SOME OBSERVATIONS ON AN APHID-BORNE VIRUS DISEASE OF RYEGRASS IN NEW ZEALAND

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ABSTRACT

Chlorotic streaks observed on spaced ryegrass plants at Palmerston North were found to be due to an aphid-borne virus. The virus was transmitted in a circulative manner by Rhopalosiphum padi (Linn.) but not by Macrosiphum miscanthi Takahashi. The host range and method of transmission of the virus indicate some similarities with barley yellow dwarf virus from which the ryegrass virus differs principally in its symptom expression on ryegrass.

INTRODUCTION

During a visit to New Zealand in spring 1960, Dr J. T. Slykhuis observed chlorotic streaks on ryegrass plants at Palmerston North (Slykhuis 1962). Streaking was more prevalent on ryegrass plants of Lolium multiflorum parentage than of L. perenne parentage. In spring 1970 we examined 2,000 8-month-old, spaced plants of various international origins grown at Palmerston North and found that 43% exhibited chlorotic streaking to varying degrees. Field symptoms were most conspicuous on tetraploid Italian or Westerwolds ryegrasses, but also occurred on more perennial types, e.g., Lolium (multiflorum × perenne) ‘Grasslands Manawa’ and Lolium (multiflorum × perenne) × perenne ‘Grasslands Ariki’. No streaking was observed on Latium perenne L. ‘Grasslands Ruanui’.

Attempts to transmit the virus by mechanical methods were unsuccessful. Transmission tests with aphids in the glasshouse showed Rhopalosiphum padi (Linn.) to be an efficient vector of the virus. This paper describes the results of tests on some aspects of the epidemiology of this virus.

EXPERIMENTAL

The isolate of ryegrass chlorotic streak virus (RCSV) used in these tests was one of several originally obtained from single, tetraploid Italian-type ryegrass plants showing severe chlorotic streaking and varying degrees of stunting and leaf distortion. The virus was transmitted by R. padi to Lolium multiflorum Lam. ‘Grasslands Tama’, a

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tetraploid Westerwolds ryegrass. Tama ryegrass was subsequently used as the virus source and the indicator plant species for the aphid transmission tests and for virus recovery tests in the preliminary host range study. Symptoms normally appeared 6–10 days after infestation with viruliferous aphids.

One major difficulty arising from the host range studies was the possible presence of barley yellow dwarf virus (BYDV) in all the ryegrass virus isolates. However, as no streaking was observed on Tama ryegrass inoculated with BYDV obtained from ryegrass plants showing typical BYDV, i.e., stunting and leaf reddening, it was concluded that any streaking symptom on Tama ryegrass would be a consequence of RCSV and not of BYDV.

Aphid colonies were developed from single, newly emerged nymphs and were reared on ryegrass plants grown from seed. Viruliferous aphids were obtained either from colonies raised on virus-infected plants grown in small insectary chambers, or by allowing non-viruliferous aphids access for 2 days to leaves detached from infected plants. For routine tests of plant susceptibility to the virus, each seedling test plant

<table>
<thead>
<tr>
<th>Acquision feeding time in hours**</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. plants infected out of 15</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Inoculation feeding time in hours***</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>No. plants infected out of 15</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

* 5 aphids per plant.
** Aphids allowed 2-day inoculation feed.
***Aphids allowed 4-day acquisition feed.

<table>
<thead>
<tr>
<th>Aphid clone</th>
<th>No. of aphids per plant</th>
<th>Transmission*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP 1</td>
<td>1</td>
<td>4/30 = 13%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11/30 = 36%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9/14 = 64%</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2/8 = 25%</td>
</tr>
<tr>
<td>RP 5</td>
<td>9/30 = 30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14/30 = 46%</td>
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<tr>
<td></td>
<td>11/16 = 63%</td>
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<tr>
<td></td>
<td>5/16 = 31%</td>
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</tr>
</tbody>
</table>

* Numerator = number of plants infected.
Denominator = number of plants infested.
was infected with 4-5 aphids. Aphids were confined to the test plants, grown singly in 4-in. pots, by means of individual pot cages. The inoculation feeding period was usually of 2 days. In all tests a number of plants were infested with non-viruliferous aphids as controls. None of the control plants developed virus symptoms.

Inoculation feeds were terminated either by fumigation with DDVP (0, 0-dimethyl 2, 2-dichlorovinyl phosphate) in a closed chamber, or by spraying the plants with the systemic methomyl insecticide Lannate (Du Pont, Wilmington, Del.).

RESULTS

Transmission studies

No transmission of RCSV occurred with either R. padi or M. miscanthi when starved aphids were allowed short (less than 10 minutes) acquisition and inoculation feeds. R. padi, but not M. miscanthi, was able to transmit the virus when allowed acquisition and inoculation feeds of 1-2 days' duration, although the proportion of plants that were infected varied among tests. In an attempt to increase the efficiency of R. padi as a vector in glasshouse tests some aspects of the virus-vector relationship were examined in more detail.

Length of time required for acquisition or inoculation feeding—Virus-free aphids were allowed acquisition feeds on detached leaves for 2, 4, 6, 8, and 12 hr, after which the aphids were transferred to batches of 15 Tama ryegrass seedlings, 3 aphids per plant, for an inoculation feeding period of 2 days (Table 1). In another test in which single aphids were allowed access to detached leaves from infected plants for ½, 1, 2, or 4 hr, followed by a 3-day inoculation feed, 3, 2, 4, and 6 of 32 test plants, respectively, became infected.

In the second experiment viruliferous aphids, after a 4-day acquisition feed period, were caged for various times on batches of 15 Tama ryegrass seedlings, 5 aphids on each plant. Maximum transmission was obtained after 4 hr (Table 1).

Aphid number and type—The influence of different numbers of aphids per test plant was investigated using 1, 2, 4, or 8 viruliferous aphids from each of two aphid clones. The aphids were reared on infected Tama ryegrass plants, and adults and late instar nymphs were transferred to seedling test plants for 2-day inoculation feeding periods. Surprisingly, infestation with 4 aphids to a plant proved to be a more effective means of transmitting the virus than with 8 aphids (Table 2). Presumably seedling vigour and susceptibility were reduced by the larger aphid population.

A comparison of vector efficiency of alate and apterous adults and first to third instar nymphs showed all forms to be equally capable of transmitting the virus.

Persistence of virus in the vector—Twenty-five Tama ryegrass seedlings were each infested with 5 viruliferous aphids reared on virus-infected plants. After 24 hr the surviving aphids were transferred
Virus disease of ryegrass

to a fresh batch of test plants. Five consecutive daily transfers to fresh seedlings were made, each batch of seedlings being sprayed at the conclusion of the inoculation feeding period to kill any newly emerged nymphs. No reduction occurred in the proportion of plants developing streaking symptoms between the first and the last transfers, showing that the virus persisted at least 5 days in the aphid vector.

Host range

To determine the experimental host range of RCSV the virus was inoculated to at least 8 plants of each species tested. Susceptibility or non-susceptibility to the virus was ascertained by back-inoculation from each plant to 4 Tama ryegrass seedlings.

RCSV was recovered by back inoculation from the following grass species: Agrostis stolonifera L., Vahl, Bromus mollis L., B. sterilis L., Cynosurus cristatus L., Dactylis glomerata L., Festuca arundinacea Schreb., Panicum miliaceum L., Phalaris canariensis L., P. minor Retz., Hordeum murinum L., Briza minor L., and Holcus lanatus L. No symptoms were observed after back-inoculation to Tama ryegrass from Agrostis tenuis Sibth., A. alba L., Echinochloa crus-galli (L.) Beauv., Anthoxanthum odoratum L., Phleum pratense L., or Zea mays L.

All ryegrasses tested were susceptible to the virus. However, in the glasshouse, streaking was apparent only on ryegrasses with some Italian or Westerwolds parentage, such as ‘Grasslands Manawa’, ‘Grasslands Ariki’, and Aberystwyth S 22. Although ‘Grasslands Ruanui’ and Aberystwyth S 24 perennial ryegrasses are highly susceptible, no symptoms were observed on them.

Several cultivars of wheat, barley, and oats were tested. All three cereals were susceptible and produced symptoms similar to those observed for BYDV. BYDV (supplied by Dr R. C. Close) tended to produce more severe symptoms on cereals than RCSV. However, RCSV symptoms appeared about a week earlier on oats and wheat than those of BYDV. In several tests on the effect of BYDV on Tama ryegrass, no streaking symptoms were observed.

DISCUSSION

Other than BYDV, which has been well documented as a widespread cereal pathogen (Smith and Wright 1964; Close 1969), ryegrass chlorotic streak virus is the first grass virus to be described from New Zealand. The host range and manner of transmission of RCSV indicate a possible relation with some isolates of BYDV. However, RCSV has sufficient distinguishing characteristics to justify its consideration as a separate virus. For example, RCSV has been recovered from Holcus lanatus, reported as not susceptible to BYDV (Oswald and Houston 1953; Bruehl and Toko 1957), but not from Phleum pratense, which is susceptible to at least some BYDV isolates (Oswald and Houston 1953; Bruehl and Toko 1957; Watson and Mulligan 1960). However, the principal difference between RCSV and BYDV is in the symptom expression of the two viruses on Italian and tetraploid ryegrasses.
RCSV produces a definite and severe chlorotic streaking along the leaf blade within 2 weeks of inoculation. In contrast, typical symptoms of BYDV on ryegrass develop slowly and eventually take the form of reddening of the leaves and general severe stunting (Wit 1956; Catherall 1966), consistent with the effects expected for plants suffering phloem necrosis and restriction of root growth. Nevertheless, these differences do not rule out the possibility that RCSV may belong in the BYDV group. Further tests on the particle morphology and serology of the two viruses will be needed to clarify this point.

Regardless of its precise identity, it is apparent that RCSV is an aphid-transmitted virus of possibly serious economic importance in New Zealand pasture production. Further investigations of the incidence of this disease in New Zealand grasses are warranted.

Acknowledgment

Dr R. C. Close, Plant Diseases Division, DSIR, Christchurch, for providing one of the BYDV isolates.

REFERENCES


