PREVALENCE OF BARLEY YELLOW DWARF VIRUS IN WINTER WHEAT IN SOUTHWESTERN ONTARIO. 1969

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Abstract

In 1969, 60 fields selected from the 250,000 acres of winter wheat in southwestern Ontario were sampled for the presence of barley yellow dwarf virus using a stratified sample of fields based on the acreage of winter wheat in each of twelve counties. A random sample of culms was selected (with no reference to disease symptoms) from each field and bulked to form a field sample which was tested for BYDV transmission with Macrosiphum avenae and Rhopalosiphum padi; samples from 50% of the fields were found to be infected. From the pattern of transmission by R. padi and M. avenae in the initial isolations, the characterization of selected isolates and from symptoms on test plants, most isolates appeared to belong to the strains transmitted specifically by R. padi or R. maidis.

Introduction

Barley yellow dwarf virus (BYDV) is probably the most prevalent of the cereal viruses, having been reported in oats, barley and wheat from most continents (13). Barley yellow dwarf (BYD) usually affects oats and barley more severely than wheat, but wheat is severely damaged in New Zealand (15). The distribution of the disease has been noted by many workers (1,2,6,11,14,16,17) usually using visible symptoms for the initial diagnosis followed by transmission tests with aphids for verification. However, most of the evidence is based on surveys where crops have not been selected at random and consequently the results may not be representative of the total acreage of the crop within the area surveyed.

Studies in Ontario, conducted mainly in the eastern part of the province, have revealed that BYD may be of economic importance on cereals grown there (11,12,14). BYDV has been isolated in early spring from winter wheat, indicating that the virus can overwinter in this host (12). Aphid vectors of different species are numerous during the growing season on cereals and wild grasses. Furthermore, many perennial species of grass have yielded BYDV when tested with aphids (12). These grasses as well as the winter cereals such as wheat and rye, therefore, could serve as reservoirs of virus. At least one species of the aphid vector, Rhopalosiphum padi (Linnaeus), overwinters locally in the egg stage, and becomes active early in the season. Aphids that migrate from distant sources probably also play an important role in the dissemination of the virus (12).

There is little information on the incidence of BYDV in cereals in southwestern Ontario. The climate is milder there than in eastern Ontario, and overwintering of the aphid vectors might, therefore, be more successful, and the incidence of the disease higher. Approximately 70% of the winter wheat acreage in Ontario is grown in the twelve counties surveyed (Table 1). Simcoe and York are the only counties outside the survey area in which an appreciable acreage of winter wheat is grown. This paper outlines an objective survey approach to assess the prevalence of BYDV in southwestern Ontario by estimating the percentage of field samples infected with the virus; no attempt was made to assess the percentage of plants infected within the fields. A stratified sample of fields, based on wheat acreage, was selected from 12 counties. Because symptoms of BYD in winter wheat may be very slight and therefore easily overlooked (3), and because recent work in England (6) has shown that a high proportion of spring barley plants may carry a symptomless infection of BYDV, sampling was made at random within the crops, without selecting for plants with symptoms. All samples were tested for BYDV by attempted transmission with aphids, and different species of aphids were used to obtain information on the predominant virus strains.

Materials and methods

The sample used for this survey was designed by the Dominion Bureau of Statistics (DBS) to determine the yield of winter wheat in 1967.

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Table 1. Distribution of winter wheat in southwestern Ontario, showing number of segments and fields sampled in 1969

County	Number of segments	Number of fields	Acreage* expressed as % of total acreage
Essex	5	11	11
Kent	8	13	12
Lambton	6	10	11
Middlesex	6	6	7
Elgin	3	3	6
Huron	5	2	4
Perth	4	3	2
Waterloo	3	1	2
Oxford	3	5	2
Norfolk	3	4	7
Brant	2	2	2
Haldimand	2	3	2
Totals	50	63	68

* Based on 1966 Census.

The sample comprised 50 segments, selected in proportion to the acreage of winter wheat in each county. Each segment, which is delineated by natural boundaries and identifiable on aerial photographs, contained approximately 1000 acres and several farms. The sample design will be reported in detail in a later publication. For the purpose of this survey one quarter of each segment was selected at random and the winter wheat acreage on each farm in the chosen quarter was surveyed. One field of winter wheat per farm was selected at random for sampling. A total of 63 farms reported winter wheat; the distribution is shown in Table 1. The lists of farms and the aerial photographs of each segment were provided by DBS so that farms were easily located. A total of approximately 750 acres was surveyed.

Within each field, twelve main culms were collected at approximately equal intervals, along a W pattern. Random numbers from 1 to 30 were used to select the position of the first culm, e.g. if 5 was selected as the random number, the first culm was picked 5 paces from the edge of the crop. Each field was sampled during the last week of May and during the last week of June. The crops were at Growth Stages 8 to 9, and 10.5.4 (grain watery ripe) to 11.1 (grain milky ripe) respectively, according to Feekes' Growth Stages (7). At the first visit leaves 3 and 4 (where flag leaf-1) were detached From the culms and sent by air mail to Winnipeg for testing. The twelve third leaves were packed together and leaves four were treated in the same way. On the second sampling leaves 1 and 2 were chosen. A preliminary mailing of healthy wheat leaves from Ottawa to Winnipeg was made before the survey started, and the best method of packaging proved to be leaves enclosed in moist paper towelling and sealed in a polyethylene bag with most of the air evacuated. The samples were in transit for 1-3 days.

The younger and older groups of leaves from each field were tested separately. Each

of the 12 leaves in a package was cut in half lengthwise, the halves being placed in separate petri dishes, so that both dishes contained 12 half-leaves. To maintain turgidity, the ends of the leaves were buried in moist sand,

Virus-free apterae or late instar nymphs of Macrosiphum avenae (Fabricius) were added to one dish, and those of R. padi to the other. The dishes were maintained at 15C for 2 days; then aphids were transferred from each dish to individually caged 'Clintland' oat (Avena sativa L.) seedlings at the rate of 10 aphids on each of four seedlings. After 3 days in the greenhouse, the aphids were killed by spraying with tetraethyl-pyrophosphate (TEPP) insecticide. The test plants were maintained in the greenhouse for 4 weeks before final counts of infected plants were taken.

Characterization of six of the isolates selected at random, was made by comparing the ability of five species of aphids to transmit the virus. Two tests were run for each isolate. Clintland oats infected by R. padi, the aphid used in the original isolation from the field sample, were used as the virus source in the first tests for each isolate. Source plants for the second test were ones infected in the first test by R. padi for isolates 1, 2, and 3, and by R. maidis (Fitch) for isolates 4, 5, and 6 (Table 4). In each trial, leaves were detached from the virus source plant, each leaf was cut into five pieces and the pieces were placed in five petri dishes at random. Virus-free apterae or late-instar nymphs of M. avenae, R. padi, Schizaphis graminum (Rondani), Acyrthosiphon dirhodum (Walker), or R. maidis, were transferred from colonies to each dish, and the dishes were placed at 15C for 2 days. Then, feeding aphids were transferred from each dish and were caged in groups of five aphids on each of ten 'Clintland' oat seedlings for 2 days in the greenhouse. The aphids were then killed by spraying with TEPP and the test seedlings were maintained on a greenhouse bench for 4 weeks, when final readings of infected plants were taken.

Colonies of virus-free aphids used in this work were raised on caged 'Parkland' barley (Hordeum vulgare L.) in a growth cabinet at 18C. Sample batches of the aphids were tested at regular intervals to ensure that the colonies were free from virus. Clintland oat test seedlings were grown in wooden flats of soil. Greenhouse temperatures were regulated for an average of 20C, but occasionally daily averages rose above this temperature.

Table 2. Number of field samples in which barley yellow dwarf virus was found

	Sampling period	
	May	June
No. of field samples tested	51	60
No. of field samples in which virus was detected	11	33
% of field samples in which virus was detected	22	55

Results

Some of the samples deteriorated during transit, and this interfered with the efficiency of testing. Usually the second youngest leaves arrived in poorer condition than the youngest leaves, and there was more deterioration of leaves sampled in the first period than in the second period. Consequently, samples representing only 34 out of 51 fields in the first period and 46 out of 60 in the second period were adequately tested, In a complete test, both the youngest and the second youngest leaves were tested with each aphid species. Some of the samples that were not fully tested, included those in which only one aphid species survived the acquisition feeding or only one age of leaf was tested. Much of the deterioration resulted from a yellowing of the leaves before they were sampled, possibly because of excessive moisture in some fields that were waterlogged during the May sampling. None of the tillers sampled in this survey exhibited BYD symptoms in the field,

The number of field samples in which BYDV was found for each of the two sampling periods is shown in Table 2. An analysis of the successful transfers of BYDV from the youngest and second youngest leaves by the two species of aphids is shown in Table 3. R. padi was considerably more efficient in transferring the virus from the samples than M. avenae, and transfers were more successful from the youngest leaves than from the second youngest. Considering both sampling periods, virus was transmitted from 35 samples by R. padi only, from 6 samples by M. avenae only, and from 3 samples by both vectors; each of these samples represented all the leaves from one field. The average number of test plants per sample that became infected in the successful transfers, of 4 plants inoculated, was 2 for R. padi and 1 for M. avenae, when feeding on the youngest leaves, and 1.3 and 1, respectively, when feeding on the second youngest leaves. Generally symptoms on the oat test plants were moderate to mild, though in a few cases symptoms were severe.

Table 3. Proportion of successful transfers of barley yellow dwarf virus from detached wheat leaves to 'Clintland' oat test plants by Rhopalosiphum padi and Macrosiphum avenae, and from younger and older wheat leaves

Aphid vector or age of wheat leaf	Sampling period: May Number*	May %	June Number*	June %	Total Number*	Total %
R. padi	20/340	5.9	59/448	13.2	79/788	10.0
M. avenae	4/344	1.2	5/452	1.1	9/796	1.1
Younger leaf†	20/356	5.6	40/480	8.3	60/836	7.2
Older leaf†	4/328	1.2	24/420	5.7	28/748	3.7

* Number of test plants infected/number inoculated.

† In the May samples leaves 3 and 4 were tested: in the June samples, leaves 1 and 2 (where flag leaf = 1).

Five of the six isolates selected for characterization were transmitted from field samples by R. padi only, while the other (isolate number 3, Table 4) was transmitted by both R. padi and M. avenae. Two of these isolates were from the first period of sampling and four from the second period. The pattern of transmission for isolates 1 and 2 (Table 4) and the symptoms they produced on oats were similar to those of the strain transmitted specifically by R. padi (4,9). R. padi was the most efficient vector for isolate 3, but the occasional transmissions by M. avenae and A. dirhodum, and a stunting effect on oats, often so severe as to kill the plants, indicated the possibility that a mixture of strains was present.

Isolates 4, 5, and 6 were transmitted with low efficiency by R. padi and S. graminum, but were transmitted much more efficiently by R. maidis. The pattern of transmission for these isolates was, therefore, similar to that of the strain specific for R. maidis (4,9). Isolates of this strain are occasionally transmitted by R. padi and S. graminum, and the high temperatures that sometimes occurred in the greenhouse during the inoculation feeding may have increased the efficiency of these two vectors (10). The mild symptoms on oats of these three isolates were also characteristic of the strain specific for R. maidis.

Fig. 1 shows the distribution of fields sampled in the survey. The fields where virus was found in the second sample occur at random throughout the area surveyed, suggesting that the geographical position of a field does not increase or decrease its chances of being infected.

Discussion

Virus was recovered from more than half of the fields sampled and this is considered to be a very conservative estimate for the following reasons.

Three of the six isolates that were examined more intensively were found to be characteristic of the R. maidis - specific strain, and it is highly likely that the use of R. maidis with the other two species would have resulted in detection of virus in a larger proportion of the samples. In many of the successful transfers of virus from field samples only one of the four test plants inoculated by a given species became infected, possibly the result of using inefficient vectors for the strains concerned as, for instance, R. padi for the R. maidis strain. On the other hand, the low percentage transmission could be due to a low number of culms infected or to a low virus content in infected leaves. Also

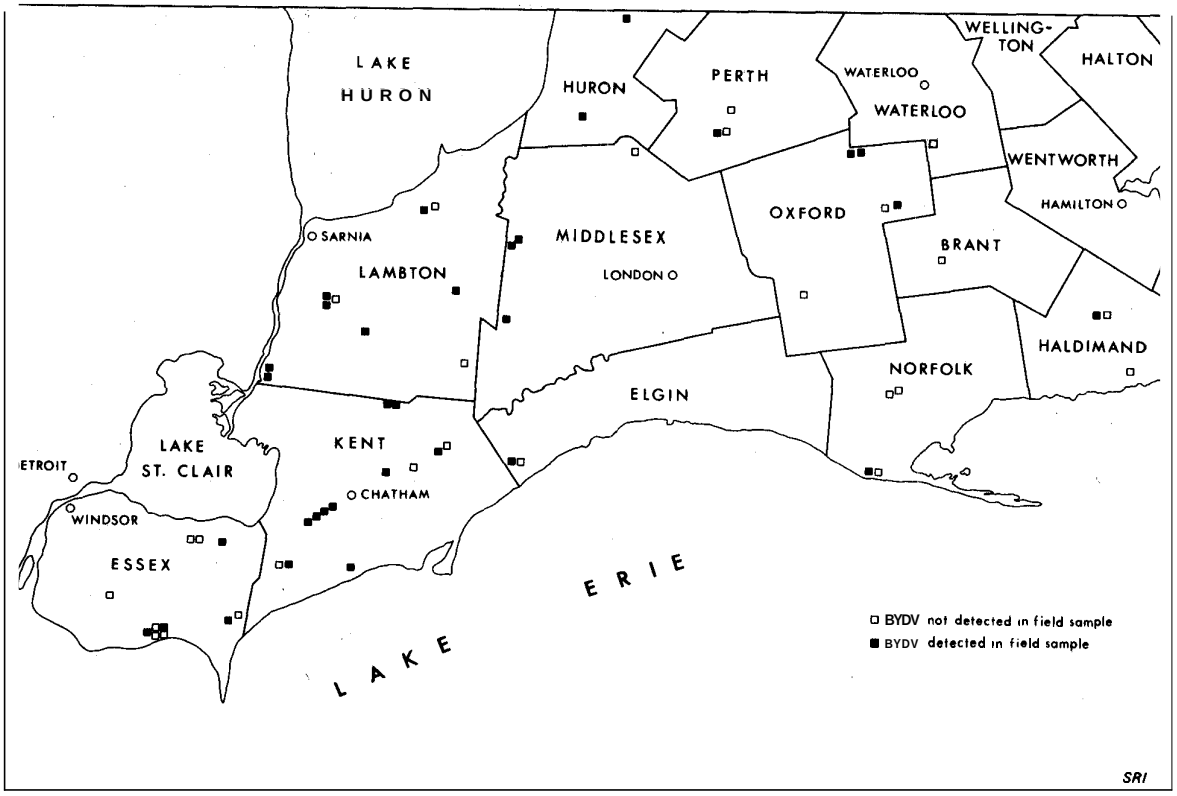

Figure 1. Distribution of barley yellow dwarf virus in winter wheot crops in southwestern Ontario, June 1969.

deterioration of leaves was probably a main factor in reducing the efficiency of transmissions. During both sampling periods (Table 3), the percentage of successful transfers was higher from the younger leaves, which showed less deterioration than the older ones. Similarly the second sampling had more successful transfers than the first sampling, when the leaves had deteriorated more due to excessive soil moisture. Consequently the increase in infection during the second sampling period is probably due to a difference in efficiency of transmission or to an increase of BYDV with the passage of time.

When comparing the distribution data for the virus from this survey with other data (1,2,11,14), it is important to note that the techniques used are different. The culms sampled in this survey were selected at random with no regard to BYD symptoms whereas other surveyors have used this as a means of diagnosing the disease. James (6) pointed out that approximately 90% of apparently 'healthy' leaf samples were infected with BYDV when random culms of barley were selected in the field. A similar result was obtained in this survey for although no obvious symptoms of BYD were observed in the field (no methodical check was conducted) more than 50% of the samples were infected. These findings stress the importance of selecting a random sample of culms when studying the distribution of the disease, especially if an attempt is being made to study the distribution of the disease within a particular crop. The sampling technique used in this survey has certain advantages over the 'ad hoc' sampling methods usually employed, because it is simple and well defined; it is therefore capable of being repeated by different people and allows valid comparisons to be made regarding the prevalence of the virus in different years, regions, varieties, etc. Although this survey was designed only to establish the number of field samples infected, some indication of the level of infection within the infected fields can be obtained. For the sample of twelve culms to be classified infected, it follows that at least 1 culm is infected; this is equivalent to 9% of culms infected. Assuming binomial distribution, the 95% confidence limits for the true infection levels are 0.2% and 39%. For the fields with infected samples, this implies that in 95 cases out of each 100 the true mean for the percentage of tillers infected will be between 0.2% and 39%. In practice,

Table 4. Transmission of six isolates of barley dwarf virus by five species of aphids

BYDV isolate	Macrosiphum avenae	Acyrthosiphon dirhodum	Rhopalosiphum padi	Schizaphis graminum	Rhopalosiphum maidis
1	0	0	15	1	0
2	0	0	18	1	0
3	1	2	14	1	0
4	0	0	1	1	10
5	1	0	3	2	13
6	0	0	3	2	13

* Figures represent number of 'Clintland' oat plants infected out of 20 plants inoculated, using five aphids per plant.

as in this survey, the amount of laboratory work involved in transmission tests severely restricts the usefulness of survey results.

Judging from the relative efficiency of transmission of the two species of aphids in the original isolations, from the symptoms of the isolates on oats, and from the characterization of six of the isolates, most of the isolates appeared to belong to the R. padi- or R. maidis-specific strains. The few isolates that were transmitted by R. padi and M. avenae, or by M. avenae only, may have been similar to the non-specific strain and the M. avenae-specific strains, respectively, but further testing of the isolates would have been necessary to prove this. Isolates similar to the latter two strains have been reported earlier from Ontario (12,14).

No estimate can be made of the losses due to BYD in winter wheat in Ontario because the data from this survey do not estimate the incidence of BYD within the crop. However, even if these data were available, present methodology on yield losses due to BYD would not allow extrapolation from survey results. Heavy losses in the yield of several winter wheat varieties have been demonstrated from controlled inoculation of experimental plots (1,5,8). In Kansas and South Dakota, infection in the autumn usually resulted in greater losses than infection in the spring (5,8). In New Zealand, however, a late infection was more injurious than an early infection (17), and in one particular year the average losses in that country on winter-sown wheat were estimated at 25% of the yield (15).

In view of these reported losses, and the fact that more than 50% of the fields were infected in southwestern Ontario in 1969, the need for meaningful methodology on yield losses due to BYD is apparent. Further sampling and testing will be necessary to determine the prevalence and incidence of the disease and to determine whether any large annual variations occur; information on the predominant virus strain is also needed, The susceptibility to BYDV of winter wheat varieties currently grown in Ontario should be determined so that recommendations can be made regarding our present varieties.

Acknowledgments

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