Protocol 16
Virus Transmission by Aphids

Introduction

Three orders in the class of Insecta contain important vectors: Homoptera (aphids, hoppers, whiteflies, mealybugs), Thysanoptera (thrips) and Coleoptera (beetles). Members of the Homoptera and Thysanoptera feed on plants by piercing cells and sucking the contents, and those of the Coleoptera by chewing leaves. Viruses are usually divided into three groups according to the way they are transmitted by vector insects: nonpersistent, persistent and semipersistent.

Nonpersistently transmitted viruses can be acquired by the vector in a few seconds after access to a virus-infected source (the acquisition access period; AAP) and can be transmitted in an equally short period to a virus-free plant to which it has access (the inoculation access period; IAP). For half an hour after leaving the infected plant, there is no loss in the vector’s ability to transmit infectious virus into a virus-free plant (inoculativity). Thereafter, its inoculativity is lost rapidly and, within a few hours, completely. When the insects are starved for approximately 1 h prior to the AAP, their transmission efficiency increases. The virus is not retained through the moult. As there is good evidence that viruses thus transmitted are retained in the food canal of the maxillary stylets and in the cuticular lining of the foregut of the vector, they are now often referred to as foregut-borne viruses.

Persistently transmitted viruses, on the other hand, are acquired by the vector in long AAPs, ranging from minimal 30 min to hours or days. After that period, the vector cannot immediately transmit the virus, as the latter has to circulate within the body of the insect to finally reach the salivary system. The time interval from the start of the AAP to the end of that IAP in which the first transmission occurred is called the latent period. The vector retains its inoculativity through the moult (transstadial transmission) and often for the remainder of its life. Viruses transmitted in this way are called circulative and when they multiply in the vector the term
propagative is used. Some of the propagative viruses are transmitted through the eggs to progeny of the viruliferous female (transovarial passage).

Semipersistent viruses have intermediate characteristics. They are acquired in AAPs and transmitted in IAPs, both of several hours, but they do not circulate inside the vector. This type of transmission is further characterised by absence of a detectable latent period, and a gradual decrease of vector inoculativity and its loss after moult. There is a positive correlation between the lengths of AAPs and IAPs, and the rates of virus transmission up to a period of 12 h.

Inclusion of protocols on transmission by representatives of all groups of insect vectors is beyond the scope of this book. Hence, a selection has been made on the basis of importance of the vector and/or specific requirements for their successful virus transmission.

Aphids are the most important group of virus vectors in temperate regions, as they can transmit a large number of different viruses. Aphid transmission may be nonpersistent, persistent or semipersistent. The mouthparts of aphids consist of two outer mandibular stylets and two inner maxillary stylets. When an aphid starts feeding on a leaf, it first produces a drop of gelling saliva whereafter its stylets penetrate the epidermis in a brief probe of approx. 18 s, thus favouring nonpersistent transmission of virus present in epidermis. Subsequently, when the host is accepted, the aphid proceeds to deeper cell layers until it finally reaches the phloem sieve tubes from which it derives its nutriment. As most of the circulative and propagative viruses are restricted to phloem tissue, these viruses are usually acquired only in longer feeding periods, characteristic of persistent transmission.

In the following protocols, the commonly occurring aphid *Myzus persicae* has been chosen as an example of a virus transmitter, as it is a vector of many viruses.

### Materials

- Glasshouse compartment at a 16 h photoperiod (light/dark: 16/8) at 22±3 °C. This light/dark regime prevents the development of winged (alate) aphids, provided the population is not too dense.
- Large aphid cage (for instance 40×40×55 cm) for virus-free stock colony.
- Cylindrical Perspex cages of different sizes (ranging from 5 to 8 cm diameter and 10 to 14 cm height) with its top and side holes covered with