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Identification of BYDV resistance in wheat

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1. Abstract

Spread by several aphid vector species, *Barley yellow dwarf virus* (BYDV) is responsible for significant yield losses in important cultivated cereal crops worldwide, including UK wheat. Reduced options for effective BYDV disease management exist. Consequently, a focus on the identification, characterisation and future exploitation of genetic sources of BYDV resistance and/or tolerance has arisen. Using a diverse range of commercial wheat, core Watkins collection and synthetic hexaploid wheat germplasm, this work conducted a strategic screening for potentially novel genetic sources of BYDV resistance/tolerance. Of the 53 wheat genotypes screened, 20 have been preliminarily identified as potentially exhibiting some level of BYDV resistance, using a recently developed BYDV qRT-PCR diagnostic assay. Validation of these observations will now be pursued, potentially resulting in the identification of genotypes that could be valuable for the future development of new elite wheat cultivars with enhanced levels of resistance/tolerance to BYDV through conventional breeding.

2. Introduction

2.1 Background to *Barley yellow dwarf virus* and the need for effective disease management

BYDV detrimentally affects normal wheat development and can cause disease symptoms including, but not limited to, reduced growth of root and shoot tissue alongside chlorosis and delayed heading and flowering, impacting overall harvest yield and grain quality (Kaddachi *et al.*, 2014). BYDV is considered to be the most destructive virus infecting cereal crops worldwide with limited effective and sustainable control strategies currently available. Accurate UK wheat harvest losses attributed to natural BYDV infection are difficult to validate due to a combination of the ways that BYDV infection is assessed in-field, variation in viral pressure occurring between years, and a large number of additional confounding variables also affecting yield, many of which convolute any assessment of direct BYDV-associated harvest yield losses.

Depending upon wheat cultivar, strain of the virus and the point during plant growth when infection occurs, the relative impacts of BYDV can vary. Harvest yield losses within field conditions due to natural BYDV infection have been estimated between 11 and 33% (Kaddachi *et al.*, 2014), and losses upwards of 60% have been recorded where BYDV was artificially introduced (Ben Ghanem *et al.*, 2018). The effects of climate change alongside shifting trends in UK wheat production will likely influence the future spread of aphid vector species and the associated incidence of BYDV. For this reason, continued study into both genetic sources of BYDV resistance and tolerance, and ecological modelling of important cereal aphid species remains of high importance for sustainable UK wheat production. This is especially true when considering recent changes to EU insecticide regulations.

Prior to the decision by the European Commission to ban the use of neonicotinoid insecticides which came into force in late 2018, neonicotinoid seed treatments were used to maintain BYDV-associated disease at a reduced level across cereal crops by targeting aphid species known to carry the virus. Total insecticide application to cereals in 2015 was 140 tonnes, applied to 3 million hectares of land, of which the neonicotinoids under the recent ban would have constituted approximately 28% (Pesticide Use Statistics, FERA, 2017). This highlights the impact of this recent decision and previous reliance on chemical measures for controlling this viral disease. Wheat growers do continue to have the option of using alternative insecticides, however the majority of these need to be applied through foliar sprays. This approach is not strictly targeted, often impacting beneficial insects alongside target pests, and is neither always possible nor effective in wet autumn conditions when winter wheat is deemed to be most vulnerable to BYDV (Choudhury *et al.*, 2018). Additionally, the emergence of resistance to major insecticides by aphid species continues to be recorded, including moderate pyrethroid resistance which is widespread in the primary vectors of BYDV (Bass *et al.*, 2014, Walsh *et al.*, 2020). The increasingly-limited armoury of effective insecticides available, and the requirements of continued sustainable use directives limiting the allowed application frequency of such insecticides mean that future management of BYDV may rely on both integrated pest management strategies and genetic sources of BYDV resistance.

2.2 *Barley yellow dwarf virus* aphid vectors and predominant UK virus strains

The two principal cereal aphid vectors of BYDV in the UK are the bird cherry-oat aphid (*Rhopalosiphum padi*) and the English grain aphid (*Sitobion avenae*). Both species efficiently spread the virus by feeding on phloem channels of an infected plant, acquiring BYDV, before transmitting the virus to new susceptible hosts upon later feeding. The virus is retained within the haemolymph of an aphid before moving to accessory saliva glands; this renders each aphid viruliferous and capable of passing on BYDV indefinitely. *Rhopalosiphum padi* and *S. avenae* reproduce asexually during the summer months, producing 10-40 nymphs each per generation and with multiple generations throughout the season (Aradottir *et al.*, 2017). This facilitates rapid population expansion, consequently increasing the likelihood of BYDV vectors and associated disease being prevalent where no effective control measures are implemented. The aphids migrate to new hosts during the autumn which increases the risk of exposure of winter wheat crop to BYDV soon after sowing. This remains a fundamental consideration for remediating the impacts of BYDV because associated disease is most severe when infection occurs during early plant development (Choudhury *et al.*, 2018). As of October 2019, during the autumn aphid migration period, approximately 24% of tested *R. padi* captured by suction traps across the Rothamsted Insect Survey network were shown to be carrying BYDV (Gia Aradottir, pers. comm./WGIN management meeting, Oct. 2019). *Rhopalosiphum padi* and *S. avenae* differ in that each aphid species primarily vectors one of the two major strains of BYDV: BYDV-PAV and BYDV-MAV, respectively. This may be due to their relative geographical distribution, or alternative biological factor(s) (Foster *et al.*, 2004). Both BYDV-PAV and

BYDV-MAV have the potential to induce disease to a similar level and to which sources of genetic resistance/tolerance are now increasingly desirable.

2.3 Development of BYDV-resistant/tolerant wheat germplasm

UK wheat yield in 2018 totalled 14 million tonnes (Farming Statistics, Defra, 2018). A 30% loss in this yield due to widespread BYDV infection would equate to 4.2 million tonnes, leading to higher wheat prices and potentially necessitating an increase in imports. Population growth, coupled with per capita increase in consumption is also rapidly increasing demand for wheat (Curtis & Halford, 2014). Using resistant/tolerant germplasm as a first line of defence in a disease management programme would be an ecologically sound strategy for reducing any future impacts of BYDV. There are no UK wheat varieties currently available, however, with resistance or tolerance to BYDV. Genetic sources of broad-spectrum resistance or tolerance to all BYDV strains are more valuable than discrete, strain-specific resistances as these would likely reduce the possibility for resistance-breaking viral mutation when used as part of an integrated disease and pest control strategy. Any and all BYDV resistances/tolerances are nevertheless still valuable, with BYDV being brought to the forefront of many wheat growers' priorities after the recent changes in neonicotinoid regulations. A number of UK wheat breeding companies now have a focus on identifying, characterising and exploiting germplasm with resistance to aphids and/or BYDV, all with the intention of breeding any such valuable trait(s) into UK AHDB Recommended List wheat varieties. The first UK wheat variety with resistance to BYDV, Wolverine developed by RAGT will become available for drilling in autumn this year (www.fwi.co.uk/arable/wheat/first-wheat-with-bydv-resistance-set-for-2020-drilling). Like many other biotic stressors, aphids and linked BYDV infection can be unpredictable. Aphid infestation levels and proportion of BYDV-carrying individuals vary between years and their interactions with different germplasm can often be complex.

2.4 *Barley yellow dwarf virus* diagnostics and research objectives

A significant limitation for monitoring the spread of BYDV, its study and control has historically been the inability to reliably detect the virus via a high-throughput assay. The biology of the virus also complicates diagnosis because BYDV movement within the plant is confined to phloem tissue, occurs in low concentrations and is not mechanically transmissible (Kaddachi *et al*., 2014). Current commercial BYDV diagnostic tools, such as antibody-based ELISA tests, are effective in detecting specific viral proteins. However, these serological tests can often require large samples from a target plant for an accurate indication of infection, primarily due to limits in assay sensitivity. ELISA tests are also reasonably low-throughput due to sample processing requirements and results can be difficult to standardise across samples and/or separate tests. A real-time quantitative reverse transcription PCR (qRT-PCR) diagnostic test allowing detection of viral genomic RNA has recently been developed at Rothamsted Research, Harpenden (Martin Williamson, unpublished). This mid/high-throughput assay is more sensitive than the current commercial ELISAs and has already

been employed for testing individual aphids captured by suction traps across the Rothamsted Insect Survey network. The new qRT-PCR assay is also capable of discriminating between BYDV-PAV and BYDV-MAV. This assay could theoretically allow rapid identification of BYDV prior to the onset of disease symptoms within both susceptible hosts and BYDV-tolerant/asymptomatic plants containing the virus, ultimately helping to monitor and limit the spread of disease by allowing growers to make informed decisions.

Given the increased recent demand for new sources of BYDV resistance/tolerance amidst changes in pesticide legislation and what is likely to be higher future incidences of BYDV-associated disease, the core aim of this 24-week research project was to identify wheat germplasm with resistance or tolerance to BYDV. Key objectives associated with this aim were to screen different types of wheat germplasm through a controlled BYDV-PAV challenge during early plant development using *R. padi* vector-based BYDV transmission. Subsequent to this, testing for BYDV infection of challenged wheat material was to be done using the newly developed qRT-PCR diagnostic assay. The wheat lines selected for evaluation came from three very different sources, namely, the Watkins landrace collection originally collected in the 1930s from 32 countries in Asia, North Africa, Australia, and Europe which have only recently become a source of pre-breeding material (Wingen *et al*. 2014), synthetic hexaploid wheat lines developed by NIAB EMR, and various current and previous UK commercial cultivars. Priorities for screening were given to lines already included in the BBSRC's Designing Future Wheat programme germplasm collection and breeders' diversity toolkit (https://designingfuturewheat.org.uk/), and/or the long-term Defra's Wheat Genetic Improvement Network variety diversity trial (www.wgin.org.uk) for which valuable data exists and is already being utilised for development of superior wheat cultivars.

3. Materials and Methods

3.1 BYDV inocula/*Rhopalosiphum padi* culturing

A clonal culture of *R. padi* originating from insecticide-susceptible individuals collected from the field was maintained under controlled environment conditions (16:8h L:D, 22 ± 1°C) in ventilated insect-proof cages within a custom-built insectary facility. Aphids were rendered viruliferous, carrying BYDV, through exclusive rearing on wheat shown previously to be infected with BYDV-PAV. Aphids, and by proxy BYDV inocula, were subsequently maintained on BYDV-susceptible wheat cv. Tybalt. Approximately every six weeks or as required, winged alate *R. padi* morphs were transferred onto fresh plants. All wheat used for aphid culturing was grown in sealed glasshouse facilities with no likelihood for inadvertent insect colonisation/introduction of alternative plant viruses. Deliberate overcrowding of *R. padi* was also implemented within each culture to generate a larger number of alate morphs.

3.2 Wheat germplasm undergoing BYDV testing, and experimental layout

A total of 53 wheat lines (Table 1) were screened for resistance to BYDV by challenging test plants with viruliferous *R. padi*. The BYDV-challenged wheat consisted of 17 lines from the core Watkins collection of hexaploid wheat landraces (Wingen *et al.*, 2014), 33 synthetic hexaploid wheat lines developed by NIAB EMR (unpublished), and three commercially available cultivars (Hereward, Paragon and Solstice).

Seeds were sown into Rothamsted Prescribed Mix and maintained under controlled environment conditions (16:8h L:D, 20 ± 1°C, 50% RH) with a daily watering regime. Growth media, supplied by Petersfield Products, Leicestershire, UK, was comprised 75% medium grade (L&P) peat, 12% screened sterilised loam, 3% medium grade vermiculite and 10% grit (5 mm screened, lime free). All the wheat lines were ordered according to a randomised blocked experimental layout and, due to the number of plants being assessed, concurrent BYDV resistance screening/phenotyping performed. A minimum of 16 lines were sown in sets (three in total, one per week with all experimental stages processed in a staggered manner). Alongside the Watkins and NIAB EMR synthetic wheat undergoing testing, the three commercial lines (the NABIM Class 1 winter wheats Hereward and Solstice, and the spring wheat Paragon) were included as controls to provide a comparative standard across all bioassays. Replicates of each wheat line were sown across the experiment, aiming for 10 plants of each line to be challenged with BYDV and three to act as unchallenged controls.

Table 1. Designations of wheat cultivars/lines challenged with bird cherry-oat aphids (*Rhopalosiphum padi*) carrying *Barley yellow dwarf virus* (PAV strain).

Commercial cultivars	Watkins collection designation	Synthetic hexaploid wheat designation
Hereward	WATKINS_0007	NIAB_SHW_012
Paragon	WATKINS_0032	NIAB_SHW_018
Solstice	WATKINS_0034	NIAB_SHW_027
	WATKINS_0042	NIAB_SHW_028
	WATKINS_0044	NIAB_SHW_029
	WATKINS_0045	NIAB_SHW_030
	WATKINS_0079	NIAB_SHW_031
	WATKINS_0103	NIAB_SHW_035
	WATKINS_0110	NIAB_SHW_036
	WATKINS_0126	NIAB_SHW_042
	WATKINS_0127	NIAB_SHW_051
	WATKINS_0139	NIAB_SHW_054
	WATKINS_0141	NIAB_SHW_071
	WATKINS_0145	NIAB_SHW_072
	WATKINS_0160	NIAB_SHW_073
	WATKINS_0468	NIAB_SHW_076
	WATKINS_0729	NIAB_SHW_077
		NIAB_SHW_080
		NIAB_SHW_082
		NIAB_SHW_083
		NIAB_SHW_084
		NIAB_SHW_085
		NIAB_SHW_086
		NIAB_SHW_087
		NIAB_SHW_090
		NIAB_SHW_091
		NIAB_SHW_093
		NIAB_SHW_094
		NIAB_SHW_095
		NIAB_SHW_096
		NIAB_SHW_099
		NIAB_SHW_137
		NIAB_SHW_138

Commercial cultivars are widely available and were used as standard test controls.

Watkins hexaploid wheat lines established and defined by Wingen *et al*. (2014).

Synthetic hexaploid wheat lines developed by NIAB EMR, Cambridge (unpublished).

3.3 Challenging wheat germplasm with BYDV/viruliferous *Rhopalosiphum padi*

At the two-leaf stage of wheat seedling growth (approximately seven days after sowing), 5 viruliferous *R. padi* were placed onto wheat lines designated for BYDV resistance testing. Purely alate *R. padi* morphs (winged aphids) were selected, ensuring all aphids chosen for challenging were taken from mature cultures where sufficient time had passed for them to uptake BYDV and be rendered viruliferous. A selection of alate aphids from mature cultures were also destructively assayed for BYDV, confirming BYDV uptake was occurring within the culture. During challenging, clip cages were used to confine aphid movement to purely the target tissue of challenged plants (Figure 1).

Figure 1. Custom-built clip cages used to contain cereal aphids, enabling directed virus resistance screening of wheat plants using viruliferous aphids.

All aphids were left within clip cages for 72 hours, effectively challenging each plant with BYDV during aphid feeding in this period, after which an insecticide treatment was applied in order to kill all *R. padi*. Seven days post insecticide treatment, the first leaf samples were taken and stored at -80°C for analyses of BYDV presence by qRT-PCR. All plants were then vernalised according to the requirements of each genotype and subsequently maintained until harvested (where possible, or plant death noted). A second set of leaf samples were taken from the flag leaf upon its emergence and stored for future BYDV testing.

3.4 *Barley yellow dwarf virus* real-time quantitative PCR diagnostic assay

BYDV present in leaf samples was assayed for in a 96-well plate mid/high-throughput format. Small leaf cuttings (<1cm^2) were macerated and heated in a sucrose extraction buffer within each preassigned well, effectively releasing viral genomic RNA from samples where BYDV was present. A reverse transcription stage was then implemented to produce BYDV-specific cDNA from viral RNA present in each sucrose extraction. Finally, a real-time qRT-PCR stage using this cDNA as a template was used to indicate the presence or absence of BYDV within each sample. The qRT-PCR assay relies on TaqMan® chemistry based on hydrolysis probe-based PCR (Lee *et al.*, 1993).

Fluorophores and an associated quencher are attached to probes designed to match specific nucleotide sequences. Here, BYDV-specific PCR primers flanking probe targets were used to displace the fluorophores away from their linked quencher via the 5'-3' exonuclease activity of the polymerase used during PCR. Multiplexing of BYDV strain-specific probes with distinct fluorophores were used for simultaneous quantification of both BYDV-PAV and BYDV-MAV in each sample. The different fluorophores linked to each probe were detected through excitation of each sample well via laser excitation with discrete wavelengths, and fluorescence linked to each probe fluorophore recorded in real-time during the PCR/cDNA amplification stage.

Quality control (QC) screening and normalisation of qRT-PCR results was done through the fluorescence of ROX, an inert fluorescent dye added to all samples which provided a background passive reference signal. Samples displaying inconsistent ROX fluorescence during the assay were disregarded as part of QC screening. After data normalisation against the ROX reference signal and the removal of suboptimal data, scored thresholds were used to qualify samples as either clearly containing BYDV-PAV or BYDV-MAV (>3), intermediate (≤3 and >1.5), or not containing BYDV (≤1.5). Control cDNA from wheat material identified as being either infected or free of BYDV-PAV was also included during assays in order to confirm validity of each 96-well plate processed and to function as an additional indicator for the reliability of qRT-PCR score standardisation.

4. Results

4.1 Overall qRT-PCR assay results for all first leaf samples

A total of 658 first leaf samples taken from BYDV-challenged and unchallenged wheat plants were assessed for BYDV using the qRT-PCR assay. Of these samples, 589 (89.5%) passed QC screening. No individual plates presented notably higher tendencies for sample QC failure, higher or lower qRT-PCR scores. Positive control BYDV-PAV cDNA reliably indicated a positive result in all instances and exhibited little variation between separate plates/assay reactions (mean normalised BYDY-PAV qRT-PCR score for positive cDNA control, 5.62 ± 0.19 SE).

As expected, all test plant sample results suggested no BYDV-MAV was present; neither clear positive nor intermediate qRT-PCR results associated with BYDV-MAV were recorded for any samples tested (Figure 2). In terms of BYDV-PAV incidence, the vast majority of all first leaf samples tested and shown to pass QC (n = 589) were considered free of BYDV, n = 528 (89.6%), whilst approximately 5% were classed as intermediate for the presence of BYDV, n = 31 (5.26%), or clearly containing BYDV, n = 30 (5.09%).

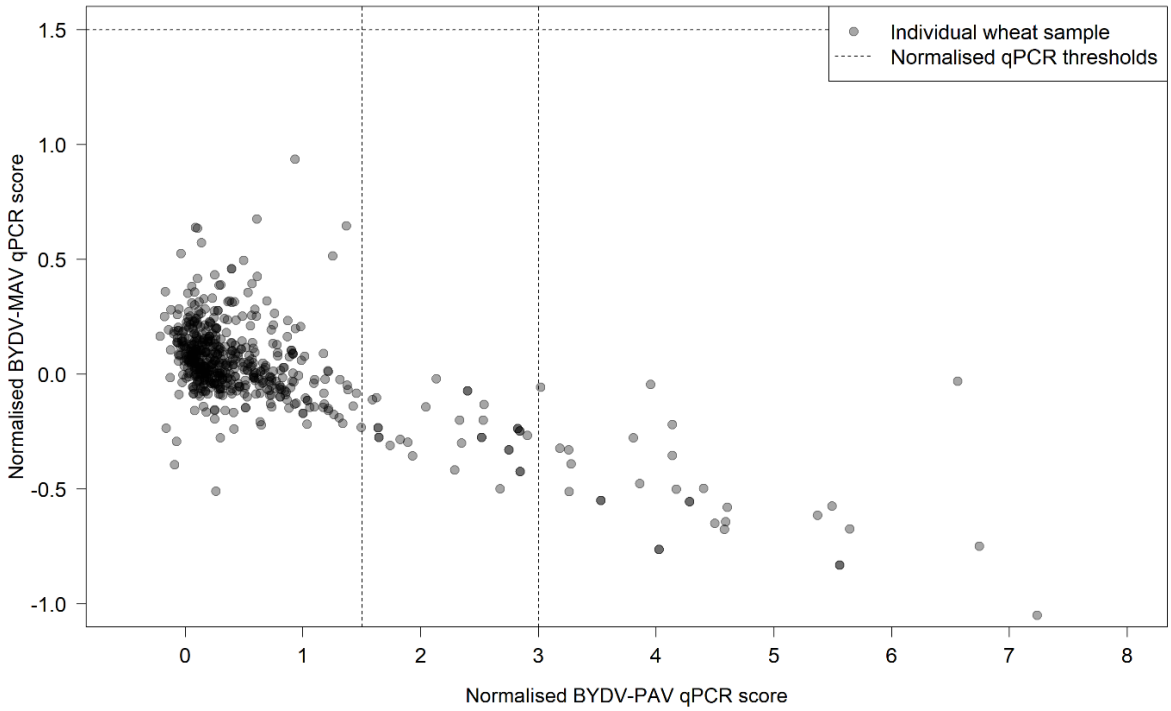

Figure 2. Scatter plot of BYDV qRT-PCR assay scores from first leaf wheat samples taken after challenging test plants with BYDV-carrying *Rhopalosiphum padi* (n = 589, also including unchallenged control samples). All presented data passed quality control screening and has been normalised against background passive ROX fluorescence. Thresholds for the presence of BYDV-MAV and BYDV-PAV also presented: >3, containing BYDV; ≤3 and >1.5, intermediate BYDV reading; <1.5, not containing BYDV.

4.2 Assay results by wheat line/cultivar

No individual wheat line screened for BYDV-PAV (including all commercial wheat germplasm) demonstrated complete BYDV-PAV susceptibility across all plants challenged. This was also the case when including intermediate BYDV qRT-PCR results. A total of 19 wheat lines presented neither a clear indication of BYDV presence nor intermediate qRT-PCR scores across any plants tested; these lines were considered to be uniformly resistant based on the samples processed in this assay (Tables 2 & 3, highlighted). A notable inclusion to the wheat lines considered to be potentially resistant was Solstice, one of the three wheat cultivars included as a standard across all testing. A total of 33 Solstice plants were challenged. Of these, one was identified as having an intermediate BYDV qRT-PCR score and none were identified as clearly containing BYDV. This is in stark contrast to Hereward and Paragon, the other two commercial wheat control cultivars, wherein multiple plants were identified as clearly containing BYDV (Table 4).

Of the total number of samples recorded as being either intermediate or clearly containing BYDV (n = 61), 12 were from the unchallenged plant material. This was unexpected and determined not be due to any possible cross contamination event(s) during the qRT-PCR assay, based on the relative distribution of these samples and times of processing. A small number of live aphids were observed during wheat harvesting, perhaps explaining this aberrant result whilst suggesting that insecticide treatments were not fully effective and/or evenly applied to plants after challenging.

Table 2. BYDV qRT-PCR assay results of first leaf samples taken from core Watkins collection wheat lines one week after being challenged with viruliferous *R. padi,* and unchallenged control plants grown in parallel.

Watkins core collection designation	Number of *R. padi*/BYDV-challenged samples			Number of unchallenged control samples		
	R	I	S	R	I	S
WATKINS_0007	10					1
WATKINS_0032	7			2		
WATKINS_0034	8			3		
WATKINS_0042	9			2		
WATKINS_0044	5	1		3		
WATKINS_0045	9			2		1
WATKINS_0079	9			3		
WATKINS_0103	7			2		
WATKINS_0110	8			2		1
WATKINS_0126	6			2		
WATKINS_0127	5			2		
WATKINS_0139	7			3		
WATKINS_0141	6			2		
WATKINS_0145	7			3		
WATKINS_0160	8			2	1	
WATKINS_0468	7	1		2	1	
WATKINS_0729	8			3		

BYDV-PAV resistance, 'R', classed as any samples where normalised BYDV qRT-PCR assay score < 1.5; intermediate, 'I', where assay score >1.5 and ≤3; susceptible, 'S', where assay score >3.

Lines where no BYDV identified (including intermediate readings) in challenged and unchallenged plants highlighted (grey).

Where no value presented, 0 samples identified.

Table 3. BYDV qRT-PCR assay results of first leaf samples taken from NIAB synthetic hexaploid wheat lines one week after being challenged with viruliferous *R. padi,* and unchallenged control plants grown in parallel.

Synthetic hexaploid wheat designation	Number of *R. padi*/BYDV-challenged samples			Number of unchallenged control samples		
	R	I	S	R	I	S
NIAB_SHW_012	9	1		3		
NIAB_SHW_018	3	1	1	3		
NIAB_SHW_027	6	1		2		
NIAB_SHW_028	7	2	1	1		
NIAB_SHW_029	7			1		
NIAB_SHW_030	10			3		
NIAB_SHW_031	5	1		3		
NIAB_SHW_035	7			1		
NIAB_SHW_036	1			1		
NIAB_SHW_042	8		1	2		
NIAB_SHW_051	6	1		2		
NIAB_SHW_054	7			3		
NIAB_SHW_071	5	1	1	1		
NIAB_SHW_072	8			3		
NIAB_SHW_073	6	3		3		
NIAB_SHW_076	7	1	1	1		
NIAB_SHW_077	6		3	3		
NIAB_SHW_080	7			2		1
NIAB_SHW_082	8		1	3		
NIAB_SHW_083	7	1		3		
NIAB_SHW_084	5			1		1
NIAB_SHW_085	5	1		1	1	
NIAB_SHW_086	4	1		3		
NIAB_SHW_087	6			2	1	
NIAB_SHW_090	11	1		2	1	
NIAB_SHW_091	8		2	3		
NIAB_SHW_093	9			3		
NIAB_SHW_094	7		2	3		
NIAB_SHW_095	7	1	1	3		
NIAB_SHW_096	8	1		1		
NIAB_SHW_099	7		3	3		
NIAB_SHW_137	4		1	2		
NIAB_SHW_138	9			3		

BYDV-PAV resistance, 'R', classed as any samples where normalised BYDV qRT-PCR assay score < 1.5; intermediate, 'I', where assay score >1.5 and ≤3; susceptible, 'S', where assay score >3.

Lines where no BYDV identified (including intermediate readings) in challenged and unchallenged plants highlighted (grey).

Where no value presented, 0 samples identified.

Table 4. BYDV qRT-PCR assay results of first leaf samples taken from commercial wheat cultivars one week after being challenged with viruliferous *R. padi,* and unchallenged control plants grown in parallel.

Wheat cultivar	Number of *R. padi*/BYDV-challenged samples			Number of unchallenged control samples		
	R	I	S	R	I	S
Hereward	16	1	2	8		1
Paragon	13	5	3	7	1	
Solstice	32	1		8		

BYDV-PAV resistance, 'R', classed as any samples where normalised BYDV qRT-PCR assay score < 1.5; intermediate, 'I', where assay score >1.5 and ≤3; susceptible, 'S', where assay score >3. Where no value presented, 0 samples identified.

Wheat cv. Solstice where no clear indication of BYDV was identified in challenged and unchallenged plants highlighted (grey).

5. Discussion

The overall presence of BYDV across first leaf samples, determined here by qRT-PCR, relative to the suggested absence of BYDV was notably lower than what may have been expected, especially considering viruliferous aphids were used to directly challenge wheat plants at an early stage of growth. This relies on an assumption, however, that any and all BYDV infections would be readily detectable when sampling was performed approximately one week post challenging. The absence of high infection rates observed at this point does not necessarily reliably indicate wheat lines presented here are BYDV-resistant. Instead, the presence of any BYDV recorded at this stage may highlight purely the sensitivity of the qRT-PCR assay and susceptibility of specific cultivars. Validation of BYDV resistance is required and will be pursued through future testing of the flag leaf samples already taken. BYDV resistance-screening strategies, where tissue blot immunoassay and ELISA-based testing are employed, generally sample germplasm much later in plant development than the first leaf samples processed here, allowing translocation and an increase in viral titre until BYDV is clearly detectable within susceptible plants, approximately six weeks after infection (Choudhury *et al*., 2018).

Results presented here correlate to data obtained previously when screening current and previous commercial wheat lines against BYDV using similar methods to all those implemented in this study (Gia Aradottir, pers. comm./WGIN management meeting, Oct. 2019, www.wgin.org.uk/information/documents/WGIN4_management/WGIN%20MM_Oct8_combined.pdf). Within the previous study, 20 wheat lines included in the Rothamsted Research/WGIN 2019 variety diversity trial were challenged with viruliferous aphids, and sampling was performed at identical stages to here with BYDV detected through the same qRT-PCR assay. Where BYDV was detected within first leaf samples of any of the plants of each of the 20 lines assayed, an average infection rate across each line of 13.8% ± 1.8 SE was recorded. This was lower than the average infection

rate per line of 21.6% ± 3.3 SE determined here with this rate being calculated in an equivocal manner; all intermediate qRT-PCR data, wheat lines where no BYDV was detected across any plants challenged, and all unchallenged plant sample data being excluded. For additional comparative purposes, no uniformly BYDV-susceptible plant lines were previously identified from purely first leaf samples, also matching observations recorded here. A cause of this may be a non-uniform level of BYDV uptake and transmission by aphids used in this screening method and/or incomplete resistance across all plants of each wheat line tested. An alternative, and perhaps more likely, reason for this observation recorded both here and previously is the duration of time after BYDV challenging when first leaf samples were taken. A near-uniform increase in detection of BYDV was recorded in the flag leaf samples versus first leaf samples taken within the WGIN-sponsored study. The future qRT-PCR testing of the flag leaf samples already taken from wheat lines assessed here will help elucidate the resistance status of the Watkins, NIAB synthetic and commercial wheat lines included in this work. This future testing of flag leaf samples will prioritise lines where no BYDV infection was recorded (Tables 2 & 3**,** highlighted).

At this stage, potentially the most notable result for any individual wheat line/cultivar assessed for BYDV resistance is that of Solstice. Previous BYDV resistance screening within WGIN included this commercial cultivar. Solstice was identified as uniformly resistant with no BYDV detected by qRT-PCR across both the first and flag leaf samples assayed (Gia Aradottir, pers. comm./WGIN management meeting, Oct. 2019, www.wgin.org.uk/information/documents/WGIN4_management/WGIN%20MM_Oct8_combined.pdf). The new data presented here reinforces the previous suggestion that Solstice may exhibit some level of resistance and, due to the randomised experimental layout employed here alongside assessments being undertaken by new staff members with no links to previous research, this noteworthy outcome can be considered increasingly robust.

In terms of unchallenged control material exhibiting an indication for BYDV presence despite not being deliberately exposed to BYDV, whilst unintended, this does not directly influence any current suggestion of wheat lines perhaps exhibiting sources of BYDV resistance. All challenged germplasm was successfully exposed to aphids maintained on BYDV-containing wheat, the aphids were confined during the 72-hour challenging window to target plants and individual aphids confirmed to be viruliferous via destructive testing (data not shown). The detection of BYDV in unchallenged material and the presence of aphids recorded during harvesting suggests that insecticide treatment may not have been completely effective and/or applied evenly. Observed ‘resistance’ to BYDV in unchallenged germplasm cannot therefore be considered robust or informative, but identification of BYDV within samples taken from material unintentionally challenged by viniferous aphids may still indicate BYDV susceptibility in the affected plant lines. The occurrence of aphids after spraying could also be considered to partially highlight the demand for genetic sources of BYDV resistance where,

even under controlled conditions, insecticide-based control may have proven to not be completely effective in eliminating the spread of BYDV.

The qRT-PCR assay used here has already been optimised for detecting the presence of BYDV and its various strains within individual aphids, a feat not previously possible by alternative mid/high-throughput serological diagnostic tools. Optimisation of the assay for plant tissue, the timings of when to sample wheat germplasm post-aphid/BYDV challenge, and specific tissues to sample/target may require further investigation in order to validate the assay and facilitate robust identification of BYDV-resistant material. Despite this, the current advantages of the assay including its high sensitivity, accuracy for the detection of specific BYDV strains and ability for efficient quality control screening remain. Indications of BYDV can be investigated further, validating current results, because of small sample sizes required and due to both the rapid and mid/high-throughput nature of the assay. Ultimately, results presented here highlight avenues for future research and discovery of wheat lines which may represent sources of BYDV-PAV resistance not previously identified. Future testing of the collected flag leaf samples will elucidate the resistance status of each wheat line challenged here and the new qRT-PCR assay may prove an advantageous alternative to the predominantly antibody-based current diagnostic tools.

6. References

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