Metabolism and Carbohydrate Composition in Barley Yellow Dwarf Virus-Infected Wheat

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ABSTRACT

Field performance factors and physiological factors of healthy and barley yellow dwarf virus (BYDV)-infected plants were compared in 10 cultivars of field-grown hard red spring wheat. Twenty-seven of 46 factors measured were significantly altered after BYDV infection. Barley yellow dwarf virus infection reduced the average yield by 53%, the photosynthetic rate by 45%, and the chlorophyll content by 80%. The soluble carbohydrate content of diseased leaf blades was increased by about 400%. Excessive carbohydrate accumulation occurred in leaf sheaths, but not in the culms or spikes. Starch accumulation followed the same pattern, but was less extensive. Barley yellow dwarf virus infection also results in stunting of all plant parts. The basic effect of BYDV infection appears to be the development of a resistance to the translocation of photosynthate from the leaf blade to leaf sheath to culm or spike.

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When seedlings of susceptible barley (*Hordeum vulgare*) C.I.666 'Black Hulless' are inoculated with barley yellow dwarf virus (BYDV), there is a rapid and massive accumulation of soluble and storage carbohydrates (6, 9, 10). This accumulation is accompanied by loss of chlorophyll, rapid decline in rate of photosynthesis, and sharp increase in the rate of respiration in lower leaves of susceptible barley seedlings (4, 6, 10). Goodman et al. (2) reported the accumulation of fructose, glucose, sucrose, and fructosans in leaves of BYDV-infected oats, and Watson & Mulligan (12) obtained a positive iodine test for starch in BYDV-infected wheat, but not in healthy wheat leaves.

We reported the effects of BYDV on components of yield and on the physiology of 10 cultivars of hard red spring wheat adapted to the Northern Great Plains of North America (7). In these studies, not only were there major differences between healthy and diseased tissues, but there were also significant differences between physiological patterns of different leaves on the same plant. In an effort to link BYDV-disrupted physiology with loss of yield, we conducted some preliminary experiments and found different levels of soluble carbohydrates and starches in different tissues above the top vegetative internode on maturing wheat. The following experiments were designed to obtain more detailed information.

MATERIALS AND METHODS.–Ten cultivars of hard red spring wheat (*Triticum aestivum* L. em Thell, North Dakota 363, North Dakota 405, Minnesota II-5430, 'Chris', 'Crim', 'Polk', 'Justin', 'Selkirk', 'Lee', and 'Thatcher') were planted in a split-plot design with four-row, 12-ft plots replicated 3 times for each treatment. Planting was done on 26 April 1968. On 27 May, the center two rows of half the plots were infested with *Rhopalosiphum padi* (Linnaeus), the oat-bird cherry aphid, carrying a severe strain of BYDV. Three days later, the aphids were killed by spraying with dimethoate. On 13 and 21 June, the plots were sprayed with maneb to reduce rust infection on the leaves. Typical symptoms of BYDV were uniformly present throughout the inoculated plot. One of the two center rows in each planting was used for physiological measurements, and the other center row for field performance determinations. On 10, 11, and 12 July, when the kernels were in the late milk or early dough stage, tissue samples were taken for analysis.

Photosynthetic and respiratory measurements.–Three average-sized culms typical of the cultivar and treatment were cut off below the top vegetative node, and the bases of the culms placed in test tubes of distilled water. The plants were placed in a sealed chamber in a water bath at 23 C, and gas exchange rates measured by an infrared CO_2 gas analyzer. The rate of photosynthesis was measured under 4,000 ft-c of illumination, and respiration was measured in total darkness. Photosynthetic rates were corrected for dark respiration. When the gas exchange measurements were complete, the fresh weight and chlorophyll content of the flag leaf was measured (1, 4), and the fresh weight and dry weight determined on the remainder.

Starch and soluble carbohydrate determination.–Separate tissue samples representing the flag leaf lamina, leaf sheath, culm, and spike were prepared for analysis of starch and soluble carbohydrates. The fresh weight was recorded; and, after drying in a vacuum drying oven for 24 hr, dry weights were determined. Dried tissue was pulverized in an oscillating ball mill, and the powder vacuum-dried and sealed for storage in a refrigerator until analyzed. Duplicate 200-mg subsamples of the dried material were extracted 3 times with hot 80% ethanol to remove the soluble carbohydrate fraction. Residue from this extraction was refluxed for 1 hr with 0.2 N H_2SO_4 to hydrolyze and extract the

starch (11). The quantity of soluble carbohydrate and hydrolyzed starch in each sample was then determined by the Shaffer-Somogyi method (3).

Field performance determinations.—Notes were taken on days to heading when 50% of the heads were fully emerged from the boot. Plant height was determined by measuring three or four average plants in each row. At harvest time, plants from two 1-ft samples were taken from each row, the spikes counted, and the weight of the threshed grain was recorded. The grain was passed through a seed counter; and number of kernels per head, weight of grain per head, and 1,000-kernel weight were calculated.

All of the data were analyzed by analysis of variance.

RESULTS.—*Field performance.*—A summary of the data and the results of analysis of variance are shown in Tables 1 to 4. Five out of seven field performance factors were altered to a highly significant degree by BYDV infection. The same five factors showed significant differences between cultivars. Only kernel plumpness and tillering were not altered to a significant degree by BYDV or cultivar effects. Among these factors, only plot yield showed a significant cultivar by treatment effect. Yield data for each of the 10 cultivars is shown in Table 4.

Photosynthesis and respiration.—When comparing photosynthetic rates of entire plant tops (Table 2), I found a 45% reduction in the diseased plants. This relationship is useful in considering the productive capacity of a plant or a field; however, when considering physiological rates, it is better to compare

TABLE 1. Effects of barley yellow dwarf virus on field performance of 10 cultivars of hard red spring wheat, and F ratios from analysis of variance

Factor measured	Avg		% of control (diseased/healthy X 100)	Cultivar effect F^a	Treatment effect F^b	Cultivar X treatment effect F^c
	Healthy	Diseased				
Kernels/spike	18.9	11.6	62	4.9**[d]	321**	1.3
Wt kernels/spike (g)	0.58	0.29	49	15.4**	1284**	1.4
1000-kernel wt (g)	32.2	24.2	75	1.3	16.2	1.0
Spikes/ft row	35.9	33.3	93	1.6	4.3	1.8
Plant height (cm)	34.3	29.9	87	4.7**	645**	1.7
Days to head	52.1	54.3	104	11.5**	159**	1.9
Plot yield (g)	107.1	50.7	47	2.6*	423**	3.3*

[a] $P_{01} = 3.60; P_{05} = 2.46$ [a,c] $P_{01} = 3.60; P_{05} = 2.46$.
[b] $P_{01} = 98.5; P_{05} = 18.5$ or [b] as shown.
[c] $P_{01} = 3.60; P_{05} = 2.46$.
[d] **, = Significant at .01 level; * = significant at .05 level.

TABLE 2. Effects of barley yellow dwarf virus on rates of photosynthesis and respiration in 10 cultivars of hard red spring wheat and F ratios from analysis of variance

Factor measured	Avg		% of control (diseased/healthy X 100)	Cultivar effect F^a	Treatment effect F^b	Cultivar X treatment effect F^c
	Healthy	Diseased				
			Photosynthesis (μliters CO_2)			
1 Total	81.4	44.5	55	1.6	133**	1.4
2 g fresh wt	20.9	15.9	76	2.6*	8.5	1.8
3 g dry wt	69.3	50.3	73	2.6*	53.7*	1.4
			Respiration (μliters CO_2)			
4 Total	21.4	14.3	67	2.2	827**	0.4
5 g fresh wt	5.5	5.1	93	1.8	0.2	0.8
6 g dry wt	18.2	16.2	89	1.5	6.2	0.5

[a] $P_{01} = 3.60; P_{05} = 2.46$ [a,c] $P_{01} = 3.60; P_{05} = 2.46$.
[b] $P_{01} = 98.5; P_{05} = 18.5$ or [b] as shown.
[c] $P_{01} = 3.60; P_{05} = 2.46$.
[d] ** = Significant at .01 level; * = significant at .05 level.

TABLE 3. Effects of barley yellow dwarf virus on the composition of the tissues of 10 cultivars of hard red spring wheat and F ratios from analysis of variance

Factor measured	Avg Healthy	Avg Diseased	% of control (diseased/healthy X 100)	Cultivar effect F^a	Treatment effect F^b	Cultivar X treatment effect F^c
			Fresh wt (g)			
1 Total	3.89	2.80	72	2.5*	274**	2.3
2 Leaf blade	1.21	0.95	79	1.6	4.4	1.9
3 Leaf sheath	0.48	0.38	79	0.9	33.6**	0.5
4 Culm	0.89	0.59	67	1.5	26.5**	0.5
5 Spike	1.31	0.88	67	3.9**	161**	0.8
			Dry wt (g)			
6 Total	1.18	0.89	75	3.3*	5427**	0.4
7 Leaf blade	0.40	0.35	87	1.2	1.4	1.5
8 Leaf sheath	0.12	0.10	86	1.5	15.9	0.6
9 Culm	0.25	0.17	68	2.7*	76.7*	0.2
10 Spike	0.41	0.27	66	4.1**	354**	0.8
			Chlorophyll (mg/g)			
11 Total	3.3	0.7	21	1.2	352**	0.6
12 g fresh wt	3.0	1.1	36	0.8	983**	0.9
13 g dry wt	8.4	2.0	25	1.5	78.6*	0.8
			Soluble carbohydrates (mg/g fresh wt)			
14 Total	22.4	34.0	155	2.5*	13.9	4.7**
15 Leaf blade	12.2	49.6	407	4.6**	31.3*	4.9**
16 Leaf sheath	13.2	19.6	149	4.8**	1089**	4.8**
17 Culm	17.4	17.5	101	4.7**	0.007	1.1
18 Spike	39.3	34.6	88	0.4	2.3	1.7
			Soluble carbohydrates (mg/g dry wt)			
19 Total	70.7	102.8	146	2.6*	15.4	3.9**
20 Leaf blade	36.3	131.2	361	4.1**	46.0*	3.7**
21 Leaf sheath	44.7	63.7	143	4.4**	132.6**	4.4**
22 Culm	58.8	59.0	100	5.5**	0.001	0.7
23 Spike	119.3	108.7	91	0.3	1.2	1.1
			Starch (mg/g fresh wt)			
24 Total	20.7	23.1	111	2.4	9.3	0.8
25 Leaf blade	17.8	25.0	140	1.3	3.2	0.8
26 Leaf sheath	16.0	18.8	118	4.9**	21.7*	0.7
27 Culm	13.5	13.7	101	1.6	0.2	1.1
28 Spike	30.1	29.1	97	1.5	0.5	1.1
			Starch (mg/g dry wt)			
29 Total	63.7	69.7	109	1.0	8.1	1.2
30 Leaf blade	48.9	68.3	140	2.3	32.0*	1.7
31 Leaf sheath	54.5	62.1	114	4.9**	12.1	0.8
32 Culm	46.5	43.3	93	1.0	0.5	2.8*
33 Spike	91.3	91.0	99	1.2	0.002	0.9

[a] $P_{01} = 3.60; P_{05} = 2.46$ [a,c] $P_{01} = 3.60; P_{05} = 2.46$.
[b] $P_{01} = 98.5; P_{05} = 18.5$ or [b] as shown.
[c] $P_{01} = 3.60; P_{05} = 2.46$.
[d] ** = Significant at .01 level; * = significant at .05 level.

equal amounts of tissue. On this basis, photosynthesis is reduced only 25% in diseased plants. From this comparison, it is apparent that one-half of the reduction in total photosynthesis in the diseased plant was due to stunting, and one-half to the decrease in the rate of photosynthesis.

Changes in total respiration of the tissues in the plant top were related only to the reduction in the amount of tissue present; and there was no significant difference between healthy and diseased plants in rates of respiration of equal amounts of fresh or dry tissue.

Fresh weight and dry weight.—The fresh and dry weight of the diseased plants were reduced by 28 and 25%, respectively (Table 3). However, this degree of stunting was not uniform over all parts of the plant top. The leaf blade and sheath were stunted about 20%, whereas the culm and spike were 33% smaller. Part of this differential effect is due to excessive accumulation of carbohydrates in the diseased flag leaf blade and sheath leading to an abnormal increase in the weight of these tissues, whereas no excessive accumulation occurs in the culm or spike. The degree of stunting of diseased plants as estimated by plant height is only about 13% as contrasted with the 25 to 28% reduction in size estimated by the weighing of the tissue.

Chlorophyll content of the flag leaf blade.—The flag leaf blades of BYDV-infected plants are usually quite chlorotic, but their general appearance belies the true loss of chlorophyll. This loss of chlorophyll greatly exceeds the loss of photosynthetic capacity. While photosynthesis per gram fresh weight of tissue was 75% of normal, there was only 35% of the normal complement of chlorophyll present.

Soluble carbohydrates.—Soluble carbohydrate content is expressed on the basis of fresh weight and then dry weight, because it is known that BYDV infection can alter the dry weight composition of the plant (4, 6). The soluble carbohydrate content is reported as the total for the composite plant top, then subdivided into the specific content of the leaf blade, leaf sheath, culm, and spike. Differences in the size of plants are not taken into consideration.

The average soluble carbohydrate content per gram fresh weight of tissue is more than 50% higher in BYDV-infected tissues, but this is not statistically significant due to the large variance resulting from differences between cultivars (Table 3). There is a highly significant cultivar by treatment interaction (Table 4). Furthermore, the excess carbohydrate is not uniformly distributed in all tissues. A diseased flag leaf lamina has from 3.5 to 7 times the normal amount of soluble carbohydrates; the infected leaf sheath has from 30% less than normal to 3 times more than normal; and the culm and spike have approximately normal soluble carbohydrate content. Results are nearly the same, whether expressed on a fresh weight or dry weight basis.

Starch.—Analysis of starch content of the tissue closely resembles analysis of carbohydrates, but with a lower magnitude of difference between healthy and diseased plants. When the whole plant top is considered as a unit, there is a nonsignificant 11% increase in starch per gram fresh weight of diseased tissue. Diseased leaf blades and leaf sheaths have 40% and 15% more starch, respectively, than do control tissues. Starch content of the infected culm, like soluble carbohydrate content of the leaf sheath, can vary from well below normal to well above normal, depending upon the cultivar selected (Table 4).

DISCUSSION.—The 10 cultivars of hard red spring wheat used in this field test differed significantly in their response to BYDV infection. The reduction in yield ranged from 70 to 40%, depending upon the cultivar. Viral invasion produced no significant difference between the cultivars in degree of stunting, effect on photosynthesis, or chlorophyll content, although all of these factors were significantly altered.

TABLE 4. Effects of barley yellow dwarf virus on 10 cultivars of field grown hard red spring wheat

	Yield in grams per 8-ft row			Soluble carbohydrate mg/g fresh wt									Starch mg/g dry wt culm		
				Total			Leaf blade			Leaf sheath					
	H[a]	D[b]	%[c]	H	D	%	H	D	%	H	D	%	H	D	%
ND 363	115.6	59.0	51	24.5	31.2	127	9.1	39.5	433	14.6	26.3	181	50.7	41.1	81
ND 405	112.3	59.4	53	20.8	30.9	149	11.4	41.0	359	11.4	16.1	141	48.3	40.4	84
Minn II-5430	119.6	52.7	44	23.2	38.6	167	14.7	56.7	386	15.1	23.0	153	48.1	45.9	95
Chris	112.3	46.1	41	24.0	35.5	148	14.6	55.5	378	17.5	15.7	90	45.8	45.1	99
Crim	104.9	60.1	57	23.6	30.0	127	9.9	35.6	358	14.4	18.6	130	43.4	51.1	118
Polk	109.7	50.0	46	17.3	43.2	250	9.0	68.7	765	11.2	34.2	306	51.5	46.9	91
Justin	83.1	51.0	61	18.0	28.2	157	10.8	38.6	359	7.0	13.1	186	42.3	46.4	110
Selkirk	101.7	30.5	30	25.4	39.6	156	16.0	62.4	391	13.8	21.2	154	45.3	65.0	143
Lee	108.2	44.9	42	26.3	32.5	124	15.9	59.7	375	15.9	10.6	67	46.2	46.9	101
Thatcher	103.5	52.9	51	21.1	29.8	141	10.4	38.5	369	11.0	17.5	160	43.6	44.9	103
Avg	107.1	50.7	47	22.4	34.0	155	12.2	49.6	407	13.2	19.6	149	46.5	47.3	102

[a] H = healthy.
[b] D = BYDV-infected.
[c] % = D/H X 100.

In diseased leaves, chlorophyll content is reduced much more than the rate of photosynthesis; therefore, the rate of photosynthesis per unit chlorophyll is much higher in diseased leaves than in healthy leaves. This implies that chlorophyll content is not rate-limiting for photosynthesis and BYDV-infected plants can compensate in part for the loss of chlorophyll. This ability may also exist in barley, where Jones & Catherall (9) observed that the degree of leaf-yellowing or chlorosis is unreliable for assessing the level of tolerance to BYDV. Total photosynthesis of a diseased plant top was reduced 25% by stunting; and another 25%, by a lowered rate of photosynthesis. Thus, the yield of grain from a diseased plant cannot be predicted by any one factor such as the degree of chlorosis, the extent of stunting, or the inhibition of photosynthesis. All of these factors are undoubtedly related to yield reduction, but the most obvious visual symptom, loss of chlorophyll, may be the least reliable in estimating yield losses.

Analysis of soluble carbohydrate and starch content of the various tissues revealed that although there were significant differences in carbohydrate content of the different cultivars, no method of comparison could be found which would relate the carbohydrate content of the tissue with the yield of healthy or BYDV-infected plants. Polk is an extreme example. Its soluble carbohydrate level was consistently among the lowest for the healthy cultivars, and highest for the diseased. Because of this trend, when the data are expressed as percentage of control, Polk always has a very high percentage accumulation of soluble carbohydrates. These observations in no way correlate with the yield of Polk, which is just average for the 10 cultivars, both healthy and BYDV-infected.

In previous experiments (6), we found very large accumulations of soluble carbohydrates and starch in the second and third leaves of barley seedlings infected with BYDV. This report supports those observations, and extends our knowledge to other tissues and other species. In the previous experiments with young barley leaves and in these experiments with more mature wheat, we have seen that soluble carbohydrates and starch do not accumulate uniformly in infected tissues; nor do the carbohydrates accumulate in proportion to the rate of photosynthetic production. There is a rather substantial rate of photosynthesis in the plant spikes and culms (8), and yet there is no apparent accumulation of soluble carbohydrates.

The simplest explanation that accounts for our results is to postulate some type of resistance to translocation of carbohydrates in BYDV-infected leaves. If there were a complete inhibition of translocation, there would be no grain fill at all, and a massive accumulation of carbohydrates in all photosynthetic tissues. This is not what was observed. The results suggest an analogy between BYDV-affected translocation and the flow of an electric current along a fine wire where the amount of electrical current flowing is inversely proportional to the length of the wire. In the case of diseased plants, it appears that the amount of translocation is inversely proportional to the distance between the source and the sink. Where the leaf blade acts as the source and the grain acts as the sink, a considerable distance must be traversed by the carbohydrates, and resistance reduces the movement to a minimal value. In the spike, where the glumes are the source and the grain is the sink, the distance of translocation is very short. Resistance to movement is inconsequential, and a nearly normal balance of flow occurs.

Cytological studies (5) of leaves show that BYDV is confined to phloem tissues, and only a few elements within a vascular bundle contain virus particles. It is difficult to account for the apparent massive disruption of translocation on the basis of the number of phloem cells showing damage.

Uninfected plants of the 10 cultivars were similar in their ability to produce carbohydrates (photosynthesis), but they differed significantly in their ability to translocate the photosynthate; hence the differing levels of carbohydrate in the tissue. The cultivars also differed significantly in their ability to store photosynthate in the grain; hence the difference in yield.

Barley yellow dwarf virus infection greatly reduces the rate of photosynthesis in the flag leaf blade and flag leaf sheath, but not in culm stem spike (8). This correlation between the reduced photosynthesis and excessive accumulation of carbohydrates suggests a feedback inhibition of photosynthesis. However, while diseased plants showed a cultivar difference in accumulation of carbohydrates, there were no cultivar differences in the amount of photosynthetic inhibition.

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