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Resistance

Virus Content as an Index of Symptomatic Resistance to Barley Yellow Dwarf Virus in Cereals

Mani Skaria, Richard M. Lister, John E. Foster, and Gregory Shaner

First, second, and fourth authors, graduate research assistant, professor, and professor, respectively, Department of Botany and Plant Pathology; third author, associate professor, Department of Entomology and research entomologist USDA, ARS, Purdue University, West Lafayette, IN 47907, USA.

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ABSTRACT

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Barley yellow dwarf virus (BYDV) content in extracts from cereals grown in the greenhouse, growth chamber, or field was measured by enzyme-linked immunosorbent assay. The results showed that, for some of the virus/host combinations tested, symptomatic "resistance" to BYDV as previously determined by plant breeders was associated with reduced virus productivity in infected plants. Although this effect was cultivar-specific and virus isolate-specific, it could be a valuable adjunct in breeding for BYDV resistance and management in cereals and deserves more extensive investigation. Suggested procedures for this are outlined.

In breeding cereals for resistance to barley yellow dwarf virus (BYDV), selection is based on symptoms such as leaf discolorations, plant dwarfing, and yield reduction (13). However, these are often difficult to distinguish from other effects and to assess accurately. They may also vary with seasonal and environmental influences. Moreover, because of technical difficulties and the scale of selection programs, little attention has been given to possible differences in reactions to different BYDV isolates (14). There is a need for a more precisely defined basis for comparing the response of breeding lines to BYDV and following the genetics of resistance.

For some viruses, symptomatic resistance has been associated with reduced virus production and such correlation has been suggested for some BYDV/host combinations (6). Virus content may, therefore, provide a basis for assessing resistance to BYDV. It has also been suggested (6) that some cultivars symptomatically resistant to BYDV are relatively poor sources of virus for transmission by vectors because of reduced virus content. If so, reduced virus content is in itself an attribute worth selecting for in breeding programs.

Enzyme-linked immunosorbent assay (ELISA) now provides a convenient measure for BYDV in tissue extracts (5,7). We report here on the use of ELISA to quantify virus content over time for three different isolates of BYDV in cultivars of oats (*Avena sativa* L.), barley (*Hordeum vulgare* L. em. Boden), and wheat (*Triticum aestivum* L. em. Thell.) previously assessed by plant breeders as symptomatically "resistant" or "susceptible." We wished to see if virus content followed these characteristics.

MATERIALS AND METHODS

Virus isolates. Isolates of three of the BYDV types distinguished

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by Rochow (14) were used to inoculate test plants. One was P-PAV (5), which is a local isolate of the PAV type, ie, transmitted nonspecifically by *Rhopalosiphum padi* L. and *Sitobion* (=*Macrosiphum*) *avenae* F. The others were the authentic MAV and RPV isolates, transmitted specifically by *S. avenae* and *R. padi*, respectively. The latter isolates were kindly supplied by W. F. Rochow (Cornell University, Ithaca, NY). All isolates and aphids were maintained on oats (cultivar Clintland 64) kept at 20 ±1 C, with a 14-hr photoperiod at 250 $\mu E \cdot m^{-2} \cdot sec^{-1}$ in growth chambers in the USDA/ARS small grains insect research laboratory.

Test plants and conditions. The test plants included pairs of barley, oat, and wheat cultivars categorized as symptomatically "resistant" (R) or "susceptible" (S) in breeding programs (8–13) conducted in California (barley) and Indiana (wheat and oats). The barley cultivars were California Mariout (S) and CM 67 (R). CM 67 contains the BYDV resistance gene Yd_2 which conditions symptomatic resistance to BYDV, while the near-isogenic cultivar, California Mariout (S), does not. The oat cultivars were Clintland 64 (S) (10) and Porter (R) (9). As in wheat (1), BYDV resistance in oats appears to be controlled by multiple genes. The wheat cultivars were Abe (S) (11) and Elmo (R) (8). The presence of a substituted chromosome pair from *Agropyron elongatum* Host (Beauv.) is considered to be the source of BYDV resistance in Elmo (J. Roberts, *personal communication*). Preliminary experiments with greenhouse-grown plants were unsatisfactory because temperatures fluctuated widely and virus contents were low, probably due to high temperatures during the day. Therefore, virus accumulation was studied in greatest detail under controlled conditions in the growth chambers. Some experiments were also conducted in the field to study the applicability of these results to the field situation.

Growth chamber experiments. As described above, growth chambers were set at 20 ±1 C, with a 14-hr photoperiod. Cultivars were seeded in a moist vermiculite-soil mixture (1:1, v/v) and given a cold treatment for 2 days at 4 C before transfer to the growth chambers.

Plants 1 wk old were infected by infestation with four to five late nymphal instars or adult aphids that had been raised on plants infected with one of the virus strains. Aphids were placed gently on the seedlings with the head oriented upwards and they settled rapidly. After 1 wk, they were killed with nicotine sulphate or malathion spray. These procedures resulted in 100% infection of the infested plants. Control plants remained uninfected and were kept separate under the same conditions during the infestation period. Each sample for ELISA consisted of tissue from five or six plants harvested at random at 4- or 6-day intervals during 1 mo. Three samples were collected at each sampling time from each set of plants. Samples were washed clean, dried by blotting, and separated into roots and shoots (= entire remaining portion). To

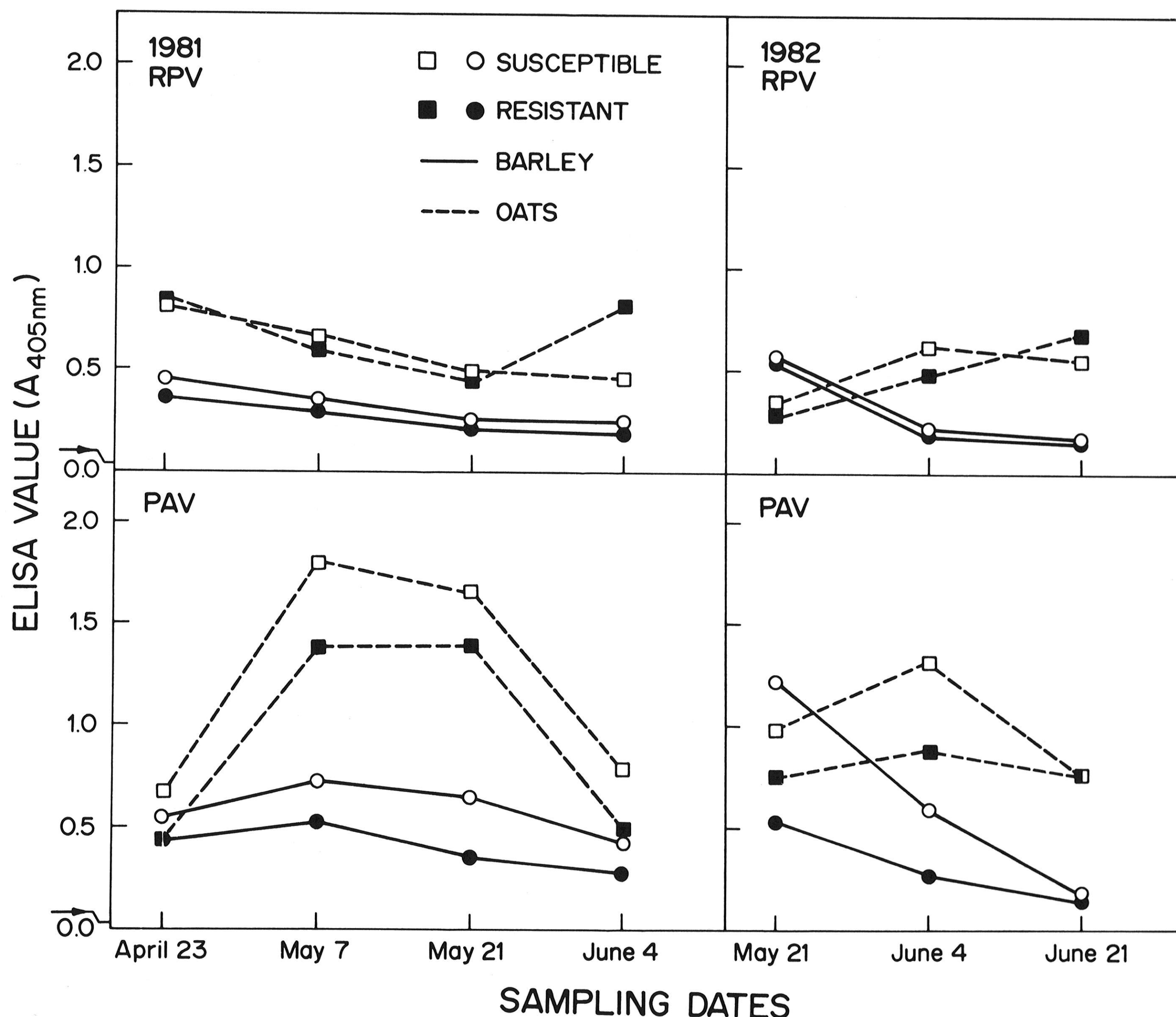

Fig. 1. ELISA values over time, for extracts from shoots of cereals grown in the field in 1981 or 1982, after inoculation with the indicated isolates of barley yellow dwarf virus. The cereal lines used are described in the text. Arrows on ordinates indicate mean values for healthy control extracts.

preserve ELISA activity, samples were stored at −20 C until extraction (usually within 2–5 wk of the last sampling date of a series). The experiments with the P-PAV and RPV isolates were repeated twice with similar results. Those described here were typical. In a separate experiment with the P-PAV isolate, the wheats were vernalized after infection, by exposing the inoculated wheat plants to 4 C for 69 days before moving them back to the growth chamber at 20 C. An additional cultivar, Caldwell (R) (12), was included in this experiment. The experiment with the MAV isolate was done once only, but Clintland 64 oats inoculated with the P-PAV isolate were included in the same growth chamber as a standard for comparison. The results for the standard corresponded closely with those in the other studies.

Field experiments. In 1981 and 1982, Clintland 64 and Porter oats, and California Mariout and CM 67 barleys, were compared after inoculation with the P-PAV or RPV isolates. Plant rearing and inoculation procedures were as described for the growth chamber experiments. After inoculation, plants were transplanted into the field (in mid-April in 1981 and late April in 1982) in a randomized complete block design with four replications of each cultivar/virus isolate combination. The experimental area was isolated by border rows of oats. Samples were of the entire aboveground parts of three plants harvested on each of several sampling dates (Fig. 1). Three samples were collected on each date except in the first and fourth harvests in 1981, when only one sample was collected for each cultivar/virus isolate combination.

Extraction and ELISA. Samples were pulverized in liquid nitrogen and then ground in a mortar with a pinch of Carborundum. Potassium phosphate buffer (0.1 M, pH 7) was added to the ground tissue (1:2, w/v) with further grinding. The resulting extract was diluted further (1:4, w/v) with phosphate-buffered saline (PBS) containing 0.05% Tween-20 and 2% polyvinyl pyrrolidone (PVP, MW. 40,000 Sigma). Virus in extracted sap was measured by ELISA, as described by Hammond et al (5), with slight modifications, ie, the alkaline phosphate-conjugated antibody was diluted in healthy sap of Clintland 64 oats (extracted as above) rather than in buffer, and incubated overnight rather than 4 hr. Substrate (*p*-nitrophenyl phosphate) reactions were stopped after 30 min by adding 50 μl of 3 M NaOH per well. Tests were done in Dynatech MicroElisa plates or Gilford EIA cuvettes. Absorbances ($A_{405\ nm}$) of the reaction products ("ELISA values") were read directly in a Gilford Instrument EIA 50 or Fisher Scientific Company EIA Reader. Each sample extract was tested in duplicate wells, and the readings obtained for the shoot or root samples of each sampling date were averaged to obtain a mean value.

RESULTS

General. Irrespective of the isolate used, symptoms included various degrees of yellowing, reddening, and dwarfing. Symptoms in plants grown in the field were more pronounced than those in

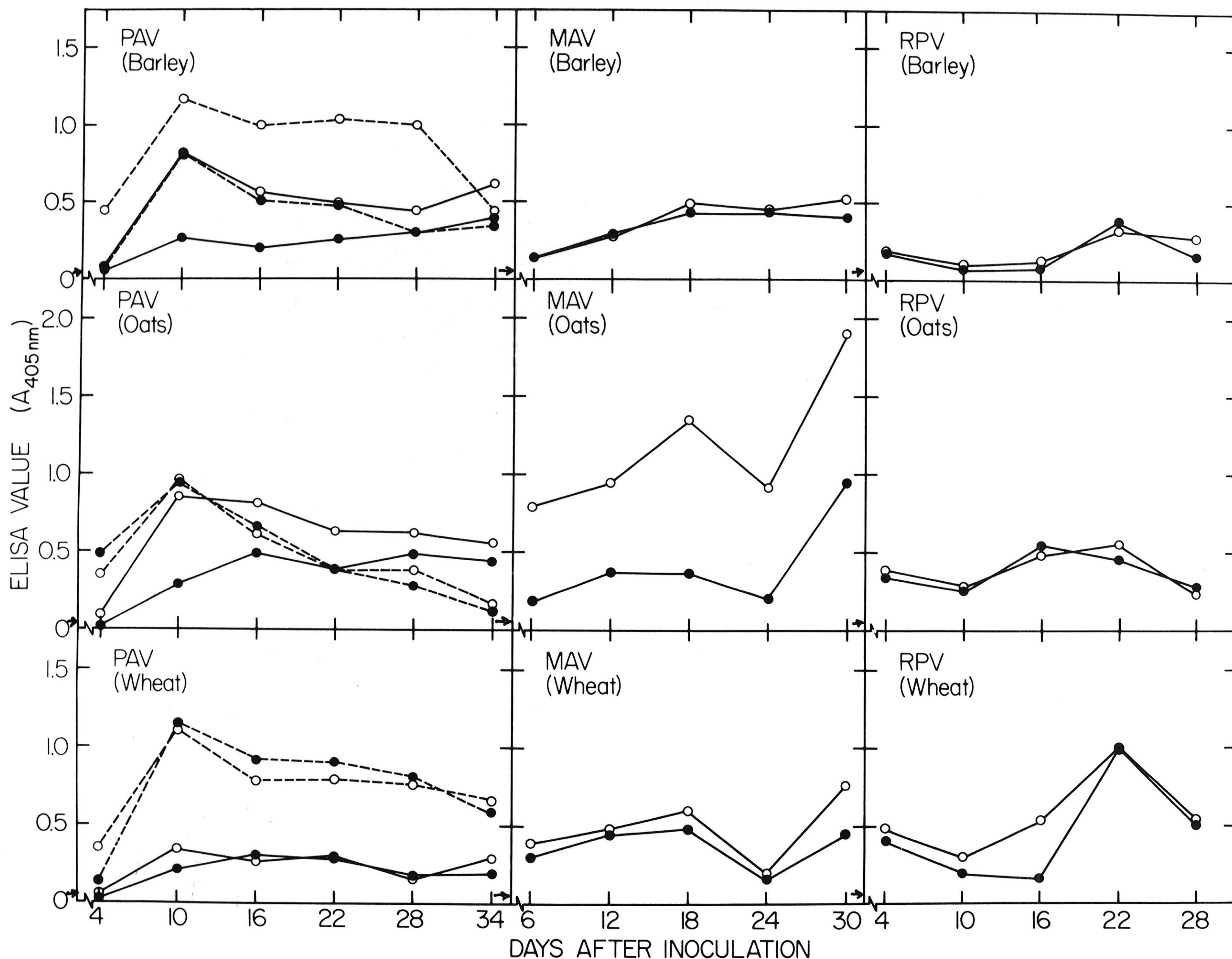

Fig. 2. ELISA values over time, for extracts from shoots (continuous lines) or roots (broken lines) of cereals grown in a growth chamber after inoculation with the indicated isolates of barley yellow dwarf virus. Values for resistant and susceptible cereal lines (described in the text) are indicated by solid circles and open circles, respectively. Arrows on ordinates indicate mean values for healthy control extracts.

plants grown in the growth chamber, but in both situations, relative symptom severity was difficult to assess. Moreover, relative symptom severities in field and growth chamber did not always coincide, and percentage weight reductions were not always consistent with symptom severity ratings (Table 1). These observations illustrate the difficulty of assessing resistances by symptom observations. Differences in virus content were as listed below and summarized in Table 2 and Fig. 2. Unless otherwise specified, results for root extracts showed trends similar to those for shoots, but they usually contained more virus.

Growth chamber experiments. *P-PAV isolate.* Shoots of the "R" barley consistently yielded significantly less virus than shoots of the "S" barley, and this was also true of root samples except for the final sample, taken 34 days after inoculation (Table 2, Fig. 2). Most root samples contained more virus per unit weight of tissue than did shoots (Fig. 2). Virus was detected in the roots, but not shoots, of the "S" barley 4 days after inoculation. Peak virus yields in both roots and shoots of both cultivars were recorded in the samples taken 10 days after inoculation.

In the oats, only the extracts from shoots showed significant differences in virus yields (Table 2, Fig. 2). Shoots of the "S" oat consistently yielded more virus than those of the "R" oat, but virus yields from roots of both cultivars were essentially similar throughout the experiment. Virus was detected in the first sample of roots collected 4 days after inoculation, but not in the corresponding shoot samples.

For the wheats, the yields of virus obtained from shoots of the two cultivars did not differ significantly (Table 2, Fig. 2), although virus yields from roots of the "S" wheat were slightly lower than those from the "R" wheat. As with barley and oats, roots consistently yielded more virus than shoots. A peak in virus yield was detected for roots of both wheats 10 days after inoculation, but peak virus yields from shoots were less well-defined. Readily detectable levels of virus developed in roots during the vernalization period (0′–0 in Fig. 3). Virus contents rose quickly when normal growth was resumed, remaining high in roots and falling in shoots. The rise in virus content appeared to be slower in the vernalized Caldwell (R) than in Abe (S) and Elmo (R). Though vernalization increased virus yields, it did not enhance disease symptoms.

MAV isolate. With the MAV isolate, there were essentially no differences in virus yields between the "R" and "S" barleys (Fig. 2). However, virus yields from the "R" and "S" oats differed significantly at each harvest (Table 2, Fig. 2). For all three cereals,

TABLE 1. Symptom severity[a] (percentage weight reduction)[b] in resistant (R) and susceptible (S) cereal cultivars infected with one of three isolates of barley yellow dwarf virus and grown in the growth chamber or the field

Cultivar	Growth chamber P-PAV	Growth chamber MAV	Growth chamber RPV	Field P-PAV	Field RPV
Barley					
California					
Mariout (S)	1 (13)	1 (22)	2 (25)	2	1
CM 67 (R)	1 (2)	1 (21)	1 (23)	1	2
Oats					
Clintland 64 (S)	2 (29)	2 (48)	2-3 (39)	2	3
Porter (R)	2 (17)	2 (22)	2-3 (19)	1	2
Wheat					
Abe (S)	2 (14)	2 (30)	2-3 (17)	...[c]	...
Elmo (R)	1 (13)	1 (25)	1 (21)	...	...

[a]Symptom severity rankings were estimated according to the scale: 1 = mild, 2 = moderate, and 3 = severe.
[b]Percentage weight reductions were determined only for the plants raised in the growth chamber.
[c]... = not tested.

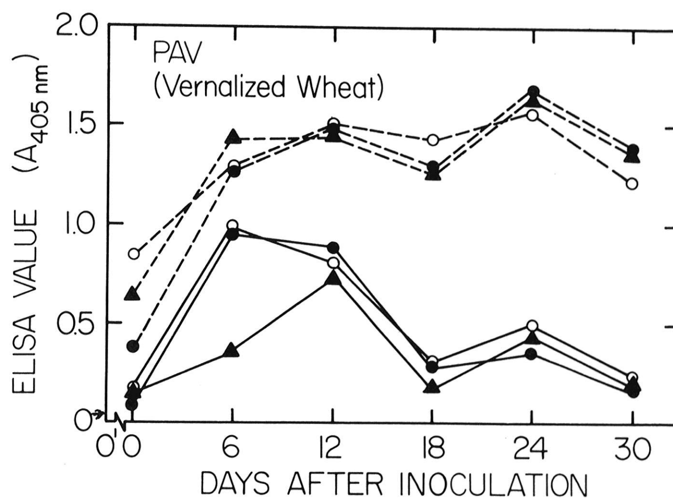

Fig. 3. ELISA values over time, for extracts from shoots (continuous lines) and roots (broken lines) of wheat lines grown in the growth chamber after inoculation with the P-PAV isolate of barley yellow dwarf virus ("0′") followed by vernalization for 69 days (to "0"). The wheats were Abe (o——o), Elmo (•——•), and Caldwell (▲——▲). Arrow on ordinate indicates mean values for healthy control extracts.

TABLE 2. Mean ELISA values[y] for extracts from resistant (R) and susceptible (S) cereals inoculated with P-PAV, MAV, or RPV isolates of barley yellow dwarf virus and grown in growth chambers or in the field

Cultivar	Growth chamber P-PAV Shoots	Growth chamber P-PAV Roots	Growth chamber MAV Shoots	Growth chamber RPV Shoots	Field 1981 Experiment P-PAV Shoots	Field 1981 Experiment RPV Shoots	Field 1982 Experiment P-PAV Shoots	Field 1982 Experiment RPV Shoots
Barley								
California								
Mariout (S)	0.501 a	0.852 a	0.382 a	0.214 a	0.589 a	0.317 a	0.671 a	0.309 a
CM 67 (R)	0.244 b	0.414 b	0.348 a	0.175 a	0.396 a	0.248 a	0.319 a	0.285 a
Oats								
Clintland 64 (S)	0.592 a	0.475 a	1.154 a	0.388 a	1.222 a	0.613 a	1.019 a	0.502 a
Porter (R)	0.357 b	0.478 a	0.420 b	0.370 a	0.929 a	0.677 a	0.797 a	0.469 a
Wheat								
Abe (S)	0.231 b	0.743 a	0.479 a	0.578 a	...[z]	...	...	...
Elmo (R)	0.196 b	0.762 a	0.339 b	0.453 a	...	...	...	...

[y]Within each column, values for cultivars of a particular crop followed by a letter in common are not significantly different, $P = 0.05$, according to Duncan's new multiple range test.
[z]... = not tested.

initial peaks in virus yields occurred later than with the P-PAV isolate. With the wheats, mean virus yields from the "S" cultivar were slightly, but significantly, higher than those from the "R" cultivar (Table 2).

RPV isolate. The "R" and "S" barleys yielded similar amounts of virus throughout the experiments. The highest virus yields for each were noted at 22 days after inoculation (Fig. 2). Similarly, the "R" and "S" oats showed little difference in virus yields, and peaks were noted at 16 and 22 days after inoculation in the "R" and "S" cultivars, respectively. With the wheats, shoot samples from each of the first three harvests from the "R" cultivar yielded significantly less virus than those from the "S" cultivar, but in later harvests both wheats yielded similar amounts of virus, with well-defined peaks at 22 days after inoculation (Fig. 2), and overall mean virus yields were not significantly different (Table 2).

Field experiments. Differences in virus yields followed the same trends as those for plants in the growth chamber experiments. Clintland 64 oat and California Mariout barley shoots infected with the P-PAV isolate consistently yielded more virus than Porter oat or CM 67 barley shoots, respectively (Fig. 1). However, because of variability, these differences were not statistically significant (Table 2). Neither pair showed such differences with respect to the RPV isolate.

DISCUSSION

In an earlier study of the correlation of BYDV content and symptom severity (6), virus content was assessed by sucrose density gradient analysis of preparations purified from greenhouse-grown oats harvested 5 wk after inoculation. Less virus was extracted from shoots of each of three "tolerant" (ie, symptomatically resistant) lines of oats than from plants of each of three symptomatically susceptible lines. This result parallels our comparative estimates of virus contents by ELISA for oats inoculated with the P-PAV isolate.

However, our use of ELISA for virus assessment has shown that the relationship between BYDV content and the symptomatic category assigned in previous plant breeding work is complex and depends both on the cultivar and on the virus isolate used for inoculation. For example, although Clintland 64 (S) shoots consistently yielded more of both the P-PAV and MAV isolates than Porter (R) shoots, this was not true with the RPV isolate. Similarly, with CM 67 (R) and California Mariout (S) barleys, virus content differences were correlated with symptomatic category in infections with the P-PAV isolate, but not in infections with the MAV and RPV isolates.

In view of other evidence of closer relationships between the PAV and MAV isolates than between these and RPV (15), the lack of correlation between the results for P-PAV and MAV is especially noteworthy, and emphasizes the need for precise identification of the virus types being used in BYDV resistance breeding programs. From our results it seems likely that, in such programs, resistance has been sought to locally prevalent types of BYDV, and that these have commonly included isolates of the PAV type. In this regard, recent surveys of cereal crops in the United States and elsewhere (2,4) suggest that PAV-like isolates predominate in several important cereal-growing areas, including the Midwest and California where the cereals used in this work were assessed for BYDV resistance.

With the wheats examined under growth chamber conditions, the evidence for association of virus content with symptomatic resistance was strongest with the MAV isolate. However, associations between symptomatic category and virus content in wheat were not as obvious as those found in barley and oats.

Field experiments gave results that showed trends consistent with those obtained under controlled conditions. Thus, with respect to infection with P-PAV (but not with RPV), Clintland 64 (S) oat yielded more virus than Porter (R) oat, and California Mariout (S) barley yielded more virus than CM 67 (R) barley, as in the growth chamber experiments. This consistency strongly suggests that it is feasible to predict field behavior in relation to virus content from the results of short-term growth chamber experiments.

Our studies have identified clear associations between virus content and symptomatic "resistance" in the barleys and oats chosen for study. Such associations were less obvious in the wheats. The basis of BYDV symptom causation is not understood, but these results suggest that correlative factors governing symptoms and virus content exist in cereals. Further search for such factors is worthwhile, because their inheritance will be easier to follow than that of factors governing symptoms alone. Also, breeding for reduced virus content in any of the cereals might expose additive effects that could be accumulated so as to approach immunity. Moreover, if plants with low virus contents are poor sources of virus for vectors, use of cereal selections with reduced virus productivity could also lead to reduced virus spread.

The results indicate that a search for cereal lines in which BYDV content is reduced could be conducted by short-term experiments in controlled conditions, and suggest several points to consider in using this approach. For example, virus content can differ as between shoots and roots. Other work suggests that it differs in different above-ground parts (3,7), but we circumvented this variable by our sampling procedure. Virus yields also fluctuated over time. In the growth chamber experiments, initial peaks in yield occurred at about 12, 18, or 22 days after inoculation with the PAV, MAV, or RPV isolates, respectively. In other work in our laboratory (5), yields of the P-PAV isolate varied with plant age at inoculation and with temperature conditions. Critical comparisons of virus yields from different cereal lines will require careful environmental and cultural control, but could be an important adjunct to current procedures for BYDV resistance breeding.

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