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Evaluating the timing of insecticide application to manage barley yellow dwarf virus and yield in winter barley

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Abstract

Barley yellow dwarf virus (BYDV) is an important viral disease of grain crops worldwide and a major cause of yield loss. The risk periods for BYDV infection coincide with milder temperature that prolongs aphid flight and facilitates viral transmission through primary and secondary aphid movement in the crop. Secondary aphid movement is associated with greater BYDV spread in winter cereals. A critical component of BYDV management is therefore delaying sowing of winter cereals and correctly timing insecticide application to maximise crop protection. Previous research in Ireland considered insecticide timing in early (September) and late (October onwards) sown cereals. Early research did not consider action thresholds around temperature, aphid flight and risk of secondary spread. This research set out to understand the optimal timing of insecticide application in October sown winter barley to reduce BYDV infection and yield impact. A critical temperature of 3°C was used as a threshold for aphid development that leads to movement and BYDV spread, and insecticide treatments were applied to the crop at predictable intervals in relation to temperature. Results show that BYDV symptoms and yield are affected by spray time, location and year, although only significant with regard to the reduction of BYDV symptoms. For both BYDV symptoms and yield, there was a significant difference between untreated (control) plots and "early" and "late" applications of insecticide, again more notable for BYDV symptoms than yield. This work indicates the value of optimising a single insecticide spray for control of October sown cereals and supports decision-making in the management of cereal crops.

Keywords

Barley yellow dwarf virus (BYDV) • sulfoxaflor • threshold • transform • yield

Introduction

Barley yellow dwarf virus

Barley yellow dwarf virus (BYDV) is a viral disease of grain crops worldwide resulting in significant yield loss (up to 80%), particularly where infection happens early (Mc Namara *et al*., 2020; Van den Eynde *et al*., 2020). Since it was first reported in 1953 (Oswald & Houston, 1953), it has remained a persistent, although low-level problem. New geographical cases of the virus are still being reported (Hamdi *et al*., 2020) and it remains an economically important problem to address. Several different strains of the virus make up the disease pathosystem (e.g. RPV, MAV, PAV) (Sõmera *et al*., 2021) transmitted by different aphid species, including *Rhopalosiphum padi* (Linnaeus) (bird cherry oat aphid), *Sitobion avenae* (Fabricius) (grain aphid) and *Metapholopium dirhodium* (Walker) (rose grain aphid), considered as the main viral vectors in Western Europe.

The risk periods for BYDV infection coincide with milder temperature (Thackray *et al*., 2009; AHDB, 2020), as this prolongs the period of aphid flight and facilitates viral transmission through secondary aphid movement in the crop. Several studies give attention to the spatial and temporal variables impacting BYDV epidemiology (Van den Eynde *et al*., 2020), including plant spacing, local weather and topography (Kendall *et al*., 1992), frequency of virus acquisition and transmission by aphids (termed the infectivity index), population dynamics such as immigration, development and survival in relation to predator dynamics (Fabre *et al*., 2006; Kendall *et al*., 1992; Morgan, 2000), aphid mobility (Gillet *et al*., 1990) and temperature-dependent aphid population dynamics (Duffy *et al*., 2017). Other models focus on BYDV showing that certain serotypes correlate well with aphid vectors (e.g. MAV and

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PAV) while others (e.g. RPV) show no correlation (Leclercq-Le Quillec *et al*., 2000). Due to an expanse of research on the topic, several review papers on control options against viral vectors (Jactel *et al*., 2019; Mc Namara *et al*., 2020) have emerged, all in an effort for better support in crop management decisions. Integrating management approaches as part of an Integrated Pest Management (IPM) programme will require the validation of cultural controls like delayed sowing, in combination with tailored insecticide applications and consideration for crop varieties that carry BYDV resistance traits (Aradottir & Crespo-Herrera, 2021) as part of assessing aphid and BYDV risk.

Climate remains an important predictor of BYDV outbreaks (Duffy *et al*., 2017; Van den Eynde *et al*., 2020). Temperature in particular influences aphid movement and as such may be a useful parameter to evaluate insecticide application. Aphid movement, development and reproduction are impacted by ambient temperature and affect when and how BYDV initially arrives in virus carrying migrating aphids, and subsequently moves through the crop via secondary spread. Since most crop damage is linked with the secondary spread of the virus by the offspring of migrant grain aphids (mild temperatures facilitate continued aphid presence in the crop with reproduction and population growth) moving out from the initial infection point (Halbert & Pike, 1985; Teulon *et al*., 1999; Foster *et al*., 2004; Williams & Dixon, 2007), decision support tools targeted at periods of aphid movement, or correlated with aphid development are likely to have greater impact. Both virus and aphid incidence may also be influenced by crop and field characteristics, in particular sowing date, geographical region and topography (Foster *et al*., 2004).

Predictive models have been developed for aphid pests using temperature as a “threshold” in decision-making (Duffy *et al*., 2017; Soh *et al*., 2018). In the UK, the Agriculture and Horticulture Development Board (AHDB) has developed a BYDV tool (see https://ahdb.org.uk/bydv) which uses a predictive temperature model to assess aphid spread and suggest control actions against the secondary spread of aphids in a crop. Like the Duffy *et al*. (2017) model it uses a baseline of 3°C, regarded as the minimum temperature associated with aphid development, movement and secondary spread in the crop (Williams & Wratten, 1987), to calculate a Degree Day (DD) threshold for foliar insecticide application.

Thresholds

Thresholds (the pest population level at which economic damage will likely occur) are an integral aspect of IPM systems used in plant protection programmes to help growers and agronomists make informed disease and pest management decisions, which reduce excessive and unnecessary pesticide applications (Oakley & Green, 2006; Ellis *et al*., 2009). Several thresholds have been developed and recently reviewed for cereal aphids (McNamara *et al*., 2020), although the time requirement and crop observation time, prevalence of outdated and obsolete thresholds and poor correlations between aphid abundance, virus transmission and yield loss also lead to thresholds often being overlooked (Ramsden *et al*., 2017). Changing pest population dynamics (e.g. resistant aphid clones identified in major pest species) and changing climate (warmer or wetter autumns) may necessitate review and adjustments to thresholds. In Ireland, there is currently no established action threshold for insecticide application.

Rational for spray regime

Critical advice in Ireland based on previous research (Kennedy & Connery, 2001) is to avoid early sowing of winter cereals where possible, no earlier than the first week of October to reduce crop exposure to migrating aphids. In autumn planted cereals the risk of BYDV is greatest in early sown crops (Kennedy & Connery, 2001). As autumn advances, the risk of BYDV declines with lower temperatures which reduce aphid movement and reproduction; however, at this time the importance of localised transfer from non-migrating aphids (secondary spread) becomes greater (Kendall & Smith, 1981; Kendall & Chinn, 1990). Evidence of this is reported in other research where 86% of aphids monitored were found in early-September plots, 12% in late-September plots and only 2% in late-October plots (Kennedy & Connery, 2001). This research found that the application of insecticides prevented yield loss due to BYDV. These trials indicated that early-September sown crops should receive two insecticide treatments, one in mid-October and the second in early-November. Crops sown in late-September and October should receive only one insecticide treatment (during the first week in November). There was no advantage to applying additional treatments throughout the winter and early spring, even in a season when aphids and virus were abundant. However, applying an insecticide in December, January or February, to plots that had not received an earlier autumn insecticide treatment did prevent yield losses in 1995–1996 of 2.4, 1.9 and 1.4 t/ha, respectively (Kennedy & Connery, 2001).

Several changes (e.g. diminishing number of insecticides available to farmers for resistance management, greater focus on IPM and the presence of pyrethroid-resistant aphid clones) warrant an evaluation of current levels of insecticide efficacy, and testing of new insecticides for resistance management and integrated control. This is because continued reliance on pyrethroid insecticides is likely to exacerbate existing resistance, and potentially drive further selection pressure for additional forms of pyrethroid resistance (Walsh *et al*., 2019). In order to develop this advice into a robust crop management programme, it is necessary to understand (1) if an insecticide application is justified after delayed sowing, and (2) is there an optimal insecticide application time post crop emergence? By treating 3°C as a critical temperature threshold for aphid development and BYDV spread (Duffy *et al.*, 2017; AHDB, 2020), insecticide treatments were applied to crops at predictable intervals post crop emergence. DD accumulation was calculated for four different periods (18, 27, 36 and 45 d above 3°C), in order to account for the effect of temperature on aphid development. The experimental plot design included a standard "Control" (which did not receive an insecticide treatment) as well as additional "Control–Monitor" plots which were used to sample aphids to confirm their presence in the field at corresponding insecticide application times. As the "Monitor" plots served the purpose of validating observations in "Control" plots, they are included in statistical analysis.

Previous research (Kennedy & Connery, 2001) recently reconfirmed by McNamara *et al*. (under review) determined that multiple insecticide applications did not offer significantly higher levels of BYDV control or yield increase. Therefore, the objective in this study was to determine if the use of DD simulations could optimise a single foliar insecticide application in October sown barley. Due to reports in Ireland of the resistant SA3 *S. avenae* aphid genotype to pyrethroid insecticides (Walsh *et al*., 2019; Walsh *et al*., 2020), a non-pyrethroid insecticide (sulfoxaflor) was selected for use in the experiment.

Although there is widespread use of sulfoxaflor in field crops in other parts of the world (e.g. USA and Australia) (Etheridge *et al*., 2019; Pirtle *et al*., 2019; Jones *et al*., 2021), a recent review by the European Union (EU) indicates it will be revoked for outdoor use due to concerns about toxicity to bees (Pesticide Action Network, 2022).

Materials and methods

Experimental design

Two locations were chosen for field trials: Minane Bridge in Co. Cork, a south-western coastal location in Ireland which is widely regarded to have milder winters, more favourable for migrating aphids leading to high BYDV pressure, and Oak Park in Co. Carlow, an inland location that experiences more extreme winters unfavourable to migrating and live overwintering of aphids, which is regarded to have low BYDV pressure.

Barley was sown in the first week of October over a 3-yr period at the two locations, although ground conditions and the forecast of prolonged inclement weather required deviations from this approach. Winter barley (var. KWS Cassia) was sown (320 seeds/m^2) in Oak Park, Carlow on 3 October 2016, 2 October 2017 and 27 September 2018. At Minane Bridge, Cork sowing took place on 12 October 2016, 12 October 2017 and 3 October 2018 (Table S1). The crops received standard fertiliser, herbicide and fungicide treatments.

All weather data were sourced from the closest Met Éireann weather stations in Carlow and Cork. Three plots of 12 m × 2.2 m in Oak Park, Carlow and 13.5 m × 2.2 m in Minane Bridge, Cork were combined into a single plot/replicate in each treatment and were laid in a fully randomised block design. There were six replicates at both sites each year with the exception of 2017, where only six (Oak Park) and four (Minane Bridge) replicates were sown.

The experimental treatment insecticide Transform (500 g/kg sulfoxaflor, rate: 0.048 kg product/ha) was applied as a foliar spray. The treatment list and timings are outlined in Table S1 and calculations of DD were undertaken from the date of crop emergence for four spray timings; (18, 27, 36 and 45 d above 3°C). Where environmental conditions (e.g. wind speed) prevented insecticide application at exact dates, this happened at the closest suitable subsequent date. For statistical analysis, treatments are grouped into early and late timings to overcome challenges in matching predicted spray dates to actual spray dates, which were in reality affected by weather and ground conditions.

Virus symptoms

The percentage of tillers displaying yellow leaves as an indication of BYDV symptoms was determined by counting tillers with and without symptoms in four quadrats per plot, as shown in Figure 1. Symptoms were recorded at growth stages (GS) 43–49 (Zadoks *et al*., 1974) using quadrats of 0.25 m^2.

Figure 1. Yellow discoloration in leaves indicative of BYDV/CYDV symptoms in barley plots in Minane Bridge, Cork in 2016.

Virus testing

Following a similar approach to earlier research (Kennedy & Connery, 2005, 2012), symptomatic leaf samples (10–15) were collected at five separate locations approximately 3 m apart in each plot at GS 41–43 (Zadoks *et al*., 1974). Sap was extracted from the leaf sample using a leaf juicer and tested for the presence of BYDV by DAS-ELISA Kits (Bioreba Ag, Reinach, Switzerland), following the standard kit protocol (negative and positive controls were included). This test also screens for the presence of the similar virus cereal yellow dwarf virus (CYDV) transmitted by aphids.

Validation of visual assessment of BYDV

In order to estimate the percentage of plants potentially infected with BYDV, characteristic visual yellowing was recorded as BYDV infection. In order to verify if this association and method of assessment was valid, 173 symptom displaying leaf samples within the 3 yr studied were analysed using an ELISA-based detection method (Bioreba).

Aphid sampling and identification

Aphid sampling was carried out using a vacuum insect net Vortis (Burkard, Rickmansworth, Hertfordshire, England) or through active searching and physical aphid removal with a paintbrush. Vortis samples consisted of five sub-samples per plot, taken in a W configuration, and each of 15-s duration. Active searching consisted of checking 50 tillers (10 tillers at five points in a W configuration) similar to Dewar *et al*. (1982). Aphids were identified using Blackman's key to the Aphidinae (Macrosiphini) (Blackman, 2010). Aphid assessments were conducted fortnightly from crop emergence until GS 31. In 2016–2017 and 2017–2018, assessments were conducted by both direct observation of 50 tillers and extraction using a Vortis suction collector. In 2018–2019, assessments were conducted by Vortis suction collector only. Aphids were not subjected to BYDV or genetic testing.

Yield

Grain yields were recorded by harvesting plots (the middle of each treatment, 2.2 × 9.1 m) using a specially modified combine harvester. Grain analyses (hectrolitre weight, percent screening, protein and 1,000 grain weight) were carried out on a grain sample from each plot as measures of yield quality. Yields were expressed as t/ha at 15% moisture.

Statistical analysis

Field trial data were analysed in RStudio Version 1.4.1103. Additional packages used included *dplyr, emmeans, ggplot2, lmerTest, performance, psych, rcompanion* and *tidyr* (Kuznetsova *et al*., 2017; Lenth *et al*., 2022; Lüdecke *et al*., 2021; Mangiafico, 2022; Revelle, 2018; Wickham, 2016; Wickham & Girlich, 2022; Wickham *et al*., 2022). Descriptive statistics for grain yield and quality are presented numerically in terms of mean ± s.d. (summarised in Table S2) and graphically using plots of the simple main effects of the estimated marginal means. For foliar insecticide applications, an early application is a combination of the 18 and 27 DD treatments and late application as a combination of 36 and 45 DD treatments. Initially, a linear mixed model on the variation in BYDV, as the response variable, was investigated with spray time (control, monitor, early application and late application), location (Carlow, Cork) and year (2016–2017, 2017–2018, 2018–2019) treated as fixed effects in the model, with replicate number as a random effect. In order for the resulting model to satisfy the assumptions of residuals being normally distributed and homoscedastic (checked by plotting and using the Shapiro–Wilk and Breusch–Pagan test, respectively) a data transformation of $\text{BYDV}^{0.275}$ was required, as determined using Tukey's transformational ladder (Tukey, 1977). Further analysis, using a second linear mixed model, on the variation in yield (t/ha), as the response variable, in terms of the above-mentioned fixed and random effects model, but with BYDV included as a covariate, was carried out. In both sets of analyses the coefficient of determination was measured using Nakagawa's R^2 and pairwise comparisons on the estimated marginal means of the main effects, and simple main effects were controlled for multiple comparisons using Tukey HSD (honestly significant difference), with adjusted *P*-values presented (Tables S3A and S3B). All formal statistical test results were interpreted using a 5% level of significance. The focus of the study was BYDV and yield; however, grain quality properties (1,000 grain weight [g], protein [%], screening [%] and hectrolitre weight [kg/hL]) were also included and subjected to inferential statistics using linear mixed model analyses (Table S3C).

Results

Validation of BYDV presence

Of the 173 samples displaying BYDV symptoms selected for testing across the two sites over 3 yr, 100.0% tested positive for BYDV (MAV and/or PAV) and 0.6% tested positive for CYDV-RPV. Broken down into individual years, in 2016–2017, 61 samples were tested with 100% testing positive for BYDV (MAV and/or PAV) and 0.0% testing positive for CYDV-RPV. In 2017–2018, 57 samples were tested with 100.0% testing positive for BYDV (MAV and/or PAV) and 0.0% testing positive for CYDV-RPV. In 2018–2019, 55 samples were tested with 100.0% testing positive for BYDV (MAV and/or PAV) and 2.9% testing positive for CYDV-RPV (there were samples testing positive for both BYDV and CYDV). Therefore, the method of visual detection of BYDV is supported by positive detections using the ELISA bioassay.

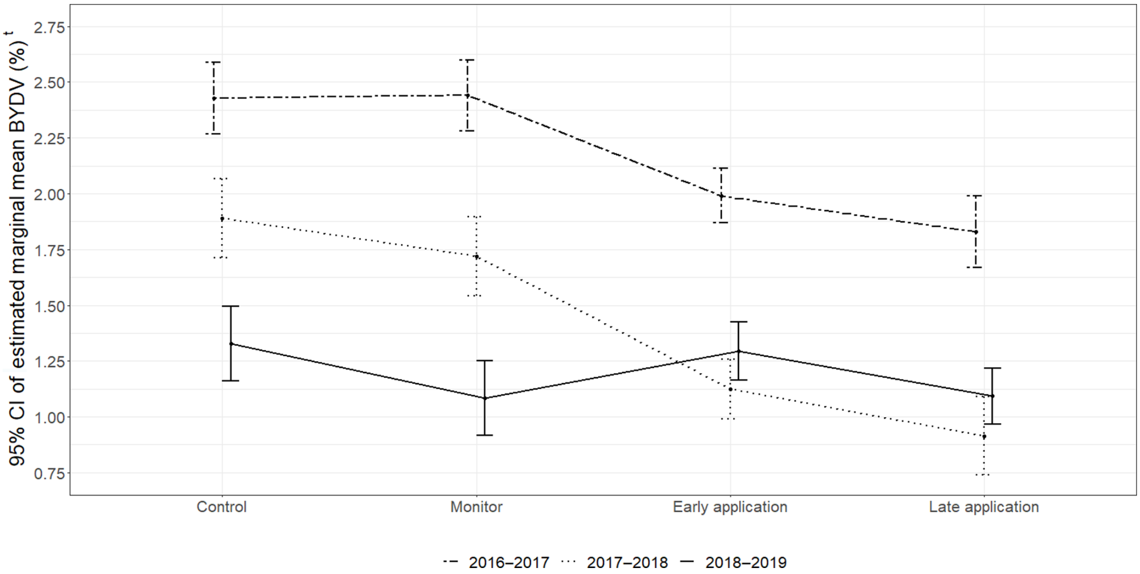

Figure 2. 95% confidence interval (CI) of estimated marginal means for percentage of BYDV^t symptoms in terms of spray time and year.

Impact of insecticide application on BYDV

The percentage of tillers displaying BYDV symptoms were subject to a data transformation of BYDV (BYDV^t) (Figure 2). BYDV^t is significantly impacted by spray time (early vs. late) ($P < 0.001$), location (Carlow vs. Cork) ($P = 0.015$) and year ($P < 0.001$). There was a significant interaction between spray time and year and also location and year ($P < 0.001$). The interaction between spray time and location, and spray time, location and year was not significant ($P > 0.05$). Differences based on mean ± s.d. are presented in Table S2.

For location, there was a significant difference between BYDV^t in Carlow and Cork ($P = 0.015$) with a higher percentage of BYDV detected in 2016–2017 and 2018–2019 across all treatment and control plots in Cork. There was a significant difference between years (2016–2017) and (2017–2018) ($P < 0.001$), (2016–2017) and (2018–2019) ($P < 0.001$), and (2017–2018) and (2018–2019) ($P < 0.001$). A significant difference was observed between untreated control plots and plots which received an "early" (18 and 27 DD treatments) application of foliar insecticide ($P < 0.001$). There was also a significant difference between untreated control plots and plots which received a "late" application (36 and 45 DD treatments) of a foliar insecticide ($P < 0.001$). There was also a significant difference between an "early" application and "late" application of foliar insecticide in terms of BYDV^t ($P = 0.003$), with the "late" treatments displaying less BYDV symptoms.

When analysed individually, an estimated marginal means analysis (Table 1) indicated that in four of the six individual experiments, an insecticide application significantly reduced the occurrence of BYDV symptoms.

Table 1: Reduction in the visual symptoms associated with BYDV within each individual experiment (*n* = 6)

Location and year	Estimated marginal means of percentage of plants displaying BYDV symptoms (untreated control plots)	BYDV symptoms reduction (%) post insecticide application[1]	*P*-value
Cork 2016–2017	5.2	47.8–69.1	0.006
Cork 2017–2018	7.0	71.6–85.1	0.001
Cork 2018–2019	5.4	(+)14.7–53.6	0.570
Carlow 2016–2017	4.3	47.9–69.0	0.006
Carlow 2017–2018	14.7	72.5–95.4	0.001
Carlow 2018–2019	2.3	23.1–67.8	0.078

[1] Percentage (%) calculations are based off untreated control plots.
(+) indicates symptoms of BYDV at greater levels than untreated control plots.
BYDV = barley yellow dwarf virus.

Impact of insecticide application on yield

Crop yield is significantly impacted by spray time ($P = 0.013$), location ($P < 0.001$) and year ($P < 0.001$) but not by BYDV ($P > 0.05$). There was a significant interaction between spray time and location ($P = 0.023$), location and year ($P < 0.001$) and spray time, location and year ($P < 0.001$). Differences based on mean ± s.d. are provided in Table S2.

In terms of location there was a significant difference between yield in Carlow and Cork ($P < 0.001$). Greater average yields (t/ha at 85% DM) were observed in Carlow in 2016–2017 and

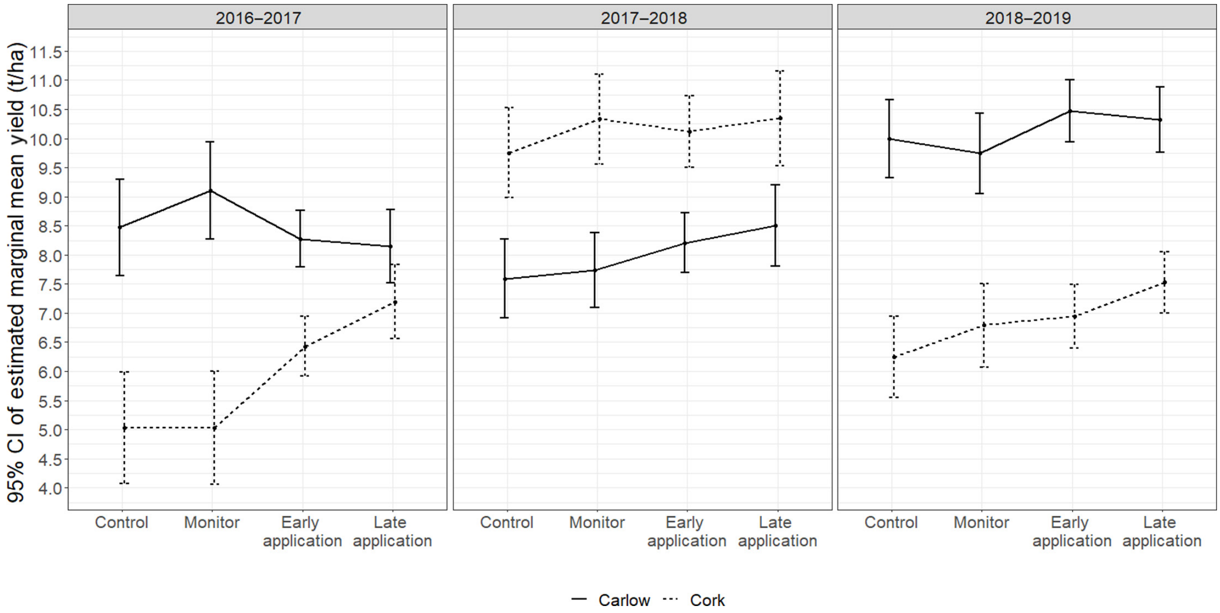

Figure 3. 95% confidence interval (CI) of estimated marginal means for yield (t/ha), with covariate BYDV, in terms of spray time, location and year.

2018–2019. There was a significant difference between years (2016–2017) and (2017–2018) ($P < 0.001$), (2016–2017) and (2018–2019) ($P < 0.001$), and (2017–2018) and (2018–2019) ($P = 0.002$). To analyse spray timings, BYDV^{t} treatments were grouped as an "early" (18 and 27 DD treatments) and "late" application (36 and 45 DD treatments) of foliar insecticide. There was a significant difference between the untreated control plots and plots that received an "early" application of sulfoxaflor ($P = 0.047$) in terms of yield. There was also a significant difference between untreated control plots and plots that received a "late" application of insecticide ($P = 0.006$). There was no significant difference between an "early" application and "late" application of insecticide in terms of yield ($P > 0.05$).

Analysis of the difference in spray time with location and year fixed (Figure 3), in Carlow (2016–2017), indicated that there was no significant difference between the untreated control plots and insecticide application, either early or late ($P > 0.05$). In Cork (2016–2017), there was a significant difference between untreated control plots and plots with an "early" insecticide application ($P = 0.014$) and between untreated control plots and plots with a "late" insecticide application ($P = 0.001$). In Carlow and Cork (2017–2018), there was no significant difference between untreated control plots and insecticide application, either "early" or "late" ($P > 0.05$). In Carlow (2018–2019), there was no significant difference between untreated control plots and insecticide application, either early or late ($P > 0.05$). In Cork (2018–2019), there was no significant difference between untreated control plots and plots with an "early" insecticide application ($P > 0.05$); however, there were between-treatment differences between the untreated control plots and plots with a "late" application of insecticide ($P = 0.011$).

Within individual experiments, the impact of insecticide application is less pronounced in terms of statistical differences than was recorded with BYDV (Table 2). The yield increases required to signify a statistically significant result are large (10.7–48.75%).

Impact of treatments on grain quality parameters

The focus of the study was BYDV and yield; however, for information purposes on grain quality, specific weight (kg/hL)

Table 2: Increase in yield associated with insecticide application within each individual experiment ($n = 6$)

Location and year	Yield increase impact of insecticide application (%)	*P*-value
Cork 2016–2017	22.4–48.8	0.014 (early and late)
Cork 2017–2018	3.5–6.7	>0.05
Cork 2018–2019	10.7–25.7	0.011 (late)
Carlow 2016–2017	(−)3.2–1.2	>0.05
Carlow 2017–2018	6.9–13.9	>0.05
Carlow 2018–2019	1.5–8.3	>0.05

1,000 grain weight (g), screenings (%) and protein content (%) were all recorded and reported descriptively (Table S2) and inferentially (Table S3C).

Aphid prevalence and identification

In the Cork monitor plots, 70 aphids were collected using a Vortis suction sampler between crop emergence and GS 31 in 2016–2017, 13 in 2017–2018 and 9 in 2018–2019. In the Carlow monitor plots, 29 aphids were collected in 2016–2017, 27 in 2017–2018 and 25 in 2018–2019. Of the 92 aphids collected in Cork, 43.5% of aphids were *S. avenae*, 19.6% were *R. padi*, 13.0% were *M. dirhodium*, 19.6% were other aphid species and 4.3% parasitised. Of the 81 aphids collected in Carlow 64.2% of aphids were *S. avenae*, 4.9% were *R. padi*, 13.6% were *M. dirhodium*, 9.9% were other aphid species and 7.4% parasitised.

Discussion

This research presents data from an early experimental field trial using the insecticide Transform (sulfoxaflor) prior to its full approval in Ireland. As such, it provides useful baseline information at two locations, which may support future evaluations of insecticide efficacy in terms of levels of BYDV and aphid control in cereal crops. Furthermore, it evaluates sulfoxaflor efficacy against a backdrop of pyrethroid resistant *S. avenae* clones within aphid populations in Ireland (Walsh *et al*., 2020). In this context, testing the efficacy of diverse insecticides with alternative modes of action is important to support a portfolio of resistance management strategies and engender more sustainable crop protection approaches.

This research set out to understand the optimal timing of insecticide application in October sown winter barley to reduce the likelihood of BYDV symptoms and yield impact. Ultimately, this contributes to the evidence base around delayed sowing and insecticide use, supporting better IPM adoption in the management of winter cereal crops.

The results indicate that visual assessment of BYDV presence correlates well with laboratory testing using DAS-ELISA Kits. This suggests that visual assessment is a valid method to evaluate the presence of infection in the crop.

This analysis shows that the presence of BYDV is significantly affected by spray timing, location and year showing the value of conducting research at multiple locations and over several years. It also demonstrates that a delayed foliar insecticide application of Transform (sulfoxaflor) (between 36 and 45 DD treatments) in December has a significantly greater effect in reducing BYDV symptoms over an early application. When analysed individually, estimated marginal means analysis indicate that an insecticide application significantly reduced the occurrence of BYDV symptoms in four of the six individual experiments.

Although the analysis shows that crop yield is impacted in a significant way by spray time, location and year, surprisingly this was not impacted by BYDV. Although an effect of spray timing on reducing BYDV symptoms in the crop was observed, overall this did not hold true for crop yield, and the analysis shows no significant difference between an “early” application and “late” application of insecticide in terms of yield ($P > 0.05$). A closer evaluation of data at the two locations and within different years showed that differences between no control and the application of a foliar insecticide were not significant in most years/locations except in Cork (a high BYDV pressure site) in a high BYDV pressure year (2016–2017). Although the data do not show significant results in terms of yield, it is still the case that single-digit yield losses can erode the profit margins of growers under current economic realities. So while the statistical significance of some results may be lacking (and potentially indistinguishable because of inherent variation within naturally infested plot experiments), the real-world implications of such losses on growers should not be dismissed as insignificant.

The relationship between BYDV and yield in a crop is not a clearly correlated one. Yield could be differently affected by the viral load and viral serotype causing infection. The appearance of BYDV may also display a non-uniform distribution, and some fields may escape detrimental levels of BYDV infection (Foster *et al*., 2004). Different BYDV/CYDV serotypes and stage of crop development at the time of infection also have a varied impact on crop yield (Foster *et al*., 2004). Observations have been made that field size, aphid and virus incidence have a parabolic relationship and aphid incidence and pressure may be lowest in larger fields (>31 ha). Another study (Vialatte *et al*., 2007) found consistent evidence of clustering and habitat selection by aphid genotypes within the landscape, which would affect the location of aphids and the BYDV pressure in a field. This suggests that insecticide trials designed as small to medium plots (the approach used in this study) may not face the same aphid and BYDV pressure as larger fields, making relationships between different variables more difficult to discern. This impact of field/plot size should be noted in the design of future trials to test insecticide efficacy in arable crops. To provide a more robust indication of insecticide efficacy for mobile species, treatment plots would need to be as large as possible with all treatments fully replicated (Ward, 2018). However, as this is often not practical or possible due to cost and field limitations, some studies suggest that larger plots with reduced replication are better than small plots for assessing the effects of insecticides on mobile species (Smart *et al*., 1989). It may also be beneficial to confirm findings on a plot scale with split field or tram line trials to capture lower levels of yield loss. Although potentially more expensive, it may help with confirming results that form IPM advice for mobile species.

The findings of this work complement previous research by Kennedy & Connery (2001), recommending a single insecticide for winter barley sown in October. However, results indicate that it may also be important to consider geographical location in decision-making on foliar insecticide application, noting that treated plots were not statistically significant in Carlow in any of the 3 yr. However, the levels of yield reductions, while not statistically significant, would be economically significant in terms of potential yield loss. Conversely, the yield losses at the Cork site would strongly support the application of an insecticide to mitigate potential yield loss. Furthermore, in four out of the six individual experiments an insecticide application significantly reduced the occurrence of BYDV symptoms.

The value of using critical temperature to simulate a DD approach as a threshold or predictor of the optimal spray time may have been diminished in combining the treatments into “early” and “late” foliar applications for statistical analysis. However, other research by this group (unpublished) using similar timings has shown that an early application had no significant difference in yield when compared with a late application, and both had a significantly positive impact on yield over a no-control option. This supports results and observations in this research.

Conclusion

This research offers four clear results to Irish growers and tillage advisors. The incidence of BYDV in the crop is not always associated with yield impact. The later application of an insecticide is more effective at reducing the appearance of BYDV symptoms over an early application, potentially explained by effective control of secondary spread (local migrants in a crop). The application of an insecticide is more beneficial at a high disease pressure location like Cork and in a high virus risk year. There were indeed yield reductions associated with the control plots (no treatment) and although these were not statistically significant, they may be economically significant to growers.

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Supplementary data

Table S1: Details of plot trial treatments at two locations

Year	Location	Treatment	Application date	Predicted application date
2016–2017	Cork	Control		
		Monitor		
		18 d[1]	7 Nov 2016	6 Nov 2016
		27 d[1]	15 Nov 2016	15 Nov 2016
		36 d[2]	25 Nov 2016	29 Nov 2016
	Carlow	Control		
		Monitor		
		18 d[1]	27 Oct 2016	28 Oct 2016
		27 d[1]	3 Nov 2016	6 Nov 2016
		36 d[2]	14 Nov 2016	14 Nov 2016
2017–2018	Cork	Control		
		Monitor		
		18 d[1]	9 Nov 2017	9 Nov 2017
		27 d[1]	24 Nov 2017	18 Nov 2017
		36 d[2]	21 Dec 2017[3]	28 Nov 2017
		45 d[2]	10 Jan 2017[3]	9 Dec 2017
	Carlow	Control		
		Monitor		
		18 d[1]	1 Nov 2017	31 Oct 2017
		27 d[1]	9 Nov 2017	9 Nov 2017
		36 d[2]	24 Nov 2017	22 Nov 2017
		45 d[2]	11 Jan 2018[3]	6 Dec 2017
2018–2019	Cork	Control		
		Monitor		
		18 d[1]	26 Oct 2018	27 Oct 2018
		27 d[1]	2 Nov 2018	5 Nov 2018
		36 d[2]	16 Nov 2018	14 Nov 2018
		45 d[2]	21 Nov 2018	23 Nov 2018
	Carlow	Control		
		Monitor		
		18 d[1]	24 Oct 2018	21 Oct 2018
		27 d[1]	30 Oct 2018	30 Oct 2018
		36 d[2]	13 Nov 2018	8 Nov 2018
		45 d[2]	19 Nov 2018	17 Nov 2018

[1] Early application. [2] Late application. [3] Insecticide applications were not possible upon the predicted dates as they were adversely impacted by the prevailing weather and ground conditions. This is accounted for in the re-designation of treatments as “early” and “late”. Aphids were sampled from monitor plots only. Yield and BYDV metrics were retained for monitor to validate observations within the control plots.

BYDV = barley yellow dwarf virus.

Table S2: Effect of aphicide application (mean ± s.d.) on prevalence of barley yellow dwarf virus (BYDV) and on parameters of grain yields of barley

Year	Location	Spray timing	t/ha at 85% DM	% BYDV	TGW (g)	Protein (%)	Screening (%)	Hectrolitre (kg/hL)
2016–2017	Carlow	Control	8.3 ± 0.87	23.0 ± 5.20	48.9 ± 2.60	–	3.6 ± 0.93	64.7 ± 3.01
		Monitor	8.9 ± 0.26	23.4 ± 4.49	51.8 ± 1.44	12.9 ± 0.64	2.7 ± 0.34	66.7 ± 0.67
		Early application	8.2 ± 0.81	11.4 ± 3.42	48.8 ± 2.35	12.3 ± 0.54	4.2 ± 1.64	64.0 ± 2.66
		Late application	8.1 ± 0.69	8.4 ± 2.64	49.1 ± 1.50	12.0 ± 0.93	4.0 ± 1.45	64.0 ± 2.21
	Cork	Control	4.8 ± 0.88	28.2 ± 6.36	41.1 ± 3.22	12.7 ± 0.94	11.6 ± 3.45	53.3 ± 7.45
		Monitor	4.8 ± 0.45	28.7 ± 5.49	40.9 ± 1.50	12.7 ± 0.95	9.5 ± 2.16	57.0 ± 1.43
		Early application	6.4 ± 0.68	13.9 ± 4.18	46.9 ± 2.38	12.1 ± 0.54	5.0 ± 2.02	60.1 ± 2.96
		Late application	7.2 ± 0.47	10.3 ± 3.23	47.8 ± 1.17	–	3.3 ± 0.56	63.3 ± 0.61
2017–2018	Carlow	Control	7.5 ± 0.28	14.7 ± 5.42	45.5 ± 2.14	10.9 ± 0.37	15.2 ± 0.36	65.6 ± 2.43
		Monitor	7.7 ± 0.35	11.1 ± 5.13	46.4 ± 1.82	10.8 ± 0.49	15.2 ± 0.82	65.0 ± 1.64
		Early application	8.3 ± 0.48	2.8 ± 2.45	47.6 ± 2.69	10.4 ± 0.35	–	–
		Late application	8.6 ± 0.63	0.7 ± 0.43	47.1 ± 1.34	10.4 ± 0.26	–	–
	Cork	Control	9.7 ± 1.06	7.0 ± 1.98	50.8 ± 5.63	11.5 ± 0.47	20.2 ± 1.14	62.3 ± 1.00
		Monitor	10.3 ± 0.87	5.9 ± 5.69	52.7 ± 2.04	–	19.5 ± 2.02	62.2 ± 0.83
		Early application	10.1 ± 1.00	1.5 ± 1.17	53.1 ± 2.59	11.9 ± 0.78	20.1 ± 1.31	62.2 ± 1.88
		Late application	10.4 ± 1.11	1.3 ± 0.64	54.2 ± 2.44	12.0 ± 0.57	–	–
2018–2019	Carlow	Control	10.1 ± 0.52	2.3 ± 1.24	58.6 ± 4.57	11.5 ± 0.58	–	67.4 ± 1.48
		Monitor	9.8 ± 1.15	1.0 ± 0.95	58.3 ± 7.22	11.4 ± 0.31	1.6 ± 0.40	67.0 ± 1.95
		Early application	10.5 ± 0.88	1.5 ± 1.12	58.4 ± 4.89	11.3 ± 0.41	1.3 ± 0.53	68.1 ± 1.36
		Late application	10.4 ± 0.91	0.9 ± 0.33	57.9 ± 5.13	11.6 ± 0.33	1.2 ± 0.46	68.5 ± 0.98
	Cork	Control	6.3 ± 0.98	5.4 ± 4.97	55.8 ± 4.55	11.6 ± 0.32	3.2 ± 1.47	55.8 ± 5.95
		Monitor	6.8 ± 0.86	2.8 ± 2.56	58.7 ± 3.18	11.3 ± 0.63	3.2 ± 1.40	57.8 ± 5.33
		Early application	6.9 ± 0.90	4.70 ± 2.43	57.1 ± 3.82	11.4 ± 0.50	2.7 ± 1.14	58.6 ± 3.82
		Late application	7.6 ± 0.91	3.2 ± 3.47	60.9 ± 5.13	11.4 ± 0.56	2.0 ± 1.05	61.6 ± 3.26

TGW = thousand grain weight.

Table S3A: Linear mixed model for BYDV$^{0.275}$ with spray time, location and year as fixed effects, replicate number as a random effect (80.1% Nakagawa's R^2)

Effects	*F*-ratio	*P*-value
Spray time	39.67	<0.001
Location	6.08	0.015
Year	211.24	<0.001
Spray time*location	1.35	0.259
Spray time*year	10.47	<0.001
Location*year	9.55	<0.001
Spray time*location*year	1.45	0.199

Table S3B: Linear mixed model for yield (t/ha) with covariate BYDV, spray time, location and year as fixed effects, replicate number as a random effect (83.2% Nakagawa's R^2)

Effects	*F*-ratio	*P*-value
BYDV	0.32	0.572
Spray time	3.74	0.013
Location	100.62	<0.001
Year	28.37	<0.001
Spray time*location	3.29	0.023
Spray time*year	0.13	0.993
Location*year	152.44	<0.001
Spray time*location*year	4.55	<0.001

BYDV = barley yellow dwarf virus.

Table S3C: Linear mixed model with BYDV as a covariate, spray time, location and year as fixed effects, replicate number as a random effect

	Response variable (Nakagawa's R^2)							
	Hectolitre (68.4%)		**Protein (63.2%)**		**% Screening (96.0%)**		**TGW (73.5%)**	
	***F*-ratio**	***P*-value**	***F*-ratio**	***P*-value**	***F*-ratio**	***P*-value**	***F*-ratio**	***P*-value**
BYDV	0.27	0.604	0.49	0.485	0.46	0.497	3.22	0.075
Spray time	2.62	0.053	3.00	0.033	3.11	0.028	0.73	0.534
Location	183.08	<0.001	10.92	0.001	215.39	<0.001	0.54	0.464
Year	3.40	0.036	21.10	<0.001	1,629.38	<0.001	59.17	<0.001
Spray time*location	5.32	0.002	1.04	0.377	10.49	<0.001	3.80	0.012
Spray time*year	1.35	0.238	2.59	0.021	4.14	0.001	0.14	0.991
Location*year	13.05	<0.001	16.35	<0.001	23.11	<0.001	24.21	<0.001
Spray time*location*year	2.65	0.018	0.86	0.523	10.33	<0.001	1.55	0.166

BYDV = barley yellow dwarf virus; TGW = thousand grain weight.