Can a PCR assay of aphids caught in-crop on yellow sticky traps inform field level barley yellow dwarf virus (BYDV) risk assessment?

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Abstract

Infection with barley yellow dwarf virus (BYDV), caused by strains of virus belonging to the family Luteovirideae including BYDV-PAV, can result in significant yield losses in autumn sown cereals following transmission by aphid vectors such as *Rhopalosiphum padi* (Homoptera: Aphididae) and *Sitobion avenae* (Homoptera: Aphididae). Spatial and temporal variance in the infectivity of alate populations influences risk to crops from the disease, which is greatest on infection at early crop growth stages. A decision support system (DSS) to guide optimised integration of crop protection strategies through risk assessment would help avoid unnecessary application of synthetic insecticides. This study contributes to the development of a DSS by exploring the viability and relevance of a methodology to detect virus levels in individual aphids trapped in-crop using yellow sticky traps. Using a reverse transcription polymerase chain reaction (RT-PCR) assay, the detectability of virus from a BYDV-PAV-positive control colony was found not to be reduced by the process of trapping, extraction and cold storage, but did drop significantly after between three and seven days of exposure on trap. This method has potential to contribute to localised risk assessment and guide optimisation of crop protection strategies.

Keywords: BYDV, *Sitobian avenae, Rhopalosiphum padi*, infectivity, winter cereals, crop protection, integrated pest management

1. Introduction

Viral diseases vectored by aphids (Aphididae) cause major crop losses worldwide (Ng and Perry, 2004; Hull, 2009; van Emden and Harrington, 2017. Barley yellow dwarf virus (BYDV) is one of the most widespread and economically damaging of these (D'Arcy 1995; Bicknell *et al.,* 2000; Jarošová *et al.*, 2016). Infection by the *Luteovirus* and *Polerovirus* genera of the family Luteovirideae typically result in qualitative and quantitative reduction in yield through chlorosis, root and shoot stunting and reduced stress tolerance (Herbert *et al.*, 1999; Riedell *et al.,* 2003), symptoms collectively described as barley yellow dwarf disease (BYDD). Three distinct strains are responsible for causing crop losses in UK cereals, all of which are phloem-restricted and transmitted in a circulative non-propagative manner by aphid vectors (Gray and Gildow, 2003). BYDV-PAV is vectored by *Rhopalosiphum padi* (Linnaeus) and *Sitobian avenae* (Fabricius) (Rochow, 1970). Crops are most vulnerable to infection before Zadoks growth stage 31 (Doodson and Saunders, 1970; Zadoks *et al*., 1974).

BYDV epidemiology is governed by a complex ecology of biotic and abiotic interactions involving virus, vector, host and climate (Miles, 1989; Irwin and Thresh, 1990; Walls *et al*., 2019). Incidence and impact of the disease consequently varies between years, areas and crops at a localised scale (Plumb, 1974; Watt, 1983; McGrath and Bale, 1989; Holland *et al.*, 2019). A major factor in disease incidence is the number of viruliferous alate aphids alighting on young crop plants (Lowles *et al.,* 1997; Bicknell *et al.*, 2000; Fabre *et al.,* 2006). Conditions including temperature that govern survival, colonisation through anholocyclic parthenogenesis and in-crop movement dictates subsequent disease development and secondary spread (Kendall *et al.*, 1992; Fabre *et al.*, 2006; Powell and Bale, 2006). With no established functional relationship between aphid infestation and virus-related damage, there have historically been no reliable economic thresholds (Oakley and Walters, 1994; Herbert *et al.*, 1999), making prophylactic chemical control the primary course of crop protection in conventional UK arable systems (Dewar and Denholm, 2017; Walls *et al*., 2019). Reduced availability and efficacy of synthetic pesticides due to growing awareness of environmental risks (Pimentel and Lehman,

1993; Pisa *et al.*, 2014; Handford *et al.*, 2015) combined with increasingly widespread insecticide resistance in aphid populations (Dewar and Foster, 2017) increases the mandate for alternative approaches to BYDV virus vector management.

Sustainable protection of crops from BYDV requires confidently informed and implemented decision support systems (DSSs) to enable land managers to appropriately integrate pest control strategies (Stern *et al*., 1959; Knight, 1997). These may include cultural control (Tatchell *et al.*, 1988), conservation biological control (Woodcock *et al.*, 2016), varietal genetic tolerance or resistance to viruses or vectors (Jarošová *et al.*, 2016; Aradottir *et al*., 2017) and pesticide application (Dewar and Denholm, 2017). Operating within an economic framework of cost benefit analysis (Shtienberg, 2013) and a regulatory framework of environmental legislation (Handford *et al.*, 2015), uptake of DSSs relies on the perception of minimal risk of false negative advice (Gent *et al.,* 2011; Gent *et al.*, 2013). Reliable forecasting of future outbreaks to inform crop protection decisions benefits from the aggregation of multiple factors into modelling and the combining of multiple forecasts (Bates and Granger, 1969; Lankin-Vega *et al.*, 2008).

Long term monitoring through national and continental suction trap networks has contributed species-specific migratory data to a range of phenomenological models (Harrington *et al.,* 2004; Harrington *et al.,* 2007; Bell *et al.*, 2015). Although correlated with in-field abundance (Fabre *et al.*, 2010), morphological variance in numbers of *R. padi* alates at differing heights (A'Brook and Dewar, 1980) results from a lower ratio of crop colonising virginoparae to alternate bird-cherry tree host-seeking gynoparae flying at high levels during the autumn migration (Tatchell *et al.,* 1988; Lowles *et al.*, 1997). For this reason, analysis of specimens from the most commonly operated 12.2m high suction trap model cannot accurately represent in-crop infectivity levels (Burgess *et al.,* 1999), undermining the relevance of a DSS based on this data alone (Gent *et al.,* 2013; Ramsden *et al*., 2017).

In-crop monitoring can help to more accurately guide management decisions at a localised level. Yellow sticky traps have been found to offer a low cost, low maintenance means of

quantifying relative abundance of aphids (Harrington *et al.,* 2007) including *S. avenae* and *R. padi* in autumn cereal crops (Holland *et al.*, 2019). Sample extraction from sticky traps using alkane solvents has been found in other entomological studies to preserve the integrity of samples for identification (Davidson *et al.*, 2015) and of viral RNA for assay (Boonham *et al*., 2002; Congdon *et al.,* 2019). Singh *et al*., 1997, found detectability of viral RNA by RT-PCR to be retained in individual aphids stored for up to seven years in 70% ethanol.

Assays to facilitate the rapid and reliable discrimination and quantification of BYDV strains including -PAV have been developed using both reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Zhao *et al.,* 2010) and real-time reverse-transcription polymerase chain reaction (RT-PCR) (Canning *et al.,* 1996; Fabre *et al.,* 2003; Malmstrom and Shu, 2004; Kundu *et al.*, 2009) techniques. RT-PCR assay through allelic discrimination using Taqman probes was found by Fabre *et al.,* (2003) to be 10^3 times more sensitive than its ELISA predecessor in the detection of BYDV-PAV. This study establishes the viability of applying a RT-PCR assay quantifying the presence of BYDV-PAV through allelic discrimination using Taqman probes (Williamson, unpublished, 2018) to aphid samples acquired through yellow sticky trapping followed by extraction using a commercial solvent and cold storage. The amount of time for which positive samples remain detectable by the assay once exposed on trap is established through a time-series field experiment.

2. Materials and methods

2.1. Control colony

To establish the viability of using in crop yellow sticky traps in combination with RT-PCR assay for detection of BYDV-PAV in aphid vectors, *R. padi* adults from a BYDV-PAV-positive colony from Rothamsted Research (Harpenden, Hertfordshire, UK) were placed onto yellow sticky traps ('Wetstick', Oecos Ltd, Hertfordshire, UK) for between 0 and 14 days prior to RT-PCR

assay. Aphids taken directly from the colony for use in the RT-PCR assay were used as a control.

2.2. Trap type and preparation

Rhopalosiphum padi from the BYDV-PAV-positive colony were placed live onto 15 20cm x 10cm yellow sticky traps. Fifty aphids were placed live on each trap in a 3mm grid pattern to ensure they could be identified from any ingressing aphids becoming stuck to the traps.

2.3. Field site

The field site used in this study was located in Wiltshire (51°18'08"N, 2°03'16"W, elevation 59m). Yellow sticky traps were erected parallel to the soil surface at a height of 25cm using rigid plastic canes inserted into holes drilled into the centre of either end of the traps and driven 10cm into the ground. The traps were positioned 5 m from the hedged eastern edge of a direct drilled winter wheat crop at GS13 (Zadoks *et al.,* 1974). The experiment was completed between December 3rd and December 17th 2018. Traps were removed from the field at 24-hour intervals but constraints of assay capacity restricted analysis to specimens removed after 0, 1, 3, 7, 10 and 14 days. Maximum and minimum air temperatures for the preceding 24 hours were recorded at each removal point using a digital thermometer (Electronic Temperature Instruments Ltd, West Sussex, UK).

2.4. Specimen extraction and storage

Aphids were extracted from traps using a commercial alkane solvent formulation of 60% aliphatic hydrocarbon and 40% glycol ether (Mykal 'De-Solv-It Sticky Stuff Remover'®, Zep UK Ltd, Cheshire, UK). Once removed from the trap, specimens were immediately placed in 5ml clear plastic vials of 95% ethanol in batches of 15 and stored at -5°C.

2.5. RT-PCR assay

A real time RT-PCR Taqman assay for detection of BYDV-PAV in cereal aphids (Williamson, unpublished, 2018) was performed on aphid samples at Rothamsted Research between February 4th and February 8th 2019 to detect levels of the virus present in individual specimens. Aphids were homogenised in a sucrose/salt buffer to lyse cells and subjected to reverse transcription with revertaid RT enzyme (ThermoFisher UK) to convert any BYDV viral RNA present into cDNA. BYDV-PAV DNA was then identified by Taqman PCR using BYDV-PAV specific primer and probe sequences. The assay was run through 30 cycles using an Applied Biosystems 7900HT real-time PCR cycler and scored according to an increase in marker dye (VIC) fluorescence when BYDV-PAV DNA was amplified in the reaction above a baseline at which little or no change in flourescence was detected. A threshold was applied to determine positive BYDV-PAV infection relative to the score for negative control samples. Control samples for negative virus infection were taken from a colony of *R. padi* that was not exposed to BYDV-PAV and consistently fell below threshold levels of detectable flourescence. Controls for positive virus infection were duplications of samples taken from *R. padi* that were from a colony exposed to BYDV-PAV and individually determined to score above the threshold, as infectivity within an infected colony varies between individuals. Control aphids (+/- virus infection) and a no-template control of sterilized H_2O were included on all assay plates.

2.6. Statistical analysis

A total of 126 samples from time points 0, 3, 7, 10 and 14 days were analysed. Twenty-eight positive controls (aphids taken directly from the same BYDV-PAV infected colony) were assayed using the same technique. Detected virus load of individual aphids compared to negative control samples at each time point were plotted in R version 3.5.3 (R Core Team,

2019). Significance of variation in the dependent variable of BYDV-PAV virus load indicated by a detected increase of the flourescence reporter signal during amplification from that of aphids taken from the same infected colony but not exposed on trap were analysed using a series of *t*-tests and overall analysis of variance (ANOVA) according to the independent variable factor of 'days on trap'.

3. Results

The detectability of BYDV-PAV by PCR assay did not vary significantly from the control (-1 days) after either 0 days ($F_{1,59} = 1.94$, $P = 0.06$) or 3 days ($F_{1,36} = 0.08$; $P = 0.8$) on trap (Figure 1.). There was a significant reduction, however, in detectability at 7 days ($F_{1,56} = 9.46$; $P = 0.003$), 10 days ($F_{1,42} = 11.55$; $P = 0.002$) and 14 days ($F_{1,63} = 15.94$; $P = <0.001$) (Figure 1.). Although the mean detectability had dropped by 7 days, it remained possible to detect virus carrying aphids. Half of the 82 individuals sampled between 7 and 14 days were still determined as BYDV-PAV-positive.

Temperatures recorded during this study were a maximum of 12.0°C, a minimum of -3.0°C, a mean maximum of 9.3°C and a mean minimum of 4.0°C. The temperatures recorded in this study were, therefore similar to the 1991-2010 averages for Southern England in November (Table 1).

4. Discussion

Results from this study indicate that the in-crop use of yellow sticky traps and subsequent use of a RT-PCR assay is a viable approach with which to assess the BYDV-PAV infectivity levels of in-crop aphid populations. The results show that the process of trapping, extraction and storage according to the protocol followed does not reduce the detectability of the virus by RT-PCR assay ($F_{1,59} = 1.94$; $P = 0.06$). The length of time that aphids remain on trap in the field

for does however affect detectability ($F_{1,151}$ =15.21; P =<0.001) with a significant drop occurring between three and seven days ($F_{1,38}$ =5.04; P =0.04).

To be utilised effectively, an optimum timeframe for trap exposure in the field is required to maximise the accuracy of virus detection whilst minimising the amount of work required by operatives in the field and cost of materials. Further study to find a more precise timeframe would be needed before the method could be confidently applied, and it would be reasonable to concentrate this on the three to seven-day window.

It is not currently known at what viral titre an individual aphid vector poses a tangible risk to crops through transmission of BYDV-PAV. Further study to determine this parameter would establish confidence in the method and help inform economic injury level thresholds for control. This could prove a valuable addition to the use of sticky traps in determination of spray thresholds (see Holland *et al.*, 2019).

Similarly, the technique used here could be used to investigate factors affecting risk variability within fields. It is established that migrating alate aphids are more likely to land at field edges due to visual cues including long-wavelength light reflected by the plant-soil boundary (Schroder *et al*., 2015) and may also be influenced by local wind currents around boundary features (Holland *et al*., 2019). If, when repeated, higher numbers of virus carrying aphids were consistently found nearer to headlands, the risk of headland crops contracting BYDV could warrant selective spraying, landscape manipulation to facilitate conservation biological control or the sowing of resistant or tolerant crop varieties at field margins.

Establishing the confidence required for uptake and application as part of a DSS would require further investigation into how air temperature during exposure on-trap affects detectability of the virus in aphid specimens. Air temperature has previously been shown to be important in determining the detectability of Tomato spotted wilt virus in sticky-trapped thrips (Okazaki *et al*., 2011). Atmospheric temperatures will vary between seasons and sites according to region, aspect, elevation and exposure. This is highlighted by extensive recognition of

temperature as a factor in the interannual variability of aphid population dynamics contributing to BYDV epidemiology (Kendall *et al.*, 1992; Fabre *et al.*, 2006; Powell and Bale, 2006). If air temperature during on-trap exposure was shown to be a factor in the reliability of the method, a calculation of optimum cumulative day degrees of trap exposure may be more appropriate than purely time-based guidance.

BYDV-PAV is just one of three causal viruses responsible for crop losses through BYDD in UK crops, and the prevalence of these varies annually (Harrington *et al.*, 1999). Although this study focusses on the distinct BYDV-PAV strain, studies such as Malmstrom and Shu (2004), Kundu *et al.* (2009) and Zhao *et al.* (2010) have established simultaneous discrimination and quantification of several viral strains is possible. The comparison of compatibility of this trapping and processing method with different assays, including those using the simpler and quicker RT-LAMP technique (Mori and Notomi, 2009; Congden *et al.*, 2019b.) could broaden the application of the method to BYDD risk from other viral strains including BYDV-MAV and CYDV-RPV. Similarly, exploration of the compatibility of the assay with other in-crop trapping techniques such as water-pan traps, which require higher levels of maintenance than sticky traps but provide specimens in better condition for identification and processing (Harrington *et al.*, 2007) may improve the method's efficiency.

Once optimised, this localised and rapid assay of aphids trapped in-crop could inform BYDV risk assessment at field level by giving a measure of infectivity of ingressing aphids. Feeding this data into an aggregated model would help to reliably forecast the economic risk posed to an individual crop following primary infection and secondary spread of the disease taking into account growth stage at vector ingression and seasonal temperatures. Understanding of the complex nature of BYDV epidemiology, including the influence of surrounding land use, could be improved using this tool and contribute to the establishment of economic thresholds for insecticide application.

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406 Figure 1. Variation over time exposed on trap in BYDV-PAV score of aphids according to florescence
407 detected above a baseline of 0 by q-PCR assay. Significance of deviation from the mean BYDV-PAV
408 score detected by the same method in aphids taken from the same infected colony but unexposed on
409 trap.
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429 Table 1. Air temperatures during trapping periods and average mean temperatures for Southern
430 England during October and November 1991-2010 (Met Office, 2019)

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	14 days to	Max°C	Min°C	Mean Max°C	Mean Min°C
Time series test	17th Dec	12	-3	9.3	4
October average England S 1991-2010				14.4	7.2
November average England S 1991-2010				10.3	4.1

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4 Table

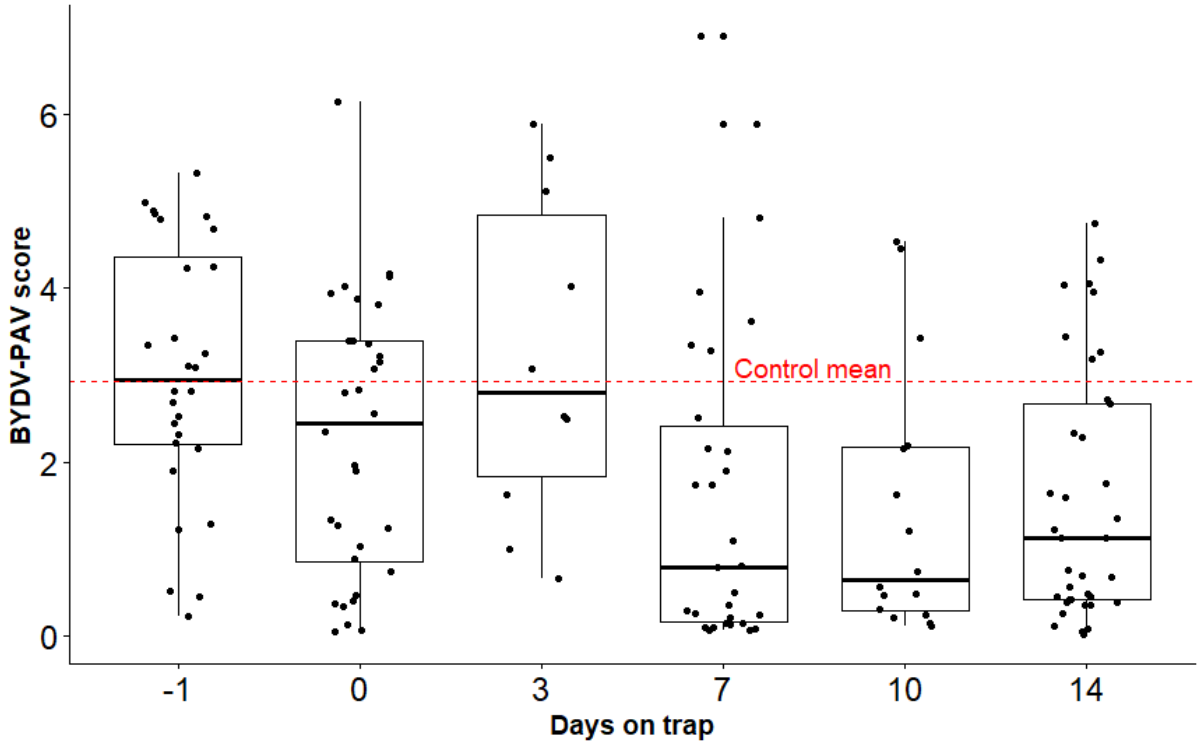

Figure 1.