Tolerance to Barley Yellow Dwarf Virus in Oats

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Cooperative Investigation, North Central and Northeast Regions, Agricultural Research Service, United States Department of Agriculture; Illinois Agricultural Experiment Station; and Cornell University Agricultural Experiment Station. Supported in part by NSF grants GB-3524 and BMS74-19814 to Cornell University.

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Accepted for publication 19 May 1977.

ABSTRACT

JEDLINSKI, H., W. F. ROCHOW, and C. M. BROWN. 1977. Tolerance to barley yellow dwarf virus in oats. Phytopathology 67: 1408-1411.

Disease severity was highly correlated with reduction in yield of three pairs of sister oat lines representing F7 generation progenies that transgressively segregated for marked tolerance and intolerance to barley yellow dwarf virus (BYDV) infection. Less BYDV was extracted from plants of each of the three tolerant lines than from plants of each of the three lines that lacked tolerance. These data show that meaningful direct tests of BYDV concentration can be used to study the mechanism of tolerance, which appears to involve a suppression of BYDV replication.

Additional key words: aphids, cereal viruses.

The use of tolerant oat (*Avena sativa* L.) cultivars is currently the only practical means of controlling the barley yellow dwarf virus (BYDV) disease. Although much progress has been made in breeding tolerant oats (5, 6, 7, 8, 18), little is known about the mechanism of this resistance to the aphid-transmitted virus. For some virus diseases an explanation for tolerance is based on suppression of virus replication (3, 4, 13, 17, 23, 25). Most such studies have involved viruses that are relatively easy to assay because they are mechanically transmissible and reach high titers in infected plants.

We used pairs of sister oat lines in preliminary tests of the thesis that a mechanism for resistance to BYDV is suppression of virus replication in the tolerant oat lines. This paper describes direct evaluations of disease severity for the paired oat lines, and shows that estimates of virus titers in such lines can be used to study the mechanism of tolerance, despite limitations of working with phloem-limited, vector-dependent luteoviruses, such as BYDV.

MATERIALS AND METHODS

Six oat lines were used in all experiments. The lines were derived from crosses made at Urbana, Illinois, from parents selected for tolerance to two vector-nonspecific isolates of BYDV described previously (18, 19). All parental oat selections were relatively tolerant and consequently differences in disease severity among the lines were small, especially in the greenhouse. Generally, heritability for BYDV reaction was high in the F2, F3, F4, and F5 generations. Genetic results, which will be published elsewhere, showed that the progenies, each derived from a different F2 plant, transgressively segregated for higher and lower levels of tolerance to BYDV than did the respective parents. The three pairs of oat lines used in these studies were quite similar; their reaction to BYDV was the major identifiable differentiating character. Consequently, each of the three pairs will be referred to as sister lines.

The disease severity and percentage yield reductions were determined at Urbana under field conditions, by using methods similar to those reported previously (19). One isolate of BYDV, Champaign-6, was used as inoculum in both years. The oat seeds were planted in the field at the end of April, with two replications in 1972, and three replications in 1974. The plantings were made in hills spaced 0.457 m (1.5 ft) apart, 12 plants per hill. To minimize contamination, we isolated plots containing plants to be inoculated from those serving as controls by a 4.57-m (15-ft) border of a mixture of different oat cultivars. The plants were inoculated at an early tillering stage by exposing them to approximately 20 viruliferous *Rhopalosiphum padi* (L.) per plant for a period of 3 days. The inoculation feeding was stopped by spraying all plants, including the border plants, with O, O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate (dimethoate) in a water emulsion at the rate of 1.24 kg active material per hectare (1.1 pounds active material per acre). Two disease severity evaluations were made: one at first symptom development; and the other, shortly before the plants matured.

The virus concentrations of BYDV extracted from each

of the three pairs of sister oat lines grown in the greenhouse were determined in Ithaca, New York. Five seeds of each of the six oat lines were planted in 60, 10-cm-diameter pots. When the seedlings were 6 days old, they were inoculated by means of *R. padi* with the PAV isolate of BYDV (26). This isolate is similar to the one used in Illinois. Both are transmitted nonspecifically by several aphid species (19, 26). The aphids had been given a 2-day acquisition feeding before a 5-day inoculation test feeding period. The oat plants then were fumigated to remove aphids, and grown for 5 wk in the greenhouse. The aboveground parts of each of the six groups of plants then were harvested, cut into small pieces, and stored in plastic bags in a freezer for about 4 mo.

Because few of the plants developed clear symptoms of barley yellow dwarf under greenhouse conditions, a leaf was selected at random from each of 10 plants from each group of the six oat lines at harvest and used in a test of virus recovery. *Rhopalosiphum padi* were allowed to feed on the detached leaves for 2 days and then were placed on seedlings of oats (*Avena byzantina* C. Koch 'Coast Black') for a 5-day inoculation test feeding. The virus was transmitted by *R. padi* from each of the 60 selected leaves to all of the 180 plants; none of six control plants became infected. Thus, plants of each of the six groups of oats were considered to be uniformly infected by PAV.

The frozen tissue was used to make a concentrated, partially purified virus preparation as described previously (27, 28). Each of the six separate groups of tissue was ground and processed separately to permit preparation of six comparable virus preparations. Because the relative amount of tissue varied for each group, the final volume of each preparation was adjusted to a concentration of 500-fold relative to the fresh weight of starting tissue. The PAV content of each preparation then was estimated by sucrose gradient centrifugation (27, 28). Two samples (0.8 ml each) were centrifuged for each preparation, the means of the two separate estimates were calculated, and the mean value was used to calculate the virus yield per 1,000 g of starting tissue.

RESULTS

Inoculation procedures used at Urbana under controlled conditions assured uniform results in the field experiments. None of the inoculated plants escaped infection. The period between the beginning of inoculation feeding by viruliferous aphids and the first appearance of symptoms was 9 days in 1972 and 10 days in 1974.

In the acute stage of the disease all inoculated plants developed symptoms typical of barley yellow dwarf. The tolerant oat lines, however, developed much milder symptoms than did the intolerant lines. As the plants approached maturity, the disease reached the chronic stage and the tolerant lines recovered, the symptoms became masked, and in some cases the infected tolerant lines yielded as well as the controls (Table 1). However, symptoms were clearly visible in the intolerant lines up to maturity. In general, disease severity was highly positively correlated with yield reduction (Table 1). The differences in yield reduction between tolerant and intolerant lines ranged from approximately twofold to sixtyfold. Yield reduction in tolerant lines ranged from 0 to 38%; that in intolerant lines, from 37 to 91%. The effect of BYDV infection on yield was much more severe and there was less variability as evidenced by coefficients of variation in 1972 than in 1974 (Table 1).

Because of the consistent differences between these pairs of sister oat lines and the experimental advantages they offer, we made concurrent tests in Ithaca to compare BYDV titers in preparations made from the lines infected with the PAV isolate. For each of the three pairs of lines, less PAV was obtained from the tolerant progeny line

TABLE 1. Disease tolerance and yield of three pairs of sister oat lines in the F7 generation infected with the vector-nonspecific isolate of barley yellow dwarf virus (BYDV)

Parental crosses[a]	Progeny no.	BYDV disease severity[b]		Yield (g)	
		1972	1974	1972	1974
		Means			
Albion × C. I. 5068	65Y2147-2	2.0	2.0	24.4	20.4
Albion × C. I. 5068	65Y1103-1	5.0	6.0	2.7	5.0
Albion × ILL 30959	65Y2034-1	1.5	2.0	31.8	16.1
Albion × ILL 30959	65Y2172-3	3.0	4.3	22.3	7.7
Albion × C. I. 1915	65Y2115-4	2.0	2.3	28.3	19.5
Albion × C. I. 1915	65Y2137-2	5.0	3.7	8.8	6.7
LSD.05		0.7	0.7	9.2	7.7
LSD.01		1.1	1.0	14.8	10.1
C.V. %[c]		8	20	19	34

r[d] = 0.91**; r = 0.97** (between disease severity and yield reduction in 1972 and 1974, respectively).
r = 0.52; r = 0.68 (between disease severity in 1972 and 1974 and virus yield in Table 2, respectively).
r = 0.65; r = 0.74* (between yield reduction in 1972 and 1974 and virus yield in Table 2, respectively).

[a]Abbreviations: C. I. refers to accession (Cereal Introduction) number of U.S. Department of Agriculture, ARS; ILL refers to Illinois selection number.

[b]Based on visual evaluation within a scale of 0 = no apparent symptoms to 10 = most severe symptoms; two replications in 1972 and three replications in 1974, with 12 plants per replication.

[c]C. V. = coefficient of variation.

[d]r = correlation coefficient; * indicates significant difference $P = 0.05$; and ** indicates significant difference, $P = 0.01$.

TABLE 2. Relative yields of the PAV isolate of barley yellow dwarf virus (BYDV) from each of six oat lines tested in the greenhouse

Parental crosses[a]	Progeny no.	BYDV tolerance	Tissue harvested (g)	Virus extracted (μg per kg tissue)[b]
Albion × C. I. 5068	65Y2147-2	Yes	1,435	22.0
Albion × C. I. 5068	65Y1103-1	No	1,298	45.0
Albion × ILL 30959	65Y2034-1	Yes	946	40.2
Albion × ILL 30959	65Y2172-3	No	1,012	53.2
Albion × C. I. 1915	65Y2115-4	Yes	958	22.4
Albion × C. I. 1915	65Y2137-2	No	887	42.6

[a]Abbreviations: C. I. refers to accession (Cereal Introduction) number of U.S. Department of Agriculture, ARS; ILL refers to Illinois selection number.

[b]Values are means of two assays by sucrose density gradient centrifugation.

than from the intolerant one (Table 2). In two of the three comparisons, the difference in virus concentration was nearly twofold.

All six of the virus preparations were infectious when bioassays were made by means of the membrane-feeding technique (28). No differences were observed among the preparations in these infectivity tests because the preparations were not diluted sufficiently, and thus all of the test plants became infected.

DISCUSSION

Barley yellow dwarf virus (BYDV) belongs to the group of phloem restricted viruses (1, 2, 10, 16, 20). The viruses cause extensive phloem degeneration that begins with necrosis of phloem parenchyma and companion cells and ultimately causes the collapse of conducting sieve and vessel elements (10, 11, 16). From this study, it appears that some oat cultivars or lines may circumvent this type of damage better than others, and infection does not cause an appreciable yield decrease in these lines.

The extent of the differences and the consistency of the pattern for each of the three combinations of tolerant and intolerant sister oat lines we tested support the idea that tolerance of oats to BYDV may result from a suppression of virus replication. To some extent, this suppression may depend on elimination or neutralization of the infectious virus within the vesicles enclosed by the membranes of the host cell (16). The data do not rule out other possibilities, such as the importance of differential systemic movement (21). Other evidence presented in the literature (9, 11) tends to support Jensen's (21) conclusion that the long distance movement of viruses does not depend on virus synthesis in the mature conducting tissues of phloem void of nuclei and normal cytoplasm. However, Gill (14, 15, 16) interpreted his data to indicate that the virus multiplies to some extent at the inoculation sites within the phloem companion cells and the phloem parenchyma before it is relatively rapidly translocated in the phloem elements. Although virus translocation and virus replication are undoubtedly related, each process may proceed independently (9). This suggests that the rate of systemic spread may depend on the factors controlling virus replication, the entry of the virus particles into the sieve and vessel elements, and their reentry into the cells and contact with the receptive multiplication sites. This concept is further supported by recent findings (24) with tobacco mosaic virus that an intact tobacco mosaic virus particle is apparently required for successful translocation in the sieve tubes and initiation of systemic infection.

Despite their preliminary nature, these data show that direct virus assays can be used in studies of the mechanism of tolerance of plants to BYDV. We do not agree with the assessment that BYDV concentrations must be estimated by indirect aphid-transmission methods because "there are no direct physical methods at present available for assaying virus concentration" (22). In addition to analytical sucrose gradient centrifugation described for BYDV in 1964 (28), a range of serological procedures also are available (1, 27). Studies of luteoviruses are more difficult than those for many plant viruses, but lack of mechanical transmission need not prevent useful virus assays.

Reduced virus titer in tolerant oats could have an important role in epidemiology in addition to its possible role in the mechanism of resistance of the infected plant. In nonpersistent aphid-virus systems, a reduced virus titer in resistant cultivars often is associated with less chance of virus transmission by aphids from resistant than from susceptible cultivars. For example, Zitter (29) recently has shown that resistant cultivars of pepper are less likely to serve as sources of pepper mottle virus for aphids than are susceptible ones, an observation that parallels relative virus titers in the two kinds of plants. There is some evidence for a similar effect with BYDV. *Macrosiphum avenae* was much more likely to transmit the PAV isolate from young leaves than from old ones in a recent study (12). This difference in BYDV transmission corresponded to the relative virus content in preparations made from the two kinds of leaves. More than three times as much virus was extracted from young leaves as from old ones. Perhaps use of tolerant oat cultivars would decrease the virus reservoir potential and thereby diminish the spread of BYDV in the field.

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