

technique compromises its utility, and no indication of biological activity can be obtained by microscopy observations alone. Therefore, when available, an infectivity assay should be used in conjunction with physical methods of detection. In the case of SYNV, LNYV, and PYDV, local lesion hosts have provided a suitable assay to monitor the amount of virus in different subcellular fractions. In a few specific cases, tissue cultures or cultured explants from insects that are vectors of the virus have been used for bioassays (12). Even though these cultures are difficult to maintain and have not been established from vectors of most rhabdoviruses, they do provide the most sensitive and rapid bioassays yet devised for plant viruses. Chemical methods may also provide a useful means of detection, especially when combined with electron microscopy and biological assays. For example, glycoprotein detection using specific lectins, in combination with Coomassie blue staining of polyacrylamide gels, has been used to assess the effectiveness of the early stages in purification (11,13). Use of an antiserum, and even antibody preparations that have some reactivity to host components when used in Western blots, can also be of enormous help for evaluating the presence and concentration of virus particles at different stages of purification.

In general, yields of rhabdoviruses from infected plants are low compared with other plant viruses, and the membrane-containing particles are often difficult to separate from host components. These problems are often compounded because of the lability of the virions. The ease with which rhabdovirus virions lose infectivity prohibits commonly used heating and freezing treatments, or the pH adjustments frequently used for clarification. Moreover, the common organic solvents and the mild detergents used to purify simple RNA viruses will solubilize the lipid membranes of rhabdovirus particles. However, several general components of the extraction media that expedite purification of plant rhabdoviruses, such as the appropriate pH, a high osmoticum, and the presence of reducing agents and divalent cations, have been known for more than 20 yr (14). More specific requirements for stability of several individual rhabdoviruses have been determined more recently, and these should provide valuable guidelines for developing purification protocols for uncharacterized rhabdoviruses. The reader is referred to Jackson et al. (2) for a more elaborate description of the requirements needed for these viruses than can be accommodated here.

Almost all purification schemes devised for the rhabdoviruses have relied on some form of centrifugation to concentrate the virus. Unfortunately, rhabdovirus preparations obtained by centrifugation without prior clarification are normally too contaminated with host components for even crude chemical characterization. The most difficult contaminants to remove are chloroplast and membrane fragments, and the presence of these components appears to be a major cause of irreversible virus aggregation following pelleting by high-speed