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Integrated management strategies for varieties tolerant and susceptible to wheat blossom midge

by

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This is the final report of a forty-five month project which started in April 2005. The work was a Defra-sponsored LINK project for £238,181 (LK0969), together with a contract of £140,857 from HGCA, plus £5,698 in-kind (project no. 3121). The following companies also contributed in-kind industrial contributions: Dow AgroSciences Ltd (£30,500); AgriSense BCS Ltd (£19,844); Elsoms Seeds Ltd (£21,130); Advanta Seeds Ltd and Nickersons UK Ltd – both now Limagrain (£19,576 and £27,513 respectively), making a total of £503,299.

Contents

Abstract

The orange wheat blossom midge (owbm), *Sitodiplosis mosellana*, is an important pest of wheat, causing severe yield loss in some years. Infestations vary from year to year depending on climatic conditions, so being able to predict the risk of damage is difficult. The major aim of this project was to develop owbm control strategies for farmers using tolerant and susceptible varieties by using pheromone traps to determine the need for, and timing of, insecticide treatments and also to identify genes for pest resistance/tolerance for further breeding.

Owbm flight was significantly reduced when humidity was lowered from 70% to 35%. Pheromone traps were highly selective and sensitive and caught over 95% male midges. Yellow sticky traps provided information on numbers of female midges. Pheromone trap catches were very variable between fields on the same farm, and more variable than catches within fields. Crops following wheat were a major source of the pest. In some years, midge infestations were best explained by pheromone trap catches in fields neighbouring the wheat field which acted as a source of the pest. Phenolic acids are believed to be responsible for the resistance of wheat varieties to owbm. However, levels barely differed between resistant and susceptible varieties, suggesting that resistance is not solely due to these compounds.

Resistance in Welford, Brompton and Carlton is due mainly to the *Sm1* gene but other genes are involved. The mechanism of *Sm1* resistance is thought to be chemical, but other genes could affect flowering time which means that the crop escapes owbm attack.

A decision flow chart was developed to help farmers predict owbm risk. When trap catches exceed 30 midges/trap/day the crop should be inspected to determine if there are sufficient to justify a spray based on existing thresholds of 1 midge/6 ears for feed varieties and 1 midge/3 ears for milling and seed varieties. If pheromone traps catch more than 120 midges/trap/day, an insecticide spray is advisable to protect wheat crops in the immediate vicinity.

Summary

Background

The orange wheat blossom midge (owbm), *Sitodiplosis mosellana*, is a common and increasingly important pest of wheat in the northern hemisphere, causing severe yield losses in some years. Larval feeding on the developing seeds causes shriveling and pre-sprouting damage and also facilitates secondary fungal attack by *Fusarium graminearium* and *Septoria nodorum*. This affects both the yield and quality of grain harvested. In an outbreak in the UK in 2004 crop losses were estimated to be 6% (1 million tonnes) nationally which was compounded by reductions in grain quality, despite insecticide application to around 500,000 ha of wheat. Owbm has a very patchy spatial distribution and numbers also vary from year to year depending on climatic conditions. In the UK, precipitation causing moist soil conditions at the end of May, followed by warm still weather in late May/early June can lead to serious owbm outbreaks. The ovipositing female is a small insect which can remain well hidden in the crop canopy. The larvae are also hidden within the wheat ear, which is a difficult spray target. Thus to achieve effective control any insecticide application has to be applied promptly before larvae burrow in-between the lemma and palea.

A previous LINK project -LK0924 (Oakley *et al*., 2005) "Integrated control of wheat blossom midge: variety choice, use of pheromone traps and treatment thresholds" identified resistance and several sources of tolerance within elite UK plant breeding lines as well as developing pheromone traps with the potential to identify fields at risk. However, as resistance is largely restricted to feed wheat varieties many farmers selected midge tolerant and susceptible varieties to satisfy demand for higher quality markets. Also it is still unclear how best to use pheromone traps to predict owbm risk.

Therefore the major aim of the current project was to develop integrated pest management strategies for varieties resistant, tolerant and susceptible to owbm by using pheromone traps to determine the need for and timing of insecticide treatment, and to identify genes for resistance/tolerance for further breeding.

This was done by undertaking the following work packages.

A. Understanding basic female biology
   A1. Wind tunnel tests (Rothamsted)
B. Understanding and interpreting pheromone trap catches
   B1. Pheromone trap calibration study (ADAS, Rothamsted, TAG, Agrisense)
   B2. Female movement study (Rothamsted)
C. Biochemistry of tolerance and resistance
   C1. Biochemical study of model varieties (Rothamsted)
   C2. Screening of germplasm and development of markers (Breeders, JIC)
D. Development of model
   D1. Develop model (Rothamsted, Dow, ADAS, Agrisense)
   D2. Model verification study (Rothamsted, ADAS, TAG, Agrisense)

A. Understanding basic female biology

Wind tunnel tests

Female owbm flight behaviour under different abiotic conditions was investigated in a specialised flight tunnel facility. Flight still occurred when relative humidity was reduced to 50%. Optimal conditions for flight were 20-25^{o}C, 70% relative humidity and 0.2m/sec wind speed. Female owbm flew at higher light intensities than previously thought possible (>30 lux). This is perhaps because under field conditions humidity and light levels are closely associated with humidity dropping in bright sunlight. Humidity could have more of a limiting effect on owbm flight than light levels.

B. Understanding and interpreting pheromone trap catches

Pheromone trap calibration study

Field experiments were done between 2006 and 2008 to assess the variability of pheromone traps between fields. Standard commercial pheromone traps were used. Traps were sited in fields which had previously been cropped with wheat

and so provided a source of midge infestation (source fields), and in fields being cropped with wheat which were under risk of midge attack (sink fields). In some years at some sites, yellow sticky traps were used to give an indication of female midge activity. Trapping was done in Herefordshire, Lincolnshire, Norfolk, Hampshire, Cambridgeshire, East Yorkshire and North Yorkshire. Traps were set just before ear emergence and removed once the crop was in flower. Ear samples were also taken to assess levels of midge infestation.

In general, levels of midge infestation were low and much less than in the previous outbreak year of 2004. There was a high level of variation in trap catches between fields. Differences in catches were sometimes as high as a hundred fold between neighbouring fields. This emphasised the need to trap in individual fields rather than picking one or two fields to be representative of a whole farm. It also became clear that it was important to consider the potential for movement of mated females from source fields in which they emerged, to sink fields containing wheat at the susceptible growth stage.

Female movement study

This was investigated using 6 x 5 grids of traps with 30m trap spacing. Pheromone traps, specifically catching male owbm were paired with yellow sticky traps, catching much lower levels of both sexes, for comparison. Traps were put out when the first wheat reached growth stage 47 (flag leaf sheath opening) and catches were recorded twice a week. Pairs of pheromone and yellow sticky traps were also put out in the adjoining fields. At the end of the season infestation levels were assessed at each point in the grid. These studies showed that although there was some variation in trap catch across a field it was dwarfed in comparison to the variation observed between fields. Infestation levels in the crop were better explained by pheromone trap catches in neighbouring source fields than by considering variation in trap catch within the field (Figure 1).

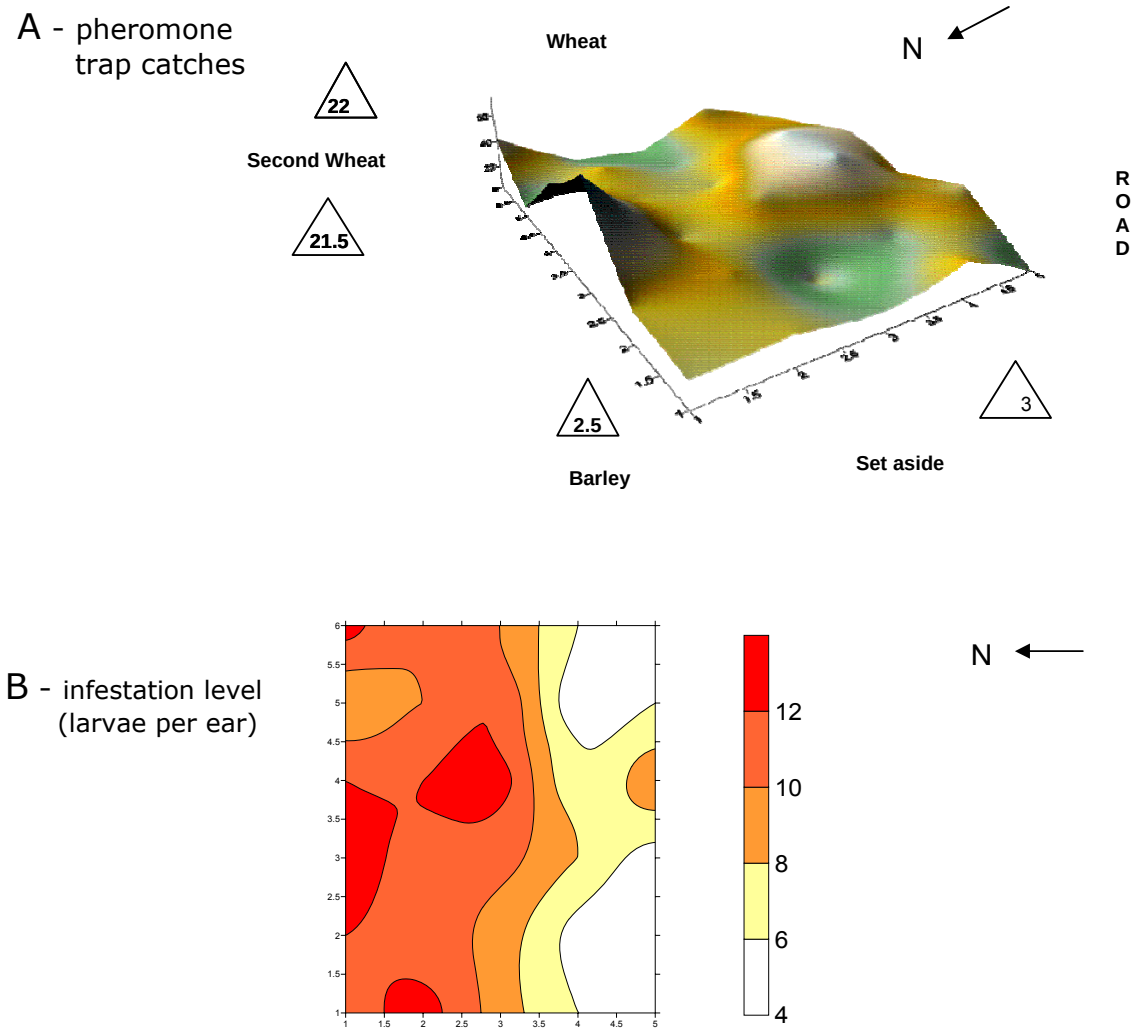


Figure 1. Relationship between (A) Pheromone trap catches within and around a wheat field during the susceptible growth period (catches in adjacent fields shown in triangles), and (B) Infestation level at the end of the season

C. Biochemistry of tolerance and resistance

Biochemical study of model varieties

A selected group of varieties with similar heading dates, but different susceptibility to owbm (Claire, ECO22, Einstein, Option, Tanker and Welford) were grown in a 6 x 6 quasi-complete Latin square design. In an additional trial, the insecticide chlorpyrifos was applied to one half of two split plots of each variety to estimate the yield loss associated with infestation. Trials were

conducted over three years (2006, 2007 and 2008). The activity of owbm was measured using pairs of pheromone traps and yellow sticky traps in a headland and a yellow sticky trap placed at the centre of each plot. In 2006, ECO22 had consistently higher levels of infestation and larval numbers than most of the other varieties, indicating a female owbm preference for this variety as seen in earlier olfactometer experiments with air entrainment samples. The resistant variety Welford had the lowest levels of infestation as expected, but there was no difference in the number of eggs laid by female owbm on this variety, compared to the others, suggesting that females do not recognise the resistance.

Analysis of phenolic acids in grain samples showed that levels of ferulic acid were higher in infested grain of Option, Welford, Einstein and ECO22 compared to uninfested grain, but there was no difference or a slight decline in levels in infested Claire and Tanker. Levels of *p*-coumaric acid were greater in the infested than in the uninfested samples of all the varieties tested indicating that owbm damage is inducing production of this acid in the seed. Although infested Welford had the highest level of *p*-coumaric acid the level of induction was insufficient to explain the big difference in owbm larval survival in Welford compared to the other varieties. This suggests that there might be another mechanism of owbm resistance.

Screening of germplasm and development of markers

Varietal variation for resistance to owbm has been observed in material from different countries, including Canada and the UK. However, there have been very few studies of the genetics of these resistance sources. The most significant demonstrated that resistance in Canadian material was conditioned by a single major gene, termed *Sm1*, on wheat chromosome 2B. Additionally, a PCR based molecular marker was developed, called *Wm1*, which was linked to the resistance gene and could be used for marker assisted selection in crosses involving the resistance source. However, there is no information on whether UK and European sources of resistance carry *Sm1* or whether there are other, independent, genes.

Therefore the objectives of the present work were to:

1. To study if *Sm1* is present in UK sources of owbm resistance
2. If *Sm1* is present, to test the utility of the *Wm1* molecular marker in identifying and tagging resistance in UK crosses
3. To identify if there are other independent genes for WOBM resistance in UK wheat germplasm.

To look at the inheritance of owbm resistance in UK material, three crosses were made between varieties/lines of high (S) and low (R) susceptibility to owbm. The three crosses were:

1. WP071 = Acess(S)/Welford(R)
2. WP151 = Brompton(R)/PBI01-0091(S)
3. WP158 = NSL WW57(S)/Carlton(R)

The F_1s of the crosses were selfed to produce F_2 seed and a sample of each of 100 individual F_2 plants were germinated and grown to maturity to produce F_3 families. The F_3 families and their subsequent bulked F_4, and F_5 generations were used in the owbm trials described below.

Three years of field trials were done to phenotype the three crosses. Ear assessments were also done to assess the level of midge infestation in each line. To test the utility of the *Wm1* molecular marker in detecting the presence of the *Sm1* gene in the parents of the crosses, the known owbm susceptible and resistant parental varieties were tested with the *Wm1* marker using primer sequences supplied by Canadian workers.

Based on the phenotyping scores, 14 lines with the highest owbm scores (Susceptible lines) and 14 lines with none or very few midges (Resistant lines) were chosen from each of the crosses for phenotypic extreme analysis. DNA samples from these 84 lines, plus the parents, were subjected to Diversity Arrays Technology (DArT) molecular marker analysis.

Based on the DArT results, putative regions of the wheat genome for each of the three crosses which were associated with the R/S divergence were identified. Simple Sequence Repeat (SSR) markers known to locate in these regions from wheat consensus genetic maps were then identified and screened for polymorphisms for mapping on the whole 100 lines of each of the populations so that QTL analysis could be carried out to confirm if the individual regions were correlated with the S/R polymorphism.

The DArT and SSR analysis has identified several genetic effects that contribute to the resistance of the lines Welford, Brompton and Carlton. The major effect is *Sm1*, but other genes are also involved, particularly the large effect of 3B in the PBI01/009 x Brompton cross.

The mechanism of *Sm1* resistance is thought to be chemical, but the effect of other chromosomes e.g. 3B, could be related to escape mechanisms associated with a difference in flowering time. If varieties were to flower early they could potentially avoid midge migration.

D. Development of the model

Develop model

The observations of variability in trap catch, and how it related to subsequent infestations, were very relevant when deciding how best to use the traps for owbm risk assessment and were used to develop a decision support model. This model is a distillation of some complicated data obtained over the project but has been framed in terms of what it means for the farmers when using the traps. With this in mind it has been kept as simple and user-friendly as possibly being based on a stepwise decision tree involving yes/no answers to questions (Figure 2).

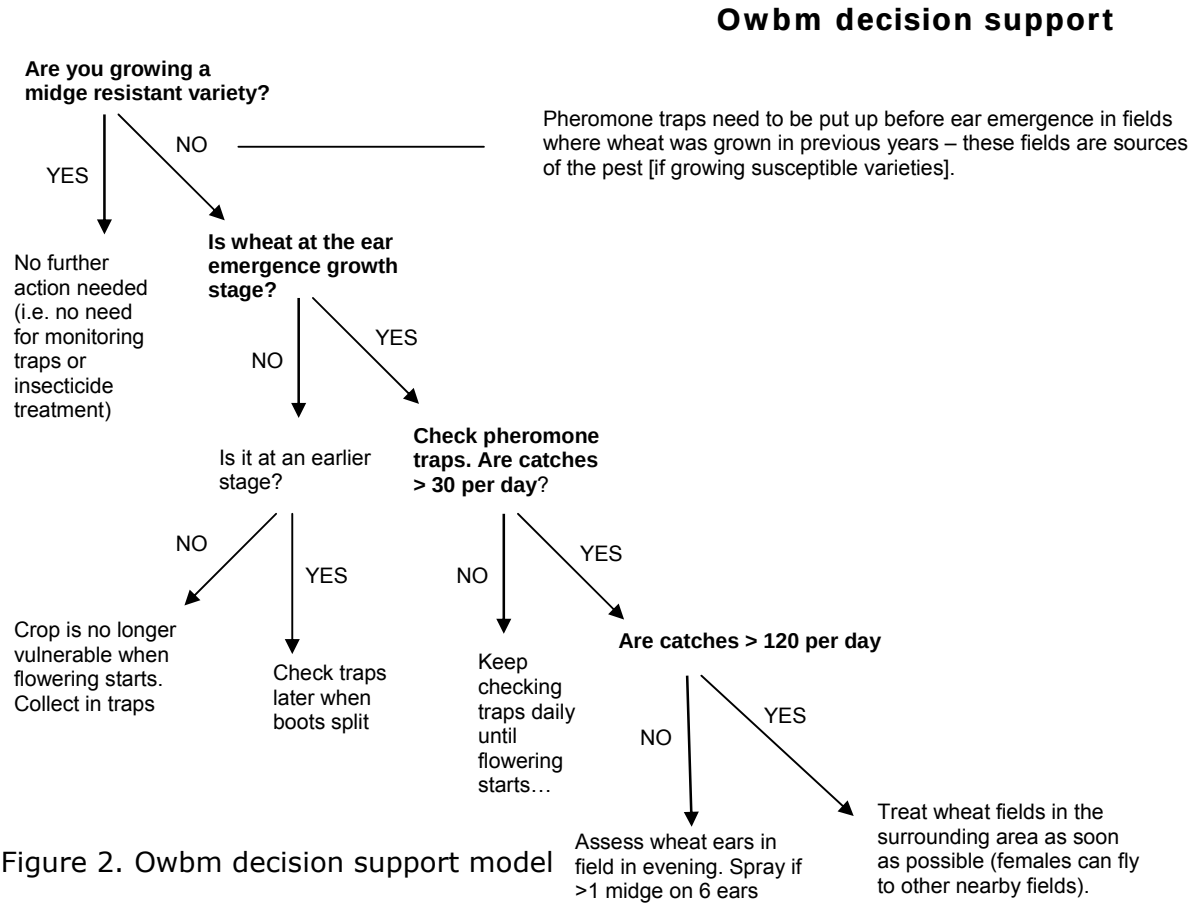


Figure 2. Owbm decision support model

Model verification study

Model verification was done at 26 sites. Male owbm numbers were monitored using pheromone traps and ear samples taken to assess the ultimate level of midge larvae infestation. In general, levels of owbm infestation were relatively low throughout the monitoring exercise and much lower than those recorded during 2004, the year of the last major outbreak of the pest. Trap catches showed that on no occasion when fewer than 30 midges/trap/day were recorded in a sink field was grain damage above the 5% threshold for seed and milling varieties. On 60% of occasions when the 120 midges/trap/day threshold was exceeded an insecticide spray was justified.

Key findings

A. Understanding basic female biology

A1. Wind tunnel tests

- Owbm flight under controlled laboratory conditions was shown to depend on humidity levels more than on light intensity

B. Understanding and interpreting pheromone trap catches

B1. Pheromone trap calibration study

- There can be large variations in trap catch from field to field
- In some years there is a good correlation between trap catch and crop damage level
- Movement of females between fields can complicate the relationship between trap catch and damage levels
- Trapping in non-wheat source fields or wheat crops can be a good indicator of owbm risk
- Traps are best sited in fields which have been damaged by owbm in the last two years, irrespective of crop

B2. Female movement study

- Infestation within a field was best explained by pheromone trap catches in neighbouring fields

C. Biochemistry of tolerance and resistance

C1. Biochemical study of model varieties

- Welford was highly resistant to larval attack although female owbm were still attracted to it and laid eggs on it
- There was evidence of induction of phenolic acids in infested seed from some varieties, but levels of these acids did not fully explain the resistance in Welford

C2. Screening of germplasm and development of markers

- The major gene influencing owbm resistance in UK varieties is *Sm1*
- Other chromosomes may also influence resistance such as 3B. The effect of this could be related to early flowering to escape midge infestation.

D. Development of model

D1. Develop model

- A simple decision flow chart was developed to provide a stepwise procedure to assessing owbm risk.

D2. Model verification study

- Low levels of midge infestation hindered model verification.
- Proposed thresholds are a good basis for predicting risk
- Further validation is required to improve risk prediction

Technical detail

Introduction

The orange wheat blossom midge (owbm), *Sitodiplosis mosellana*, is a common and increasingly important pest of wheat in the Northern Hemisphere, causing severe yield losses in years of high infestation. For example, in 2004, when wheat prices were about £60 per tonne, an outbreak in the UK was estimated to have caused crop losses in excess of £60 million. Larval feeding on the developing seeds causes shriveling and pre-sprouting damage and also facilitates secondary fungal attack by *Fusarium graminearium* and *Septoria nodorum* (Oakley, 1994). This affects both the yield and quality of grain harvested. Due to difficulties in detection of owbm the degree of damage to crops is often underestimated. The sex pheromone of owbm has been identified (Gries *et al.* 2000) and a pheromone trap system for monitoring the pest was developed in the previous LINK project - LK0924 (Oakley *et al*., 2005).

Owbm has a very patchy spatial distribution and infestations vary from year to year depending on climatic conditions. Owbm larvae hibernate in the soil and each spring a proportion develop and pupate. It is possible for larvae to hibernate for several years if conditions are unfavourable for development of adult midges (Barnes, 1956). Adult owbm mate at the emergence site and females fly in search of a wheat crop at the ear emergence growth stage on which to lay their eggs (Oakley *et al.*, 1998). In the UK, precipitation causing moist soil conditions at the end of May, followed by warm still weather in late May/early June can lead to serious owbm outbreaks. The ovipositing female is a small insect which can remain well hidden in the crop canopy (Lamb *et al.,* 2002, Pivnick & Labbe, 1993). Eggs take approximately 4-10 days to hatch depending on the temperature. The larvae feed on the grain and being well hidden within the wheat, ear are a difficult spray target. Any insecticide application has to be applied promptly before larvae burrow in-between the lemma and palea or it will not give good control. As midges are difficult to detect it is hard to predict when infestations that would warrant insecticide treatment have built up and there is considerable grower demand for a reliable monitoring system.

The LINK project - LK0924 (Oakley *et al*., 2005) "Integrated control of wheat blossom midge: variety choice, use of pheromone traps and treatment thresholds" exceeded expectations by identifying resistance and several sources of tolerance within elite UK plant breeding lines. In addition, pheromone traps were developed which had the potential to identify fields at risk from owbm. Currently the resistant material is restricted to feed wheat varieties and will not be fully available in the short-term due to the need to scale up stocks. To satisfy demand, particularly for higher quality market requirements, tolerant and susceptible varieties will still be the necessary choice of many farmers. Owbm which caused widespread damage and much insecticide use in 2004, continues to be a major and repeated threat, although risks vary between season, locality and individual crop. A longer term breeding goal is to introduce resistance into milling wheat.

The overall aim of this project is to provide sustainable control of the problem by utilising resistant and tolerant varieties in high risk situations and understanding how best to use pheromone traps in a monitoring system to help manage susceptible crops. A robust integrated control strategy will reduce the direct economic and indirect environmental impacts of this increasingly important pest. Alongside this is a need to understand, in more detail, the genetic control of resistance/tolerance with a target of marker-assisted selection for key genes in the next generations of varieties.

Therefore the major aim of the project was as follows. To develop robust integrated pest management (IPM) strategies for farmers using varieties resistant, tolerant or susceptible to owbm, by establishing new technologies for risk assessment and the use of pheromone traps to determine need for and timing of insecticide treatment, and to identify genes for resistance/ tolerance for further breeding.

Building on LK0924 will provide a basis for plant breeders to solve the problem, for milling as well as feed varieties, in the longer term. Owbm is now a recurrent and very destructive pest against which insecticides are routinely used. Therefore a short to medium term solution is also necessary for control of the

pest, by developing IPM strategies based on pest monitoring and rational pesticide use for the more susceptible varieties which are still needed to meet market demands.

The main objectives of the project were:

1. To characterise the biotic, abiotic and landscape factors influencing movement and egg laying of female owbm so as to identify the number and disposition of pheromone traps needed to estimate crop risk on a farm scale.
2. Develop a greater understanding of the genetics of resistance/tolerance and its biochemical basis.
3. Prioritisation of needs for and timing of insecticide treatment taking into account owbm risk and varietal tolerance.

These objectives have been met by completing a series of tasks or work packages which are listed below. Different members of the consortium contributed to different work packages and this information is also indicated.

Tasks or work package

A. Understanding basic female biology
   - A1. Wind tunnel tests (Rothamsted)

B. Understanding and interpreting pheromone trap catches
   - B1. Pheromone trap calibration study (ADAS, Rothamsted, TAG, Agrisense)
   - B2. Female movement study (Rothamsted)

C. Biochemistry of tolerance and resistance
   - C1. Biochemical study of model varieties (Rothamsted)
   - C2. Screening of germplasm and development of markers (Breeders, JIC)

D. Development of model
   - D1. Develop model (Rothamsted, Dow, ADAS, Agrisense)
   - D2. Model verification study (Rothamsted, ADAS, TAG, Agrisense)

The main body of this report will be divided into sections dealing with each of the 'Tasks or Work packages' in turn.

Task A Understanding basic female biology

A1. Wind tunnel tests

T J A Bruce, L E Smart, J A Martin - Rothamsted Research

The LINK project – LK0924, "Integrated control of wheat blossom midge: variety choice, use of pheromone traps and treatment thresholds" (Oakley *et al*., 2005), identified resistance and several sources of tolerance within elite UK wheat breeding lines as well as developing pheromone traps (Bruce *et al*., 2007) with the potential to identify fields at risk. Sex pheromone traps provide a solution to the detection problem and enable more accurate and effective spray timing. Our objective in the current project was to develop further the potential of the pheromone traps for monitoring this pest in order to predict crop risk accurately and, where insecticides are justified, to target their use effectively.

Introduction

Female owbm flight behaviour under different abiotic conditions was investigated in a specialised flight tunnel facility. In particular, the impact of varying light intensity and humidity were studied. For these experiments midges were reared from soil collected from fields known to have suffered from severe owbm damage.

Materials & Methods

Insect rearing

Soil samples were taken in the autumn after crop harvest from sites with severe owbm damage. These contained owbm larval cocoons. The samples were transferred to shallow seed trays and stored at 5°C. After at least three months vernalisation, trays were moved to a cabinet (22°C, 75%RH, 16:8 Light:Dark) and watered, to bring adult owbm out of diapause. Midges were then available for experiments all year round rather than just in May-June as would have been the case if adults were collected when they emerged in the field.

Wind-tunnel bioassay

A specialised Perspex wind tunnel (dimensions 90 × 30 × 30 cm) was used to investigate owbm flight under controlled but variable conditions. Parameters that were varied were light intensity (85 – 800 lux) and humidity (35 – 70% R.H.). Temperature was 25±1^{o}C and wind speed 0.2 m / s. A panicle of an attractive wheat variety, 'Tanker', was positioned at the upwind end of the tunnel to provide stimulus for the midges. Aluminium plates were fitted on either side of the stem to enable the use of a live wheat plant rather than a cut one. Mated female owbm were released individually and observed over a 20 minute period. The maximum distance flown upwind by each insect was recorded.

Results

Optimal conditions for flight were 20-25^{o}C, 70% relative humidity and 0.2m/sec wind speed. Flight still occurred when relative humidity was reduced to 50% but was significantly reduced when it was reduced to 35% (unpaired *t*-test comparing means: $P = 0.024$) (Figure 3). Female midges flew at higher light intensities than previously suspected (Figure 4) and there was an indication that there was more orientated upwind flight at higher light intensity although this trend was not significant. It has been observed that under field conditions humidity and light levels are closely associated with humidity dropping in bright sunlight. Humidity could have more of a limiting effect on owbm flight than light levels.

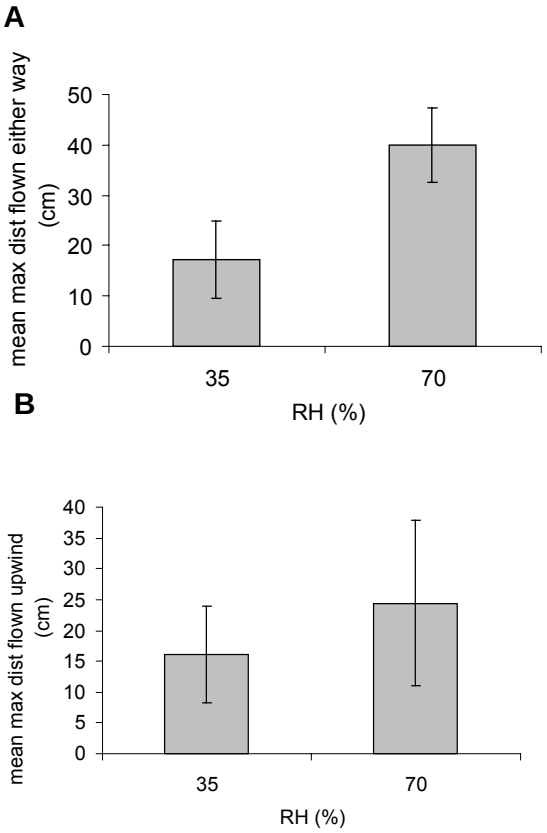


Figure 3. Effect of humidity on *S. mosellana* flight (A) in either direction and (B) orientated upwind (n = 9)

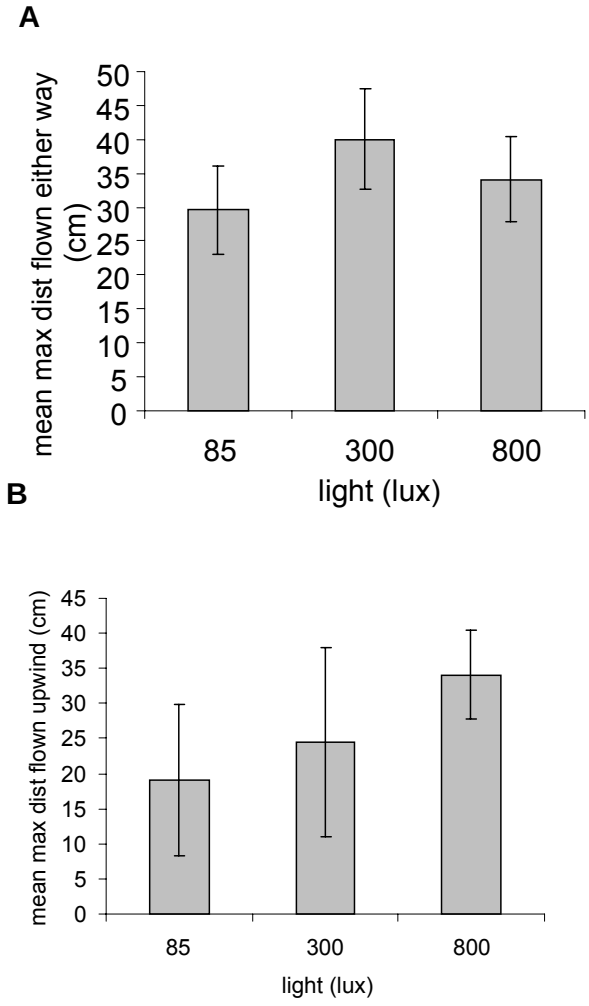


Figure 4. Effect of light intensity on *S. mosellana* flight (A) in either direction and (B) orientated upwind (n = 9)

Discussion

There was a significant effect of humidity on owbm flight but light intensity had less of an impact. Flight still occurred when relative humidity was reduced to 50% but was significantly reduced when it was reduced to 35%. It has been observed that under field conditions humidity and light levels are closely associated with humidity dropping in bright sunlight. Humidity could have more of a limiting effect on owbm flight than light levels.

Task B Understanding and interpreting pheromone trap catches

B1. Pheromone trap calibration

T J A Bruce, L E Smart, J A Martin - Rothamsted Research
J N Oakley, S A Ellis – ADAS
M M Self – The Arable Group TAG

Introduction

Field Experiments were conducted to assess the variability of pheromone trap catches between fields. Standard commercial pheromone traps supplied by Agrisense (Pontypridd, Mid-Glamorgan, UK) (www.agrisense.co.uk) were used in all experiments as these are the ones used by farmers and developed in the previous LINK project. Traps were sited in fields which had previously been in wheat and so provided a source of midge infestation (source fields) and also in fields being cropped with wheat which were under risk of midge attack (sink fields). In some years at some sites yellow sticky traps were also used to give an indication of female midge activity in sink fields.

Materials & methods

Rothamsted Research

Two pheromone traps and two yellow sticky traps were put out at various sites around Rothamsted farm to investigate spatial variability in pest distribution, calibrate traps with subsequent infestation levels and investigate differences between distributions of males (caught with pheromone traps) and females (caught with yellow sticky traps). The trapping points consisted primarily of wheat fields but also included sites in other crops that followed wheat in the rotation and set-aside fields. GPS co-ordinates of each trapping location were recorded to enable spatial mapping of trap catch data. Traps were put out when the first wheat on the farm reached growth stage 47 and catches were recorded twice a week. At the end of the season infestation levels were assessed at the

milky ripe growth stage at each wheat site. The following sites were monitored between 2005 and 2008.

2005: 18 sites comprising: 11 wheat fields, four set-aside fields and three fields of other crops.
2006: 13 sites comprising: nine wheat fields, one set-aside field and four fields of other crops.
2007: 17 sites comprising: 10 wheat fields, one set-aside field and six fields of other crops.
2008: 15 sites comprising: 12 wheat fields and three fields of other crops.

A line of ten emergence traps were used in 2006, 2007 and 2008 to monitor the exact timing of emergence and the sex ratio of owbm at one site on Rothamsted farm. The trap consisted of a circular, metal cone skeleton (ground area $0.5m^2$) covered with fine black netting that allowed rain to penetrate. A clear plastic collection cup, containing 70% ethanol preservative, was mounted at the top. The traps were partially embedded in the soil and emerging insects moved up towards the light and were captured in the collection cup. Two pairs of pheromone traps and yellow sticky traps were also deployed at the same site. Traps were changed twice weekly.

Meteorological data recorded by Rothamsted weather station were used to help interpret owbm emergence and distribution. Conditions including air and soil temperature, rainfall, soil moisture and wind strength for each year were compared to those of 2004, the year of the last serious outbreak.

ADAS
Studies were conducted in 2006, 2007 and 2008 to monitor owbm incidence. The work was conducted at High Mowthorpe in all three years, Boxworth in 2007 and 2008 and Grindale in East Yorkshire in 2008 only.

In 2006 a total of 22 fields were selected across High Mowthorpe at which to monitor owbm. A total of 10 of these were in susceptible wheat varieties and potential sink fields. The other 12 were in a range of non-wheat crops but had

been wheat in the previous two years and so potentially provided a source of owbm. During the monitoring exercise all fields received the standard herbicides, fungicides, nitrogen and growth regulators but no summer insecticides.

Monitoring was done using pheromone traps and yellow sticky traps. Traps were first located in fields on 8 June, 2006 with crops at about GS 49. Traps were located at least 25 m into the crop at a convenient point of access. There were two pheromone traps and two sticky traps in each field. These were arranged along a diagonal line with alternate pheromone and sticky traps. Traps were at least 5 m apart and were supported on a fibreglass pole at crop height and were moved up the pole as the crop grew. Traps were inspected at intervals of between one and four days and the number of both male and female owbm recorded. Owbm numbers were monitored until crops reach GS61. At GS71 a total of 25 randomly sampled ears were collected from each wheat field. These were dissected and the number of owbm larvae recorded. If ywbm were present their numbers were also noted.

In 2007 and 2008 owbm were monitored at a number of sites. In 2007 six fields were monitored at High Mowthorpe and six at Boxworth. At each site three wheat (sink) fields were selected and three non-wheat (source) fields. Owbm numbers were again monitored using pheromone and sticky traps and wheat ears sampled to determine the level of pest infestation. In 2008 six fields were monitored at High Mowthorpe as in 2007 and a further six fields at Grindale, East Yorkshire, a site which had experienced owbm problems in the past. At both sites three source and three sink fields were again selected. At Boxworth four fields were monitored, two source and two sink fields. Only pheromone traps were used in 2008. Ears were also sampled to assess levels of midge infestation. A list of all monitored fields at each site is given in Tables 1-3.

Table 1. Fields monitored for owbm at High Mowthorpe 2006-2008. (All sink fields were winter wheat, the crop for each source field is given.)

2006			2007			2008		
Source	Crop	Sink	Source	Crop	Sink	Source	Crop	Sink
Stonechair	Organic spring beans	Wether Plain	Wether Plain	WOSR	Stonechair NE	Stonechair NE	W Wheat	Wether Plain
Stonechair NE	WOSR	Smithfield	Smithfield	W Barley	Homefield	Homefield	WOSR	Front Field
Old Type	W barley	Office Field W	Kirby Grass	S barley	Tommy Ireland	Tommy Ireland	W barley	Crow Tree
Malton Road	S wheat	Home Field						
Office Field	WOSR	Front Field E						
Elbow North	WOSR	Crow Wood						
Bugdale	S barley	Crow Tree						
Front Field West	W barley	Kirby Field NE						
Warren	WOSR	Kirby Grass N						
Duggleby Side	Organic spring beans	Kirby Grass S						
Kirby Field W	W barley							
Tommy Ireland	Set-aside							

Table 2. Fields monitored for owbm at Boxworth 2007 and 2008. (All sink fields are winter wheat, the crop for each source field is also given.)

2007			2008		
Source	Crop	Sink	Source	Crop	Sink
Sykes	W wheat	Long field	Side Hill	W beans	Knapwell
Gow Leys	W beans	40 Acres	40 Acres A	WOSR	40 Acres S
Childerley	WOSR	Pamplins S			

Table 3. Fields monitored for owbm at Grindale, E Yorkshire in 2008. (All sink fields are winter wheat, the crop for each source field is also given.)

2008		
Source	Crop	Sink
Cottage Field	W barley	White Dyke
West Hill	W barley	Argham Gates
West Field	W barley	Chalk road

TAG

A study was conducted in 2007 and 2008 to monitor owbm incidence by measuring the numbers of males in pheromone traps in potential source and sink fields. The study was conducted at three farms with mixed cropping across England in Lincolnshire, Norfolk and Hampshire. These farms had a known history of owbm infestation and wheat crops included a mixture of varieties susceptible and resistant to owbm.

In each year six fields were selected on each farm, three first or subsequent wheat crops, preferably drilled with a susceptible variety and three potential source fields for owbm. The source fields were identified as those which were close to the selected wheat fields and were growing a crop other than wheat, and ideally had been infested by owbm in recent years.

During the study all fields received normal husbandry treatments of herbicides, nitrogen, growth regulators and autumn applied insecticides. Summer

insecticides were not applied on any of the farms throughout the duration of the study.

Trapping of adult owbm was done using pheromone traps supplied by Agrisense (with a lure of owbm female sex pheromone to attract male adults) and yellow sticky traps (non selective trapping) supplied by Oecos.

Traps were positioned in each monitored field from the end of May, or when the first wheat crop reached GS51 (first spikelets visible), whichever was the sooner and left in place until the latest developing wheat crop reached GS 59 (full ear emergence). The sticky inserts and yellow sticky traps were changed a minimum of three times per week, the exact interval between trap servicing depended upon the level of infestation.

The traps were located at least 25m into the crop at a convenient point for access. Two pheromone and two sticky traps were placed in each field in a diagonal line comprising pheromone trap, yellow sticky trap, pheromone trap and yellow sticky trap, each 5m apart from the other. Traps were placed with their top at canopy height, and were moved up the holding cane to maintain this position as the crop grew. An Ordnance Survey map reference was noted for the position of each set of traps.

Numbers of male owbm on the pheromone trap inserts and male and female owbm on the yellow sticky traps were recorded on each occasion the traps were changed. Each time the traps were changed in the wheat fields the percentage emergence of 25 ears of wheat was assessed and recorded. Ears were selected at random, these data represented the range of growth stages present in the crop.

Samples of 25 wheat ears per trapping area were collected at GS 71 (grain watery ripe), or just before if heavy rain was forecast. The ears were dissected under a binocular microscope to establish the numbers of owbm and yellow wheat blossom midge (ywbm) eggs and larvae present. The number of fertile grain sites was also recorded to enable an estimate of larvae per 100 grains per ear.

Results

Rothamsted Research

2005

Two pairs of pheromone and yellow sticky traps were put out at each of 18 sites around Rothamsted farm when the first wheat reached growth stage 47 (23 May) and catches were recorded twice a week. Infestation levels were assessed on 25 ears at each wheat site on 4 July. There was considerable variation in pheromone trap catch from site to site (Figure 5). For example on the 10th June a mean of 0.5 midges was caught at the New Zealand field site whereas a mean of 85 midges was caught at the nearby Long Hoos field.

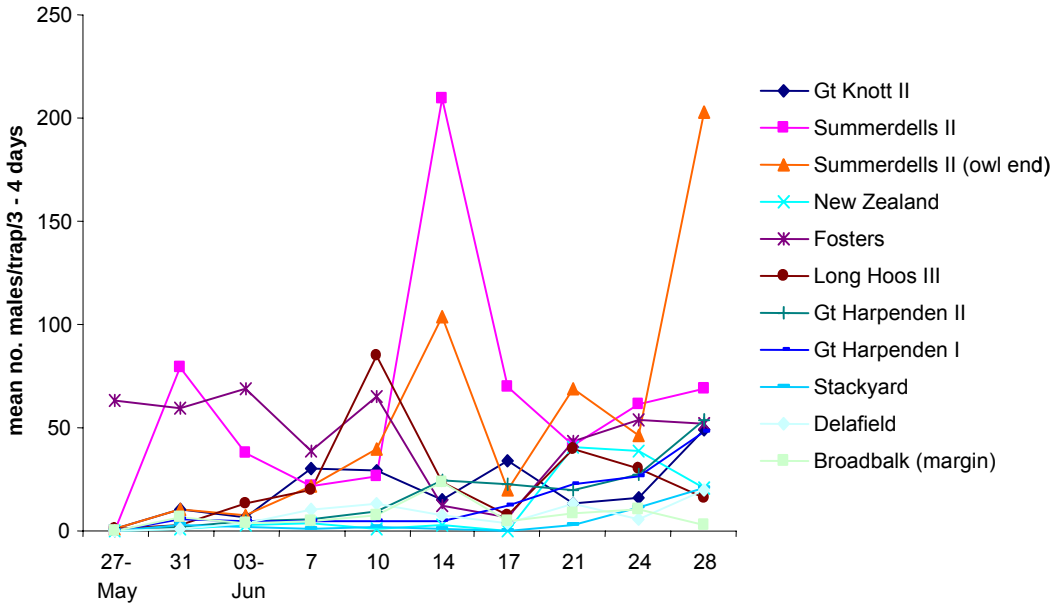


Figure 5. Pheromone trap catches at wheat sites in 2005

Trap catch variations from field to field were one to two orders of magnitude. At most of the sites numbers of males on the pheromone traps and females on the yellow sticky traps appeared to be linked. However, there were two sites (Stackyard and New Zealand) where females appeared before males (data not shown).

GPS co-ordinates were used to map trap catches across the farm and pheromone and yellow sticky trap catches for 7th June and 10th June are shown in Figures 6 and 7. These were the critical dates when most wheat crops were at the susceptible growth stage. A discrepancy between male and female numbers on respective traps was observed, which suggested that there could be movement of females from emergence sites to wheat fields. Four of the sites where there appeared to be female immigration (Delafield, Great Knott, New Zealand and Great Harpenden 2) were sown with the owbm resistant variety Robigus leading us to suspect this variety may be very attractive to female owbm, since other varieties were at the susceptible growth stage at the same time.

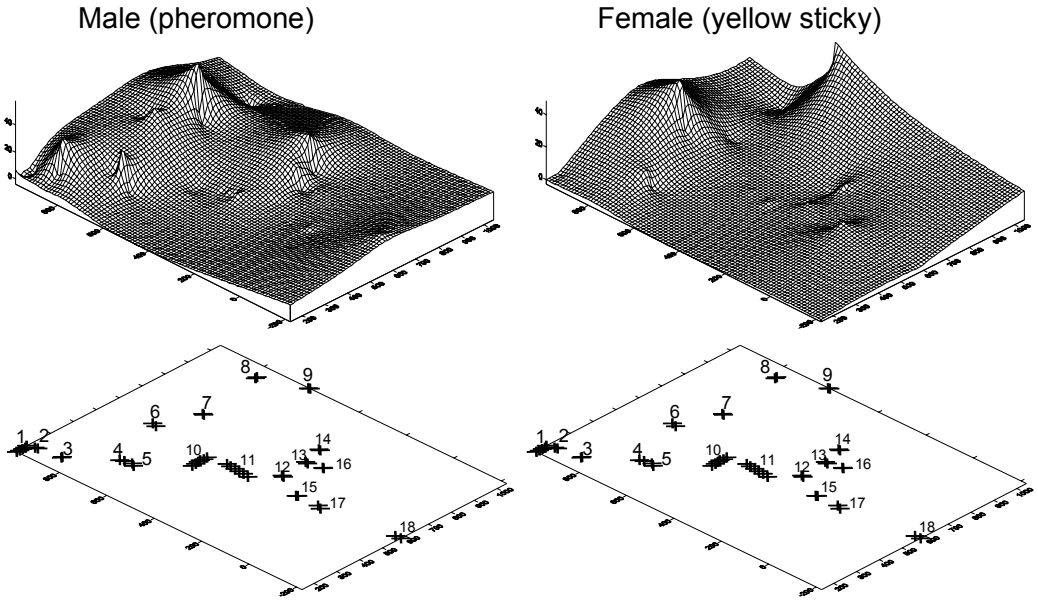


Figure 6. Whole farm trap catches 07/06/05 1=Whitehorse I, 2=Whitehorse II, 3=Summerdells II (by Owl Box), 4=Summerdells II (by Appletree), 5=Appletree, 6=Great Knott, 7=Fosters, 8=Whitlocks, 9=Delafield, 10=Bones Close, 11=Broadbalk, 12=Great Harpenden I, 13=Little Hoos, 14=Long Hoos, 15=Stackyard, 16=Great Harpenden II, 17=New Zealand; 18=Delharding

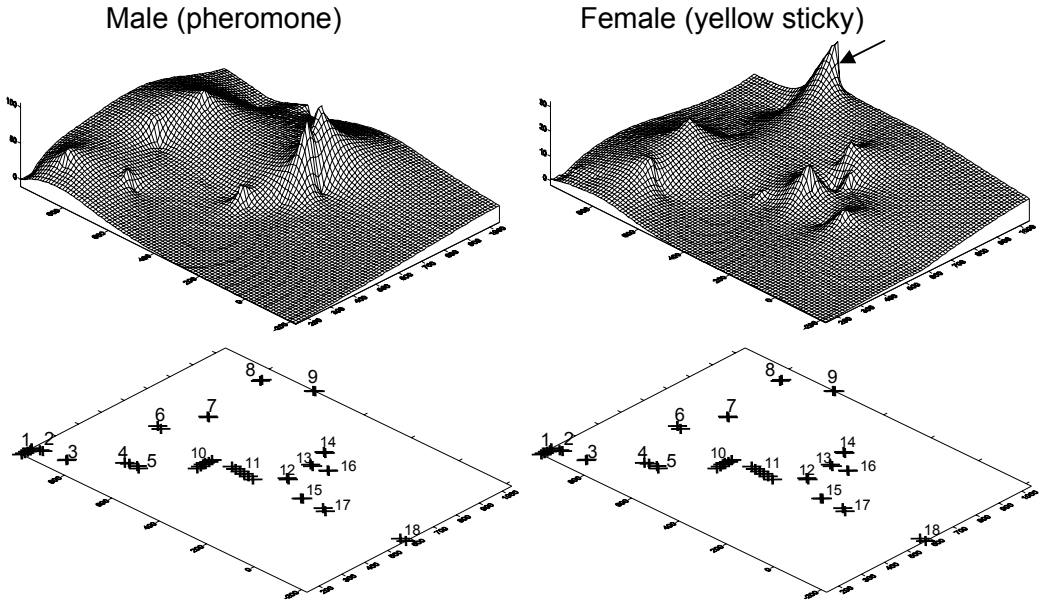


Figure 7. Whole farm trap catches 10/06/05 1=Whitehorse I, 2=Whitehorse II, 3=Summerdells II (by Owl Box), 4=Summerdells II (by Appletree), 5=Appletree, 6=Great Knott, 7=Fosters, 8=Whitlocks, 9=Delafield, 10=Bones Close, 11=Broadbalk, 12=Great Harpenden I, 13=Little Hoos, 14=Long Hoos, 15=Stackyard, 16=Great Harpenden II, 17=New Zealand; 18=Delharding

There was only a weak correlation between peak pheromone trap catch during the susceptible growth period and subsequent infestation level (r^2=0.22) and between yellow sticky trap catch during the susceptible growth period and subsequent infestation level (R^2=0.10) (Figures 8 & 9). This contrasts with 2004 when there was a significant correlation between peak pheromone trap catch and subsequent infestation (R^2=0.03).

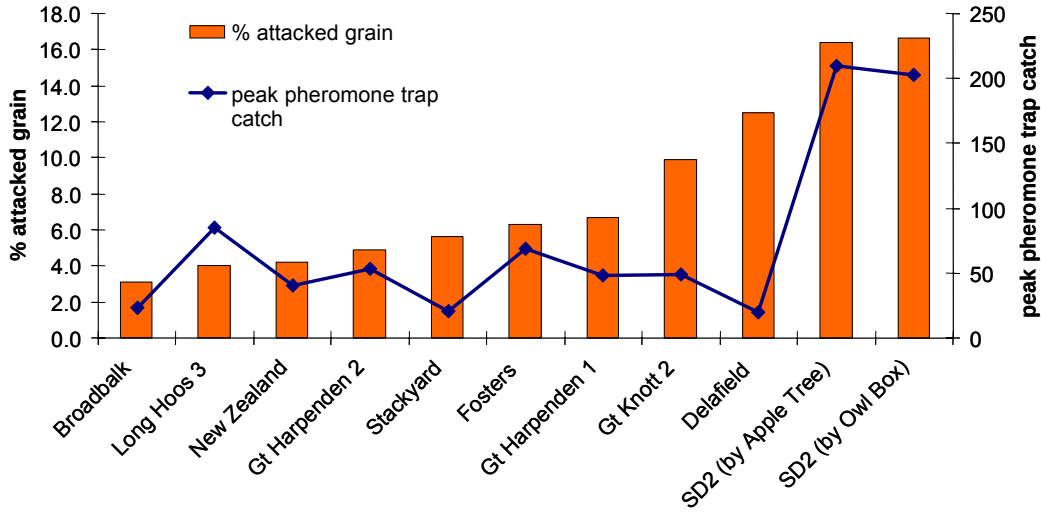


Figure 8. Peak pheromone trap catch and subsequent infestation level at the different sites 2005

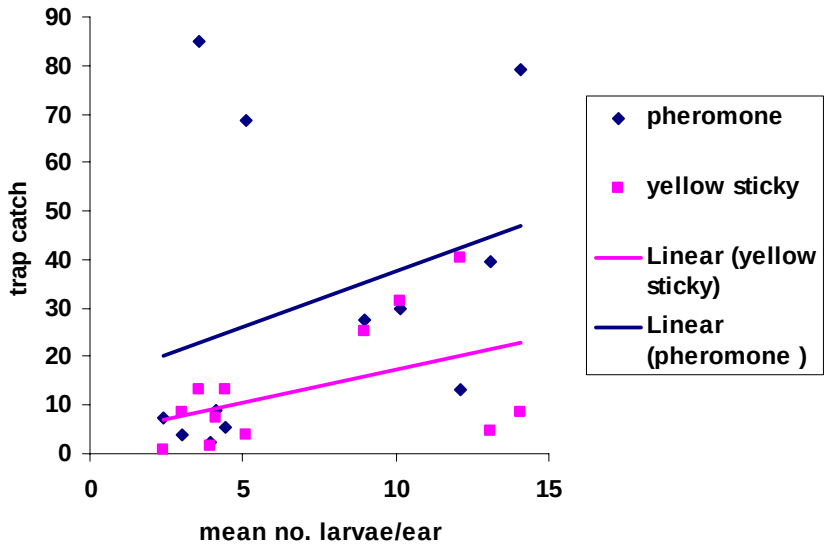


Figure 9. Peak pheromone trap catch and subsequent infestation level 2005

At most sites the peak pheromone trap catch for the whole season occurred when the wheat was past the susceptible growth stage, but for setting the economic threshold the peak catch during the susceptible period is more relevant. The two Summerdells sites (SD2 in Figure 8) had 16% attacked grain, a level of attack which was above the threshold. The mean (n=4) pheromone trap peak catch during the susceptible growth stage of the crop was 60 midges over a 4 day period (i.e. 15 midges per trap per day). However, pheromone trap

catches at Delafield, which also had a high level of attack (12.5% attacked grain), were considerably lower than this. The mean pheromone trap peak catch during the susceptible growth stage at Delafield was only 10 midges over a four day period. This is below the preliminary threshold for the traps of 20-30 midges over a three day period in the susceptible growth stage. We have good evidence that there was emigration of females to this site because of the high numbers caught on the yellow sticky traps. Another factor that could have influenced this anomalous finding is that secondary tillers lower down in the crop entered the susceptible growth stage later and thus were exposed to more owbm.

In terms of the impact of meteorological conditions, although soil moisture levels were favourable, weather conditions in the 2005 season were colder than usual, which delayed the emergence of owbm. Furthermore, the windy conditions at the time of emergence meant that their movement was impeded. As a consequence, although the pheromone traps indicated that owbm had emerged, the time of arrival of many of the egg-laying females in the crop was not synchronised with the susceptible growth stage. This explains why there was a poorer correlation between pheromone trap catches and subsequent infestation than in 2004.

2006

In 2006, two pairs of pheromone and yellow sticky traps were put out at each of 13 sites around Rothamsted farm when the first wheat reached growth stage 47 (24 May). Catches were recorded twice a week and infestation levels were assessed at each wheat site during the milk grain growth stage on 27 June. As found in previous years there was considerable variation in trap catch from field to field (Figure 10). The peak owbm emergence was late and trap numbers were below the suggested threshold in most fields during the ear emergence period (30 May to 6 June).

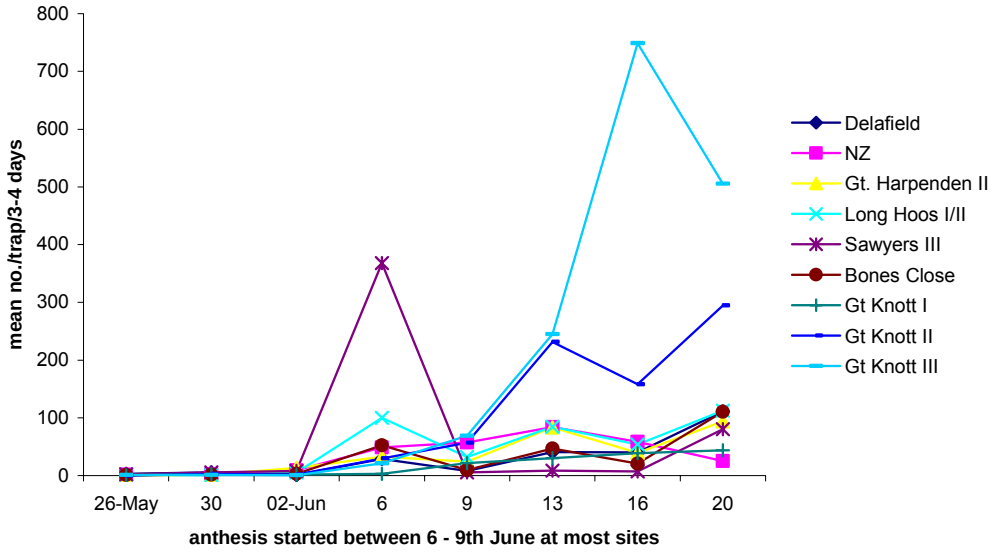


Figure 10. Pheromone trap catches on wheat sites 2006

GPS co-ordinates were used to map trap catches and infestation levels across the farm. Pheromone and yellow sticky trap catches for 2 June are shown in Figures 11 and 12 and infestation levels in Figure 13. Pheromone trap catches were approximately

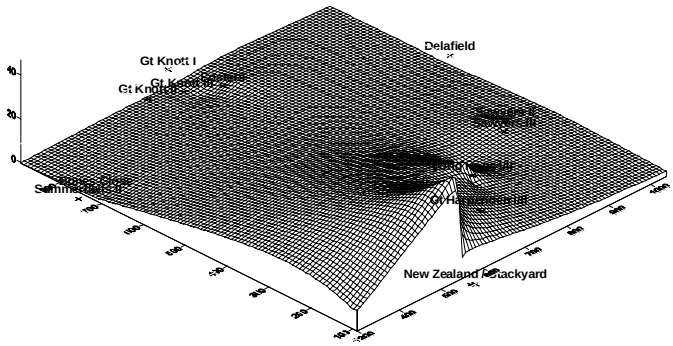


Figure 11. Pheromone trap catches across the farm (2 June 2006)

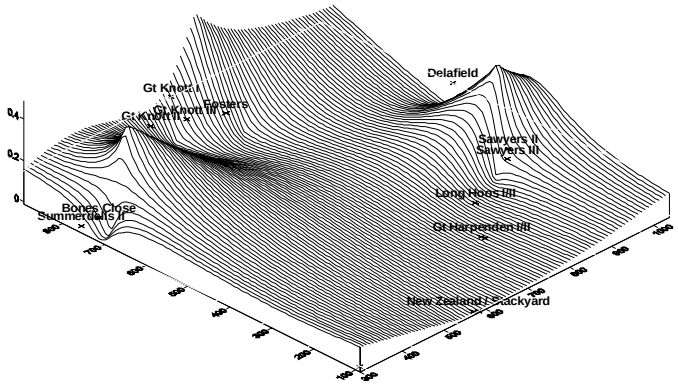


Figure 12. Yellow sticky trap catches across the farm (2 June 2006)

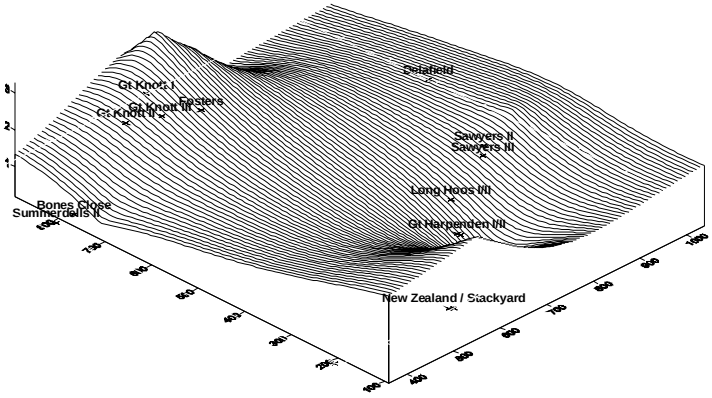


Figure 13. Percentage infested grain (27 June 2006)

100 times higher than yellow sticky trap catches and provided a better indication of subsequent infestation levels at the New Zealand site. However, pheromone trap catches were less predictive of the levels of infestation at the Great Knott fields (Figure 14), but this was not a serious problem because infestation was below the economic threshold with levels lower than had been recorded for the last five years.

Yellow sticky trap catches on 30 May did indicate infestation at the Great Knott fields (Figure 12). Other than this there was no obvious evidence of the large-scale female movement between fields in 2006, which had occurred in 2005.

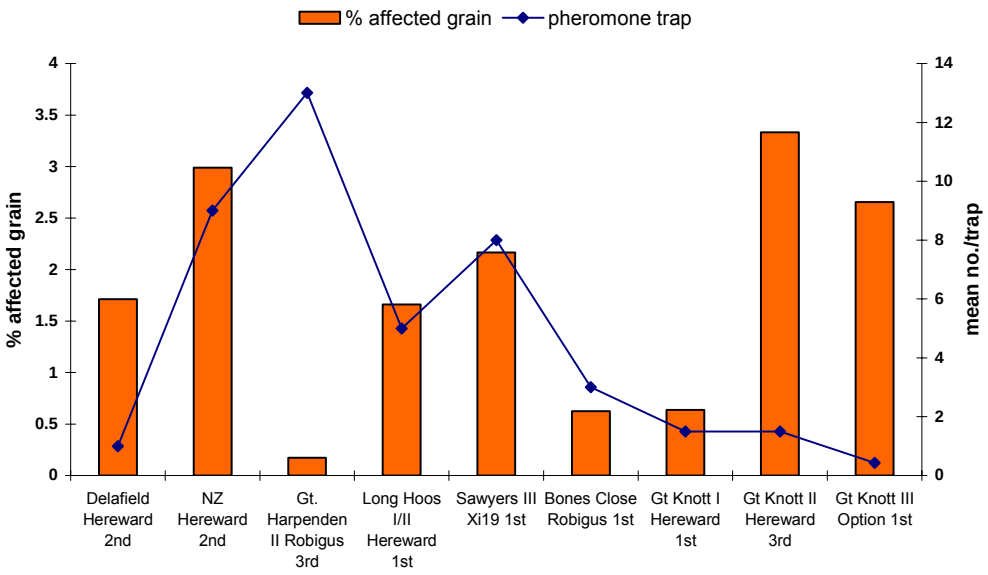


Figure 14. Affected grain on 27 June 2006 and mean number of males in pheromone traps on 2 June 2006 (all 2nd and 3rd wheats follow Robigus)

The data on relationship of pheromone trap catch with subsequent infestation shown in Figure 14 are not very useful for setting the economic threshold because infestation levels were less than 4%. The site where pheromone trap catch was highest (Gt. Harpenden 2) had negligible infestation because it was sown with the resistant variety Robigus.

Ten emergence traps were put out on 24 May 2006 on Summerdells 2, which was in set-aside after second wheat. Two pairs of pheromone and yellow sticky traps were also deployed alongside and traps were changed every three to four days. At peak emergence there were approximately twice as many female midges as males (Figure 15), but as seen across the rest of the farm (Figure 10) the peak was well after the susceptible growth stage of the wheat crops. Yellow wheat blossom midge (ywbm, *Contarinia tritici*) emergence was much earlier and females outnumbered males to an even greater extent than with owbm (Figure 15).

Numbers of female owbm caught on yellow sticky traps at the Summerdells 2 site were very low and reflected the pattern seen in the emergence traps. However, numbers of males caught in the pheromone traps showed a false peak

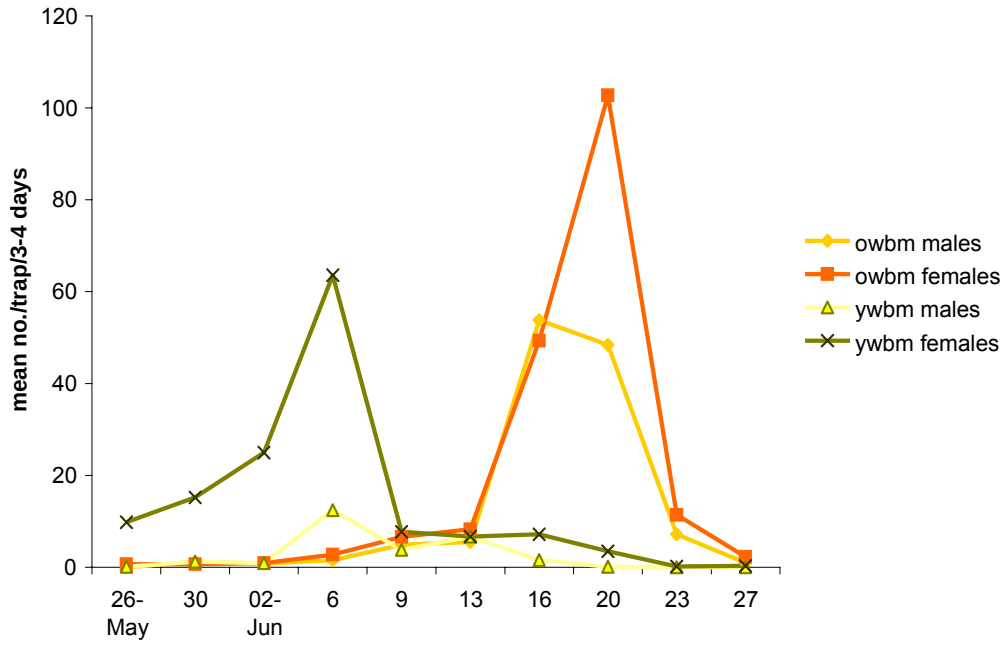


Figure 15. Orange wheat blossom midge and yellow wheat blossom midge emergence Summerdells 2 set-aside after wheat 2006

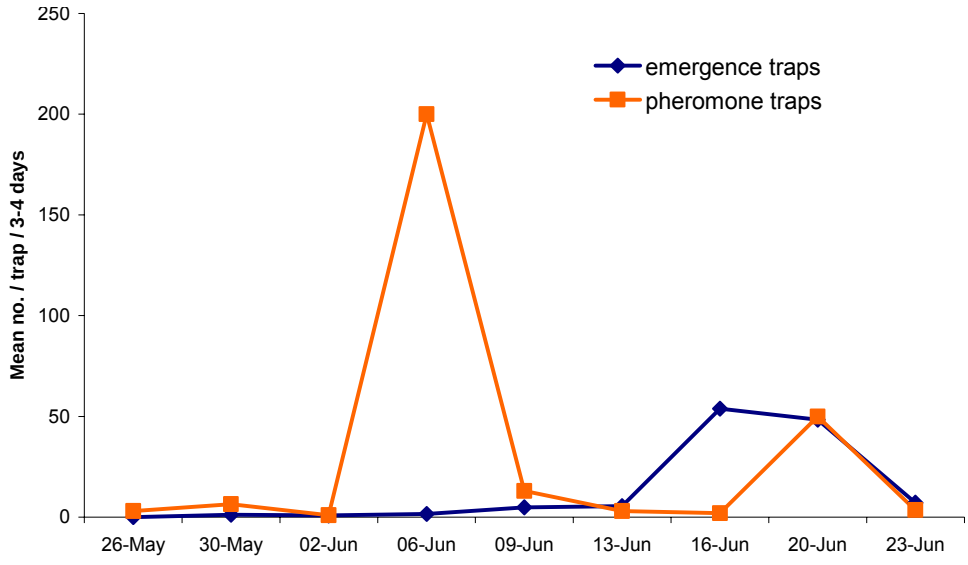


Figure 16. Male owbm caught in pheromone traps next to emergence traps

on 6 June (Figure 16) and a secondary peak coinciding with the peak in the emergence traps on 20 June. The mean emergence trap catch was 1.6 males/0.5m^2 on 6 June, and 49 males/0.5m^2 on 20 June. The early peak suggests that the pheromone traps had recruited males from the neighbouring area (approximately 62 m^2 /trap), possibly due to lack of competition from

natural sources of sex pheromone due to the low numbers of female owbm present. On 20 June, when female numbers were high, numbers of males caught in the pheromone traps were the same as the emergence trap catch. This could account for the discrepancies seen on some of the wheat sites in 2005 and 2006 where pheromone trap catches were high during the susceptible growth stage, but subsequent infestation levels were low.

In terms of the impact of meteorological conditions, rainfall at the critical late April, early May time was less than in 2004, the year of the previous outbreak, and there was a 20-30% potential soil moisture deficit in early May, which probably contributed to the delayed owbm emergence. In addition, 2006 was a cooler year than 2004 with lower soil temperature at 20cm, which also would have delayed midge emergence.

2007

Two pairs of pheromone traps and yellow sticky traps were put out at each of 17 sites around Rothamsted farm on 17 May when wheat sites reached growth stage 47 and catches were recorded twice a week. Infestation levels were assessed in 25 ears at each wheat site during the milk grain growth stage on 20 June.

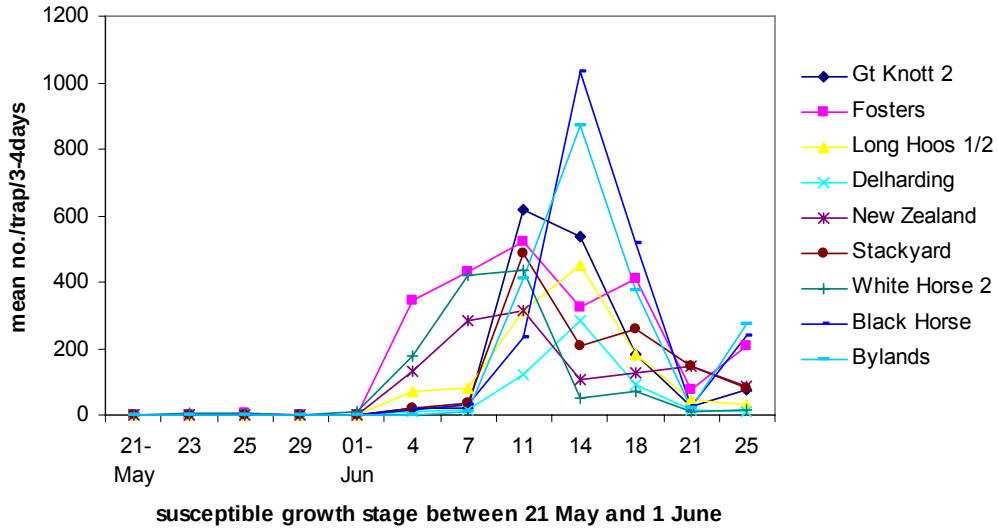


Figure 17. Pheromone trap catches at sites where wheat was grown 2007

As found in previous years there was considerable variation in pheromone trap catch from field to field (Figure 17). In 2007, numbers were well below the economic threshold in all fields during the ear emergence period (21 May to 1 June) and showed clearly that application of insecticide was unnecessary. Emergence on crop sites following wheat peaked a little earlier than on the wheat sites, as seen in previous seasons, but was also outside the susceptible growth stage. As seen in 2006, infestation levels were very low (Figure 18) except at one site Great Knott 2, a susceptible 4th wheat, variety Hereward. The site was bordered by crops of oats and field beans in Great Knott 1 and Great Knott 3 respectively, which were both in wheat in 2006. The Great Knott fields had low levels of owbm emergence, but Great Knott 2 probably recruited females from the neighbouring Great Knott 1 & 3 fields. However, numbers of females on yellow sticky traps was also very low and could not confirm this hypothesis. From the GPS mapping of the pheromone and yellow sticky trap catches across the Farm there was no evidence of movement of female owbm between fields.

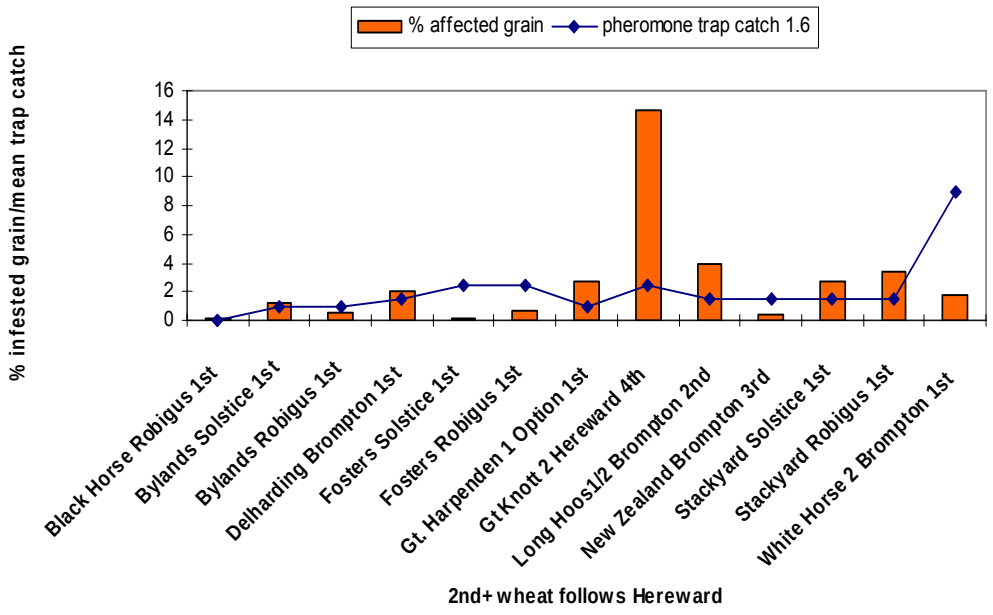


Figure 18. Affected grain 20/06/07 and males on pheromone traps 01/06/07

Ten emergence traps were put out on 16 May 2007 on Great Knott 3, which was sown to spring field beans following wheat. Two pairs of pheromone and yellow sticky traps were also deployed alongside and traps were changed every three to four days. The pattern of owbm emergence in these traps (Figure 19) was similar to that shown by the pheromone traps across the farm (Figure 17). As seen in 2006, at peak emergence, there were more than twice as many female owbm as males, and the peak was well after the susceptible growth stage of wheat crops on the farm confirming pheromone trap data. Ywbm emergence was much later than seen in 2006 and again females outnumbered males.

Additionally, emergence of the midge egg parasitoid, *Macroglenes penetrans* was also recorded with females outnumbering males (Figure 19). Numbers of female owbm caught on the yellow sticky traps were very low (Figure. 20). However, numbers of males caught in the pheromone traps were high and peaked just before the peak of females in the emergence traps. However, unlike 2006 there was no false early peak in the pheromone traps.

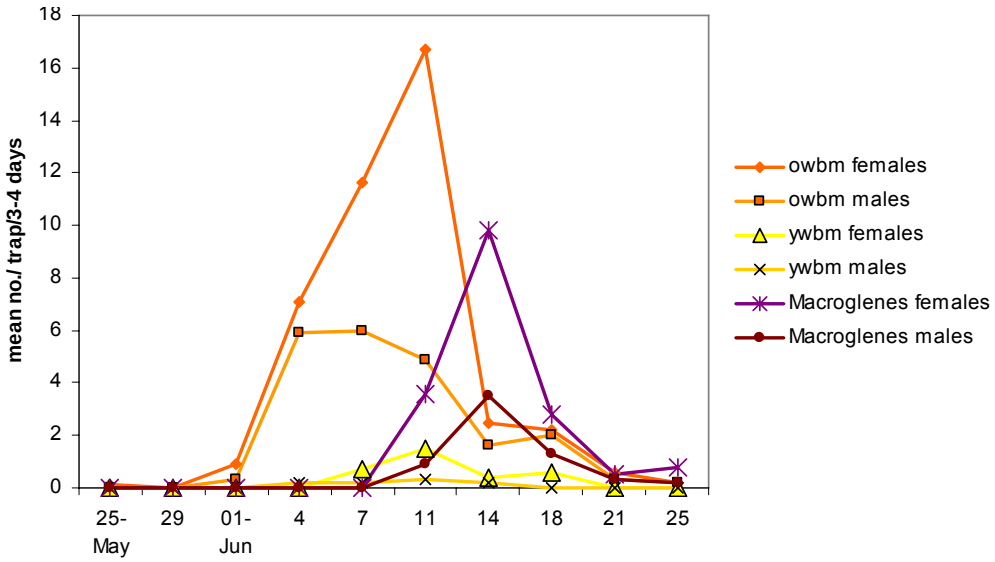


Figure 19. Orange wheat blossom midge, yellow wheat blossom midge and *Macroglenes* emergence trap catches at Rothamsted in 2007

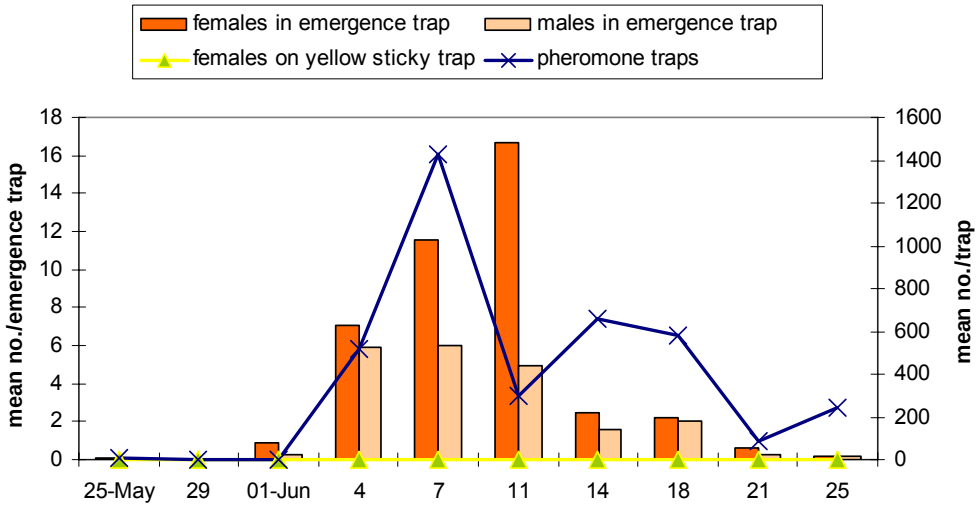


Figure 20. Comparison of emergence trap catches with pheromone and yellow sticky trap catches at the same site

In terms of the impact of meteorological conditions, 2007 was a much warmer year than 2004, which was the last year to have a substantial owbm outbreak. This resulted in a much earlier development of the crop (susceptible growth stage between 1 and 15 June in 2004 compared to 21 May to 1 June in 2007). Additionally, there was less early rainfall in 2007 compared to 2004 resulting in a potential 50-100% soil moisture deficit during mid-April to late May. This delayed owbm larval movement and pupation and the late emergence of adults completely missed the susceptible growth stage of most wheat crops. However, late secondary tillers on some sites were infested.

2008

Two pairs of pheromone and yellow sticky traps were put out at each of 15 sites around Rothamsted farm on 21 May 2008 when wheat sites reached growth stage 47 and catches were recorded twice a week. At the end of the season infestation levels were assessed in 25 ears at each wheat site during the milk grain growth stage on 26 June. As found in previous years there was considerable variation in pheromone trap catch in wheat from field to field (Figure 21).

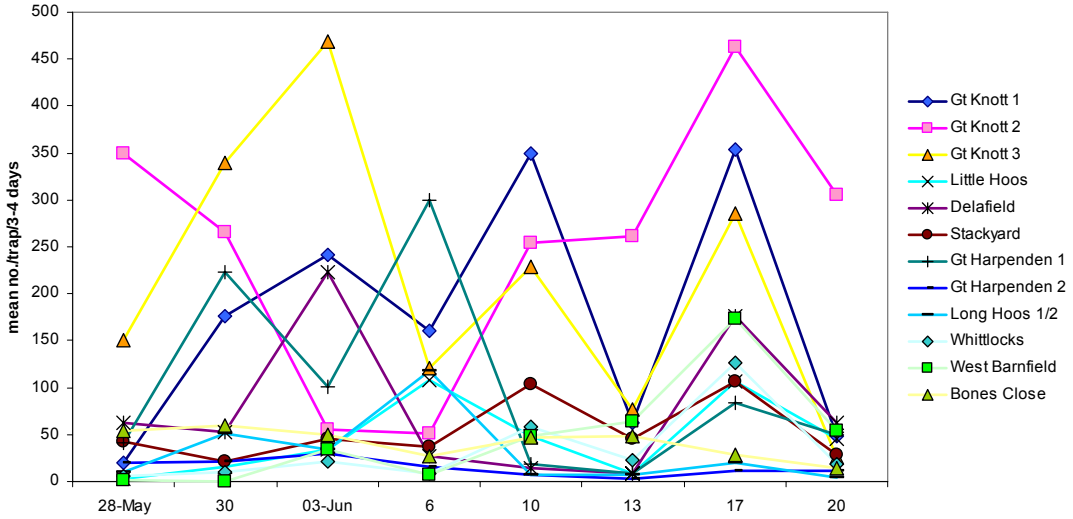


Figure 21. Male owbm caught in pheromone traps on wheat sites 2008

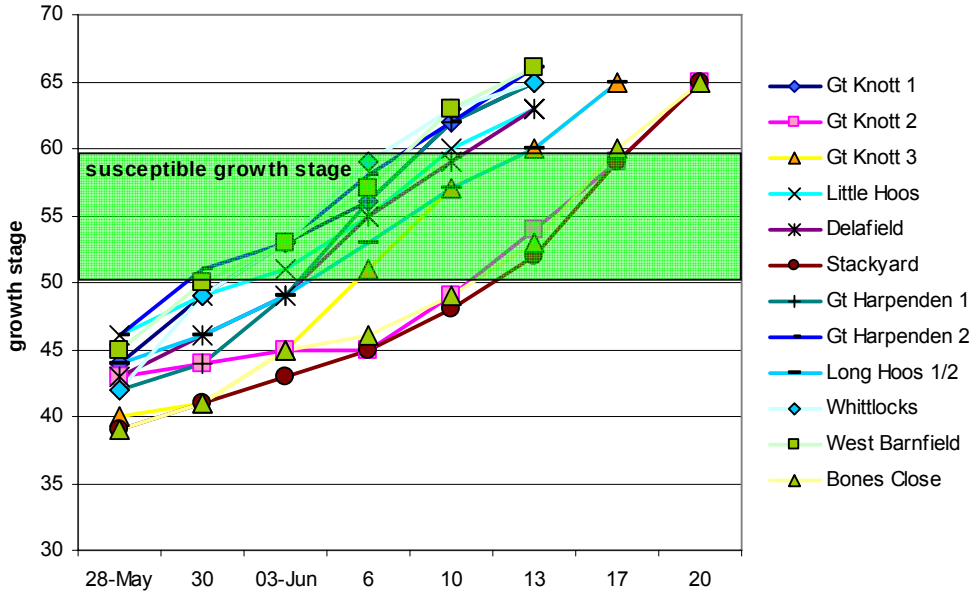


Figure 22. Development of wheat on sites at Rothamsted 2008

At many of the sites, numbers of males caught were at or well above the suggested threshold of 50 per trap over three nights during the susceptible ear emergence period (Growth Stage 53-59). However, ear emergence was very variable across the farm and occurred over a prolonged period (30 May to 17 June; see Figure 22). Despite the positive indication of the threat of a serious owbm outbreak, infestation levels were low in the few susceptible crops (Figure 23). From 2006-2008 there has been an increasing trend for most of the non-experimental wheat fields on Rothamsted farm to be sown with owbm resistant

varieties, in 2008 mostly variety Brompton. However, female owbm do not discriminate against resistant varieties and are attracted to oviposit on them

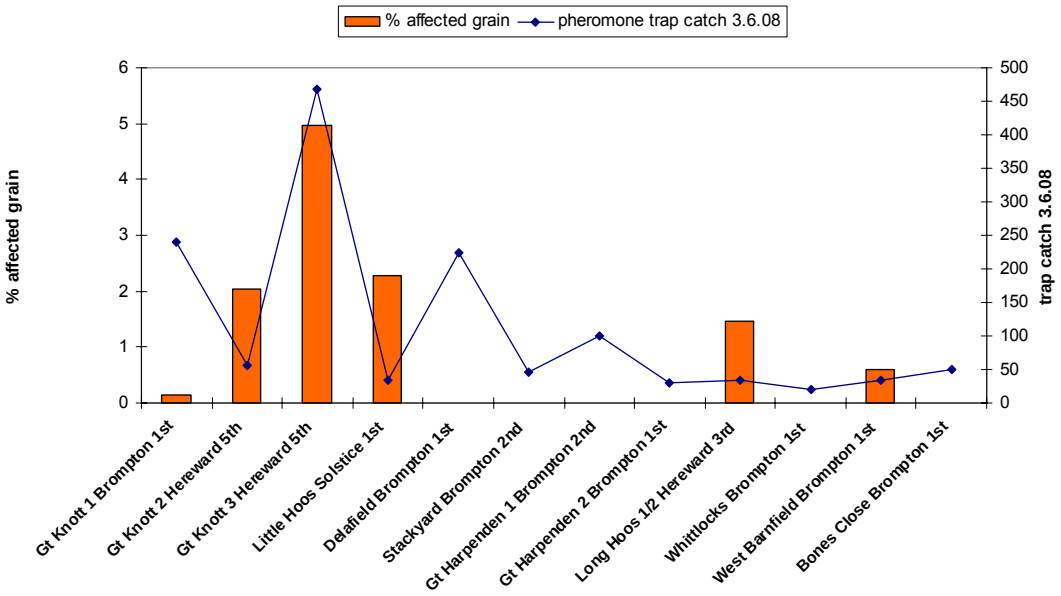


Figure 23. Owbm infestation and pheromone trap catch on wheat sites 2008

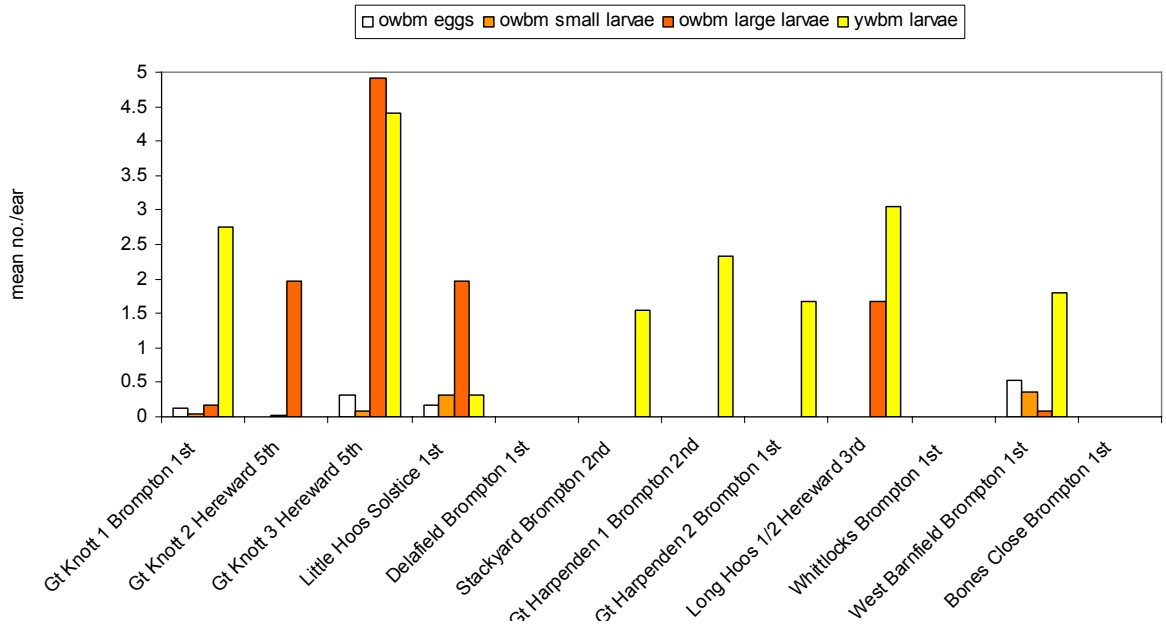


Figure 24. Owbm eggs and larvae and ywbm larvae on infested wheat sites on 26 June 2008

even though the resulting larvae will not survive (Figure 24). Many of the Brompton sites (particularly Great Knott 1 and West Barnfield) were in ear

earlier than the neighbouring susceptible varieties (Figure 22) and may have attracted females emerging in the susceptible wheat, which was not yet at ear emergence. The numbers of females caught on yellow sticky traps within the crops and the occurrence of eggs and small larvae at these sites would seem to support this hypothesis. However, this does not entirely explain the comparatively low infestation on Great Knott 2, where, although the peaks in emergence occurred just before and just after the susceptible growth stage, there were large catches in the pheromone traps on 10 and 13 June during the susceptible growth stage (Figure 21).

The gradual rise in the area sown to resistant varieties at Rothamsted will eventually deplete the local population of owbm, although mean peak pheromone trap catches have not yet begun to fall since residues of owbm larvae may remain in the soil for several years. In contrast, the resistant varieties are not resistant to ywbm and their numbers may increase as owbm numbers decline (Figure 24).

As in previous years, ten emergence traps were put out (19 May), this year on Great Knott 2, which was in a fifth year of wheat and had the highest level of infestation (13% grain attacked) in 2007. Two pairs of pheromone and yellow sticky traps were also deployed alongside and traps were changed every 3-4 days.

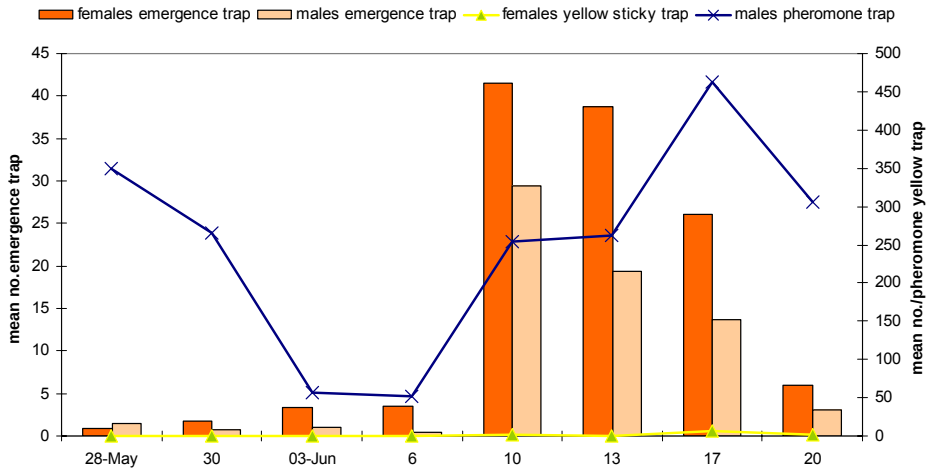


Figure 25. Owbm in emergence traps compared to pheromone and yellow sticky trap catches at the same site 2008

The pattern of owbm emergence (Figure 25) does not compare directly to the numbers of males caught in the accompanying pheromone traps suggesting that, as in 2006, the early peak in the pheromone traps was due to males recruited from the neighbouring area, possibly because of lack of competition from natural sources of sex pheromone since the numbers of female owbm present was low. On 28 May there was a mean emergence trap catch of 1.5 males per 0.5 m^2 while the mean pheromone trap catch was 349.5, which would indicate that males were being recruited from an area of 116m^2 around the trap. This effect could account for the discrepancies seen on some wheat sites where pheromone trap catches were high during the susceptible growth stage, but subsequent infestation levels were low. However, it does not account for the low infestation on Great Knott 2 since the peak emergence trap catches occur during the susceptible growth stage (10 to 17 June).

As seen in previous years, at peak emergence there were more females owbm emerging than males. Very few Ywbm emerged at this site and this was reflected in the lack of infesting larvae found in the ear sample (Figure 24).

In terms of the impact of meteorological conditions, temperatures in 2008 were very similar to 2004. However, in 2004, there was greater early rainfall compared to 2008. This resulted in a 20% soil moisture deficit from mid-April to early May rising to a 60% deficit by late May in 2008, but apparently the late deficit did not delay owbm emergence, although it may have prolonged the ear emergence period (susceptible growth stage between 1 and 15 June in 2004 compared to 30 May to 17 June in 2008). In addition, higher rainfall in late May to early June in 2008 may have extended the owbm and ear emergence periods.

ADAS

2006

High Mowthorpe
Most male midges were caught between 11 and 19 June. Catches were very variable between fields. In wheat fields catches in excess of 120 midges/trap/day were recorded in Homefield and Crow Wood whereas in Kirby Grass S numbers never exceeded 32/trap/day (Figure 26). Male midge emergence tended to coincide with the susceptible period of the crop (12-19 June). Catches were also very variable in the potential source fields. Highest catches were recorded in Tommy Ireland, Old Type and Bugdale. In these fields over 300 male midges/trap/day were trapped during the susceptible period. In contrast, in Elbow North numbers never exceeded 8 midges/trap/day (Figure 27).

Catches of female midges on yellow sticky traps were much lower than of males in pheromone traps. In wheat crops, numbers caught never exceeded 3/trap/day during the susceptible period of the crop (Figure 28). Peak catches were recorded in Malton Road and Smithfield between 25 and 27 June, well beyond the susceptible growth stage of the crop. In the non-wheat crops, peak catches of females were recorded during the susceptible period of the crop, particularly in Old Type, although numbers never exceeded 4 midges/trap/day (Figure 29).

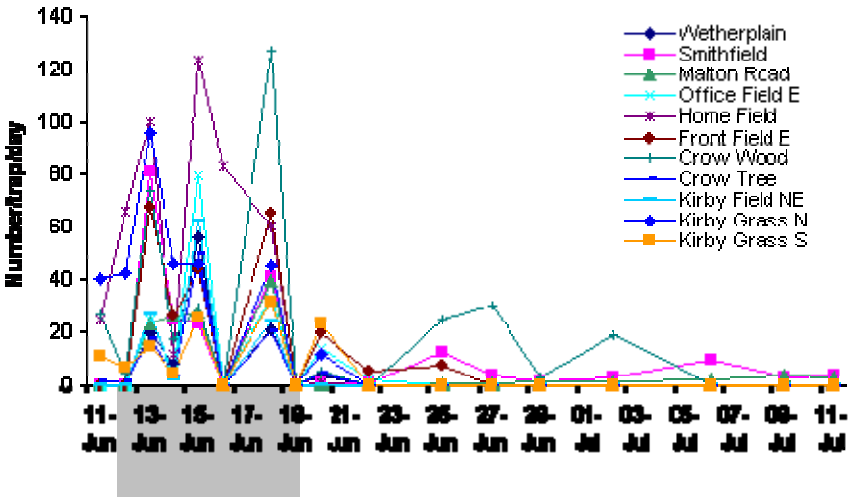


Figure 26. Mean number of male owbm caught in pheromone traps in wheat (sink) fields at High Mowthorpe, 2006 (shaded grey area = susceptible period of the crop).

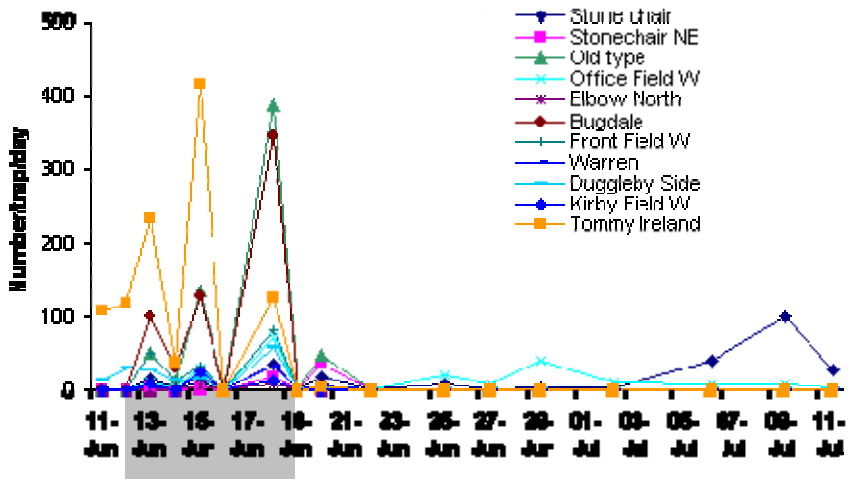


Figure 27. Mean number of male owbm caught in pheromone traps in non-wheat (source) fields at High Mowthorpe, 2006 (shaded grey area = susceptible period of the crop).

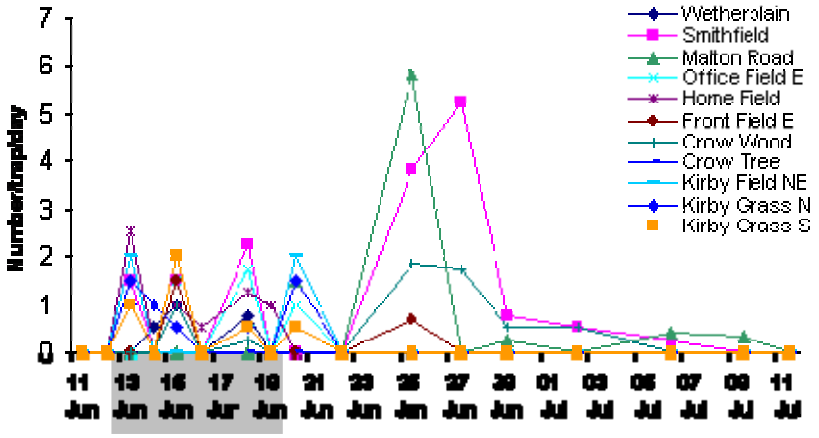


Figure 28. Mean number of female owbm caught on yellow sticky traps in wheat (sink) fields at High Mowthorpe, 2006 (shaded grey area = susceptible period of the crop).

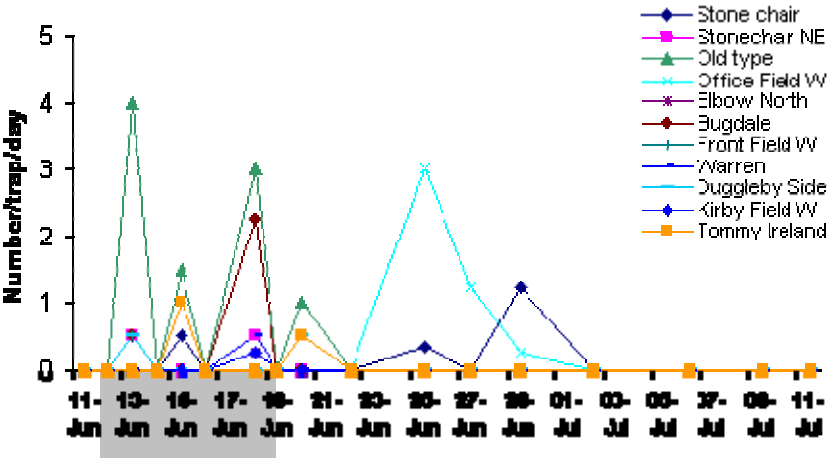


Figure 29. Mean number of female owbm caught on yellow sticky traps in non-wheat (source) fields at High Mowthorpe, 2006 (shaded grey area = susceptible period of the crop).

Table 4. Mean numbers of owbm larvae/ear and % damaged grain at High Mowthorpe, June 2006

Field	Mean owbm larvae/ear	% damaged grain
Wether Plain	3.9	3.3
Smith Field	2.9	5.4
Malton Road	0	0
Office Field E	0	0
Home Field	5.7	5.5
Front Field E	4.5	5.2
Crow Wood	0.3	0.6
Crow Tree	0.2	0.1
Kirby Field NE	0.5	0.9
Kirby Grass N	2.5	2.3
Kirby Grass S	5.2	3.0

Levels of midge infestation in the grain were relatively low (Table 4). Only in Smith Field, Home Field and Front Field did levels of % grain infestation exceed the threshold of 5% for milling and seed crops (Jon Oakley, Pers. comm.). All of these fields caught between 60 and 120 midges/trap/day during the susceptible period of the crop. Female midges were also caught in these fields during the susceptible period of the crop although numbers never exceeded 3/trap/day.

2007

High Mowthorpe

Peak catches of male midges occurred on 12 June during the susceptible period of the wheat crop. Most midges were caught in Tommy Ireland in a crop of wheat when 357/trap/day were recorded (Figure 30). The next highest catch was in wheat in Home Field with 147 midges/trap/day. In general, lower numbers of male midges were caught in the potential source than sink fields.

Peak catches of female midges on yellow sticky traps were also recorded on 12 June with Home Field recording 10.5 midges/trap/day (Figure 31). Numbers in all other fields were less than 2/trap/day.

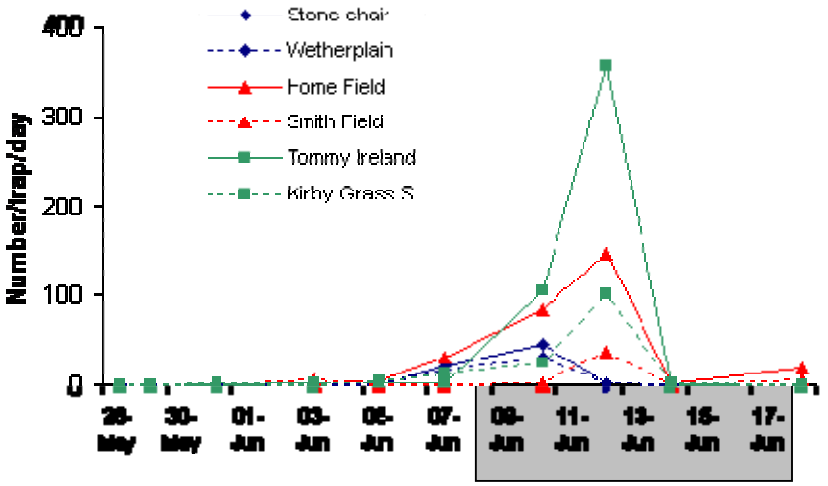


Figure 30. Mean number of male owbm caught in pheromone traps in both source (dotted line) and sink (full line) fields at High Mowthorpe in 2007 (shaded grey area = susceptible period of the crop).

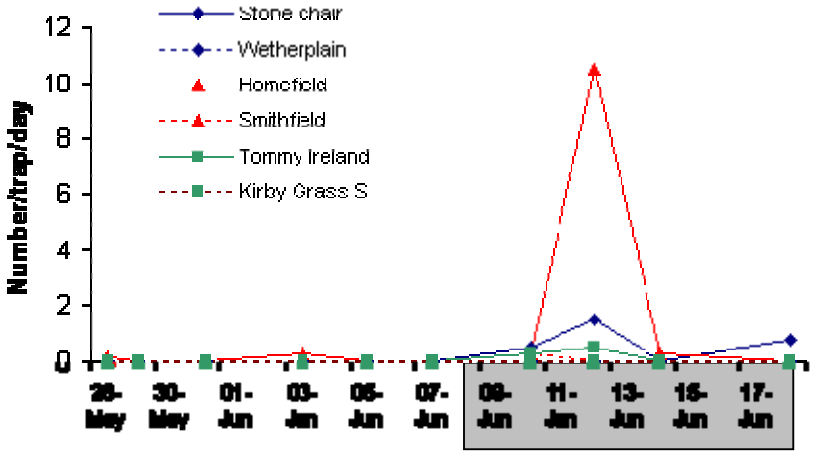


Figure 31. Mean number of female owbm caught on yellow sticky traps in both source (dotted line) and sink (full line) fields at High Mowthorpe in 2007 (shaded grey area = susceptible period of the crop)

Table 5. Mean number of owbm larvae/ear and % damaged grain at High Mowthorpe, June 2007.

Field	Mean owbm larvae/ear	% damaged grain
Stonechair NE	2.1	2.7
Home Field	0	0
Tommy Ireland	1.2	1.4

Levels of % grain infestation were generally low in all monitored fields (Table 5).

Boxworth

Catches of male wbm at Boxworth in 2007 were very low and never exceeded 12/trap/day (Figure 32). Between 25 May and 1 June very few midges were caught. Catches were increasing when monitoring ended on 4 June. In general, numbers of male midges caught in source fields were greater than in sink fields with the exception of Long field between 23 and 25 May.

Catches of female owbm in sticky traps were very low and never exceeded 2/trap/day and were generally less than 0.5/trap/day throughout the monitoring period.

Table 6. Mean number of owbm larvae/ear and % damaged grain at Boxworth, June 2007.

Field	Mean owbm larvae/ear	% damaged grain
Pamplins South	0.5	0.5
Long Field	0.4	0.5
40 Acres	1.2	1.3

There was a low level of grain infestation in all monitored fields and never more than a mean of 1.2 larvae/ear (Table 6).

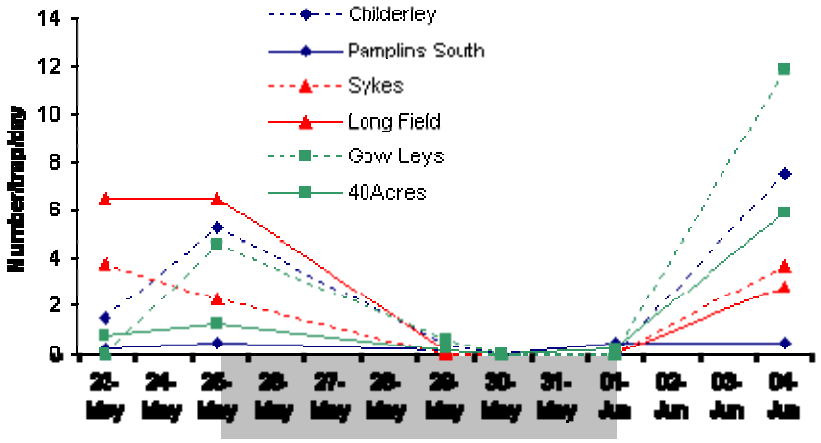


Figure 32. Mean number of male owbm caught in pheromone traps in both source (dotted line) and sink (full line) fields at Boxworth in 2007 (shaded grey area = susceptible period of the crop)

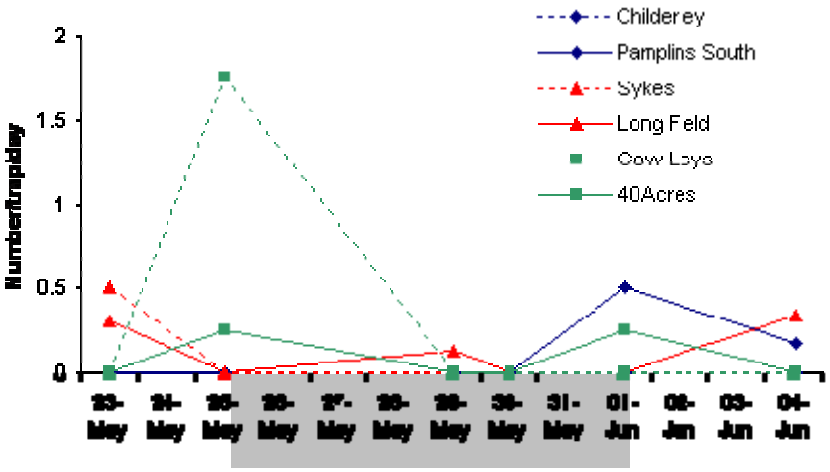


Figure 33. Mean number of female owbm caught on yellow sticky traps in both source (dotted line) and sink (full line) fields at Boxworth in 2007 (shaded grey area = susceptible period of the crop)

2008

High Mowthorpe

Peak catches of male owbm were recorded between 3 and 10 June before the susceptible stage of the crop (Figure 34). A peak catch of 278 midges/trap/day was recorded in Stonechair a source fields on 8 June. Catches of between 180 and 260 midges/trap/day were also recorded on 5 June in Wether Plain, Crow Tree and Front Field West. Catches declined dramatically on 10 June but a second peak of activity was recorded on 12 June at the start of the susceptible period. On these data catches were very variable and ranged between 2 and 148 midges/trap/day. Peak numbers were found in Crow Tree, a sink field. There was no clear difference in numbers of midges caught in source or sink fields.

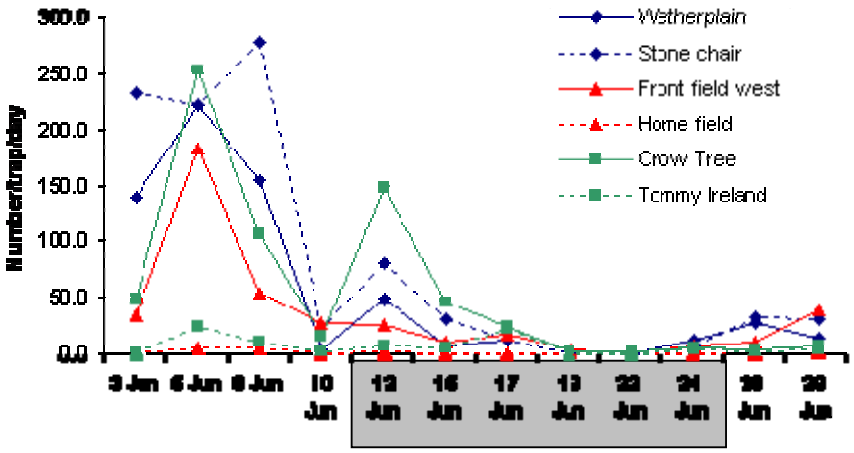


Figure 34. Mean number of male owbm caught in pheromone traps in both source (dotted line) and sink (full line) fields at High Mowthorpe in 2008 (shaded grey area = susceptible period of the crop)

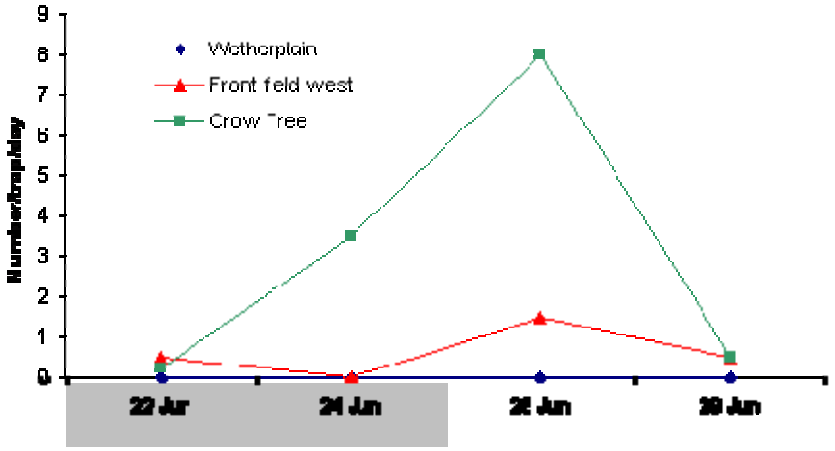


Figure 35. Mean number of female owbm caught on yellow sticky traps in both source (dotted line) and sink (full line) fields at High Mowthrope in 2008 (shaded grey area = susceptible period of the crop)

Peak catches of female owbm were recorded on 26 June in Crow Tree with 8 midges/trap/day (Figure 35). Catches in Front Field West were much lower and none were caught in Wether Plain.

Table 7. Mean numbers of owbm larvae/ear and % damaged grain at High Mowthorpe,, June 2008

Field	Mean owbm largae/ear	% damaged grain
Stonechair	0.7	1.0
Wether Plain	0	0
Front Field	0.4	0.7
Crow Tree	10.9	8.4

The highest level of midge infestation was recorded in Crow Tree with 8.4% of grain damaged by the pest (Table 7). In this field there was a mean of 10.9 larvae/ear. In all other fields there were low numbers of midge larvae and low levels of damaged grain.

Boxworth

There were some high catches of male owbm at Boxworth in 2008. Catches varied between 36 and 274/trap/day during the susceptible period (Figure 36). The peak catch was recorded in 40 Acre N with 274 midges/trap/day on 2 June. Catches in 40 Acre S were also high and 266 midges/trap were caught on 2 June. In Side Hill, 156 midges/trap/day were caught on the same date. Catches declined slightly on 4 June but peaked again on 6 June when 273, 226 and 181 male midges/trap/day were recorded in 40 Acre N, Side Hill and 40 Acre S respectively. In general, catches in source fields were higher than in sink fields, with the exception of 40 Acre S.

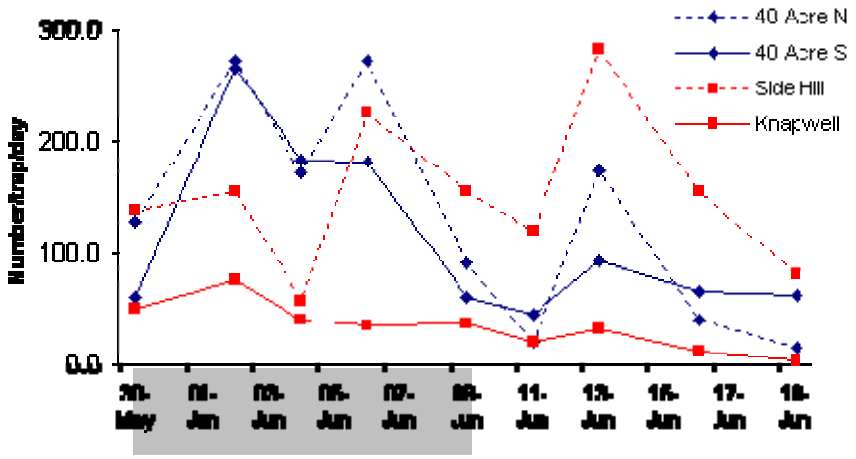


Figure 36. Mean number of male owbm caught in pheromone traps in both source (dotted line) and sink (full line) fields at Boxworth in 2008 (shaded grey area = susceptible period of the crop)

Table 8. Mean number of owbm larvae/ear and % damaged grain at Boxworth, June 2008

Field	Mean owbm larvae/ear	% damaged grain
40 Acre S	7.9	8.9
Knapwell	3.8	4.8

The highest level of ear infestation by owbm larvae was recorded in 40 Acre S (Table 8). The % damaged grain was also highest in this field. Levels of damaged grain and numbers of owbm larvae/ear were lower in Knapwell than in 40 Acre S. These results reflect the differences in catches of owbm males between the two fields.

Grindale

Peak male owbm catches occurred outside the susceptible period of the crop on 8 June. Catches were generally low with the exception of White Dyke field where about 51 midges were recorded per trap per day (Figure 37). Numbers of midges in other fields varied between 3 and 16/trap/day. After 8 June very few midges were caught in any field. There was no obvious relationship between the numbers of midges trapped in source or sink fields.

Table 9. Mean number of owbm larvae/ear and % damaged grain at Grindale, East Yorkshire, June 2008

Field	Mean owbm larvae/ear	% damaged grain
White Dyke	0.7	0.8
Argham Gates	0.1	0.2
Chalk Road	0	0

Numbers of owbm larvae/ear and % damaged grain were very low in all fields at Grindale (Table 9). White Dyke had the highest catch of male midges and this was reflected in the level of damaged grain and number of larvae/ear.

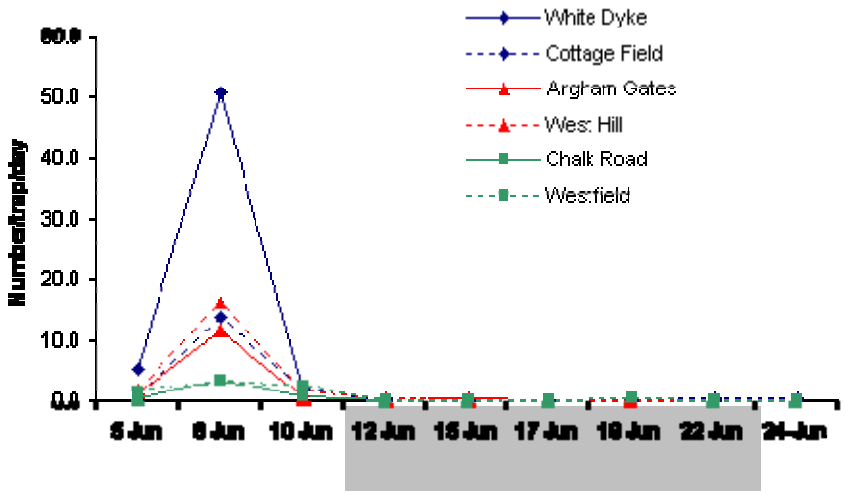


Figure 37. Mean number of male owbm caught in pheromone traps in both source (dotted line) and sink (full line) fields at Grindale, East Yorkshire in 2008 (shaded grey area – susceptible period of the crop)

TAG

2007

In 2007 TAG conducted three farm scale studies at Morley Farms, Morley St Botolph in Norfolk, Lower Norton Farm, Sutton Scotney in Hampshire and Biscathorpe Farm House, Louth, Lincolnshire. Summer insecticides were not applied to the wheat fields at any of the sites.

Soils were moist from the second to third week in May due to frequent rain. However, soil temperatures remained relatively cool during this period fluctuating between 10 and 13^{o}C which may have delayed the onset of pupation. Although a background population of midge were present at the beginning of the susceptible stage numbers remained low peaking towards the end of this period. Therefore, in 2007 owbm infestation was most severe on secondary tillers and later-developing wheat crops as supported by observations in other wheat crops.

Morley Farms, Norfolk

Table 10. Cropping of selected fields at Manor, Morley, Norfolk, 2007. Table shows pairing of sink and potential source fields which were adjacent e.g. Hacketts (sink), Bullswood (source). All wheat varieties were susceptible to owbm.

Field Name	Cropping 2008 (variety)	Cropping 2007	Cropping 2006	Cropping 2005
Hacketts	Winter wheat (Claire)	Winter oilseed rape	Winter barley	Winter wheat
Bullswood	Winter beans	Winter wheat	Winter wheat	Winter oilseed rape
Ravens Grove A	Winter wheat (Einstein)	Winter wheat	Winter wheat	Winter wheat
Home Close	Spring barley	Sugar beet	Winter wheat	Winter beans
Myll	Winter wheat (Alchemy)	Fallow	Sugar beet	Winter wheat
Wheate Close	Winter oilseed rape	Spring barley	Sugar beet	Winter wheat

In the wheat fields low levels of male owbm were first trapped on 21st May during the early stages of ear emergence (first spikelets visible). Trap catches in all three wheat fields remained very low until the ears were approximately 50% emerged. Adult midge activity peaked in Ravens Grove (continuous wheat) between 8th and 11th June with 53.5 midge trapped during this period (Figure 38).

In the "non wheat" fields very low levels of male owbm were trapped on 21st May. Trap catches in Bullswood (winter beans following winter wheat) and Home Close (oilseed rape following spring barley) remained low during the susceptible stages of the adjacent wheat crops. In Hacketts (oilseed rape following spring barley) peak adult activity occurred simultaneously with that in the near by wheat crop in Ravens Grove. In Hacketts the highest mean catch from two traps during this period was 45.0 midge caught between 6th and 8th June (Figure 39).

At this site very low levels of owbm (male and female) were caught on the yellow sticky traps and these traps were not deemed to be a reliable indicator of adult midge activity.

Following low trap catches the number of larvae per ear in each wheat field was very low at less than one larva per ear.

Trap catches and adult infestation at this site were too low to determine which fields were a potential source and which were a potential sink for adult midge. The influence of previous cropping is not clear from these data, fields with the highest level of midge activity were Ravens Grove a continuous wheat and Wheate Close which was cropped with oilseed rape and last sown with winter wheat in 2003.

Figure 38. Percentage ear emergence and owbm trap catches (adult males) during the susceptible period of each winter wheat field at Morley, Norfolk, 2007

Figure 39. Owbm trap catches (adult males) from adjacent potential "source" fields during the susceptible period of each winter wheat field, Morley, 2007

Lower Norton Farm, Sutton Scotney, Hampshire

Table 11. Cropping of selected fields at Lower Norton Farm, Sutton Scotney Hampshire, 2007. Table shows pairing of sink and potential source fields which were adjacent e.g. Rookery (sink), Bassingstoke (source). Einstein is susceptible to owbm, Robigus is resistant to owbm

Field Name	Cropping 2008 (variety)	Cropping 2007	Cropping 2006
Rookery	Winter wheat (Robigus)	Winter oilseed rape	Spring barley
Bassingstoke	spring barley	Winter wheat	Winter wheat
Home Field	Winter wheat (Robigus)	Winter oilseed rape	Winter wheat
Bullington	Winter oilseed rape	Spring barley	Spring barley
Pattersons	Winter wheat (Einstein)	Fallow	Spring barley
Footpath	Spring barley	Spring barley	herbage/winter wheat

At Sutton Scotney the crops reached ear emergence relatively early compared with the other sites and the monitored wheat crops reached the end of the susceptible stage (early flowering) just prior to the emergence of the adult owbm as shown by Figure 40. Consequently the level of larval infestation in the ear was very low (less than one larva per ear). The later timing of the midge compared with ear emergence was fortunate as large numbers of adults were trapped after the susceptible stage (e.g. between 12th and 13th June, 1,587.0 adult males were trapped on Home Field). Had the susceptible stage of the crop and the midge emergence coincided the level of damage to untreated crops could have been significant. These data should give growers confidence that prophylactic or "revenge" insecticides are not required once crops are beyond the susceptible stage.

In the "non wheat" fields high levels of male owbm were also trapped after the susceptible stage of the neighbouring wheat fields. However, peak emergence in these fields occurred slightly earlier than in the wheat fields. Between 8th and

12th June 406.5, 894.0 and 891.5 midge were trapped respectively in Footpath (spring barley, previously wheat in 2005), Basingstoke (spring barley, previously wheat in 2006) and Bullington (winter oilseed rape, previously wheat in 2005). These fields where owbm appeared to emerge or congregate earlier could have provided a source of midge to the neighbouring wheat fields. These data suggests that trapping in neighbouring non-wheat fields can provide a guide to the onset and magnitude of midge activity. If this result is typical then monitoring in these fields may give advanced warning of activity in susceptible neighbouring wheat fields.

Figure 40. Percentage ear emergence and owbm trap catches (adult males) during the susceptible period of each winter wheat field at Sutton Scotney, Hampshire, 2007

Figure 41. Owbm trap catches (adult males) from adjacent potential "source" fields during the susceptible period of each winter wheat field, Hampshire, 2007

Biscathorpe Farm House Louth, Lincolnshire

Table 12. Cropping of selected fields at Biscathorpe Farm House, Louth, Lincolnshire, 2007. Table shows pairing of sink and potential source fields which were adjacent e.g. Long Field (sink), Platts (source). All wheat varieties were susceptible to owbm.

Field Name	Cropping 2008 (variety)	Cropping 2007	Cropping 2006	Cropping 2005
Long Field	Winter wheat (Alchemy)	Winter oilseed rape	Winter wheat	Winter wheat
Platts	Spring barley	Winter wheat	Winter wheat	Winter oilseed rape
Mill Field	Winter wheat (Einstein)	Winter wheat	Winter oilseed rape	Winter wheat
FB Walk	Winter barley	Winter wheat	Winter oilseed rape	Winter barley
Home Close	Winter wheat (Einstein)	Winter oilseed rape	Spring barley	Winter wheat
23 Acre	Winter barley	Winter wheat	Winter wheat	Winter oilseed rape

Very few midges were trapped in the wheat fields during the susceptible stages of these crops. Midge activity "peaked" between 8th and 11th June when just two midges were trapped in Long Field. Similar levels of midge were trapped in the other crops that were monitored. Very few larvae were found in the ears (less than one larva per ear).

2008

In 2008 TAG conducted three farm scale studies at Morley Farms, Morley St Botolph in Norfolk, Borough Farm, Micheldever in Hampshire and Biscathorpe Farm House Louth, Lincolnshire. Summer insecticides were not applied to the wheat fields at any of the sites.

Soils were relatively moist below the surface during the first two weeks in May due to of 9.4 mm of rain on May 1st. During this period soil temperatures were

warm enough to trigger pupation as they hovered around 13°C before falling slightly to 11°C during the third week of the month. This season adults were active earlier than in 2007, with activity occurring at the onset of the susceptible stage and peaking around 6th June at mid-ear emergence. The level of larval infestation in the ears was low due to low levels of adult midge and unfavourable conditions for egg laying during the susceptible period of the crop.

Morley Farms, Norfolk

Table 13. Cropping of selected fields at Manor and Wood Farm, Morley, Norfolk, 2008. Table shows pairing of sink and potential source fields which were adjacent e.g. Bullswood (sink), Ravens Grove (source). All wheat varieties were susceptible to owbm.

Field Name	Cropping 2008 (variety)	Cropping 2007	Cropping 2006	Cropping 2005
Bullswood	Winter wheat (Alchemy)	Winter beans	Winter wheat	Winter wheat
Ravens Grove B	Winter beans	Winter wheat	Winter wheat	Winter wheat
Shadwells	Winter wheat (Humber)	Fallow	Sugar beet	Winter wheat
Home Close	Winter barley	Spring barley	Sugar beet	Winter wheat
Hastings	Winter wheat (Oakley)	Winter beans	Winter wheat	Winter oilseed rape
Holmes	Winter oilseed rape	Winter wheat	Winter beans	Winter wheat

In the wheat fields low levels of male owbm were first trapped on 28th May during the early stages of ear emergence (first spikelets visible). Trap catches in Bullswood (winter wheat following winter beans) remained low during the susceptible stages of the crop. However, small peaks of adult midge activity were detected in Hastings (winter wheat after beans) and Shadwells (winter wheat after fallow) between 3rd and 6th June. The highest mean pheromone catch was 86.5 midges recorded in Hastings between the 4th and 6th June (Figure 42).

In the "non-wheat" fields low levels of male owbm were also first trapped on 28th May. Trap catches in Ravens Grove (winter beans following winter wheat) and Holme Close (winter barley following spring barley) remained low during the susceptible stages of the adjacent wheat crops. In Holmes (winter oilseed rape after winter wheat) a peak of adult activity (109.0 midges) occurred simultaneously with that in the neighbouring wheat crops between June 4th and June 6th (Figure 43).

At this site very low levels of owbm (male and female) were caught on the yellow sticky trap. A maximum of 1.5 adults/ trap/day were caught when assessed on 3rd June in Holmes whilst during the same time period a mean of 54.0 male adults were caught on pheromone traps in the same position. This could indicate that the pheromone traps were drawing midges from a distance to the trap thus giving an exaggerated impression of the risk of midge damage. Alternatively. the yellow sticky traps are not reliable in indicating the presence of adult midges, or most likely a combination of these factors. The traps do signal the onset of male emergence which should trigger farmers to monitor vulnerable crops. If the pheromone traps give "false positive" readings they may lead to unnecessary sprays, however, from an agronomic perspective this is considered better than a "false negative" which would not detect a midge population that should be treated.

Following low levels of adult midge infestation and trap catches the number of larvae per ear in each wheat field was very low at 0.02 per ear in Bullswood, 0.01 per ear in Shadwells and 0.01 in Hastings.

Trap catches and adult infestation at this site were too low to determine which fields were a potential source and which were a potential sink for adult midge. The influence of previous cropping is not clear from these data.

Figure 42. Percentage ear emergence and owbm trap catches (adult males) during the susceptible period of each winter wheat field at Morley, Norfolk, 2008

Figure 43. Owbm trap catches (adult males) from adjacent potential "source" fields during the susceptible period of each winter wheat field, Norfolk, 2008

Borough Farm, Micheldever, Hampshire

Table 14. Cropping of selected fields at Borough Farm, Micheldever, Hampshire, 2008. Table shows pairing of sink and potential source fields which were adjacent e.g. HG8903 (sink), HG3204 (source). All wheat varieties were susceptible to owbm.

Field Name	Cropping 2008 (variety)	Cropping 2007	Cropping 2006
HG8903	Winter wheat (Timber)	Winter oilseed rape	Spring barley
HG3204	Spring barley	Winter wheat	Winter oilseed rape
Dewdales	Winter wheat (Exsept)	Winter oilseed rape	Spring barley
HG4864	Winter oilseed rape	Spring barley	Winter wheat
Reservoir	Winter wheat (Exsept)	Winter oilseed rape	Spring barley
HG7247	Spring barley	Winter wheat	unknown

At the Hampshire site midge activity was variable (low to high) between the monitored fields during the susceptible period of the wheat crops. Trap catches in Dewdales and Reservoir (both winter wheat following oilseed rape) remained low during the susceptible stages of the crop. However in HG8903 (winter wheat after oilseed rape), midge activity peaked between 28th May and 30th May with 466.0 adults recorded followed by a smaller peak of activity (225.5 midge) between 6th June and 9th June (Figure 44).

In the "non-wheat" fields low levels of male owbm were also first trapped on 28th May. Trap catches in HG4864 (oilseed rape following spring barley) and HG7247 (spring barley following winter wheat) remained relatively low during the susceptible stages of the adjacent wheat crops. In HG3204 (spring barley after wheat) a peak of adult activity occurred simultaneously with the beginning of ear emergence in the wheat crops. On the first day of trapping (27th May) activity peaked (790.0 midge) and remained high (above 100 midge/trap) until 2nd June (Figure 45).

At this site low levels of owbm (male and female) were caught on the yellow sticky traps. A maximum of 13.0 adults/trap/day were caught on 4th June in HG7247 whilst during the same time period 228.5 male adults were caught on pheromone traps in the same position. These data suggest that the yellow sticky traps were not a reliable indicator of male midge activity.

Following low levels of adult midge infestation and trap catches the number of larvae per ear in Dewdales and Reservoir was low with 0.96 and 1.44 respectively. In HG8903 where adult catches were high 4.00 larvae per ear were recorded, this level of infestation could significantly reduce grain yield and quality.

These data suggests that HG3204 (spring barley after wheat) was a potential source field as activity was high in this field and the adjacent wheat field HG8903. The influence of previous cropping is not clear from these data. HG3204 was cropped with wheat in 2007 and 2005.

Figure 44. Percentage ear emergence and owbm trap catches (adult males) during the susceptible period of each winter wheat field at Micheldever, Hampshire, 2008

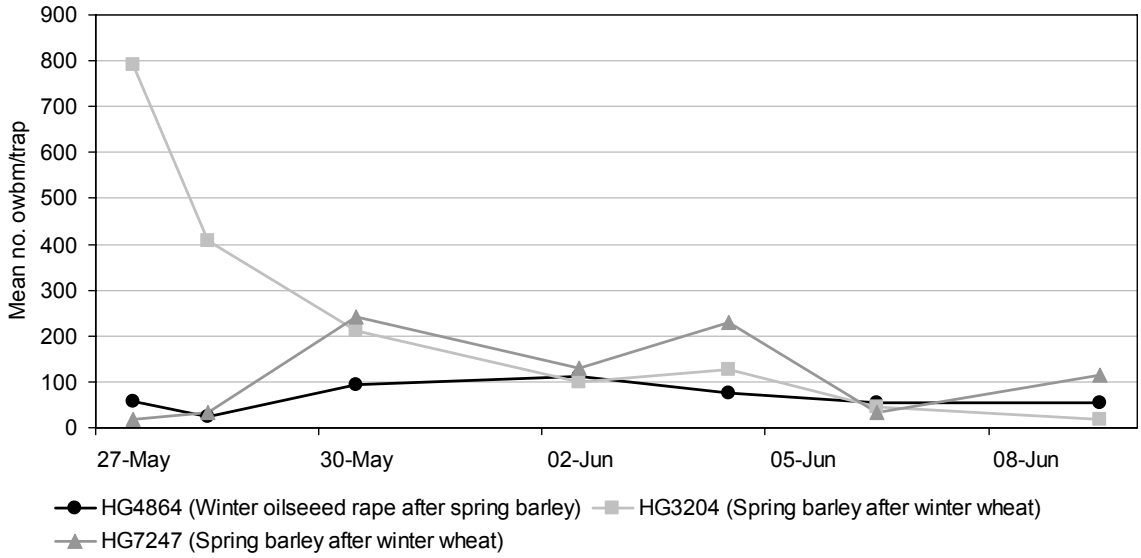


Figure 45. Owbm trap catches (adult males) from adjacent potential "source" fields during the susceptible period of each winter wheat field, Hampshire, 2008

Biscathorpe Farm House Louth, Lincolnshire

Table 15. Cropping of selected fields at Biscathorpe Farm House, Louth, Lincolnshire, 2008. Table shows pairing of sink and potential source fields which were adjacent e.g. 21 Acre (sink), Smithsons (source). All wheat varieties were susceptible to owbm

Field Name	Cropping 2008 (variety)	Cropping 2007	Cropping 2006
21 Acre	Winter wheat (Glasgow)	Winter oilseed rape	Spring barley
Smithsons	Winter barley	Winter barley	Winter wheat
Long Field	Winter wheat (Einstein)	Winter wheat	Winter oilseed rape
23 Acre	Winter barley	Winter barley	Winter wheat
Big Rounds	Winter wheat (Glasgow)	Winter oilseed rape	Spring barley
Little Rounds	Winter barley	Winter wheat	Winter wheat

In the wheat fields low levels of male owbm were first trapped on 30th May just prior to the beginning of ear emergence. Trap catches in all the wheat fields remained low during the susceptible stages of the crop with less than 25 midges per trap caught between 6th and 9th June when activity peaked in Long Field (Figure 46).

In the "non-wheat" fields field low levels of male owbm were also first trapped on 30th May. Trap catches remained very low during the susceptible stages of the adjacent wheat crops reaching a peak of less than 12 adults per trap caught between 6th and 9th June (Figure 47).

At this site very low levels of owbm (male and female) were caught on the yellow sticky traps. A maximum of 1.4 adults/ trap/day were caught between 6th and 9th June in Long Field whilst during the same time period 23.0 male adults were caught on pheromone traps in the same position.

Following low levels of adult midge infestation and trap catches the number of larvae per ear in each wheat field was very low at 0.16 in 21 Acres, 0.24 in Long Field and none in Big Rounds.

Trap catches and adult infestation at this site were too low to determine which fields were a potential source and which were a potential sink for adult midge.

Figure 46. Percentage ear emergence and owbm trap catches (adult males) during the susceptible period of each winter wheat field at Louth, Lincolnshire, 2008

Figure 47. Owbm trap catches (adult males) from adjacent potential "source" fields during the susceptible period of each winter wheat field, Lincolnshire, 2008

When the relationship between the number of trapped male midges and larvae per ear from all nine wheat fields (3 fields x 3 sites) in 2009 was investigated there was a surprisingly strong correlation between these factors (R^2=0.92), (Figure 48). However, these data should be treated with caution as most of the data from the sites represents low male catches and a low number of larvae/ear. When the data point (as circled on Figure 48) from the Hampshire site representing field HG8903 with 4.0 larvae per ear and a total of 2275 adults caught during the susceptible period of the crop was removed the correlation produced an R^2 value of 0.55.

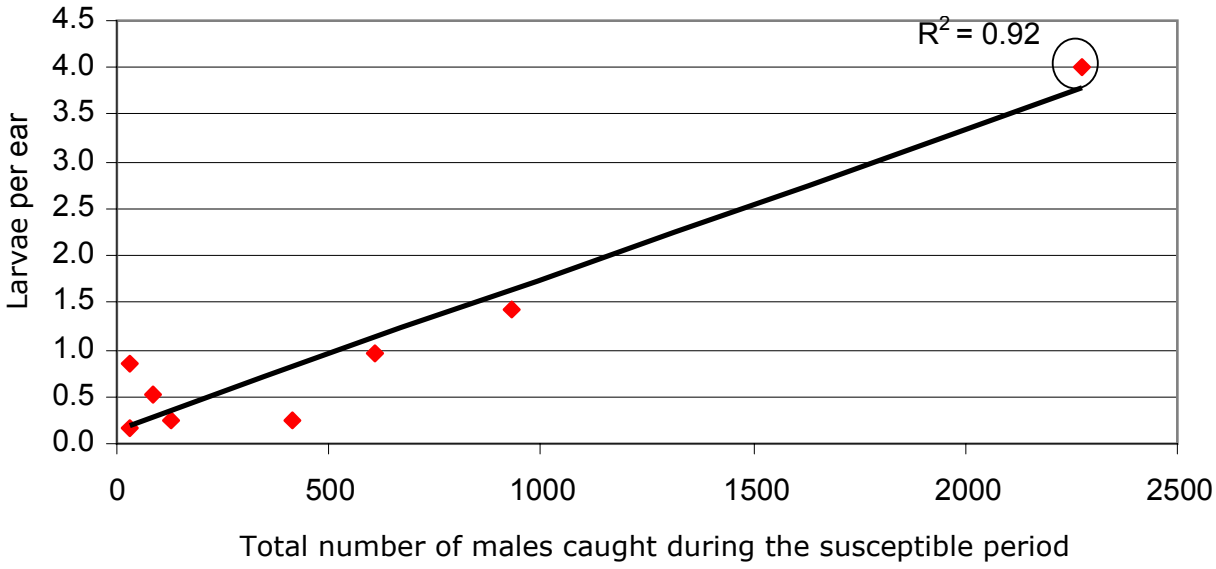


Figure 48. The relationship between larvae per ear and male adult emergence from all wheat fields at three sites (n=9) in 2008.

Discussion

In farm scale studies undertaken by Rothamsted, ADAS and TAG, there was much variation in pheromone trap catches from field to field. This emphasises the need to trap in individual fields rather than relying on data from small numbers of fields to be representative of the whole farm. It is also important to consider the movement of mated females from source fields where emergence

levels are higher. The variability in trap catches in the Rothamsted studies between fields was much greater than the variability observed within fields in the grid experiments. This suggests that it is more useful for farmers to put traps in neighbouring fields of second wheats or crops following wheat rather than in wheat fields.

In general, in 2006, 2007 and 2008 the peak of midge flight did not coincide with the susceptible stage of the crop. This meant that damage levels tended to be low. Pheromone traps were very valuable in indicating when to visit crops to look for female midges. This is a significant benefit over previous systems when much time could be wasted monitoring crops for midges unnecessarily.

B2. Female movement study

T J A Bruce, L E Smart, J A Martin - Rothamsted Research

Introduction

This experiment was conducted in 2005, 2006 and 2007 at Rothamsted farm to investigate the variability of pheromone trap catches within a field.

Materials & methods

The grid experiment was conducted between 2005-2007 in three different fields. An array of traps was set up on a uniform wheat field comprising a 6 x 5 grid with traps spaced 30 m apart. Pheromone traps were paired with yellow sticky traps for comparison. Traps were put out when the first wheat on the farm reached growth stage 47 (flag leaf sheath opening) and catches were recorded twice a week. Two pairs of pheromone and yellow sticky traps were also put out in each of the adjoining fields. At the end of the season infestation levels were assessed at each point in the grid. A total of 25 ears were taken at each trap site on the grid at growth stage 73 (milky ripe). Infestation across the field was related to pheromone trap catches within the field and in the adjacent fields.

2005 The field site was Summerdells I, in which the variety Consort was grown. It was a undulating field flanked by set aside, barley and wheat (Consort) fields on three sides and a hedgerow and road on the other side.

2006 The field site was Great Knott III, and the variety was Option. It was a flat field, with a slight downward slope to the south, flanked by wheat (Hereward) fields on two sides, a wheat headland (Option) on the third side and a track beyond which was a pea field following wheat on the fourth side.

2007 The field site was Great Harpenden I, and the variety was Option again. It was a flat field, with a slight downward slope to the south, flanked by wheat fields, varieties Hereward and Brompton, to the south and east respectively, and winter and spring sown beans following wheat to the north and west.

Results

2005

Figure 50 shows the layout of the grid experiment and the surrounding area. Mean pheromone trap catches were below 10 midges per trap per day until 21st June (Figure 51). There was a small peak on the 10th June. The crop was in the susceptible ear emergence growth stage (53-59) from 3rd-10th June after which it started anthesis. This meant that the bulk of owbm emerged too late to cause serious damage to the
crop.

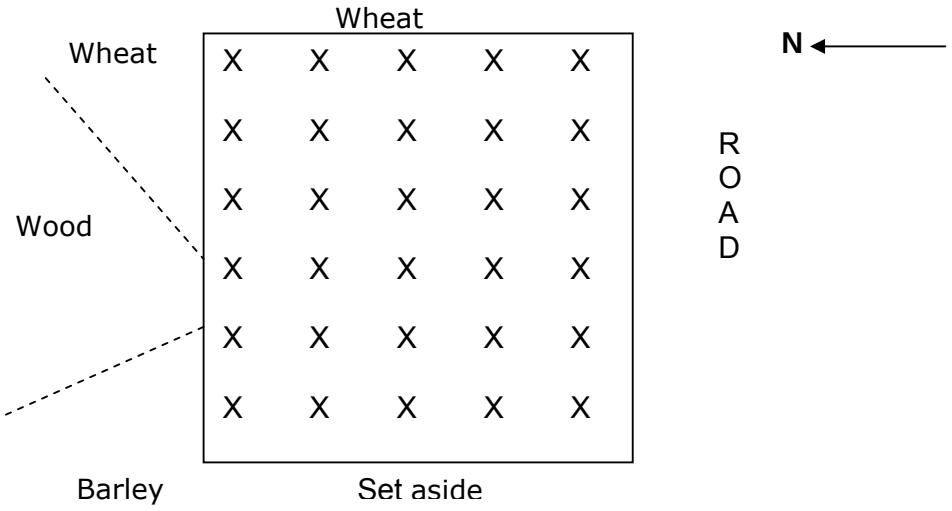


Figure 50. Grid experiment site Summerdells 1, 2005

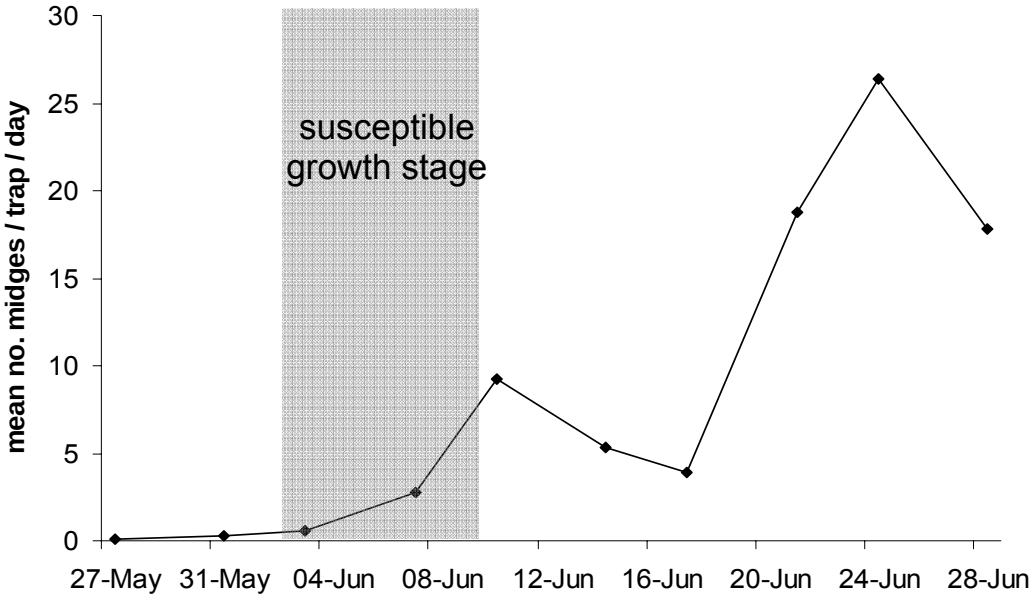


Figure 51. Mean trap catches in the Summerdells 1 grid experiment 2005

Trap catches for each date were mapped on 3D charts and the spatial pattern was observed. Catches were variable to start with when the numbers were low but from 10th June onwards they were more uniform across the field. The crucial time, however, was when the crop was in the susceptible growth stage and the spatial plot for this period is shown in Figure 52. From this it appeared that there were more owbm on the south side of the grid but trap catches only varied by a

factor of two or three across the grid. This level of variation was far less than that observed in going from field-to-field in the farm scale study.

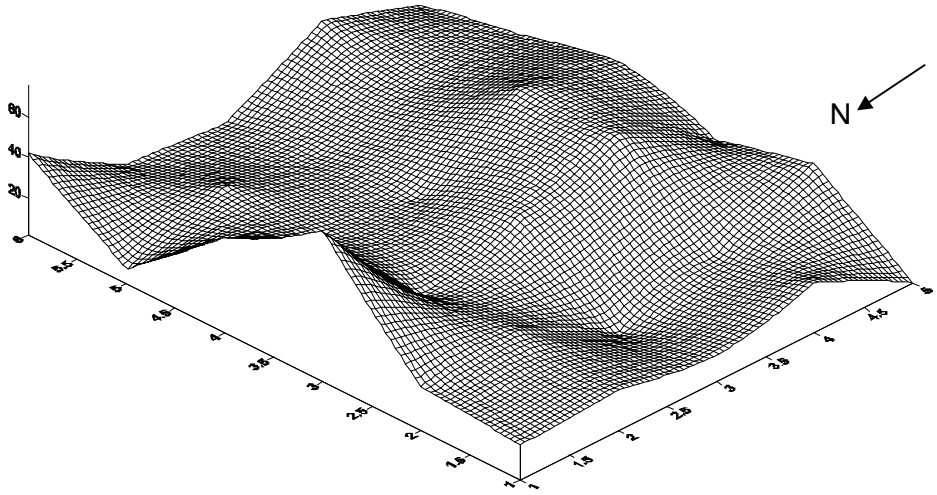


Figure 52. Pheromone trap catches in the grid experiment 7-10/06/05

Trap catch data for both pheromone and yellow sticky traps were analysed by SADIE (Perry *et al.* 1999) and presented as red-blue plots (Figure 53 and 54) during the period in which the crop was at the susceptible growth stage. In these, the red areas show clusters where catches are higher than average, which is white, and the blue areas show where catches are lower than average. For the pheromone traps on both sampling occasions during the susceptible growth stage, the analysis showed quite a uniform pattern across the grid. The yellow sticky traps showed a patch cluster on both occasions but this was in a different place each time.

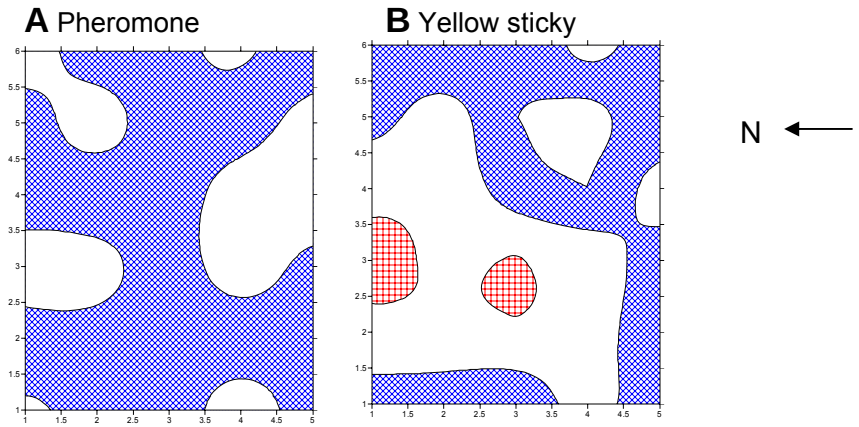


Figure 53 SADIE analysis of pheromone and yellow sticky trap catches on the grid experiment 3-7/6/05

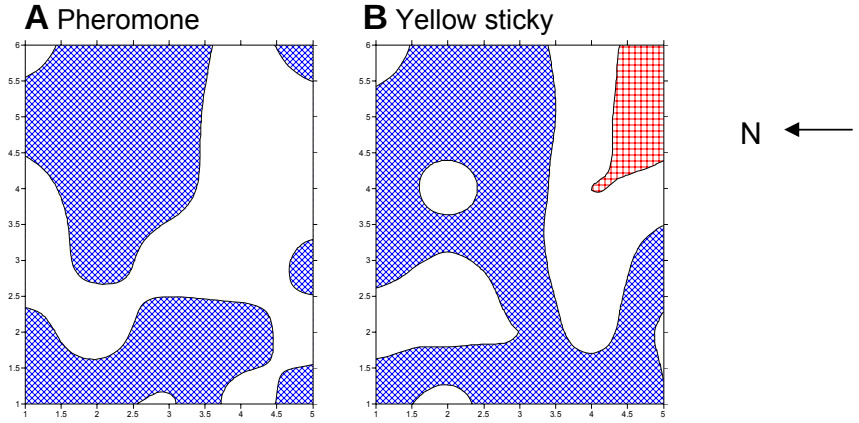


Figure 54. SADIE analysis of pheromone and yellow sticky trap catches on the grid experiment 7-10/6/05

These data were then related to infestation level at the end of the season, which was highest on the north side of the grid (Figure 55). There was a good agreement between the areas with the highest numbers of females on 7 June on the yellow sticky traps and the infestation level. However, there was no apparent relationship with males caught on pheromone traps within the grid. Pheromone traps situated in the adjoining wheat fields (Figure 50) caught high numbers of males but the yellow sticky traps paired with them did not catch many females suggesting that emigration occurred from these fields into the grid experiment. Infestation levels in the crop were better explained by pheromone trap catches

in neighbouring source fields than by considering variation in trap catch within the field.

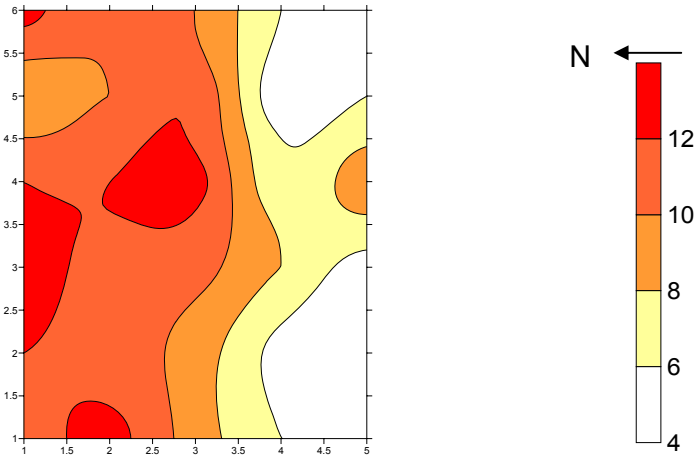


Figure 55 Infestation level in 2005 grid experiment (mean no. larvae per ear)

2006

As in the 2005 experiment, a 6 x 5 grid of pairs of pheromone and yellow sticky traps was put out at growth stage 47 on 23rd May and catches were recorded twice a week until 20 June. Two pairs of pheromone and yellow sticky traps were also put out in each of the adjoining fields (Figure 56). On 27 June, during the milky ripe growth stage, 25 ears were taken at each point in the grid to assess infestation levels.

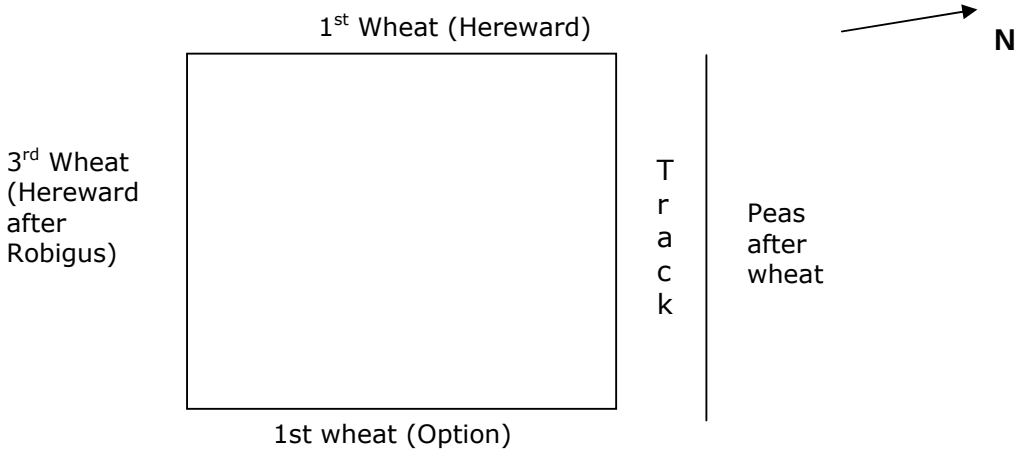


Figure 56. Grid experiment site Gt. Knott 3, 2006

The Option in the grid was at the susceptible growth stage from 30 May to 6 June, when anthesis began. However, at this time pheromone trap catches were well below the peak (Figure 57) indicating that the vast majority of emergence occurred too late for the insect to threaten the crop.

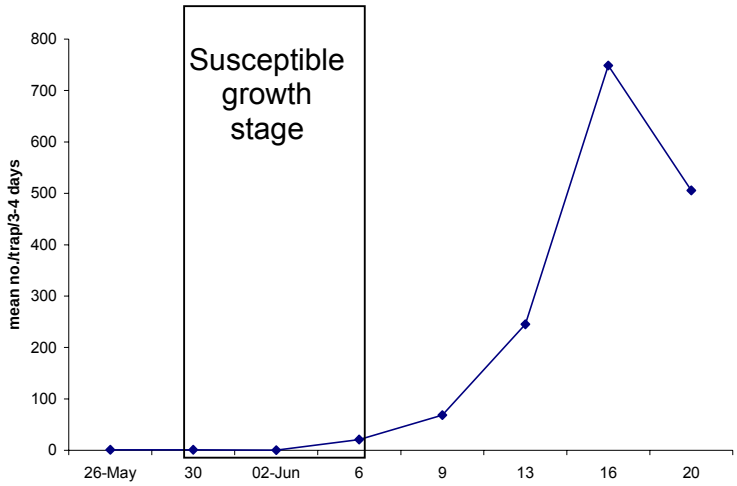


Figure 57. Mean pheromone trap catches in the 2006 Grid Experiment

Contour maps of pheromone trap catches during the susceptible growth stage (2 June, Figure 58) and subsequent infestation levels during the milky ripe stage after egg hatch (Figure 59) were plotted and were generally in agreement. The strongest trend was the high trap catch and infestation level along the northern edge of the field, which was bordered by a pea crop following wheat. Pheromone trap catches in the pea field were many times higher than in the wheat, demonstrating that this field was a probable source of owbm moving into the grid experiment. This again showed that more useful information was obtained from monitoring traps in adjacent fields than from additional traps in the grid field. Infestation levels were well below the economic threshold.

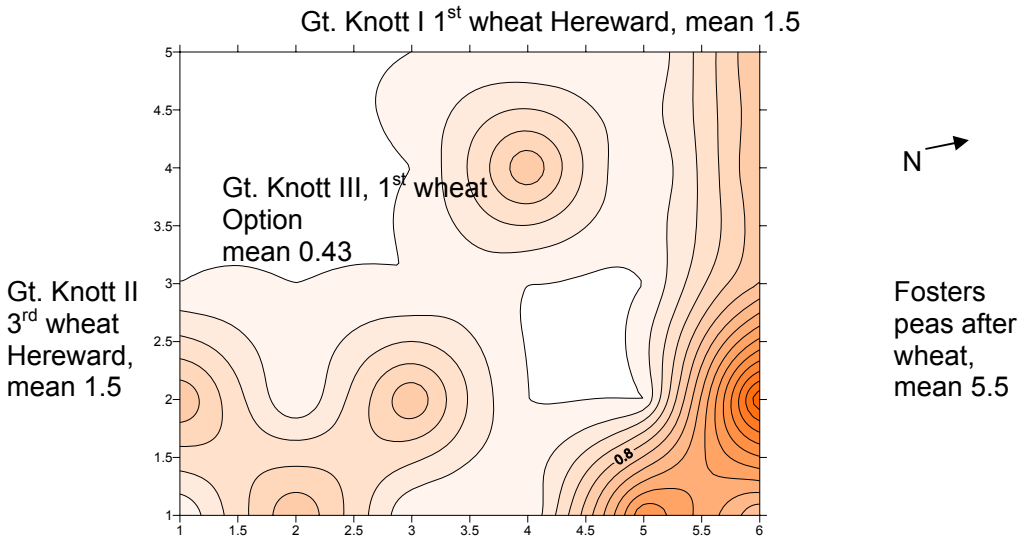


Figure 58. Mean number male of owbm in pheromone traps 2.6.06 (ear emergence)

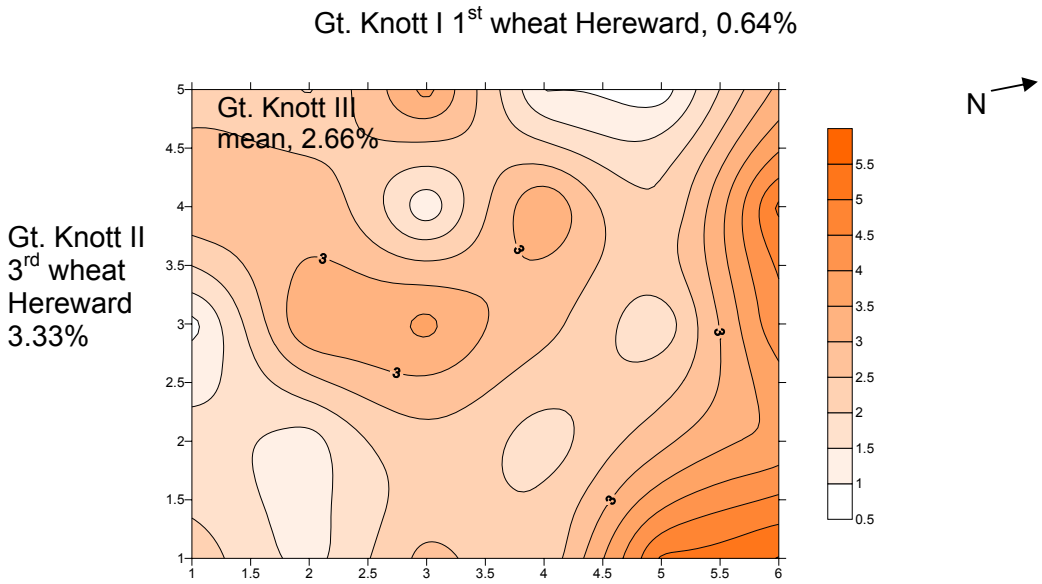


Figure 59. Percentage affected grain in grid experiment 27.06.06

2007

In 2007, the 6 x 5 grid experiment was repeated with paired traps in Great Harpenden I with the variety Option. The field was flanked by wheat fields with the varieties Hereward and Brompton, to the south and east respectively, and winter and spring sown beans following wheat to the north and west. Traps were

put out at growth stage 47 on 16 May and catches were recorded twice a week until 25 June (Figure 60). Two pairs of pheromone traps and yellow sticky traps were also put out in each of the adjoining fields. On 20 June, during the milky ripe growth stage, 25 ears were taken at each point in the grid to assess infestation levels.

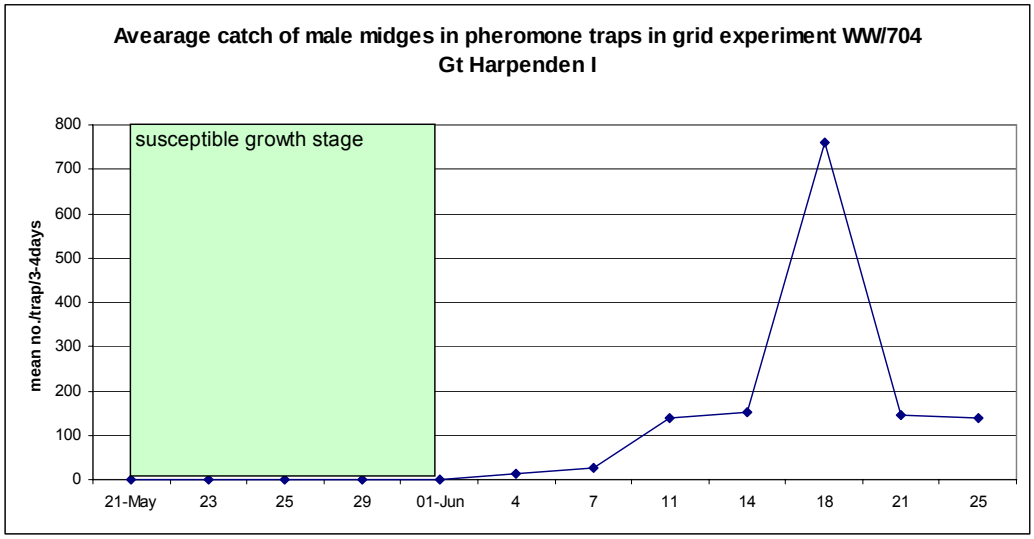


Figure 60. Mean pheromone trap catches in the 2007 grid experiment, Gt. Harpenden 1

The susceptible growth stage of Option was earlier than in 2006 and the main peak of owbm emergence was even later, occurring much too late for the insect to threaten the crop and resulting in no infestation. This meant that in 2007 comparisons of trap catch data with subsequent infestation levels could not be made. In addition, a change in the design of the sticky inserts in the pheromone traps resulted in an increase in trap catches where these inserts were used and confounded the mapping of emergence. This was the final year of the grid experiment.

Overall, the grid experiments showed that trap catch did not vary very much across individual fields. Results from 2005 and 2006 clearly demonstrated the value of monitoring adjacent fields that could act as sources of infestation and this information was built into the decision support model.

Discussion

In experiments with a grid of pheromone traps in one wheat field plus additional traps in adjacent fields, infestation levels in the crop were better explained by pheromone trap catches in neighbouring source fields than by considering variation in trap catch within the field. It was found that in the grid experiment that there was not very much trap catch variation across individual fields. Results from 2005 and 2006 clearly demonstrated the value of monitoring adjacent fields that could act as sources of infestation and this information was built into the decision support model.

Task C Biochemistry of tolerance and resistance

C1. Biochemical study of model varieties

T J A Bruce, L E Smart, J A Martin – Rothamsted Research

Introduction

In order to explore the apparent differences in varietal characteristics leading to differential susceptibility to owbm infestation, a replicated field experiment was done at Rothamsted using six varieties (Claire, ECO22, Einstein, Option, Tanker and Welford) with similar heading dates but different characteristics in terms of susceptibility, tolerance and resistance. Also, as it has been suggested that wheat resistance to owbm may be due to phenolic acids, grain samples were taken from the variety trial to determine the levels of these products in susceptible and resistant varieties.

Materials & Methods

Variety trial

In order to explore the apparent differences in varietal characteristics leading to differential susceptibility to owbm infestation, a replicated plot field trial was done at Rothamsted, in 2006, 2007 and 2008. A group of six varieties with similar heading dates (Claire, ECO22, Einstein, Option, Tanker and Welford), but

with different characteristics in terms of susceptibility, tolerance and resistance to owbm attack, were grown in 12 x 12m plots in a 6 x 6 quasi-complete Latin square design. In an additional trial, the insecticide chlorpyrifos was applied to one half of two split plots of each variety to estimate the yield loss associated with infestation.

The activity of owbm was measured using a pheromone trap and a yellow sticky trap placed at the centre of each plot in the first year. Subsequently, paired pheromone and yellow sticky traps were deployed in a headland, and just a single yellow sticky trap at the centre of each plot. Traps were changed twice weekly throughout late May to mid-June and the number of larvae developing to the second instar was assessed on 25 ears per plot at the early milky ripe stage (GS 73).

Assessment of phenolic acids in grain samples from variety trials

It has been suggested that wheat resistance to owbm may be due to naturally high or inducible levels and rates of production of the phenylpropanoids, specifically phenolic acids, in particular ferulic and *p*-coumaric acids (Ding *et al.*, 2000). Thus, resistant varieties such as Welford may produce constitutively high levels of phenolic acids in the seed coat and are therefore unsuitable hosts at all growth stages, exhibiting an antibiosis to the larvae, which fail to develop and eventually die. Alternatively, larval feeding may induce increased production of phenolic acids resulting in similar antibiotic effects. Ding *et al.* (2000) demonstrated that levels of ferulic and *p*-coumaric acids change rapidly as grain develops, the former rising as the latter declines, until both levels stabilise 15 days after the onset of anthesis. In order to understand the biochemical basis of varietal tolerance/resistance of the wheat in the variety trials, ears were labelled at the onset of anthesis and the grain samples were collected 10 and 17 days later. The grain was examined and separated into the following categories: i) grain from uninfested ears, and ii) infested grain (including those of Welford showing callous development). Once categorised, the grain samples were placed in liquid nitrogen and then stored deep frozen at -80°C until extraction.

To extract grain samples for each variety, three, approximately 200 mg samples of grain of similar size were weighed and placed into a mortar, to which 3ml of 2M NaOH was added. The sample was then ground until the tissue was fully crushed. The solution was transferred to a 50ml centrifuge vial and the mortar was rinsed twice with 2ml 2M NaOH solution. The vials of extracted samples were transferred to a water bath at 37°C and left for two hours after which 2M HCl was added to each vial slowly until the pH reached 1. Ten ml of AR diethyl ether was added to each vial, which was shaken gently to extract the sample. The vials were then centrifuged for four minutes at 2000rpm after which the ether layer was transferred to a round bottomed flask. The sample was extracted twice more with 10 ml ether and the ether layers were combined. A spoonful of $MgSO_4$ was then added to remove any water and the ether was transferred to a fresh round bottomed flask. Samples were then evaporated to dryness on a rotary evaporator and taken up in three lots of 1 ml of diethyl ether and transferred to a 4 ml glass vial from which the ether was evaporated to dryness under nitrogen. After problems with samples taken in 2006, all samples from the 2007 and 2008 trials were diluted by adding 500 µl of diethyl ether and shaking gently to take up the dry sample. 50 µl of this was then transferred to a fresh vial and evaporated to dryness under nitrogen. In order for samples to be analysed by Gas Chromatography (GC) they were derivatised by adding 100 µl of MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) to each dry, diluted sample and the vials were then heated in a dry heat block at 60°C for 1hour. Standard solutions of ferulic and *p*-coumaric acids were made up and derivatised in the same way. Samples were analysed directly by GC-FID (1 µl injections) and compared to the ferulic and *p*-coumaric acid standards to determine whether levels of these phenolic acids varied between varieties and in response to owbm larval feeding and thus, whether they are inducible. Co-injection with standard was used to confirm the ferulic and p-coumaric acid peaks in the samples.

Results

Variety trial

2006

In 2006 the experiment was in Little Hoosfield. The activity of owbm was measured using a pheromone trap and a yellow sticky trap placed at the centre of each plot on 24 May. Traps were changed every three-four days throughout late May to mid-June. The number of eggs laid was assessed on 20 ears per plot on 14 June, and the number of second instar larvae was assessed on 25 ears per plot on 27 June. ECO22, Einstein and Option started ear emergence on 29-30 May and began anthesis on 6 June, while Claire, Tanker and Welford started ear emergence on 2 June and anthesis on 9 June.

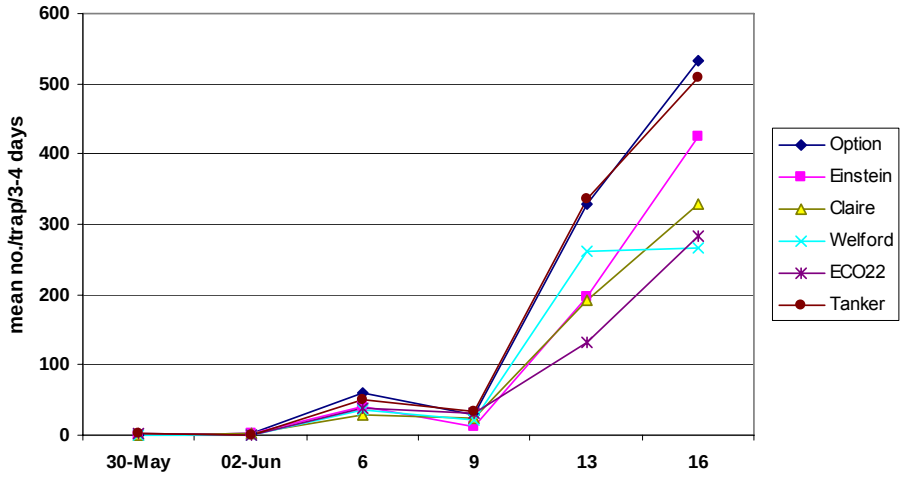


Figure 61. Pheromone trap catches on variety trial 2006

Catches of male owbm in pheromone traps were very low until 6 June, when the more advanced varieties were already starting anthesis (Figure 61). Mean trap catches differed between the varieties, but there were no significant differences when tested by ANOVA. The peak emergence of males did not occur until well after the susceptible growth stage, which coincided with the peak seen in the emergence traps (see Task B). The map of pheromone trap catch (Figure 62) showed evidence of immigration of males into the trial site because trap catches

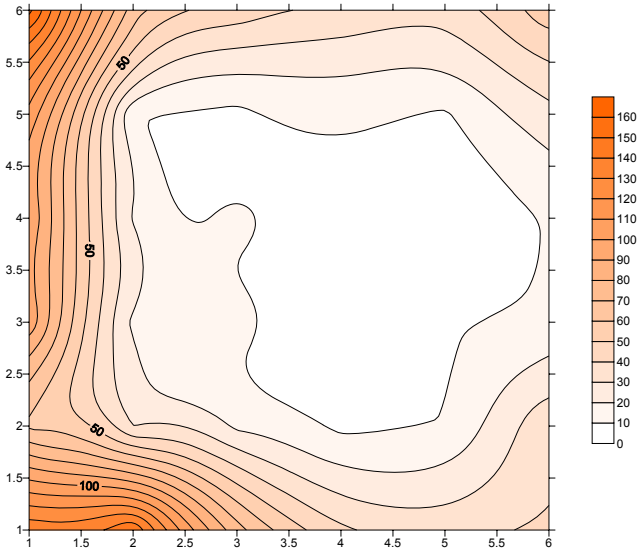


Figure 62. Map of males in pheromone traps on the variety trial on 6 June 2006

were higher around the edges of the field. It is more likely that this was from within the same field because physical barriers such as high hedges, a road and a row of houses in between would have reduced attraction from neighbouring fields. The ultimate trap catches on the variety trial site were so large (Figure. 62), compared to the mean peak trap catch in wheat fields over the farm (153 males), that it is likely that the trial was achieving mass trapping of males and possibly some mating disruption during the susceptible growth stage.

Catches of female owbm on yellow sticky traps were variable, very low and peaked well after the susceptible growth stage (Figure 63). They reflected the overall low infestation levels in the experiment (Figure 64), but did not relate well to infestation levels per variety.

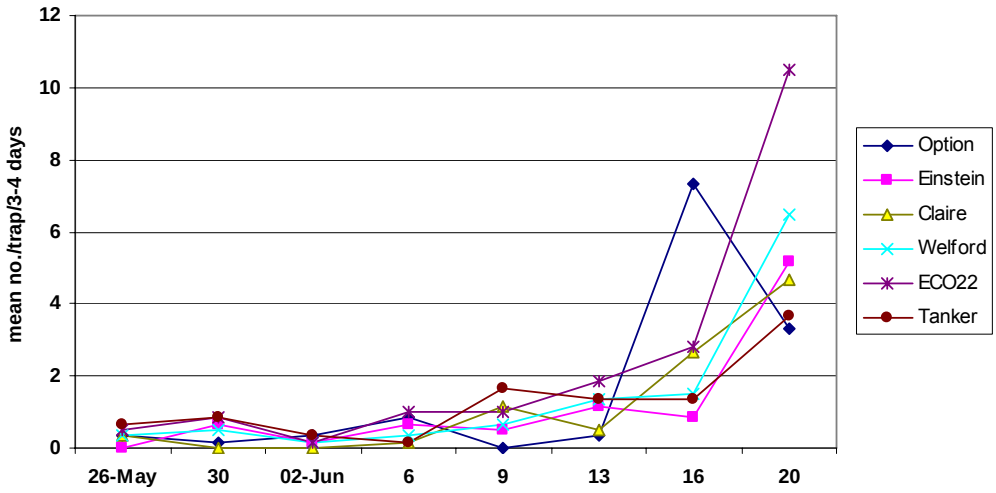


Figure 63. Female owbm on yellow sticky traps on the variety trial 2006

Infestation assessment 14 June 2006

Despite the low infestation, ECO22 had significantly more infested grain than Claire and Welford (Figure 64). It also had a larger number of eggs than any of the other varieties (Figure 65), but this difference was not significant due to variability in egg distribution. The resistant variety Welford had similar numbers of eggs compared to the other varieties, but significantly fewer owbm larvae than the other varieties except for Tanker (Figure 65). ECO22 had the largest number of owbm larvae and Option and ECO22 had the most ywbm larvae (Figure 65).

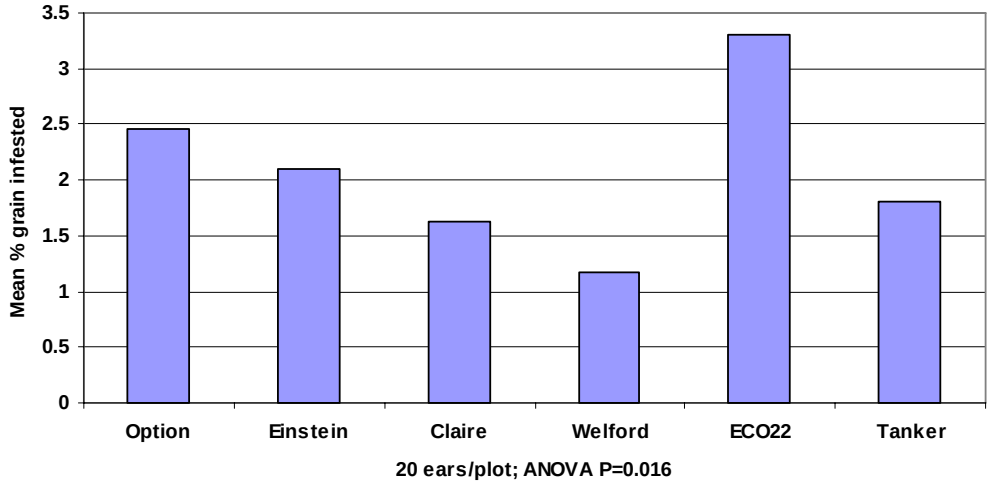


Figure 64. Percent infested grain on variety trial, 14 June 2006

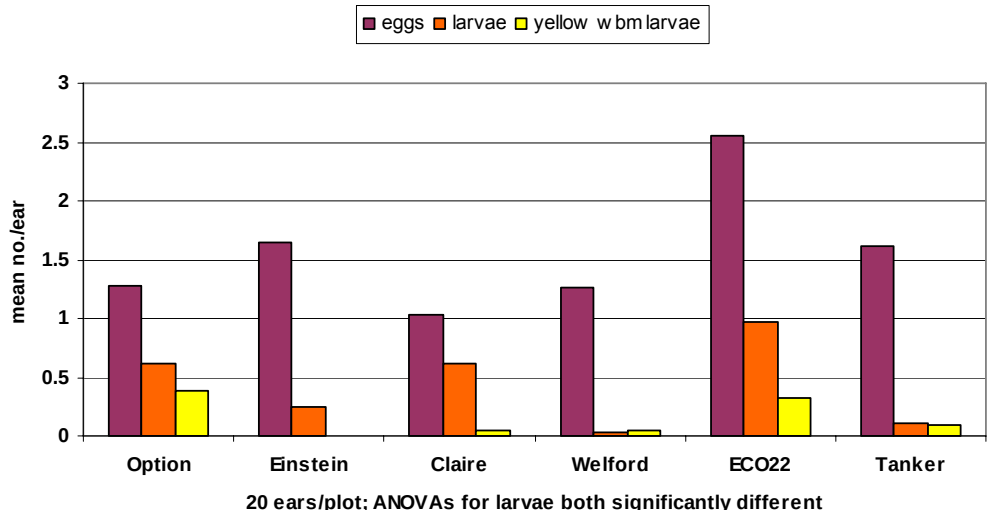


Figure 65 Eggs and larvae on variety trial 14 June 2006

Infestation assessment 27 June 2006

ECO22 had significantly more infested grain than Einstein, Claire and Welford on 27 June (Figure 66). There was no significant difference in infestation between Claire and Welford. ECO22 and Option had significantly more second instar owbm larvae than Einstein, Claire and Welford, the latter having significantly

fewer large larvae than all the other varieties (Figure 67). However, there were no significant differences in egg numbers and ywbm larvae between varieties.

ECO22 had consistently higher levels of infestation and larval numbers than most of the other varieties, indicating a female owbm preference for this variety as seen in earlier olfactometer experiments with air entrainment samples. The resistant variety Welford had the lowest levels of infestation as expected, but there was no difference in the number of eggs laid by female owbm on this variety, compared to the others, suggesting that females do not recognise the resistance. Claire and to a lesser extent Einstein had comparatively lower levels of infestation than the remaining three varieties.

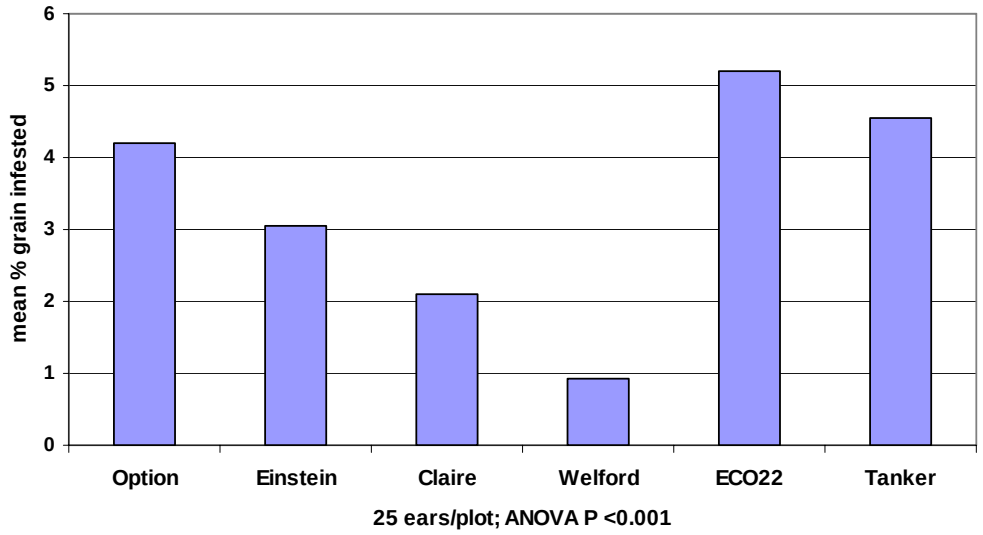


Figure 66. Infested grain on the variety trial 27 June 2006

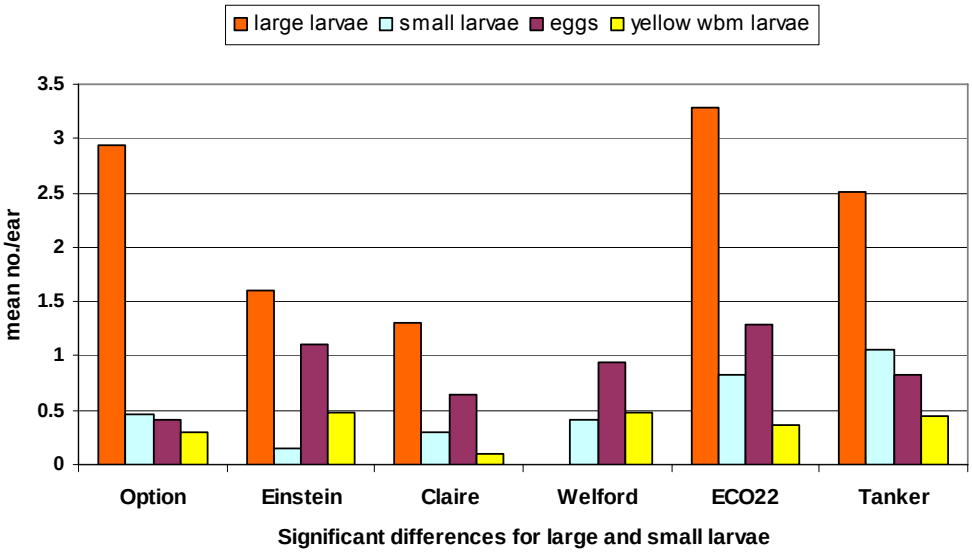


Figure 67. Egg and larval infestation on the variety trial 27 June 2006

Yield

Since the infestation levels were so low (maximum 5.2% for ECO22) there were no significant differences for yield due to owbm damage between the varieties and thus no yield improvement with insecticide treatment (Figures 68 & 69). ECO22 yielded significantly less than the other varieties in the main trial due to severe lodging (Figure 68), but was not significantly different in the trial with chlorpyrifos (Figure 69).

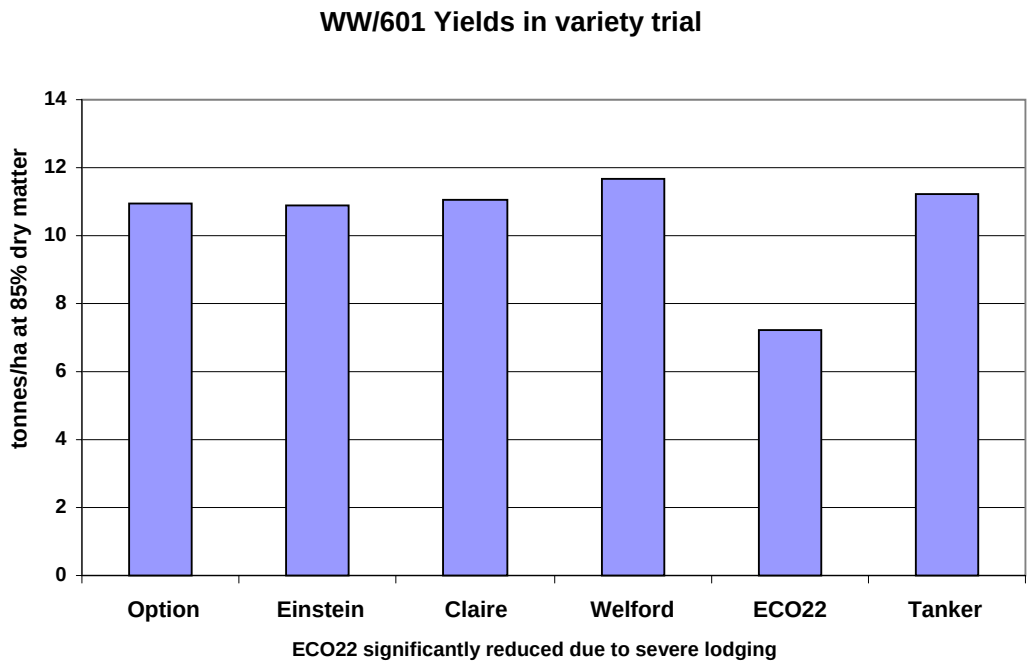


Figure 68. Yield in the variety trial WW/601 2006

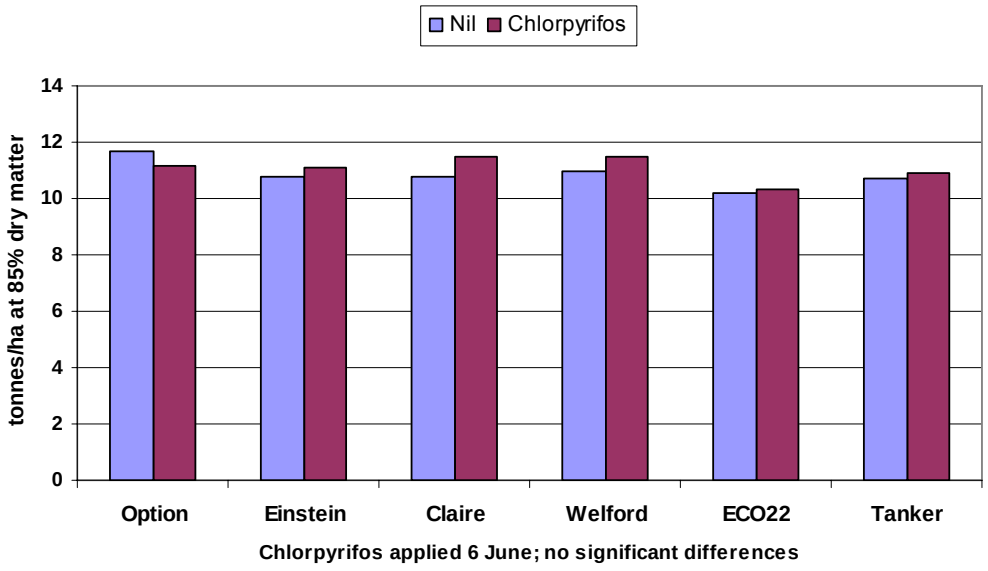


Figure 69. Comparison of yields with and without insecticide treatment 2006

2007

In 2007 (WW/703) infestation levels were extremely low with a mean infestation well below 1% attacked grain and there were no significant differences between varieties, although Claire had the lowest rate of attack. Due to this, data for 2007 are not included in this report. Thirty ears per variety were labelled at the start of anthesis for the phenolic acid study but, due inadequate infestation on labelled ears, this study was eventually conducted on late developing secondary tillers (see below).

2008

A third replicated plot variety trial (WW/804) was conducted on Great Harpenden 1 field at Rothamsted in 2008 to explore differential susceptibility to owbm infestation. The same group of wheat varieties (Claire, ECO22, Einstein, Option, Tanker and Welford) were again grown in a 6 x 6 quasi-complete Latin square design. In an additional trial, the insecticide chlorpyrifos was applied to one half of two split plots of each variety to estimate the yield loss associated with infestation.

The activity of owbm was measured using a yellow sticky trap placed at the centre of each plot on 21 May. Traps were changed every three to four days throughout up to mid June. Thirty tillers of each variety were labelled at the onset of anthesis. Half of the ears from the labelled plants were collected after 10 days and the other half after 17 days and the number of eggs and larvae present and % attacked grain was assessed. Infested and uninfested grain samples from each variety at each sample date were placed in liquid nitrogen and stored at -80°C for subsequent assessment of phenolic acid content (see below). A further 24 ears per plot were taken and assessed for infestation on 26 June.

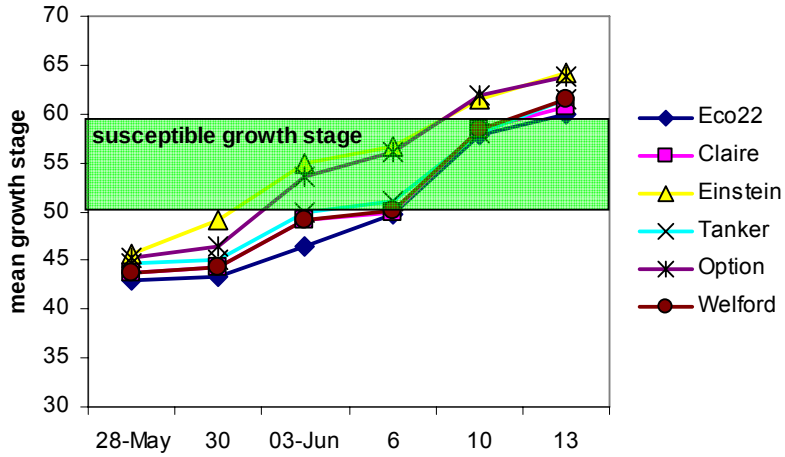


Figure 70. Development of varieties in WW/804 2008

Einstein and Option started ear emergence earlier than the other four varieties (2 June compared to 6 June) and began anthesis on 9 June compared to 12 June for the other varieties (Figure 70). Numbers of female owbm caught on yellow sticky traps peaked on 6 June when Einstein and Option were at the susceptible growth stage, but the other varieties were just at ear emergence (Figure 71).

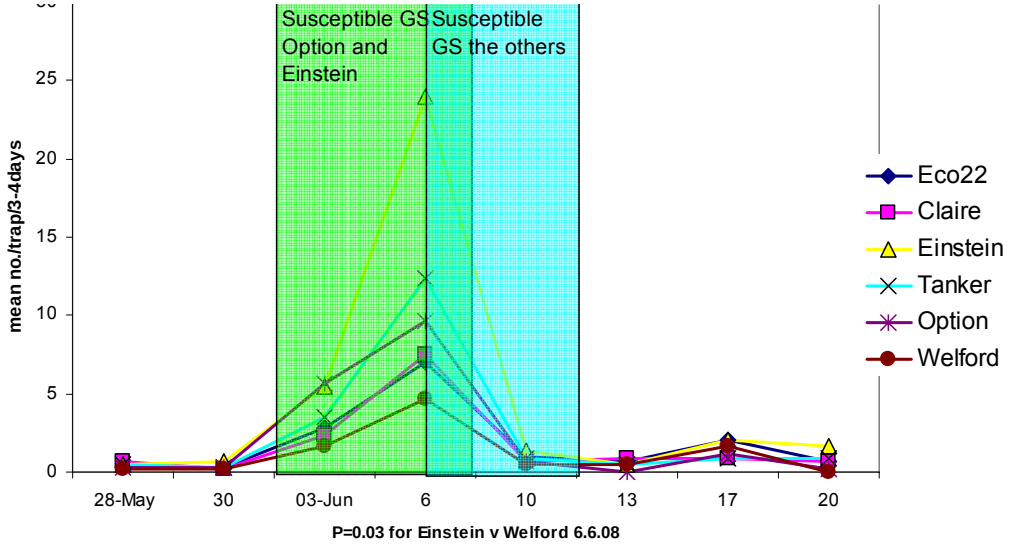


Figure 71. Female owbm caught on yellow sticky traps in 2008 variety trial

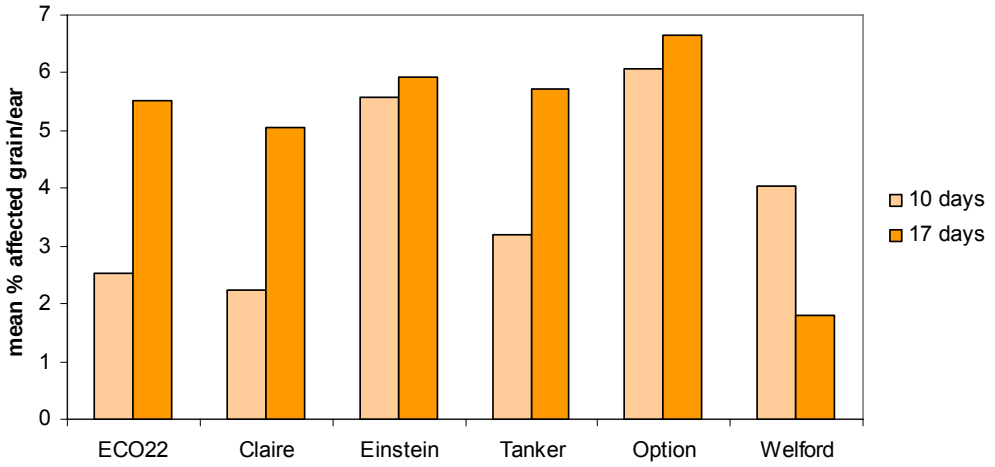


Figure 72. Percentage infestation 10 and 17 days after start of anthesis

Levels of infested grain in these small samples were similar at 10 and 17 days for the two earlier varieties, but there was an increase in infested grain at 17 days for Claire, Tanker and ECO22 (Figure 72). There was no difference in infestation at 17 days between varieties except for Welford, which showed a decrease in infestation probably because it had no large larvae and the small larvae that were found were dead (Figures 73 & 74). It did have eggs on both dates confirming that females were equally attracted to this resistant variety. The two early developing varieties, Einstein and Option, had larger numbers of ywbm larvae than the others (Figures 73 & 74).

There was a good correlation between numbers of female owbm caught on yellow sticky traps on the 6th June and subsequent infestation levels (Figure 75).

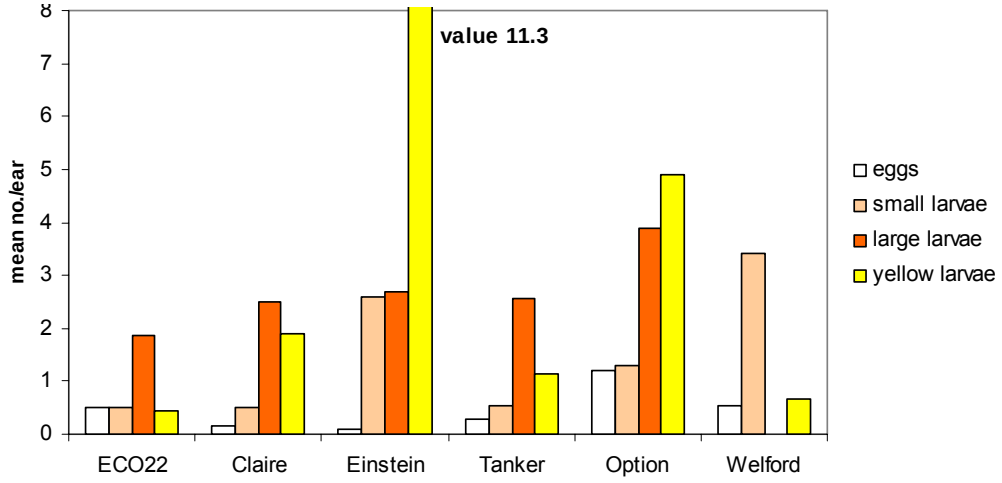


Figure 73. WW/804 Eggs and larvae 10 days after start of anthesis (19-23.6.08)

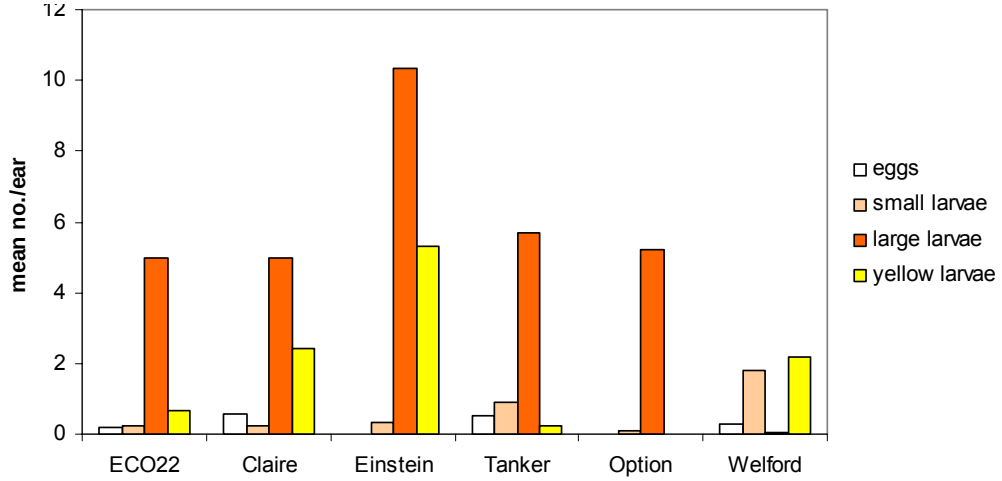


Figure 74. WW/804 Eggs and larvae 17 days after start of anthesis (26-30.6.08)

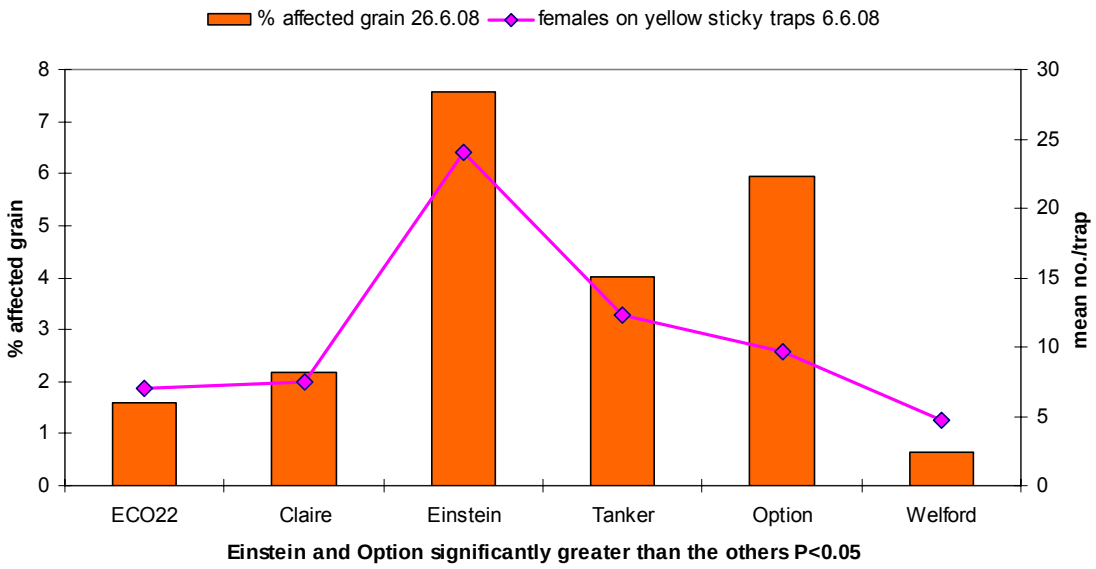


Figure 75. WW/804 owbm females on sticky traps (6.6.08) and subsequent infestation

Yield

There was no significant difference between the yields (Figure 76) of the varieties and no significant effect of chlorpyrifos treatment on yield in the split plot experiment (Figure 77), confirming that levels of infestation were below the damage threshold.

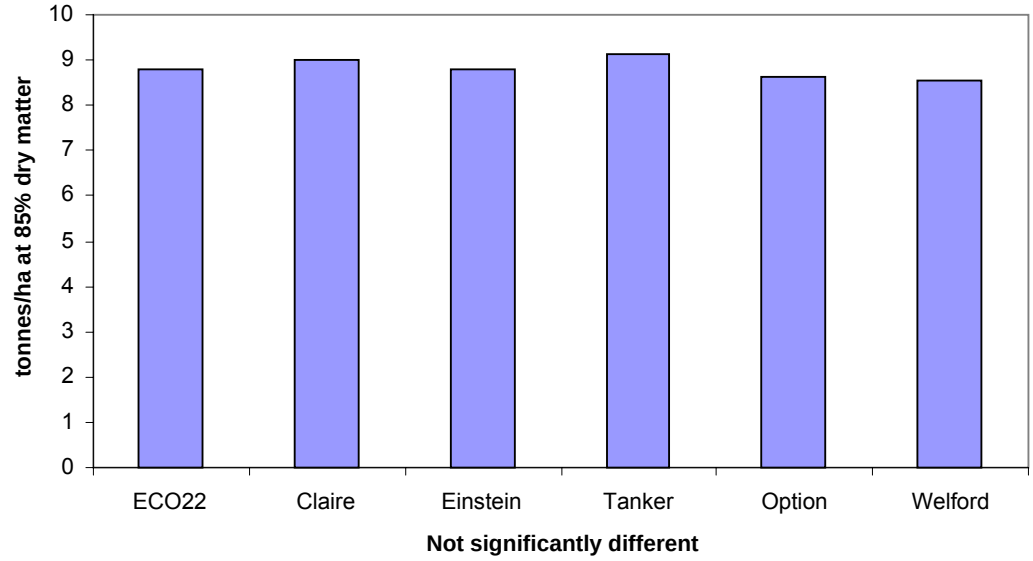


Figure 76. Yield in varieties trial WW/804 2008

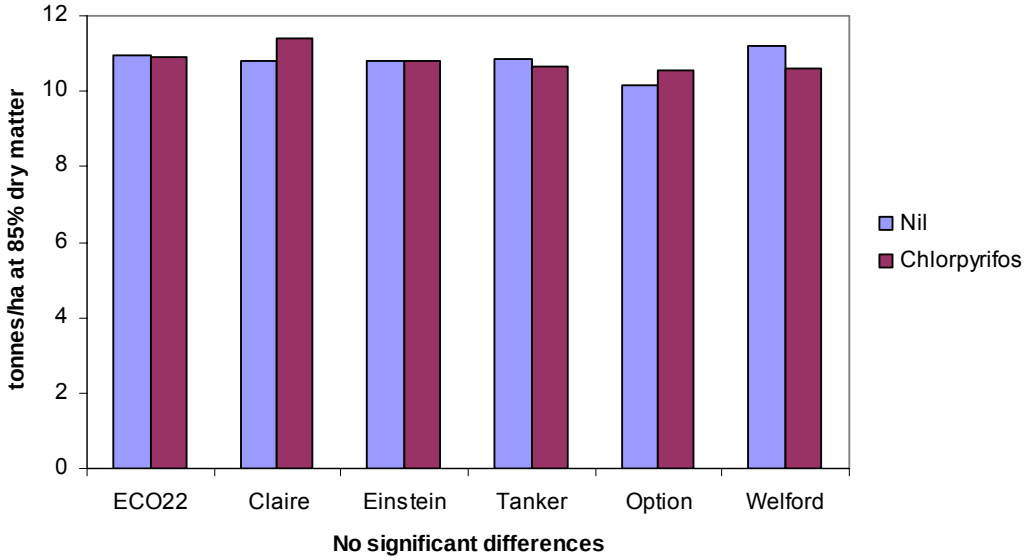


Figure 77. Yield response in split-plot varieties trial WW/804 2008

Assessment of phenolic acids in grain samples from variety trials

In 2006, the whole extracted samples of grain taken from the variety trial WW/601 were derivatised. However, analysis of these samples was delayed and they were left in a deep freezer for some time after which they were found to have deteriorated and results were therefore unreliable. The derivatised standard solutions were also shown to deteriorate after only a few days. From then on, all extracted grain samples were diluted before derivatisation and analysed immediately afterwards and fresh standards were prepared for each batch.

In 2007, there was almost no owbm infestation, resulting in insufficient grain to extract from the labelled ears. However, there were late flowering secondary tillers in most plots, which were lightly infested and these were collected on 21 June and extracted, although the timing of anthesis was not known. There was an apparent induction of levels of *p*-coumaric acid in infested seed of Option, Claire and to a lesser extent Tanker (Figure 78) and an induction of ferulic acid levels in infested Option and Welford (Figure 79). However, since the period of development of these seed batches after anthesis was unknown, no firm conclusions can be drawn from these results.

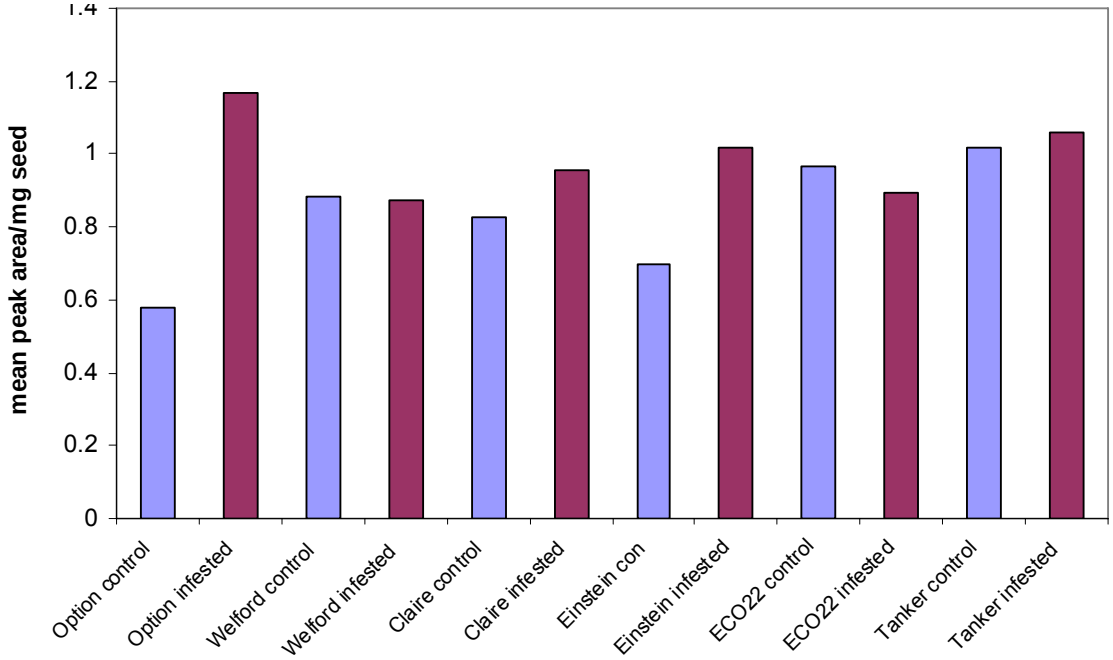


Figure 78. GC analysis of *p*-coumaric acid in wheat seed extracts WW/703 (2007)

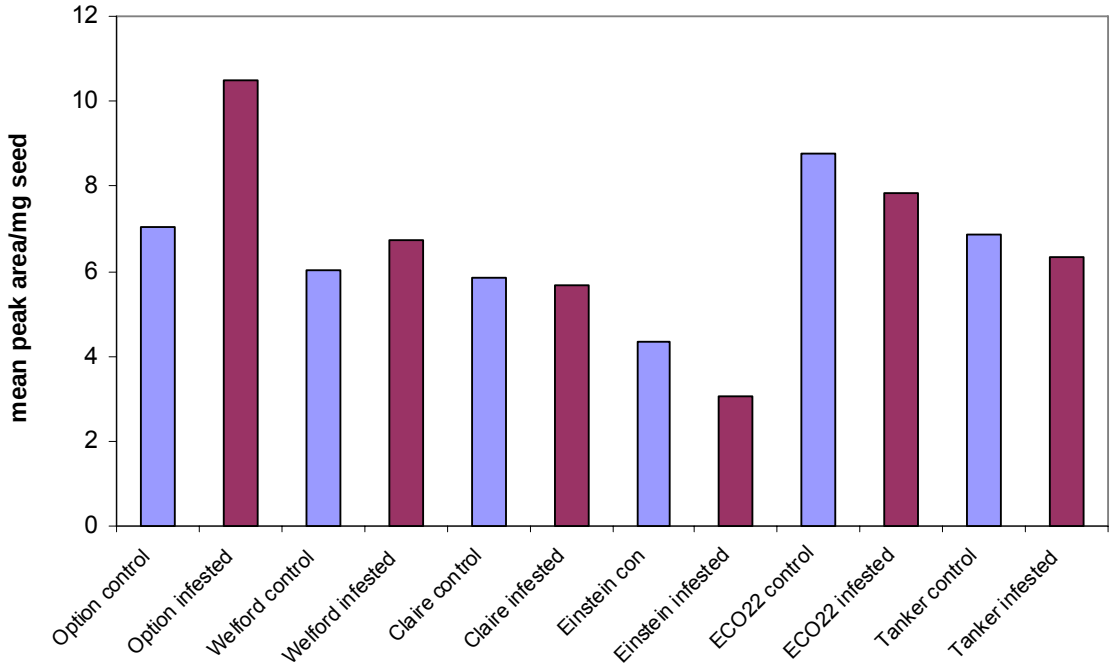


Figure 79. GC analysis of ferulic acid in wheat seed extracts WW/703 (2007)

In 2008, infestation was low, but sufficient infested grain was obtained for extraction. Analysis concentrated on the 10 day grain samples as these should

give the greatest difference between lines (Ding *et al.*, 2000). Levels of *p*-coumaric acid were greater in the infested samples of each variety than in the uninfested (Figure 80), indicating that owbm damage is inducing production of this acid in the seed of both the susceptible and resistant varieties. Infested Welford had the highest level of *p*-coumaric acid. Levels of ferulic acid were higher in infested grain of Option, Welford, Einstein and ECO22 compared to uninfested grain, but there was no difference or a slight decline in levels in infested Claire and Tanker (Figure 81). This confirmed the result for Option and Welford from the 2007 grain samples. The small levels of induction of phenolic acids are probably insufficient to explain the big difference in owbm larval survival in Welford compared to the other varieties and these data suggest that there might be other additional mechanisms resistance.

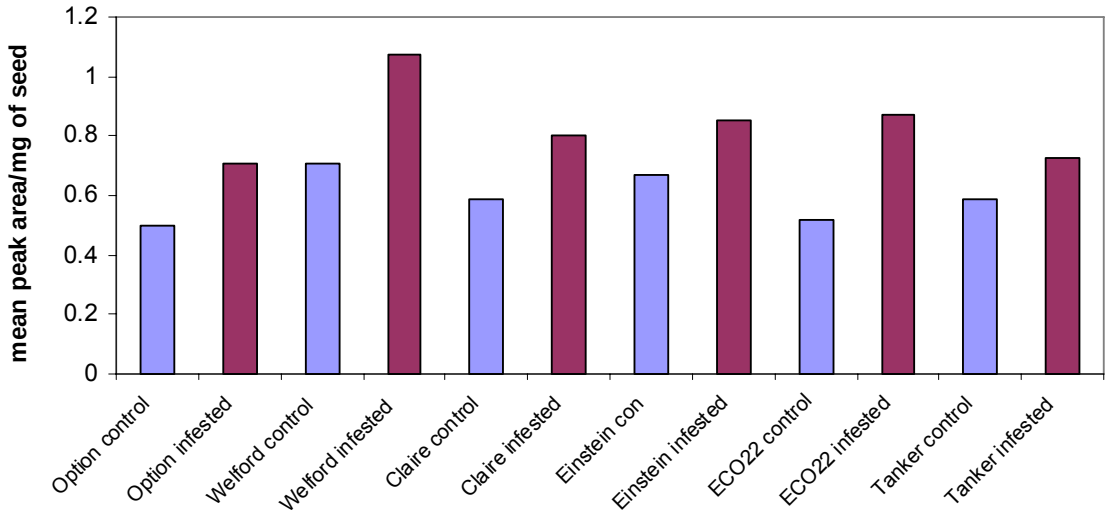


Figure 80. GC analysis of *p*-coumaric acid in wheat seed extracts WW/804 (2008)

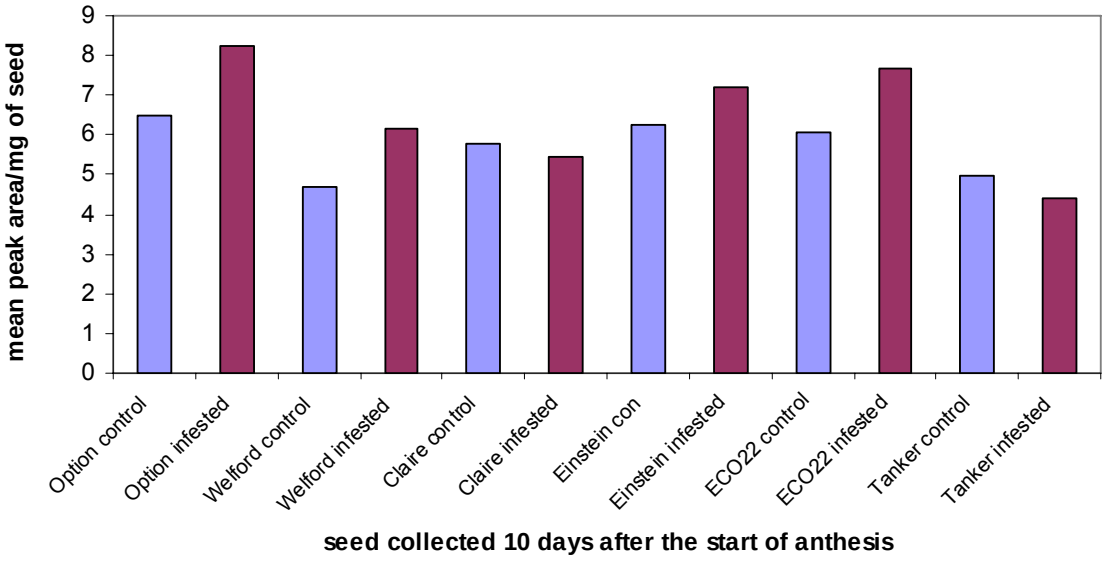


Figure 81. GC analysis of ferulic acid in wheat seed extracts WW/804 (2008)

Discussion

Variety trials showed consistently low owbm larval infestations on the resistant variety Welford. In contrast, infestation levels on other varieties varied between seasons. Synchronicity between the susceptible ear emergence stage of the crop and the peak of owbm flight activity was the key factor in determining larval infestation levels. The resistant variety Welford had the lowest levels of owbm larval infestation as expected. However, there was no difference in the number of eggs laid by female owbm on Welford compared with other varieties suggesting that female midges do not recognise the resistance. Levels of midge infestation between 2006-2008 were generally too low to be able to demonstrate any yield benefit from applying chlorpyrifos sprays. This result confirms the importance of monitoring pest numbers in order to decide on the need for insecticide treatment.

Analysis of phenolic acids in grain samples showed that levels of ferulic acid were higher in infested grain of Option, Welford, Einstein and ECO22 compared to uninfested grain, but there was no difference or a slight decline in levels in infested Claire and Tanker. Levels of *p*-coumaric acid were greater in the

infested than in the uninfested samples of all the varieties tested indicating that owbm damage is inducing production of this acid in the seed. Although infested Welford had the highest level of *p*-coumaric acid the level of induction was insufficient to explain the big difference in owbm larval survival in Welford compared to the other varieties. This suggests that there might be another mechanism of owbm resistance.

C2. Screening of germplasm and development of markers

J Snape, L Sayers – John Innes Centre

Introduction

Varietal variation for resistance to owbm has been observed in material from different countries, including Canada and the UK. However, there have been very few studies of the genetics of these resistance sources. The most significant study was by the Canadian group in Winnipeg (Thomas *et al*., 2005), which demonstrated that resistance in Canadian material was conditioned by a single major gene, termed *Sm1*, on wheat chromosome 2B. Additionally, these workers developed a PCR based molecular marker, called *Wm1*, which was linked to the resistance gene and could be used for marker-assisted selection in crosses involving the resistance source. However, there is no information on whether UK and European sources of resistance carry *Sm1* or whether there are other, independent, genes.

Thus the objectives of the present work were:

1. To study if *Sm1* is present in UK sources of owbm resistance
2. If *Sm1* is present, to test the utility of the *Wm1* molecular marker in identifying and tagging resistance in UK crosses
3. To identify if there are other independent genes for owbm resistance in UK wheat germplasm.

Materials & methods

Materials

To look at the inheritance of owbm resistance in UK material, three crosses were made by Elsoms Seeds Ltd. between varieties/lines of high (S) and low susceptibility (R) to infestation by owbm. The crosses are:

1. WP071 = Access (S) / Welford (R)
2. WP151 = Brompton (R) / PBI 01-0091 (S)
3. WP158 = NSL WW57 (S) / Carlton (R)

The F_1s of the crosses were selfed to produce F_2 seed and a sample of each of 100 individual F_2 plants were germinated and grown to maturity to produce F_3 families. The F_3 families and their subsequent bulked F_4, and F_5 generations were used in the WOBM trials described below.

Field Trials

Three years of trials were carried out by JIC to phenotype the three crosses for owbm infestation, over the growing seasons 2005/06, 2006/07, and 2007/08. In 2005/06 there were 100 F_3 lines of each population, which were sown in two replications at TAG, Morley. Unfortunately no parents were supplied with the populations initially, so these were not included in the first year of trials. Parental varieties were obtained from the appropriate breeders during the first year, and bulked up to enable them to be included in the following years.

All experiments were treated with standardized programmes of herbicides and fungicides, but not insecticides.

The 2005/06 trial contained the F_3 bulks of each individual line and was grown at Little Gymballs field, Morley. The 2006/07 trial contained individual plots of the bulked F_4 lines pooled from random years harvested from each F_3 plot, and was grown at Raven's Grove field, Morley. The 2007/08 trial contained the bulk F_5 lines of each separate line from random heads harvested from the F_4 plots the previous year, and was grown at Mylls field, Morley. In each of these fields the trials were drilled as 2nd or 3rd wheats in order to increase the chance of midge infestation.

Each experiment used a randomised plot design with two replications, drilled using dressed seed, (Latitude), in a Hege 90 drill to produce a plot of 1m x 1m. All individual plots were scored for ear emergence, and time of anthesis (growth stage 61). Three weeks after anthesis, 10 random ears were collected, as a group of five ears from two different locations within the plot. These were taken back to the laboratory and frozen until they could be assessed for midge infestation. For 2005-06, and 2006-07, a full set of ears were taken for assessment, but in 2007-08, only the lines used for DArT molecular marker analysis, plus the parents, were harvested.

At maturity, the plots were harvested using a Wintersteiger plot combine and yields recorded. In 2005-06, due to poor weather at harvest time, most plots suffered from pre-harvest sprouting, so no yield values were taken. However, 20-30 ears were harvested by hand from each of the lines to ensure seed for drilling the trial for the following year.

Midge assessments

Each ear was observed under a low powered binocular microscope and scored for the presence and number of orange wheat blossom midge by examining each individual floret separately and noting the total number of midge larvae in each ear. Data were recorded as the number of larvae per 10 ears. Ywbm were also recorded when seen in many ears and numbers noted.

Molecular marker analysis

Testing for a Sm1/Wm1 relationship

To test the utility of the *Wm1* molecular marker in detecting the presence of the *Sm1* gene in the parents of the crosses, the known owbm susceptible (S) and resistance (R) parental varieties were tested with the *Wm1* marker using the primer sequences supplied by the Canadian group. Ten seeds of each variety examined were germinated and seedlings grown for DNA analysis. When there was sufficient green leaf they were harvested as a bulk, and put in a single bag. Some of the leaf material was taken and put straight into a 96 well tray for extraction of the DNA via a 'Quiagen DNeasy kit'. Everything was placed in a

freezer at -20C ready for extraction. The extra bulk was placed into a -80C freezer before it was freeze-dried, and the freeze-dried material was stored in a sealed container in the fridge. Once the DNA was extracted it was diluted for use in PCRs.

PCR was used to identify polymorphism for the *Wm1* marker using primers associated with the presence of the *Sm1* gene that produce a 214bp amplicon band:
Forward primer: WM1F3: 5'-CACCTGGAATGTTGGACTG-3'
Reverse primer: WMR214 5'-ACATCATCTGTCAACGCACTA-3'

PCRs were carried out in a total of 15 µl, each containing 100 ng of genomic DNA, 0.2 µM forward and reverse primers, 0.25mM dNTPs, 1X PCR reaction buffer with MgCl2 and 0.07µl (5units/µl) Taq DNA polymerase (Roche). The variety Augusta was used as a positive control. The amplification conditions used were 35 cycles with an initial denaturation of five minutes, followed by one minute at 94°C, one minute at 61°C, and one minute at 74°C with a final extension of five minutes. 4 µl of PCR product was mixed with 4 µl of formamide loading dye and denatured at 100°C for three minutes. Electrophoresis was carried out on 5% polyacylamide gels in TBE at 90W for 1.5 hours. The silver staining technique was used to visualise fragments (Bassam *et al*., 1991).

Mapping of the segregating populations using phenotypic extreme analysis and DArT markers

Marker analysis on the F_2 plants from the three crosses was carried out by evaluating pooled DNA samples from their F_3 progeny. Plants of each line, from each population, were grown in the greenhouse in pots, with six plants per pot, and two pots per line. Leaf material was bulked from both pots for DNA extraction, carried out as described above.

Based on the phenotyping scores from the 2005/06 trial and confirmed on the 2006/07 field trial, 14 lines with the highest owbm scores (Susceptible lines) and 14 lines with none or very few midges in both seasons (Resistant lines) were chosen from each of the crosses for phenotypic extreme analysis. DNA samples from these 84 lines, plus the parents, were sent to the company 'Triticarte' in

Australia for Diversity Arrays Technology (DArT) molecular marker analysis (www.triticarte.com.au).

Based on the DArT results, putative regions of the wheat genome for each of the three crosses which were associated with the R/S divergence, were identified. Simple Sequence Repeat (SSR) markers known to locate in these regions from wheat consensus genetic maps were then identified and screened for polymorphisms for mapping on the whole 100 lines of each of the populations so that QTL analysis could be carried out to confirm if the individual regions were correlated with the S/R polymorphism. The PCR conditions and SSR marker analysis methodology were as described in Simmonds et al, 2008.

Results

Field phenotyping

Levels of infestation over the three years were recorded by TAG with both pheromone and sticky traps, and they were generally very low. In the populations, the levels of midge found in years 2006 and 2007 were reasonably high but did drop in 2008. This is probably due to the poor weather conditions during anthesis in 2008, which were not favourable for midge to lay eggs whilst the plants were most vulnerable to attack.

Figure 82, 83, and 84 show the distributions of levels of infestation in 2006 for the 100 lines in each population (82= WP071, 83= WP151, 84= WP158). From these we can see that there is a segregation pattern into lines which have midge and those lines that do not, but with a continuous change from resistant to high levels of infestation. The parents were not available for testing in 2006 but were scored in 2007 and 2008 (Table 16). This shows that the parental lines showed the expected phenotypic behaviour with the resistant parents Welford, Brompton and Charlton having no midge infestation whilst the susceptible parents Access, PBI 01-0091, and NSL WW57 all had high levels of infestation. However, PBI 01-0091 is, apparently, more susceptible than the other two 'susceptible' parents, Access and WW57, which may have some background resistance. This also shows up in the mean for the segregating populations in 2006 where the WP151

population has a higher mean than the other two. Infestation levels in 2007 were much higher than 2008, and no infestation was observed on the resistant parents in 2007 or 2008.

These segregation patterns were repeated in the 2007 scores. Since none of the distributions is bimodal indicating the clear segregation of a single gene, the inheritance patterns cannot be directly inferred as there is a continuous change from no infestation to a few lines with high infestation (>60 midge per ten ears). Consequently, more detailed dissection of the distributions using marker-assisted quantitative trait locus (QTL) analysis is necessary to understand the number and location of the genes involved. The crosses also differed in their absolute levels of infestation (Table 1) with the Access x Welford and NSL WW57 x Carlton lines being, on average, more resistant than the Brompton x PBI 01-0091 cross in both 2007 and 2008.

In 2006 and 2007, a note was also made of the presence of ywbm. In 2006, WP071 had ywbm present in 30 lines, WP151 had none present in 21 lines, and WP158 had ywbm present in 15 lines.

Table 16. Mean parental infestations (midge infestation) in 2007 and 2008 and population means for 2006

	2007		2008		Population values
	S parent	R Parent	S parent	R Parent	2006
WP071	43.3	0.0	7.0	0.0	18.2
WP151	66.7	0.0	10.0	0.0	24.4
WP158	43.3	0.0	8.0	0.0	12.0

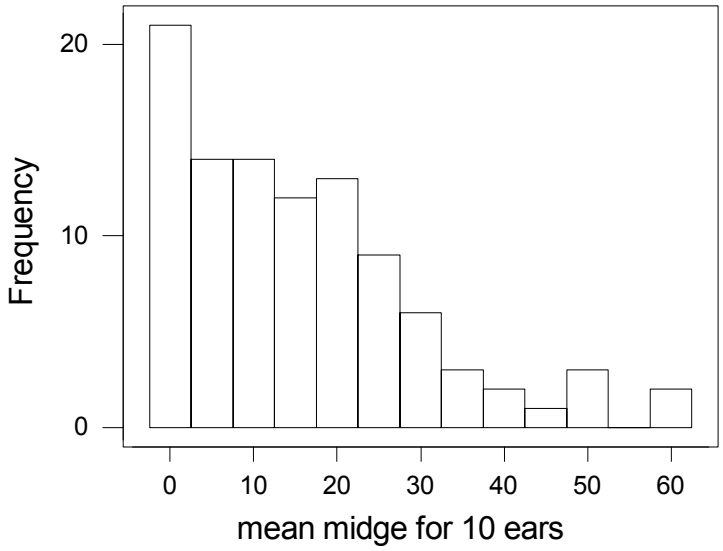


Figure 82. Histogram showing the distribution of phenotypic scores for owbm mean number of larvae per 10 ears for the 100 lines of the WP071 cross, Access (S) / Welford (R)

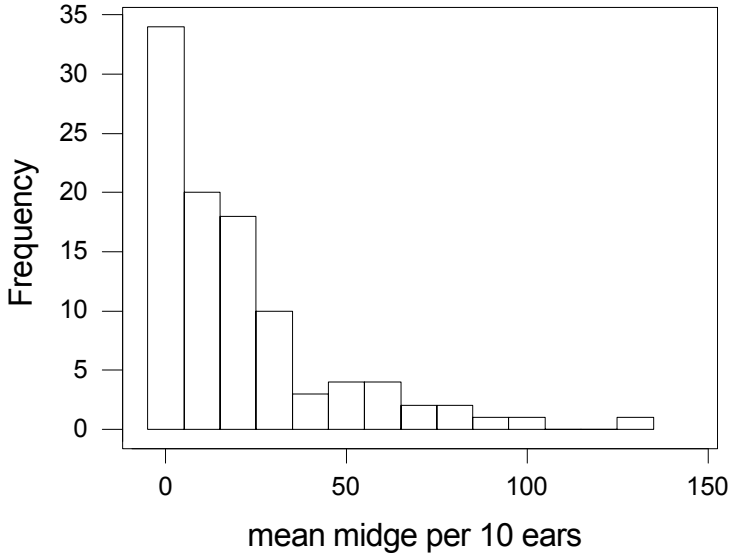


Figure 83. Histogram showing the distribution of phenotypic scores for owbm mean number of larvae per 10 ears for the 100 lines of the WP151 cross, Brompton (R) / PBI 01-0091 (S)

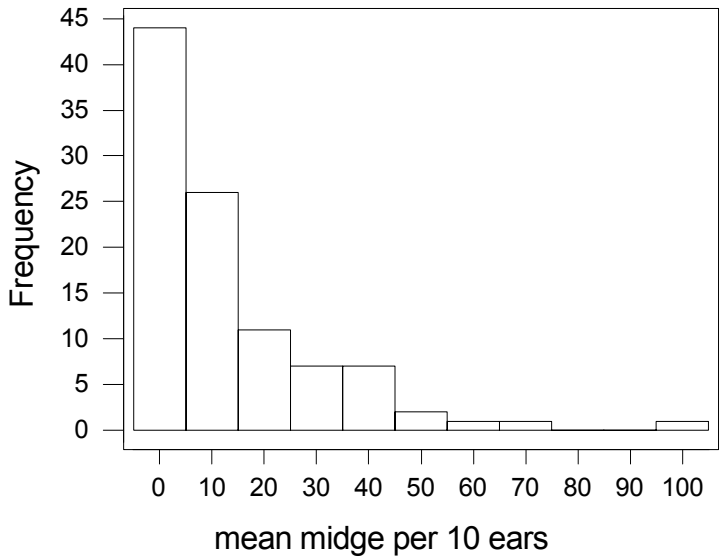


Figure 84. Histogram showing the distribution of phenotypic scores for owbm mean number of larvae per 10 ears for the 100 lines of the WP158 cross, NSL WW57 (S) / Carlton (R)

Testing for the presence of Sm1 using the Wm1 molecular marker

The primer sequence obtained from the Winnipeg group was used to screen the parents of the crosses for the presence of the *Wm1* marker using PCR. In WOBM project I, for a broad sample of European varieties, the marker was generally unreliable in predicting whether a resistant line carried *Sm1* as some susceptible varieties also had an amplicon of the same size. However, in the crosses being investigated, Figure 85 shows that all the three resistant Elsoms Seeds Ltd varieties have the *Wm1* band whilst the susceptible lines do not. From this result, it can be inferred that Welford, Brompton and Charlton probably carry *Sm1*, although this needed to be confirmed by further genetic analysis using SSR and DArT markers.

To confirm that these resistant parents do indeed carry *Sm1*, the *Wm1* marker and SSR markers presumed to be near the locus from consensus maps were used to genotype all 100 lines in each of the three populations, and the marker data were then used for QTL analysis by single marker ANOVA using the OBM scores from the 2006 field trial. Table 17 shows the marker means from the

ANOVA for each population, and indicates a highly significant association between *Wm1* and owbm resistance for the Access x Welford, and PBI01/009 x Brompton populations, but surprisingly not for the WW57 x Carlton population. The latter may be due to difficulties of accurately scoring the amplicon in this population. However, in this population there is a significant difference for the polymorphic marker *Xgwm614* known to be in the presumed *Sm1* region. Thus, all three resistant parents Welford, Brompton and Charlton are confirmed as carrying *Sm1*. This was also confirmed using DArT analysis (see below). However, since the crosses also differ in their mean levels of resistance, it is likely that additional factors are present. Analysis of the phenotypic extremes using DArT marker differences was used to identify these additional factors.

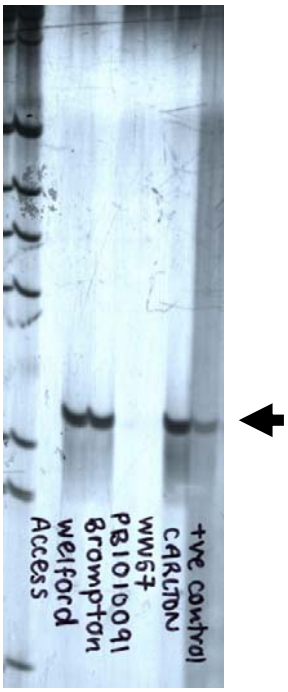


Figure 85. Gel photo showing the amplification of the *Wm1* band associated with the *Sm1* owbm resistance gene in the resistant parents of the cross, but not in the susceptible parents

Table 17. Marker means, differences, and significance of differences from single marker ANOVA on each population for an association between mean midge per ten ears (2006 data) and *Wm1* and SSR markers on 2B.

Cross	Marker	Chromosome	S parent allele	R parent allele	Difference	Significance level
WP071	*Wm1*	2B	27.0	16.2	10.8	**
WP151	*Wm1*	2B	57.0	18.8	38.2	***
WP158	*Wm1*	2B	11.5	12.0	-0.5	ns
WP158	*Xgwm 614*	2B	20.9	3.6	17.3	***

'Phenotypic extremes' analysis

Choice of phenotypic extremes

The phenotyping data from 2006 and 2007 were used to identify 14 lines with no midge infestation and 14 lines with the highest levels of infestation over years, in each cross. Table 18 shows the lines for WP071; the lines from crosses WP151 and WP158 were similarly chosen.

Table 18. OWBM scores for lines selected for 'phenotypic extreme' analysis in the WP071 cross, Access x Welford

Low **2006** **2007**

Access / Welford															Access / Welford													
		code	1	2	3	4	5	6	7	8	9	10	total	midge/10			code	1	2	3	4	5	6	7	8	9	10	total	midge/10		
WP - 071	13	1-14	0	0	0	0	0	0	0	0	0	0	0	**0**	WP - 071	13	3-16	0	0	0	0	0	0	0	0	0	2	2	**2**		p
WP - 071	20	1-21	0	0	0	0	1	0	0	0	0	0	1	**1**	WP - 071	20	2-2	0	0	0	0	0	0	0	0L	0	0	0	**0**	19 lemon	X
WP - 071	27	2-5	0	0	0	0	0L	0	0	0	0	0	0	**0 17lem**	WP - 071	27	3-6	0	0	0	0	0	0	0	0	0	0	0	**0**		X
WP - 071	45	3-1	0	0	0	0	0	0	0	0	0	0L	0	**0 lem 11**	WP - 071	45	2-4	0	0	0	0	0	0	2	0	0	0	2	**2**		p
WP - 071	49	3-5	0	0	0	0	0	0	0	0	0	0	0	**0**	WP - 071	49	1-10	0	0	0	0	0	0	0	0	0	0L	0	**0**	9 lemon	X
WP - 071	57	3-13	0	0	0	0	0	0	0	0	0	0	0	**0**	WP - 071	57	2-22	0	0	0	0	0	0	0	0	0	0	0	**0**		X
WP - 071	61	3-18	0L	0	0	0	0	0	0	0	0	0	0	**0 12lem**	WP - 071	61	2-17	0	0	0	0	0	0	0L	0	0	0	0	**0**	23 lemon	X
WP - 071	62	3-19	0	0	0	0	0	0	0	0	0	0	0	**0**	WP - 071	62	1-6	0	0	0	0	0	0	0	0	0	0	0	**0**		X
WP - 071	66	3-23	0	0	0	0	0	0	0L	0	1	0	1	**1 14lem**	WP - 071	66	1-19	0	0	0	0	0	0	0	0	0	0	0	**0**		p
WP - 071	68	4-2	0	0	0L	0	0	0	0	0	0	0	0	**0lem15**	WP - 071	68	5-6	0	0	0	0	0	0	0	0	0	0	0	**0**		X
WP - 071	71	4-5	0	0	0	0	0	0	0	0	0	0	0	**0**	WP - 071	71	1-12	2	0	0	0	0	0	0	0	0	0	2	**2**		p
WP - 071	77	4-12	0	0	0	0	0	0	0	0L	0	0	0	**0 8lem**	WP - 071	77	3-2	0	0	0L	0	0	0	0	0	0	0L	0	**0**	23 lemon	X
WP - 071	90	5-3	0	0	0	0	0	0	0	0	0	0	0	**0**	WP - 071	90	2-1	0	0L	0L	0	0	0	0	0	0	0	0	**0**	17 lemon	X
WP - 071	98	5-11	0	0	0	0	0	0L	0	0	0	0	0	**0 9lem**	WP - 071	98	4-1	0	0L	0	0	0	0	0	0	0	0	0	**0**	15 lemon	X

High **2006** **2007**

Access / Welford															Access / Welford													
		code	1	2	3	4	5	6	7	8	9	10	total	midge/10			code	1	2	3	4	5	6	7	8	9	10	total	midge/10		
WP - 071	3	1-3	9	3									**12**	60	WP - 071	3	4-7	0	9	1								**10**	33.33		X
WP - 071	16	1-17	9	0									**9**	45	WP - 071	16	5-11	5	0	4	1							**10**	25		X
WP - 071	17	1-18	0	1	5								**6**	20 5lem	WP - 071	17	1-22	4	0	8								**12**	40		X
WP - 071	31	2-9	0	0	1	0	0	0	0	3	0	0	**4**	4	WP - 071	31	2-3	0	0	0	7	0	0	1	0	14	0	**22**	22		X
WP - 071	32	2-10	5	3									**8**	40	WP - 071	32	4-2	0	3	0	0	0	3					**6**	10		p
WP - 071	36	2-15	2	0	0	6							**8**	20	WP - 071	36	2-20	4	0	0	6							**10**	25		p
WP - 071	41	2-20	0	0	0	0	0	0	1L	0	5L	8	**14**	14 lem21	WP - 071	41	3-5	6	2	3								**11**	36.66		X
WP - 071	44	2-23	5	0									**5**	25	WP - 071	44	5-9	0	2	0	3	2						**7**	14		p
WP - 071	52	3-8	3	0	0	6							**9**	22.5 lem12	WP - 071	52	2-23	0	6	0	15							**21**	52.5		X
WP - 071	64	3-21	1	0	0	0	0	2	7				**10**	14.29	WP - 071	64	2-7	7	0	0	9							**16**	40		X
WP - 071	69	4-3	3	3									**6**	30	WP - 071	69	2-13	2	1	0	1	4						**8**	16		p
WP - 071	80	4-15	0	6									**6**	30	WP - 071	80	3-8	8	10									**18**	90		X
WP - 071	89	5-2	0	1	12								**13**	43.33	WP - 071	89	4-3	0	0	7	0	0						**7**	14		X
WP - 071	95	5-8	0	2	2								**4**	13.33	WP - 071	95	5-12	0	2	1	13	18						**34**	68		X

As can be observed, it was not possible to identify 42 lines with complete absence of midge for the resistant population, and some had very small levels of infestation. Nevertheless, there can be seen to be quite significant phenotypic divergence between the groups.

DArT data analysis

DArT is a dominant marker system which provides a plus-minus signal for the presence/absence of particular genomic sequences in a variety, and these are chosen to be dispersed throughout the wheat genome. Generally, in UK crosses, they reveal between 250-350 presence/absence polymorphisms between any pair of different wheat lines (Snape *et al*., unpublished). In a segregating generation of a cross, each individual is examined for these sequence polymorphisms by hybridizing DNA of the parents and each recombinant individual/line to a chip containing the target sequences. Thus, DArT can be used to develop a detailed *de novo* genetic map covering the whole genome, especially when combined with a few anchoring SSR co-dominant markers. By examining the phenotypic extremes for a phenotype in a segregating population, it is hoped that DArT polymorphisms associated with genes controlling the trait will show frequency differences between the high and low groups which match the parental marker classifications.

In the present populations, DArT revealed approx 250 polymorphisms between the parents, and these were reflected in polymorphisms within and between the phenotypic high and low owbm groups of the 28 chosen lines in each cross. To identify those markers associated with differences in marker frequencies between the high and low groups, the whole genotype file of all DArT marker scores for each cross was sorted to identify those markers with frequency differences between the groups. The tables below (Tables 19, 20 and 21) show the results of sorting the DArT markers by differences between the high and low groups of the three populations, WP071, WP151 and WP158, respectively. In this analysis, it was important to only choose those markers which also reflected the parental direction for the frequency changes, and also that the frequencies differences were in opposite directions in the high and low groups. The threshold

for selection of a marker was chosen so that a ratio of at least 5:9 presence/absence or absence/presence was observed in one of the groups.

Table 19. DArT marker scores (1=presence, 0= absence, x= missing value) from hybridization of DNA of each high or low phenotypic extreme line of the WP071 cross (Access x Welford), and the parents, to the wheat DArT chip, for markers selected as showing differences between groups. Left hand columns show marker name, chromosome location from consensus maps, and stringency statistics for hybridization. Two independent hybridizations for the parents are in the right hand columns. Columns to right of scores show ratios of 0:1 within phenotype groups.

High owbm (Sus) | **Low owbm (Res)** | **Parents**

MarkerName	Chromosome	Dif Pools	WP071-3-H	WP071-16-H	WP071-17-H	WP071-31-H	WP071-32-H	WP071-36-H	WP071-41-H	WP071-44-H	WP071-52-H	WP071-63-L	WP071-64-H	WP071-69-H	WP071-80-H	WP071-89-H	WP071-95-H	no. of 0	no. of 1	WP071-13-L	WP071-20-L	WP071-27-L	WP071-45-L	WP071-49-L	WP071-57-L	WP071-61-L	WP071-62-L	WP071-68-L	WP071-71-L	WP071-77-L	WP071-90-L	WP071-98-L	no. of 0	no. of 1	S WP071-ACC	S WP071-ACC	R WP071-WEL	R WP071-WEL
wPt-8398	-	0.6	0	0	0	0	1	1	1	0	0	1	1	0	0	1	0	9	6	1	X	1	1	1	1	1	1	1	1	1	1	1	0	12	0	0	1	1
wPt-9859	-	0.8	1	1	1	X	0	1	X	1	1	0	1	X	1	X	X	2	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	1	1	0	0
wPt-3792	-	0.6	1	1	1	1	1	0	1	1	X	0	0	1	1	1	1	3	11	0	0	0	0	1	0	0	0	0	X	1	X	1	8	3	1	1	0	0
wPt-1867	-	0.2	1	1	X	1	1	X	0	X	1	1	1	1	0	1	0	3	9	0	0	0	X	1	1	1	0	0	X	0	1	1	6	5	1	1	0	0
wPt-3901	-	0.3	1	1	1	1	0	X	0	1	1	0	1	1	1	0	1	4	10	0	1	0	1	0	1	1	0	1	0	0	0	1	7	6	1	1	0	0
wPt-6938	-	0.4	1	0	0	0	0	0	0	0	1	X	X	0	1	0	0	10	3	0	0	1	1	1	X	0	0	1	1	1	1	1	4	8	0	0	1	1
wPt-5014	-	0.2	1	1	1	0	0	1	1	1	1	1	X	1	0	X	0	4	9	0	1	X	0	0	0	X	0	1	0	1	0	1	7	4	1	1	0	0
wPt-0838	-	0.3	1	0	1	1	1	1	1	0	0	0	1	1	0	1	0	6	9	0	0	1	1	0	0	0	0	1	0	0	0	1	9	4	1	1	0	0
wPt-5374	1A|2B	0.5	1	1	0	1	0	1	0	X	1	0	X	1	1	0	1	5	8	0	1	0	0	X	0	0	0	0	X	0	0	1	9	2	1	1	0	0
wPt-5195	2B	0.5	0	0	0	1	1	X	1	0	X	1	0	0	0	X	X	7	4	1	1	1	0	1	X	0	1	1	1	X	1	1	2	9	0	0	1	1
wPt-6311	2B	0.7	1	1	1	0	1	1	0	X	1	1	1	1	1	X	1	2	11	0	0	0	0	0	0	X	0	X	0	0	0	0	11	0	1	1	0	X
wPt-9268	3A	0.3	1	1	1	1	0	0	1	1	1	0	1	0	1	0	X	5	9	1	0	1	1	0	0	0	0	1	0	1	0	0	8	5	1	1	0	0
wPt-4131	5A	0.1	0	0	1	1	1	0	1	1	1	1	0	1	1	0	0	6	9	0	0	0	1	0	X	0	0	0	0	1	1	1	8	4	1	1	0	0
wPt-6048	5A	0.3	1	0	0	0	1	0	0	0	X	0	1	0	0	X	1	9	4	1	1	1	0	0	1	1	X	1	1	0	X	1	3	8	0	0	1	1
wPt-1179	5B	0.4	0	0	0	0	1	1	1	X	X	1	0	0	1	1	0	7	6	1	1	1	1	0	X	X	1	1	1	1	1	0	2	9	0	0	1	1
wPt-8094	5B	0.3	0	1	0	1	1	0	0	0	0	1	0	1	1	X	0	8	6	1	1	1	1	0	X	1	1	0	1	0	1	1	3	9	0	0	1	1
wPt-4880	7A	0.3	0	0	0	0	1	1	1	0	0	1	0	0	1	1	0	9	6	1	1	1	1	0	0	X	1	X	1	1	1	0	3	8	0	0	1	1
wPt-8473	7A	0.3	0	0	0	0	1	1	1	0	0	1	0	0	1	X	0	9	5	1	1	1	1	0	0	X	1	0	1	1	1	0	4	8	0	0	1	1

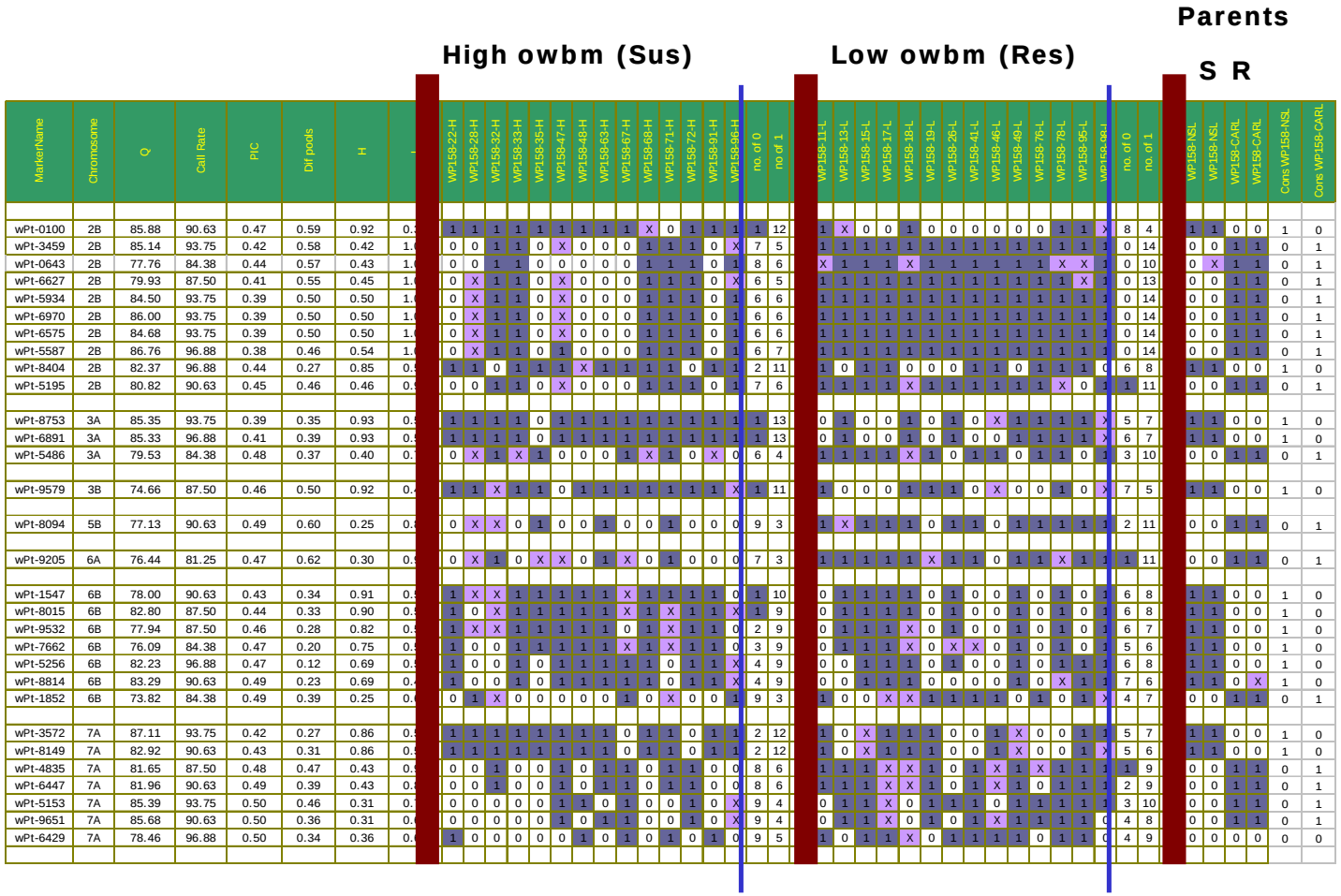
Table 20. DArT marker scores (1=presence, 0= absence, x= missing value) from hybridization of DNA of each high or low phenotypic extreme line of the WP151 cross (Brompton x PBI 01-0091), and the parents, to the wheat DArT chip, for markers selected as showing differences between groups. Left hand columns show marker name, chromosome location from consensus maps, and stringency statistics for hybridization. Two independent hybridizations for the parents are in the right hand columns. Columns to right of scores show ratios of 0:1 within phenotype groups

Parents

High owbm (Sus) **Low owbm (Res)** **S R**

MarkerName	Chromosome	Dif Pools	H		WP151-7-H	WP151-8-H	WP151-12-H	WP151-44-H	WP151-47-H	WP151-48-H	WP151-57-H	WP151-60-H	WP151-65-H	WP151-68-H	WP151-73-H	WP151-85-H	WP151-87-H	WP151-94-H	no. of 0	no. of 1	WP151-2-L	WP151-4-L	WP151-9-L	WP151-16-L	WP151-19-L	WP151-26-L	WP151-27-L	WP151-28-L	WP151-32-L	WP151-49-L	WP151-82-L	WP151-89-L	WP151-95-L	WP151-97-L	no. of 0	no. of 1	WP151-PB1	WP151-PB1	WP151-BROM	WP151-BROM
wPt-9607	-	0.37	0.17	0.	0	0	1	0	1	X	X	0	0	0	0	0	0	0	10	2	1	0	1	0	0	1	X	1	1	0	0	1	0	1	6	7	0	0	1	1
wPt-9058	-	0.36	0.18	0.	0	0	X	1	0	0	0	0	X	X	1	0	0	0	9	2	X	0	0	1	0	1	0	1	1	1	1	1	0	0	6	7	0	0	1	1
wPt-6015	-	0.35	0.77	0.	0	0	1	1	1	X	1	1	1	1	0	1	1	1	3	10	1	1	0	0	1	X	X	0	1	0	0	0	0	1	7	5	1	1	X	0
wPt-7513	-	0.40	0.82	0.	1	1	1	X	1	X	0	1	1	0	1	X	1	1	2	9	1	1	0	X	0	X	0	0	0	0	1	0	1	1	7	5	1	1	0	0
wPt-7186	-	0.35	0.40	0.	X	X	1	0	0	0	0	0	1	0	X	1	X	1	6	4	1	X	1	0	0	1	0	1	1	1	1	1	X	1	3	9	0	0	1	1
wPt-4719	-	0.50	0.83	0.	1	X	1	1	1	1	X	0	1	1	1	1	1	0	2	10	0	1	0	1	0	X	1	0	0	X	0	0	1	0	8	4	1	1	0	0
wPt-9342	-	0.30	0.57	0.	0	1	1	1	1	0	1	0	1	0	1	1	0	0	6	8	X	0	X	0	0	1	X	0	0	1	1	0	0	0	8	3	1	1	0	0
wPt-1106	-	0.57	0.77	0.	1	0	X	1	1	1	0	0	1	1	1	1	1	1	3	10	0	X	0	X	0	X	1	0	X	0	0	0	1	0	8	2	1	1	0	0
wPt-9714	-	0.34	0.64	0.	1	0	0	1	1	1	0	0	1	1	1	1	1	0	5	9	0	0	0	1	0	X	1	0	X	X	0	X	1	0	7	3	1	1	0	0
wPt-8569	1A|2B	0.33	0.83	0.	1	X	0	1	0	1	1	1	X	1	1	1	1	1	2	10	1	1	0	1	1	X	1	0	0	0	X	0	1	0	6	6	1	1	0	0
wPt-6179	1A|2D	0.31	0.23	0.	0	0	0	0	1	0	0	X	1	0	0	0	0	1	10	3	1	1	1	1	X	0	0	1	0	1	0	0	0	1	6	7	0	0	X	1
wPt-4453	2B	0.92	1.00	0.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	14	X	1	0	0	0	0	0	0	0	0	0	0	0	0	12	1	1	1	0	0
wPt-8235	2B	0.92	1.00	0.	1	1	1	1	1	1	1	1	1	1	X	1	X	1	0	12	1	X	0	0	0	0	0	0	0	0	0	0	0	0	12	1	1	1	0	0
wPt-4916	2B	0.68	0.75	0.	1	1	1	1	1	X	1	X	1	1	0	0	1	0	3	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	13	1	X	1	0	0
wPt-7212	3B	0.06	0.77	0.	0	1	1	0	0	1	1	1	X	1	1	1	1	1	3	10	1	1	1	1	1	0	1	X	X	1	0	1	1	1	2	10	0	0	1	1
wPt-5704	3B	0.23	0.82	0.	1	1	1	1	0	X	X	0	1	1	X	1	1	1	2	9	1	X	1	0	0	1	X	0	1	1	1	0	0	1	5	7	0	0	1	1
wPt-9088	3B	0.40	0.83	0.	1	1	X	1	1	1	0	X	1	1	1	1	1	0	2	10	0	0	1	1	0	1	0	0	1	1	0	1	0	0	8	6	1	1	0	0
wPt-3107	3B	0.23	0.46	0.	0	1	1	0	0	0	1	0	X	0	1	1	0	1	7	6	X	0	1	0	0	1	0	1	1	1	1	1	1	1	4	9	0	0	1	1
wPt-8886	3B|4A	0.50	0.08	0.	0	0	0	0	X	0	0	0	X	0	0	1	0	0	11	1	1	1	1	0	X	1	1	1	0	1	0	X	0	0	5	7	0	0	X	1
wPt-6904	6A	0.38	0.77	0.	0	1	1	1	1	X	1	0	1	0	1	1	1	1	3	10	1	1	1	0	0	1	0	X	0	0	1	0	0	0	8	5	1	1	0	0
wPt-7108	7B	0.42	0.92	0.	1	1	1	X	1	1	1	1	X	1	1	1	1	0	1	11	1	0	0	0	0	1	1	1	1	1	1	0	0	0	7	7	1	1	0	0
wPt-6320	7B	0.15	0.45	0.	0	X	X	0	X	0	1	1	1	0	0	0	1	1	6	5	1	1	0	0	0	0	0	1	1	X	0	0	0	0	9	4	0	0	1	1
wPt-0600	7B	0.44	0.71	0.	1	1	0	0	1	1	1	1	1	1	0	0	1	1	4	10	1	1	X	0	0	0	0	1	X	0	0	0	0	X	8	3	1	1	0	X

Table 21. DArT marker scores (1=presence, 0= absence, x= missing value) from hybridization of DNA of each high or low phenotypic extreme line of the WP158 cross (NSL WW57 x Carlton), and the parents, to the wheat DArT chip, for markers selected as showing differences between groups. Left hand columns show marker name, chromosome location from consensus maps, and stringency statistics for hybridization. Two independent hybridizations for the parents are in the right hand columns. Columns to right of scores show ratios of 0:1 within phenotype groups.



Sorting the data in this way identified the markers shown in the tables as locating to genomic areas associated with group phenotypic differences. Many, but not all, of these DArT markers have been mapped to chromosomes. By using the mapping information on the Triticarte website, it was possible to allocate specific markers into groups relating to specific chromosomes or intra-chromosomal regions, as shown in the tables. Table 22 provides a summary, by cross, of the chromosomes identified as possibly containing QTL for WOBM response, and the number of DArT markers associated with each chromosome.

From Tables 19, 20 and 21, it can be clearly seen that the 'extreme phenotype' analysis is working correctly, since it identifies the *Sm1* region in each cross through associated DArT markers, and this complements the data on directly mapping *Sm1* from its linkage to *Wm1*. However, in addition to *Sm1*, QTL affecting WOBM resistance are provisionally detected on chromosomes 3A, 5A, 5B, and 7A in the WP071 cross, on chromosomes 3B, 6A, and 7B in the WP151 cross, and on chromosomes 3A, 3B, 5B, 6A, 6B and 7A in the WP158 cross. Clearly, some of these are very tentative since only one marker is associated. Nevertheless, several are consistent across crosses, such as 3A, 5B, 6A and 7A. The greatest number of 'hits' is for chromosome 7A.

Table 22. Location and numbers of polymorphic DArT markers associated with difference between the phenotypic extreme groups in each cross

Population	**Chromosome**	**No. markers**
WP071 = Access / Welford	**2B**	**3**
	3A	1
	5A	2
	5B	2
	7A	8
WP151 = Brompton / PBI 01-0091	**2B**	**4**
	3B	6
	6A	1
	7B	3
WP158 = NSL WW57 / Carlton	**2B**	10
	3A	3
	3B	1
	5B	1
	6A	1
	6B	7
	7A	7

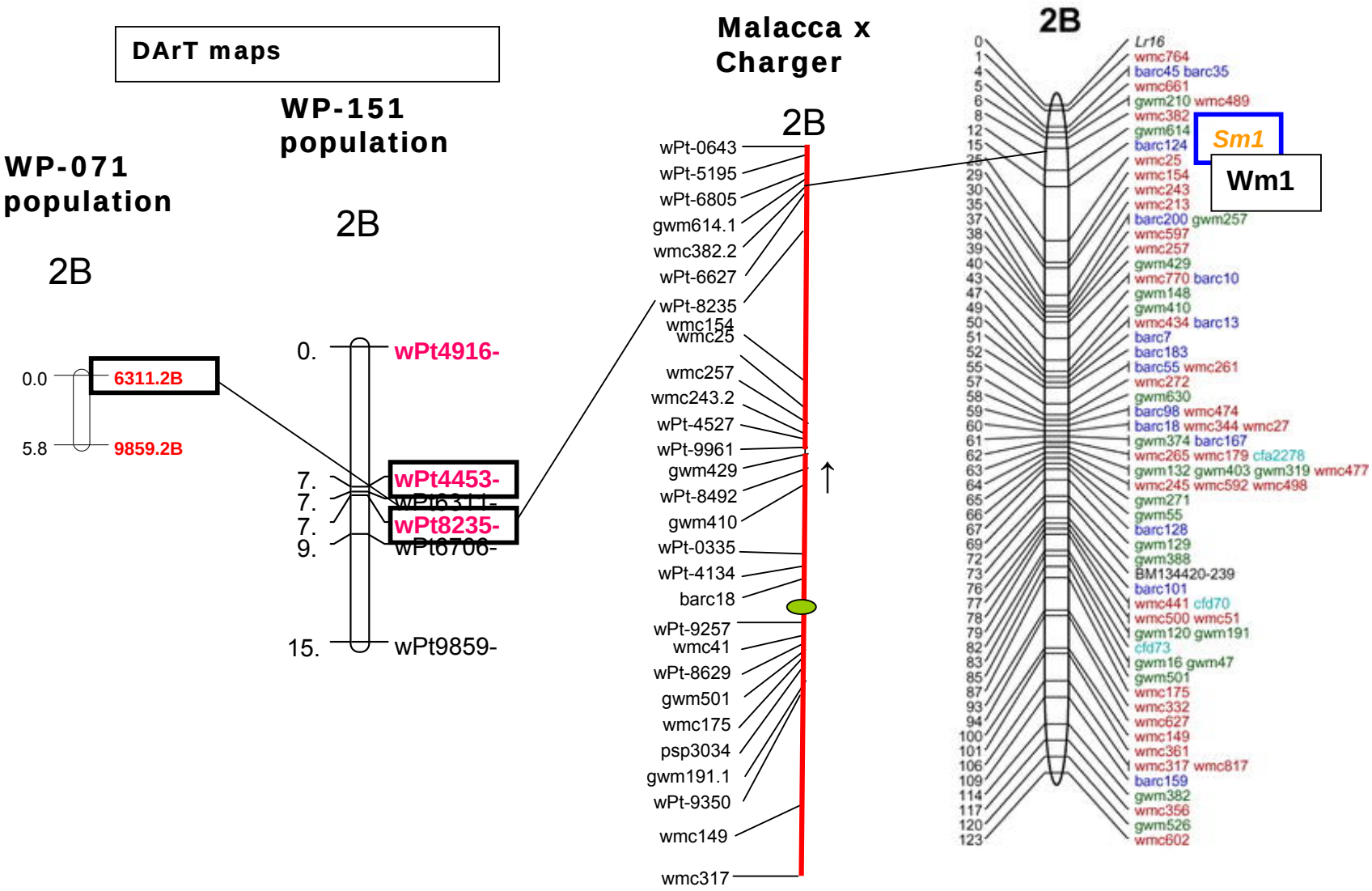
Comparative mapping of the Sm1 region

By comparing the known locations of DArT markers on the Triticarte consensus DArT map with the recently developed Malacca x Charger map at JIC (Snape *et al*., unpublished), which contains both DArT and SSR markers, and the wheat SSR consensus map (Somers *et al*., 2004), it is possible to align all the available marker information together to establish a more accurate location for *Sm1*. This is shown in Figure 86, and locates *Sm1* near to the end of the short arm of chromosome 2B, near to the SSR marker *Xgwm614*.

Figure 86. (Over) Alignment of the DArT genetics maps developed from the 28 lines within the WP071 and WP151 populations (markers associated with differences between phenotype extremes highlighted in red) with the Malacca x Charger map and the wheat consensus map, showing the presumed location of *Sm1* in the three crosses.

Figure 86

Wheat SSR consensus map



Mapping and comparative mapping of non-specific owbm QTL

To more accurately analyse and locate the non-*Sm1* effects of owbm resistance QTL that appear to be segregating in these crosses, the DArT data for all markers in the presumptive chromosomal regions in each cross were used to develop a skeleton DArT map for each population using only the 28 extreme lines. These are shown in the Figures 87, 88 and 89 for WP071, WP151, WP158, respectively, where the DArT markers associated with group differences are highlighted in red. In cross WP071 (Figure 87), it can be seen that the markers associated with differences between the high and low groups on 7A show significant linkage, giving confidence that this is a 'real' effect, but also with weaker effects associated with 3A, 5B and 7B. Similarly, in cross WP151 (Figure 88) chromosomal regions on 3B were strongly suggested, with a weak association also with 7B; and in cross WP158 (Figure 89), regions on 3A, 6B and 7A strongly suggested, and with weaker effects on 3B. Putting all these data together, suggests that the strongest associations of owbm QTL are with markers on chromosomes 3A, 3B, 6B and 7A.

To align these effects onto the wheat consensus maps, and with other data on locations of QTL controlling other traits, particularly known QTL for flowering time, these maps were then aligned with consensus DArT maps, JIC DArT/SSR maps, and the consensus wheat SSR map to align effects from the different crosses to known map locations. Because of known DArT locations, it was also possible to align the effects detected on 5B and 6A. These comparative maps are shown in Figures 90 (3A), 91 (3B), 92 (5B), 93 (6A), 94 (6B), 95 (7A).

<u>Chromosome 3A</u>

The most frequent association of DArT markers for chromosome 3A were exhibited in the WP158 cross, and this DArT map was aligned with the Malacca x Hereward 3A map (Snape *et al*., unpublished) and the wheat SSR consensus map – Figure 87. This located a presumptive WOBM QTL to the distal part of the short arm of 3A, in the vicinity of SSR markers *Xwmc11*, *Xcfd79* and *Xwmc532*.

Chromosome 3B

The DArT maps for WP151 and WP158 were aligned with the consensus DArT maps and through this to the Malacca x Charger 3B map, although there was a large group of linked DArT markers that could not be definitively aligned, Figure 8. By comparative mapping, the most likely location of a possible owbm QTL is in a region near to the centromere on the short arm of 3B, in the vicinity of SSR marker *Xgwm533*.

The putative 3A and 3B owbm QTL both locate to slightly different locations on the short arms of their respective chromosomes (Figures 90 and 91), and are unlikely to be homoeologous loci.

Chromosomes 5B

In cross WP071 it was possible to align the single 5B DArT marker *wpt8094* with the Cranbrook x Halberd DArT map and then onto the consensus wheat SSR map. This places a putative owbm QTL distal on the long arm of chromosome 5B, Figure 92.

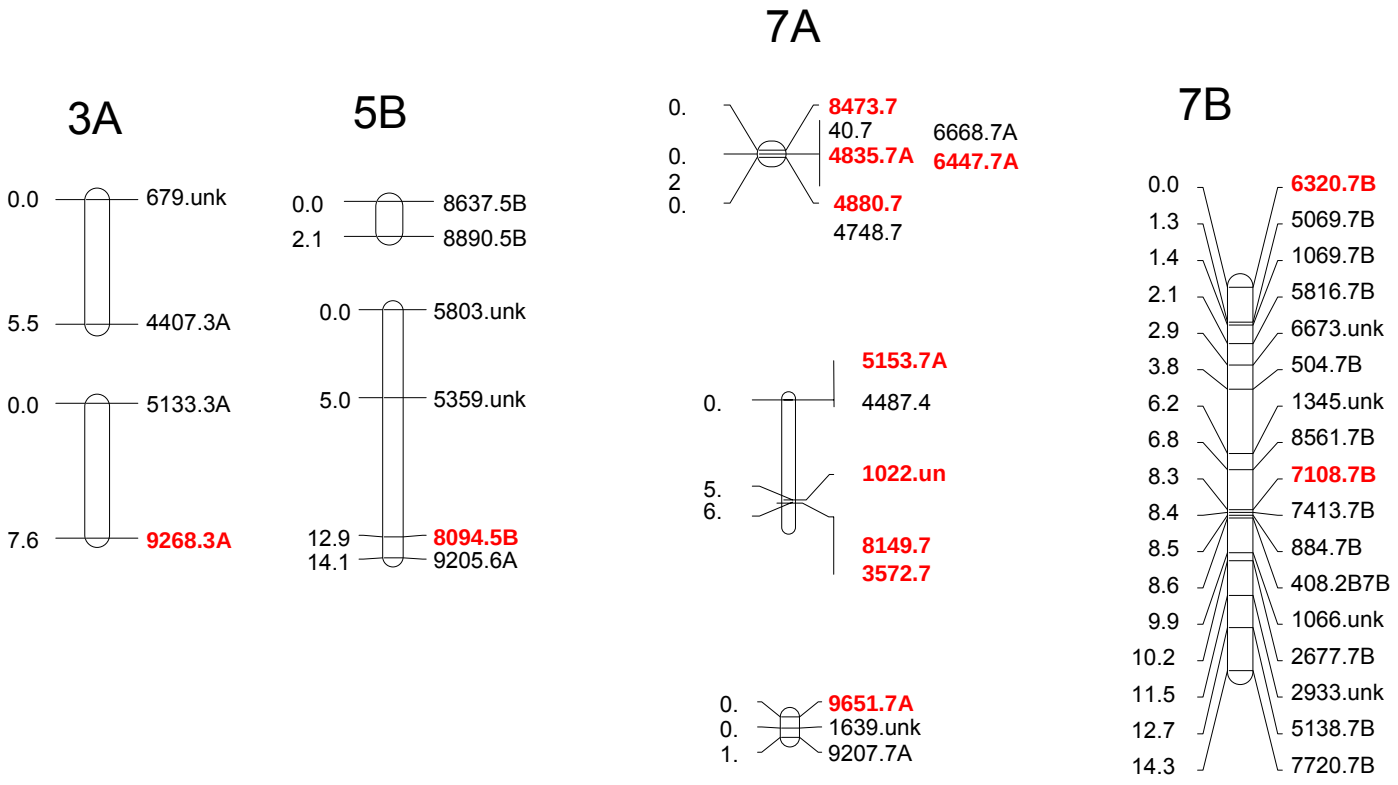


Figure 87. Skeleton DArT maps for chromosomes associated with possible owbm QTL in the WP071 population, Access x Welford

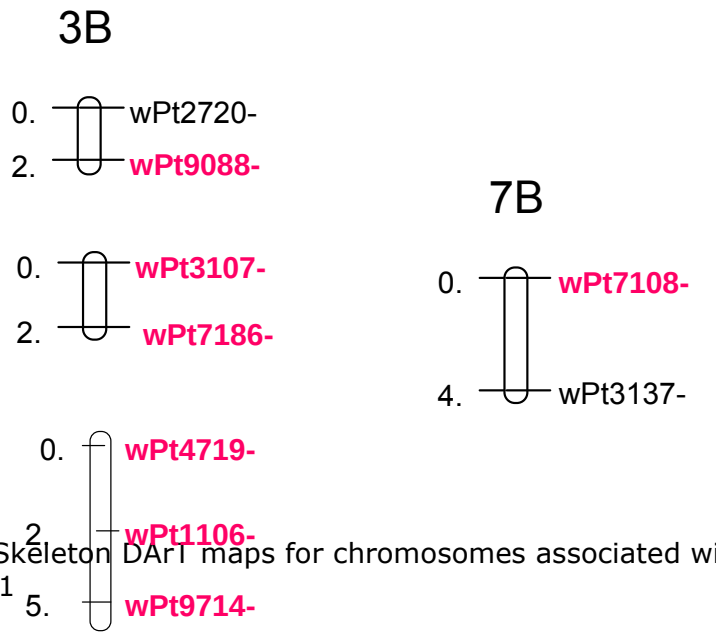


Figure 88. Skeleton DArT maps for chromosomes associated with possible owbm QTL in the WP151 population, Brompton x PBI 01-0091

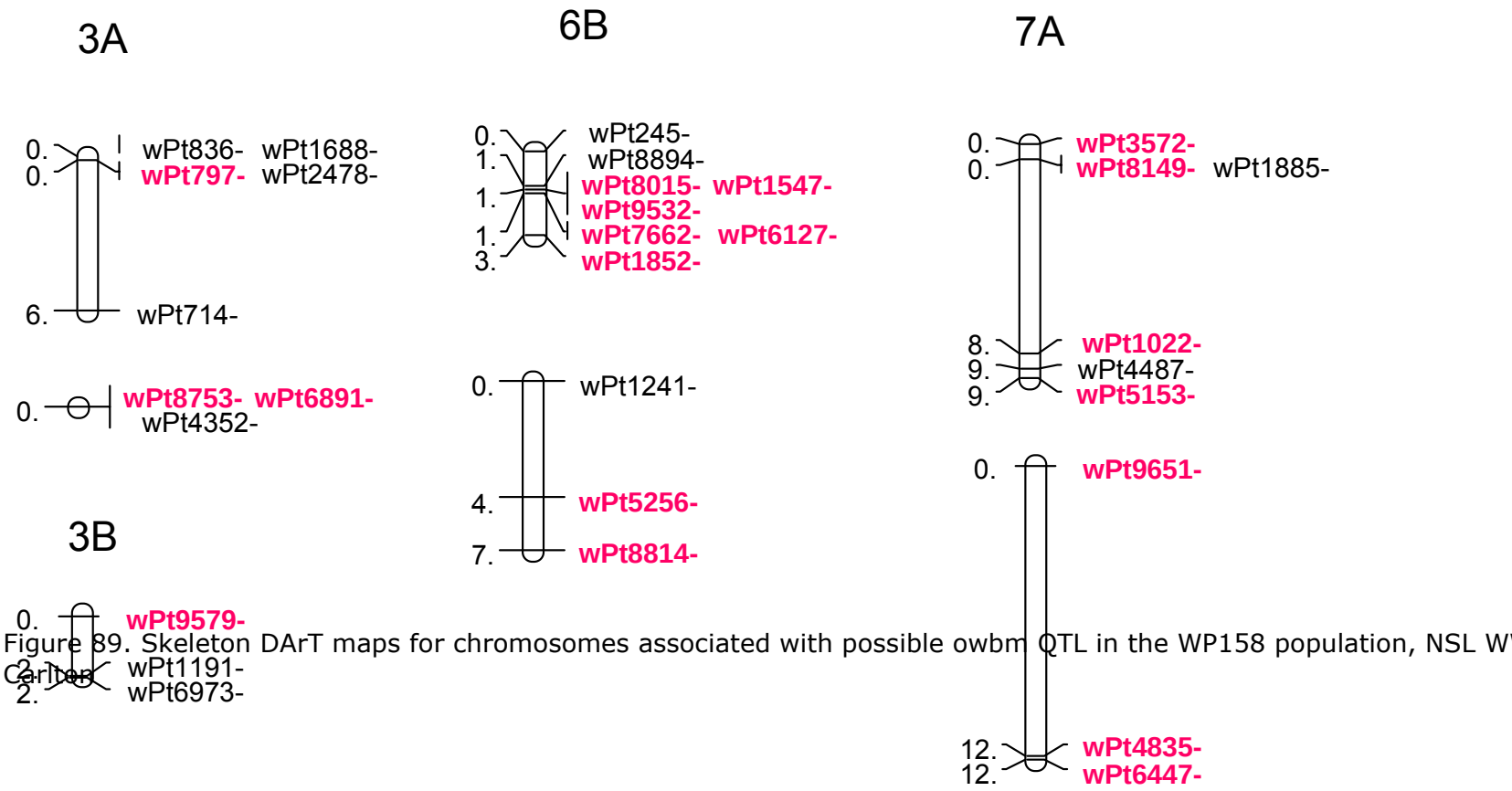


Figure 89. Skeleton DArT maps for chromosomes associated with possible owbm QTL in the WP158 population, NSL WW57 x Carlton

Chromosome 6A

In both crosses WP151 and WP158, there was a single marker from each that could be aligned to the Cranbrook x Halberd DArT map, and were closely linked and located to the distal end of the short arm, near to SSR loci *Xgwm459*, *Xgwm334*, Figure 93.

Chromosome 6B

In cross WP158, a large group of DArT markers associated with differences between the owbm extremes map together, and these can be aligned onto the Malacca x Charger map and through this to the distal part of the short arm of chromosome 6B, Figure 94. This suggests this region as the location of a possible WOBM QTL near SSR locus *Xcfd13*.

Chromosome 7A

Because of the large number of DArT markers significantly associated with differences between the owbm extreme lines, it was possible to align the DART maps for this chromosome in both the WP071 and WP158 crosses. This locates a possible owbm QTL to the middle of the short arm of chromosome 7A, near SSR locus *Xwmc479*, Figure 95.

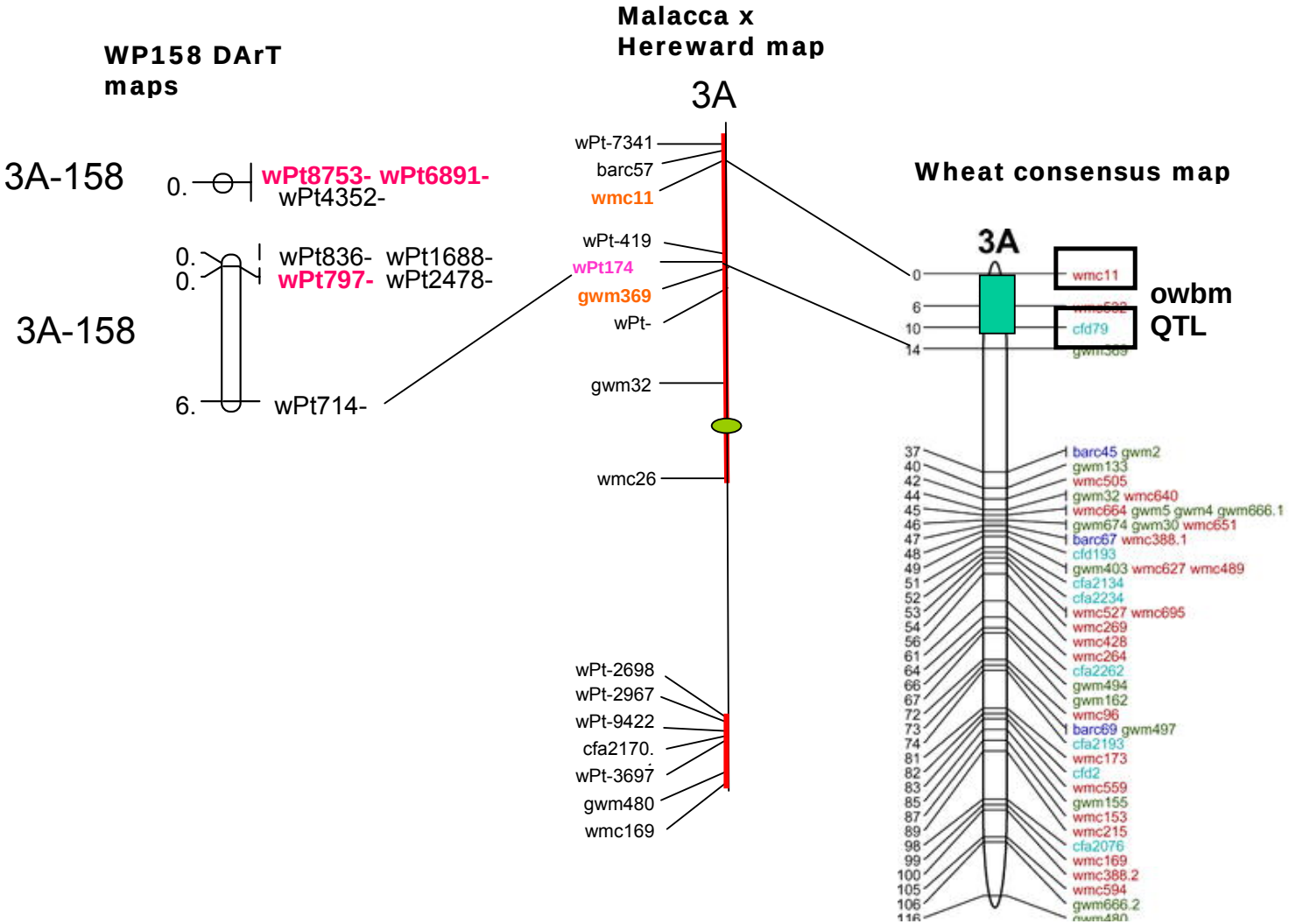


Figure 90. Comparative genetic maps of chromosome 3A developed from the WP158 DArT maps, Malacca x Hereward SSR/DArT map and the wheat consensus SSR map (black box indicates locus significant from single marker ANOVA)

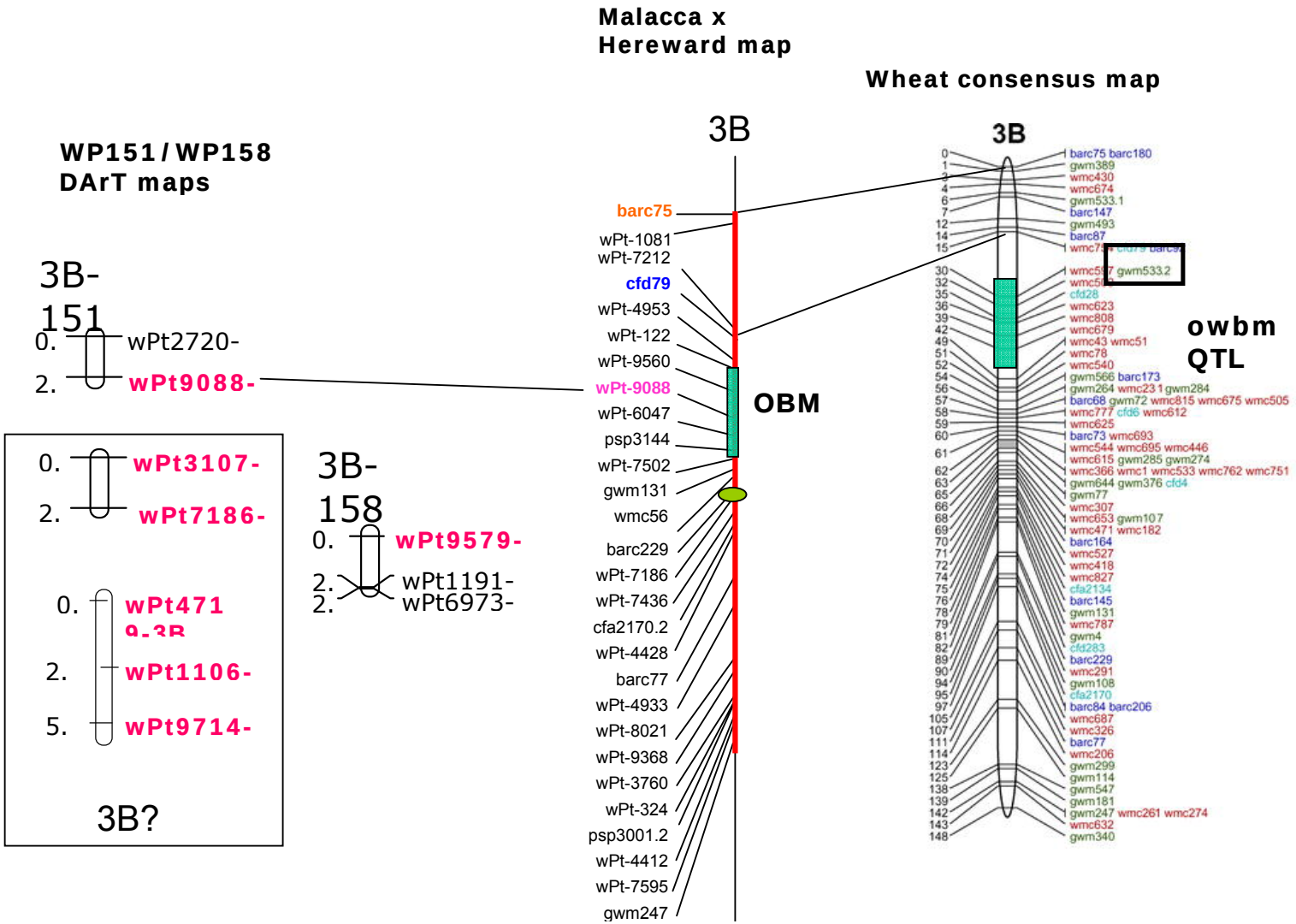


Figure 91. Comparative genetic maps of chromosome 3B developed from the WP151 and WP158 DArT maps, Malacca x Hereward SSR/DArT map and the wheat consensus SSR map (black box indicates locus significant from single marker ANOVA)

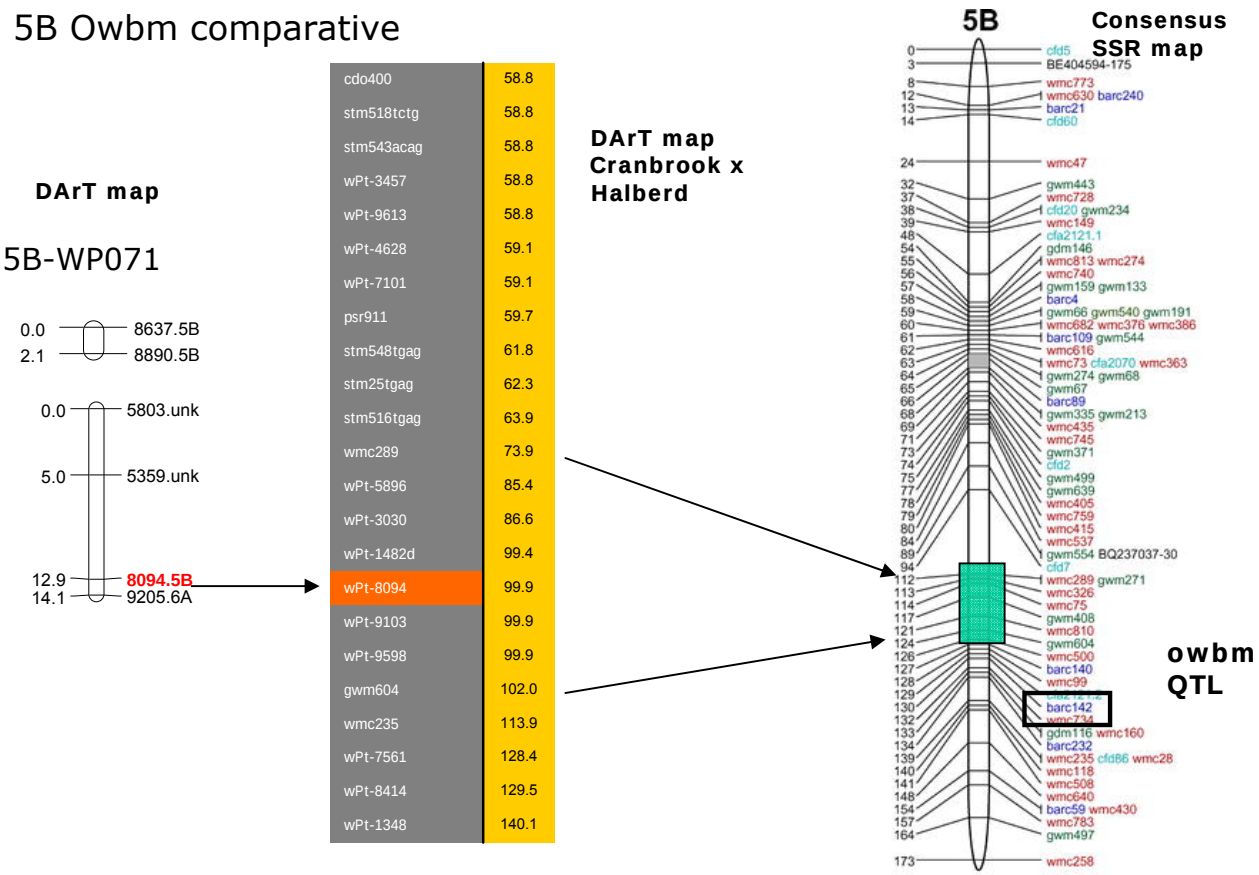


Figure 92. Comparative genetic maps for chromosome 5B developed from the WP071 Dart map, Cranbrook x Halberd DArT map and the wheat consensus SSR map (black box indicates locus significant from single marker ANOVA)

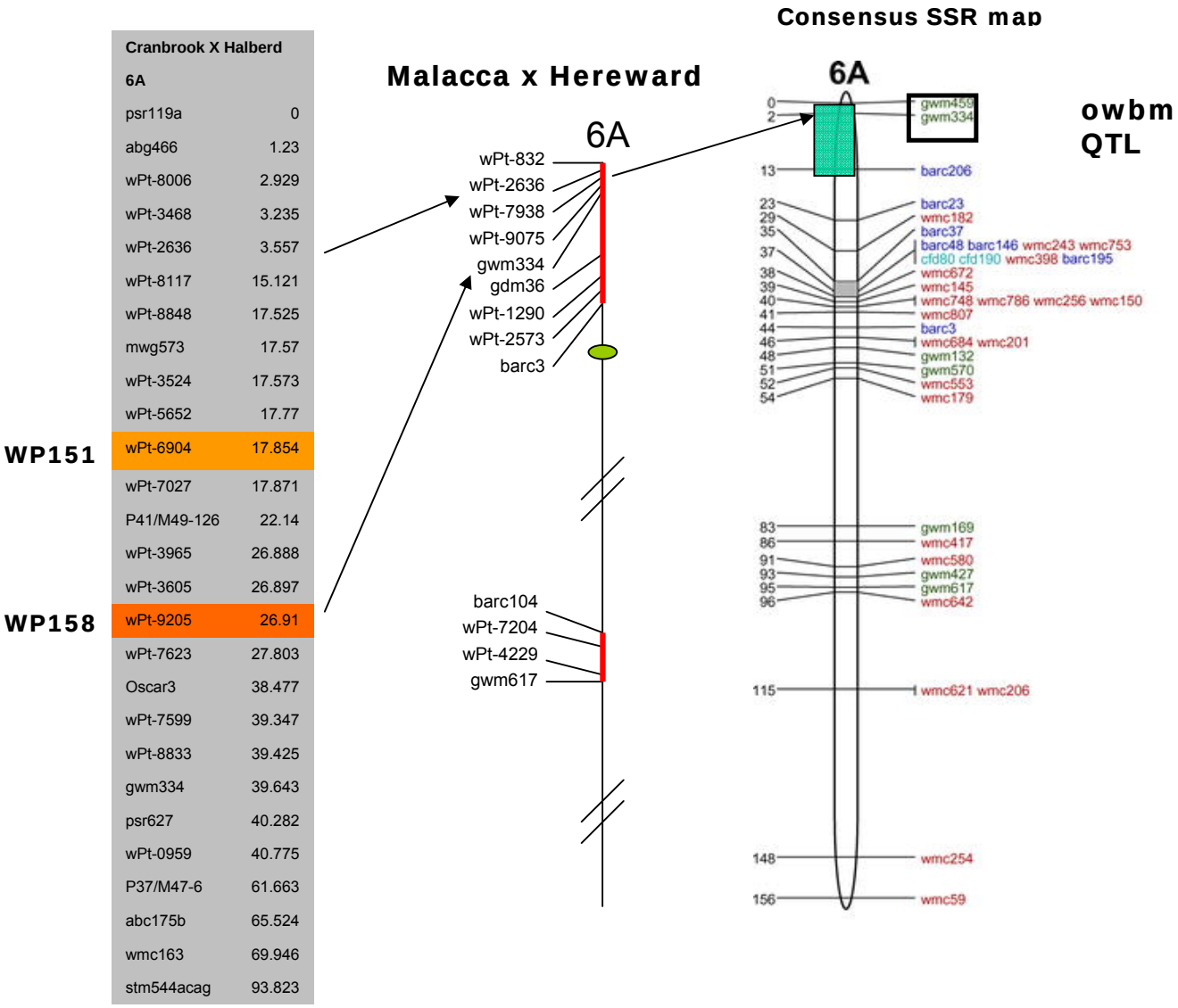


Figure 93. Comparative genetic maps for chromosome 6A developed from the Cranbrook x Halberd DArT map and the wheat consensus SSR map (black box indicates locus significant from single marker ANOVA)

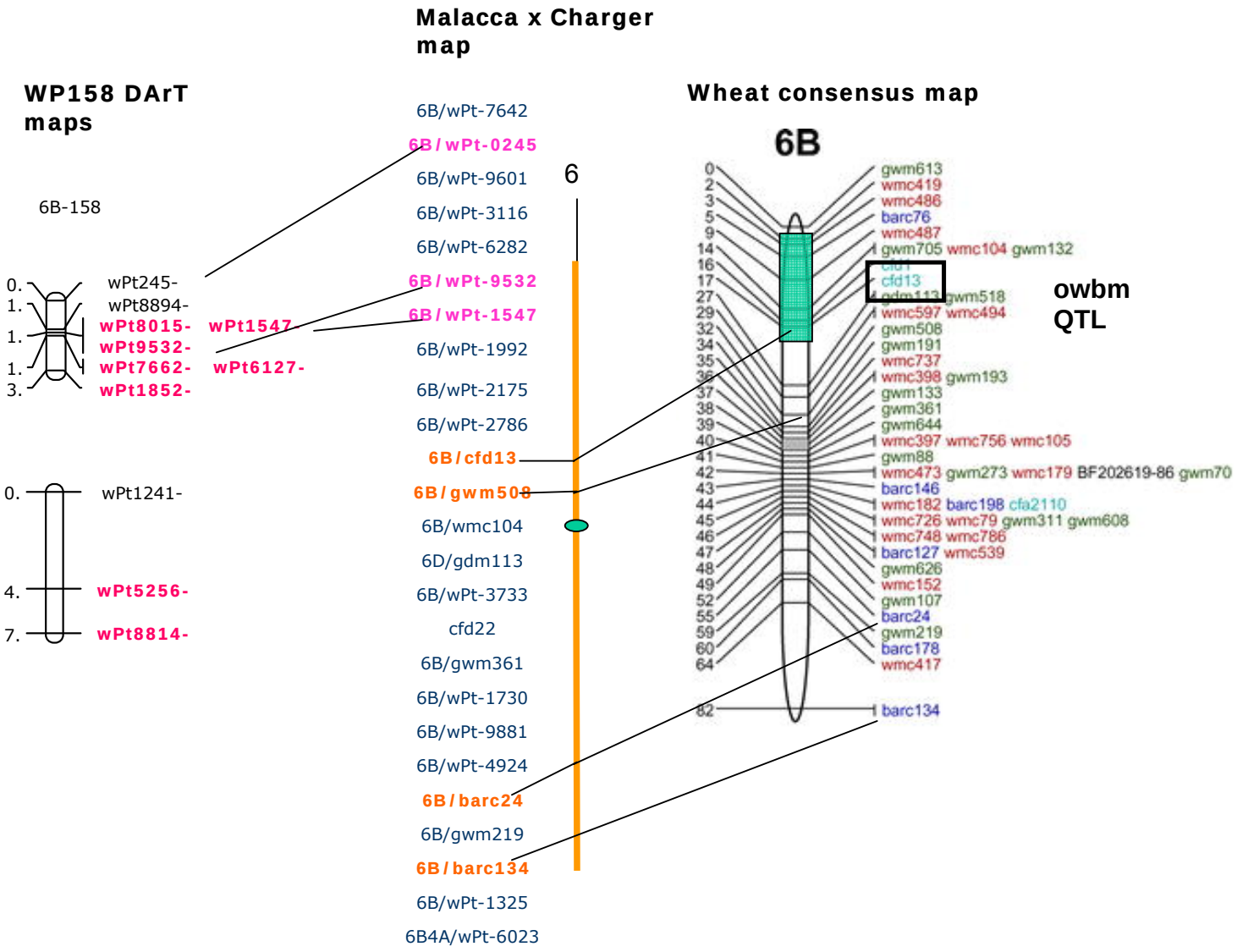


Figure 94 Comparative genetic maps of chromosome 6B developed from the WP158 DArT maps, Malacca x Charger SSR/DArT map and the wheat consensus SSR map (black box indicates locus significant from single marker ANOVA)

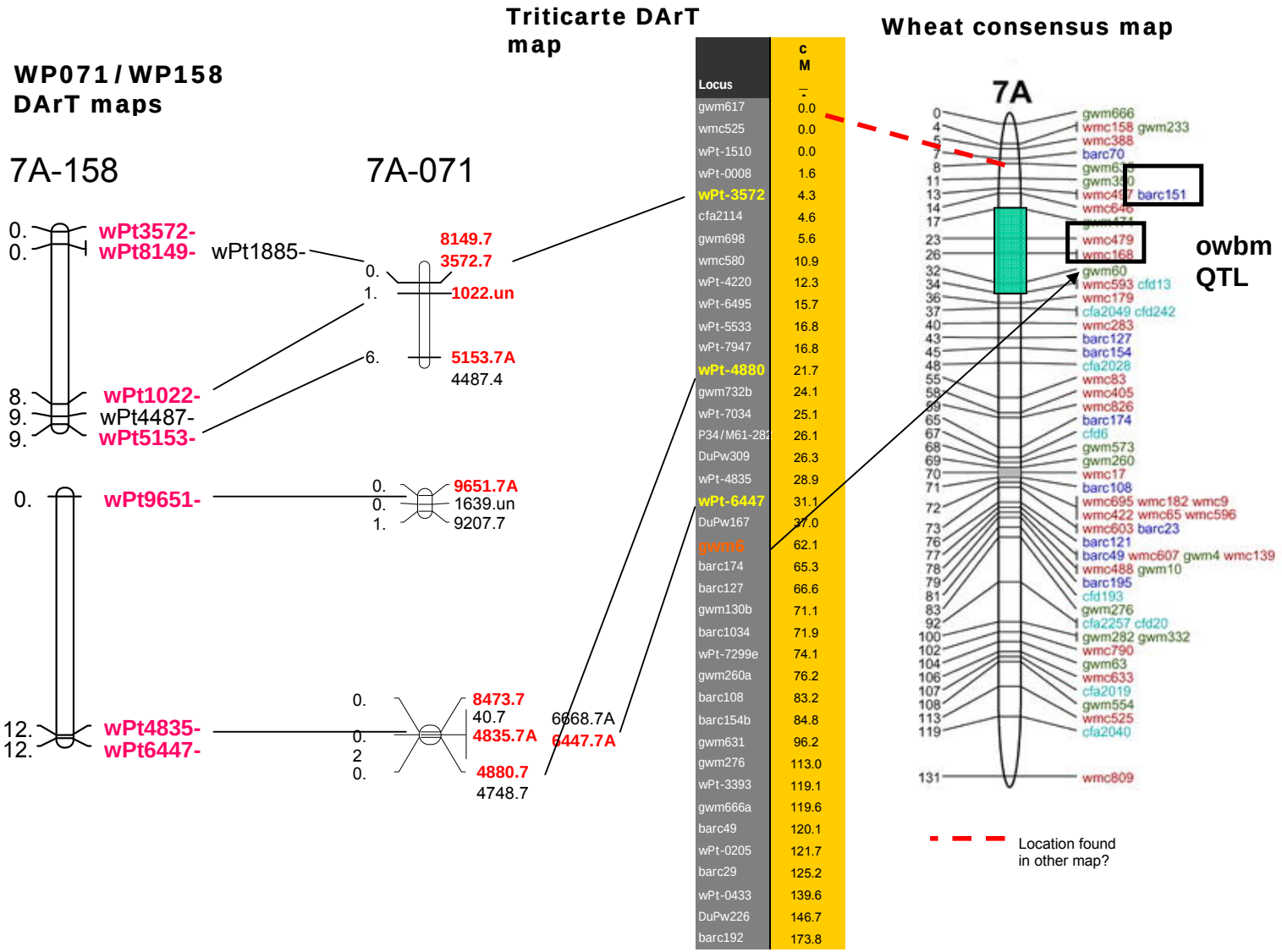


Figure 95. Comparative genetic maps of chromosome 7A developed from the WP071/WP158 DArT maps, Triticarte consensus DArT map and the wheat consensus SSR map (black boxes indicate loci significant from single marker ANOVA)

SSR ANOVA Results

The analyses above have located possible owbm QTL to specific chromosomal regions. However, these QTL are tentative due to the small number of lines, 28, (the phenotypic extremes) used in the comparisons, and the associations were not tested for statistical significance. Thus, to extend the analysis to the whole population so that single marker ANOVAs could be carried out to validate these QTL, known SSR markers located within these regions were assessed for polymorphism so that they could be genotyped on the whole population. At least one marker was found that could be used on the whole population for each region. Additionally, other markers were tested and used as controls. The analysis was, however, slightly complicated by the fact that several of the SSRs revealed more than one polymorphic band, presumed to be from homoeologous chromosomes.

Using the genotype scores on each line and the 2006 WOBM field scores, single marker ANOVAs were carried out marker by marker for each population. The full results are shown in Table 23 for populations WP071, WP151 and WP158, respectively, which shows the markers, chromosome locations, the means of the allele groups, and the significance of the differences. The significant and 'tentative' effects are summarised in Table 24. In all cases, except for a marker on 5D, the mean of the S group has a higher infestation level than the R group.

For WP071, none of the allele comparisons reached statistical significance, so that the putative, DArT located, QTL could not be definitely confirmed with these data. However, there were tentative, but non-significant associations with *Xbarc142* on 5B and *Xwmc479*, on 7A. The former aligns with the suggested DArT location of a WOBM QTL (Figure 92) and appears in Table 20 for the extremes analysis. The latter also appears in Table 19 and 21 and in Figure 94. For both markers, the susceptible allele group has a higher mean infestation than the allele from the resistant parents.

For WP151, there were three significant effects, for loci *Xgwm533* on 3BS, *Xgdm63* on 5D, and *Xgwm334* on 6A. The 3B effect confirms the phenotypic extremes DArT analysis, Table 20, although this marker is more distal from the

expected location, Figure 91. The 6A effect also confirms the putative location by aligning the significant DArT markers from the WP151 and WP158 phenotypic extremes analysis (Tables 20 and 21) with the Cranbrook x Halberd DArT map and the wheat consensus SSR map, Figure 93. The 5D effect is new, and may be a false positive, since the direction of the effect suggests that the allele from the R parent is associated with great infestation.

For population WP158, three markers give significant differences between S and R groups, *Xwmc11* on 3A, Xcfd13 on 6B and *Xbarc151* on 7A. All three confirm the putative locations given by the DArT phenotypic extremes analysis (see Figure 90, 94, 95).

Table 23. Marker means for SSR allelic groups for each population, difference and significance of difference estimated by single marker ANOVA using the 2006 orange blossom midge field scores

WP071					
Marker	**Chromosome**	**Access allele**	**Welford allele**	**Difference**	**Significance level**
cfd79	3A	19.9	17.5	2.4	ns
gwm291	5A	16.2	17.9	-1.7	ns
barc142	5B	22.7	16.4	6.3	*'?
barc151	7A	19.8	17.6	2.2	ns
barc70	7A	16.7	18.6	-1.9	ns
wmc479	7A	18.4	14.2	4.2	*'?

WP151					
Marker	**Chromosome**	**PBI01/009 allele**	**Brompton allele**	**Difference**	**Significance level**
gwm312	2A	16.9	28.0	-11.1	ns
gwm539	2D	31.6	23.1	8.5	ns
psp3144	3BL	20.9	32.4	-11.5	ns
cfd79	3BS	27.2	19.3	7.9	ns
gwm533	3BS	47.4	20.3	27.1	*
gdm63	5D	18.4	32.6	-14.2	*
gwm334	6AS	29.4	16.2	13.2	*
gwm63	7A	21.3	23.5	-2.2	ns
gwm577	7BL	24.1	18.9	5.2	ns

WP158					
Marker	**Chromosome**	**WW57 allele**	**Carlton allele**	**Difference**	**Significance level**
gwm 312	2A	15.3	16.9	-1.6	ns
gwm 614	2B	20.9	3.6	17.3	***
gwm 539	2D	12.1	14.7	-2.6	ns
wmc11	3AS	17.8	9.9	7.9	*
cfd79	3AS/3BS/3D	15.8	10.7	5.1	ns
psp 3144	3BLC	13.9	11.1	2.8	ns
barc68	3BS/3DS/4B	17.6	8.7	8.9	ns
psp 3103	4D	10.4	12.9	-2.5	ns
gwm 291	5A	8.9	13.6	-4.7	ns
gdm 63	5D	5.7	10.9	-5.2	ns

Table 24. SSRs showing significant allelic differences for each population, estimated by single marker ANOVA using the 2006 orange blossom midge field scores

Cross	Marker	Chromosome	S allele	R allele	Difference	Significance level
WP158	wmc11	3AS	17.8	9.9	7.9	*
WP051	gwm533	3BS	47.4	20.3	27.1	*
WP158	cfd13	6BS	17.5	9.3	8.2	*
WP071	wmc479	7A	18.4	14.2	4.2	*'?
WP158	barc151	7AS	13.2	4.7	8.5	*
WP071	barc142	5B	22.7	16.4	6.3	*'?
WP051	gdm63	5D	18.4	32.6	-14.2	*
WP051	gwm334	6AS	29.4	16.2	13.2	*

Discussion

The DArT and SSR analysis has identified several genetic effects that contribute to the resistance of the lines Welford, Brompton and Carlton. The major effect is clearly *Sm1*, but other genes are also involved, particularly the large effect of 3B in the PBI01/009 x Brompton cross. Interestingly, this is the cross with the highest mean susceptibility, so this QTL may be a QTL for greater susceptibility in PBI01/009, rather than resistance only in Brompton, and may be fixed in the other parents. The significant QTL effects vary between 27.1 midge/10 ears for 3B to 8.2 midge/10 ears for 6B, compared to the *SM1* effect measured at the marker of 10.8 in the WP071 cross, 38.2 in the WP151 cross and 17.3 in the WP158 cross.

Figure 36 summarises the probable owbm QTL locations using all the information from the DArT and SSR analyses. From looking at the intra-chromosomal locations, the 3A and 3B QTL are probably not homoeologous, but it is possible that the 6A and 6B QTL are homoeologous loci.

The mechanism of *Sm1* resistance is thought to be chemical, and it will be interesting to see what forms of resistance/tolerance are imparted by these QTL by future studies. It could be that they are related to escape mechanisms

associated with a difference in flowering time. The relationship to flowering time QTL is thought to be important, since one obvious mechanism by which QTL for resistance can work is by infestation escape due to a mis-match between flowering time in wheat and emergence of the midges from the soil. This was examined by comparisons of the present discovered QTL with a meta-QTL analysis of flowering time in adapted UK crosses (Griffiths *et al.*, in press). This suggests that the owbm QTL on chromosomes 3B, 5B and 7A may be associated with flowering time QTL, but those on 3A, 6A and 6B probably not.

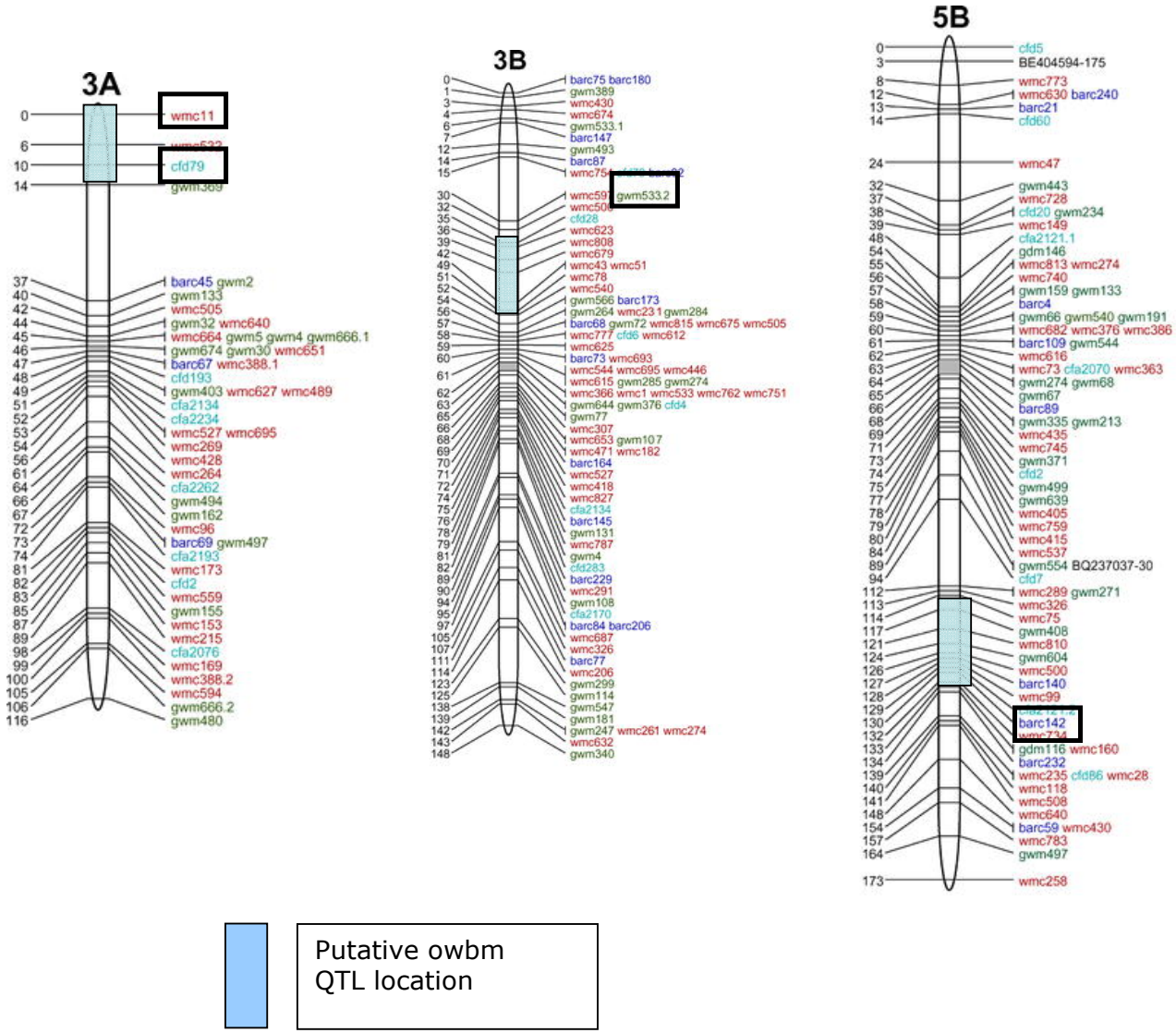
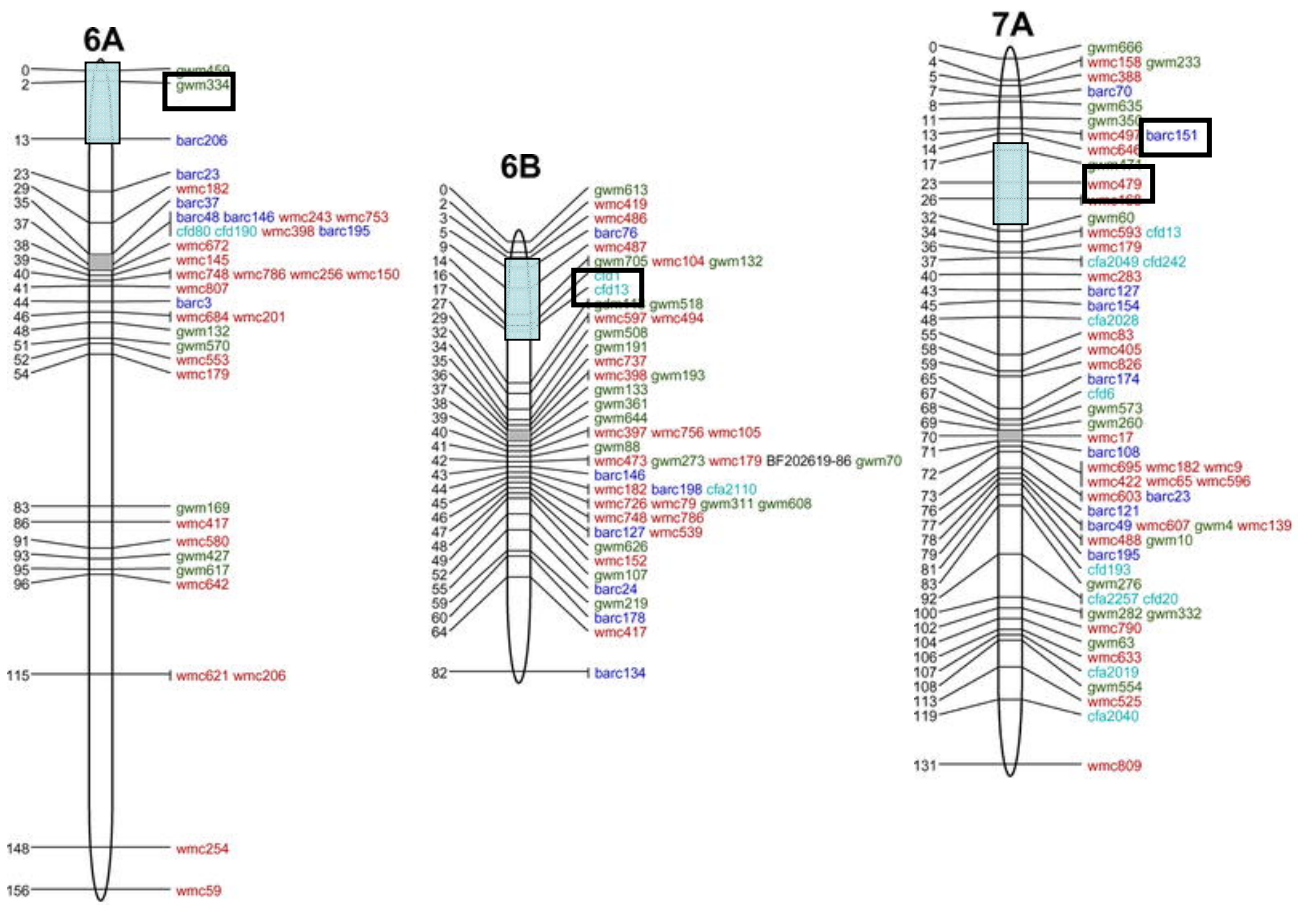


Figure 96. Summary of nearest markers for putative locations of the owbm QTL mapped onto the SSR consensus maps


6A
0 gwm334
13 barc206
23 barc23
29 wmc182
35 barc37
37 barc48 barc146 wmc243 wmc753
cfd80 cfd190 wmc398 barc195
38 wmc672
39 wmc145
40 wmc748 wmc786 wmc256 wmc150
41 wmc807
44 barc3
46 wmc684 wmc201
48 gwm132
51 gwm570
52 wmc553
54 wmc179
83 gwm169
86 wmc417
91 wmc580
93 gwm427
95 gwm617
96 wmc642
115 wmc621 wmc206
148 wmc254
156 wmc59
6B
0 gwm613
2 wmc419
3 wmc486
5 barc76
9 wmc487
14 gwm705 wmc104 gwm132
16 cfd1
17 cfd13
27 gwm518
29 wmc597 wmc494
32 gwm508
34 gwm191
35 wmc737
36 wmc398 gwm193
37 gwm133
38 gwm361
39 gwm644
40 wmc397 wmc756 wmc105
41 gwm88
42 wmc473 gwm273 wmc179 BF202619-86 gwm70
43 barc146
44 wmc182 barc198 cfa2110
45 wmc726 wmc79 gwm311 gwm608
46 wmc748 wmc786
47 barc127 wmc539
48 gwm626
49 wmc152
52 gwm107
55 barc24
59 gwm219
60 barc178
64 wmc417
82 barc134
7A
0 gwm666
4 wmc158 gwm233
5 wmc388
7 barc70
8 gwm635
11 gwm35
13 wmc49 barc151
14 wmc646
17
23 wmc479
26
32 gwm60
34 wmc593 cfd13
36 wmc179
37 cfa2049 cfd242
40 wmc283
43 barc127
45 barc154
48 cfa2028
55 wmc83
58 wmc405
59 wmc826
65 barc174
67 cfd6
68 gwm573
69 gwm260
70 wmc17
71 barc108
72 wmc695 wmc182 wmc9
wmc422 wmc65 wmc596
73 wmc603 barc23
76 barc121
77 barc49 wmc607 gwm4 wmc139
78 wmc488 gwm10
79 barc195
81 cfd193
83 gwm276
92 cfa2257 cfd20
100 gwm282 gwm332
102 wmc790
104 gwm63
106 wmc633
107 cfa2019
108 gwm554
113 wmc525
119 cfa2040
131 wmc809

The fact that the crosses differ in their absolute resistance levels suggests that the resistant parents have cumulative effects of *Sm1* plus the other QTL. The difference between WP071 and WP158 on the one hand and WP151 on the other appears to be mainly due to QTLs on 3A, 3B and 7A QTL (Table 24). Thus in using these and other parents in crosses it will be important to ensure that *Sm1* is pyramided with the other effects to ensure immunity in new derived varieties. This may also provide insurance against *Sm1* resistance breaking down.

Task D Development of model

D1. Develop model

T J A Bruce, L E Smart, J A Martin - Rothamsted Research
S A Ellis - ADAS

The exhaustive field trapping experiments described Task B provided information on how best to use the pheromone traps. It was apparent that fields had grown wheat before were important sources of infestation and that it was necessary to consider movement of female midges from these fields into those currently being cropped with wheat. The grid experiments had shown that infestation patterns were best predicted by pheromone trap catches in source fields. Thus the decision support model (Figure 97) specifies that traps should be set up in these fields. Discussions with farmers at the Cereals event had shown that some are not aware that even susceptible wheat varieties become resistant to owbm once flowering starts and thus the model emphasises that monitoring is only necessary during the ear emergence growth period.

During the course of this project it was decided that the provisional threshold of 20 midges per trap per day during ear used at the end of the previous LINK project (LK0924) was too low. It led to too many occasions on which a false positive was obtained i.e. insecticide treatment recommended when pest levels were not damaging. However, it is better to allow a margin for error because if the threshold is set too high false negatives (insecticide not applied when damaging levels of pest are present) would occur, which would erode farmer confidence in the traps.

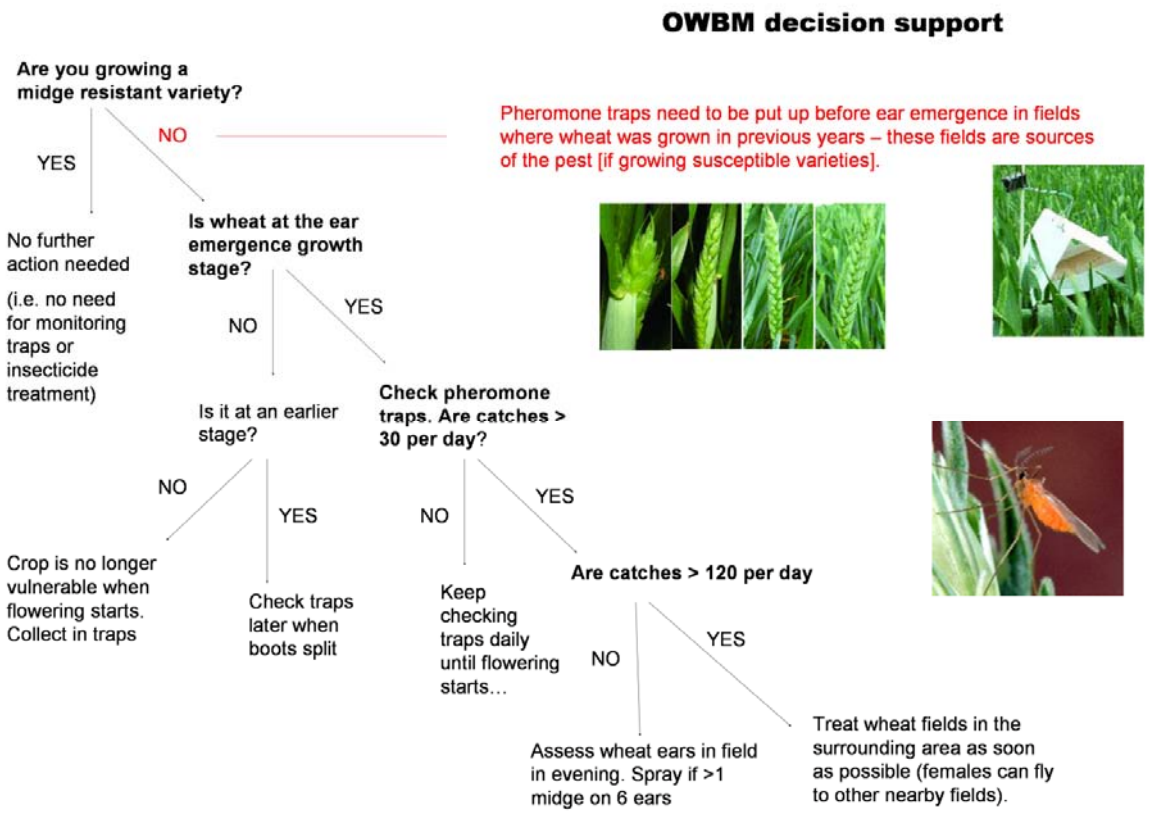


Figure 97. Owbm decision support model

Also pheromone traps only monitor male midges populations whereas it is female owbm that lay eggs from which the damaging stage of the pest emerges. Therefore, it is not possible to set a simple trap catch threshold above which economic damage occurs and below which it does not. It was decided to implement a two step threshold in which crop inspections are carried out when a lower threshold of 30 midges per trap per day is exceeded and insecticide treatment is triggered if a higher threshold of 120 midges per trap per day is exceeded. Although the second threshold is quite high it is comparable with the one used for pea midge (Biddle *et al.*, 2002). It is advantageous that the pheromone traps are so sensitive and catch so many owbm because they provide an early warning of midge flight thus avoiding situations in which insecticide sprays are applied too late when they are needed.

A decision support model that can be used by farmers was developed using a stepwise decision tree involving yes/no answers to questions. When growing a susceptible wheat variety pheromone traps need to be put out before ear emergence in fields where wheat was grown in previous years and provide a source of the pest. These traps should be monitored daily during the susceptible growth stage. When trap

catches exceed a lower threshold (30 midges per day) crop inspections provide additional information to help decide whether to treat a field. When a higher threshold (120 midges per day) is exceeded insecticide treatment is needed to protect against damage.

D2. Model verification study

T J A Bruce, L E Smart, J A Martin - Rothamsted Research
S A Ellis - ADAS

Introduction

Model verification involved analysis of pheromone trap monitoring data collected at ADAS sites and data on the percentage of grain damage by owbm larvae.

Materials & methods

Pheromone trapping was undertaken at three ADAS sites throughout the experiment, Boxworth Cambridgeshire, High Mowthorpe North Yorkshire and Grindale East Yorkshire. High Mowthorpe was monitored in 2006, 2007 and 2008, Boxworth in 2007 and 2008 and Grindale in 2008 only. For all of these sites the highest catch of male midges in pheromone traps was recorded and compared with the % of damaged grain. A level of 5% grain damage was considered a threshold above which an insecticide would be justified for a milling or seed variety and 10% grain damage for a feed variety (J Oakley, pers comm.). Regression analysis was used to investigate any relationship between peak male midge catch and the ultimate level of grain damage.

Results

The peak trap catch for each wheat field (sink fields) monitored between 2006 and 2008, together with the percentage grain damaged, is given in Table 25. Catches of owbm males in pheromone traps in source fields are also given, although the source fields are not named.

Table 25. Peak catch of owbm males in pheromone traps and % grain damaged at a range of sites between 2006 and 2008

Year	Site	Field	Peak catch Source	Peak catch Sink	% grain damage
2006	HM	Wetherplain		56	3.3
2006	HM	Smithfield		80.5	5.4
2006	HM	Malton Road		39	0
2006	HM	Office Field E		79.5	0
2006	HM	Home Field		123.5	5.5
2006	HM	Front Field E		67	5.2
2006	HM	Crow Wood		127.3	0.6
2006	HM	Crow Tree		49.5	0.1
2006	HM	Kirby Field NE		62	0.9
2006	HM	Kirby Grass N		96	2.3
2006	HM	Kirby Grass S		31.3	3
2007	HM	Stonechair	31	46	2.7
2007	HM	Homefield	37	146.5	0
2007	HM	Tommy Ireland	101	357	1.4
2007	Box	Pamplins South	5.3	0.5	0.5
2007	Box	Long Field	3.7	6.5	0.5
2007	Box	40 Acres	11.8	5.8	1.3
2008	HM	Wetherplain	81	48	1
2008	HM	Stonechair	81	81	1
2008	HM	Front Field W	1.5	24.5	0.7
2008	HM	Crow Tree	22.5	147.5	8.4
2008	Box	40 Acres S	273.7	266	8.9
2008	Box	Knapwell	225.8	75.7	4.8
2008	Grindale	White Dyke	0.2	0.3	0.8
2008	Grindale	Argham Gates	0.3	0.2	0.2
2008	Grindale	Chalk Road	0.3	0.3	0

There was a poor correlation between % grain damage and peak numbers of male owbm in wheat (sink) fields during the susceptible period (% grain damage = 1.21 +

0.0134 sink, R^2 = 0.43) and also between peak catches in source fields and % grain damage (% damage = 0.854 = 0.0221 source, R^2 = 0.65).

As a result, data were summarised in Tables 26-27 which indicate the number of occasions when a midge catch of less than 30/trap/day, 30-120/trap/day and more than 120/trap/day, resulted in levels of grain damage above the 5% threshold for yield loss in seed and milling varieties.

Table 26. Number and % of sink sites with less or more than 5% grain damage at a range of pheromone trap catches of male owbm

	<30 midges		30-120 midges		>120 midges		Total no of sites
	No of sites	% of sites	No of sites	% of sites	no of sites	% of sites	
<5% damage	7	100	10	77	3	50	20
>5% damage	0	0	3	23	3	50	6
Total	7	100	13	100	6	100	

Table 27. Number and % of source sites with less or more than 5% grain damage at a range of pheromone trap catches of male owbm

	<30 midges		30-120 midges		>120 midges		Total no of sites
	No of sites	% of sites	No of sites	% of sites	no of sites	% of sites	
<5% damage	7	88	5	100	1	50	14
>5% damage	1	12	0	0	1	50	1
Total	8	100	5	100	2	100	

Data from sink sites showed that on no occasion when there were fewer than 30 male owbm/trap/day was grain damage greater than 5%. When midge numbers were between 30 and 120/trap/day then 77% of sites had less than 5% damage. With this number of midges, the decision flow chart would have recommended inspecting the crop for female midges and using the results of this assessment to predict the need for treatment. On approximately 75% of occasions when male midge catches ranged from between 30 and 120/trap/day, an insecticide was unnecessary. This emphasises the need to examine susceptible crops for female midges. Where more than 120

midges/trap/day were caught, the decision flow chart recommends insecticide treatment. This would have been the correct decision in 50% of cases. Sprays would have been applied unnecessarily to half of fields, but this is preferable to not applying a treatment and suffering yield loss.

In source fields where midge catches were less than 30/trap/day results suggest that on 12% of occasions grain damage would have exceeded the 5% threshold for seed and milling varieties. However, this result is due to a single site in which crop inspections would have been advised if the corresponding sink field (Crow Tree) was also monitored. None of the source sites with between 30 and 120 midges/trap/day resulted in greater than threshold levels of grain damage. At more than 120 midges/trap/day 50% of sites would have been sprayed as advised by the decision flow tree. However, these data only relates to two sites, and in one the level of grain damage was very close to the 5% threshold for seed and milling varieties.

Discussion

In general, levels of midge infestation between 2006 and 2008 were much lower than recorded in the previous outbreak year of 2004. The low levels of midge infestation hindered the verification of the decision flow tree as it was not possible to examine the impact of a range of midge catches on grain damage. Also, verification has only been based on 26 sink and 15 source fields and further monitoring would allow more robust analysis of the proposed thresholds.

There can be some confidence in the proposed threshold of greater than 30 midges/trap/day (but less than 120/trap/day) to indicate a need to inspect crops for the pest. When midge numbers were below this level in wheat fields, there was no occasions when the level of grain damage was above the 5% threshold for seed and milling crops. There was one occasion when midge catches in a source field were below 30/trap/day and the level of grain damage in the nearby wheat field was above the 5% threshold. However, this risk would have been detected if traps were also located in the sink field (Crow Tree) where 147.5 midges/trap/day were recorded.

There were only five occasions when peak male midge catches were greater than 120/trap/day in either the wheat (sink) or source field. In three of six wheat fields an

insecticide spray would have been justified, and in a fourth field the level of grain damage was only just below the 5% threshold.

Although levels of midge infestation were generally low between 2006 and 2008, and only 26 sites were monitored, there is evidence to suggest that the proposed thresholds in the decision flow chart are a good basis with which to predict the risk of midge attack. In general they err on the side of caution, but this is preferable to a "false negative" where crops would not be sprayed and yet suffer significant midge attack.

Key findings and general conclusions

The key findings for this study are summarised below.

A. Understanding basic female biology

A1. Wind tunnel tests

- Owbm flight under controlled laboratory conditions was shown to depend on humidity levels more than on light intensity

B. Understanding and interpreting pheromone trap catches

B1. Pheromone trap calibration study

- There can be large variations in trap catch from field to field
- In some years there is a good correlation between trap catch and crop damage level
- Movement of females between fields can complicate the relationship between trap catch and damage levels
- Trapping in non-wheat source fields or wheat crops can be a good indicator of owbm risk
- Traps are best sited in fields which have been damaged by owbm in the last two years, irrespective of crop

B2. Female movement study

- Infestation within a field was best explained by pheromone trap catches in neighbouring fields

C. Biochemistry of tolerance and resistance

C1. Biochemical study of model varieties

- Welford was highly resistant to larval attack although female owbm were still attracted to it and laid eggs on it
- There was evidence of induction of phenolic acids in infested seed from some varieties, but levels of these acids did not fully explain the resistance in Welford

C2. Screening of germplasm and development of markers

- The major gene influencing owbm resistance in UK varieties is *Sm1*
- Other chromosomes may also influence resistance such as 3B. The effect of this could be related to early flowering to escape midge infestation.

D. Development of model

D1. Develop model

- A simple decision flow chart was developed to provide a stepwise procedure to assessing owbm risk.

D2. Model verification study

- Low levels of midge infestation hindered model verification.
- Proposed thresholds are a good basis for predicting risk
- Further validation is required to improve risk prediction

In general, the current project has improved understanding of the owbm problem. Specifically, a commercial pheromone trap is now available which is effective at indicating when midge emergence is underway, and a decision tree has been developed to improve the process of risk assessment for the pest. Owbm resistance has been confirmed as being linked with the *Sm1* gene, although other chromosomes may also influence resistance. The induction of phenolic acids in the grain did not fully explain resistance to owbm.

Although economically damaging outbreaks of owbm are sporadic, pesticide usage figures indicate that about 15% of UK wheat crops are treated against the pest. This suggests that the perceived risk of attack is greater than occurs in practice and has important environmental implications as the favoured product is chlorpyrifos, a broad spectrum insecticide. To minimise the impact of unnecessary insecticide applications against non-target species, further refinements of owbm management are now required to promote rational control of the pests.

This work should concentrate on the following:

- Understanding the biochemical basis of resistance: although it is clear that the *Sm1* gene is responsible for resistance, as in Canada, the mechanism of resistance is still not understood. Canadian research suggested a correlation between increased levels of ferulic acid and resistance, but work with UK varieties does not support this. Further investigation is required to help future breeding programmes.

- Improving risk prediction: the decision flow chart proposes thresholds to help predict the need for insecticide treatment against owbm. The verification study suggested that these are a good basis for risk management. However, thresholds are based on data from a limited number of sites and further work is

required to confirm the initial findings and improve the precision with which it is possible to predict the risk of pest attack. Risk of damage is also primarily dependent upon the coincidence between midge activity and the susceptible stage of the crop. Being able to predict the likely timing of the susceptible growth stage in relation to midge emergence would be a significant development, and help to limit unnecessary insecticide treatment.

- Understanding natural enemies of owbm: rational use of insecticides involves understanding how chemical control affects beneficial parasitic or predatory species. An improved understanding of which parasites and predators influence owbm numbers is required to allow promotion of integrated strategies of control against the pest.

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