Lack of Barley Yellow Dwarf Virus Dosage Effects on Virus Content in Cereals

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ABSTRACT

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No effects of inoculum dosage on symptoms or on virus content (as assessed by enzyme-linked immunosorbent assay) were observed in experiments with cultivars of wheat, oats, and barley inoculated with two PAV isolates of barley yellow dwarf virus (ie, nonspecifically transmitted by *Rhopalosiphum padi* and *Sitobion avenae*). Typically, virus contents peaked about 12 days after inoculation before declining but were similar at each sampling time during 1 mo after inoculation whether the plants had been infested with two or 10 aphids. Similarly, no significant differences were noted in symptoms or tissue weight between plants infested with two or 10 aphids.

The term "barley yellow dwarf virus" (BYDV) encompasses a group of variously related aphidborne, phloem-restricted luteoviruses of cereals (15). They are not sap-transmissible and occur in very low amounts in their hosts, hence virus assay was difficult and imprecise until the applicability of enzyme-linked immunosorbent assay (ELISA) was established (9). Because of such assay restrictions, assessment of cultivar resistance or susceptibility to BYDV, as in breeding work, has usually been based on symptomatic effects alone (12).

In some investigations (1,2,20), the use of increasing numbers of aphids for inoculation has been correlated with increasing symptom severity and yield reduction. Though the relative amounts of virus inoculum involved are unknown, this is reasonably regarded as an inoculum dosage effect, ie, increased inoculum leads to increased symptom severity. Such effects could have important implications in interpreting the reactions of plants in breeding programs (2). By contrast, in other work, no similar effects of dosage were observed (7,23,24). We have now investigated dosage effects in relation to their possible impact in more extensive comparative studies of the influence of BYDV on symptomatic reaction and virus content (assessed by ELISA) in various cereal hosts (19; *unpublished*). In this paper, we report results indicating that with inoculation procedures similar to those used by other workers, there were no effects of inoculum dosage on virus content or on symptoms when two or 10 aphids were used for inoculation. A preliminary report has been published (18).

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MATERIALS AND METHODS

Viruses used. Two isolates of BYDV of the PAV type (ie, transmitted nonspecifically by *Rhopalosiphum padi* L. and *Sitobion* (*Macrosiphum*) *avenae* Fabr. (13) were used to inoculate test plants in separate experiments. An isolate from wheat (P-PAV) was obtained locally (8) and the type isolate (R-PAV) was supplied by W. F. Rochow. Both isolates were maintained on Clintland 64 oats (*Avena sativa* L.) in the USDA, ARS, controlled-environment cabinets at Purdue University, set at 20 ± 1 C with a 14-hr photoperiod. Effects of the R-PAV isolate were studied in most detail.

Test plants. Test plants were paired barleys (*Hordeum vulgare* L. em Boden), wheats (*Triticum aestivum* L. em Thell), and oats, each pair consisting of a cultivar or line previously assessed by plant breeders as symptomatically resistant (R) and one previously assessed as susceptible (S). The barleys were California Mariout (S) (CI 1455) and CM 67 (R) (CI 13782). CM 67 is near-isogenic to California Mariout but contains a gene (Yd_2) imparting symptomatic resistance to BYDV (12). The wheat pair consisted of Elmo (R) (CI 17887) and Abe (S) (CI 15375). The presence of a substituted chromosome pair from *Agropyron elongatum* Host (Beauv.) is considered the source of symptomatic resistance in Elmo (J. Roberts, *personal communication*). The oat pair consisted of Porter (R) (CI 19412) and Clintland 64 (S) (CI 7639). Symptomatic resistance to BYDV in oats is probably controlled by multiple genes, as suggested for wheat (4).

Infestation and ELISA. Cultivars were seeded in flats in a 1:1 vermiculite-soil mixture. Flats were moistened, subjected to a cold treatment of 2 days at 4 C, and transferred to growth chambers at 20 ± 1 C with a 14-hr photoperiod. Plants were infested when 1 wk old with two or 10 late-instar *R. padi* of uniform size from cultures raised for virus acquisition on infected Clintland 64 oats. Aphids were transferred with a damp camel's-hair brush and placed with the head oriented upward on test plants. With such placement, aphids moved up and quickly settled on the seedlings. Infection feeding was allowed for 3 days, during which plants were inspected to make sure the aphids were still present. The aphids were then killed by spraying with insecticide. These procedures resulted in 100% infection of infested plants. Control plants were removed from the chamber during the infestation period and remained uninfected during the experiment.

Samples for testing consisted of eight individual plants harvested at each of several intervals from each treatment during 30 days. Roots were separated from shoots (= all remaining parts) and the samples were blotted with paper towels, weighed, and stored at −20 C until extracted. For extraction, individual shoot or root samples were pulverized in a mortar in liquid nitrogen, then ground with a pinch of Carborundum and 1:2 (w/v) potassium phosphate buffer (0.1 M, pH 7). The extract was reground after further dilution to 1:6 (w/v) with phosphate-buffered saline (PBS) containing 2% polyvinyl pyrrolidone (PVP, mol wt 40,000, Sigma), and 0.05% Tween 20 (5). Virus content of extracts was measured by ELISA as described previously (6,9), except the conjugated antibody was diluted in extract from healthy Clintland 64 oats rather than buffer, and incubated overnight at 4 C rather than 4 hr at 37 C. These procedures gave reduced background values. The solid-phase formats used were Gilford EIA cuvettes (Gilford Instrument Laboratories, Oberlin, OH) or Dynatech microelisa plates (Dynatech Laboratories Inc., 900 Slater Lane, Alexandria, VA). Substrate reactions were stopped by adding 50 µl of 3 M NaOH after 30 min. Absorbances ($A_{405\ nm}$) of the reaction products (ELISA values) were read directly in appropriate ELISA readers

(Gilford EIA 50 or Fisher Scientific Company EIA Reader). Dilution curves showed that over the range of concentrations used, halving virus concentration about halved the ELISA values. Data for root samples were more variable but generally confirmed the trends of data for shoots.

RESULTS

Results with the R-PAV isolate. Results with the R-PAV isolate were confirmed in three experiments done at different times. Peak levels of virus were detected in samples taken 12 days after infection. This effect was remarkably consistent. Later on, dilution effects caused by reduced virus synthesis during rapid growth probably contributed to the fall in amounts of detectable virus per unit of tissue weight. Figure 1 illustrates typical results obtained in one experiment.

Wheat. Symptoms on wheat inoculated with R-PAV first appeared 8–9 days after infestation as chlorotic areas on older leaves. Later, 11–12 days after infestation, these areas coalesced and leaves become completely yellow. Symptoms developed 1 or 2 days sooner on Abe (S) than on Elmo (R) and were more obvious and severe, regardless of the numbers of aphids used for infection. In general, average fresh weights of infected plant samples did not differ significantly from the control values (Fig. 1). ELISA values indicated that both wheat cultivars contained similar amounts of virus per unit weight of tissue over the period. These effects were independent of the numbers of aphids used for inoculation.

Oats. Symptoms in oats were first seen 9–10 days after inoculation in both cultivars as a reddish yellow coloration at the tip of the first leaf. Later, the entire leaf became reddish yellow to red and the second leaf developed longitudinal reddish yellow stripes. Symptom severity was similar in both cultivars and was independent of the numbers of aphids used. Average shoot weights of infected plants did not differ significantly in relation to the numbers of aphids used for inoculation but were consistently somewhat lower than those of healthy controls (Fig. 1). ELISA values showed no significant effects of inoculum dosage on virus content. Indeed, the data (Fig. 1) indicated a slightly lower virus content for shoot samples from plants inoculated with 10 aphids. Virus production was more rapid in Clintland 64 (S) than in Porter (R) but peaked at a similar level after 12 days. Thereafter, virus content per unit of shoot weight fell more rapidly in Porter (R) than in Clintland 64 (S).

Barley. Symptom appearance and development in barley was similar to that in wheat. Weights of inoculated California Mariout (S) samples were lower than those of healthy controls, but with both California Mariout and CM 67, sample weights and symptoms were similar regardless of whether two or 10 aphids were used for inoculation (Fig. 1). ELISA values indicated that, 24 and 30 days after infection, California Mariout plants inoculated with 10 aphids contained more virus than plants inoculated with two aphids, whereas with CM 67 (R), the situation was reversed. Overall differences were not significant, however, and virus contents were essentially independent of the numbers of aphids used.

Results with the P-PAV isolate. Comparisons involving the P-PAV isolate were made in a fourth experiment, which was less detailed than those done with R-PAV but gave similar results. Virus contents and sample weights were examined 15, 20, and 30 days after infestation, and for the oat and barley pairs only (Table 1). Symptoms and sample weights were independent of the numbers of aphids used for inoculation. Differences in virus content between

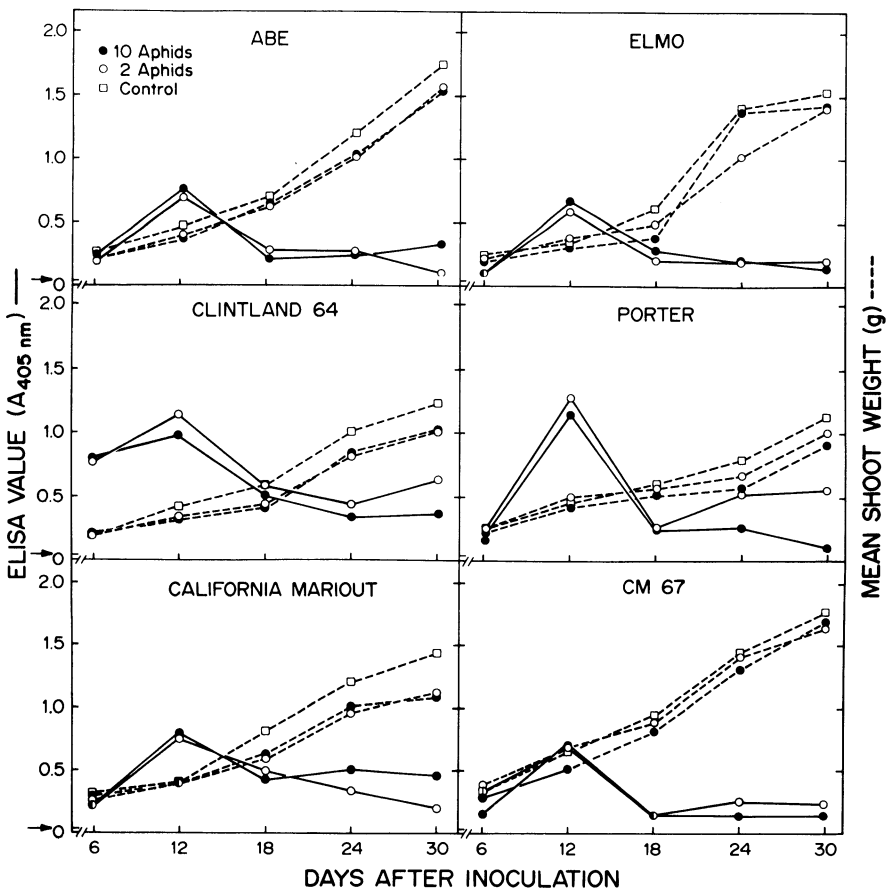

Fig. 1. Variations in mean shoot weights and enzyme-linked immunosorbent assay (ELISA) values of extracts for cereal samples collected at intervals after inoculation with two or 10 aphids with the R-PAV isolate of barley yellow dwarf virus. Arrows on the ELISA value axis indicate background values for healthy control tissue. As assessed in plant breeding experiments, Abe wheat, Clintland 64 oat, and California Mariout barley were symptomatically susceptible and Elmo wheat, Porter oat, and CM 67 barley were symptomatically resistant. Scale values are the same for both ordinates.

Table 1. Enzyme-linked immunosorbent assay (ELISA) values[a] for extracts of oats and barley shoots inoculated with P-PAV by two or 10 aphids per plant and harvested 15, 20, and 30 days after inoculation[b]

Number of aphids used	Cultivars and days after inoculation											
	California Mariout			CM 67			Clintland 64			Porter		
	15	20	30	15	20	30	15	20	30	15	20	30
2	0.877	0.478	0.561	0.326	0.271	0.296	0.783	1.015	0.864	0.533	0.635	0.833
10	0.717	0.415	0.483	0.427	0.287	0.250	0.714	0.739	0.959*	0.639	0.526	0.619

[a] Values are means for the eight individual plants harvested on each date (see text).
[b] ELISA values for extracts of samples inoculated with two or 10 aphids were compared by the t test wherever the 10-aphid value was greater. * = Differences significant (5%) only in Clintland 64 at 30 days after inoculation, with ($t = 2.48$, 14 df). The overall means showed no significant differences.

samples inoculated with two or 10 aphids were not significant, although these data also seemed anomolous in consistently indicating somewhat lower virus contents for samples of the oat pair and of California Mariout barley inoculated with 10 aphids. The data also indicated a greater difference in virus content between Clintland 64 (S) and Porter (R) than occurred with the R-PAV isolate (compare Table 1 and Fig. 1).

DISCUSSION

Previous investigations of inoculum dosage effects with BYDV concentrated on comparing symptoms and yields in plants inoculated with different numbers of aphids. As far as we know, this is the first systematic attempt to detect differences in virus content as well as symptoms. Relationships among these are not understood, but variations in symptoms indicate the likelihood of variations in virus content. Overall, however, our results indicate no significant effect of the number of aphids used for inoculation on either factor. Furthermore, although our estimates of virus content relate only to the first month of growth in controlled conditions, more extensive comparisons (19; *unpublished*) indicate that such data are predictive of the relative virus contents of infected plants to maturity in the field. Despite uncertainty regarding the amount of inoculum introduced by individual aphids, acquisition and test feeding times were long enough to ensure that each aphid was capable of infecting, thus dosage effects should have been exposed. Therefore, the major point requiring discussion is that we did not find evidence of dosage effects despite those reported in some previous investigations.

In fact, results indicating dosage effects with BYDV seem associated with inoculations with larger numbers of aphids than used here. For example, Boulton and Catherall (1) infected plants grown outdoors with groups of 5, 10, 20, or 50 aphids taken from a stock culture maintained on infected oats and allowed a 24-hr access to test plants. Their results indicated some dosage effects in the time required for heading and in the degree of stunting, but grain yield was the same regardless of the numbers of aphids used. Burnett and Gill (2) studied dosage effects in greenhouse-grown plants infested with 1, 20, or 100 aphids from colonies exposed for about 1 wk on infected plants, then allowed a 2-day access to test plants. Their results showed progressive effects on infection rates, incubation period, symptom severity, and yield components as between the use of 1 or 100 and 20 or 100 aphids for inoculation. Smith (20) found more severe symptoms in field plants infected with high numbers of aphids (150 per plant) than with low numbers of aphids (15 or 20 per plant).

In contrast, Toko and Bruehl (24) found no differences in symptom severity in plants inoculated with one, two, four, or eight viruliferous aphids. Similarly, Tetrault et al (23) found no differences in symptom severity or transmissibility in plants inoculated with 1, 3, 5, or 10 aphids. Gill (7) reported no difference in time taken for symptom appearance in plants inoculated with three or 12 aphids. Lack of progressive inoculum dosage effects on symptoms has also been reported with other luteoviruses (21). In a single unreplicated experiment, we extended the results reported here by comparing symptoms and virus content in Clintland 64 oats inoculated with P-PAV with 2, 10, and 20 aphids per plant (five plants per treatment) and found no differential effects in severity during 1 mo and no differences in virus content at the end of that time.

In insect vector studies in general, earlier suggestions that infections could result from an accumulation of sub-minimal doses of virus (22) have been discounted in the face of evidence against such a "mass-action hypothesis" (11,17). It is reasonable to assume, therefore, that this effect should not occur with BYDV; however, increase in the number of infective doses might be cumulative by hastening systemic movement (10). Thus, with a phloem-restricted virus, the efficiency of inoculation and infection of the phloem system could conceivably vary according to the number of sites inoculated. But against this, BYDV movement is rapid (3) and vascular interconnections throughout the plant should ensure uniform spread, especially when, as our detailed investigations of BYDV kinetics in infected cereals (*unpublished*) suggest, virus accumulates rapidly in roots and moves from them into shoots, as in other systems (16).

BYDV isolates can be categorised by their vector relationships and symptomatology, but within each category, a continuum of variants may occur (14). The differences reported here between accumulation of R-PAV and P-PAV in Clintland 64 and Porter oats probably reflect this. Progressive dosage effects on symptom severity may therefore arise through the increased likelihood, when very large numbers of aphids are used, that some transfers will include mutant variants occurring in the source plants, giving rise to mixed infections with additive or synergistic effects.

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