

centrifugation. Therefore, host components must be selectively removed without substantial virus loss before concentration of the virions. Selective filtration normally provides the most effective method to separate rhabdoviruses from plant host contaminants. For this purpose, rhabdovirus preparations are often filtered through thin pads of Celite before concentration (2). When Celite pads of appropriate thickness are used, and the washing steps are optimized, greatly enriched virus particles pass through the Celite pads before significant elution of host chloroplast and mitochondrial membrane fragments occurs. However, filtration through other supports may be useful with certain rhabdoviruses. For example, LNYV has been clarified by shaking extracts with DEAE cellulose and decolorizing charcoal or bentonite before Celite filtration (14). Unfortunately, these clarification procedures are not equally effective with all rhabdoviruses, because DEAE cellulose, bentonite, and various clays can adsorb other rhabdoviruses (A. O. Jackson, unpublished observations).

After clarification, rhabdoviruses are usually concentrated from homogenized plant brei by differential centrifugation. Unfortunately, because of particle lability, the reduction in biological activity caused by compression forces during ultracentrifugation is more pronounced with rhabdoviruses than with simpler RNA viruses. Even with SYNV, with which we have routinely used differential centrifugation, the infectivity of the virus is reduced when clarified extracts are centrifuged at high speed (8). Therefore, we have also employed a simple polyethylene glycol (PEG) precipitation procedure as an alternative to differential centrifugation to prepare highly infectious SYNV (15). Thus, when it is important to maintain infectivity, precipitation with PEG, followed by low-speed centrifugation, provides a suitable alternative to ultracentrifugation. However, PEG concentrations required for optimum precipitation of rhabdoviruses vary somewhat (12), and this will need to be evaluated with individual viruses.

Following concentration, rhabdoviruses are normally subjected to rate-zonal and equilibrium centrifugation in sucrose gradients during the final stages of purification. However, various types of chromatography have been used in a few limited cases in the final stages of purification (2). Chromatography over calcium phosphate is routinely used as a step in purification of LNYV (7). However, this method is somewhat limited in utility, since different batches of calcium phosphate may not be uniform (R. I. B. Francki, personal communication), and our experiments have also shown that there is considerable variation in the absorption of SYNV and PYDV by calcium phosphate (A. O. Jackson, unpublished observations). Electrophoresis into sucrose gradients has also been used, but major disadvantages with this method are the time involved and possible losses in infectivity. Therefore, although these alternative steps may have some utility for specific purposes, neither method is really satisfactory for general use with rhabdoviruses.