Variations in Virus Content Among Individual Leaves of Cereal Plants Infected with Barley Yellow Dwarf Virus

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ABSTRACT

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Infected cereal plants, whether symptomatically tolerant or sensitive to barley yellow dwarf virus (BYDV), showed wide variations in virus content (as assessed by enzyme-linked immunosorbent assay [ELISA]) among different leaves on the same plant, and also among leaves in the same position (i.e., of the same age) on different plants. Variations were more pronounced with Clintland 64 oats than with several barley and wheat cultivars, but in all cases were sufficient to indicate that comparisons of virus acquisition efficiency by allowing aphids unrestricted access to intact plants would be difficult. Comparisons involving acquisition feeding on individual leaves of known virus content should be more reliable. The results also illustrate the danger of relying on ELISA of individual leaves or leaf samples for estimating differences in virus productivity between cultivars, and that even qualitative ELISA diagnosis requires adequate samplings of different leaves.

Barley yellow dwarf luteoviruses (BYDV) are specifically transmitted in a persistent, circulative manner by various species of aphids (13). Tolerance (sensu Cooper and Jones, 2) to BYDV is cultivar- and virus-specific, but it can be associated with relatively reduced virus productivity in infected plants, as compared with that in sensitive cultivars (5,17). For some viruses, reduced virus titer has been correlated with reduced vector efficiency (18,19,22), and, if this were true with BYDV, a reduced virus concentration in tolerant plants might reduce virus availability to vectors, providing an important constraint on virus spread (5).

Here we describe preliminary investigations of this possibility with whole plants, which led to a detailed study, based on ELISA (enzyme-linked immunosorbent assay), of variations in virus content between individual leaves. The results are relevant to the study of effects of virus concentration on transmissibility, to sampling for diagnosis, and also to prospects for comparing BYDV productivity between hosts by sampling leaves. Comparisons of the relative efficiencies of different leaves with differing virus contents as sources of virus for acquisition and transmission by aphids are dealt with in a companion paper (12).

MATERIALS AND METHODS

Cereal cultivars. The distribution of BYDV in individual leaves and plants of selected cereal cultivars (Table 1) was studied in the oat (*Avena sativa* L.) cultivar Clintland 64 (11); in two wheat (*Triticum aestivum* L. em. Thell.) cultivars, Abe (10) and Elmo (9) and in three pairs of barley (*Hordeum vulgare* L. em Boden), Briggs (Yd2−) (15) and Prato (Yd2+) (16), Atlas 57 (Yd2−) (20) and Atlas 68 (Yd2+) (14), and California Mariout (Yd2−) (21) and CM 67 (Yd2+) (14), respectively. (The members of each barley pair are near-isogenic except that the first member of each pair, marked "Yd2−," lacks a genetic factor, Yd2, conferring resistance to some types of BYDV.) All cultivars were grown in sterilized soil in Styrofoam cups in growth chambers at 20 ± 1 C with 14-hr illumination.

Virus isolates and vectors. The three BYDV isolates tested were the MAV and RPV isolates of Rochow (13) and the P-PAV isolate (4), which is an Indiana isolate of the PAV type. For MAV and RPV, their respective specific vectors *Sitobion* (*Macrosiphum*) *avenae* F. and *Rhopalosiphum padi* L. were used for transmissions. For P-PAV, its efficient vector *R. padi* was used. The virus isolates, with their vectors, were maintained on Clintland 64 oats in separate growth chambers at 20 ± 1 C with a 14-hr photoperiod.

ELISA of extracts and statistical analysis. Samples for ELISA consisted of freshly harvested roots, shoots (= all aboveground parts), or individual leaves frozen at −80 C for not longer than a week. All comparisons were of tissue stored for the same period of time. Each shoot or root sample was extracted at 1:5 (w/v) dilution in 0.1 M potassium phosphate buffer, pH 7.0, in a Polytron homogenizer (Brinkman Instruments, Westbury, NY) with the PT 20 ST probe at setting 6 for 20 sec. Extracts were then further diluted in the same buffer to a final dilution of 1:20 (w/v). Each individual leaf sample was ground to a powder in liquid nitrogen with a mortar and pestle, a method equally efficient, but more effective for small samples. The powder was further ground to a paste in a small amount of 0.1 M potassium phosphate buffer, pH 7.0, and then diluted in the same buffer to a final dilution of 1:20 (w/v) and reground. All extracts were tested quantitatively by double antibody sandwich (DAS)-ELISA with polyclonal rabbit antisera as described elsewhere (6,7,17), but with Immulon 2 ELISA plates (Dynatech MicroELISA) instead of Immulon 1 plates. To eliminate possible plate variation, comparative tests between paired cultivars were done in the same plate, and duplicate wells were used for all samples.

A standard dilution curve was developed in all quantitative ELISA tests to determine the relationship between reaction and antigen dilution. Mean ELISA values (± standard deviation, SD) were calculated for each experiment, as well as the total mean ELISA value (± SD) for all the repetitions of each treatment, i.e., for each cereal cultivar and virus incubation period tested. For individual leaves, the range of ELISA values for each leaf position was also noted. We recognize that serological tests do not necessarily detect only intact virions. However, because comparative virus contents in our purifications are generally consistent with those indicated by ELISA (4), and because our antisera

do not appear to react with dissociated virus in DAS-ELISA (3), it is reasonable to assume that ELISA values obtained in these experiments were an index of virus content.

Virus acquisition from source plants and transmission to test plants. The efficiency with which *R. padi* acquired and transmitted P-PAV after access to various source plants for various times was examined in several preliminary experiments as follows. Twelve days after inoculation of 6-day-old seedling source plants, *R. padi* were placed on each for acquisition access periods of 2, 6, or 48 hr. These aphids were then transferred to 6-day-old Clintland 64 test plants (five test plants per source plant and two aphids per test plant). After a test feed of 2 days the test plants were sprayed with an insecticide containing pyrethrins (Ortho Tomato and Vegetable Spray, Chevron Chemical Company, San Francisco, CA) and kept in the greenhouse for 10 days more, to allow virus propagation before qualitative ELISA to determine the percentage of plants infected. Control plants were kept separately but in the same growth conditions.

After transferring the aphids to test plants, roots and shoots (= all aboveground parts) of all source plants used for acquisition of P-PAV by *R. padi* were individually tested by quantitative ELISA.

Virus content of individual leaves. For each isolate, eight aphids (apterous adults and nymphs) per plant of the appropriate viruliferous aphid vector were used to infest 5–6-day-old seedlings of the cereal cultivars to be tested. After a test feed of 2 days, the aphids were killed with nicotine sulfate, and the plants were kept in growth chambers at 20 ± 1 C with a 14-hr photoperiod for the completion of the virus incubation period. The plants were then harvested, and individual leaves separated and used in transmission experiments (12). The leaves were then stored individually at −80 C until tested by quantitative ELISA. Control plants were kept separate, but in the same growth and storage conditions. Selected leaves of Clintland 64 oats and of the barley pair Briggs (Yd2−) Prato (Yd2+), inoculated with P-PAV, were tested individually 12, 18, 24, and 30 days after inoculation. Leaves of the other two barley pairs, Atlas 57 (Yd2−) - Atlas 68 (Yd2+), and California Mariout (Yd2−) - CM67 (Yd2+), also inoculated with P-PAV, were tested 12 days after inoculation. Leaves of Clintland 64 oats inoculated with MAV were tested 18 days after inoculation, and leaves of Clintland 64 oats, and the wheat cultivars Abe and Elmo, inoculated with RPV, were tested 22 days after inoculation. Virus incubation periods of 12, 18, and 22 days in inoculated plants were previously found to give peak virus contents for cereal seedlings inoculated with the P-PAV, MAV, and RPV isolates, respectively (17).

RESULTS

Transmissibility of P-PAV from cereal plants with relatively high or low overall virus contents. As previously observed by Skaria et al (17), virus content 12 days after inoculation with the P-PAV isolate was higher in roots than shoots of the same plants, for all cultivars (Table 2). The relative virus contents of the cultivars examined were also consistent with the findings of Skaria et al (17 and *unpublished*). Clintland 64 oat extracts gave the highest ELISA values, and values for Yd2− barleys were higher ($P \leq 0.01$) than those for the corresponding Yd2+ members of each near-isogenic barley pair (Table 2).

For all cultivars, acquisition efficiency (as measured by the percentages of test plants infected) increased as the aphid feeding time on the source plant increased (e.g., Fig. 1). For the three barley pairs, differences in the percentages of test plants infected also suggested improved acquisition efficiency from sources with relatively high virus contents (= Yd2−) as compared with that from sources with relatively low virus contents (= Yd2+), except with respect to the 6-hr acquisition access for the Atlas 57(Yd2−) - Atlas 68(Yd2+) pair (Fig. 1). However, although differences in virus acquisition efficiency were noted for all acquisition access times tested, they were statistically significant ($P \leq 0.05$) only for the Briggs(Yd2−) - Prato(Yd2+) and Atlas 57(Yd2−) - Atlas 68(Yd2+) pairs, and only with respect to the 2-hr acquisition

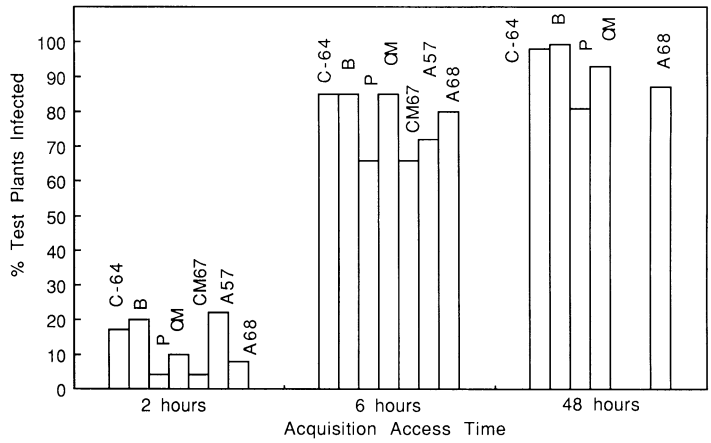

Fig. 1. Relative transmissibility by *Rhopalosiphum padi* of barley yellow dwarf virus (P-PAV isolate) from source plants with relatively high or low total virus content, to Clintland 64 test plants. Acquisition access times on source plant were 2, 6, and 48 hr and access for the transmission test feed was 48 hr. Ten source plants per cultivar and five test plants per source plant were used. C-64 = Clintland 64, B = Briggs (Yd2−), P = Prato (Yd2+), CM = California Mariout (Yd2−), CM67 = CM67 (Yd2+), A57 = Atlas 57 (Yd2−), A68 = Atlas 68 (Yd2+). CM67 and Atlas 57 barley were not tested for the 48-hr acquisition access time. For statistical analysis see text.

TABLE 1. Classification of cereal cultivars used by susceptibility and relative virus content when infected with the isolates of barley yellow dwarf virus (BYDV) indicated

Cultivar	Symptomatic reaction to BYDV	Relative virus content when infected with BYDV (isolate)[a]	
Oats			
Clintland 64	S[b]	H[c]	(PAV)
Barley			
Briggs (Yd2−)	S	H	(PAV)
Prato (Yd2+)	R	L	(PAV)
Atlas 57 (Yd2−)	S	H	(PAV)
Atlas 68 (Yd2+)	R	L	(PAV)
California Mariout (Yd2−)	S	H	(PAV)
CM67 (Yd2+)	R	L	(PAV)
Wheat			
Abe	S	H	(RPV)
Elmo	R	L	(RPV)

[a] Data from Skaria et al (17).
[b] S = susceptible (i.e., sensitive, 2); R = resistant (i.e., tolerant, 2), to BYDV (unknown isolates), as described in the literature (17).
[c] H = high; L = low.

TABLE 2. Mean enzyme-linked immunosorbent assay (ELISA) values[a] for extracts of tissues from Clintland 64 oats and near isogenic pairs of barley inoculated with the P-PAV isolate of barley yellow dwarf virus[b]

Cultivar	Shoots	Roots
Oats		
Clintland 64	1.100 a	1.520 b
Barley		
Briggs (Yd2−)	0.963 b	1.870 c
Prato (Yd2+)	0.362 a	0.772 b
Atlas 57 (Yd2−)	0.755 b	1.064 c
Atlas 68 (Yd2+)	0.298 a	0.499 ab
California Mariout (Yd2−)	0.524 b	1.703 c
CM67 (Yd2+)	0.244 a	0.328 ab

[a] Mean ELISA values for each barley tissue followed by a letter in common are not significantly different, $P \leq 0.01$, according to Duncan's multiple range test. Mean ELISA values between shoots and roots of Clintland 64 oats were compared by a Student's *t*-test.
[b] Shoots and roots were extracted 1:20 (w/v) in 0.1 M potassium phosphate buffer, pH 7.0. All cultivars were tested twice, and each time extracts from 10 plants were tested in duplicate wells.

access times. Indications of differences that were observed with 6- and 48-hr acquisition access times were overshadowed by variability and increased acquisition efficiency overall.

Acquisition efficiency from Clintland 64 oats was similar to acquisition efficiency from the barley cultivars with higher virus contents (Atlas 57 and Briggs), but was only significantly different ($P \leq 0.05$) to acquisition efficiency from the cultivars Prato and CM67 and then only for the 2-hr acquisition access. Though ELISA values indicated highest virus concentrations in Clintland 64 oats and Briggs barley, virus acquisition from Atlas 57 was as efficient as acquisition from these for a 2-hr access, and from California Mariout for a 6-hr access (Table 2, Fig. 1).

In a subsequent experiment, virus acquisition by *R. padi* from all available leaves of P-PAV infected Briggs (Yd2−) and Prato (Yd2+) barley plants was compared (Fig. 2). The results indicated improved acquisition efficiency from Briggs for the 1- and 2-hr acquisition access periods, but not for the 3-, 4-, and 6-hr acquisition access periods, for which Prato was a better source of virus. Similarly, for the California Mariout (Yd2−) and CM67 (Yd2+) pair, results for a 3-hr acquisition access suggested that California Mariout (Yd2−) might be a better source of virus than CM67, but with the Atlas 57 (Yd2−) and Atlas 68 (Yd2+) pair, the inverse situation occurred (results not shown). These differences were statistically significant ($P \leq 0.05$) only for the Briggs-Prato pair, and then only in the experiments with 1- and 6-hr acquisition access times.

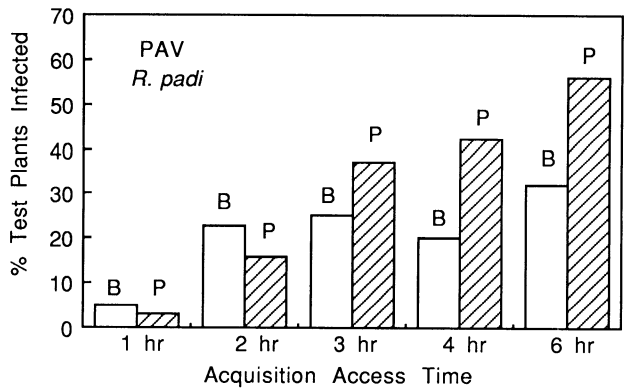

Fig. 2. Variations in relative transmissibility of barley yellow dwarf virus (P-PAV isolate) by *Rhopalosiphum padi* from individual leaves of (B) Briggs (Yd2−) and (P) Prato (Yd2+) barleys to Clintland 64 oats test plants. (Differences in leaf virus contents are ignored.) *R. padi* acquisition access times on source leaves were 1, 2, 3, 4, and 6 hr, and access for the transmission test feed was 48 hr. Totals of 15 individual leaves (five source plants) were used for each of the 2, 4, and 6 hr experiments with Briggs (Yd2−) and 1, 2, 4, and 6 hr experiments with Prato (Yd2+). Totals of 35 individual leaves (10 source plants) were used for the other experiments with each barley. The number of test plants used for each individual source leaf varied from four to two. For statistical analysis see text.

Comparative concentration of P-PAV in different leaves of oats. Clintland 64 oats showed typical BYDV symptoms 12 days after inoculation with the P-PAV isolate. Symptoms started at the leaf tip as yellowish-green areas that coalesced and turned dark-red to brown. Later, the first leaf turned completely brown and the second and third (young) leaves developed a yellowish green color. For all the virus incubation periods studied, virus content as measured by ELISA was not uniform among the different leaves of the same plant. For example, 12 days after inoculation the first (inoculated) leaves averaged about four times more virus than the youngest leaves (Table 3). Later, virus developed in the younger leaves of the plant, and 30 days after inoculation, the average ELISA value for extracts from the youngest leaves was significantly higher than that for extracts from the older ones. However, as is clear from the ranges of ELISA values obtained, the virus contents of individual leaves in the same position varied greatly among plants.

Comparative concentration of P-PAV in different leaves of barley. Symptoms of P-PAV in barley cultivars were not so clear as in oats, and were evident only 3 wk or more after inoculation. Initial symptoms appeared at the leaf tip and consisted of a yellowing that spread progressively to the base of the leaf. Five weeks after inoculation the first (inoculated) leaf was dead, and the second had turned brown. Checks by ELISA of extracts of the three barley pairs confirmed that the Yd2+ cultivars (Tables 4 and 5) had on average a lower virus content than the corresponding Yd2− cultivars. For all the barley cultivars tested, P-PAV distribution among individual leaves of the same plant was in general much more uniform than in Clintland 64 oats; but as with Clintland 64, the virus contents of individual leaves in the same position varied greatly from plant to plant. On average, each time tests were done, the indicated virus contents for different leaves of Briggs (Yd2−) plants were similar (Table 4). Prato (Yd2+) plants also showed a similar distribution of virus in the different leaves of the same plant, except that 12 days after inoculation, inoculated leaves had, on average, a much lower virus titer than that indicated for other leaves on the same plants (Table 4). Similar results were also obtained with Atlas 68 (Yd2+) barley (Table 5), but not with CM67 (Yd2+) barley (Table 5). The other two barley cultivars, Atlas 57 (Yd2−) and California Mariout (Yd2−), showed a more uniform distribution of P-PAV in the different leaves of the same plants (Table 5).

TABLE 3. Variations in enzyme-linked immunosorbent assay (ELISA) values over a 30-day period among individual leaves of Clintland 64 oats infected with the P-PAV isolate of barley yelllow dwarf virus

Days after inoculation	Leaf position (old) 1	2	3	4	5	(young) 6
12	0.966[a] (0.366)[c] 1.834–0.014[d]	0.599 (0.310) 1.720–0.101	0.407 (0.176) 0.938–0.030	...[b]	...	...
18	1.142 (0.288) 1.347–0.474	0.409 (0.119) 0.727–0.248	1.054 (0.278) 1.649–0.584	0.711 (0.195) 1.088–0.416	...	...
24	...	0.308 (0.136) 0.577–0.120	0.561 (0.373) 1.755–0.175	0.656 (0.150) 1.007–0.408	0.770 (0.226) 1.185–0.441	...
30	...	0.271 (0.194) 0.904–0.102	0.410 (0.090) 0.613–0.276	0.503 (0.093) 0.656–0.324	1.114 (0.161) 1.368–0.749	1.164 (0.206) 1.378–0.777

[a] Mean ELISA values for duplicate wells in six separate experiments on a total of 144 plants for the 12-day incubation period, and two separate experiments on totals of 18, 16, and 16 plants for the 18-, 24-, and 30-day incubation periods, respectively. Mean ELISA value (± SD) for healthy Clintland 64 oats was 0.032 (± 0.004). When antigen concentration was reduced by one-half, ELISA values were reduced by about one-third.
[b] Leaves not available.
[c] Standard deviation (SD).
[d] Range of ELISA values for individual leaves.

Comparative concentration of MAV in different leaves of oats. Clintland 64 oats had developed obvious symptoms by 18 days after inoculation with the MAV isolate. There was an intense red color in the first (oldest) leaf, and similar symptoms started to appear at the tip of the second leaf. Average MAV content of the oldest leaf (inoculated leaf) was significantly higher than that of the other leaves on the same plant (Table 5), but again ELISA values for extracts from individual leaves at the same position varied from plant to plant.

Comparative concentration of RPV in different leaves of oats. When leaves were harvested 22 days after inoculation, plants of Clintland 64 inoculated with the RPV isolate showed intense reddening on the first and second leaves, and symptoms had also started to develop at the tip of the third leaf. Virus distribution in individual leaves of Clintland 64 oats sampled at this time showed similar variation to that in plants inoculated with the other two BYDV isolates. On average, in infections with RPV the oldest leaves of Clintland 64 oats had approximately four times as much virus as the youngest leaves (Table 5). Also, the range of ELISA values obtained indicated that the virus content of individual leaves at the same position varied greatly from plant to plant.

Comparative concentration of RPV in different leaves of wheat. Twenty-two days after inoculation, the first (inoculated) leaves of both wheat cultivars were dead, and no symptoms were observed on the other leaves. Abe showed significant differences in virus content among the three different leaf positions available, with the highest virus content in the oldest leaves (Table 5). In Elmo, virus was much more uniformly distributed among leaves of different ages (Table 5). Again, large variations occurred in the ELISA values for extracts from individual leaves in the same position on the plant.

DISCUSSION

Overall, the results of transmissions from intact plants, or from all available leaves separated from groups of plants, suggested that in many cases sufficient variability occurred to overshadow possible differences in acquisition efficiency between different cultivars. A basis for this variability was found in the detailed comparisons of virus concentrations in individual leaves of various cultivars infected with various isolates. Thus, in all the combinations of host, virus isolate, and time after infection that were examined, virus concentrations in individual leaves as indicated by ELISA varied greatly with leaf position and age. With the three BYDV isolates used virus concentrations in individual leaves followed the sequence of symptom severity only with Clintland 64 oats. In other work with persistently transmitted viruses (8), different leaves of *Physalis floridana* differed as sources of potato leaf roll virus, and there was no relation between the availability of virus to aphids and symptom severity; however, for potato virus Y, symptom development in tobacco leaves reflected the availability of virus to aphids (1).

In previous work (17), correlations of BYDV content and tolerance were assessed by ELISA of extracts from whole plants. Our results with the P-PAV isolate indicated that, overall, virus concentrations in the leaves of Yd2+ barleys were lower than those of the corresponding Yd2− barleys, confirming the similar trends found by Skaria et al (17). It is difficult to generalize regarding virus distribution among leaves, but at each time tested, it was generally more uniform for the barleys than for the Clintland 64 oats. With the latter, early samplings 12 and 18 days after inoculation indicated highest average virus concentrations in the lower (oldest) leaves, but in later samplings, highest average virus concentrations were present in the younger leaves. With the barley

TABLE 4. Variations in enzyme-linked immunosorbent assay (ELISA) values over a 30-day period among individual leaves of Briggs (Yd2−) and Prato (Yd2+) barleys infected with the P-PAV isolate of barley yellow dwarf virus .

Cultivar (Yd2 status)	Days after inoculation	Leaf position (old) 1	2	3	4	5	(young) 6
Briggs (Yd2−)	12	0.320[a] (0.248)[c] 1.018–0.025[d]	0.402 (0.217) 0.891–0.055	0.383 (0.189) 0.845–0.024	0.440 (0.177) 0.740–0.214	...[b]	
	18	0.811 (0.363) 1.305–0.169	0.637 (0.189) 0.968–0.260	0.715 (0.136) 0.950–0.476	0.807 (0.101) 0.920–0.587	...	
	24	0.506 (0.211) 0.851–0.229	0.555 (0.203) 0.879–0.045	0.458 (0.228) 0.814–0.049	0.609 (0.320) 1.363–0.048	...	
	30	...	0.443 (0.097) 0.560–0.283	0.471 (0.086) 0.658–0.365	0.507 (0.126) 0.801–0.359	0.522 (0.076) 0.678–0.418	
Prato (Yd2+)	12	0.086 (0.098) 0.506–0.006	0.223 (0.121) 0.416–0.101	0.283 (0.123) 0.752–0.030	0.269 (0.075) 0.390–0.073	...	...
	18	0.455 (0.408) 1.549–0.112	0.211 (0.151) 0.499–0.047	0.276 (0.181) 0.627–0.077	0.236 (0.131) 0.359–0.044	...	...
	24	0.325 (0.200) 0.631–0.115	0.240 (0.182) 0.470–0.049	0.258 (0.114) 0.399–0.055	0.303 (0.127) 1.481–0.061	...	...
	30	...	0.331 (0.108) 0.485–0.200	0.329 (0.054) 0.399–0.278	0.397 (0.187) 0.699–0.247	0.420 (0.207) 0.727–0.286	0.503 (0.084) 0.613–0.429

[a] Mean ELISA values for duplicate wells in, for Briggs (Yd2−) barley, three separate experiments on a total of 61 plants for the 12-day incubation period, and one separate experiment on totals of 11, 12, and 11 plants for the 18-, 24-, and 30-day incubation periods, respectively, and, for Prato (Yd2+) barley, in three separate experiments on a total of 49 plants for the 12-day incubation period, and one separate experiment on totals of 11, 7, and 5 plants for the 18-, 24-, and 30-day incubation periods, respectively. Mean ELISA values (± SD) for healthy barleys (both cultivars) were 0.033 (± 0.006). When antigen concentration was reduced by one-half, ELISA values were reduced by about one-third.

[b] Leaves not available.

[c] Standard deviation (SD).

[d] Range of ELISA values for individual leaves.

TABLE 5. Variations in enzyme-linked immunosorbent assay (ELISA) values among individual leaves of several cereal cultivars infected with the P-PAV, MAV, or RPV isolates of barley yellow dwarf virus

Isolate (days after inoculation)	Cultivar (Yd2 status)	Leaf position (old) 1	2	3	4	(young) 5
P-PAV (12 days)	Atlas 57 (Yd2−)	0.201[a] (0.222)[c] 0.706–0.017[d]	0.517 (0.314) 0.799–0.013	0.478 (0.187) 0.721–0.151	0.186 (0.093) 0.368–0.029	...[b]
	Atlas 68 (Yd2+)	0.051 (0.030) 0.112–0.017	0.351 (0.152) 0.623–0.038	0.309 (0.132) 0.702–0.088	0.214 (0.086) 0.357–0.031	...
	California Mariout (Yd2−)	0.481 (0.162) 0.664–0.163	0.327 (0.114) 0.541–0.015	0.251 (0.143) 0.525–0.112	0.483 (0.137) 0.639–0.319	...
	CM 67 (Yd2+)	0.205 (0.129) 0.452–0.027	0.194 (0.088) 0.467–0.097	0.129 (0.089) 0.425–0.027	0.174 (0.042) 0.239–0.134	...
MAV (18 days)	Clintland 64	1.199[e] (0.299)[c] 0.772–0.593[f]	0.305 (0.151) 0.611–0.103	0.525 (0.293) 1.419–0.203	...	...
RPV (22 days)	Clintland 64	0.940[e] (0.510)[d] 1.709–0.033[e]	0.859 (0.501) 1.366–0.025	0.618 (0.315) 1.297–0.033	0.512 (0.249) 1.037–0.040	0.362 (0.207) 0.946–0.117
RPV (22 days)	Abe	...	1.215 (0.469) 1.767–0.425	0.767 (0.365) 1.367–0.163	0.286 (0.131) 0.614–0.157	
RPV (22 days)	Elmo	...	0.328 (0.331) 1.071–0.059	0.541 (0.334) 1.107–0.075	0.505 (0.158) 0.779–0.263	

[a]Numbers are: mean ELISA values for duplicate wells in two separate comparisons of totals of 17 plants for each barley cultivar; two experiments with totals of 20 plants each for Clintland 64 oats; one comparison of 10 plants for each wheat cultivar. Mean ELISA values (± SD) for healthy controls were 0.029 (± 0.004) for the Atlas 57-Atlas 68 pair, 0.017 (± 0.006) for the California Mariout-CM 67 pair, 0.020 (± 0.008) for the Clintland 64 oats with MAV, 0.047 ± 0.006 for the Clintland 64 oats with RPV, and 0.048 (± 0.012) with the wheat pair.
[b]Leaves not available.
[c]Standard deviation (SD).
[d]Range of ELISA values for individual leaves. (When antigen concentration was reduced by one-half, ELISA values were reduced by about one-third).
[e]Mean ELISA values for duplicate wells in tests of 20 plants. Mean ELISA value (± SD) for healthy controls was 0.020 (± 0.008).
[f]Mean ELISA values for duplicate wells in tests of 20 plants. Mean ELISA value (± SD) for healthy controls was 0.047 (± 0.006).

cultivars, such relationships were less clear, but there were indications with two of the Yd2+ cultivars (Atlas 68 and Prato) that the oldest leaves had least virus when tested 12 days after inoculation. Differences in virus concentration between individual leaves occurred also with respect to wheat and oats infected with the RPV and MAV isolates. Thus, for Abe wheat inoculated with the RPV isolate, the oldest leaves, examined 22 days after inoculation, had on average a much higher virus than the youngest ones, whereas the reverse was true for Elmo wheat. High values for both RPV and MAV content were also seen in the oldest leaves of Clintland 64 oats sampled 22 or 18 days after inoculation, respectively. These data suggest relatively rapid development of virus in the inoculated leaves of the more susceptible cultivars. They are consistent with the observations of Skaria et al (17) of more rapid development of virus in "susceptible" (i.e., sensitive) cultivars than in "resistant" (i.e., tolerant) cultivars. The data clearly indicate that because of the wide variations in virus content among leaves of the same plants, comparisons of virus acquisition efficiency by aphids feeding on whole plants are likely to be invalid, and that comparisons involving acquisition feeds on individual leaves of known virus content should be more reliable. The results also indicate the danger of relying on ELISA of individual leaves or leaf samples for estimates of differences in virus productivity between cultivars, and suggest that even for qualitative ELISA diagnosis, adequate samplings of different leaves are required.

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