Soil-borne viruses occurring in nursery soils and infecting some ornamental species of Rosaceae

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SUMMARY

Soil from twelve of twenty-six sites on ten nurseries growing ornamental trees and shrubs contained viruliferous nematodes, transmitting arabis mosaic virus (AMV) at eight sites, tomato black ring virus at three and tobacco rattle virus at one site. Tobacco necrosis virus was detected at two sites. Xiphinema spp. were found at nine sites, Longidorus spp. at sixteen and Trichodorus spp. at thirteen sites. Thirty-one ornamental species of Rosaceae were tested for virus infections and AMV was found in Spiraea bumalda, Spiraea japonica alba and Kerria japonica.

INTRODUCTION

Nematode-borne polyhedral viruses (nepoviruses) occur in a wide range of woody plants. Cadman (1963) reported nepovirus infections of Robinia and Ulmus spp. and Harrison (1964) found roots of Chamaecyparis lawsoniana and Picea sitchensis infected with nepoviruses after feeding by viruliferous nematodes. Thomas (1970) showed that the host range of Xiphinema spp. included many woody plants and that Acer pseudoplatanus, C. lawsoniana and Fraxinus excelsior could be infected with nepoviruses transmitted by X. diversicaudatum.

In East Germany Schmelzer (1971), in a summary of his previous studies of hardy ornamentals, reported arabis mosaic virus (AMV) in eight genera, including Spiraea. He also found tomato black ring virus (TBRV) in eight genera and strawberry latent ringspot virus (SLRV) in three genera of hardy ornamentals.

Investigations of shrub roses in the United Kingdom revealed infections by SLRV and AMV (Cammack, 1966), the former virus being particularly important in the vegetatively propagated Rosa rugosa rootstocks used in the production of standard roses (Ilkin & Frost, 1974). These authors found no evidence of nematode transmission of either virus in outdoor roses though Osborne (1964) reported X. diversicaudatum in some old rose beds. However, Harrison (1967) and Sweet (1974) suggested that Xiphinema was primarily responsible for the transmission of SLRV in glasshouse roses.

Tapio (1972) made a survey of nurseries in Finland and found nepoviruses at several sites. No such survey has been done in the U.K., and little is known of the virus status of hardy ornamental trees and shrubs here despite these numerous reports of nepoviruses infecting woody plants. A study was therefore made of the soil from a number of sites on ten nurseries in the south and in the east midlands of England.
where ornamentals were grown and propagated. The soil was 'baited' to test for the presence of virus and examined specifically for the presence of *Xiphinema* and *Longidorus* spp., though *Trichodorus* spp. were counted when observed. In addition thirty-one species of rosaceous ornamentals, excluding roses, collected from these and other nurseries were tested for infection by sap-transmissible viruses.

**MATERIALS AND METHODS**

*Herbaceous indicator plants*

Plants of *Chenopodium quinoa*, *C. amaranticolor*, *C. murale*, *Nicotiana tabacum* cv. White Burley, *N. clevelandii*, *Phaseolus vulgaris* cv. Prince, *Cucumis sativus* cv. Butcher's Disease Resister, and *Petunia hybridu* cv. Blue Dandy grown under a 16 h photoperiod in an insect-proofed glasshouse were kept in the dark for 24 h prior to inoculation.

*Antisera and serological tests*

Viruses were identified by gel-double-diffusion tests against specific antisera. Antisera to AMV and TBRV were supplied by Dr B. D. Harrison, a second antiserum to AMV by Dr J. A. Tomlinson, antiserum to tobacco necrosis virus (TNV) by Dr J. I. Cooper and antiserum to tobacco rattle virus (TRV) by Dr M. Hollings.

*Soil baiting and nematode extractions*

Soil samples of at least 600 ml were taken to a depth of 20 cm from each nursery site. Some of the samples were taken randomly, others were taken from around specific plants or in areas of a field where plants were either not growing well or had been established for a number of years. Each soil sample was split into two sub-samples. The first sub-sample was planted with young seedlings of *Chenopodium quinoa*, *N. tabacum* cv. White Burley, or *Cucumis sativus* which were grown for 4 wk in plastic pots in a glasshouse. The roots of the seedlings were then ground in 0.1 M phosphate buffer at pH 7.5 and the homogenate was rubbed on to the leaf surfaces of healthy test plants.

The other sub-samples were subjected to a nematode extraction process based on a modification of Cobb's decanting and sieving technique (Flegg, 1967) to assess numbers of *Xiphinema* and *Longidorus* spp. present in each soil sample. This technique is not designed to extract *Trichodorus* spp., but where this nematode was observed the numbers were recorded. Nematodes were identified according to their genera only, though *X. diversicaudatum* was positively identified in three soil samples.

*Detection of sap-transmissible viruses*

Woody rosaceous ornamentals were tested for infection with sap-transmissible viruses by the following methods.

**Method 1.** 2–3 g of petals or young leaves from each plant to be tested were ground in a pestle and mortar with 0.5 g Celite (Johns Manville Ltd) in 10–15 ml of one of the following media.

(a) 0.1 M potassium phosphate buffer at pH 7.5.

(b) 2% nicotine solution.
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(c) A buffer (pH 7.5) containing 0.1 M potassium phosphate, 0.02 M sodium diethyl-dithiocarbamate, 0.02 M sodium thioglycollate, 5% (w/v) polyvinyl-pyrrolidone (mol. wt. = 44000) and 0.02 M ethylenediamine tetra-acetic acid.

The herbaceous indicator plants were inoculated by rubbing their leaves with a finger dipped in the homogenates.

Method 2. 10–12 g of young leaves or petals were comminuted in a liquidizer with 40–50 ml of one of the three media listed above. The extract was filtered and centrifuged at 3850 g for 10 min. The supernatant fluid was decanted and centrifuged at 140 × 10³ g for 1 h in an MSE Superspeed 50 ultracentrifuge. The pellet was washed in distilled water and resuspended in 1–2 ml of 0.01 M phosphate buffer at pH 7.4. The preparation was inoculated into the herbaceous indicator plants by rubbing it on to leaf surfaces that had been lightly dusted with Celite.

Extractions by either method were done as quickly as possible in a cold room, using buffers and equipment at 1–2 °C. Inoculations were made to at least three plants of each herbaceous test species and most inoculations were repeated several times during the growing season.

RESULTS

Soil bait tests and nematode extractions

The soil-baiting tests revealed that nepoviruses were present in eleven of the twenty-six soil samples taken from nurseries growing trees and shrubs (Table 1). Serological tests showed that AMV was present in eight soil samples and TBRV in three. TRV was detected in one soil sample and TNV in two. TNV was probably transmitted into the bait plants by its chytrid fungus vector, Olpidium brassicae (Teakle, 1962), though the soil was not tested for this fungus. Trichodorus spp., vectors of TRV (Taylor & Cadman, 1969), were not detected in the soil from which TRV was isolated, probably due to the inefficiency of this technique for extracting them. However, Trichodorus spp. were found in thirteen of the twenty-four soils tested, thirty nematodes per 1 of soil being recorded at one site. Xiphinema spp. were found at nine sites, six of these populations contained viruliferous Xiphinema. AMV was found at one site where no Xiphinema were detected, suggesting that there were in fact a small number of nematodes in the soil. Three of the populations of Xiphinema were examined and identified as X. diversicaudatum. Longidorus spp. were found at sixteen sites and were associated with TBRV at two of the sites. TBRV was also found at one site where no Longidorus spp. were detected, again suggesting low numbers of the nematode vector in the soil.

Several of the soil samples came from old-established plantings or hedges and these had the highest counts of Xiphinema and Longidorus, often associated with nepovirus.

The old bed site in nursery 4 and the standard rose site 1 in nursery 9 both contained some SLRV-infected rose plants but the virus was not detected in the soil by baiting, even though a large population of Xiphinema was present at the first site.

Virus isolation from ornamentals

Plants belonging to the following thirty-one species of woody rosaceous ornamentals were tested to the range of herbaceous indicator plants: Amelanchier canadensis,
Table 1. A survey of nursery soils for soil-borne viruses and nematode virus vectors

<table>
<thead>
<tr>
<th>Location</th>
<th>Nursery no.</th>
<th>Source</th>
<th>Xiphinema Longidorus</th>
<th>Tricho-</th>
<th>Virus isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somerset</td>
<td>1</td>
<td>Old mother trees</td>
<td>45*</td>
<td>25</td>
<td>5</td>
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<tr>
<td></td>
<td>1</td>
<td>Cotoneaster bed</td>
<td>10*</td>
<td>22</td>
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<tr>
<td></td>
<td>2</td>
<td>Rose bed</td>
<td>0</td>
<td>15</td>
<td>30</td>
</tr>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>Mixed planting</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Hants</td>
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<td>Sorbus</td>
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<tr>
<td></td>
<td>3</td>
<td>Cherry</td>
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<td>17</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Potentilla</td>
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<tr>
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<td>N/T</td>
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<tr>
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<td>3</td>
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<td>Glos</td>
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<td>Old bed – roses</td>
<td>35*</td>
<td>55</td>
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<td></td>
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<td>25</td>
<td>5</td>
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<td>4</td>
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<td>25</td>
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<td>R. rugosa hedge</td>
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<td>16</td>
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<td>Surrey</td>
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<td>18</td>
<td>15</td>
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<td>5</td>
<td>0</td>
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<tr>
<td></td>
<td>9</td>
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<td>0</td>
<td>5</td>
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<tr>
<td>Derby</td>
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<td>Shrub roses</td>
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<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

* Identified as X. diversicaudatum.  N/T, not tested; —, no virus isolated.

AMV, Arabis mosaic virus; TRV, tobacco rattle virus; TNV, tobacco necrosis virus; TBRV, tomato black ring virus.

A. laevis, Chaenomeles lagenaria, Chaenomeles lagenaria cv. Pink Lady, Cotoneaster franchetii, Cotoneaster rotundifolia, Cotoneaster simonsii, Crataegus carrierei, Crataegus korolkowii, Crataegus mollis, Crataegus monogyna stricta, Crataegus prunifolia, Deutzia gracilis rosea, Exochorda racemosa, E. serratifolia, Kerria japonica (six plants), Mespilus germanica (three plants), Photinia flava, Photina serrulata, Photinia villosa, Potentilla fruticosa, Potentilla grandiiflora, Potentilla pumila, Potentilla parvifolia, Pyracantha crenulata, Pyracantha crenulata rogersiana, Sorbus hupehensis, Sorbus sargentiana, Sorbus scalaris, Sorbus tianshanica, Spiraea bumalda cv. Anthony Waterer (four plants), Spiraea japonica alba (two plants). The majority induced no symptoms using either Method I or Method II. However, two plants of Spiraea bumalda cv. Anthony Waterer, one from Long Ashton and the other from a nursery in Surrey, and a single plant of Spiraea japonica alba (S. albiflora (Miq.) Zab.) from the Bristol University Gardens at Churchill Hall yielded virus isolates which induced obvious symptoms in several herbaceous indicators. In addition, extracts from two plants of Kerria japonica, one from Long Ashton and the other from a site in nursery 1 (Table 1), induced virus symptoms in a number of the herbaceous indicators.
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Virus was more consistently isolated using Method II than Method I and buffers (b) and (c) were more efficient than buffer (a). The infected Spiraea and Kerria plants appeared symptomless, though the S. bumalda cv. Anthony Waterer showed the characteristic variegation that Smolak (1949) suspected to be a virus symptom. However, Sweet (1974) in transmission and heat therapy experiments found no evidence of a pathological agent inducing this variegation. All four virus isolates from these shrubs produced similar severe systemic symptoms in the Chenopodium species and a faint chlorotic mottle and ring pattern in N. tabacum cv. White Burley, suggestive of infection with nepoviruses. All were identified as AMV by serological tests, giving reactions with both of the AMV antisera. No reaction occurred with antisera to tomato black ring, strawberry latent ringspot and raspberry ringspot viruses.

To fulfil Koch’s postulates, apparently virus-free Spiraea japonica alba seedlings and Kerria japonica plants were approach-grafted respectively with Chenopodium amaranticolor plants infected with AMV isolates from Spiraea and Kerria. Others were approach-grafted with infected Spiraea and Kerria plants. No symptoms were observed in any of these grafted plants 1 yr later. However, when they were tested by inoculation of sap to C. quinoa, AMV was detected in two S. japonica alba seedlings and two K. japonica plants inoculated by grafting with infected plants of the same species. However, inoculations by approach-grafting with C. amaranticolor induced detectable infection in Kerria but not in Spiraea, probably due to difficulties in establishing satisfactory graft unions.

DISCUSSION

These results suggest that nepoviruses and their vectors occur frequently in southern England in the soils of nurseries growing hardy ornamental plants. Although nepoviruses were detected in only a few of the rosaceous ornamental plants tested, preliminary studies of trees and shrubs in other families have revealed nepovirus infections of Fraxinus, Cornus and Hedera spp. in the U.K. (J. I. Cooper & J. B. Sweet, unpublished data). It is apparent that plants introduced into nursery soils are liable to be exposed to populations of Xiphinema and Longidorus and to the risk of infection by nepoviruses, if these populations are viruliferous. Conversely, if the plant is infected by nepoviruses before being introduced into a nursery the nematode population may become viruliferous, creating a threat to nearby plants susceptible to nepoviruses.

Many hardy ornamental plants, including Spiraea and Kerria, are vegetatively propagated and it is likely that most infected material is disseminated in this way. This is particularly so where there is no selection against virus-infected material by the nurseryman due to the ‘latent’ nature of the infection. Many of the root-stocks used in the propagation of ornamental trees and shrubs are seedlings but, because some nepoviruses are seed-transmitted in several genera of plants (Murant & Lister, 1967), infected material could also be propagated in this way.

Sweet (1974) tested a wider range of rosaceous plants for pome fruit viruses (Posnette, 1963) by double-budding with sensitive indicator cultivars (Posnette & Cropley, 1954, 1961). He found that one plant of Crataegus korolkowii, three of
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*Mespilus germanica* and one of *Sorbus tianshanica* induced symptoms indicative of apple chlorotic leaf spot virus (CLSV) in the sensitive indicators and a *Chaenomeles lagenaria* plant induced symptoms indicative of apple mosaic virus. The identity of these viruses has yet to be confirmed. It is likely that the *Mespilus* plants were grafted on to pear rootstocks infected with CLSV, but the means of infection of the other plants is unknown. It therefore appears that many hardy ornamental plants are likely to be infected with any of a wide range of viruses, but further examination of the distribution and effects of these viruses will be needed before recommendations for the improvement of the health of hardy nursery stocks can be made.

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REFERENCES


