Neth. J. Pl. Path. 74 (1968) 12–16

Chenopodium quinoa, a herbaceous test plant for chlorotic leaf spot virus in apple

Hillegonda J. Pfaeltzer

Institute of Phytopathological Research (IPO), Wageningen

Accepted 17 August, 1967

Abstract

The chlorotic leaf spot virus which occurs latent in apples, is widespread in Dutch orchards. In the present paper sap inoculation on *Chenopodium quinoa* is recommended for rapid indexing of trees. Young leaves gave satisfactory results when tested during the first 4–6 weeks after expansion of the buds.
The best results were obtained when homogenated petals were used as inoculum.

Introduction

One of the latent viruses of widespread occurrence in apple varieties and rootstocks is chlorotic leaf spot virus (CLSV), which causes chlorotic leaf spots in indicator plants (Mink and Shay, 1959; Luckwill and Campbell, 1959). Cropley et al. (1963) demonstrated that the virus is identical with the virus causing ring spot in pear.
From pears with ring pattern mosaic Pfaeltzer (1962) transmitted a virus by sap inoculation onto *Chenopodium quinoa*. The symptoms on *C. quinoa* are local lesions on the inoculated leaves (Fig. 1) followed by a systemic infection. Similar symptoms were obtained by sap inoculation from apples (Pfaeltzer, 1962, 1964). Cropley (1963, 1964) was successful in transmitting the virus from *C. quinoa* back into woody host plants, including some indicator varieties.
The testing of fruit trees for the presence of latent viruses is generally done by grafting onto woody indicators. However, indexing of apple trees for CLSV can be simplified considerably by using the sap inoculation method on *C. quinoa*. The author investigated how the most reliable results could be obtained with this method.

Materials and methods

Fifty trees of various varieties ('Ellison's Orange', 'Stark's Earliest', 'Belle de Boskoop', 'James Grieve', 'Winston', 'Ingrid Marie', 'Tydeman's Early Worcester', 'Lord Lambourne', 'Cox's Orange Pippin') were used as virus sources. The trees consisted of grafts from selected mother trees from different parts of The Netherlands, worked on rootstock MM 111 known to be free from CLSV. The Dutch Plant Protec-

Fig. 1. Symptoms of chlorotic leaf spot virus on *Chenopodium quinoa*

Fig. 1. Symptomen van chlorotische-bladvlekkenvirus op Chenopodium quinoa

tion Service had tested the mother trees and found them free from apple mosaic and rubbery wood viruses.

At the beginning of the growing season buds which were just sprouting were used as sources of inoculum; later young leaves were used. The apple leaves were macerated in two or three ml of a 2.5% nicotine solution. The *C. quinoa* test plants were dusted with carborundum and rinsed immediately after inoculation. Local lesions began to appear on the fourth or fifth day after inoculation. Counts of the local lesions were made seven days after inoculation.

The *C. quinoa* plants were inoculated 5 – 6 weeks after sowing. The plants were grown in the glasshouse and might have been affected by varying day-lengths. Another batch of test plants was therefore grown entirely under artificial light. During the experiment each apple leaf sample was inoculated on one test plant grown under glasshouse conditions and on one plant grown under artificial light. No difference in the reactions of the plants of the two groups was noticed.

Results

Several leaves of each test plant were inoculated. The numbers of local lesions on the leaves varied greatly. No local lesions developed on leaves which were not fully grown. The leaves which were just fully grown generally reacted strongly, with numerous local lesions. The older leaves – the third to the sixth on the plant – reacted with a few local lesions only. These leaves, however, were very sensitive and seldom failed to show symptoms if virus was present.

Effect of season on the transmission of virus

In order to find out in which period of the growing season the testing on *C. quinoa* gives the most reliable results, 50 trees of the varieties already mentioned were tested regularly. The experiment was started at the budding stage of the trees. The samples

Fig. 2. Percentages of plants of *Chenopodium quinoa* showing the presence of chlorotic leafspot virus in apple trees at various sampling dates during the growing season of 1964

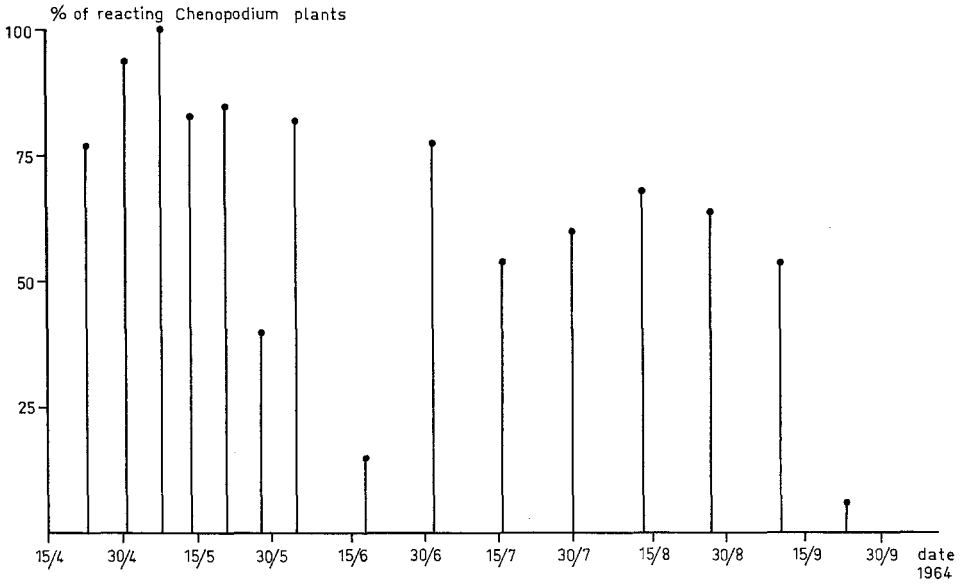

Fig. 2. Het verloop van de aantoonbaarheid van het chlorotische-bladvlekkenvirus in appelbomen door middel van toetsing op Chenopodium quinoa in 1964

were taken weekly until the beginning of June and thereafter once a fortnight. Each sample was inoculated on two *C. quinoa* plants. The percentages of these 50 samples which resulted in at least one distinct local lesion on one of the two test plants are shown in Fig. 2. The graph shows that for five to six weeks after development of the leaves the virus can be easily transmitted to *C. quinoa* and thus its presence in the apple tree established.

No explanation can be given for the very low percentages of transmission on 28 May and 18 June. As we expected from our experience with other viruses and with the apple virus the previous year, the latter virus was most readily transmitted during the spring.

The graph shows that the virus transmission, after a quick increase to nearly 100%, gradually decreased. After 4 – 6 weeks the average number of local lesions per plant decreased from several hundreds to one or two.

Effect of age of the apple leaf on the transmission of virus

In the experiment described we used young and very young apple leaves. When the experiment started there were only buds and very young leaves. During the season the buds grew out to become shoots. At the end of September it became difficult to find shoots which had not stopped growing and still had some reasonably young leaves. In June, therefore, an experiment was set up to investigate whether transmission of CLSV is affected by the age of the apple leaves used for inoculation. Forty shoots about 40 cm in length were collected. The leaves on these shoots were divided into

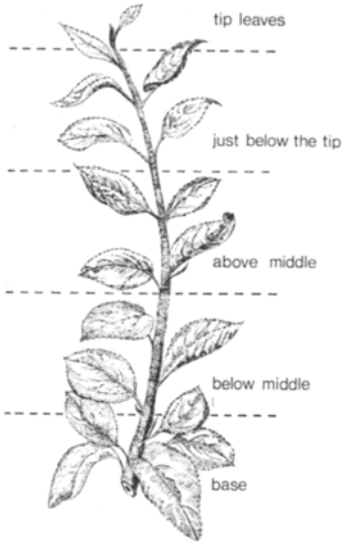

Fig. 3. Showing the groups of apple leaves of different age, used in tests for the presence of virus by indexing on *Chenopodium quinoa*
Fig. 3. Groepen appelbladeren van verschillende leeftijd, gebruikt voor virustoetsing op Chenopodium quinoa

the five groups shown in Fig. 3. The percentages of virus transmission from these groups were as follows:

tip leaves	60%	below middle	62%
just below the tip	98%	base	80%
above middle	80%		

These results indicate that the young leaves just below the tip are the best with which to get virus transmission.
In May 1964 the transmission rate of virus from macerated leaves was compared with the transmission rate from macerated petals. The number of local lesions obtained with inoculum from the petals was at least 10 times as high as that obtained with inoculum from the leaf samples.

Conclusion

The method of sap inoculation on *C. quinoa* is a quick method to test fruit trees for the presence of CLSV. If, for instance, CLSV were still present in heat-treated material, testing on *C. quinoa* could reveal the presence of the virus within a week, thus saving prolonged indexing on woody indicators.

Samenvatting

Chenopodium quinoa, een kruidachtige indicator voor chlorotische-bladvlekkenvirus van appel

Het chlorotische-bladvlekkenvirus komt in Nederland zeer algemeen latent voor in alle appelrassen. Het virus kan door middel van sapinoculatie op *Chenopodium quinoa* worden aangetoond. Een week na de inoculatie reageren de toetsplanten met lokale vlekken op de bladeren. Deze methode is veel sneller dan de indicatie met behulp van

houtige toetsplanten. Hoewel gedurende het gehele seizoen virusoverdracht mogelijk is, kan het virus goed worden aangetoond met jong blad in de eerste 4 tot 6 weken na het uitlopen van de bladknoppen. Nog betere resultaten worden verkregen met bloembladjes.

References

Cropley, R., 1963. The association of a sap-transmissible virus with apple chlorotic leaf spot. Pl. Dis. Reptr 47: 165–167.

Cropley, R., 1964. Transmission of apple chlorotic leaf spot virus from Chenopodium to apple. Pl. Dis. Reptr 48: 678–680.

Cropley, R., Wolfswinkel, L. D. and Posnette, A. F., 1963. The identification of some viruses infecting apple, pear and quince. (Proc. 5th Eur. Symp. Fruit Tree Virus Dis.) Phytopath. Medit. 2: 32–136.

Luckwill, L. C. and Campbell, A. I., 1959. Malus platycarpa as an apple virus indicator. J. hort. Sci. 34: 248–252.

Mink, G. I. and Shay, J. R., 1959. Preliminary evaluation of some Russian apple varieties as indicators for apple viruses. Suppl. Pl. Dis. Reptr 245: 3–17.

Pfaeltzer, H. J., 1962. Mechanical transmission of virus from diseased pear trees to herbaceous hosts. Pl. Dis. Reptr 46: 338–339.

Pfaeltzer, H. J., 1964. Virusziekten in appel en peer. Jrvsl. Inst. Plziektenk. Onderz. 1963: 81–82.