Cross-protection among strains of barley yellow dwarf virus

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ELISA, cDNA dot blot hybridization and transmission by vector aphids were used to investigate the occurrence and degree of cross-protection produced in oat plants by virus isolates representing five strains or serotypes of barley yellow dwarf virus, namely PAV, MAV, SSV, RPV and RMV. Generally, the degree of cross-protection was positively correlated with the serological relatedness between the isolates. A high degree of cross-protection occurred between NY-MAV and MAV-PS1, two isolates of the MAV serotype; cross-protection was moderate between MAV-PS1 and either P-PAV (a Purdue isolate of the PAV serotype) or NY-SGV; cross-protection between P-PAV and NY-SGV was low. Cross-protection did not occur in other paired inoculations and did not persist in some plants, the challenge virus eventually becoming detectable. The persistence of cross-protection depended on the interval between inoculations with protecting and challenge viruses; longer inoculation intervals enhanced the persistence of cross-protection. Results obtained by ELISA and dot blot hybridization were usually consistent, indicating that cross-protection affected both viral capsid and RNA synthesis.

Introduction

Cross-protection is a phenomenon whereby prior infection with one (protecting) plant virus will prevent or interfere with superinfection by another, usually related virus (challenge) (Fulton, 1982; Hamilton, 1980; Sherwood, 1987). Cross protection has been used to investigate relationships among viruses and as a practical means of protecting plants against virus infection (Oshima, 1975; Costa & Muller, 1980; Yeh & Gonsalves, 1984). Interest in cross-protection has also been stimulated by recent successes in obtaining genetically engineered protection by transforming plants with viral genes, such as those encoding the viral coat protein (Beachy et al., 1987).

Cross-protection has been investigated in most detail using sap-transmissible viruses that are readily assayed. It has been more difficult to study using vector-borne viruses that are not sap-transmissible, many of which are difficult to manipulate or to identify by sensitive specific assays. This problem is exemplified by the barley yellow dwarf virus group (BYDV), which consists of a cluster of luteoviruses which infect graminaceous hosts and which are transmitted by a range of aphid vectors (Rochow & Duffus, 1981). Typical isolates of BYDV, originally named for their predominant aphid vectors, can be separated into two groups based on their serological relatedness (Rochow, 1970; Aapola & Rochow, 1971; Rochow & Carmichael, 1979; Rochow & Duffus, 1981) and other features (Gill & Chong, 1979; Gildow et al., 1983). Based on these criteria, BYDV group 1 includes isolates representative of three distinct serotypes, NY-MAV, transmitted principally by Macrosiphum avenae Fabr., NY-PAV, transmitted principally by Rhopalosiphum padi L. and M. avenae, and NY-SGV, transmitted principally by Schizaphis graminum Rond. (Rochow, 1970). Group 2 includes NY-RMV, transmitted principally by R. maidis Fitch., and NY-RPV, transmitted principally by R. padi (Rochow, 1970). [The prefix NY (New York) indicates geographical origin, according to the suggestion of Rochow (1984)]. Serological relationships within these groups are closer than relationships between them (Waterhouse et al., 1988).

Evidence of cross-protection among isolates of BYDV has so far focused on the effects of challenge inoculations on symptom expression and plant growth, and on the ability of vectors to recover challenge virus from mixed infections. By such means, interference has been demonstrated between some pairs of isolates, whereas synergism and lack of interference have been reported among other combinations (Smith, 1963; Aapola & Rochow, 1971; Halstead & Gill, 1971; Jedlinski & Brown, 1965; Gill & Comeau, 1977). However, such experiments are not quantitative and their results can be ambiguous, especially because of transcapsidation which occurs in mixed infections with some isolates of BYDV (Rochow, 1982). Extensive transcapsidation could interfere with the use of vector specificity as a means of...
mixed infections because vector specificity appears to be
distinguishing between the viruses able to propagate in
mixed infections as vector specificity appears to be
based on capsid properties (Rochow, 1977).

Specific serological and cDNA probes are now
available that can distinguish BYDV antigens and
RNAs in mixed infections. Here we describe the use of
such probes to quantify cross-protection among various
combinations of BYDV isolates in terms of the inhibi-
tion of establishment and multiplication of challenge
virus. Abstracts of some of the information have been

Methods

Viruses and inoculations. Isolates NY-SGV, NY-RPV and NY-RMV
were obtained from the collection at Cornell University, Ithaca, New
York, U.S.A. (Rochow, 1970); P-PAV was a Purdue PAV serotype
isolate from wheat in Indiana (Hammond et al., 1983). A Purdue
subculture of NY-MAV (MAV-PS1) was also used; this is distinguish-
able from NY-MAV by its reactions with monoclonal antibodies
(Lister & Sward, 1988; Lei & Lister, 1988). Each isolate was propagated
in oat seedlings, Avena sativa L. cv. Clintland 64, and grown in a growth
chamber inoculated by mass infestation with appropriate viruliferous
aphids. Apterous aphids from cultures established on these plants for 1
to 2 weeks were used for virus inoculation.

Clintland 64 plants were also used for the cross-protection
experiments. Uniform 5- to 7-day-old seedlings (early one-leaf stage),
grown singly in soil in 8 cm diameter polystyrene cups, were doubly
inoculated with paired viruses, simultaneously or at intervals, by
infesting each plant with five viruliferous aphids for each virus and
allowing a 24 h inoculation access feeding period.Positive controls for
each experiment were similar groups of plants inoculated singly with
either protecting or challenge viruses at the time corresponding to that
at which the doubly inoculated plants were inoculated with the same
virus. Healthy plants which had been mock-inoculated with non-
viruliferous aphids were also used as negative controls. Aphids were
killed after inoculation access feeding, either mechanically (for
treatments involving short inoculation intervals) or with 0.4%
Malathion. The plants were then randomly arranged and maintained in
a growth chamber at 20 _+ 1 °C with a 14 h photoperiod (200 ~E/m²/s).
Between 10 and 14 doubly inoculated plants and between five and 12
plants from each of the control sets were taken randomly in each
sampling at various times after the second (challenge) inoculations.
Samples were individually tested by ELISA and in some cases by
cDNA dot blot hybridization tests.

Sample processing. Harvested plants were washed with tap water and
divided into roots and all above-ground parts (shoots). These were
ground in liquid nitrogen with a mortar and pestle and then extracted in
0.1 M-potassium phosphate buffer pH 7.0 at a dilution of 1:20 (w/v) and
ground further. The extracts were used in ELISA or, for dot blot
hybridization tests, were clarified with 1:1 (v/v) chloroform for 1 min
by shaking in microfuge tubes, followed by centrifugation for 2 min at
low speed in a microfuge. The aqueous phase was used for the tests.

ELISA procedures. Double antibody sandwich (DAS-) or indirect
ELISA procedures (Clark et al., 1986) were used to detect virus in
experiments involving distantly or closely related isolates of BYDV,
respectively. For DAS-ELISA, monoclonal antibodies made in rabbits
in this laboratory were used (Clement, 1984; Lister et al., 1983; Webby
et al., 1989). For indirect ELISA, rabbit polyclonal antibodies were
used as first antibodies (antigen trapping) and mouse monoclonal
antibodies (Diaco et al., 1986; Hsu et al., 1984) were used as second
antibodies. Goat anti-mouse IgG conjugated with alkaline phospha-
tase (Sigma) was used to detect bound monoclonal antibodies. All tests
were done in duplicate. Reactions were considered positive if ELISA values
(A405) equaled or exceeded twice the mean ELISA value for healthy
control extracts.

ELISA data processing. To compare virus levels in doubly and singly
inoculated plants, relative changes in virus levels in serial samplings
and the degree of cross-protection in different experiments, we
calculated a 'D/S ratio' and determined the virus detection frequency.
The D/S ratio for each isolate is defined as [(mean ELISA value for
doubly infected plants - mean ELISA value for healthy plants)/(mean
ELISA value for singly infected plants - mean ELISA value for healthy
plants)]. Thus, if the replication of one virus was inhibited by the
other the D/S ratio for the inhibited virus should be less than 1. If the
challenge virus was undetectable (complete cross-protection), the
D/S ratio of the challenge virus was considered to be 0. Where
necessary, t-tests were used to determine the likelihood that the
differences in virus levels between doubly and singly inoculated plants
(reflected by the D/S ratio) were due to the treatments.

Dot blot hybridization tests. Dot blot hybridization tests were
restricted to experiments with the three isolates for which cDNA
libraries were available. The cDNAs used were P26 S4 (PAV-specific),
M7+ S5 (MAV-specific) and R23 (RPV-specific) (Wen, 1990); the
procedures used were as described by Barbara et al. (1987). In
comparative tests, dot blot hybridization tests were four- to eightfold
more sensitive than ELISA in detecting purified virus (Wen, 1990), as
was also found by Barbara et al. (1987).

Recovery of infectious virus by vector aphids. The first half-expanded or
fully expanded leaves at the top of protected and unprotected plants
were detached and each detached leaf was split at the midrib into
halves. The two halves of each leaf were placed individually in test
samples or P-PAV (l, shoot samples; I, root samples) or P-PAV (l, shoot samples; I, root samples).

Fig. 1. ELISA values for extracts from samples collected at intervals
from batches of oat plants inoculated at 5 days old with MAV-PS1 (l, shoot samples; l, root samples) or P-PAV (l, shoot samples; I, root samples).
Table 1. Effects of the interval between MAV-PS1 (protecting) and NY-MAV (challenge) virus inoculation on the persistence of cross-protection

<table>
<thead>
<tr>
<th>Inoculation interval (days)</th>
<th>NY-MAV detection*</th>
<th>Persistence of cross-protection after</th>
<th>10 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoots</td>
<td>Roots</td>
<td>Shoots</td>
</tr>
<tr>
<td></td>
<td>D/S ratio</td>
<td>0-9</td>
<td>0.8</td>
<td>0-9</td>
</tr>
<tr>
<td></td>
<td>Detection frequency</td>
<td>1-0</td>
<td>1-0</td>
<td>1-0</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0-1</td>
<td>0-0</td>
<td>0-6‡</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0-0</td>
<td>0-0</td>
<td>0-5‡</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0-0</td>
<td>0-0</td>
<td>0-2‡</td>
</tr>
</tbody>
</table>

* Samples were tested by indirect ELISA. Only the analytical results for NY-MAV detection are shown; D/S ratios and detection frequencies for MAV-PS1 were close to 1.0.
† Only the ELISA values for samples that also showed NY-MAV infection were used in calculating D/S ratios.
‡ The mean ELISA value of NY-MAV in doubly infected plants differed significantly from that in singly inoculated plants at P < 0.05.

Results

Cross-protection between MAV-PS1 and NY-MAV

Based on viral antigen production as detected by ELISA, a high degree of cross-protection occurred between MAV-PS1 and NY-MAV (Table 1). Time course experiments indicated, however, that the cross-protection was overcome in some of the protected plants, as indicated by the increased number of plants showing detectable amounts of challenge virus accumulation and by the increased amounts of challenge virus in these plants (Table 1). The degree of cross-protection increased as the inoculation interval between protecting and challenge isolates increased. Complete cross-protection persisted for at least 30 days when the inoculation interval was increased to 7 or 9 days (data not shown), a period during which virus accumulated to high levels in plants singly infected with MAV-PS1 (Fig. 1).

Cross-protection between P-PAV and MAV-PS1

Tables 2 and 3 summarize ELISA results for time course studies of reciprocal cross-protection experiments with P-PAV and MAV-PS1. Cross-protection was complete in all the protected plants within 5 days after the challenge inoculation when the inoculation interval was between 3 and 5 days or more. Although cross-protection was progressively overcome in a proportion of plants by 15 days post-challenge inoculation, longer inoculation intervals clearly tended to result in longer persistence of cross-protection. For example, 30 days after P-PAV challenge inoculation (Table 2), the challenge virus was detected in 100% of plants when the inoculation interval was 3 days or less, but when the inoculation interval was 5, 7 or 15 days, the challenge virus could be detected in only 60%, 40% and 10% of the plants, respectively.

Cross-protection was not maintained in some of the protected plants; in these plants, detectable amounts of challenge virus gradually developed within 15 to 30 days

Table 2. Effects of the interval between MAV-PS1 (protecting) and P-PAV (challenge) virus inoculation on the persistence of cross-protection

<table>
<thead>
<tr>
<th>Inoculation interval†</th>
<th>Virus tested</th>
<th>5 days*</th>
<th>15 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>0</td>
<td>MAV-PS1</td>
<td>1-0 (0-8‡)</td>
<td>1-0 (0-7)</td>
<td>1-0 (0-5‡)</td>
</tr>
<tr>
<td></td>
<td>P-PAV</td>
<td>1-0 (1-2)</td>
<td>1-0 (0-8)</td>
<td>1-0 (0-9)</td>
</tr>
<tr>
<td>2</td>
<td>MAV-PS1</td>
<td>1-0 (0-9)</td>
<td>1-0 (0-7‡)</td>
<td>1-0 (0-6‡)</td>
</tr>
<tr>
<td></td>
<td>MAV-PS1</td>
<td>0-2 (0-4)</td>
<td>0-7 (0-4)</td>
<td>0-8 (0-8‡)</td>
</tr>
<tr>
<td>3</td>
<td>MAV-PS1</td>
<td>1-0 (0-9)</td>
<td>1-0 (1-0)</td>
<td>1-0 (0-7‡)</td>
</tr>
<tr>
<td></td>
<td>P-PAV</td>
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<td>0-1 (0-1)</td>
<td>0-8 (0-6‡)</td>
</tr>
<tr>
<td>5</td>
<td>MAV-PS1</td>
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<td>1-0 (0-7)</td>
<td>1-0 (0-8‡)</td>
</tr>
<tr>
<td></td>
<td>P-PAV</td>
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<td>0-0 (0-0)</td>
<td>0-1 (0-2)</td>
</tr>
<tr>
<td>7</td>
<td>MAV-PS1</td>
<td>1-0 (1-2)</td>
<td>1-0 (0-8)</td>
<td>1-0 (0-6‡)</td>
</tr>
<tr>
<td></td>
<td>P-PAV</td>
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<td>0-0 (0-0)</td>
<td>0-3 (0-2‡)</td>
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<tr>
<td>15</td>
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<td>1-0 (1-1)</td>
<td>1-0 (1-0)</td>
</tr>
<tr>
<td></td>
<td>P-PAV</td>
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<td>0-0 (0-0)</td>
<td>0-1 (0-6)</td>
</tr>
<tr>
<td>25</td>
<td>MAV-PS1</td>
<td>1-0 (1-0)</td>
<td>1-0 (1-0)</td>
<td>1-0 (1-0)</td>
</tr>
<tr>
<td></td>
<td>P-PAV</td>
<td>0-0 (0-0)</td>
<td>0-0 (0-0)</td>
<td>0-0 (0-0)</td>
</tr>
</tbody>
</table>

* Days after challenge inoculation. Samples were tested by indirect ELISA.
† Interval (days) between protecting (MAV-PS1) and challenge (P-PAV) inoculations.
‡ Mean ELISA values of the virus from doubly and singly infected plants, which were used in calculating the D/S ratio (in parentheses), were significantly different at P < 0.05.
Table 3. Effects of the interval between P-PAV (protecting) and MAV-PS1 (challenge) virus inoculation on the persistence of cross-protection

<table>
<thead>
<tr>
<th>Inoculation interval</th>
<th>Virus tested</th>
<th>Virus detection frequency (and D/S ratio) after</th>
<th>5 days*</th>
<th>15 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>1</td>
<td>P-PAV</td>
<td>1.0 (1.0)</td>
<td>1.0 (0.9)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>MAV-PS1</td>
<td>0.4 (0.3)</td>
<td>0.1 (0.4)</td>
<td>0.7 (0.6)</td>
<td>0.6 (0.4)</td>
</tr>
<tr>
<td>2</td>
<td>P-PAV</td>
<td>1.0 (0.9)</td>
<td>1.0 (1.0)</td>
<td>1.0 (0.9)</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>MAV-PS1</td>
<td>0.0 (0.0)</td>
<td>0.1 (0.0)</td>
<td>0.5 (0.3)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>3</td>
<td>P-PAV</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>MAV-PS1</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.5 (0.3)</td>
<td>0.5 (0.2)</td>
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<tr>
<td>7</td>
<td>P-PAV</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
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<tr>
<td></td>
<td>MAV-PS1</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.4 (0.2)</td>
<td>0.4 (0.1)</td>
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<tr>
<td>15</td>
<td>P-PAV</td>
<td>1.0 (0.9)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>MAV-PS1</td>
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<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>

* Days after challenge inoculation. Samples were tested by indirect ELISA.
† Interval (days) between protecting (P-PAV) and challenge (MAV-PS1) virus inoculations.
‡ Mean ELISA values of the virus from doubly and singly infected plants, which were used in calculating the D/S ratio (in parentheses), were significantly different at P ≤ 0.05.

Fig. 2. Detection of P-PAV viral antigen and RNA by indirect ELISA (lanes 1, 4, 7 and 10) and cDNA dot blot hybridization (P26-S4 probe) (lanes 2, 3, 5, 6, 8, 9, 11 and 12), respectively, in extracts of shoot (lanes 1, 2, 3, 7, 8 and 9) and root (lanes 4, 5, 6, 10, 11 and 12) samples collected 15 (lanes 1 to 6) and 30 (lanes 7 to 12) days after challenge inoculation, from oat plants inoculated first with MAV-PS1 followed 15 days later with P-PAV (D plants). Extracts of plants singly inoculated with either MAV-PS1 (MAV) or P-PAV (PAV) and non-infected plants (H) were included as positive and negative controls, respectively. Two dilutions [1/20 (lanes 2, 5, 8 and 11) and 1/40 (lanes 3, 6, 9 and 12) in 0.1 M-phosphate buffer pH 7.0] were tested for each sample in cDNA dot blot hybridizations. A 1/20 dilution only was used in ELISA.

Fig. 3. Detection of MAV-PS1 viral antigen and RNA by indirect ELISA (lanes 1, 4, 7 and 10) and cDNA dot blot hybridization (M7+S5 probe) (lanes 2, 3, 5, 6, 8, 9, 11 and 12), respectively, in extracts of shoot (lanes 1, 2, 3, 7, 8 and 9) and root (lanes 4, 5, 6, 10, 11 and 12) samples collected 15 (lanes 1 to 6) and 30 (lanes 7 to 12) days after challenge inoculation, from oat plants inoculated first with P-PAV followed 15 days later with MAV-PS1 (D plants). For other details, see the legend of Fig. 2.

infected plants when MAV-PS1 was the protecting virus and P-PAV the challenge virus (Table 2). In plants containing detectable amounts of both protecting and challenge viruses, the concentrations of both were usually less than in singly infected-plants; this effect was more marked in roots than in shoots. However, more P-PAV than MAV-PS1 eventually accumulated in most of these plants. The latter effect is clearest in the experiment with a zero inoculation interval (i.e. simultaneous inoculation; Table 2) and was also detected in the experiments with P-PAV as the protecting virus (Table
Table 4. Comparison of aphid transmission, indirect ELISA and cDNA dot blot hybridization in detecting viruses in plants inoculated reciprocally with MAV-PS1 and P-PAV at an inoculation interval of 15 days

<table>
<thead>
<tr>
<th>Inocula†</th>
<th>MAV-PS1</th>
<th>P-PAV</th>
<th>MAV-PS1</th>
<th>P-PAV</th>
<th>MAV-PS1</th>
<th>P-PAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphid transmission‡</td>
<td>8/8</td>
<td>0/8</td>
<td>10/10</td>
<td>1/10</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>ELISA</td>
<td>8/8</td>
<td>0/8</td>
<td>10/10</td>
<td>1/10</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Dot blot</td>
<td>8/8</td>
<td>0/8</td>
<td>10/10</td>
<td>2/10</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>P-PAV/MAV-PS1</td>
<td>0/9</td>
<td>9/9</td>
<td>0/10</td>
<td>10/10</td>
<td>1/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Aphid transmission‡</td>
<td>0/9</td>
<td>9/9</td>
<td>0/10</td>
<td>10/10</td>
<td>2/10</td>
<td>10/10</td>
</tr>
<tr>
<td>ELISA</td>
<td>0/9</td>
<td>9/9</td>
<td>0/10</td>
<td>10/10</td>
<td>2/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Dot blot</td>
<td>0/9</td>
<td>9/9</td>
<td>0/10</td>
<td>10/10</td>
<td>2/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* Number infections detected/number of plants tested.
† Protecting virus/challenge virus.
‡ MAV-PS1 was transmitted by *S. avenae* and P-PAV was transmitted by *R. padi*. P-PAV and MAV-PS1 were recovered from all of the plants singly inoculated with either isolate by their corresponding vector aphids.

3), in which multiplication of P-PAV was rarely affected by the multiplication of MAV-PS1.

Reciprocal cross-protection between these isolates was also demonstrated by dot blot hybridization assay in two other sets of experiments with inoculation intervals of 7 and 15 days, respectively. Hybridization results from these experiments were similar, so only the results of the second experiment (15 day inoculation interval) are shown (Fig. 2 and 3). For shoot samples, the results were generally consistent with the corresponding ELISA results; challenge virus RNA was not detectable in most samples which showed negative reactions in ELISA. However, a few root samples for which ELISA detected no viral antigen production gave positive reactions in the dot blot hybridization assay (e.g. D1 and D6 in Fig. 2). Such discrepancies also occurred in other experiments which compared the results of ELISA and dot blot hybridizations and sometimes hybridization signals for root samples were greater than those for shoot samples which showed similar ELISA values (Fattouh, 1988; Wen, 1990). Taken together, these discrepancies may indicate that significant challenge virus nucleic acid accumulation can precede virion production, especially in roots.

Cross-protection between P-PAV and MAV-PS1 was also confirmed in the experiments on virus recovery by vector aphids. Challenge virus was recovered by vector aphids only from test plants in which the viral RNAs and antigens were also detectable, though not from all. No infectious virus was recovered from two test plants in which low levels of challenge viral antigen and/or RNA could be detected by ELISA and dot blot hybridization, respectively (Table 4).

Cross-protection between NY-SGV and either MAV-PS1 or P-PAV

Some degree of cross-protection was detected between NY-SGV and MAV-PS1 when NY-SGV was used as the protecting virus (Table 5). Prior infection of plants with NY-SGV significantly delayed and reduced the accumulation of MAV-PS1. The results also indicated mutual inhibition between NY-SGV and MAV-PS1, because less NY-SGV accumulated in the doubly infected plants than in the singly infected ones. A similar trend of interference was also found in cross-protection experiments with NY-SGV and P-PAV (Table 5).

Cross-protection was greater when either MAV-PS1 or P-PAV was the protecting virus and NY-SGV was the challenge virus. Relative detection frequencies as well as accumulation levels of NY-SGV were lower in doubly inoculated plants than in the plants singly inoculated with NY-SGV and indicated that cross-protection persisted in some of the protected plants for at least 15 to 30 days. A comparison of NY-SGV detection frequencies in the two experiments (Table 5) indicated that the efficiency of cross-protection given by MAV-PS1 against NY-SGV was somewhat higher than that of the cross-protection produced by P-PAV against NY-SGV. However, owing to the low transmission efficiency of NY-SGV by its vector aphids, the interpretation of these results is complicated by the difficulty in determining what proportion of the protection observed was due to inefficiency of inoculation rather than to a real cross-protection effect.

For inoculations in which NY-SGV was the protecting virus and either P-PAV or MAV-PS1 was the challenge
Table 5. Relative detection frequencies and D/S ratios of viruses in plants inoculated reciprocally with NY-SGV and MAV-PS1

<table>
<thead>
<tr>
<th>Viruses inoculated</th>
<th>Time after challenge inoculation (days)</th>
<th>5 days</th>
<th>10 days</th>
<th>15 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>NY-SGV/MAV-PS1 (15)*</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NY-SGV Detection frequency</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NY-SGV D/S ratio</td>
<td>0.6‡</td>
<td>0.9 ‡</td>
<td>0.7‡</td>
<td>0.7‡</td>
<td>0.6‡</td>
</tr>
<tr>
<td>MAV-PS1 Detection frequency</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MAV-PS1 D/S ratio</td>
<td>0.2‡</td>
<td>0.7‡</td>
<td>0.7‡</td>
<td>0.7‡</td>
<td>0.8‡</td>
</tr>
<tr>
<td>MAV-PS1/NY-SGV (9)*</td>
<td>NT§</td>
<td>NT</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MAV-PS1 Detection frequency</td>
<td>NT</td>
<td>NT</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>NY-SGV Detection frequency</td>
<td>NT</td>
<td>NT</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>NY-SGV D/S ratio</td>
<td>NT</td>
<td>NT</td>
<td>0.4‡</td>
<td>0.5‡</td>
<td>0.4‡</td>
</tr>
<tr>
<td>NY-SGV Detection frequency</td>
<td>NT</td>
<td>NT</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>NY-SGV/NY-SGV (9)*</td>
<td>NT</td>
<td>NT</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>NY-SGV Detection frequency</td>
<td>NT</td>
<td>NT</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>NY-SGV/NY-SGV (9)*</td>
<td>NT</td>
<td>NT</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Protecting virus/challenge virus. Number in parentheses is inoculation interval (days).
† The transmission efficiency of NY-SGV by its vector aphids is low. So, before MAV-PS1 or P-PAV inoculation, the plants were selected by symptoms to obtain 100% NY-SGV infection. This pre-selection was impossible in the reverse inoculation sequence.
‡ Mean ELISA values of the virus from doubly and singly infected plants are significantly different at P < 0.05.
§ NT. Not tested.

virus, the viral RNAs of P-PAV or MAV-PS1 were also assessed by dot blot hybridization. As in other experiments, the results were generally in agreement with those of ELISA. Viral RNAs were readily detected in the samples in which viral antigens were detectable, but not in most samples in which viral antigens were not detectable (Wen, 1990).

Cross-protection in other paired inoculations

No evidence of cross-protection was observed in plants inoculated with other pairs of viruses, in either order. The pairs used were NY-RMV and NY-RPV, P-PAV and NY-RPV, MAV-PS1 and NY-RPV, and NY-RMV and MAV-PS1. A 9 day inoculation interval was chosen to enhance the likelihood of cross-protection because all these viruses accumulated to relatively high levels during this time in singly infected plants (Wen, 1990). All the doubly inoculated plants showed challenge virus infection at all sampling times (5, 15 and 30 days after challenge inoculation) as detected by ELISA and, where tested, by dot blot hybridization. The virus accumulation levels of either protecting virus or challenge virus in doubly inoculated plants were mostly similar to those in the singly inoculated plants. However, in some samplings in the experiments with the NY-RMV and NY-RPV combination, virus concentrations in root and shoot samples of the doubly infected plants differed significantly and accumulation of challenge virus was sometimes reduced in the shoots but enhanced in the roots of the same plants (Table 6).

Discussion

Previous observations on cross-protection in BYDV were based only on aphid transmission and the induction of symptoms. Similarly, our experiments with MAV-PS1 and P-PAV confirmed that vector aphids were unable to transmit challenge virus from protected plants. However, the use of serological and nucleic acid probes in our studies provided the first unequivocal quantitative
Table 6. Relative virus levels (D/S ratios) of NY-RPV and NY-RMV in plants reciprocally inoculated with NY-RPV and NY-RMV*

<table>
<thead>
<tr>
<th>Reciprocal inoculation†</th>
<th>Post-challenge period (days)</th>
<th>NY-RPV</th>
<th>NY-RMV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoots</td>
<td>Roots</td>
</tr>
<tr>
<td>NY-RPV/NY-RMV</td>
<td></td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1.0</td>
</tr>
<tr>
<td>NY-RMV/NY-RPV</td>
<td></td>
<td>5</td>
<td>0.3‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.4‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Interval between protecting and challenge inoculations was 9 days. The detection frequency of each virus in doubly inoculated plants was 1-0 (100% infection).
† Protecting virus/challenge virus.
‡ Mean ELISA values for viruses from doubly and singly infected plants, used in calculating the D/S ratio, were significantly different at \( P < 0.05 \).
§ NT, Not tested.

evidence that cross-protection occurs between isolates of BYDV within group 1, but not between isolates within group 2 or between isolates in group 1 and group 2. Moreover, use of the two kinds of probes indicated that virion production and the production of viral RNA in infections were generally mutually consistent, although dot blot hybridization sometimes gave relatively stronger reactions for root samples than for corresponding shoot samples (Fig. 2), perhaps suggesting that significant asynchrony in nucleic acid and virion production can occur, especially in roots.

Cross-protection efficiency was greatly affected by the inoculation interval. For MAV-PS1 and NY-MAV, efficient cross-protection occurred when the inoculation interval was 2 days or more, but for P-PAV and MAV-PS1 cross-protection was efficient only after an inoculation interval of 3 to 5 days or more. These results suggest that no matter how closely related the protecting and challenge viruses, a minimum time interval between protecting and challenge inoculations is required to establish cross-protection. Beyond this minimum interval, the degree of cross-protection increases as the inoculation interval increases.

Although the mechanism is not known, the results suggest that for cross-protection to occur the protecting virus must be present in cells at the time of challenge inoculation. Time course studies with P-PAV and MAV-PS1 (Fig. 1) showed that both accumulated in infected plants within the time required for efficient protection to be established. However, although both viruses reached maximum concentrations within 5 to 8 days after inoculation, efficient cross-protection required an inoculation interval of 15 days or more. Other factors such as translocation of the protecting virus and the site of aphid feeding may be involved.

One current explanation of cross-protection is that the presence of protecting virus coat protein inhibits the uncoating of challenge virus RNA (Sherwood, 1987). A comparison of serological relationships (Waterhouse et al., 1988) and the degree of cross-protection we observed among different BYDV isolates indicates that, in general, the more closely related the protecting and challenge viruses, the greater the degree of cross-protection. These results are consistent with the notion that details of protein structure affecting serological relatedness can play a role in cross-protection. However, NY-RMV and NY-RPV did not cross-protect plants against each other, although both are in group 2 and are reportedly serologically related. A possible explanation of this is that viruses in group 1 are more closely related to each other than are viruses in group 2 (Webby & Lister, 1989). Alternatively, some features of protein structure important in cross-protection may not be reflected by serological relatedness.

The efficiency of cross-protection sometimes depended on the order of inoculation of the two cross-protecting viruses. For example, cross-protection was more efficient with paired viruses inoculated in the sequences P-PAV/MAV-PS1, P-PAV/NY-SGV and MAV-PS1/NY-SGV (protecting virus/challenge virus), than when these inoculation sequences were reversed. However, greater mutual inhibition between the viruses was observed with the latter inoculation sequences (i.e. lower cross-protection efficiency). How such differences in the ability of viruses to interfere with each other relate to their ability to cross-protect is not clear, but possible explanations include differences in uncoating ability and differences in virus replication and translocation efficiency.

Neither cross-protection nor mutual inhibition was observed between other pairs of viruses, such as P-PAV and NY-RPV, MAV-PS1 and NY-RPV, NY-RPV and NY-RMV, etc. Although early samplings sometimes indicated reduced challenge virus production, the reduction was not consistently indicated in later samplings, and the virus levels usually varied irregularly between shoots and roots of the plants. Possibly transcapsidation, non-specific resistance induced by the protecting virus infection, or interference with virus translocation influenced these observations.

Time course studies of cross-protection among P-PAV, MAV-PS1 and NY-MAV indicated that the challenge virus eventually developed in some protected plants. In other words, cross-protection was progressive-
ly overcome in these plants. This suggests that cross-protection requires continuous interference between protecting and challenge viruses; presumably challenge virus may become established in cells not already occupied by the protecting virus. This, and simultaneous or near simultaneous inoculations, could explain why multiple infections with viruses that would normally cross-protect against each other do occur in the field. Incomplete cross-protection was also indicated in previous studies in which the challenge virus could be recovered from some of the protected plants by their vector aphids (Aapola & Rochow, 1971; Gill & Comeau, 1977) and this was eventually the case in our experiments with MAV-PS1 and P-PAV as challenge and protecting viruses.

Mutual exclusion has been reported between isolates of the PAV and MAV serotypes when they are simultaneously inoculated into oat plants in the field (Jedlinski & Brown, 1965). No such mutual exclusion was observed in our investigation. This discrepancy may be due to different analytical methods, experimental conditions and virus isolates used.

Because cross-protection typically occurs between related viruses or strains, it has traditionally been regarded as a useful criterion of relationship. Perhaps more important from a practical standpoint is that it has also been used successfully in strategies for protecting plants against infection with severe virus strains by infecting these plants with mild strains (Oshima, 1975; Costa & Muller, 1980; Yeh & Gonsalves, 1984). Similar strategies based on transferring coat protein genes of viruses into target plants are now receiving considerable attention (Beachy et al., 1987). Our results suggest that for BYDV, viral coat protein genes from isolates within group 1 might be capable of eliciting a cross-protection-like effect in transgenic plants against other viruses in group 1. Of the three strains or serotypes in group 1, MAV might be a more promising candidate than PAV because MAV-PS1 gave slightly better cross-protection against NY-SGV. However, it seems less likely that viral coat protein genes from isolates in either group 1 or group 2 would elicit cross-protection in transgenic plants against other viruses in group 2.

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References


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