Future virus problems?

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SUMMARY

Of seventeen viruses that infect British grasses several have been reported only recently; others that occur in Europe have hosts and vectors in Britain and could become a problem. Ryegrass bacilliform virus and ryegrass spherical virus are described and maize dwarf mosaic virus, maize rough dwarf virus and brome mosaic virus are considered as possible pathogens.

INTRODUCTION

Species composition and changes in husbandry affect the occurrence and prevalence of diseases. Thus the predominance of ryegrass in sown leys may have increased the incidence of ryegrass mosaic virus (RMV) and diminished the importance of the severe cocksfoot viruses. Some crops previously insignificant have become prominent, particularly maize in southern England which occupied about 20000 ha in 1974, mostly grown for forage.

Seventeen viruses have now been described from British grasses (Table 1); nine of them for the first time during the last 5 yr. Consequently, the distribution, transmission and effects of the latter are imperfectly understood but they apparently differ greatly in agricultural importance. Spartina mosaic virus and Agropyron mosaic virus seem unimportant. Others occur in important crop species causing symptoms and damage (ryegrass spherical virus and oat sterile dwarf virus) but some, although widespread, remain symptomless (Lolium latent virus and ryegrass bacilliform virus). Much more, but by no means everything, is known about the epidemiology and effects of barley yellow dwarf virus (BYDV), RMV and the viruses of cocksfoot. However, this paper emphasizes viruses recently recognized in Lolium in the British Isles and some known elsewhere that might be or become important here.

Spherical or isometric particles infecting ryegrass

Several such viruses have recently been reported, Lolium mottle virus (LMV) (O'Rourke, 1968; A'Brook, 1972), Lolium latent virus (LLV) (A'Brook, 1971) and ryegrass spherical virus (RSV) (Plumb, 1973). They appear distinct, but future work may suggest that they should be grouped as strains of a single virus.

Ryegrass spherical virus was first detected in samples from Wye, Kent which were suspected of being infected with RMV. Leaves with patchy chlorosis contained many isometric particles 29.5 ± 1.63 nm in diameter as well as RMV. Mechanical inoculation did not transmit RSV to wheat, oats, maize, timothy, Avena elatior or Chenopodium quinoa, C. amaranticolor, Nicotiana glutinosa, N. tabacum, and N. clevelandii. However, the chlorotic symptoms and both particles developed in S.22 Italian ryegrass (L. multiflorum) inoculated with a mixture of RSV and RMV. Pot tests showed dry matter yields of these S.22 plants was halved, damage greater than that usually caused by RMV alone (Heard, 1971). Subsequent experiments, including infection with RMV alone or in the presence of RSV, all showed a 30% decrease in yield. However, electron microscopy of sap from a sample of plants showed that RSV occurred in small amounts in uninoculated plants and in as many inoculated with RMV alone as with both viruses. Further tests showed that seedlings of the cvs S.22 and RVP, grown insect-free in soilless compost, confirmed the presence of a few particles, apparently of RSV, in uninoculated and symptomless plants. When plants of S.22 were inoculated with RMV from oats, which are not susceptible to RSV, the first symptoms were typical of RMV and RMV particles predominated in these leaves. Subsequently a chlorosis developed that was not typical of RMV infections and these leaves contained many particles of RSV as well as of RMV. The widespread occurrence of RSV in Italian varieties casts doubt on the accuracy of the losses attributed to RMV and questions whether some of it is not attributable to RSV.
We have not found RSV in seedlings of perennial ryegrass or been able to infect them. Perhaps the susceptibility of Italian ryegrass to RSV may explain why it suffers more from RMV infection. Although plants of Italian ryegrass from swards at Rothamsted contained RSV there have been no confirmed reports of damaging dual infections of RSV and RMV although the widespread occurrence of both viruses makes this possible.

No vector is known but the presence of several nematodes, especially *Longidorus macrosoma*, in soil from areas where ryegrass was infected with RSV increased the possibility that nematodes may be the vectors, as suggested for LLV (Catherall, 1971).

Table 1. Grass viruses in Britain

<table>
<thead>
<tr>
<th>Virus</th>
<th>Vectors</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocksfoot streak</td>
<td>Aphids</td>
<td><em>Dactylis, Lolium spp.</em> Storey (1953); Smith (1952)</td>
</tr>
<tr>
<td>Ryegrass mosaic</td>
<td>Eriophyid mites</td>
<td><em>Lolium, oats</em> Slykhuis, Watson &amp; Mulligan (1957)</td>
</tr>
<tr>
<td>Barley yellow dwarf</td>
<td>Aphids</td>
<td>All cereals and most grasses Watson &amp; Mulligan (1957)</td>
</tr>
<tr>
<td>European wheat striate</td>
<td>Planthoppers</td>
<td>Wheat, <em>Lolium</em> Slykhuis &amp; Watson (1958)</td>
</tr>
<tr>
<td>Barley stripe mosaic</td>
<td>Seed and pollen</td>
<td>Wheat, barley, maize Kassanis &amp; Slykhuis (1959)</td>
</tr>
<tr>
<td>Cocksoot mottle</td>
<td>Beetles</td>
<td>Cocksoot, wheat Serjeant (1963)</td>
</tr>
<tr>
<td>Phleum mottle</td>
<td>Beetles</td>
<td>Timothy Catherall (1966)</td>
</tr>
<tr>
<td>Anthoxanthum mosaic</td>
<td>—</td>
<td><em>Anthoxanthum, wheat oats, barley</em> Catherall (1967)</td>
</tr>
<tr>
<td>Tobacco rattle</td>
<td>Nematodes</td>
<td>Oats Catherall (1971)</td>
</tr>
<tr>
<td><em>Lolium</em> latent</td>
<td>—</td>
<td><em>Lolium</em> spp. A'Brook (1971)</td>
</tr>
<tr>
<td>Ryegrass seedborne</td>
<td>—</td>
<td><em>Lolium multiflorum</em> Plumb (1973)</td>
</tr>
<tr>
<td>Ryegrass bacilliform</td>
<td>—</td>
<td><em>Lolium</em> spp. Plumb (1973)</td>
</tr>
<tr>
<td>Festuca mottle?</td>
<td>Aphids</td>
<td><em>Festuca, Oats</em> Gibson (1974)</td>
</tr>
<tr>
<td>Spartina mosaic</td>
<td>—</td>
<td><em>Spartina</em> P. Jones &amp; R. T. Plumb (unpublished)</td>
</tr>
<tr>
<td>Agropyron mosaic</td>
<td>Mites</td>
<td><em>Agropyron, wheat</em> P. L. Catherall (personal communication)</td>
</tr>
</tbody>
</table>

**Ryegrass bacilliform virus (RBV)**

These particles were first found in 1972 in plants from trial plots of the National Institute of Agricultural Botany at Stoneleigh and later from Wye (Plumb, 1973). In leaf dips the particles were bullet-shaped but in thin sections were bacilliform, with morphology (Plumb & James, 1975) similar to the group of rhabdoviruses (Francki, 1973). At both localities the variety infected was *Grasslands Manawa, a L. multiflorum x L. perenne* hybrid but particles have since been found in Italian and perennial varieties. Infected plants show no symptoms that reliably indicate the presence of RBV and there is no interaction with RMV. Particles of RBV have also been found in plants from swards at Cannington (Somerset), Pluckley (Kent), Bangor (North Wales) and at Rothamsted. Thus RBV seems widespread but there is no evidence that it causes damage. The morphology of the particles suggests transmission by hoppers or aphids but neither the aphid *Rhopalosiphum padi*, or the planthopper *Javesella pellucida* transmitted, nor was it transmitted mechanically. At present RBV is not a problem in British grassland but as similar viruses damage other members of the gramineae (Francki, 1973) RBV should not be forgotten.

**Grass viruses in Europe that may appear in Britain**

Table 2 lists three of the viruses known in Europe but not in Britain that are potential pathogens of our crops because their vectors and hosts occur here naturally. Two of these viruses are a particular threat to our increasing acreage of maize (Maize dwarf mosaic virus
Maize dwarf mosaic virus (MDMV)

This virus is usually considered a strain of sugarcane mosaic virus which infects a number of perennial grasses, notably Johnson grass (Sorghum halepense). Infection usually causes a mosaic but symptoms vary between the numerous strains and hosts. Serious losses from MDMV have occurred in the United States of America (Williams & Alexander, 1965; Toler, Hobbs & Bockholt, 1967) although there has been some success in breeding resistant varieties (Grogan & Teakel, 1969).

Two grasses Digitaria sanguinalis and Setaria spp. are also perennial hosts of MDMV and have been introduced and become established along the south coast. Two of the most important vectors in France, Macrosiphum (Sitobion) avenae and Myzus persicae occur in Britain although they are not common on maize, and Rhopalosiphum padi, which is common on maize, is a vector in the United States of America. Aphid transmission is of the non-persistent type and introduction by aerial vectors is unlikely. However, there is one report of a little (0.04%) seed transmission (Shepherd & Holdeman, 1965). The great variability of MDMV and the presence of hosts and vectors makes this perhaps the most likely virus to be introduced.

Table 2. Some grass viruses in Europe

<table>
<thead>
<tr>
<th>Virus</th>
<th>Vectors</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize dwarf mosaic</td>
<td>Aphids. <strong>Rhopalosiphum padi</strong>, Myzus persicae, Macrosiphum (Sitobion) avenae</td>
<td>Maize, Digitaria sanguinalis, Setaria spp.</td>
</tr>
<tr>
<td>Maize rough dwarf</td>
<td>Planthoppers. <strong>Laodelphax striatella</strong>, (Jaisella pellucida)</td>
<td>Maize, Digitaria sanguinalis, Echinochloa crus-galli</td>
</tr>
<tr>
<td>Brome mosaic</td>
<td>Nematodes. <strong>Xiphinema diversicaudatum</strong>, X. coxi</td>
<td>Maize and many grasses</td>
</tr>
</tbody>
</table>

Maize rough dwarf virus (MRDV)

This virus occurs in France and Italy where it dwarfs young maize plants and causes enations on leaves and ears. It is damaging in Italy because sowing of maize coincides with the dispersal of its principal vector, the planthopper **Laodelphax striatella**. It is difficult to predict the damage that might follow the introduction of this virus to Britain because although **L. striatella** does occur it is uncommon where maize is grown (Le Quesne, 1960). However, another planthopper shown to be a vector, **J. pellucida**, is one of the most numerous on British grasses. Furthermore two introduced grasses **D. sanguinalis** and **Echinochloa crus-galli** are natural hosts and **L. perenne** is susceptible.

Brome mosaic virus (BMV)

This virus is known in Germany (Ohmann-Kreutzberg, Pawlitschek & Schmidt, 1960; Ohmann-Kreutzberg, 1963) and is causing concern in Finland (Bremer, 1973). BMV can kill young maize plants but usually causes only a mild mosaic unless it occurs with other viruses which synergizes to cause severe damage (M. Tosic personal communication). Its host range is wide, including maize, wheat, barley, oats, Phleum, Agrostis, Agropyron, Lolium as well as some dicotyledons. It is readily transmitted by sap and grass-cutting machines (McKinney, 1953). Nematodes (Xiphinema spp.) are natural vectors (Schmidt, Fritsche & Lehmann, 1963) and occur in small numbers in old pastures (Pitcher & Jha, 1961) and grass-sower swards (Peacock, 1959) in Britain.

The effects of BMV do not appear to be as serious as those of cockfoot mottle virus and cockfoot streak, but by analogy with the rapid transmission of these viruses by cutting machinery BMV, if introduced, has the properties necessary for a similarly rapid dispersal. The presence of soil-inhabiting vectors capable of retaining the virus during the absence of the crop would increase the problems of control and eradication.
CONCLUSION

The motive for reporting two viruses hitherto unrecorded in Britain and three that might be introduced is not to be alarmist but to help prevent serious consequences by recognizing them early and taking correct preventive action. The time for such a warning seems opportune because maize is being grown increasingly both for grain and especially forage and the economics of producing and protecting crops for stock-feed are changing rapidly.

REFERENCES

Recent reports indicate that fungal infection may elicit a multiple phytoalexin response by resistant plants (Higgins & Smith, 1972; Bailey, 1974). Two closely related phytoalexins have been recognized in the broad bean, wyerone and wyerone acid (Letcher, Widdowson, Deverall & Mansfield, 1970; Fawcett, Firn & Spencer, 1971). To avoid confusion we shall refer to wyerone as wyerone ester in this article (Fig. 1).

It has been suggested that the differential pathogenicities of Botrytis fabae (virulent) and B. cinerea (avirulent) towards Vicia faba depend on their sensitivities to wyerone acid and their abilities to degrade this inhibitor to inactive forms. B. fabae, unlike B. cinerea, is able to metabolize the phytoalexin to reduced wyerone acid (Fig. 1) which lacks antifungal activity (Mansfield & Widdowson, 1973; Mansfield, Porter & Widdowson, 1973; Mansfield & Deverall, 1974).

Here we report the detection of seven antifungal compounds produced by Vicia faba in response to fungal infection, and preliminary experiments into their role in controlling infection of the broad bean by species of Botrytis.

Detection of Phytoalexins

Methanol extracts of infected tissues were dried in vacuo then partitioned between water and diethyl ether. Antifungal compounds present in the ether phase were separated by thin layer chromatography (TLC) on silica gel pre-coated plates (Merck 5715) and detected by spraying developed chromatograms with spores of Cladosporium herbarum in Czapek dox solution (pH 5.0). Inhibitory bands were recognized as white areas of silica gel where the dark green fungus had failed to grow. The TLC plate bioassays revealed the accumulation of wyerone acid, wyerone ester and up to five as yet unidentified phytoalexins, in infected tissues. None of the inhibitors were detected in tissues inoculated with sterile distilled water. Concentrations of purified wyerone derivatives and one unidentified inhibitor, named PA1, were determined by ultra violet (u.v.) spectrophotometry. Estimates of phytoalexin concentrations were also obtained from areas of inhibition on TLC plates.

The relative concentrations of inhibitors detected depended on the tissue infected and fungal species used as inoculum. Wyerone acid, wyerone ester and two inhibitors named PA1 and PA2 were found consistently in all infected tissues examined. Two days after inoculation with B. cinerea similar relative concentrations of these inhibitors accumulated in infected pod and leaf tissues, but the accumulation of wyerone ester was most striking in cotyledons (Fig. 2).