

prepared from samples of the original extracts of these preparations. The samples of the original extracts were held on ice throughout each CVC procedure, and all of the grids for each comparison were then made up at the same time. The CVC ratios (final supernatant: starting exudate) obtained in the trials presented here ranged from 114:1 to 6:1, with an average increase of 39:1. All told, over 2400 viewing fields were examined and 18,700 particles were counted.

Cowpea Mosaic. In Experiment 1, the two procedural variations were processed simultaneously using leaves from a pooled source so that the 50% higher counts of particles obtained from the standard procedure as compared to Variation A (Table 1) reflect a real difference in particle yields. It was also noted that the Step 7 supernatant for the standard procedure was clear and colorless, while that from Variation A was also clear, but yellow in color.

Cucumber Mosaic. A smaller increase in virion numbers was realized by the CVC procedure for this virus than any other studied here. Still, Variations B and C resulted in increases of 6- and 11-fold, respectively, as compared to the number of virions found in the original extracts. No virions could be found in CVC preparations when the standard procedure was attempted.

Lettuce Virus. The virus-like particles associated with a disease of Florida lettuce (Figure 4A) closely resemble those depicted in micrographs of negatively stained ilarvirus particles that may be found in numerous publications (e.g. 8). These ilarvirus-like particles were unstable and were found only when sodium sulfite and EDTA were added to the buffer solutions.

Nandina Stem-pitting. Greater numbers of particles were obtained with the insertion of a simple step into the CVC procedure: the addition of uranyl acetate to the supernatant at Step 7 (Variation F). Although the addition of uranyl acetate dramatically lowers the pH value of the sample, it is unlikely that this factor alone accounted for the additional clarification that occurred, as buffers of similarly low pH values failed to produce the same effect when they were substituted for uranyl acetate. Unfortunately, this technique cannot be universally applied, as viruses typically precipitate at low pH values. Indeed, NSPV was the only virus in this study that was amenable to this treatment, although several others were tested. In the other cases, either no virions were found on the specimen grids or they were found to be aggregated. The technique is simple, however, and may be worth trying when all other attempted methods fail to yield the degree of clarification desired. No negative staining effect was obtained after the use of uranyl acetate for clarification. Because all of the yellow color of the uranyl acetate sedimented with the pellet following centrifugation, it seems likely that the uranyl ions were