

require that a fraction of their CP subunits contain a readthrough domain that provides a C-terminal extension to the CP which resides externally on the assembled virion (e.g., luteoviruses).

The study of virus–vector relationships is often among the most tedious of subjects in the field of plant virology. Initial identification of candidate vector species may be extremely time-consuming, even if the virus associated with a particular disease has been characterized as to group. Different members of a virus group may have unrelated vectors. For example, different members of the *Potyviridae* may be vectored by insects (e.g., aphids, mealybugs, whiteflies), other arthropods (e.g., mites), or fungi. Transmission studies are often complicated by inefficiency of the vectors involved. If a virus is transmitted inefficiently, many individuals of the candidate vector species may be required for transmission. This difficulty may be compounded for study of virus transmission in woody perennial plants if no suitable alternate herbaceous host for the virus is identified. Furthermore, the small size of many vector species (e.g., mites, thrips) renders studies directed toward determining such information as minimum acquisition time, retention time, and whether or not a virus replicates in its vector a difficult task. Although little can be done to reduce the time involved in direct demonstration of vector transmission, advances in detection sensitivity have allowed detection and localization of viruses in their invertebrate vectors.

## 6. Purification and Detection of Plant Viruses

Most viruses are easily purified from plant tissue. Commonly, plant tissue is homogenized with an appropriate buffer and the brei is strained to remove wall materials, and so on. Low pH or organic solvents are often used to help clarify the extract by centrifugation. Virus is collected from the supernatant by polyethylene glycol precipitation, or by ultracentrifugation. Gradients based on sedimentation through sucrose or buoyant density in cesium chloride are often used for further purification. Through steps such as these, which can be accomplished within a few hours, milligram quantities of many plant viruses can be obtained (*see* Chapters 4–24 for examples).

Control of plant viruses has always depended on accurate determination of the causal agent involved in a disease. Early experiments at the turn of the twentieth century depended on needle inoculations of filtered sap preparations. The next major advance was the development of the local lesion assay by Holmes (*15*), which still remains a useful method for inoculum quantitation and purification (*see* Chapter 49). The development of electron microscopy allowed the images of these submicroscopic pathogens to be seen, and has provided an irreplaceable tool for diagnostics and many other aspects of plant virology (*see* **ref. 16** for review).