

(9) and a polymerase complex from nuclei of plants infected with SYNIV (10). These techniques each permit analysis of transcription and translation of SYNIV genes *in vivo*.

### **1.1. Problems Encountered During Purification of Plant Rhabdoviruses**

The single most important factor limiting studies of plant rhabdoviruses is the difficulty of devising simple and reproducible purification protocols suitable for recovery of adequate amounts of highly purified virus for biochemical analyses. Therefore, we have provided a synopsis emphasizing the factors that have proven to be important for optimizing recovery of highly purified virus that retains its infectivity.

Before embarking on development of a purification procedure, several factors related to the interaction of the virus with the host should be considered in order to obtain good virus yields. If the virus is virulent on several hosts, the particular hosts or cultivars that give the highest infectivity titers should be investigated further. Identification of suitable hosts for purification was particularly important for development of purification protocols for SYNIV, potato yellow dwarf (PYDV), and LNYV. In some cases, the choice of the host was critical. For instance, strawberry crinkle virus (SCV) was successfully purified (11) only after it was transferred from strawberry into *Physalis floridana*. Since SCV could be mechanically transmitted from *Physalis*, this eliminated the necessity of using the aphid vector for routine serial transfers, and it also permitted mechanical transmission to a range of experimental hosts that are not preferred by the vector. Even so, we were unable to purify the virus from *Nicotiana edwardsonii* or *N. glutinosa*, both of which had strikingly intense symptoms. In addition to the host used for purification, the age of the plants, and the light and temperature requirements, as well as the length of infection, are critical factors that can drastically alter the amount of virus recovered from tissue. As described below, these factors are extremely important for purification of optimal amounts of SYNIV. Thus, to obtain the highest yields and purity of rhabdoviruses, one must consider a number of host and environmental variables. However, the time invested in a systematic analysis of host and biological factors that contribute to optimum recovery of rhabdoviruses can help minimize numerous problems that otherwise might arise during subsequent development of purification protocols (2).

A reliable procedure for virus detection during different stages of purification is a second important consideration that can help in determining the efficacy of procedures used for virus separation, and minimize the effort necessary to optimize a purification protocol. Electron microscopy is an obvious choice for following particle enrichment, but the labor, precision, and expense of this