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Crop management guidelines for minimising wheat yield losses from wheat bulb fly

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1. Abstract

Wheat bulb fly (WBF) is one of the most serious pests of winter wheat and is particularly prevalent in the east of England and Scotland. WBF lays eggs in bare ground during the summer and its larvae hatch during winter and can reduce the yield of wheat by killing shoots and reducing final ear number. The potential yield loss depends on the shoot population in winter, the size of pest population and how much damage an individual larvae can cause. The aim of this project is to develop a pest threshold scheme to predict the minimum plant population, latest sowing date and need for an insecticide treatment (seed or foliar) to minimise the risk of yield losses to WBF.

A WBF threshold scheme was developed that used information from the autumn survey of WBF incidence in September, egg viability, the maximum shoot number the crop could achieve by late winter, and the number of shoots that a single WBF larvae could destroy. This model showed that typical variation in the maximum shoot number had a large effect on the chance of yield loss because well grown crops produce excess shoots which can be sacrificed without affecting yield. A model of shoot production was developed based on thermal time and plant population that was embedded within the WBF threshold scheme. This was done to allow a prediction of yield loss from WBF to be made in time for decisions about sowing date and seed rate. A review of literature showed that most WBF mortality occurs in the larval stage between egg hatch and plant invasion. The lowest level of mortality recorded was 56% and this value was used to help calculate the numbers of shoots likely to be lost to the pest. The literature also suggested that the number of shoots destroyed by an individual WBF larva was typically four.

Five winter wheat field experiments with combinations of sowing date, seed rate, variety, seed or foliar insecticide treatments were set up in the 2015-16 and 2016-17 seasons to calibrate and test the WBF threshold scheme and the shoot production model. Independent tests showed the shoot production model performed reasonably well, but it should be recognised that it does not deal with site specific factors that may limit tillering (e.g. soil capping). Some field experiments were deliberately done at sites which historically have been at high risk of WBF damage, however there was insufficient pest pressure against which to effectively test the threshold scheme due to nationally low levels of pest oviposition. The potential to estimate WBF prevalence using water trapping, rather than laborious egg counts from soil samples, was assessed by reviewing literature and testing at 12 sites. The literature on this topic demonstrated that this approach should work, however the field tests were inconclusive due to low WBF egg levels in the seasons of testing.

The project has developed prototype guidelines summarising how sowing date and plant population should be adjusted, and insecticide seed treatments targeted, for different WBF risk situations. Further work is required to field test the WBF threshold scheme in situations of high WBF pressure, and to develop it to deal with varietal differences in shoot number and site specific factors.

2. Introduction

Taking account of crop tolerance is fundamental to improving pest risk assessment and achieving a rational approach to pest control which is cost effective and minimises the impact on the environment and the potential for the development of resistance to pesticides (Ellis *et al*., 2009). This approach was discussed in CRD project PS2814 'A desk study to review the potential for crop physiology based thresholds for invertebrate feeding groups' (Ellis *et al*., 2013) and has been pivotal in AHDB Cereals and Oilseeds project RD-2005-3242 'Re-evaluating thresholds for pollen beetle in oilseed rape' (Ellis & Berry, 2012) and CRD projects PS2805 'Assessing tolerance to slugs in winter wheat and oilseed rape by simulating pest damage' (Ellis *et al*., 2012), PS2821 'Economic and agronomic analysis of reducing the risk of slug induced crop losses by increasing crop seed rate' (Kendall *et al*., 2014) and PS2820 'Further investigation of the tolerance of winter wheat and oilseed rape to slugs' (Ellis & Berry 2014). The pollen beetle project showed that oilseed rape generally produces more buds than are required to achieve potential yield and therefore some can be sacrificed to pollen beetle without any yield loss. A study to quantify the number of buds that may be sacrificed to pollen beetle and the number of buds that can be destroyed by pollen beetles enabled the development of more robust thresholds to limit unnecessary insecticide application (Ellis & Berry, 2012). Work on the tolerance of oilseed rape and wheat to slugs indicated that both crops were able to compensate for the loss of some leaf area but that increasing seed rate to combat the loss of plants was uneconomic in most situations. This project on wheat bulb fly (WBF, *Delia coarctata*) will use a similar approach to that used for pollen beetle in oilseed rape by determining how many excess shoots a wheat crop develops and how many shoots are destroyed by WBF. Bryson *et al.* (2005) investigated control of gout fly (*Chlorops pumilionis*) in winter wheat. They concluded that in spite of high levels of the autumn generation of gout fly, in some cases (50-60% plants infested), there was no significant reduction in yield even where there was a low seed rate, thin crops and reduced early nitrogen. There was no relationship between the percentage of plants infested and yield and it was suggested that in the majority of cases the crop can compensate for the early loss of tillers due to the autumn damage by the pest. It is possible that similar crop tolerance occurs for wheat against WBF.

In general the project will develop further our understanding of crop tolerance to dipterous stem borer pests, together with the damage done by this pest, and how this can be used to develop crop management strategies for minimising yield losses and to achieve effective control techniques including the sustainable use of pesticides.

2.1. Wheat bulb fly – a major UK pest

WBF reduces the yield of wheat by killing shoots and reducing final ear number. The potential yield loss therefore depends on the plant population and number of shoots per plant, the size of pest population and whether the pest destroys single or multiple shoots/plant.

WBF ranks amongst the most serious pests of winter wheat in Great Britain and is particularly prevalent in the east of England and Scotland. There is considerable annual variation in the intensity of the attacks but occasionally these are severe. Until recently, the WBF threshold above which an egg hatch insecticide spray of chlorpyrifos was recommended was 2.5 million eggs/ha. However, in 2016 the use of chlorpyrifos as an egg hatch spray was lost and therefore there is currently no chemical control available beyond the application of a pyrethroid seed treatment. This casts some doubt over the value of the 2.5 million eggs/ha threshold although it does represent the level at which damage sufficient to impact on the yield might be expected in crops sown up until the end of October. The threshold for use of a seed treatment is 1 million eggs/ha for late sown crops sown after 1st November only (AHDB Information Sheet No. 51). However, recent thinking (Ellis & Berry 2012) has suggested that pest thresholds should be related to the ability of individual crops to tolerate pest attack and so are likely to be represented by a sliding scale of pest risk rather than a single value. This project will attempt to develop this thinking for WBF and present it in such a way that it is easily applicable by farmers and agronomists.

Monitoring of WBF egg populations in England and Wales from 1984-2015 showed that, on average, 21% of fields in areas at risk from the pest had egg numbers above the previous 2.5 million eggs/ha threshold. The area of England and Wales potentially at risk from WBF annually can be estimated from the area of crops that potentially provide bare ground as egg laying sites for the pest. These include potatoes, sugar beet, vining peas, onions, dry harvested peas and fallow. This represents an area of 479,000 ha. WBF is only a problem in the east of England and this accounts for about 70% of the total area of crop area that provides egg laying sites, but only about 80% of these potential egg laying sites will be followed by wheat in the rotation. Young (1992) measured an average yield response to WBF control of 17% where the threshold was exceeded so at an average wheat yield of 8 t/ha at £120/t the total cost of WBF in England and Wales is approximately £10 million per year.

An estimate of potential annual risk from WBF can be gained from the AHDB Cereals and Oilseeds autumn survey of WBF incidence which assesses the annual level of egg laying and is published in early October. This information is then used with the current WBF thresholds (1.0 million eggs/ha for late sown/backward crops, sown November onwards) to decide whether to apply a seed treatment to late sown crops. However, these thresholds do not take account of the crop's shoot number which is crucial for determining how effectively it can tolerate damage. Wheat crops require a minimum of 400 to 450 fertile shoots/m^2 to achieve potential yield (Spink *et al*., 2000a), but typically produce more than 1000 shoots/m^2 (and up to 2000 shoots/m^2) by GS30 which is usually in March (AHDB Cereals & Oilseeds HGCA Wheat Growth Guide). From March to May there is a decline in shoot numbers in most crops as the weakest shoots die leaving a final number of 400 to 700 shoots/m^2. In most cases the majority of the shoots are produced during autumn and are therefore present at the time when WBF can damage the crop in January/February. Crops sown late (after mid-October) or with low plant populations produce a greater proportion of their shoots after winter, and tend to produce fewer excess shoots and therefore have a lower tolerance to shoot loss.

Chemical control of WBF is currently solely reliant on insecticidal seed treatments. Austral Plus (fludioxinil + tefluthrin) and Signal (cypermethrin) are available for crops sown after November. From 31 March 2016 products containing chlorpyrifos-ethyl such as Dursban WG and Equity could no longer be sold by distribution and storage and disposal and relabelling of any existing stocks had to be completed by 1st October 2016. Up until this date chlorpyrifos was an important product for WBF control and could be applied between December and February as an egg hatch spray which is designed to kill larvae before they enter the plant. With the loss of this use it has become increasingly important to develop alternative control strategies that are less reliant on insecticides. Under the Sustainable Use Directive (2009/128/EC) there is a requirement to increase the use of Integrated Pest Management (IPM) techniques and this is an option that should be explored for WBF.

As chlorpyrifos is no longer available it is no longer possible to control the number of shoots killed per larva by chemical control using an egg hatch spray. Therefore it is essential for growers to understand the risk of WBF damage before a crop is drilled, as the number of shoots could be manipulated using earlier sowing dates and higher seed rates to improve crop tolerance to WBF. Seed treatments could also be used to control WBF in late sown cereals. Figure 1 shows the key timings for development of early sown and late sown wheat crops, and demonstrates how the timing of tillering varies around the likely timing of WBF damage depending on when the crop is sown. It also shows the main timing of WBF egg laying, which occurs on bare soil before a crop is drilled, demonstrating that there may be opportunities to monitor the risk from WBF before drilling, and then modify management practices through seed rate or sowing date to enhance the crop’s tolerance to the pest. The project therefore aimed to provide guidelines for how farmers should manage crops by optimising sowing date, seed rate and the use of seed treatments to maximise crop tolerance to pests and minimise the need for insecticides.

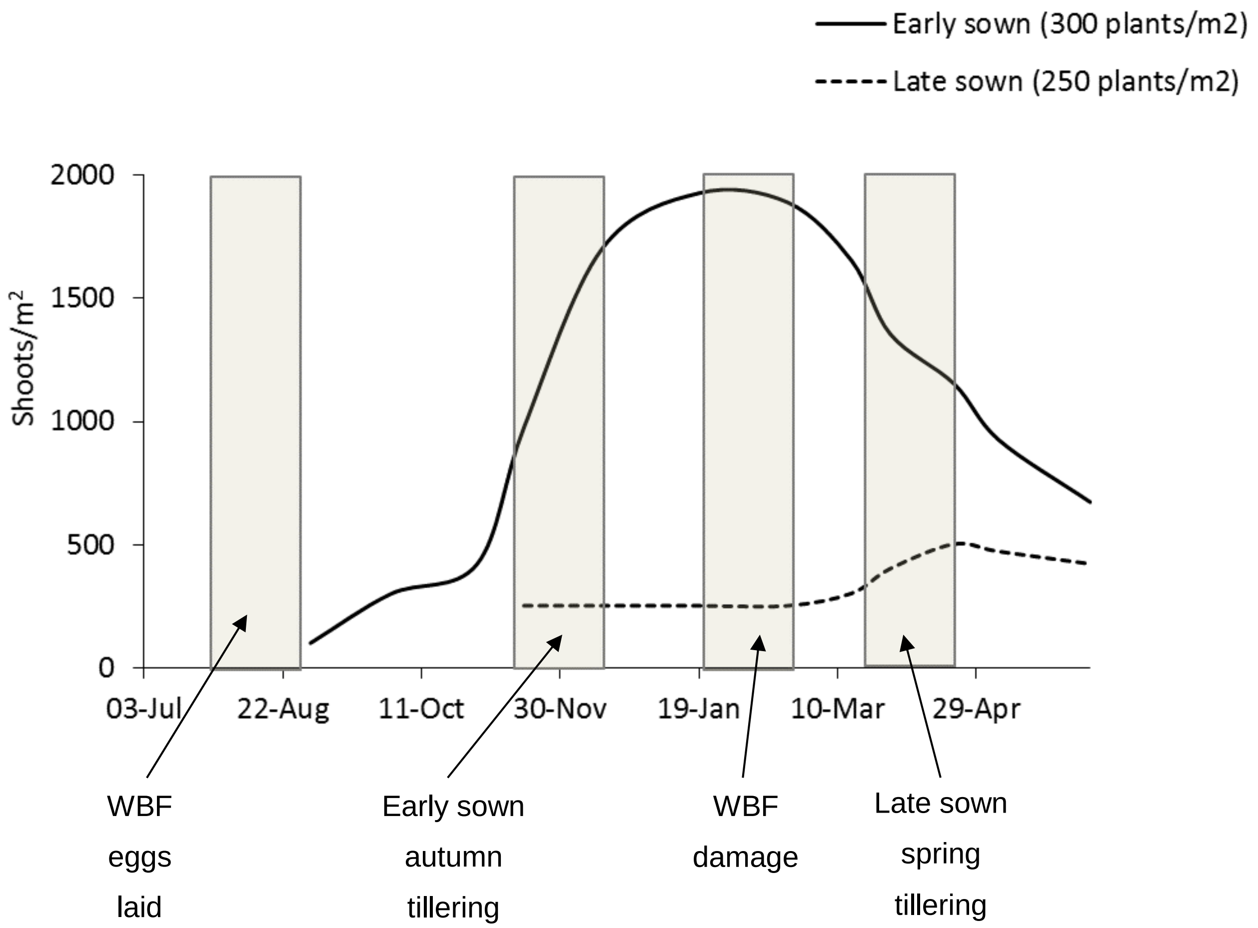

Figure 1. The number of shoots/m^2 produced for an early or late sown crop respectively and timings of WBF egg laying and damage.

2.2. Developing a new threshold for wheat bulb fly

The impact of WBF on the crop and the need for treatment is dependent upon a number of factors as listed below.

1) How many shoots each WBF larva destroys.
2) The minimum number of shoots a crop requires to achieve yield potential.
3) Actual number of shoots in the crop.
4) Value of the crop yield.
5) Cost of the control measure.
6) Efficacy of the control measure.

Ellis *et al*. (2013) outlined a science-based threshold scheme for WBF and concluded that it should include, as a minimum, components 1 to 3, and would have additional value if it included components 4 to 6. The economic injury level (EIL, the lowest pest population density that will cause economic damage) for WBF (million eggs per hectare) can be calculated using the equation below in which SN is the number of shoots/m^2 measured in winter, SN_{MIN} is the minimum number of shoots/m^2 required to achieve potential yield, SN_{KILL} is the number of shoots killed by an individual larva and Egg viability is the proportion of eggs that produce larvae.

$$\boldsymbol{EIL = \frac{(SN - SN_{MIN})/SN_{KILL}}{Egg\ Viability \times 100}}$$ **Equation 1**

By assuming a minimum number of shoots/m^2 for potential yield (SN_{MIN}) of 400, a winter shoot number (SN) of 1100/m^2, a wheat bulb fly larvae kill rate (SN_{KILL}) of three shoots and an egg viability of 0.5 the above formula gives an EIL of 4.7 million wheat bulb fly eggs per hectare. This is significantly above the previous value of 2.5 million eggs per hectare and suggests that at typical wheat crop has the potential to tolerate WBF attack and still yield well.

A sensitivity analysis was done to test the impact on the EIL of changing each parameter used in the above equation from its likely minimum value to its maximum value (Figure 2). This shows that the known variation in winter shoot number of 600 to 1600 shoots/m^2 has a very large effect on the EIL and changes it from 1 to 8 million eggs per hectare.

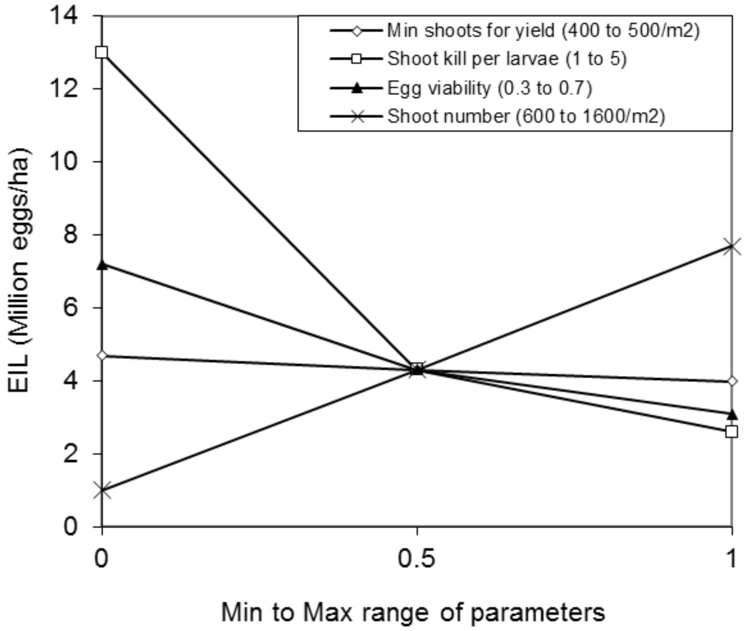

Figure 2. Sensitivity analysis for the economic injury level (EIL) of wheat bulb fly for the minimum and maximum ranges of each of the parameters used to calculate the EIL.

Altering the minimum shoot number required for potential yield has relatively little effect on the EIL. The range of values for the number of shoots killed by an individual larva and the egg viability will be reviewed as part of this project, so the ranges used here should be regarded as preliminary estimates. If the range of shoots killed per larva is 1 to 5 and the range of egg viability of 0.3 to 0.7 are realistic then variation in these parameters is predicted to have a large impact on the EIL.

2.3. An alternative to soil sampling for WBF risk assessment

Sampling soil for eggs of WBF is very time consuming and involves collecting large quantities of soil (approximately 20kg). Consequently few farmers or agronomists use soil sampling to predict the risk of WBF attack. AHDB Cereals & Oilseeds fund ADAS to undertake an annual survey to assess WBF egg numbers at 30 sites in the east of England and a similar survey is undertaken by Scotland's Rural College (SRUC). This provides data to predict the need for seed treatments for late sown crops. However, results are not usually available until the end of September and this can be too late to make decisions on the need for sowing date, seed rate and seed treatment. Cooper (1981) used water traps to catch WBF adults. The numbers of females caught and subsequent egg numbers, estimated from soil cores, were linearly related. Water trapping ran between mid-July and the end of August so potentially data on the WBF risk was available earlier than where soil sampling is used to assess egg numbers. This early indication of WBF risk will be very valuable for determining the latest sowing date, lowest seed rate and need for an insecticide seed treatment for wheat crops sown from September onwards in WBF high risk situations.

2.4. Remote sensing of tiller numbers

Given the large potential impact of winter shoot number, egg viability and the number of shoots killed by an individual WBF larva on the yield impact of the pest, understanding these variables will form the main focus of this project. Whilst there is a lot of data on the minimum number of tillers required to achieve potential yield and the yield loss that occurs if shoot numbers fall below this critical level, the methodology for assessing shoot number is laborious and unlikely to be something that would be undertaken by the majority of farmers/agronomists. This project will therefore also investigate developing methods for rapid assessment of tiller populations. An estimate of winter shoot number will guide the use of egg hatch insecticide sprays should any become available.

2.5. Project aims and objectives

With regard to developing an integrated pest management strategy for dipterous stem borers in line with implementation of the Sustainable Use Directive (Directive 2009/128/EC), this project will improve risk assessment by providing further guidance which farmers/agronomists can use to rationalise insecticide use in winter wheat for control of WBF. It will also provide guidelines for how farmers should manage crops by optimising sowing date and seed rate to maximise crop tolerance to pests. Whilst WBF is the most serious dipterous stem boring pest, others such as frit fly (*Oscinella frit*), gout fly (*Chlorops pumilionis*) and yellow cereal fly (*Opomyza florum*) also cause 'deadhearts' and can reduce yield in some situations. This project will concentrate on WBF to develop crop specific treatment thresholds which would also be applicable to these other stem borers with minimal modification.

In the absence of chlorpyrifos, being able to predict and remotely sense shoot number would add to any product stewardship programme for any new or existing actives that may become available as an egg hatch treatment. The project will enable farmers and agronomists to determine the minimum seed rate and latest sowing date combinations, and the need for seed treatments, to minimise the risk of yield losses to WBF based on numbers of eggs or adults of the pest, egg viability and crop tolerance. Crop tolerance will be measurable in terms of the number of excess shoots produced by individual crops. In general, the project will significantly improve understanding of the relationship between WBF and crop yield. This in turn will help to develop IPM strategies for this pest which minimise reliance on chemical control.

Once the potential WBF risk is known the next stage is to predict the seed rate, sowing date, and requirement for a seed treatment necessary to produce a crop which is sufficiently robust in terms of its shoot population to tolerate pest attack. To achieve this the project developed a model of shoot production to estimate shoot number for a given seed rate and sowing date. This was used to develop a simple threshold scheme, which will help growers to manage the WBF risk.

Overall, the project aimed to improve understanding of the relationship between WBF and crop yield. This in turn helps to develop IPM strategies for this pest which minimise reliance on chemical control.

The project objectives were:

- To develop a method for predicting the maximum number of shoots and the number of excess shoots in wheat that could be lost to WBF pests without affecting yield.
- To quantify the proportion of WBF eggs or adults measured in early autumn that become shoot-damaging larvae and the number of shoots a single larva can destroy.
- To evaluate water trapping adult WBF as an alternative to counting eggs in soil samples for assessing WBF risk.
- To develop a scheme to predict the minimum seed rate (target plant population), latest sowing date and need for a seed treatment to minimise the risk of yield losses to WBF pests, based on egg or adult numbers, egg viability and crop tolerance.
- To develop a scheme to predict threshold egg or adult WBF numbers that justify insecticide treatment based on egg viability and crop tolerance.
- To field test the prediction schemes
- To produce crop management guidelines for minimising the risk of yield losses to WBF.

3. Materials and methods

3.1. Modelling shoot number in winter wheat

3.1.1. Background

Model development involved two separate processes. Firstly the potential shoot production of a single wheat plant growing in isolation without any competition from neighbouring plants was determined. Secondly this calculation was refined by using field data to indicate how plant completion and environmental stress factors limit potential shoot production. These data were then used to apply a correction factor to the single plant model in order to account for plant competition.

3.1.2. Model development

Published principles of wheat shoot development were used to develop a thermal time based model of shoot production (Klepper *et al*., 1984). Production of the first tiller from the main shoot occurs approximately when the 3rd leaf of the main shoot has fully emerged. Production of the second tiller from the main shoot occurs approximately when the 4th leaf of the main shoot has fully emerged. Production of subsequent tillers from the main shoot follow the same pattern. Tillers also develop on tillers according to the same principals as described for the main shoot (Klepper *et al*., 1984). Tillering generally continues until the terminal spikelet has formed in the developing ear (Thorne & Wood, 1987). This developmental stage coincides approximately with the start of stem extension (Kirby *et al*., 1994). Terminal spikelet tends to occur just before the start of stem extension for sowing before mid-October and around stem extension for later sowing dates (Kirby *et al*., 1994). It is likely that the competition for resources between the extending stem and formation of new tillers causes the cessation of new tiller production. The terminal spikelet developmental stage is relevant because previous studies have described how its date is influenced by factors such as sowing date. Sowing date has an important effect on potential tillering through two mechanisms; i) later sowing reduces the thermal time interval between the emergence of successive leaves (phyllochron) and ii) reduces the thermal time interval between sowing date and terminal spikelet. The development of new tillers takes place in the logical order described above when growing conditions are good. However, if stressful growing conditions occur during the thermal time period when tiller production is expected then this tiller will often not grow. For example, poor seed bed quality often causes the non-emergence of the first (coleoptile) tiller (Peterson *et al*., 1982). Other stressful conditions include low sunlight, drought, waterlogging and disease. Competition for resources (e.g. light) between tillers also inhibits the production of tillers per plant. This is often due to high plant density (Darwinkel, 1978). However, higher numbers of plants/m^2 can counteract this and crops with many plants/m^2 often have greater maximum numbers of shoots/m^2 (AHDB Wheat Growth Guide).

Thermal time

The model is based on relationships between thermal time and plant development. Thermal time is the sum of all daily temperatures (mean of minimum and maximum temperature each day) above a base temperature at which wheat growth stops (0^{o}C). For August until February, typical values of thermal time per day for Central England were taken from values reported in the AHDB Wheat Growth Guide (original AHDB report; Sylvester-Bradley *et al*., 1998). The reported monthly values were divided by the number of days per month and this value was assumed to be the value for the middle date in that month. The values for interim days were then linearly decreased or increased as relevant to match with the following month's peak value. This provided an estimate of thermal time per day. It should be recognised that the shoot number model can use any thermal time data to best represent the growing conditions of any particular field.

Time between sowing date and plant emergence

To estimate the length of time between sowing and plant emergence, the relationship between soil temperature and emergence time as described by Lindstrom *et al*., 1976 (Equation 2 where t = emergence time (days) and T = soil temperature (Kelvin) was used.

$$\boldsymbol{t = \frac{T}{3.19T - 877.1}}$$ **Equation 2**

Thermal time to the end of tillering

The end of tillering generally coincides with the start of stem extension and the formation of the terminal spikelet within the developing ear. The thermal time between sowing date and terminal spikelet production for early and late sown winter wheat crops was reported in Kirby *et al*., 1999. The difference between an early (1st Sept) and late (8th November) sowing was used to estimate the effect of sowing date to reduce the thermal duration of the period between sowing and terminal spikelet, assuming that it decreased linearly over time. The thermal time from sowing to emergence was then subtracted from this value, leaving the total thermal time available for leaf and shoot production.

Phyllochron length

The phyllochron length is the time between emergence of successive leaves and is measured in thermal time above a base temperature of 0^{o}C. The phyllochron length reported by Kirby *et al*., 1985 was shown to decrease with later sowing dates (Equation 3) where d = the number of days after 1st September when the crop was sown.

$$\boldsymbol{Phyllochron\ length = -0.5383 \times d + 140.3}$$ **Equation 3**

The phyllochron length, along with the thermal time since sowing corrected for thermal time between sowing date and emergence, was then used to estimate the number of leaves and shoots that could be produced over time on an individual plant basis. The following assumptions were made based on Klepper *et al*., (1984).

- The first primary shoot emerges three phyllochrons after plant emergence
    - The primary shoot is a tiller emerging directly from the main shoot
- The second primary shoot emerges four phyllochrons after plant emergence and so on for subsequent primary shoots.
- The first secondary shoot emerges on the first primary shoot five phyllochrons after plant emergence
    - The secondary shoot is a tiller emerging directly from a primary shoot
- The second secondary shoot emerges on the second primary shoot six phyllochrons after plant emergence
- The first tertiary shoot emerges on the first secondary shoot seven phyllochrons after plant emergence
    - The tertiary shoot is a tiller emerging directly from a secondary shoot
- The first tertiary shoot emerges on the second secondary shoot eight phyllochrons after plant emergence

Shoot number, plant competition and environmental factors.

For a given sowing date, the mechanism of shoot production described above provided an estimate of the shoot number per plant in the absence of competition or the effect of environmental factors on shoot production. However, under field conditions, wheat plants are typically grown under much higher plant densities of ca. 100 to 300 plants/m^2. The model was therefore modified using field trial data from seed rate experiments (described in Section 3.2) to account for both competition and environmental factors.

3.2. Field experimentation

3.2.1. Seed rate experiments

Experimental design

Two winter wheat seed rate experiments were sown on 2nd October 2015 in Towthorpe, East Yorkshire and 20th September 2015 in Rosemaund, Herefordshire. The site details are summarised in Table 1. These locations were selected as having a very low WBF risk to prevent the pest from influencing the measured shoot numbers and provide reliable data for the shoot production model. In order to confirm the low WBF risk, an assessment of egg numbers was done at each site in December 2015. Approximately 20 kg soil was collected from a part of the field considered highest-risk (bare soil prior to sowing). A 10 cm diameter golf hole borer was used to take 20 cores across the site. Samples were then extracted using a Salt and Hollick apparatus

(Salt and Hollick, 1944) and the number of WBF eggs counted after flotation in saturated magnesium sulphate solution. Eggs were confirmed as being WBF by microscopic examination of the egg case. No WBF eggs were recorded at either the Rosemaund or Towthorpe sites.

Table 1. Site details for winter wheat seed rate experiments.

Location	Grid Reference	WBF egg count (million eggs/ha)	Sowing date
Towthorpe, East Yorkshire	SE 90944 62406	0	2nd October 2015
Rosemaund, Herefordshire	SO 55793 48437	0	20th September 2015

At each site the winter wheat variety Evolution (Limagrain UK) was sown at six seed rates (40, 80, 160, 320, 480 and 640 seeds/m^2). Three additional varieties were also drilled at either 80 or 320 seeds/m^2. These varieties were selected as having variation in their tillering ability and included Butler (Limagrain UK, shy tillerer), Horatio (Limagrain UK, profuse tillerer), Revelation (Limagrain UK, standard tillerer). The experiment was arranged in a split plot design, with variety as the main plot factor and seed rate as the sub plot factor. Each treatment combination was replicated four times. All crop protection and nutrient inputs were managed as per the farm crop.

Assessments

Once 50% of the plants in the 320 seeds/m^2 Evolution treatment reached GS31 digital photographs were taken from approximately 1 m above the crop prior to destructive plant sampling. Each plot was also scored for visual ground cover by estimating the percentage of ground covered by crop within the photographed area to the nearest 5%. At the end of each plot, all plants were dug up from a quadrat area measuring 0.7 m x 0.7 m (High Mowthorpe) or two 0.5 x 0.5 m quadrats (Rosemaund) which were placed diagonally so that one row ran through the opposite corners. Samples were not taken within a meter of the plot end or two outer rows of the plot. Samples were then returned to the laboratory, washed, weighed and an approximate 25% subsample collected and weighed. The number of plants in the subsample were counted, the roots cut off and the number of fertile tillers counted. There was a low level of gout fly infestation recorded in these experiments, and so any tillers that were infested with gout fly were also recorded.

Prior to harvest (July), the number of fertile shoots (shoots with an ear) in each plot was recorded from 10 locations spread throughout the plot in a ‘W’ shaped path by counting shoots within a 0.5 m length of row. The plots were assessed for lodging at harvest and the % area of each crop that was lodged to 46-90 degrees past the vertical, or was leaning (5 to 45 degrees past the vertical) was recorded. The plots were then combined using a Sampo small plot combine harvester to measure fresh weight grain yield. Plot length and width was recorded and used to calculate grain yield on a tonnes per hectare basis. Samples of grain were collected and assessed for moisture content using a Dickey John grain analyser. Plot yield values were then corrected to 15% moisture content.

3.2.2. Sowing date experiments

Experimental design

One site in 2015-16 and two sites in 2016-17 were established with Evolution winter wheat sown at six seed rates (40, 80, 160, 320, 480, 640 seeds/m^2), over two sowing dates (normal or late) either with or without application of chlorpyrifos insecticide, an egg hatch spray for WBF (e.g. Dursban WG). Each treatment combination was replicated three times. The experiments were arranged in a randomised split plot design with sow date as the main plot factor, and insecticide and seed rate as sub plot factors. The chlorpyrifos treatment was applied at a rate of 1 kg/ha in 200 L of water, and timed to coincide with the start of egg hatch at each site (ca. January-February). The start of egg hatch was assessed by taking soil samples at weekly intervals and examining the extracted eggs under the microscope. An administrative experimental authorisation (AEA) for use of this insecticide was required since it was no longer registered for use on UK winter wheat crops. An additional treatment of Evolution sown at 480 seeds/m^2 was included which had also been seed treated with Austral Plus (10 g/litre fludioxonil and 40 g/litre tefluthrin) before sowing. This was to assess the impact of the seed treatment on WBF infestation. The site details are summarised in Table 2. All other inputs were managed as per the farm crop.

Table 2. Site details for the winter wheat sowing date experiments.

Location	Grid Reference	WBF egg count (million eggs/ha)	Sowing date
Huggate, East Yorkshire	SE 90944 62406	3.1	Normal: 10th October 2015 Late: Not drilled due to wet soil conditions
Foxholes, North Yorkshire	TA 01400 72200	0.8	Normal: 24th October 2016 Late: 16th November 2016
Bardwell, Suffolk	TL 93095 73956	0.3	Normal: 29th September 2016 Late: 25th October 2016

Assessments

Historical egg hatch data was used to select sites which had a high risk of WBF. A sample was also taken during the trial season to confirm the WBF egg count, the values are summarised in Table 2. Egg numbers at the Huggate site were above 2.5 million/ha the level at which damage might be expected to impact on yield. Egg numbers at Foxholes and Bardwell were lower than this level and the threshold for late sowings (1.0 million eggs/ha) but autumn 2016 was generally low risk for WBF (Ellis, 2016) and sites with significant egg numbers were difficult to locate. The Foxholes and Bardwell sites had suffered high levels of damage in the past. The WBF samples were collected and processed as described in Section 3.2.2.

In December assessments were done on insecticide treated and untreated plots at 80 and 320 seeds/m^2. Due to poor over winter establishment, at the Foxholes site in 2017 these were combined with the GS30 assessments in April. Photos was taken from approximately 1 m above the crop near the end of the plot (but at least 1 m from the end and two rows from the edge). The area photographed was that over which a quadrat was later placed to take a destructive sample. A visual ground cover score was made, estimating the percentage of ground covered by the crop within the photographed area to the nearest 5%. Three repeat readings light reflected from the crop were taken using the CropScan sensor (Cropscan Inc. Minnesota, USA) above the same region from which the photograph was taken. Light reflected at 640 and 810 nm wavelengths were collected which was later used to estimate the spectral reflectance index normalised difference vegetation index (NDVI). At the Bardwell site a RapidScan sensor (Holland Scientific, Lincoln NE, USA) was used to measure NDVI. The instrument was pointed in the same direction each time and readings were only taken on bright, clear days, between 10am-2pm to ensure the brightest light conditions were used. Readings were not taken when the crop was wet or in frosty conditions. A quadrat (0.7m x 0.7m at Huggate and Foxholes, 0.5 x 0.5 m at Bardwell) was placed in the plot so that one row ran through its opposite corners. A destructive sample was taken from this area and returned to the laboratory where it was washed and the fresh weight recorded. A 25% representative sub-sample was then collected. The number of plants was counted, the roots cut off and then the number of fertile, dead and dying tillers were also counted. The combined green area of the leaves and stems was measured in the sub-sample using a Li-Cor leaf area meter. The above ground material was then dried until there was no further weight loss and the dry weight recorded.

From March onwards (pre-GS30), 20 plants were sampled from the site weekly to assess larval invasion and crop growth stage. Once all the larvae reached the second instar (when further plant invasion was unlikely), 25 plants were randomly collected from each plot at 80 and 480 seeds/m^2 sowing dates. These were dissected to check for the presence of WBF larvae. The number of live, infested and dead/dying tillers were recorded. The identity of the extracted larva was recorded and assigned to instar I, II or III.

At GS30-31 all plots were sampled for shoot number assessments by destructively taking a 0.7 x 0.7 m quadrat placed diagonally so that one row ran through opposite corners. The plants were dug up, returned to the lab and washed before taking the fresh weight. A 25 % representative subsample was weighed, the number of plants counted and roots cut off before counting the total number of fertile, dead and dying tillers. The number of tillers showing signs of WBF damage was also recorded.

In July, prior to harvest, the number of fertile tillers (tillers with ears) was assessed for all plots by counting tillers within a 0.5 m length of row at 10 locations randomly selected by sampling in a W shaped path for each plot. Lodging assessments were done before harvest where the % area of each crop that had lodged 46 to 90 degrees past the vertical or was leaning 5 to 45 degrees past the vertical was recorded. Each plot was then combine harvested using a Sampo small plot combine harvester and the fresh weight grain yield recorded. The plot length and width was measured and used to calculate grain yield on a tonnes per hectare basis. The moisture content of a grain sample was analysed using a Dickey John moisture meter. The fresh weight yields were then corrected to 85% dry matter.

3.2.3. Statistical analysis

The data were analysed using a two or three way ANOVA. Where sowing date was included in the experiment, these data were analysed using a split plot ANOVA, with sowing date as the main plot factor, and insecticide and seed rate as sub plot factors. At Towthorpe in 2016 two plots which should have been drilled with Revelation were drilled with the wrong variety, so were excluded from the analysis. The December tiller number data were correlated with GAI, NDVI and % ground cover for the Bardwell site. Standard error of the difference (SED) and least significant difference (LSD) values are reported with all statistics where relevant.

3.3. Quantifying the risk from wheat bulb fly

3.3.1. The number of shoots that a wheat bulb fly larva can destroy

This was determined by desk based study. A literature search of peer reviewed papers was used to quantify the number of tillers destroyed by a single WBF larva and this value was used in the threshold model.

3.3.2. Using water trapping as an alternative to soil sampling for wheat bulb fly eggs

In mid-July 2016 four water traps were set in each of eight fields (four in Cambridgeshire, four in North/East Yorkshire). A range of levels of wheat bulb fly oviposition had been recorded at these sites in previous years of the ‘AHDB Autumn survey of wheat bulb fly incidence’ (RD-2011-3578). The site details are summarised in Table 3.

Table 3. Sites used for WBF water trapping and egg counts.

Year	Site	Grid Reference	Previous crop
2015	Duggleby, North Yorkshire	SE 888726853	Potatoes
2015	Southburn, East Yorkshire	SE 9815154760	Vining peas
2015	Tibthorpe, East Yorkshire	SE 9503455583	Potatoes
2015	Terrington St Clement, Norfolk	TF 5404019386	Sugar beet
2015	Terrington St Clement, Norfolk	TF 5341623595	Sugar beet
2016	Duggleby, North Yorkshire	SE 888726853	Potatoes
2016	Foxholes, North Yorkshire	TA 0137672340	Potatoes
2016	Eastburn, East Yorkshire	SE 9846857239	Vining peas
2016	Tibthorpe, East Yorkshire	SE 9478155600	Vining peas
2016	Terrington St Clement, Norfolk	TF 5381024049	Sugar beet
2016	Walpole St Peter, Norfolk	TF 5051716198	Sugar beet
2016	Terrington St Clement, Norfolk	TF 5429419451	Sugar beet

Water trapping

In each of the selected fields, four WBF water traps were set following the methodology developed by Cooper (1981). Four white trays (355 x 255 x 50 mm) were placed in different parts of the field at soil level. Each tray had a small hole drilled at the top of each corner to allow the water to overflow without the loss of the catch. Each tray was then filled with water and a small amount of detergent. On a weekly basis, the water was collected and the trap re-filled. The catch was collected using a plastic kitchen sieve (approximately 15 cm diameter) lined with muslin. The trap contents was poured into the muslin and through the sieve. Any large (non-WBF) insects were removed and the folded muslin stored inside a screw topped container. The samples were then stored in 70% alcohol and returned to the laboratory where the number of male and female WBF were recorded. This process was repeated weekly until the end of August.

Egg count assessments

The same sites at which water traps were located were re-visited in September and soil samples taken to assess the number of WBF eggs laid as part of the AHDB Autumn survey of wheat bulb fly incidence. Approximately 20 kg of soil was collected from across the field. The diameter of the core was either 10 cm (Yorkshire sites) or 7.6 cm (Cambridgeshire sites). Where the 10 cm core was used, 20 samples were collected across the site, whereas 32 cores were collected when the 7.6 cm corer was used. The soil samples were extracted using the Salt and Hollick apparatus (Salt & Hollick, 1944) as described in Section 3.2.1.

4. Results

4.1. Field experimentation

4.1.1. Seed rate experiments

Towthorpe, 2016

There was a significant difference among seed rates for plant number (P<0.001), shoot number (P<0.001) and the number of shoots per plant (P<0.01) at growth stage 31 (GS31, Table 4) for winter wheat variety Evolution. There was also a significant difference in final ear number (P<0.01) and yield (P<0.001) among the six seed rates of Evolution (Table 4; Figure 3). There was no significant level of leaning or lodging reported in the Towthorpe 2016 experiment. There were also no gout fly recorded in the shoots at the GS31 sampling timing. Whilst GS31 is generally considered optimal timing for measuring maximum shoot number, the increase in shoot number in the final shoot number when compared to the GS31 shoot number in the lower seed rate plots is a good demonstration that crops can continue to tiller post GS31 if the plant population is low.

Table 4. Seed rate experiment results for Evolution plots at Towthorpe 2016.

Variety	Seed rate (seeds/m^2)	GS31 plant number (plants/m^2)	GS31 tiller number (shoots/m^2)	GS31 no. shoots per plant	Final ear number (shoots/m^2)	Yield at 85% DM (t/ha)
Evolution	40	38	254	6.7	721	8.18
Evolution	80	53	266	5.0	704	10.54
Evolution	160	77	527	7.0	663	12.15
Evolution	320	138	595	4.7	590	13.07
Evolution	480	202	723	3.6	602	13.58
Evolution	640	232	733	3.4	598	13.62
Grand mean		123	516	5.1	647	11.9
P		<0.001	<0.001	0.002	0.004	<0.001
SED		27.7	76	0.82	34.3	0.191
LSD		59.0	162	1.74	73.1	0.406

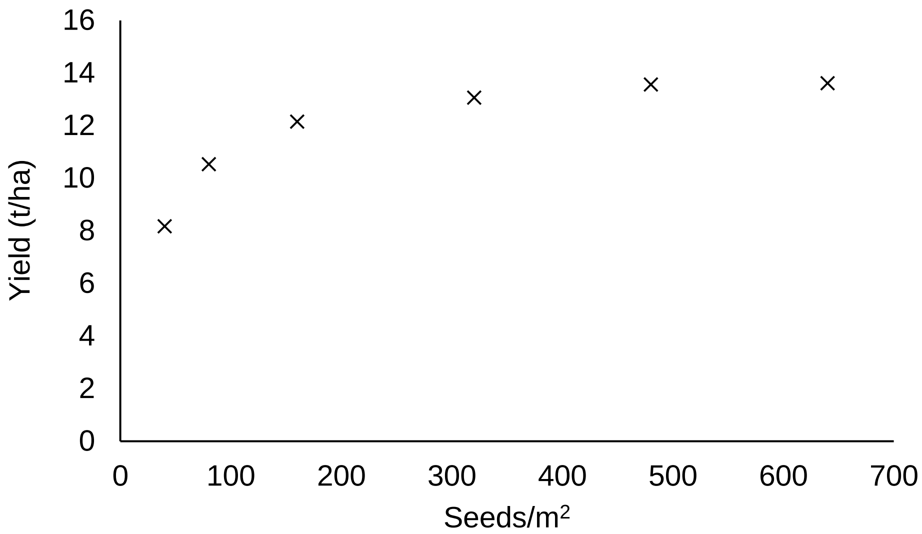

Figure 3. Yield of evolution plots at Towthorpe 2016 in response to varying seed rate.

There was a significant difference in shoots/m^2 at GS31 between seed rates as well as between varieties when all four varieties were included in the analysis ($P<0.001$; Figure 4; Table 5). On average, across the two seed rates revelation had the highest shoot number (561 shoots/m^2) and Butler the lowest (347 shoots/m^2), with Evolution and Horatio producing similar number of shoots (430 and 448 shoots/m^2 respectively). There was almost a significant interaction between seed rate and variety ($P = 0.064$), which was because Evolution produced few shoots at low seed rate (266 shoots/m^2) and many shoots at high seed rate (595 shoots/m^2), relative to other varieties. Seed rate had a significant effect on all parameters (Table 5), whereas there was only a significant difference among varieties for shoot number at GS31 and yield. There was also almost a significant interaction between seed rate and variety for yield and a significant interaction for GS31 plant number, although variety was not significant for this parameter therefore this result may not be real.

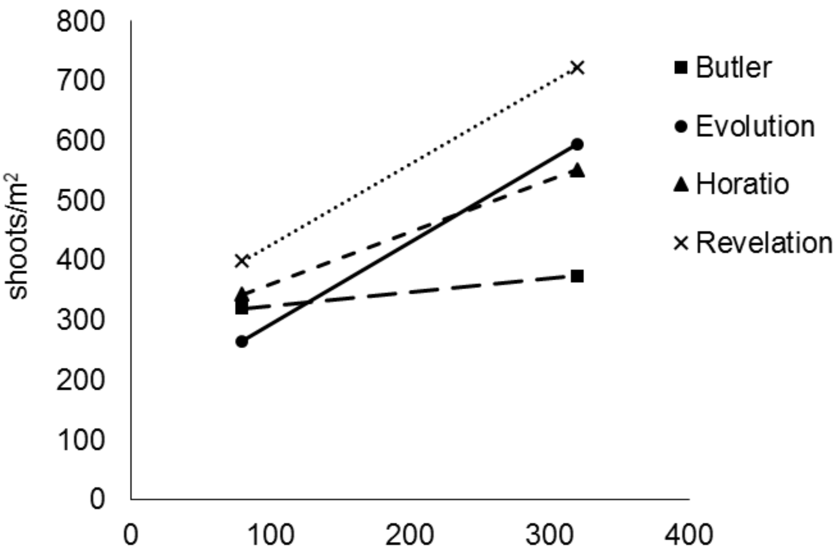

Figure 4. Shoot number at GS31 (shoots/m^2) for four winter wheat varieties sown at either 80 or 320 seeds/m^2 at Towthorpe 2016.

Table 5. Seed rate experiment results for the winter wheat varieties Butler, Evolution, Horatio and Revelation at two seed rates (80 and 320 seeds/m^2) at Towthorpe 2016.

Variety	Seed rate (seeds/m^2)	GS31 plant number (plants/m^2)	GS31 tiller number (shoots/m^2)	GS31 no. shoots per plant	Final ear number (shoots/m^2)	Yield at 85% DM (t/ha)
Butler	80	68	320	5.5	642	12.17
Butler	320	77	375	5.3	535	13.95
Evolution	80	53	266	5.0	704	10.54
Evolution	320	138	595	4.7	590	13.07
Horatio	80	62	344	5.6	601	12.20
Horatio	320	144	552	4.0	590	13.88
Revelation	80	59	399	7.6	602	10.69
Revelation	320	198	723	3.7	570	12.78
Grand mean		98	443	5.2	603	12.43
P (Seed rate)		**<0.001**	**<0.001**	**0.015**	**0.033**	**<0.001**
SED		13.2	45	0.568	28.6	0.112
LSD		27.6	94	1.189	59.9	0.234
P (variety)		0.12	**0.041**	0.533	0.365	**<0.001**
SED		18.6	64	0.804	40.5	0.158
LSD		39.0	133	1.682	84.7	0.331
P (seed rate * variety)		**0.02**	0.14	0.102	0.491	0.064
SED		26.4	90	1.137	57.2	0.224
LSD		55.1	188	2.379	119.8	0.468

Rosemaund 2016

There was a significant effect of seed rate on the number of plants/m^2 ($P<0.05$), shoots/m^2 ($P<0.001$), final ear number ($P<0.01$) and yield at GS31 ($P<0.001$) (Table 6; Figure 6). There was no effect of seed rate on the number of shoots per plant. This site was infested with low levels (<3%) of gout fly larvae at GS31, but the number of healthy tillers infested did not differ between seed rates and therefore this is unlikely to have confounded the results for the other parameters.

Table 6. Seed rate experiment results for Evolution plots at Rosemaund 2016.

Variety	Seed rate (seeds/m^2)	GS31 plant number (plants/m^2)	GS31 shoot number (shoots/m^2)	GS31 no. shoots per plant	Final ear number (shoots/ m^2)	Yield at 85% DM (t/ha)
Evolution	40	86	429	5.4	260	8.91
Evolution	80	104	440	4.8	236	10.14
Evolution	160	124	572	4.7	264	11.32
Evolution	320	123	479	3.9	286	12.22
Evolution	480	117	630	5.7	275	12.40
Evolution	640	176	768	4.4	290	12.41
Grand mean		122	553	4.8	269	11.23
P		**0.023**	**<0.001**	0.479	**0.006**	**<0.001**
SED		22.3	65.5	0.96	12.4	0.48
LSD		47.6	139.7	2.05	26.4	1.03

There was a significant difference among varieties in the number of shoots/m^2 at GS31 $P<0.01$ Table 7; Figure 6) and yield ($P<0.001$, Table 7). Revelation had the highest shoot number at GS31 with 566 shoots/m^2 across the two seed rates, and Butler the lowest with 442 shoots/m^2, although there was very little difference between Butler, Evolution and Horatio with a range of 442-493 shoots/m^2. In contrast, the highest yielding crop was Evolution which at 11.18 t/ha yielded significantly higher than both Butler (10.0 t/ha) and Revelation (10.09 t/ha). There was also a significant effect of seed rate on GS31 shoots/plant ($P<0.05$), final ear number ($P < 0.001$) and yield ($P<0.001$), but no interaction between seed rate and variety for any parameter except for the number of tillers infested by gout fly ($P<0.05$). This was probably because gout fly infestation increased with increasing seed rate for Butler but decreased with increasing seed rate for all other varieties. Overall the gout fly infestation was low with <3% tillers affected on average.

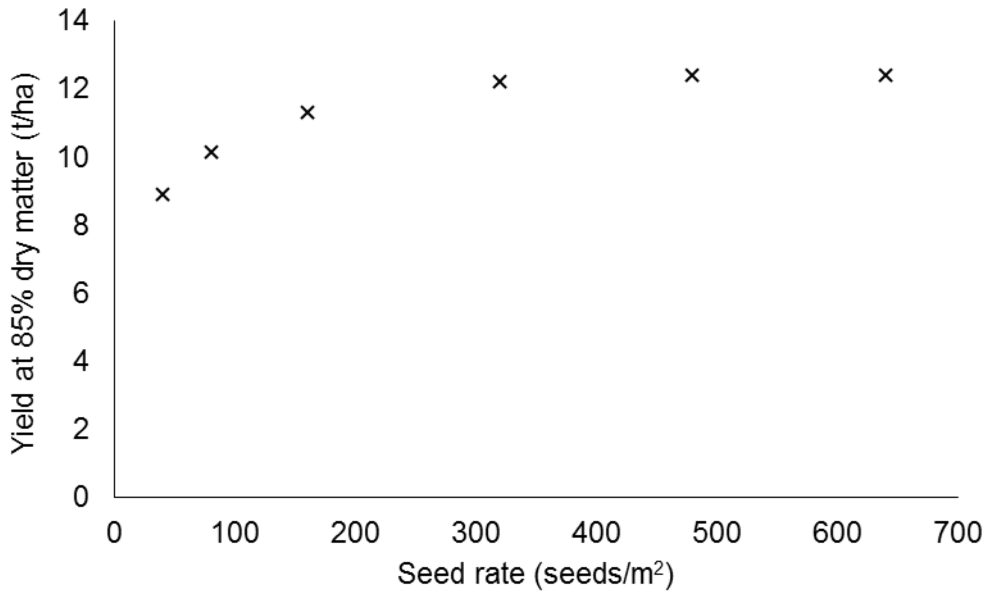

Figure 5. Yield of evolution plots at Rosemaund 2016 in response to varying seed rate.

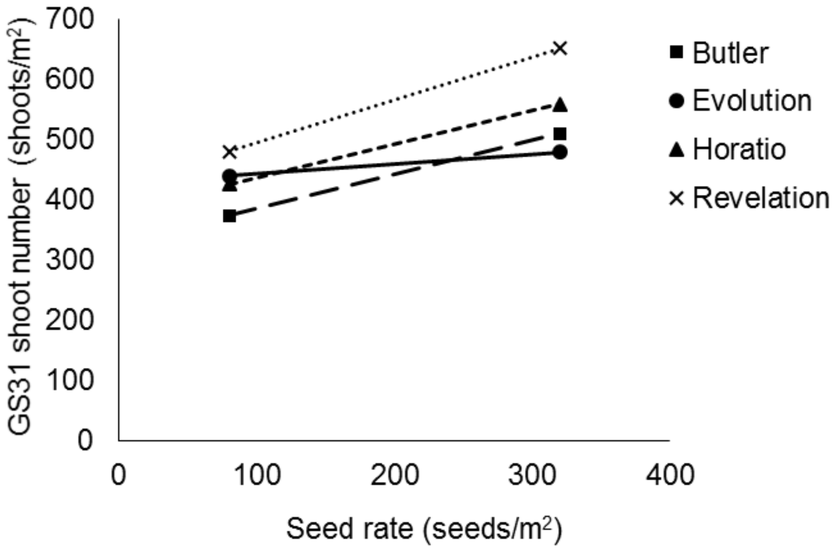

Figure 6. Shoot number at GS31 (shoots/m^2) for four winter wheat varieties sown at either 80 or 320 seeds/m^2 at Rosemaund 2016.

Table 7. Seed rate experiment results for the winter wheat varieties Butler, Evolution, Horatio and Revelation at two seed rates (80 and 320 seeds/m^2) at Rosemaund 2016.

Variety	Seed rate (seeds/m^2)	GS31 plant number (plants/m^2)	GS31 tiller number (shoots/m^2)	GS31 no. shoots per plant	Final ear number (shoots/m^2)	Yield at 85% DM (t/ha)
Butler	80	70	375	6.1	250	9.42
Butler	320	86	510	6.3	266	10.80
Evolution	80	104	440	4.8	236	10.14
Evolution	320	123	479	3.9	286	12.22
Horatio	80	107	426	4.4	250	10.78
Horatio	320	145	559	3.9	284	11.51
Revelation	80	84	480	5.8	242	9.32
Revelation	320	99	652	8.0	287	10.86
Grand mean		102	490	5.39	263	10.63
P (Seed rate)		0.057	0.118	**0.035**	**<0.001**	**<0.001**
SED		12.7	36.8	0.73	5.6	0.209
LSD		26.4	76.6	1.51	11.7	0.434
P (variety)		0.095	**0.004**	0.747	0.694	**<0.001**
SED		18.0	52.1	1.02	8.0	0.295
LSD		37.4	108.4	2.14	16.6	0.613
P (seed rate * variety)		0.908	0.626	0.478	0.168	0.183
SED		25.4	73.7	1.45	11.3	0.417
LSD		52.82	153.2	3.02	23.5	0.867

4.1.2. Sowing date experiments

Huggate 2016

The number of tillers infested by WBF at GS30 was low (on average < 1%) and there was no significant effect of insecticide or seed rate on these levels (P > 0.05). There was a significant effect of seed rate on GS31 plant and shoot number (P<0.001 and P=0.02 respectively), the number of shoots per plant at GS31 (P<0,001), final ear number (P=0.008) and yield (P<0.001, Table 8). However, there was no effect of insecticide and no significant interactions between insecticide and seed rate for any of these parameters. There was a significantly lower percentage ground cover in the lower seed rate plots at Huggate in December 2016 (P < 0.001; Table 8). There was also a significant interaction (P = 0.013) between ground cover and insecticide, possibly driven by the lower ground cover in the higher seed rate insecticide treated plots.

Table 8. GS31 and harvest results for Huggate 2016.

Insecticide treatment	Seed rate (seeds/m^2)	GS30 % ground cover	GS31 plant number (plants/m^2)	GS31 shoot number (shoots/m^2)	GS31 Shoots per plant	Final ear number (shoots/m^2)	Yield at 85% DM (t/ha)
Untreated	40	-	49	849	19.9	745	11.08
Untreated	80	32	48	919	19.4	716	11.71
Untreated	160	-	106	996	9.7	671	12.57
Untreated	320	52	176	897	5.1	588	12.79
Untreated	480	-	188	845	4.5	667	12.79
Untreated	640	-	249	1,057	4.4	693	12.79
Treated	40	-	39	883	24.6	765	10.74
Treated	80	37	59	1,011	17.4	749	12.25
Treated	160	-	104	1,052	10.2	694	13.01
Treated	320	45	168	899	5.4	657	12.80
Treated	480	-	181	728	4.1	638	13.10
Treated	640	-	310	1,038	3.4	649	13.08
Grand mean		41.3	139	930	10.7	686	12.39
P value (Insecticide)		0.635	0.604	0.892	0.743	0.541	0.129
SED		1.67	11.6	43.9	1.03	19.7	0.135
LSD		4.08	24.2	91.7	2.15	40.9	0.280
P value (Seed rate)		**<0.001**	**<0.001**	**0.02**	**<0.001**	**0.008**	**<0.001**
SED		1.67	20.1	76.1	1.79	34.2	0.234
LSD		4.08	41.9	158.8	3.72	70.9	0.485
P value (Insecticide*Seed rate)		**0.013**	0.459	0.811	0.54	0.607	0.475
SED		2.36	28.4	107.6	2.52	48.3	0.331
LSD		5.77	59.2	224.5	5.27	100.2	0.686

Foxholes 2017

There was a significant interaction between sow date and seed rate for GS30 shoots/plant (P<0.001) GS30 shoots/m^2 (P = 0.001), GAI (P = 0.013) and ground cover (P<0.001; Table 9; Appendix 1, Table 18). This suggests that the response to seed rate varied between sowing dates, which may be driven by the GS30 shoot number increasing over 20 times from 12 to 260 shoots/m^2 for the 40 up to the 640 seeds/m^2 rate for the late sown crop, whereas it only increased by five times for the normal sowing date crop, although this had a higher seed rate overall, from 176 to 900 shoots/m^2 respectively. NDVI differed significantly between seed rates (P = 0.006; Appendix 1, Table 18) and sow dates (P = 0.011; Appendix 1, Table 18). Most parameters tended to increase with increasing seed rate (except shoots/m^2) and earlier sow dates. There was no significant interaction between seed rate and sow date for NDVI.

There was a significant effect of sow date and seed rate on final ear number (Table 9), and the interaction was almost significant between sow date and seed rate. There was a significant interaction between sowing date and seed rate for the final yield, possibly because the late sown crops generally produced lower yields, particularly in the lower seed rates. There was no effect of insecticide application on any of the measured parameters, which is not surprising given the low level of WBF pressure at the site (Table 2). Also there were no differences in the levels of larval infestation measured between treatments (Table 9). The seed treatment also seemed to have little effect on the measured parameters, although there was a slightly increased yield in the seed treated plots (Table 9; Appendix 1, Table 19).

Table 9. Foxholes 2017 GS30 and harvest results.

Sow date	Insecticide treatment	Seed rate (seeds/m^2)	GS30 no. shoots per plant	GS30 shoots/m^2	GS30 GAI	GS30 % shoots infested by WBF	Final ear number (shoots/m^2)	Yield at 85% DM (t/ha)
Normal	Untreated	40	-	-	-	-	285	8.83
Normal	Untreated	80	-	-	-	4.87	456	10.23
Normal	Untreated	160	-	-	-	-	511	11.86
Normal	Untreated	320	-	-	-	-	605	12.75
Normal	Untreated	480	-	-	-	2.55	482	13.29
Normal	Untreated	640	-	-	-	-	565	13.72
Normal	Treated	40	7.2	176	0.1	-	337	8.62
Normal	Treated	80	7	324	0.27	3.45	469	10.95
Normal	Treated	160	5.9	542	0.51	-	438	12.18
Normal	Treated	320	4.3	795	0.66	-	471	13.05
Normal	Treated	480	3.3	895	0.84	2.37	538	13.07
Normal	Treated	640	3.1	900	0.56	-	531	13.4
Late	Untreated	40	-	-	-	-	212	5.15
Late	Untreated	80	-	-	-	0.8	256	6.84
Late	Untreated	160	-	-	-	-	362	8.87
Late	Untreated	320	-	-	-	-	499	10.74
Late	Untreated	480	-	-	-	2.12	394	10.84
Late	Untreated	640	-	-	-	-	558	11.85
Late	Treated	40	2.7	12	0.004	-	128	4.73
Late	Treated	80	2.3	42	0.02	1.71	249	7.43
Late	Treated	160	2.6	73	0.03	-	453	9.2
Late	Treated	320	3.9	159	0.07	-	471	11.13
Late	Treated	480	3.6	297	0.16	2.54	519	11.49
Late	Treated	640	3	260	0.12	-	512	11.02
	Grand mean		4.09	373	0.278	2.55	429	10.47
	P value sowing date		0.052	**0.02**	**0.017**	0.212	**0.02**	**0.002**
	SED		0.501	66.4	0.0559	0.839	12.76	0.127
	LSD		2.155	285.9	0.2406	3.611	54.91	0.545
	P value (Insecticide)		-	-	-	0.918	0.773	0.396
	SED		-	-	-	614	19.66	0.125
	LSD		-	-	-	1.351	39.62	0.252
	P value (Seed rate)		**0.03**	**<0.001**	**<0.001**	0.621	**<0.001**	**<0.001**
	SED		0.563	56.3	0.0772	0.614	34.05	0.216
	LSD		1.174	117.4	0.1611	1.351	68.63	0.436
	P value (sowing date * Insecticide)		-	-	-	0.257	0.476	0.932
	SED		-	-	-	1.04	23.44	0.178
	LSD		-	-	-	2.769	49.22	0.415
	P value (sowing date * seed rate)		**<0.001**	**0.001**	**0.013**	**0.045**	0.064	**<0.001**
	SED		0.882	98.5	0.1143	1.04	45.78	0.307
	LSD		1.901	225	0.2408	2.769	92.22	0.624
	(Insecticide*Seed rate)		-	-	-	0.77	0.229	0.065
	SED		-	-	-	0.868	48.16	0.306
	LSD		-	-	-	1.91	97.06	0.616
	Sow date * insecticide * seed rate		-	-	-	0.497	0.317	0.707
	SED		-	-	-	1.354	66.45	0.433
	LSD		-	-	-	3.05	133.75	0.872

Table 10. Foxholes 2017 GS30 and harvest results for seed treatments only sown at 480 seeds/m^2.

Sowing date	Seed treatment	Insecticide treatment	Plants/m^2 GS30	Shoots/m^2 GS30	Shoots/plant GS30	GAI	% tillers infested	Final Ear number	Yield (t/ha) at 85% DM.
Normal	Untreated	Untreated	-	-	-	-	2.6	482	13.29
Normal	Untreated	Treated	282	895	3.30	0.84	2.4	538	13.07
Normal	Treated	Untreated	-	-	-	-	5.8	478	13.84
Normal	Treated	Treated	278	815	2.90	0.81	3.1	501	13.52
Late	Untreated	Untreated	-	-	-	-	2.1	394	10.84
Late	Untreated	Treated	82	297	3.60	0.16	2.5	519	11.49
Late	Treated	Untreated	-	-	-	-	31.1	457	12.10
Late	Treated	Treated	94	360	3.80	0.17	4.2	546	12.47
		Grand Mean	184	592	3.43	0.49	6.70	490	12.58
		P value (Sow date)	**0.02**	**0.02**	**0.03**	**0.01**	0.41	0.057	**0.022**
		SED	30.2	70.5	0.11	0.06	7.69	5.1	0.25
		LSD	130.1	303.4	0.49	0.26	16.50	22.1	1.10
		P value (Seed trt)	0.86	0.85	0.78	0.84	0.28	0.659	**0.001**
		SED	19.7	43.4	0.23	0.05	7.69	27	0.19
		LSD	54.6	120.5	0.65	0.14	16.50	58.8	0.41
		P value (Insecticide)	-	-	-	-	0.36	**0.019**	0.534
		SED	-	-	-	-	7.69	27.0	0.19
		LSD	-	-	-	-	16.50	58.8	0.41
		P value (Sow date * Seed trt)	0.69	0.17	0.32	0.71	0.40	0.252	0.128
		SED	36.1	82.8	0.26	0.08	10.88	27.5	0.32
		LSD	103.2	242.0	0.65	0.21	23.33	59.5	0.84
		P value (Sow date * Insecticide)	-	-	-	-	0.46	0.237	0.059
		SED	-	-	-	-	10.88	27.5	0.32
		LSD	-	-	-	-	23.33	59.5	0.84
		P value (Seed trt * Insecticide)	-	-	-	-	0.35	0.529	0.632
		SED	-	-	-	-	10.88	38.2	0.27
		LSD	-	-	-	-	23.33	83.2	0.58
		P value (Sow date * Seed trt * Insecticide)	-	-	-	-	0.43	0.984	0.817
		SED	-	-	-	-	15.39	47	0.42
		LSD	-	-	-	-	33.00	102.2	0.93

Bardwell, 2017

There was no significant effect of sow date on any of the GS31 or harvest parameters (Table 11). Insecticide application at egg hatch resulted in increased shoot numbers (P = 0.042 and P = 0.008), but did not affect yield (Table 11). However, increasing seed rate resulted in increased values for all parameters (P > 0.05 in each case; Table 11). There was a significant interaction between sow date, insecticide and seed rate for the number of shoots/plant at GS31. This was possibly because insecticide increased shoot number per plant to a greater extent in the lowest seed rates at the normal sowing date (P = 0.007; Table 11). There was also a significant effect of sow date on ground cover (P=0.01; Appendix 1, Table 20), shoots/plant (P=0.01), and GAI (P=0.03) in December, with the normal sow date crops generally having higher values for these parameters (Table 11). There was also a significant effect of seed rate on ground cover (P<0.001), plants/m^2, (P<0.001) shoots/m^2 (P<0.001), and GAI (P = 0.013), with the higher seed rates again producing higher values (Table 11). Although almost significant for GAI and shoots/m^2 the only significant interaction between sow date and seed rate was for ground cover (P < 0.001; Appendix 1; Table 20). Whilst the higher seed rates produced higher levels of ground cover in both sowing dates, there were higher values and a greater range of ground covers found for the normal sowing date crops as expected (Appendix 1, Table 20).

There was a significant interaction (P < 0.05) between seed rate, sow date and insecticide treatment for larval infestation. This was probably because there was no consistent ranking in larval infestation between seed rates for both insecticide treated and untreated plots. In general, insecticide reduced larval infestation (P < 0.01, Table 11) but the number of larvae was not consistent between seed rates, sow dates and insecticide treatments as indicated by the interaction (Table 11). Seed treatment and insecticide application also appeared to increase the number of shoots/m^2 (P = 0.031 and P = 0.012 respectively), although did not result in a higher yield (Table 12). However there was a significant interaction between sow date and seed treatment for yield (P = 0.024).

Table 11. Early season and harvest results for Bardwell 2017 site.

Sowing date	Insecticide treatment	Seed rate (seeds/ m²)	Dec. no. plants/ m²	Dec. no. shoots / plant	Dec. shoots/ m²	Dec. GAI	GS30 % shoots infested by WBF	GS31 plant number (plants/ m²)	GS31 shoot number (shoots/ m²)	GS31 Shoots per plant	Final ear number (shoots/ m²)	Yield at 85% DM (t/ha)
Normal	Untreated	40	-	-	-	-		44	127	7.0	158	4.66
Normal	Untreated	80	-	-	-	-	6.5	77	160	3.0	213	5.98
Normal	Untreated	160	-	-	-	-		55	192	4.0	272	8.07
Normal	Untreated	320	-	-	-	-	2	65	231	4.0	338	8.71
Normal	Untreated	480	-	-	-	-		96	251	3.0	392	9.43
Normal	Untreated	640	-	-	-	-		142	363	3.0	487	9.18
Normal	Treated	40	14	2.7	37	0.02		11	96	8.0	177	5.02
Normal	Treated	80	127	1.5	158	0.04	1.5	30	267	9.0	223	6.92
Normal	Treated	160	156	2.1	211	0.07		36	197	5.0	276	7.70
Normal	Treated	320	274	2.3	633	0.24	1.3	70	235	4.0	346	9.13
Normal	Treated	480	453	1.7	634	0.2		105	372	4.0	371	9.42
Normal	Treated	640	448	1.4	632	0.23		218	473	2.0	461	9.37
Late	Untreated	40	-	-	-	-		12	29	2.0	171	2.21
Late	Untreated	80	-	-	-	-	3.9	12	41	4.0	218	2.98
Late	Untreated	160	-	-	-	-		36	102	3.0	228	5.56
Late	Untreated	320	-	-	-	-	8.8	37	104	3.0	356	6.49
Late	Untreated	480	-	-	-	-		64	134	2.0	398	6.18
Late	Untreated	640	-	-	-	-		108	235	2.0	382	6.86
Late	Treated	40	30	1.0	30	0.00		7	40	5.5	145	2.15
Late	Treated	80	67	1.0	67	0.01	2.4	21	69	2.9	193	4.05
Late	Treated	160	106	0.9	90	0.02		27	74	2.7	259	4.83
Late	Treated	320	236	0.9	221	0.02	2.4	61	154	2.6	309	6.88
Late	Treated	480	343	0.9	301	0.04		101	208	2.1	351	6.40
Late	Treated	640	381	0.9	349	0.04		112	260	2.4	414	7.91
Grand mean			222	1.4	282	0.08	3.6	64	182	3.73	298	6.52
P value (Sow date)			0.543	**0.01**	0.101	**0.033**	**0.047**	0.221	0.124	0.177	0.26	0.232
		SED	65.3	0.1	70.5	0.021	0.35	16.3	48.7	0.815	15.36	1.51
		LSD	281.1	0.44	303.5	0.09	1.51	70.2	209.4	3.51	66.11	6.49
P value (Insecticide)			-	-	-	-	**0.006**	0.761	**0.042**	**0.008**	0.409	0.116
		SED	-	-	-	-	1.03	9.6	17	0.323	8.66	0.2
		LSD	-	-	-	-	2.25	19.4	34.4	0.65	17.47	0.41
P value (Seed rate)			**<0.001**	0.347	**<0.001**	**0.013**	0.971	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
		SED	80.1	0.32	76.9	0.04	1.03	16.7	29.5	0.56	15	0.35
		LSD	168.2	0.68	161.6	0.083	2.25	33.7	59.5	1.13	30.25	0.71
P value (Sow date * Insecticide)			-	-	-	-	0.595	0.509	0.482	**0.038**	0.484	0.73
		SED	-	-	-	-	1.09	18.9	51.6	0.877	17.64	1.52
		LSD	-	-	-	-	2.34	54.9	183.4	2.99	52.16	6.33
P value (Sow date * seed rate)			0.962	0.37	0.092	0.074	**0.039**	0.541	0.672	0.139	0.172	0.517
		SED	122.3	0.43	121.8	0.055	1.09	27	61.8	1.09	24.7	1.57
		LSD	261.5	0.9	264.4	0.115	2.34	58.3	157.9	2.65	53.86	5.84
P value (Insecticide*Seed rate)			-	-	-	-	0.903	0.291	0.356	0.372	0.616	0.369
		SED	-	-	-	-	1.46	23.6	41.7	0.79	21.21	0.5
		LSD	-	-	-	-	3.18	47.6	84.2	1.6	42.78	1
P value (Sow date*Insecticide*Seed rate)			-	-	-	-	**0.044**	0.461	0.849	**0.007**	0.375	0.974
		SED	-	-	-	-	1.82	35.9	74.5	1.35	32.57	1.65
		LSD	-	-	-	-	3.94	73.5	165.8	2.91	67	5.39

Dec. = December; ‘-‘ = Parameter not measured.

Table 12. Seed treatment results from Bardwell 2017 sown at 480 seeds/m^2.

Sowing date	Seed treatment	Insecticide treatment	Plants/ m^2 GS31	Shoots/m^2 GS31	Shoots/plant GS31	Final Ear number	Yield (t/ha) at 85% DM.
Normal	Untreated	Untreated	96	251	2.7	392	9.43
Normal	Untreated	Treated	105	372	3.7	371	9.42
Normal	Treated	Untreated	100	336	3.4	369	8.63
Normal	Treated	Treated	132	418	3.4	413	7.89
Late	Untreated	Untreated	64	134	2.2	398	6.18
Late	Untreated	Treated	101	208	2.1	351	6.40
Late	Treated	Untreated	96	222	2.4	394	7.31
Late	Treated	Treated	111	215	1.9	408	6.77
		Grand mean	101	269	2.71	387	7.79
		P value (Sow date)	0.06	0.209	0.203	0.987	0.289
		SED	3.93	82.5	0.62	12.5	1.463
		LSD	16.9	355	0.267	53.6	6.294
		P value (Seed treatment)	0.274	**0.031**	0.629	0.411	0.75
		SED	15.93	22.3	0.281	20.1	0.396
		LSD	35.06	49.1	0.619	44.2	0.873
		P value (Insecticide)	0.177	**0.012**	0.744	0.859	0.643
		SED	15.93	22.3	0.281	20.1	0.396
		LSD	35.06	49.1	0.619	44.2	0.873
		P value (Sow date * Seed trt)	0.884	0.66	0.722	0.728	**0.024**
		SED	16.4	85.4	0.68	23.7	1.515
		LSD	35.7	325	2.222	51.7	5.765
		P value (Sow date * Insecticide)	0.868	0.141	0.18	0.462	0.642
		SED	16.4	85.4	0.68	23.7	0.561
		LSD	35.71	325	2.222	51.7	5.765
		P value (Seed trt * Insecticide)	0.996	0.188	0.206	0.157	0.481
		SED	22.53	31.5	0.398	28.4	0.561
		LSD	49.59	69.4	0.876	62.6	1.234
		P value (Drill date * Seed trt * Insecticide)	0.484	0.619	0.597	0.934	0.856
		SED	27.87	91	0.79	37	0.793
		LSD	61.07	292.7	2.045	80	5.189

4.1.3. Cross site analysis

Varietal differences in shoot number

The two seed rate x variety experiments at Towthorpe and Rosemaund in 2016 were grouped in a cross site analysis. This showed no significant difference in shoot number between sites, but a significant effect of both varieties ($P = 0.002$; LSD = 81.6) and seed rate ($P < 0.001$, LSD = 57.7), and no significant interactions. Across sites and seed rates, Revelation produced the highest shoot number of 560 shoots/m^2, followed by Horatio with 470 shoots/m^2, evolution with 445 shoots/m^2 and Butler with 395 shoots/m^2.

Yield and shoot number under insecticide treated conditions

The yield and shoot number under insecticide treated conditions were analysed across all five sites for the normal sow date treatments. There was a significant interaction between site and seed rate for all parameters including plants/m^2 ($P = 0.003$; LSD = 71), shoots/m^2 ($P < 0.001$; LSD = 213), shoots/plant ($P < 0.001$; LSD = 2.74), final ear number ($P < 0.001$; LSD = 91.2) and yield (P =

0.001; LSD = 3.150).There was also a significant effect of both site and seed rate ($P < 0.01$ or lower in each case) for all parameters except yield (site) and final ear number (seed rate). Although there was a significant interaction between site and seed rate, this was partly driven by different sites producing overall higher shoot numbers or yields. In order to update the model and produce guidelines that are applicable generally across sites, these data have therefore been averaged across sites and are summarised as such in Figure 7. As expected there was a linear relationship between seed rate and plant number (Figure 7a), and an associated negative relationship between shoots/plant and seed rate (Figure 7c). Shoots/m^2 also increased with seed rate (Figure 7b), as did yield (Figure 7e) whereas there was no relationship between final ear number and seed rate (Figure 7d), indicating that even the lowest seed rate crops on average produced over 500 shoots/m^2 and yielded well at > 8t/ha.

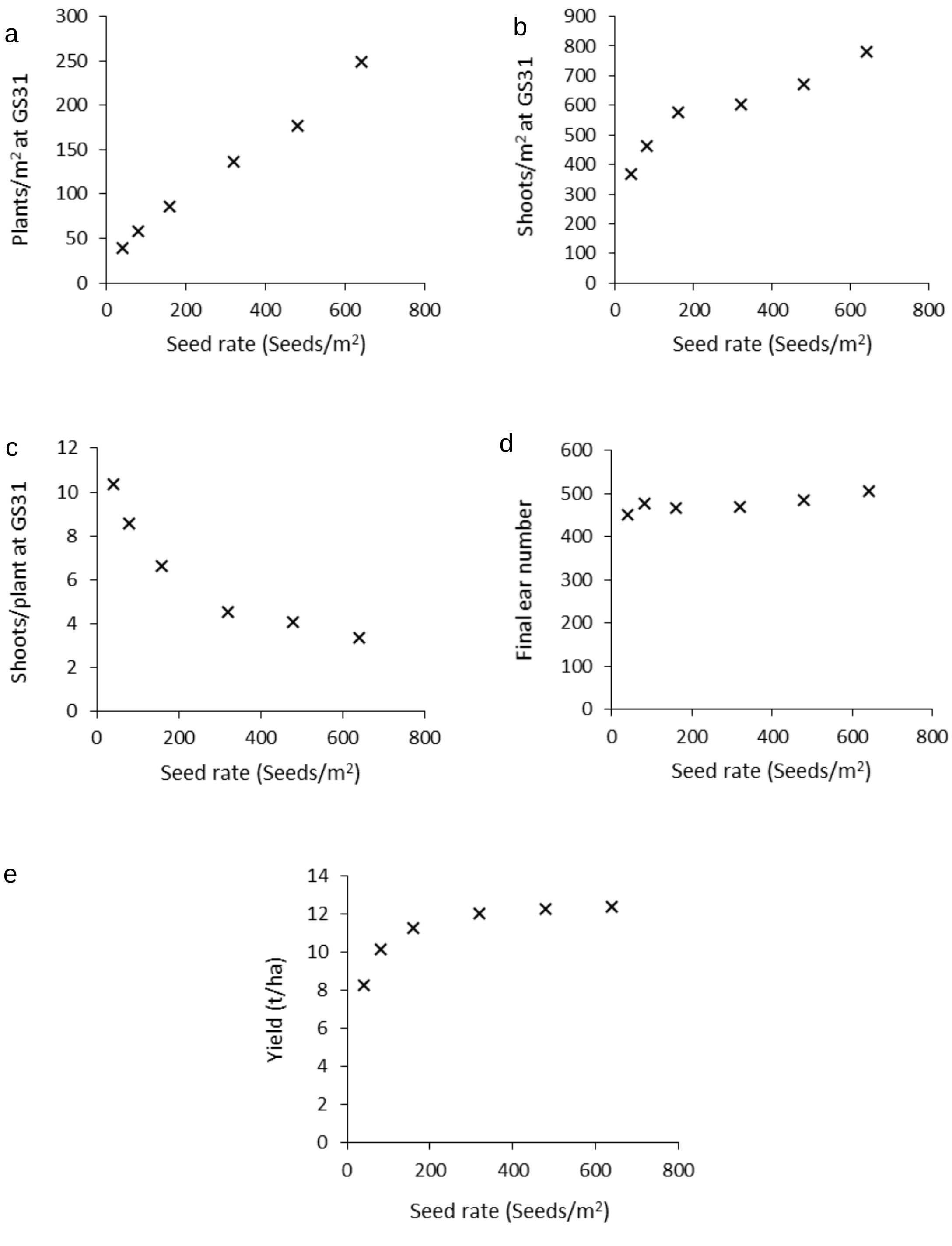

Figure 7. Mean plants/m², shoots/m², shoots/plant, final ear number and yield at GS31 across the five insecticide treated sites plotted against seed rate (0 to 640 seeds/m²). The winter wheat variety was Evolution in all five experiments.

4.2. Quantifying the risk from wheat bulb fly

4.2.1. The number of shoots a WBF can destroy and the number of eggs that become shoot damaging larvae

A literature search was conducted to determine the number of shoots a WBF larva is likely to destroy and also the level of pest mortality between oviposition and plant invasion (Table 13). The general consensus was that a larva will destroy between three and five shoots, so an average value of four shoots lost per larva was used in the threshold calculation. The highest level of WBF mortality occurs between egg hatch and plant invasion although there is limited data on the number of larvae that fail to find a host. Gough (1946) estimated that 56-81% of larvae die between hatching and plant invasion so the lowest value of 56% was used in the threshold calculation to give the most risk averse estimate of pest mortality.

Table 13. Estimates of numbers of shoots killed by a single wheat bulb fly larva and the level of pest mortality between egg hatch and plant invasion.

Variate	Value	Source
Number of shoots killed by a larva	3-5	Ellis *et al.*, 2014
	3-5	Oakley, 2003
	Up to 5	Young & Ellis, 1995
	5	Ryan, 1973
Larval mortality between egg hatch and plant invasion	56-81%	Gough, 1946
	>50%	Raw, 1967

4.2.2. Water trapping evaluation

In his 1981 study Cooper monitored adult WBF numbers at 16 oviposition sites. The period of water trapping for adult flies was the same at each site so data were plotted as egg numbers against total female fly numbers. In the current study the period of water trapping varied between sites so egg numbers were plotted against the total catch of female flies per day in order provide comparable data between oviposition sites. In general, catches of female flies were low and there was no significant relationship between the number of WBF female flies caught in water traps and the number of WBF eggs counted at the site (Figure 8).

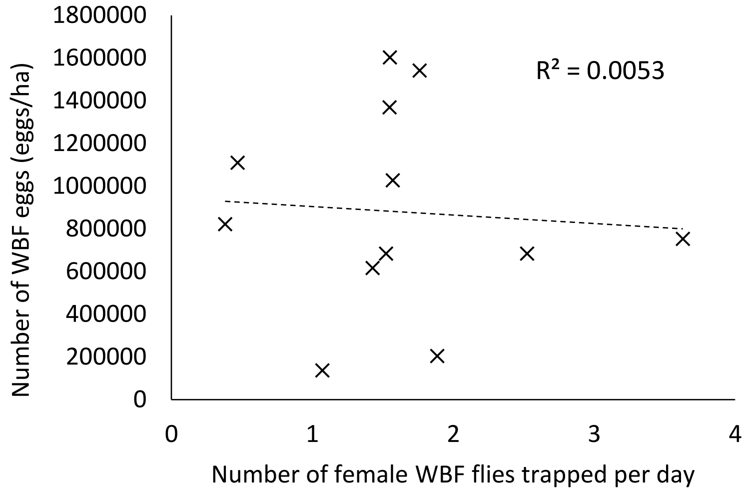

Figure 8. Number of WBF eggs determined by soil sampling against WBF female flies/day caught using water traps.

4.2.3. Shoot number assessments

This project aimed to develop a method for rapidly assessing the shoot number remotely in December using either percentage ground cover, GAI and/or NDVI as a proxy to provide guidance on whether the crop was likely to survive the level of WBF risk it would encounter or whether it was justified to apply an egg hatch treatment in the spring. During the life of the project the use of chlorpyrifos as an egg hatch spray was withdrawn but the analysis is still presented here in case chemical control options change in future.

At Bardwell in December 2016 there was a significant positive correlation between GAI and shoot number ($P < 0.001$; $R^2 = 0.80$; Figure 9), % ground cover and shoot number ($P < 0.001$, $R^2 = 0.80$; Figure 11) and between NDVI and shoot number, although this relationship was poor ($P < 0.001$; $R^2 = 0.19$; Figure 10).

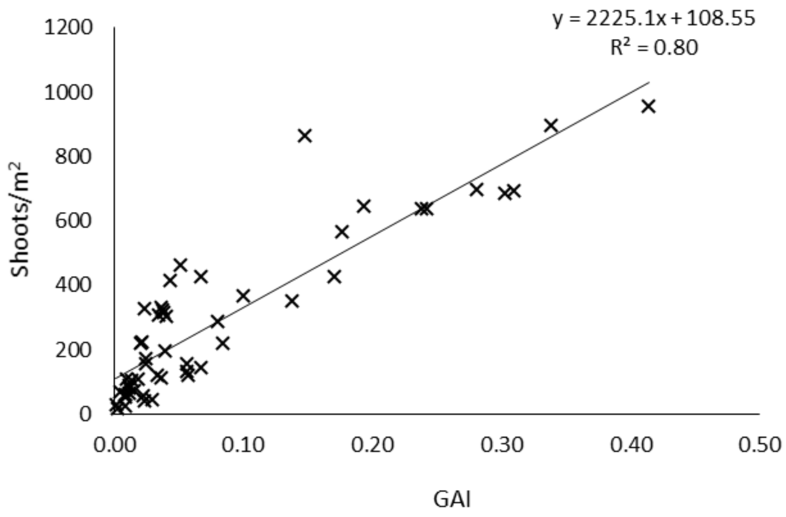

Figure 9. Green area index plotted against the number of shoots/m^2 for the Bardwell 2017 site.

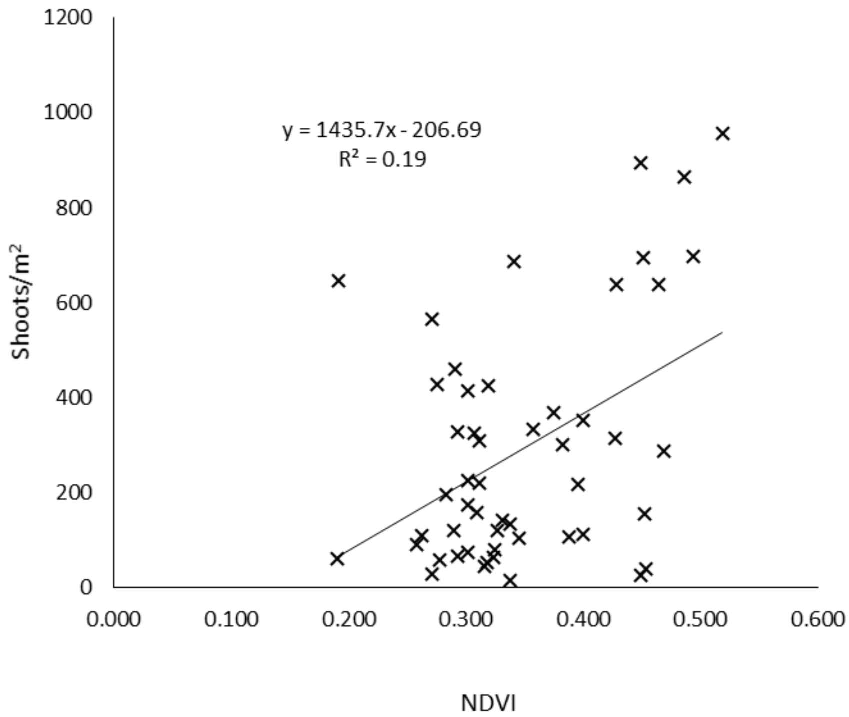

Figure 10. Normalised difference vegetation index (NDVI) plotted against the number of shoots/m^2 for the Bardwell 2017 site.

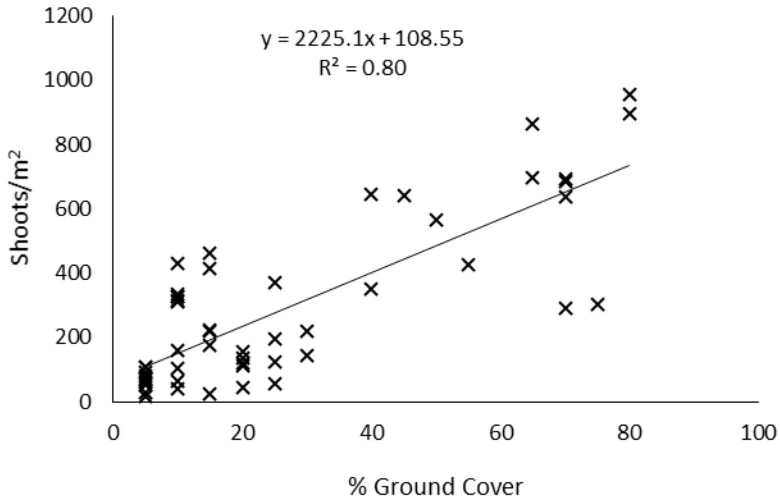

Figure 11. Visual assessment of % ground cover plotted against the number of shoots/m^2 in December at the Bardwell site.

4.3. Development and testing of a new threshold scheme for wheat bulb fly

4.3.1. Modelling shoot number production in winter wheat

Developing the model

The results from the model to predict maximum shoot production of a single plant grown in isolation at a range of sowing dates between 1st September and 30th December is shown in Figure 12. This predicted that a single plant sown on 1st September has the potential to produce 47 shoots by terminal spikelet (which approximates to the start of stem extension), whereas at the other extreme a single plant sown on 30th December would produce only two shoots.

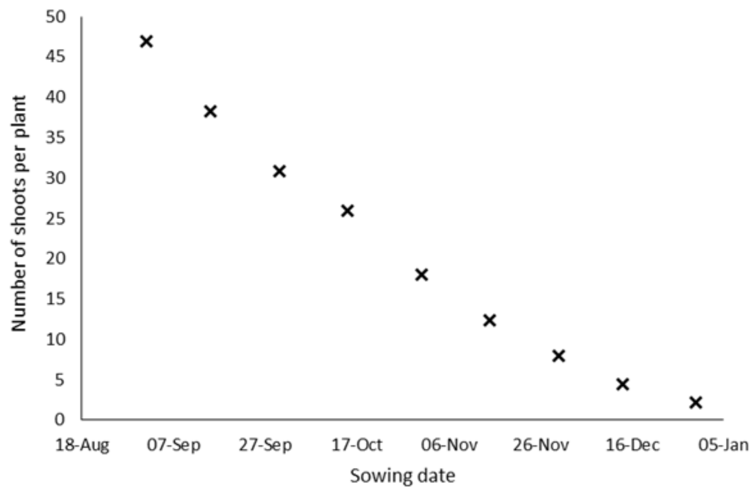

Figure 12. Predicted maximum number of shoots per winter wheat plant for a given sowing date when grown in the absence of competition and environmental stress factors.

Calibrating the model

It was recognised that achieving a shoot population of 47 shoots per plant was unrealistic under field conditions primarily due to competition between shoots for limited resources, but also due to environmental stress factors (e.g. soil capping, pests, disease). The field study results from the low WBF sites (seed rate experiments; Section 4.1.1) and chlorpyrifos treated plots from the normal sowing date plots (sowing date experiments; Section 4.1.2) were therefore used to calibrate the model to ensure that it provided a more realistic estimation for the number of shoot number that could be achieved for a given sowing date and seed rate. Given that competition between shoots is likely to be the primary factor preventing the realisation of the potential shoot number, the calibration was developed between the potential shoots/m^2 as predicted by the model against the ratio of predicted : measured shoots/m^2 from the experiments described above (Figure 13). The relationship from Figure 13 was then used to calibrate the predicted shoot numbers.

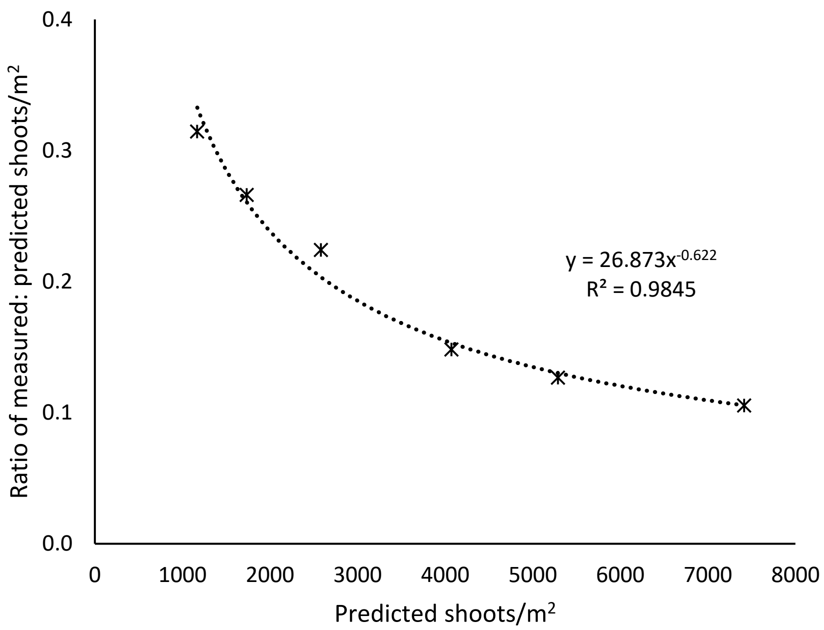

Figure 13. Modelled maximum shoots/m^2 for crops sown in the first week of October plotted against the ratio of measured: predicted maximum shoots/m^2.

Results from the updated calibrated model can be seen in Figure 14. This illustrates that the shoot number model correctly predicts a decline in maximum shoot number due to reduced plant population and delayed sowing date. A crop sown at a typical plant population of 200 plants/m^2 at the end of September is predicted to produce a maximum shoot number of approximately 750 shoots/m^2. This figure may be regarded as being relatively low, given that the AHDB benchmark shoot number at GS30 is 1040 shoots/m^2 for a plant population of 260 plants/m^2. The slightly low estimate reflects the field experiments that were used to calibrate the model, and suggests that the shoot number prediction maybe conservative. The model can be used to estimate the minimum plants/m^2 required to achieve 500 shoots/m^2 (the minimum number of shoots required to achieve a high potential yield). The model estimates minimum plant numbers of 80 plants/m^2 for late September sowing, 100 plants/m^2 for early October sowing, 120 plants/m^2 for mid-late October sowing and 180 plants/m^2 for mid-November sowing. These figures are similar, or slightly greater, than estimates of the economic optimum plant density reported by Spink *et al*. (2000b) who estimated 60-70 plants/m^2 for late September sowing, 90-100 plants/m^2 for mid-late October sowing and 140 plants/m^2 for mid-November sowing. This gives further confidence that the model of shoot production is generally realistic, the only potential shortcoming being that it may underestimate the maximum shoot number. It should be recognised that the optimum plant densities and minimum plant number to achieve 500 shoots/m^2 described above represent the minimum plant population before yield is likely to be lost. They do not represent target commercial target plant populations which have a degree of insurance built in and are consequently higher.

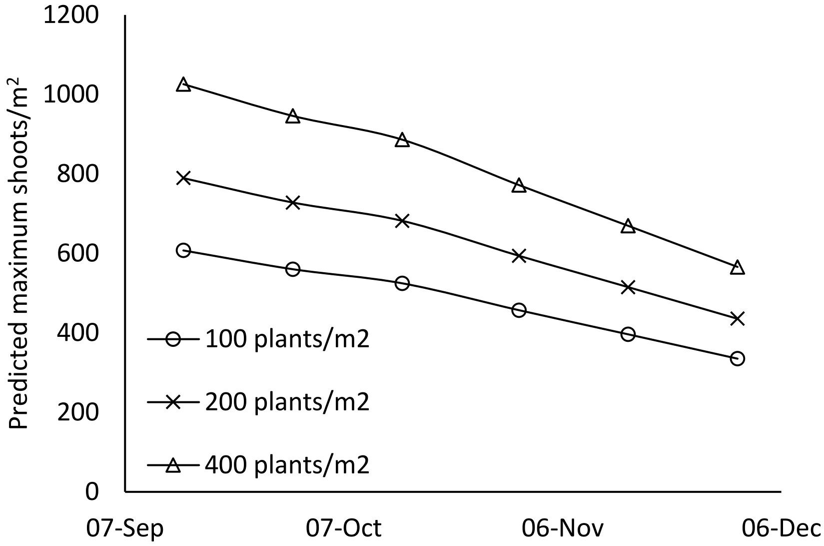

Figure 14. Predicted maximum number of shoots per m^2 for plant populations of 100, 200 and 400 plants/m^2 after calibration for environmental and plant competition factors.

The shoot production model shows that each day that the crop is sown earlier increases the maximum shoot number by approximately 4, 5 or 6 shoots/m^2 for 100, 200 or 400 plants/m^2 respectively (valid for sowing dates after 1st September) (Figure 15) and each additional plant/m^2 increases the maximum shoot number by approximately 1.5, 1.3 and 0.9 shoots/m^2 for 1st September, 30th September and 15 November sowing dates respectively (valid between 100 and 400 plants/m^2) (Figure 16). This information can be used to help estimate changes in sowing date and seed rate to minimise the risk of yield loss to WBF.

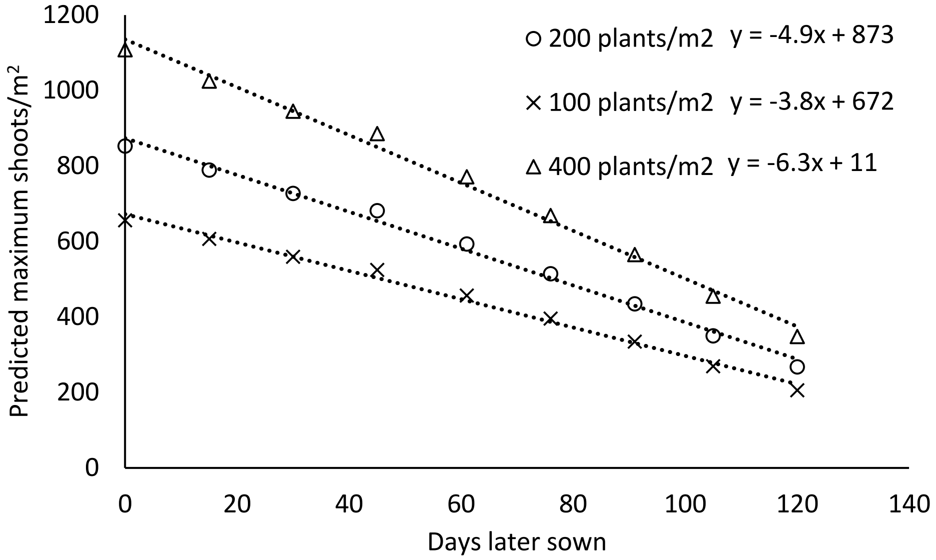

Figure 15. Effect of sowing after 1st September on predicted maximum shoots/m².

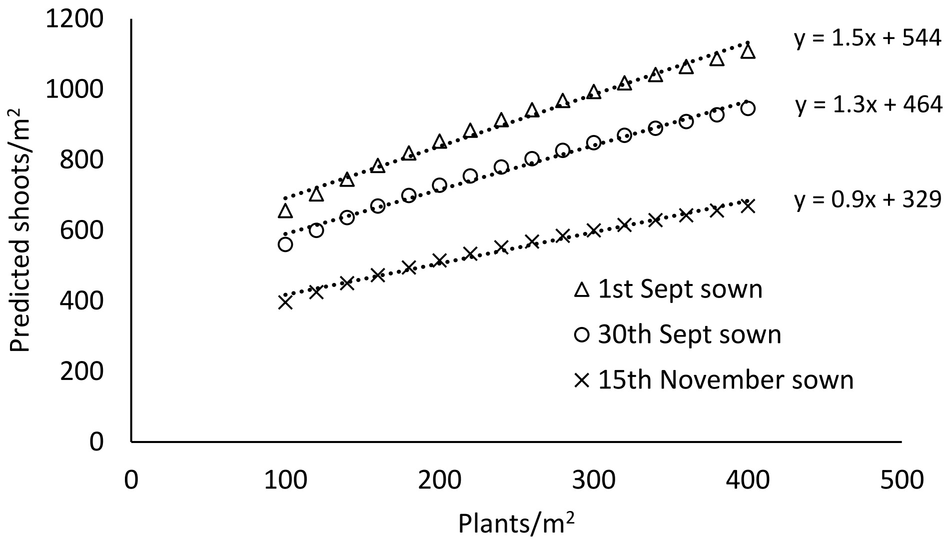

Figure 16. Effect of increasing plant population on predicted maximum shoots/m².

Research from the literature has shown that 44% of WBF eggs survive to produce larvae and each larva can destroy 4 shoots (Section 4.2.1). This information can be used to calculate the minimum number of shoots required to tolerate WBF damage without losing yield (Table 14). A typical wheat crop which has been sown on the 1st October, established 180 plants/m^2 and expected to achieve 1200 shoots/m^2 in the absence of pest attack would be expected to tolerate WBF levels up and including 2.5 million eggs/ha, but would not tolerate a WBF level of 5 million eggs/ha. Figure 17 illustrates the combinations of changes to sowing date and seed rate that would be required to produce sufficient shoots to withstand a severe WBF level of 5 million eggs/ha. Figure 18 illustrates the combination of changes in plant population and sowing date to increase the maximum shoot number by 100 to 400 shoots/m^2.

Table 14. Minimum shoot number to tolerate different levels of WBF.

Egg count (million per ha)	Minimum shoot number/m^2
1.25	720
2.50	940
5.00	1380
7.50	1820

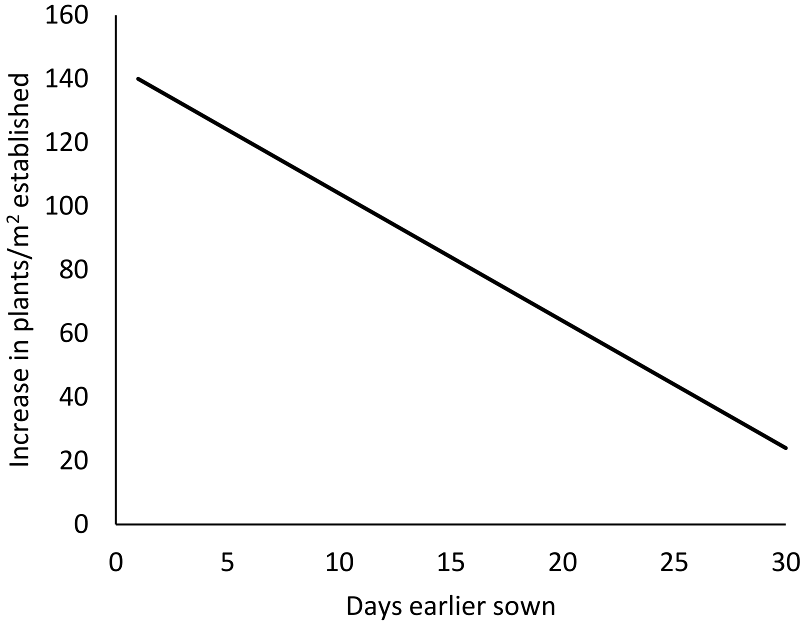

Figure 17. Combination of changes to sowing date and plant population to enable a crop able to produce 1200 shoots/m^2 without pest attack to tolerate a WBF pressure of 5 million eggs/ha.

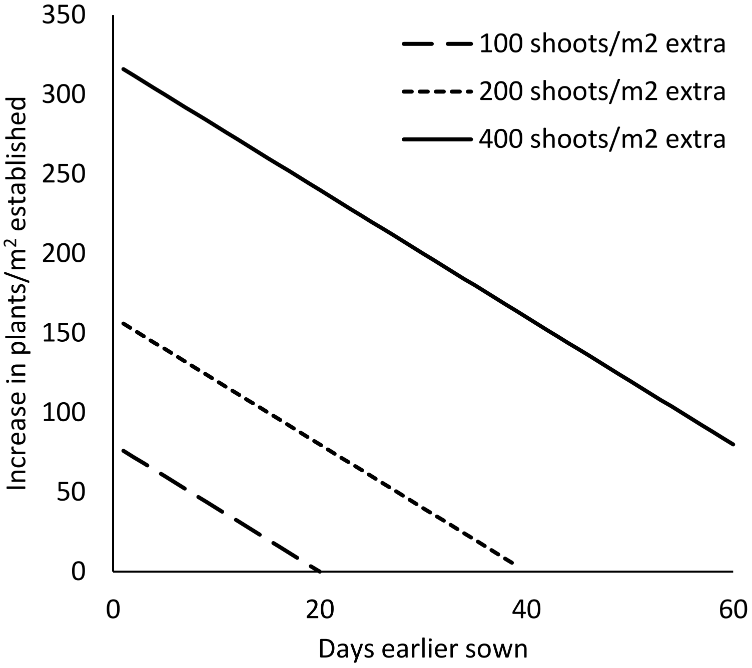

Figure 18. Combination of changes to sowing date and plant population to enable a crop able to increase its maximum shoot number by 100, 200 or 400 shoots/m^2.

4.3.2. Testing the threshold scheme

Data not used in model development from field studies described in Section 4.1 and from the literature (Spink *et al*. 2000b) were used to test the model and are summarised in Table 15. The predicted shoots/plant were estimated using the shoot number model. This value was multiplied against the measured plant population to predict the number of shoots/m^2. These were then corrected using the model correction factor described in Section 4.3.1 (Calibrating the model) to produce the corrected predicted shoots/m^2 values. The predicted shoots/m^2 values were then plotted against the actual measured GS31 shoots/m^2 values, as shown across sites in Figure 19 and as individual sites in Figure 20. There was no apparent effect of insecticide treatment or sow date on these relationships. Whilst this provides a good test of the models ability to predict shoot number under no WBF pressure, it does not test whether the shoot number estimates under high WBF scenarios are accurate. Unfortunately there were no sites with a high WBF risk during the course of the study, and therefore the scheme could not be tested as thoroughly as had been hoped so this remains as a potential topic for future work.

Table 15. Details of experimental sites used in the shoot number model testing. For each experiment maximum (GS30-32) shoot number and plant population (plants/m²) was used along with the model to predict and test the maximum shoot number.

Experiment	Sow date	Treatment	Seed rates included
Bardwell 2017	29/9/2016	No egg hatch insecticide (Normal sown)	40, 80,160, 320, 480, 640
Bardwell 2017	25/10/2016	With egg hatch insecticide (Late sown)	40, 80,160, 320, 480, 640
Bardwell 2017	25/10/2016	No egg hatch insecticide (Late sown)	40, 80,160, 320, 480, 640
Foxholes 2017	24/10/2016	No egg hatch insecticide (Normal sown)	80, 320
Foxholes 2017	16/11/2016	With egg hatch insecticide (Late sown)	40, 80,160, 320, 480, 640
Foxholes 2017	16/11/2016	No egg hatch insecticide (Late sown)	80, 320
Huggate 2016	10/10/2015	No egg hatch insecticide (Normal sown)	40, 80,160, 320, 480, 640
Spink *et al*. 2000b	29/09/1996	NA	40, 80, 160, 320, 640
Spink *et al*. 2000b	23/09/1997	NA	40, 80, 160, 320, 640

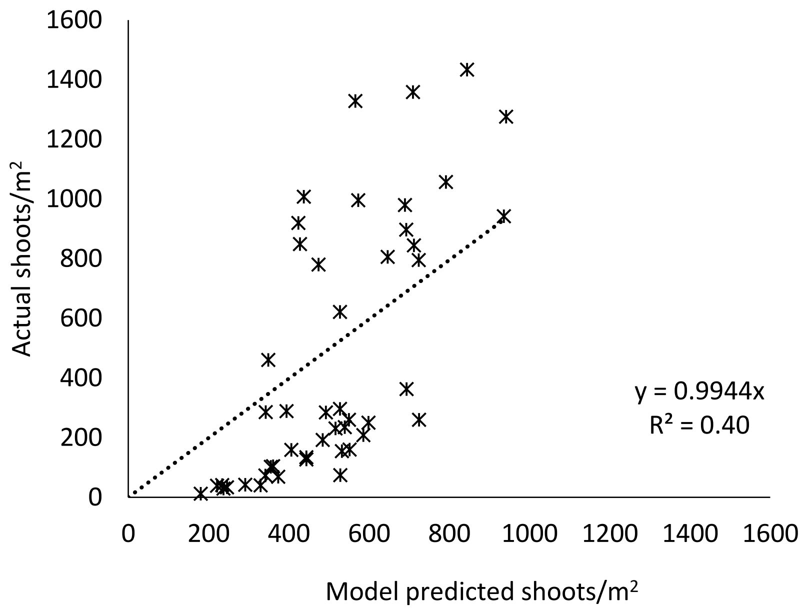

Figure 19. Model predicted shoots/m² plotted against actual measured shoots/m² for data from the experiments described in Table 15. Trend line fitted through the origin.

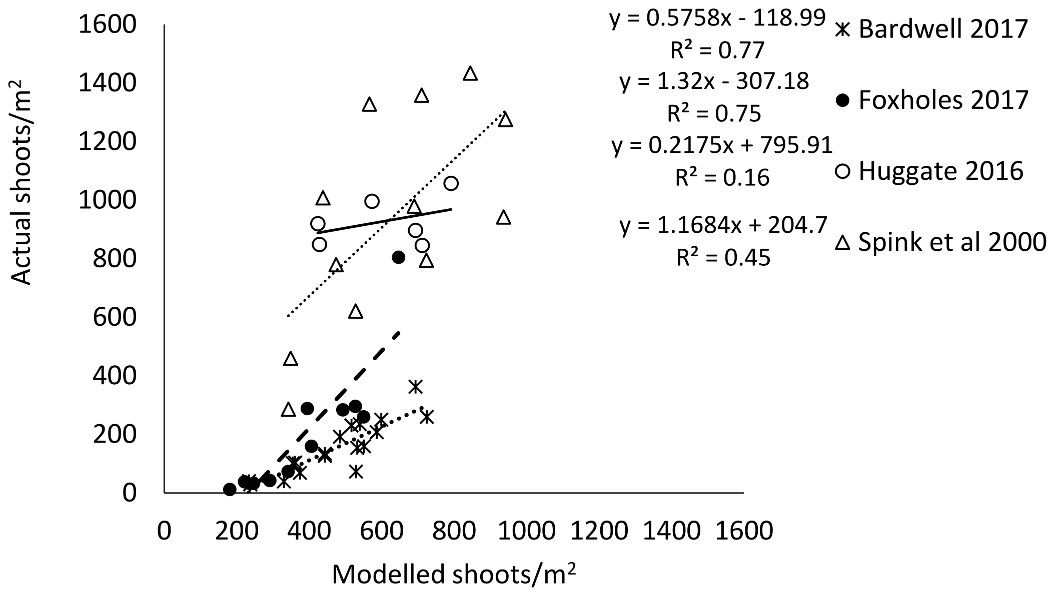

Figure 20. Model predicted shoots/m^2 plotted against actual measured shoots/m^2 separated by experiment as described in Table 15.

5. Discussion

5.1. Field experimentation

5.1.1. Shoot number and yield

Across the five experimental sites, there was a typical response at GS31 for shoot number and yield to increase with increasing seed rate as found in previous studies (Spink *et al*. 2000b; Spink *et al*., 2005). The shoot numbers measured at the range of seed rates and plant populations were comparable to those reported in Spink *et al*., 2000b and Spink *et al*., 2005, although lower than the benchmark value given in the AHDB Wheat Growth Guide, and comparable yields ranging from 2.15 to 13.95 t/ha were also consistent with those seen in previous seed rate experiments. For example, Spink *et al*., 2005 reported a range of 4.2 t/ha to 12.9 t/ha for similar seed rate experiments in 2001-2. The lower yields were generally achieved from late sown, lower seed rate plots (e.g. 5.15 t/ha and 2.21 t/ha for untreated late sown 40 seeds/m^2 at Foxholes and Bardwell respectively). In contrast, when lower seed rates were used at early or more typical sow date timings, the yields were higher, ranging from 5.02 t/ha at Bardwell up to 10.74 t/ha at Huggate, with a mean of 8.29 t/ha across the five insecticide treated (or absence of WBF) sites used to calibrate the model. Spink *et al*., (2005) reported average first wheat yields of 7.27 t/ha for sites where N was applied at normal timings for 15 different sites in experiments between 2001-2003. Similarly, Spink *et al*., 2000b measured yields ranging from 7-8 t/ha and 8-9 t/ha for plant populations of < 20 plants/m^2 and just less than 40 plants/m^2 respectively, demonstrating the ability of very low plant populations of winter wheat to produce large numbers of compensatory tillers and ultimately yield.

The Bardwell site had relatively lower shoot numbers and yield compared to the other experimental sites, particularly for the later sown treatments, reflecting the lower plant establishment of the later sown crop. However, even in the late sown crops, the maximum yield reached 7.91 t/ha from a final ear number of 414 ears/m^2 and a GS31 shoot number of 260 shoots/m^2. Similar effects were seen at the Towthorpe and Foxholes sites, particularly for the late sown or lower seed rate crops. This demonstrates the ability of later sown or lower plant population crops to produce additional tillers after GS31. This is currently not accounted for in the threshold scheme, partly because it is difficult to predict, but also because the WBF damage would have occurred before this growth stage (Ellis *et al*., 2014). If a primary tiller is lost to WBF, all subsequent potential tillers growing from this tiller would also be lost. Therefore, including the potential for tillering after GS31 may over-estimate the plant's capacity to compensate for WBF damage. Additionally, not including this additional potential late tillering capability in the model provides an additional level of insurance when utilising the scheme.

5.1.2. Foliar insecticide treatment

Across the three sites which included an egg hatch spray for control of WBF larvae, there was no significant benefit for yield. This is unsurprising given the low levels of WBF found at Foxholes and Bardwell (Table 2), although a population of 3.1 million eggs/ha at Huggate would have been considered above the level at which yield might be expected to suffer (2.5 million eggs/ha). In this case, the insecticide would not have improved yield at this site and demonstrates that a higher threshold may have been more appropriate.

5.1.3. Seed insecticide treatment

There was a small positive (0.1 t/ha), but significant, effect of seed insecticide treatment on yield across both sowing dates at Foxholes in 2017. This was despite the WBF egg count for the site being low at 0.8 million eggs/ha, and below the level (1.0 million eggs/ha) at which a seed treatment would normally be advised. However, this result should not be considered as evidence to justify seed treatment for late sown crops, irrespective of the WBF egg count, for a number of reasons. Firstly, the early sowing was on 24 October 2016 so only seven days before November when a revised threshold of 1.0 million eggs/ha is applied. Secondly, plant dissections indicated that the site was also attacked by yellow cereal fly (*Opomyza florum*). This pest has a very similar life cycle to WBF in that larvae hatch in the new year and so contribute to tiller loss even though yellow cereal fly only attacks a single tiller. Approximately one third of larvae recovered at the site were yellow cereal fly so it is likely that the combined oviposition of this pest and WBF would have exceeded the 1.0 million eggs/ha threshold. Thirdly, seed treatment only affected crop yield and had no effect on plant or shoot number. An effect on these variables would be expected as the seed treatment is designed to reduce plant invasion by larvae of WBF or yellow cereal fly. This casts some doubt on the validity of the yield response to seed treatment and it is possible that it was simply due to experimental error. Finally, the effect of seed treatment on yield was from a single trial. Ideally, further work would be needed to confirm this result before any decision could be made on changing advice on when seed treatments should be used.

At the Bardwell site there was a significant interaction between sow date and seed treatment with only the late sown crops appearing to benefit from the seed treatment. This result appears to support the advice that seed treatments are only beneficial for late sown crops. However the large SEDs meant it was not possible to state which treatments differ, suggesting this result may have come about by chance. Therefore in the absence of conclusive evidence to the contrary it is recommended that seed treatments should still be considered to be an important component of an integrated pest management strategy against WBF.

5.1.4. Varietal differences in shoot number

Revelation produced higher shoot final numbers across sites and seed rates of 560 shoots/m^2, whereas Butler, Evolution and Horatio produced 395, 445 and 470 shoots/m^2 respectively. In a study comparing between 15-26 winter wheat varieties running from 1997-1999 Spink *et al*., (2000b) concluded that the variety had very little impact on the optimum plant population. Nonetheless, they reported significant differences in GS32 shoot number among varieties within each sowing date treatment, although this had no effect on the final yield. Similarly, whilst Revelation produced the highest number of shoots/m^2 in the present study it was not the highest yielding variety at either site (11.74 t/ha at Towthorpe and 10.09 t/ha at Rosemaund), with Horatio yielding well at both sites (Towthorpe: 13.04 t/ha, Rosemaund: 11.14 t/ha) followed by Evolution (Towthorpe: 11.08 t/ha, Rosemaund: 11.18 t/ha) and Butler (Towthorpe: 13.06, Rosemaund: 10.00 t/ha). Whilst varietal differences in final shoot number may not be important for yield, a variety that produces a higher maximum shoot number may be beneficial to crops which are under attack from stem boring pests prior to GS31. These variety experiments were purposely run on sites with no WBF to prevent WBF damage from confounding the results, however, future research would be beneficial in order to understand whether varieties with higher shoot numbers can be identified, and if so, whether using these as part of an IPM scheme to reduce WBF risk is justified. If it is possible to quantify the level of increased shoot number for certain varieties, it would also be possible with additional further work to add a varietal factor into the shoot production model and subsequent WBF threshold scheme.

5.2. Quantifying the risk from wheat bulb fly

5.2.1. Water trapping as an alternative to soil sampling to predict WBF risk

If numbers of WBF adult females caught in water traps is correlated with oviposition by the pest this would provide an earlier indication of WBF risk in any particular season than soil sampling for eggs. This has the advantage that it would give an early indication of the need for seed treatments for crops to be sown later that autumn. Also an early indication of WBF risk would allow appropriate seed rates to be calculated for crops sown at conventional timings for which seed treatments would not be sufficiently persistent to protect against larvae hatching in January/February of the following year.

Cooper (1981) showed a good correlation between egg numbers and numbers of female flies with egg numbers increasing linearly with increasing catches of female flies. In contrast in the current study the correlation was poor. It is possible that was due to the very low number of eggs recovered from the monitoring sites in both 2015/16 and 2016/17. Egg numbers ranged from 0.2-1.4 million/ha whereas in Coopers work numbers ranged between about 0 and 8.0 million/ha. The differences in egg numbers between the two studies is illustrated in Figure 21. In Coopers study an egg population of 2.5 million/ha was associated with about 50 females but in the current study egg numbers never exceeded 1.4 million/ha.

It would be useful to determine if Cooper's relationship between egg numbers and female flies could be replicated in seasons when wheat bulb fly numbers are high. Alternative methods of achieving an early indication of WBF risk have also been studied. Bowden & Jones (1979) studied the capture of WBF adults in light-traps over a nine-year period. They found that catches of females were correlated with the number of eggs later recorded in the area near the light-traps. They therefore suggested that light-traps sited at ground level near fields at risk from WBF could be a useful method of predicting egg numbers.

A potential criticism of trapping female WBF is that their ability to lay eggs is influenced by the availability of fungi within the cereal ears on which they feed. If there is a plentiful supply of fungal food, as might be the case if harvest is delayed by wet weather, then many more eggs are likely to be produced than in a dry season when food for females flies is more limiting. Young and Cochrane (1993) produced a multiple regression model to predict egg numbers from meteorological data. Egg numbers were negatively correlated with departure from average July temperatures and positively correlated with August rainfall. By using met data this model is likely to take better account of any variability in levels of food for WBF and so provide a more precise estimate of risk. As a result this model should be investigated further as it also has the potential to provide a much earlier indication of risk.

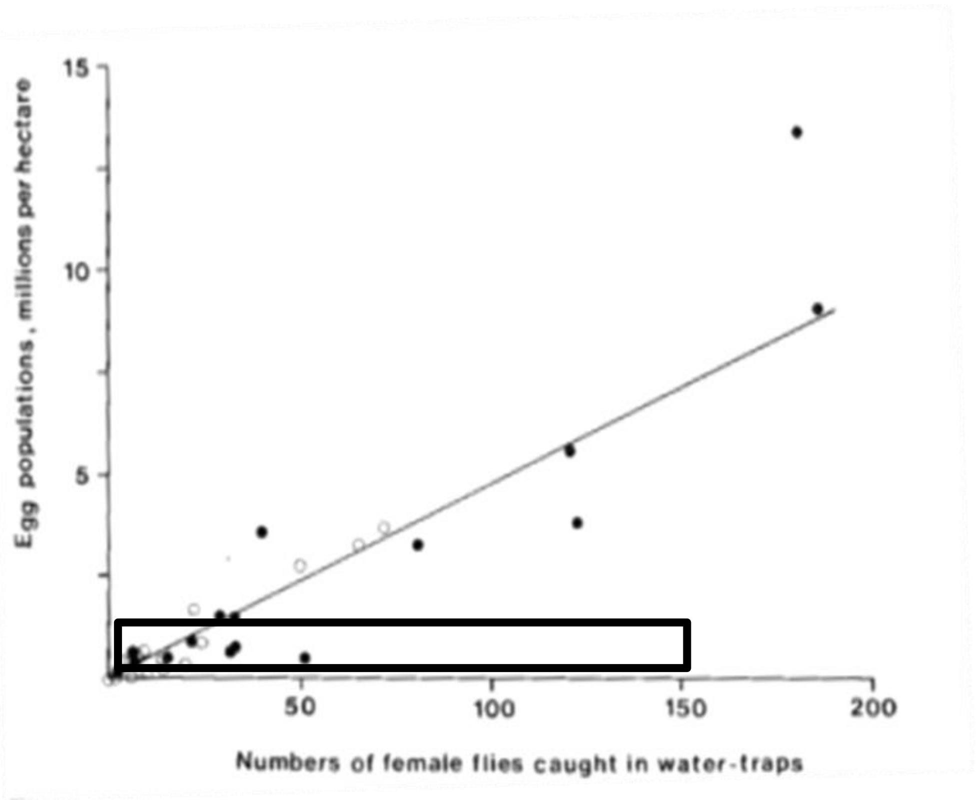

Figure 21. Relationship between numbers of wheat bulb fly (WBF) eggs at oviposition sites. Estimated from 20 soil cores per field, and the numbers of adult females caught in four water traps/site. o 1977; ● 1978 (from Cooper, 1981). The black rectangle indicates the range of egg and fly numbers recorded in the current study.

5.2.2. Shoot number assessments

The significant positive correlation between December shoot number and GAI or ground cover demonstrates that it is possible to estimate the shoot number of an established crop remotely using either GAI prediction schemes or aerial imagery. However, the relationship between shoot number and NDVI was not as strong, possibly because the crop was small. It has been demonstrated in previous research that spectral reflectance indices such as NDVI can accurately sense crop GAI in the range of small to moderate sized canopy (Haboudane *et al*., 2004). Before stem extension variation in crop GAI is primarily driven by variation in shoot number, so this indicates that it should be possible to sense shoot number using spectral reflectance indices. The original aim of this work was to provide an in season estimate of shoot number so that growers could use this to decide whether they would need to apply an egg hatch spray in January/February depending on the autumn WBF egg counts from their site and the timing of egg hatch. However, since the project started the egg hatch spray is no longer available and therefore this information is no longer useful in current WBF management schemes. Nonetheless, it has been included in case future chemical control of egg hatch becomes available. A method of remotely sensing shoot number will also be useful for farmers to benchmark their crop so they can understand how many shoots their crops typically achieve. This information can then be used to estimate tolerance that their crops have to WBF damage.

5.3. Development of a new threshold scheme for wheat bulb fly

The WBF threshold scheme developed in this project requires information about WBF egg count and the maximum shoot number that would be expected from the crop in the absence of significant pest damage. Most growers and agronomists will not be familiar with the maximum number of shoots their crops typically produce. In time we hope that remote sensing techniques trialled in this project could provide a way for growers and agronomists to get a better understanding of this crop characteristic. The Wheat Growth Guide Benchmark maximum shoot number is 1020 shoots/m^2 at GS30/31. This was the average for a number of reference crops which were sown late September to early October and had an average establishment of approximately 250 plants/m^2. A simple guide about how the maximum shoot number may vary with different sowing dates and plant populations is described in Table 16. To generate this information the shoot production model was used to calculate the relative changes in shoot number that will be caused by changing sowing date and plant population. These relative changes were then used to adjust the Wheat Growth Guide benchmark shoot number. This approach was used because the shoot production model estimates shoot numbers that are slightly low compared with typical values (due to the nature of the crop data it was calibrated against) but it has been shown to estimate the relative effects of sowing date and plant population well. This approach therefore enables the more representative figures to be provided. The data in Table 16 indicates that sowing in mid-September and establishing 400 plants may achieve a maximum shoot number of 1323 shoots/m^2. In contrast sowing in mid-November and establishing 150 plants/m^2 is estimated to only achieve 596 shoots/m^2. It should be recognised that site specific factors such soil capping or different autumn temperatures could have a significant effect on these shoot number estimates.

The steps for following the threshold scheme are as follows;

1. Use the WBF egg count to estimate the minimum number of shoots required to tolerate the pest damage using figures in Table 17.
2. Estimate the maximum shoot numbers your crop is likely to reach in the absence of significant pest damage, either based on your own experience (accounting for both expected sowing date and plant population), using the Wheat Growth Guide benchmark as a default value, or the estimated values given in Table 16.
3. Subtract the minimum shoot number estimated in step 1 from the expected shoot number estimated in step 2. If the result is above zero then your crop has a good chance of tolerating any loss of shoots due to WBF. If the result is negative then changes to crop husbandry will be required to produce a crop with sufficient shoots to tolerate the WBF (see step 4).

If the answer from step 3 is less than zero then use

4. Figure 22 to estimate the changes to sowing date and number of plants established required to increase the shoot number by a sufficient amount to exceed the minimum number of shoots required to tolerate WBF estimated in step 1*. Note that the changes described in*

5. Figure 22 *are appropriate for a crop sown around early October with 200 plants/m^2. Different sow date/plant number combinations are required from crops sown around mid-September or November, or with <150 or >250 plants/m^2. These are described in Figure 15 and Figure 16.*

6. If the crop must be sown in November or later, and the answer to step 3 was less than zero, then this crop may benefit from a seed treatment.

The WBF threshold scheme describes changes in target plant population required. Growers must make a judgement about how many seeds to sow to achieve the target plant population. Guidance about how factors affect plant establishment are summarised in AHDB Research Review 51 (Blake *et al*. (2003)). This review concludes that plant establishment averages 70% in September / early October, falling to 60% in late October and 50% or less in November.

Table 16. Maximum shoot number (shoots/m^2) estimated by using the shoot production model to adjust the Wheat Growth Guide benchmark shoot number for different sowing dates and plant numbers.

	Sowing date				
	15 September	1 October	15 October	1 November	15 November
150 plants/m^2	913	842	789	687	596
200 plants/m^2	1018	939	880	766	664
250 plants/m^2	1196	1020	957	834	607
300 plants/m^2	1187	1095	1025	893	775
350 plants/m^2	1258	1160	1087	947	821
400 plants/m^2	1323	1220	1143	996	863

Table 17. Minimum shoot number to tolerate different levels of WBF

Egg count (million per ha)	Minimum shoot number/m^2
1.25	720
2.50	940
5.00	1380
7.50	1820

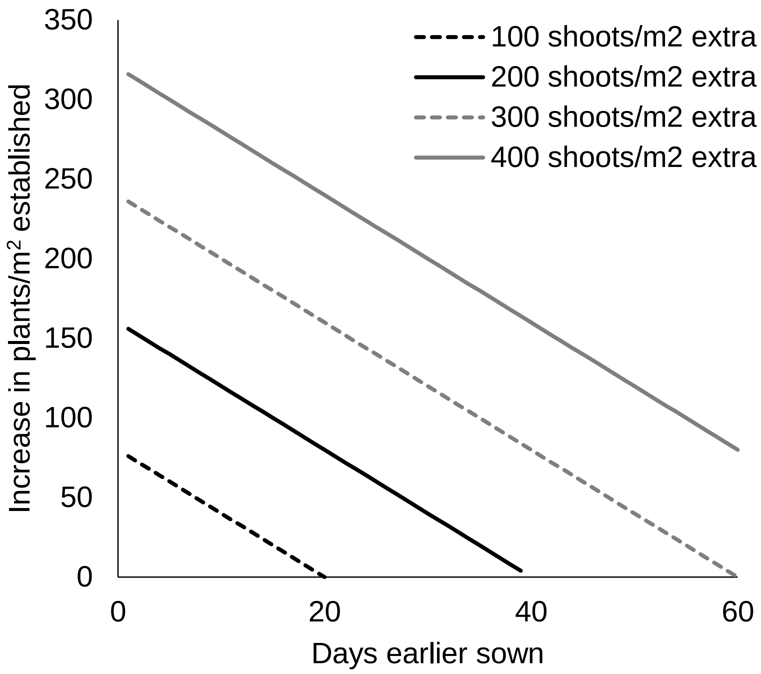

Figure 22. Combination of changes to sowing date and plant population to enable a crop able to increase its maximum shoot number by 100, 200, 300 or 400 shoots/m^2. These changes are appropriate for a crop sown in early October with 200 plants/m^2.

At present in order to use the threshold scheme growers would need to assess the WBF risk level, predominantly relying on egg count data from their own site or from the AHDB Autumn survey of WBF incidence. Ultimately the aim would be to develop the water trapping methodology to enable July water trapping or an alternative pest monitoring method/model to provide an earlier estimate of the WBF risk.

While the current version of the threshold scheme is tabulated, in the future it would be possible with additional continued development to convert the scheme into a more interactive format, for example via a website or mobile application. It is possible to develop the scheme into a code based format which can provide site specific guidance on the plant population recommended based on local conditions including soil type, variety, and local weather data. This would require further development as described in the future research section below in order for it to be reliable but it has the potential to be more site specific.

5.4. Further work

This project took place in seasons with nationally low levels of WBF which restricted testing. The WBF threshold scheme requires testing in situations with moderate and high levels of WBF. There is also a need to provide more maximum shoot number data from both seed rate and sowing date experiments in order to calibrate it against a wider range of environments, to test how sowing date impacts on the maximum shoot number more thoroughly and to test the water trapping technique for estimating adult WBF.

To achieve greatest utility the WBF threshold scheme should be made into an interactive tool online and as an app. This would also benefit from incorporating an economic assessment about impact of changing husbandry.

Results from the current study suggest that varieties vary in their capacity to produce tillers. The current shoot production model assumes no difference in tillering capacity between varieties and this would need to be modified if such varietal differences exist. This would enable the estimate of maximum shoot number to be improved. Further work should quantify varietal differences in shoot number in a range of environments. It would also be important to assess whether this data could be collected from AHDB funded recommended list trials, possibly using remote sensing techniques. It has also been suggested that varieties that show apical dominance would be disproportionately affected if the main shoot was lost due to WBF attack in comparison with varieties which do not show apical dominance. This question could be answered using experiments in which different tillers are pruned.

The project has shown that tiller number in December could be assessed rapidly using remote sensing techniques. The relationships developed in the project should be tested more widely including on different varieties.

It has been shown that 400 shoots/m^2 is sufficient to achieve typical UK yield of 8 t/ha (Spink *et al*., 2000a). However, the minimum shoot number required to achieve high yields (>12 t/ha) is unknown. The current threshold increased the minimum shoot number from 400 to 500 shoots/m^2 to provide insurance, but there is uncertainty about whether this is correct. The Yield Enhancement Network (YEN, www.yen.adas.co.uk) has collated final ear number and yield data from several hundred high yielding winter wheat over several seasons. The average ear number for this dataset is close to 500 ears/m^2, however it is not known whether this is linked with yield. Future work could use the YEN dataset to estimate minimum final shoot number required for high yielding crops and whether different ear numbers are required for different yield levels.

The shoot production model assumes that if a crop achieves the minimum shoot number of 500 shoots/m^2 by GS31, then each of these shoots produces an ear and contributes to the ultimate yield. However, it is possible that, even in crops with low shoot populations at GS31, shoots are lost between GS31 and harvest and this would potentially reduce the compensatory ability of the crop. In contrast, data from the experiments reported here have shown that low plant populations can continue to tiller after GS31, with final ear number increasing. The relationship between shoot number and final ear number needs to be investigated further and taken account of in the model if necessary. This should include investigating the relationship between plant population, ear number and yield using information from ADAS studies and previous AHDB studies.

Assessing WBF risk as early as possible will help in determining the need for seed treatments or the need to consider changing sowing date and/or seed rate in order to produce a crop which is sufficiently robust to tolerate the pest. It would be important to determine if Cooper's relationship between egg numbers and female flies could be replicated in seasons when WBF numbers are high. Also Young and Cochrane (1993) produced a multiple regression model to predict egg numbers from meteorological data. Egg numbers were negatively correlated with departure from average July temperatures and positively correlated with August rainfall. Such a model would also provide an early indication of WBF risk and should be investigated further. This model could be run with historic met data to determine WBF risk and compared with the results of the AHDB Autumn survey of wheat bulb fly incidence. The Rothamsted Suction traps catch a wide range of insects. It would be worth determining if WBF adults are caught and if so, whether these catches can be correlated with egg counts in the autumn.

This project has concentrated on WBF but the model can easily be adapted for other dipterous stem borers by simply changing the number of tillers that can be killed by the larva of the pest. For example, WBF moves between tillers and is thought to kill between three and five during its life cycle. Other stem borers such as gout fly (*Chlorops pumilionis*) and yellow cereal fly (*Opomyza florum*) complete their life cycle within a single tiller and so pose a significantly lesser threat than either WBF or frit fly.

6. Conclusions

The range of insecticides available for control of insect pests is declining. A number of products have been lost, few replacements are coming onto the market and those that have been registered are generally more expensive than synthetic pyrethroids that have been the primary method of controlling pests in arable crops since the 1980's. Reliance on pyrethroids has also caused problems as resistance among the pest population is now widespread. In future and in compliance with the Sustainable Use Directive pest control will become increasingly reliant on integrated pest management (IPM) with reduced reliance on chemicals.

Seed treatments are the only chemical control option currently available for WBF and these are only effective for crops sown after November. As only a small proportion of wheat crops are sown after this date alternative control strategies are urgently required for this pest. This project has gone some way to filling this gap by developing a threshold scheme for the pest which is reliant on manipulating sowing date and/or target plant population (through seed rate) in order to produce a crop which is sufficiently robust to tolerate pest attack. Such an approach has involved the collaboration of both plant physiologists and entomologists and takes advantage of the fact that wheat crops often produce more shoots than are required to achieve potential yield. Consequently these excess shoots can be sacrificed to pests without impacting on yield. It should be stressed that the threshold model used to predict sow dates and changes in target plant population is a prototype and is likely to go through further iterations of testing and development before it is finalised. Nonetheless it is an important step in the sustainable management of WBF and follows on from the work to re-evaluate thresholds for pollen beetle in oilseed rape which also took advantage of the fact that this crop produces more buds than it needs to achieve potential yield.

Being able to predict sowing date and target plant population to produce crops that are tolerant of WBF attack effectively provides another control method for this pest. It is conceivable that manipulation of sowing date and/or plant population could be used instead of seed treatments for late sown crops although the relative economics of these control options will need to be evaluated. In those situations where cropping decisions are made too late for application of seed treatments the current threshold model will at least provide the opportunity to assess the likely cost of manipulating seed rate so that it could be compared with potential options for spring cropping.

Predicting the annual risk of WBF attack is crucial to making early decisions on WBF control, whether this be to use a seed treatment for late sown crops of manipulate sowing date and/or seed rate for those sown at a more conventional timing. Soil sampling is effective but laborious and often too late to influence decisions for winter wheat crops. Risk prediction for WBF is an important area for future research and could involve water trapping for adult WBF, analysis of suction trap data or even the development of pheromone traps for the pest.

An important component of IPM is knowing when not to treat and understanding the inherent tolerance of crops to pests. The presence of damage does not always equate to loss of yield. This concept is likely to become increasingly important as the insecticidal armory continues to decline. Whilst the science involved with the development of models to predict sowing date and target plant population in the case of WBF or re-evaluation of thresholds for pollen beetle may be complex it is vital that its implementation is ultimately simple for farmers and agronomists. This will ensure wide scale adoption of these novel ideas. This is likely to involve a more interactive format, for example via a website or mobile application. This will be the challenge for future research.

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8. Appendix 1.

Table 18. Foxholes 2017 ground cover and NDVI results.

Sow date	Insecticide treatment	Seed rate (seeds/m^2)	% ground cover	NDVI
Normal	Untreated	40	8	0.38
Normal	Untreated	80	17	0.39
Normal	Untreated	160	27	0.33
Normal	Untreated	320	37	0.4
Normal	Untreated	480	47	0.38
Normal	Untreated	640	50	0.54
Normal	Treated	40	8	0.23
Normal	Treated	80	15	0.38
Normal	Treated	160	35	0.47
Normal	Treated	320	43	0.54
Normal	Treated	480	47	0.53
Normal	Treated	640	45	0.55
Late	Untreated	40	5	0.09
Late	Untreated	80	3	0.11
Late	Untreated	160	3	0.11
Late	Untreated	320	8	0.17
Late	Untreated	480	8	0.25
Late	Untreated	640	8	0.18
Late	Treated	40	5	0.11
Late	Treated	80	4	0.12
Late	Treated	160	7	0.13
Late	Treated	320	10	0.15
Late	Treated	480	10	0.15
Late	Treated	640	45	0.3
	Grand mean		19	0.29
	P value sowing date		**0.01**	**0.011**
	SED		2.46	0.028
	LSD		10.58	0.121
	P value (Insecticide)		0.298	0.335
	SED		1.37	0.028
	LSD		2.77	0.056
	P value (Seed rate)		**<0.001**	**0.006**
	SED		2.38	0.048
	LSD		4.79	0.098
	P value (sowing date * Insecticide)		0.968	0.465
	SED		2.82	0.04
	LSD		8.36	0.093
	P value (sowing date * seed rate		**<0.001**	0.874
	SED		3.93	0.069
	LSD		8.58	0.14
	(Insecticide*Seed rate)		0.541	0.678
	SED		3.36	0.069
	LSD		6.77	0.138
	Sow date * insecticide * seed rate		0.851	0.207
	SED		5.17	0.097
	LSD		10.64	0.195

Table 19. Foxholes 2017 seed treatment results for NDVI and % ground cover.

Sowing date	Seed treatment	Insecticide treatment	NDVI	% Ground cover
Normal	Untreated	Untreated	0.38	47
Normal	Untreated	Treated	0.53	47
Normal	Treated	Untreated	0.41	47
Normal	Treated	Treated	0.64	43
Late	Untreated	Untreated	0.25	8
Late	Untreated	Treated	0.15	10
Late	Treated	Untreated	0.24	15
Late	Treated	Treated	0.20	13
		Grand Mean		29
		P value (Sow date)	**<0.001**	**0.012**
		SED	0.008	3.7
		LSD	0.036	15.9
		P value (Seed trt)	0.481	0.482
		SED	0.060	2.3
		LSD	0.131	5.0
		P value (Insecticide)	0.343	0.723
		SED	0.060	2.3
		LSD	0.131	5.0
		P value (Sow date * Seed trt)	0.652	0.172
		SED	0.061	4.4
		LSD	0.132	12.4
		P value (Sow date * Insecticide)	**0.05**	0.723
		SED	0.061	4.4
		LSD	0.132	12.4
		P value (Seed trt * Insecticide)	0.548	0.482
		SED	0.085	3.3
		LSD	0.186	7.1
		P value (Sow date * Seed trt * Pesticide)	0.981	1.0
		SED	0.105	5.4
		LSD	0.228	12.7

Table 20. Ground cover and NDVI results for Bardwell 2017.

Sowing date	Insecticide treatment	Seed rate (seeds/m²)		Ground cover (%)	NDVI
Normal	Treated	40		15.0	0.41
Normal	Treated	80		23.3	0.35
Normal	Treated	160		25.0	0.33
Normal	Treated	320		55.0	0.40
Normal	Treated	480		58.3	0.41
Normal	Treated	640		75.0	0.44
Late	Treated	40		4.7	0.30
Late	Treated	80		6.7	0.32
Late	Treated	160		5.0	0.32
Late	Treated	320		10.0	0.31
Late	Treated	480		10.0	0.34
Late	Treated	640		11.7	0.31
Grand mean				25	0.35
P value (Sow date)				**0.01**	0.06
			SED	3.43	0.02
			LSD	14.756	0.08
P value (Seed rate)				**<0.001**	0.71
			SED	3.23	0.04
			LSD	6.76	0.08
P value (Sow date * seed rate)				**<0.001**	0.58
			SED	5.4	0.05
			LSD	12.03	0.11