Resistance

Aphid Feeding Behavior: Relationship to Barley Yellow Dwarf Virus Resistance in *Agropyron* Species

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ABSTRACT


The infectivity of various *Agropyron* species (wheatgrasses) was examined with respect to three isolates of barley yellow dwarf virus (BYDV) by infesting them with appropriate vector aphids, followed by testing by enzyme-linked immunosorbent assay. Feeding behavior of the vectors *Rhopalosiphum padi* and *Sitobion avenae* was also electronically monitored to determine their ability to inoculate phloem. The results indicated that resistance to BYDV infection occurs in several *Agropyron* species. For most species tested, resistance seemed due to failure in virus increase, but in some species a major constraint on infection was the inability of vectors to locate phloem. Two potential approaches to breeding for reduced BYDV in wheat by crossing with *Agropyron* species may thus be: incorporating factors reducing or preventing virus production and incorporating factors reducing the ability of vectors to inoculate plants successfully.

Additional key words: BYDV-resistant germ plasm, luteoviruses, wheat improvement.

The term barley yellow dwarf virus (BYDV) includes at least five variously interrelated types of luteoviruses (9,18) that are apparently restricted to gramineous plants. They cause yellow-dwarf diseases in cereals that affect the supply of wheat, oats, and barley throughout the world (6,18). Like the other luteoviruses, they are obligatorily transmitted by aphids in a circulative manner and are restricted to phloem.

Two important vectors of BYDV are the English grain aphid, *Sitobion avenae* F., and the bird cherry oat aphid, *Rhopalosiphum padi* L. *S. avenae* transmits the MAV and PAV isolates (sensu Rochow, 17), whereas *R. padi* transmits the PAV and RPV isolates (17). Recent results in experiments with electronic monitoring of feeding behavior have provided direct evidence that penetration of phloem sieve elements is a prerequisite for BYDV transmission by these vectors (19, Lampe et al, unpublished). Infection with BYDV might therefore be avoided in cereal cultivars by resistance to phloem contact and ingestion.

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Currently, tolerance (sensu Cooper and Jones, 3) for BYDV infection occurs in wheat, *Triticum aestivum* L. em. Thell. (14, 15), but there are no clear-cut examples of immunity or of the resistance (reduced virus production) that occur in some cultivars of barley (16). If such were available in wheat, or in species that can intercross with wheat, it could have considerable significance for BYDV resistance breeding programs.

In this regard, high levels of resistance (i.e., virus could not be detected by enzyme-linked immunosorbent assay [ELISA] in plants infested by viruliferous aphids) were recently reported with respect to two BYDV isolates, PAV and RMV, in several *Agropyron* species (23). There is, however, no information concerning such resistance with other isolates of BYDV, or whether it may actually reflect the inability of vectors to inoculate plants efficiently.

The objectives of the present study were: to determine if various *Agropyron* species show high levels of resistance to virus production with a PAV-like isolate of BYDV (P-PAV, 7) or with the MAV and RPV isolates of BYDV and to electronically monitor the feeding behavior of aphids on the *Agropyron* species to determine whether they are readily able to locate and ingest from phloem tissue.

**MATERIALS AND METHODS**

**Insects and virus isolates.** Viruliferous *R. padi* carrying the P-PAV or RPV isolates and *S. avenae* carrying the MAV isolate of BYDV, respectively, were obtained from cultures maintained on infected oats, *Avena sativa* L. 'Clinton 64', in isolated chambers at 21°C with a 16-hr photoperiod. Viruliferous aphids used to infest *Agropyron* species and oats and to monitor feeding behavior were aperious, vigorous adults. The RPV and MAV isolates were subcultures maintained from cultures initially supplied by Dr. W. F. Rochow (24); the P-PAV isolate has been described (7).

**Plants.** Seeding plants of six wheat grasses, *Agropyron intermedium* (Host) Beauv., *Agropyron elongatum* (Host) Beauv. 'Jose', *Agropyron sibiricum* (Willd.) Beauv., and *Agropyron smithii* Rydb., 'Arriba', 'Barton', and 'Rosanna' were grown from seed under greenhouse conditions beneath sodium lights (930 μEinstein/m²/sec) with a 16-hr photoperiod. Seeds of the *Agropyron* species were obtained from commercial sources. Plants were grown in sterile soil mix and irrigated with water containing a soluble NPK fertilizer (15-30-15). Tests for BYDV infectibility and aphid feeding behavior were conducted with seedling plants in the third-leaf stage (14–18 days after emergence).

**Tests for infectibility with BYDV isolates.** Plants were tested for infectibility with the BYDV isolates by infestation with 10 viruliferous, adult aphids (vide supra) per plant. Ten plants were used for each *Agropyron* species tested. Infested plants were maintained in isolation at 21°C (16-hr photoperiod) for 1 wk. Aphid survival was recorded and aphids were then killed by spraying with malathion. The plants were then grown for an additional 14 days under greenhouse conditions. Noninfested control plants and infested Clinton 64 oats were treated similarly.

**Tissue samples for ELISA.** Tissue samples for ELISA consisted of each entire plant (including roots), washed clean and dried by blotting.

**Extraction and ELISA.** Plant samples were first pulverized in liquid nitrogen in a mortar. Potassium phosphate buffer (0.1 M, pH 7) containing 0.05% Tween-20 and 2% polyvinyl pyrrolidone (MW 40,000) was then added (1.6, w/v) along with a pinch of Carborundum, and the tissue ground further. The resulting extract was filtered through cheesecloth and stored for 1–2 wk at −76°C until assayed for virus. Virus in extracts was assayed by ELISA as described by Hammond et al (7) with the modifications of Skaria et al (24).

**Monitoring aphid feeding behavior.** Aphid feeding behavior was electronically monitored using a system similar to that described by McLean and Kinsey (10,11). In our system (19), a 12-μm gold wire attached to the dorsal side of the aphid with silver conducting paint serves as one electrode. After a 1-h starvaton period, the aphid is given access to the leaf of a plant and a 20 mV, 330 Hz signal is transmitted via the electrode attached to the aphid to a

**RESULTS**

**ELISA test results.** Aphids settled well on all the seedlings tested. Surviving apterae and newly deposited nymphs were noted on all of them 7 days after infestation. ELISA test results for the infested plants are summarized in Table I. They indicated that in general, few infections had occurred among the *Agropyron* species tested. Exceptions to this were the high number of infections detected in *A. sibiricum* plants inoculated with the P-PAV isolate, and in *A. intermedium 'Jose'* inoculated with the RPV isolate. Among the *Agropyron* species inoculated with the MAV isolate, the only infections detected were in *A. elongatum 'Jose'* and *A. sibiricum* (one infection each). No infections with any of the isolates were detected in *A. intermedium* or in *A. smithii 'Barton'* and 'Rosanna'. Contrasting susceptibilities occurred to the different isolates. These were especially noticeable with *A. elongatum 'Jose'* and *A. sibiricum*.

**Waveforms recorded during feeding.** Typical waveforms recorded during feeding on Clinton 64 4 B. *S. avenae* and *R. padi* are shown in Figure 1. These waveforms resemble those recorded for other species of aphids and host plants in that salivation (S), sieving element penetration (X), and ingestion (I) waveforms can be distinguished. In our system, the X-waveform corresponds to penetration of a sieve element generally is a sequence of M-shaped waves, although the shape and number of waves varies somewhat with the aphid species. The sequence of waveforms generated during probing and ingestion by *S. avenae* and *R. padi* was the same, except for the X-waveform generated upon penetration of a
TABLE 1. Enzyme-linked immunosorbent assay (ELISA) test results for seedlings of *Agropyron* species and Clingland 64 oats infested with aphids carrying the P-PAV, RPV, or MAV isolates of barley yellow dwarf virus

<table>
<thead>
<tr>
<th>Host</th>
<th>P-PAV isolate</th>
<th>RPV isolate</th>
<th>MAV isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>Mean (Range)</td>
<td>No. positive</td>
</tr>
<tr>
<td><em>A. intermedium</em></td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>A. sibiricum</em></td>
<td>9</td>
<td>0.27 (0.10-0.72)</td>
<td>4</td>
</tr>
<tr>
<td><em>A. elongatum</em></td>
<td>2</td>
<td>0.16 (0.11-0.20)</td>
<td>10</td>
</tr>
<tr>
<td>cv. Jose</td>
<td>1</td>
<td>0.10</td>
<td>2</td>
</tr>
<tr>
<td>cv. Arriba</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>cv. Barton</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>cv. Rosanna</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>Avena sativa</em></td>
<td>5</td>
<td>0.90 (0.36-1.51)</td>
<td>4</td>
</tr>
</tbody>
</table>

*ELISA values exceeding twice those for healthy control values were rated as positive. Control values ranged from 0.03 to 0.04 for *Agropyron* spp. and 0.01 to 0.02 for Clingland 64. All tests were of 10 seedlings, except with Clingland 64, for which five, four, and six seedlings were tested with the PAV, RPV, and MAV isolates, respectively. All seedlings had surviving apterous and most had nymphs when inspected 7 days after infestation.

TABLE 2. Means of various electronically recorded events in feeding behavior of *Sitobion avenae* and *Rhopalosiphum padi* on different *Agropyron* species or Clingland 64 oats during 180 min of feeding

<table>
<thead>
<tr>
<th>Host</th>
<th>Probes (no.)</th>
<th>Salivation (min)</th>
<th>Phloem ingestion (min)</th>
<th>Nonphloem ingestion (min)</th>
<th>Non-probing (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. avenae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. intermedium</em></td>
<td>4 bc</td>
<td>63 c</td>
<td>91 a</td>
<td>10 a</td>
<td>19 bc</td>
</tr>
<tr>
<td><em>A. sibiricum</em></td>
<td>3 c</td>
<td>65 c</td>
<td>94 a</td>
<td>10 a</td>
<td>11 c</td>
</tr>
<tr>
<td><em>A. elongatum</em></td>
<td>(cv. Jose)</td>
<td>10 ab</td>
<td>126 a</td>
<td>8 cd</td>
<td>22 a</td>
</tr>
<tr>
<td><em>A. smithii</em></td>
<td>(cv. Arriba)</td>
<td>11 a</td>
<td>93 abc</td>
<td>2 d</td>
<td>17 a</td>
</tr>
<tr>
<td>(cv. Barton)</td>
<td>12 a</td>
<td>78 bc</td>
<td>6 d</td>
<td>24 a</td>
<td>75 a</td>
</tr>
<tr>
<td>(cv. Rosanna)</td>
<td>6 abc</td>
<td>76 bc</td>
<td>44 bc</td>
<td>29 a</td>
<td>28 bc</td>
</tr>
<tr>
<td>Clingland 64 oats</td>
<td>8 abc</td>
<td>109 ab</td>
<td>51 ab</td>
<td>6 a</td>
<td>15 e</td>
</tr>
<tr>
<td><em>R. padi</em></td>
<td>6 a</td>
<td>58 a</td>
<td>41 b</td>
<td>49 ab</td>
<td>32 b</td>
</tr>
<tr>
<td><em>A. sibiricum</em></td>
<td>6 a</td>
<td>73 a</td>
<td>24 b</td>
<td>69 a</td>
<td>14 a</td>
</tr>
<tr>
<td><em>A. elongatum</em></td>
<td>(cv. Jose)</td>
<td>6 a</td>
<td>72 a</td>
<td>67 ab</td>
<td>19 ab</td>
</tr>
<tr>
<td><em>A. smithii</em></td>
<td>(cv. Arriba)</td>
<td>8 a</td>
<td>61 a</td>
<td>0 c</td>
<td>30 ab</td>
</tr>
<tr>
<td>(cv. Barton)</td>
<td>7 a</td>
<td>79 a</td>
<td>48 ab</td>
<td>14 ab</td>
<td>39 ab</td>
</tr>
<tr>
<td>(cv. Rosanna)</td>
<td>4 a</td>
<td>80 a</td>
<td>60 ab</td>
<td>18 ab</td>
<td>22 b</td>
</tr>
<tr>
<td>Clingland 64 oats</td>
<td>4 a</td>
<td>55 a</td>
<td>100 a</td>
<td>7 b</td>
<td>18 b</td>
</tr>
</tbody>
</table>

*Means (N = 6) followed by different letters within columns for *S. avenae* and *R. padi*, respectively, are significantly different, *P* < 0.05, Duncan's multiple range test with transformed scores (\(\sqrt{X + 1}\)).

sieve element (Fig. 1). *S. avenae* generated two to six X-waves before commencing phloem ingestion, whereas *R. padi* generated only one. Additionally, the shape of the X-wave varied slightly with the two aphid species.

Association of waveforms with stylet tip location. Histological preparations of six aphids of both species feeding on Clingland 64 oats showed that when the X-waveform or the S-X-1 sequence were recorded, aphids were invariably in contact with phloem. By contrast, in 12 preparations of aphids of both species where only the S-waveform or the S-I sequence were recorded, styles were not in contact with phloem. It was sometimes possible to determine that stylet tips were inside a cell during the I-waveform (Fig. 2), thereby confirming that this waveform was recorded during ingestion, whereas S-waveforms were recorded during salivation.

Aphid feeding behavior. Means of various electronically recorded events in the probing/ingestion behavior of *S. avenae* and *R. padi* during 180 min of feeding on seedlings of the *Agropyron* species or Clingland 64 oats are shown in Table 2. Cultivars Arriba and Barton of *A. smithii* apparently resisted phloem contact by *S. avenae*. On both of these cultivars only two of the six aphids (33%) whose feeding behavior was monitored contacted phloem, and the subsequent ingestion was for a short time (mean of 2 min for Arriba and 6 min for Barton). With cultivar Rosanna of *A. smithii*, however, all six *S. avenae* tested contacted phloem and ingested for relatively long periods (mean of 44 min).

*A. elongatum* 'Jose' also appeared resistant to phloem contact and ingested by *S. avenae*, though not by *R. padi*. Only two of six *S. avenae* contacted phloem on this *Agropyron* species, and the subsequent ingestion of phloem sap was for a short time (mean of 8 min). *A. intermedium* and *A. sibiricum*, however, were both susceptible to phloem contact by *S. avenae*.

Within the species *A. smithii*, cultivar Arriba also seemed highly resistant to phloem contact by *R. padi* (i.e., none of the aphids tested contacted phloem). Phloem contact and ingestion was observed, however, with *A. smithii* cultivars Barton and Rosanna, as well as with the other *Agropyron* species tested (Table 2).

**DISCUSSION**

Sharma et al (23) reported that they were unable to detect the PAV and RMV isolates of BYDV by ELISA in plants of several species in the genus *Agropyron* infested with viruliferous aphids. Similarly, we have here identified several *Agropyron* species in which we could not detect P-PAV, RPV, or MAV isolates of BYDV by ELISA in a high proportion of plants infested with viruliferous aphids under conditions in which this and many other experiments 100% of Clingland 64 oat plants become infected (Table 1). Interpretation of the results requires caution, but in general they clearly indicate considerable resistance (sensu Cooper and Jones, 3), if not immunity, to BYDV among the *Agropyron* species tested.

Monitoring the feeding behavior of *S. avenae* and *R. padi* (Table 2) provided direct evidence that the resistances to the P-PAV, RPV, and MAV isolates of BYDV apparent in those *Agropyron* species where aphids easily contacted and ingested from phloem were unlikely to result from inability of aphid vectors to inoculate the plants. Resistance in these plants would seem, therefore, to occur at the level of some virus-host interaction that prevents the virus from establishing or, perhaps, from replicating effectively. An example of this is illustrated by *A. intermedium*. For this species, ELISA indicated resistance to infection with the P-PAV, RPV, and MAV isolates (Table 1), but monitoring of feeding behavior (Table 2) showed that both *R. padi* and *S. avenae* contacted phloem easily and ingested for long periods. In contrast to this, resistance to phloem contact by *R. padi* and *S. avenae* seems likely to be a major component of the apparent resistance to BYDV observed with cultivar Arriba of *A. smithii* (Table 1), for which phloem contact was drastically reduced (Table 2). Though in tests for resistance with this cultivar a few plants gave positive ELISA values (Table 1), these may have resulted from occasional successful phloem contact by a few aphids.

*A. smithii* 'Barton' and *A. elongatum* 'Jose' demonstrated...
Fig. 1. Typical waveforms recorded from (A) *Sitobion avenae* and (B) *Rhopalosiphum padi* feeding on Clingland 64 oats. Arrows indicate initiation of probes. S: salivation waveform, X: penetration of sieve element, I: continuous ingestion. The sequence S-X-Ip indicates ingestion from phloem, whereas the sequence S-Inp indicates ingestion from nonphloem tissue.

Fig. 2. Stylet tips located in a phloem cell from *Sitobion avenae* feeding on Clingland 64 oats. Aphid had generated an S-X-I waveform sequence. P: phloem cells, X: xylem vessel, ST: stylet tips, SS: salivary sheath, S: stylets within salivary sheath.
reduced phloem contact by *S. avenae*, but not by *R. padi*. Resistance to infection with the MAV isolate by *S. avenae* would therefore be expected with these cultivars, but ELISA also indicated this for the P-PAV isolate transmitted by *R. padi*. Contrast in resistance to phloem contact by *S. avenae* and *R. padi* raises the question of why one aphid species can contact phloem, while another has difficulty. Both aphid species probe these cultivars and ingest from tissues other than phloem, as they do with all of the Agropyron species tested. Thus, the resistance to phloem contact is not because aphids do not attempt to probe and feed. Perhaps it is due to differences in enzymes secreted during stylet penetration of plant tissues or in gutulatory responses that limit the ability of *S. avenae* to contact phloem, but not that of *R. padi*.

Resistance to phloem contact by *Schizaphis graminum* (Rondani) has been suggested to be associated with the chemical composition of intercellular pectins through which aphids probe (4,5,12). Resistance to location of phloem and ingestion of phloem sap by aphids could also be due to other factors such as lack of appropriate gutulatory stimuli or the presence of repulsive agents.

Our results suggest that “resistance” to the P-PAV, RPV, and MAV isolates of BYDV in the genus *Agropyron* can occur not only at the level of virus increase but also as a result of impaired vector efficiency due to failure to locate phloem. Because *Agropyron* species have been successfully crossed with wheat (21,22), there is a potential for introducing either or both of these kinds of “resistance” into wheat. *Agropyron* species have in fact been used successfully as sources of disease-resistant germ plasm for wheat improvement in the past (1,8,20,25).

**LITERATURE CITED**