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Barley yellow dwarf virus: effects on carbohydrate metabolism in oat (*Avena sativa*) during cold hardening

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SUMMARY

Barley yellow dwarf virus (BYDV) causes significant losses in yield and in overwintering ability of winter cereals. Mechanisms by which the physiology of plants is affected by the virus are not clear. To see how carbohydrates in the crown of winter cereals were affected by BYDV, fructan isomers of degree of polymerization (DP) 3–5, fructan DP > 6 and the simple sugars, glucose, fructose and sucrose, were measured before and during cold hardening in three oat (*Avena sativa* L.) cultivars, 'Wintok', 'Coast Black' and 'Fulghum'. On a fresh weight basis fructan DP > 6 decreased by 50% in infected 'Wintok' and 'Coast Black' and by 25% in 'Fulghum'. Two DP3, one DP4 and one DP5 isomer were significantly higher than non-infected controls. The percentages of simple sugars in infected crowns were significantly higher than controls in all three cultivars in every week except the first week of hardening. Crude enzyme extracts from BYDV infected plants incubated with sucrose suggested higher invertase and lower sucrose-sucrosyl transferase activity. When incubated with 1-kestose and neokestin, no significant difference was found in fructose fructosyl transferase or in hydrolase activity. The activity of unidentified enzymes catalysing the synthesis of larger (DP > 5) fructan was altered by BYDV. The decrease of carbohydrates in the crown induced indirectly by BYDV may alter the plant's capacity to regenerate tillers in the spring. The ability of plants to prevent or tolerate carbohydrate fluctuations induced by BYDV infection may be an important genetically regulated characteristic for developing virus-resistant cultivars.

Key words: Barley yellow dwarf virus, carbohydrate metabolism, cold hardening, *Avena sativa* L. (oat), fructans.

INTRODUCTION

Barley yellow dwarf is a disease of small grains induced by phloem-specific, aphid-transmitted viruses (Gildow, 1987) belonging to the luteovirus group (Waterhouse Gildow & Johnstone, 1987). Barley yellow dwarf virus (BYDV) can significantly reduce grain yield (Andrews & Sinha, 1991; Endo & Brown, 1963) and freezing tolerance (Andrews & Sinha, 1991; Andrews & Paliwal, 1986) in cereal crops.

Photosynthesis is lower in leaves of BYDV-infected plants (Jensen, 1968*a*) than healthy plants. This may be due to feedback inhibition from sucrose accumulation (Williams *et al.*, 1992) in leaves resulting from virus-induced phloem sieve-element necrosis, disrupting assimilate transport. Effects of BYDV on host plant respiration are inconclusive. On the basis of either f. wt or leaf area, Jensen (1968*a*) reported that respiration rates were higher in infected barley plants but were unchanged when expressed on a d. wt basis. He also reported that respiration rates on a d. wt basis were higher than controls in plants grown at 21 and 27 °C but lower when grown at 10 and 15 °C (Jensen, 1968*b*). Andrews & Sinha (1991) reported an increase in the respiration rate of two wheat cultivars infected with two strains of BYDV. The effect was greater in plants infected with the more virulent strain.

As expected from measurements of photosynthesis and respiration, BYDV infection significantly alters

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Abbreviations: SST, sucrose-sucrosyl transferase – EC 2.4.1.99; FFT, fructose fructosyl transferase – EC 2.4.1.100.

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carbohydrate concentrations in cereals. Jensen (1972) and Fereres *et al.* (1990) found significantly higher carbohydrate concentrations in leaves of BYDV infected wheat. Goodman, Watson & Hill (1965) reported higher fructan per unit f. wt in leaves of oat infected with BYDV than is uninfected controls. However, since at some stages after infection the water content of infected leaves is lower than uninfected controls (Jensen, 1968*b*), there may have been little or no difference in fructan if Goodman's data had been expressed on a d. wt or per unit area basis. On a d. wt basis Andrews & Sinha (1991) found lower fructan concentrations in leaves of infected wheat. They also reported (1991) lower fructan in the below ground portion of BYDV-infected wheat stems (crown); additionally, if total fructan had been subtracted from total nonstructural carbohydrate, the remainder (presumably simple sugars) would have been higher in infected plants than in non-infected plants. In a similar study with oats, the percentage of all size classes of fructan greater than DP (degree of polymerization) 4 was significantly lower in crowns but % sucrose, glucose and fructose was significantly higher in infected plants (Livingston & Gildow, 1991).

The ability of a winter cereal to accumulate fructan may affect physiologically important phenomena such as tolerance to environmental stress and tiller regeneration in spring. Therefore, how fructan synthesis is altered in BYDV-infected plants could be relevant to selecting agronomically desirable germplasm. The purpose of this study was to compare infected and non-infected oat plants for differences in sugar and fructan oligomers and to see if those differences were related to the activity of fructan-metabolizing enzymes.

MATERIALS AND METHODS

Plant material

Three cultivars of oat (*Avena sativa* L.) were tested: 'Fulghum', 'Wintok', (both check cultivars in the Uniform Winterhardiness Oat Nursery, coordinated by the US Department of Agriculture in Raleigh, North Carolina) and 'Coast Black', a BYDV susceptible cultivar. Seeds were planted in plastic tubes filled with a mixture of sand, sphagnum peat moss and perlite. Tubes were placed in racks and suspended over plastic pans that had holes drilled in the base to allow drainage. A 5-cm layer of the soil mixture was placed in the pans allowing contact with the tube bases. Plants were watered twice weekly with a complete nutrient solution (Livingston, 1991) and were flushed three times weekly with tap water. Plants were grown for 10 d in a growth chamber at 10–13 °C and a 12-h photoperiod with 180 μmol m^{-2} s^{-1} light (80% cool fluorescent and 20% incandescent).

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Viral inoculation

Ten d after planting, individual plants at the two-leaf stage were infested with 10 viruliferous aphids (*Rhopalosiphum padi* L.), which had been given a 48-h acquisition feeding on detached leaves from oat infected with either the PAV or RPV isolates (Rochow, 1969) of BYDV or on healthy plants as a control.

Seedlings were individually caged and the aphids allowed a 3-d inoculation feeding in a growth chamber at 18–20 °C with a 24-h photoperiod. The aphids were then killed by removing the cages and fumigating the seedlings overnight with DDVP (*o,o*-dimethyl-2,2-dichlorovinyl phosphate). Sources and methods for maintaining the BYDV isolates and aphid vectors have been described (Gildow *et al.*, 1987).

Growing conditions

After killing the aphids, individual tubes containing plants were randomized within tubs and returned to the original growth chamber. Five wk after planting, (3.5 wk after infection) pans containing plants were transferred to a chamber at 2 °C with an 18-h photoperiod at 200 μmol m^{-2} s^{-1} for a cold-hardening treatment.

Dry weight measurements

At the end of week 4, week 5 (1 wk of hardening), week 7 (2 wk hardening) and week 9 (4 wk hardening), individual plants were removed from tubes, washed in iced water and trimmed of roots. Plants were cut into four parts: 1 cm of the stem base closest to the roots (crown); the adjacent 2 cm (lower stem); the upper 2 cm of stem (upper stem); and leaves. Tissue from individual plants was placed in aluminum dishes and weighed before and after drying for 16 h (constant weight) at 90 °C.

Carbohydrate extractions

Plants grown and harvested as described for d. wt measurements were ground at room temperature using a stainless-steel grinder (Livingston, 1990). Crown tissue from six plants (one replicate) was bulked and completely ground in 30 s. The ground tissue was rinsed into a beaker with 15 ml of water and then heated to 90 °C for 15 min. The water was decanted from the beaker and the ground tissue was washed twice with water at room temperature; the three washings were combined. Carbohydrates were not detectable in a fourth extraction. Two aliquots from each sample (one for quantification of F, G, S, DP6 and DP > 6 and one for quantification of DP3–5 oligomers) were filtered through a 0.45 μm filter in preparation for HPLC analysis.

Chromatography

Sugars, and size-classes of Fructan, were separated by HPLC using a modified Bio-Rad (Richmond, CA, USA) Aminex HPX-42A (silver based) analytical column (7.8 × 300 mm) preceded by an anion and a cation exchange guard column. The column was permanently modified by passing 0.5 M $NaNO_3$, at a rate of 2 ml min^{-1}, through the column for approx. 18 h. This treatment eliminated on-column hydrolysis of smaller fructans and sucrose and improved the resolution of smaller (DP3 and 4) fructans, sucrose and simple sugars; resolution of larger (DP5–7) fructans, however, was slightly reduced in the modified column. The mobile phase consisted of water and the flow rate was 0.4 ml min^{-1}.

In a second aliquot DP3 to DP5 fructan oligomers were separated by an Altech Absorbosphere-HS analytical column (4.6 mm × 250 mm) (Altech, Deerfield, IL, USA) with water at a flow rate of 0.4 ml min^{-1}. A heater maintained the column at 35 °C.

Carbohydrates separated by both columns were detected by a Waters 410 refractive index (RI) detector (Waters, Milford, MA, USA). Unknown peaks were identified by co-chromatography with external standards containing the 10 oligomers, sucrose and a mixture of glucose and fructose. Oligomers were quantified by comparison of unknown peak areas to peak area curves of standards. Peak area was measured by a Waters Baseline 810 chromatography workstation on a microcomputer.

The structure of each oligomer was determined using a combination of gas chromatography–mass spectrometry (GC–MS) of partly methylated alditol acetates, analysis of the products of a partial acid hydrolysis and co-chromatography with oligomers whose structure had previously been determined (Livingston, Chatterton & Harrison, 1993) (Table 1).

Table 1. *The structure of fructans of DP3–DP5 in oat* (Avena sativa); *the sucrose moiety in each isomer is designated by* ***G–F*** *nomenclature*

Fructan structure	Sucrose moiety
DP3	
3a: 1-kestotriose (1-kestose)	**G–F**$_{1\text{-}2}$F
3b: 6^G-kestotriose (neokestose)	F$_{2\text{-}6}$**G–F**
DP4	
4a: 6^G,6-kestotetraose	F$_{2\text{-}6}$F$_{2\text{-}6}$**G–F**
4b: 1,1-kestotetraose (nystose)	**G–F**$_{1\text{-}2}$F$_{1\text{-}2}$F
4c: 6^G,1-kestotetraose	F$_{2\text{-}1}$F$_{2\text{-}6}$**G–F**
4d: 1 & 6^G-kestotetraose	F$_{2\text{-}6}$**G–F**$_{1\text{-}2}$F
DP5	
5a: 6^G,6,6-kestopentaose	F$_{2\text{-}6}$F$_{2\text{-}6}$F$_{2\text{-}6}$**G–F** / F$_2$
5b: 6^G,1 & 6-kestopentaose	F$_{2\text{-}6}$F$^1_{2\text{-}6}$**G–F**
5c: 6 & 6^G,6-kestopentaose	F$_{2\text{-}6}$F$_{2\text{-}6}$**G–F**$_{6\text{-}2}$F
5d: 1 & 6^G,6-kestopentaose	F$_{2\text{-}6}$F$_{2\text{-}6}$**G–F**$_{1\text{-}2}$F

Nomenclature is after that proposed by Waterhouse & Chatterton (1993). Methods used to determine structures are described in detail elsewhere (Livingston, Chatterton & Harrison, 1993).

Statistics

Standard errors were calculated from a two-way ANOVA with three replicates, and three cultivars. The statistical significance of differences of carbohydrates from controls were determined using a three-way ANOVA with three replicates, three cultivars and three treatments (control, infection with PAV and infection with RPV).

Virus determinations

The infected or non-infected condition of the test plants in each treatment was verified by enzyme immunosorbant assay (ELISA) (Gildow *et al.*, 1987). A 1 g sample of leaf tissue was powdered in liquid nitrogen, homogenized in 3 ml of ELISA sample buffer, and filtered through Miracloth (CalBiochem, San Diego, CA, USA). The detecting antiserum was polyclonal IgG to the PAV–NY or RPV–NY isolates of BYDV originally obtained from W. F. Rochow (Ithaca, NY, USA). Procedures for coating the microtitre plate, sample incubation, and alkaline phosphatase detection were as described previously (Gildow *et al.*, 1987). Absorbance of each sample was determined at 405 nm.

Enzyme extraction and incubation

Roots and leaves from plants at the identical stage as for carbohydrate analysis were removed. Approx. 3 cm of stem material were homogenized with 50 mM of acetate buffer (pH 5.5) in a Waring® Blender at 2 °C. The ratio of buffer to plant material was 4.25 ml to 1 g f. wt.

Solid material was removed first by pressing through a commercial coffee press then by centrifugation at 12000 ***g*** at 5 °C for 15 min. Protein was precipitated from the supernatant solution by the gradual addition of solid ammonium sulphate to 100% saturation while stirring at 5 °C. After centrifugation (12000 ***g*** at 5 °C for 15 min) the pellet was re-suspended in acetate buffer; 3 ml of protein solution was desalted by passing through a 10 cm bed of Biogel® P6DG (Econo-Pac 10 column) equilibrated with acetate buffer.

The reaction mixture consisted of 80 µl of enzyme preparation and 20 µl of substrate in 50 mM acetate buffer. The final concentration of substrates was sucrose: 20 mM (to assay for SST and invertase activities), 1-kestose: 10 mM (to assay for FFT

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Table 2. *Carbohydrates (mg g^{-1} f. wt) in 'Wintok' oat crowns just before and during a 5 wk cold hardening period*

Week		F	G	S	3a	3b	4a	4b	4c	4d	5a	5b	5c	5d	DP6	DP > 6	Total
5	Control	1.28	2.60	3.66	0.27	0.34	0.27	0.15	0.13	0.35	0.03	0.26	0.13	0.20	0.37	0.71	10.75
	Δ	0.03	0.14	−0.03	0.02	−0.02	−0.01	0.00	−0.03	−0.07	0.01	−0.04	−0.01	−0.05	−0.14	−0.23	−0.44
									*								
6	Control	2.23	5.38	20.03	5.26	4.20	1.86	0.69	0.92	3.71	0.17	2.38	0.64	1.14	2.70	3.64	54.94
	Δ	0.27	0.45	−2.29	−0.95	−0.30	−0.21	−0.15	−0.17	−0.25	−0.01	−0.24	−0.16	−0.05	−0.35	−0.26	−4.66
		*	*	**	*				*								
8	Control	2.64	5.89	13.84	3.14	4.15	4.18	1.82	0.87	2.90	0.67	3.90	1.95	2.14	8.36	14.70	71.15
	Δ	0.21	0.81	−0.27	−0.42	−0.24	−1.26	−0.49	−0.13	−0.39	−0.30	−0.45	−0.69	−0.71	−2.56	−5.23	−12.12
					*		*	*			*		**	*	**	**	*
10	Control	3.33	7.14	14.76	3.26	3.94	4.78	1.65	1.44	2.12	0.82	3.18	2.15	2.06	9.49	24.83	84.93
	Δ	−0.18	−0.30	−1.02	−0.16	−0.04	−1.14	−0.41	−0.42	−0.13	−0.21	−0.05	−0.71	−0.55	−3.39	−11.14	−19.87
															*	*	*

*, **: Δ was significantly different from controls at $P < 0.05$, 0.01, respectively.

Differences (Δ) between controls (not infected) and plants infected with the PAV isolate of the Barley yellow dwarf virus are also shown. Structures of fructan isomers are shown in Table 1.

activity) and neokestin (a neokestose-based mixture of fructan isomers, purified from oat, with an average DP of 12): 250 mg ml^{-1} (to assay for hydrolase activity). Relative proportions of DP 3–5 oligomers synthesized in a crude enzyme preparation at 30 °C for up to 3 h were identical to those found *in vivo* in plants exposed to 3 °C for 3 wk (Livingston, Knievel & Gildow, 1994). Therefore, to avoid lengthy incubation times at 3 °C extracts were incubated at 30 °C for 1.5 h with sucrose and 1-kestose and for 3 h with neokestin. Samples were heated to 100 °C for 4 min to stop the reaction. Reaction products were analysed by HPLC with the Absorbosphere-HS column as described previously.

RESULTS

Carbohydrates

Results from 'Fulghum' and 'Coast Black' were similar to those of 'Wintok' and results of RPV similar to those of PAV. Thus for simplicity only data for 'Wintok' infected with PAV are presented. Total carbohydrate in crowns of infected plants was lower (especially in week 8 and 10) whilst fructose and glucose were generally higher (Table 2); week 10 was an exception. Some DP3–5 fructan isomers were lower and some were higher than controls. Decrease of isomers in infected plants was not always related to initial amount. For example at week 8, even though the amount of 3b in controls was higher than that of 3a, in infected plants 3a decreased by nearly twice the amount that 3b decreased (Table 2). A similar result occurred with 5b and 5d (Table 2). Fructan of DP > 6 had an average DP of 12 and was a mixture of isomers of different size and structure based on neokestose (Livingston *et al.*, 1993); it was the carbohydrate affected most by BYDV with approx. a 35 % decrease of DP > 6 g^{-1} f. wt in week 8 and 10 (Table 2). DP > 6 was decreased by nearly 50 % in 'Coast Black' and by 25 % in 'Fulghum' (data not shown).

The percentage of individual carbohydrates relative to total carbohydrate was used to determine the proportion of each sugar and fructan isomer; carbohydrate percentages in infected crowns were then subtracted from that of their respective controls to determine how carbohydrate proportions were affected by BYDV (Fig. 1). In all three cultivars and in plants infected with both isolates the percentage of DP6 and DP > 6 was significantly lower than controls while glucose, fructose, sucrose and both DP3 isomers were higher (Fig. 1: 'Wintok' and PAV only); sucrose and 3a at week 6 were lower than controls. With some exceptions the effect was higher in plants hardened for 3 and 4 wk.

Within oligomers DP3–5 the magnitude of the effect was smaller but in a number of cases was statistically significant. For example, 3a, 3b, 4d and

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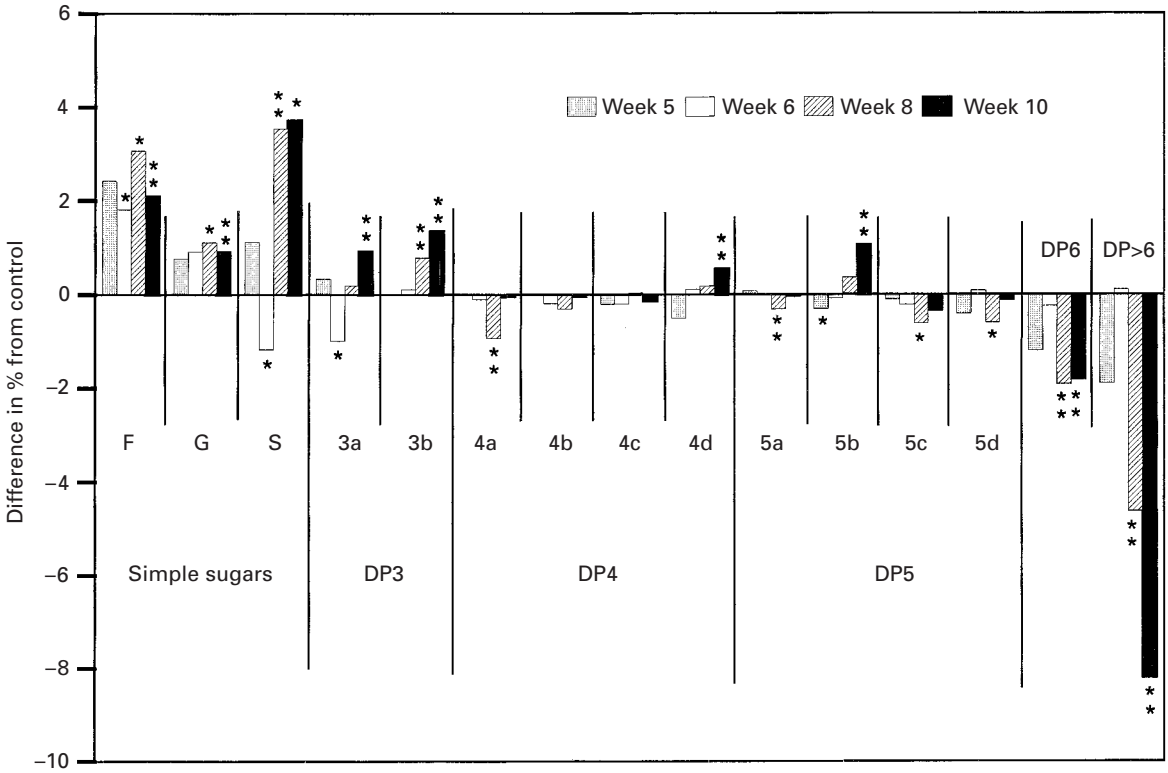

Figure 1. The effect of the PAV isolate on the percentage of carbohydrates in 'Wintok' oat before (week 5) and during (weeks 6–10) cold hardening. Bars are the difference between the percentage of carbohydrates in crowns of infected and non-infected plants. Values are significantly different from controls at **, $P = 0.01$; *, $P = 0.05$.

Table 3. *Products of crude enzyme* (μg g^{-1} *f. wt*) *incubated for 1.5 h with Sucrose* (*S*), *1-kestose* (*1-K*) *and neokestin* (*N*)

		Fructan isomers														
Substrate		F	G	S	3a	3b	4a	4b	4c	4d	5a	5b	5c	5d	DP6	DP > 6
S	C	33.3	37.3	36.6	13.0	4.2	—	—	—	1.2	—	—	—	—	—	—
	Δ	5.3	4.8	−2.3	−2.8	−1.0				−0.4						
		**	*	n.s.	**	**				**						
1-K	C	15.5	10.4	9.4	44.4	8.5	—	2.4	—	8.1	—	—	—	—	—	—
	Δ	1.1	0.71	−0.4	1.5	−0.8		−0.1		−0.9						
		n.s.	n.s.	n.s.	n.s.	n.s.		n.s.		n.s.						
N	C	7.1	—	—	—	—	—	—	—	—	—	—	—	—	—	90.1
	Δ	1.0														−0.3
		n.s.														n.s.

*, ** Δ was significantly different from controls at $P < 0.06$, 0.01, respectively.
n.s., Not significantly different at $P = 0.06$.
—, None detected.
Differences (Δ) between controls (C) and plants infected with the PAV isolate of the Barley Yellow Dwarf Virus are also shown. Values are the means of two experiments, $n = 5$. Structures of fructan isomers are shown in Table 1.

5b in week 10 were all significantly higher in infected plants but, 4a, 5a, 5c, 5d in week 8 were significantly lower than controls (Fig. 1).

At the end of week 6 (the first wk of hardening) many BYDV-induced changes were unlike those observed in other weeks. For example, sucrose and 1-kestose, were lower than controls and most other carbohydrates, including DP6 and DP > 6, were essentially unchanged (Fig. 1). The same or a similar effect was observed in the other two cultivars and in RPV infected plants but the magnitude of the effect was different (data not shown).

Enzymes

Invertase, sucrose-sucrosyl transferase (SST), fructose fructosyl transferase (FFT) and hydrolase activities were measured in the same enzyme prep-

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Table 4. *Average absorbance at 405 nm, indicating level of infection, over 4 wk in 2 isolates of BYDV infected oats* (Avena sativa)

	Absorbance		
Cultivar	Control	RPV	PAV
Wintok	0.02	1.46	1.66
Coast Black	0.03	0.81	1.65
Fulghum	0.03	0.94	1.65
	n.s.	*	n.s.

*, n.s.: Cultivars are, or are not significantly different from each other at $P = 0.05$, respectively.

aration. Therefore, values are relative and do not take into account competition for substrates, especially SST and invertase for sucrose.

When enzyme extracts from infected plants were incubated with sucrose they produced more glucose and fructose but less 1-kestose (3a) and neokestose (3b) than extracts from non-infected plants (Table 3). When the same extract was incubated with 1-kestose no difference was found in the production of DP4 isomers. When incubated with neokestin more fructose was produced in extracts from infected plants but this difference was not significant ($P = 0.1$).

Symptoms of infection

Of the three cultivars inoculated with PAV or RPV isolates of BYDV, 'Coast Black' was the only one that showed typical visual symptoms of leaf reddening. However, ELISA tests indicated significant virus concentration differences between control and inoculated tissue and confirmed that virus was present in all three cultivars (Table 4). 'Wintok' infected with RPV had the highest virus concentration in every week (data not shown) and its average concentration over the 4 wk was significantly higher than that of 'Coast Black' or 'Fulghum' (Table 4). The three cultivars had similar PAV concentrations (Table 4).

Even though the characteristic leaf reddening symptom of BYDV infection was obvious only in 'Coast Black', infected plants of all three cultivars had a lower crown f. wt during hardening (Fig. 2) and were obviously stunted. The effect of the two isolates was greatest in 'Coast Black' and by week 10, crowns of that cultivar were 50% of the weight of crowns of uninfected controls. 'Fulghum' was the only cultivar which showed a difference in f. wt depending on the isolate but the difference was only significant ($P = 0.05$) in week 6 (Fig. 2). The decrease of f. wt in infected plants was caused by a simultaneous decrease in d. wt and water content in all three cultivars and all 4 weeks (data not shown). This concurrent decrease resulted in a nearly identical

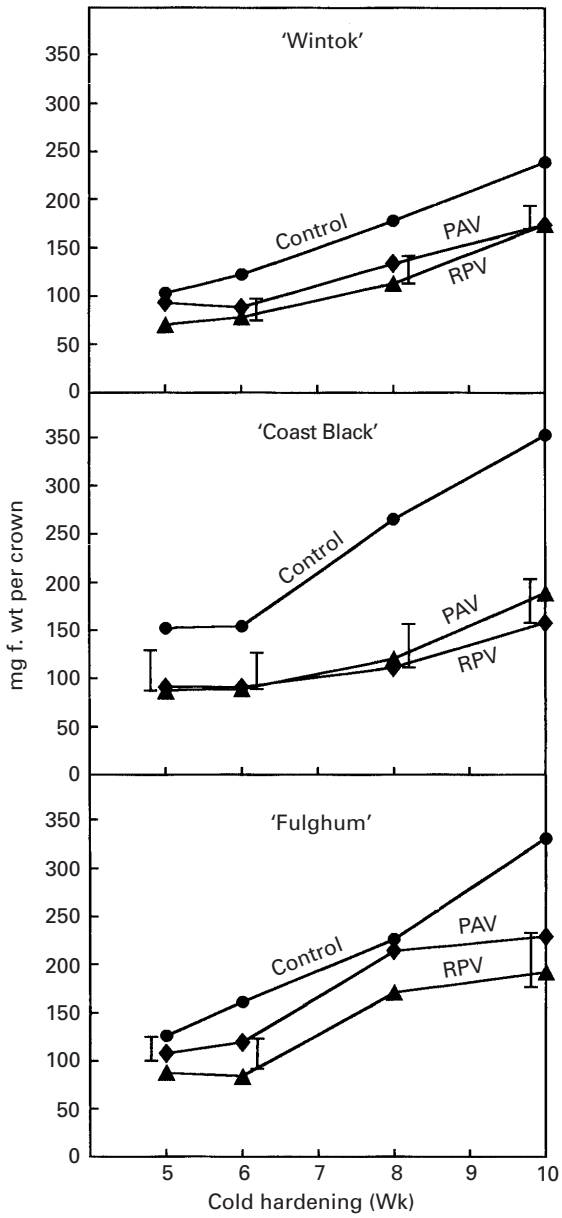

Figure 2. The effect of two isolates of barley yellow dwarf virus on the f. wt of crowns of three oat cultivars before (week 5) and during (weeks 6–10) cold hardening. Bars represent the least significant difference at $P = 0.05$. Where no bars are shown differences between treatments at that week were not significantly different.

d. wt proportion of total f. wt in infected (20.8% d. wt) and non-infected (20.9% d. wt) crowns of 'Wintok' by week 10. No significant difference in d. wt percentage of crowns was found in any week for any cultivar (data not shown).

DISCUSSION

Carbohydrates

During the first week of hardening (week 6) several carbohydrates were affected in a different manner or

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to a different extent than in other weeks. For example, sucrose was lower in infected plants after week 6 than it was in any other week. The lower amounts of sucrose did not result in more glucose and fructose than in other weeks which suggests that in week 6 sucrose might have been utilized in transferase rather than hydrolysis reactions. In fact, the BYDV effect on DP6 and DP > 6 percentages was less in week 6 than in any other week. It is possible that during week 6 fructan synthesis reactions, with higher rates than in other weeks (Livingston *et al.*, 1994), may have competed with whatever mechanism the virus induced to lower fructan amounts.

The relatively large change in DP6 and DP > 6 in comparison to the other fructan isomers was probably because of an additive effect of a number of isomers in each size class. The accumulation of these isomers DP > 5 may have been affected by the supply of substrate (DP3–5) or by an alteration of the activity of enzymes (not yet described) for synthesis of larger fructan. However, if accumulation of DP6 and DP > 6 fructan was lower in infected plants due to lower substrate then both should have been higher in infected plants in week 10 since substrates were higher. Instead they were lower than in any other week, supporting the theory that the activity of an as yet undescribed fructan synthesis enzyme(s) was altered in infected plants.

In uninfected plants, 3a, 3b, 4d and 5b were apparently the first isomers synthesized in their respective DP size classes (Livingston *et al.*, 1994). After the first week (or week 3 in the case of 5b) these isomers all decreased; they might be preferred as substrates in the synthesis of larger fructans (Livingston *et al.*, 1994). Concentrations of these isomers were all higher in infected plants, primarily in week 8 and 10. The isomers 4a, 4b, 4c, 5a 5c and 5d, all lower in infected plants, accumulated during hardening (Livingston *et al.*, 1994). If the decrease in isomers 3a, 3b, 4d and 5b after their initial increase is an indication of substrate preference (Livingston, *et al.*, 1994) then their higher proportion in infected plants implies that they may be preferred to a lesser extent when the plants are infected with BYDV. This again suggests that the activity of synthesis enzymes could be altered by virus infection, reducing enzyme preference for specific substrates and causing the substrates to accumulate in infected plants.

Enzymes

The significant increase in glucose and fructose production when crude extracts from infected plants were incubated with sucrose suggests that invertase activity was higher than in healthy plant extracts. This would explain the higher concentrations of glucose and fructose *in vivo* in infected plants (except for week 10). Higher concentrations of sucrose *in vivo* at first appear to contradict this but if photosynthate supply exceeded conversion to monomers by invertase, sucrose concentrations *in vivo* would increase. Higher invertase activity in wheat infected with powdery mildew may supply the fungus with carbohydrates needed for growth (Zulu, Farrar & Whitbread, 1991). Because viruses do not require a carbohydrate energy source more information will be needed on the bioenergetics of BYDV infection to explain reasons for the increasing sugar content.

In addition to hydrolysis of sucrose, invertase catalyzes synthesis of 1- and 6-kestose as well as neokestose (Pollock & Cairns, 1991) so it is possible that the transferase activity observed in controls was due to invertase not SST. If sucrose-hydrolysing activity competed with transferase activity this would explain the higher glucose and fructose, but lower 3a and b, concentrations. The lower amount of 4d in the sucrose incubation was probably a result of less substrate, presumably 3a and/or 3b.

The lower amount of 3a and b in incubations of extracts from infected plants imply an inhibition of SST. However, amounts of 3a and b *in vivo* were mostly higher in infected plants, not lower as would be expected from SST inhibition. Differences between the results *in vivo* and *in vitro* may be related to differences in pH or temperature optima for the two enzymes. The temperature of plants during hardening (*in vivo*) was 2 °C at an unknown pH but was 35 °C *in vitro* at a single pH. Invertase activity in crude enzyme extracts from *Lolium rigidum* shoots was significantly higher when incubated at 30 °C than at 25 °C but SST activity was lower (Smouter & Simpson, 1991); similar differences in pH optima were reported. Much larger differences between invertase and SST activities at different assay temperatures were reported in crested wheatgrass (Chatterton *et al.*, 1988). Further purification and characterization of invertase and SST activities from oats will be necessary to determine precisely how activity is affected by the virus.

The significant decrease of some DP5 isomers, DP6 and DP > 6 in infected plants *in vivo* at first suggested an increased hydrolase activity and/or lower FFT activity. However, differences between infected and non-infected plants for FFT and hydrolase activities in crude extracts were not significant. The possibility that fructan content of the crown was lower in infected plants because of phloem blockage restricting photosynthate supply was previously suggested (Livingston & Gildow, 1991) because sucrose levels were slightly higher in infected crowns, rather than lower, as would be expected if phloem blockage was restricting carbohydrate allocation to crowns in infected plants. Similar results were found here. Only after the first week of hardening (week 6) was sucrose lower in infected plants; at that time fructan concentration in infected plants was not different from controls.

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Therefore, even if phloem blockage caused lower sucrose after week 6 this had no effect on DP6 or DP > 6 accumulation. As suggested earlier, viral inhibition of an enzyme(s) involved in the synthesis of larger fructans would explain lower fructan concentrations in the crown in infected plants reported here and elsewhere (Andrews & Sinha, 1991).

Fructan decreased by 80% in *Festuca pratensis* during spring re-growth (Pollock & Jones, 1979). The amount of fructan metabolized in spring re-growth of oat is not known. But a reduction of nearly half in energy reserves by BYDV, as reported here, will undoubtedly have a severe impact on re-growth. In fact, the lower cold and ice tolerance of BYDV-infected barley reported by Andrews & Sinha (1991) could have been due to the lack of sufficient reserves to regenerate tillers.

Visual symptoms of infection

The absence of leaf reddening in 'Wintok' plants with high BYDV concentration might indicate a certain degree of tolerance to the virus despite the lack of resistance to virus replication. Leaf reddening can be the result of anthocyanin accumulation caused by carbohydrate build-up. Carbohydrate increase in leaves of infected plants is thought to be caused by virus-induced phloem deterioration (Jensen, 1968*a*; Esau, 1957). It is possible that phloem damage was less severe in 'Wintok' than in 'Coast Black'. The difference between 'Wintok' and 'Coast Black' in f. wt decrease in infected plants also suggests some tolerance in 'Wintok'.

Many of the measurements in this study and that of Andrews & Sinha (1991) were taken from the crown (the below ground portion of the stem) which functions as the overwintering organ containing meristematic tissue and energy reserves used in spring re-growth. During winter above-ground tissue and roots of cereals can be killed; if the crown survives, an intact plant can be regenerated in spring. Therefore, the effect of BYDV on crown physiology is critical to determining the ability of winter cereals like rye, wheat, barley and oats to over-winter and regenerate successfully. Andrews & Sinha (1991) reported that crowns of wheat and barley infected with BYDV weighed less than uninfected crowns but that their water content was higher. The decrease in f. wt they reported was similar to our finding in oat crowns but we found no difference in the water content of crowns of infected and non-infected plants. This suggests a slightly different mechanism induced by the virus between species. We found that water and d. wt decreased by equal amounts in infected oat, but in wheat and barley the decrease of d. wt was higher than that of water. Additional studies are necessary to access the significance of the differences between the species.

Grain yield in wheat infected with BYDV was reduced by as much as 60% and winter survival was reduced by 40% compared with uninfected plants. At present the only practical method of control for this potentially devastating disease is growing BYDV resistant cultivars. Identifying mechanisms whereby the virus affects agronomically important characteristics will help breeders develop methods to screen germplasm for resistance genes. Measuring the effect of BYDV on carbohydrate reserves in storage organs might be a viable method for determining resistance.

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