Apparently, washing the tissue is not necessary (5), and tissues have been successfully fixed in mixtures of GA and osmic acid (2, 9), although this practice has not been widely followed.

Better preservation by fixation in 5 GA for 5 min, followed by 1% buffered osmic acid, than with other fixative methods tested here is attributed to the reduction in time that tissue is subjected to possible cellular lytic

action following death of the cells. In our previous paper (4), we touched upon some reports that GA fixation has been shown to preserve enzymatic activity. In addition, glutaraldehyde has been used to bind enzymes to antibodies to produce an immunosorbent (1).

Our suggestion of enzymic breakdown as the reason for the disappearance of LA's and the general reduction in density of the cytoplasm after conventional GA fixation, remains a hypothesis until a specific enzyme, or chain reaction, can be shown to be responsible. In the meantime, the possibility of cellular lytic activity during fixation has been strengthened by the fact that very brief fixations show the same result as fixations where inhibitors were added (4).

There seems to be no need for lengthy fixation and washing procedures for plant tissues that lend themselves to vacuum infiltration. Lengthy fixation procedures may unwittingly introduce serious artifacts in the study of the ultrastructure of diseased plants.

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