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Abstract and summary

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

by

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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°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).

A - pheromone trap catches

Wheat

N

Second Wheat

22

21.5

ROAD

2.5

3

Barley

Set aside

B - infestation level (larvae per ear)

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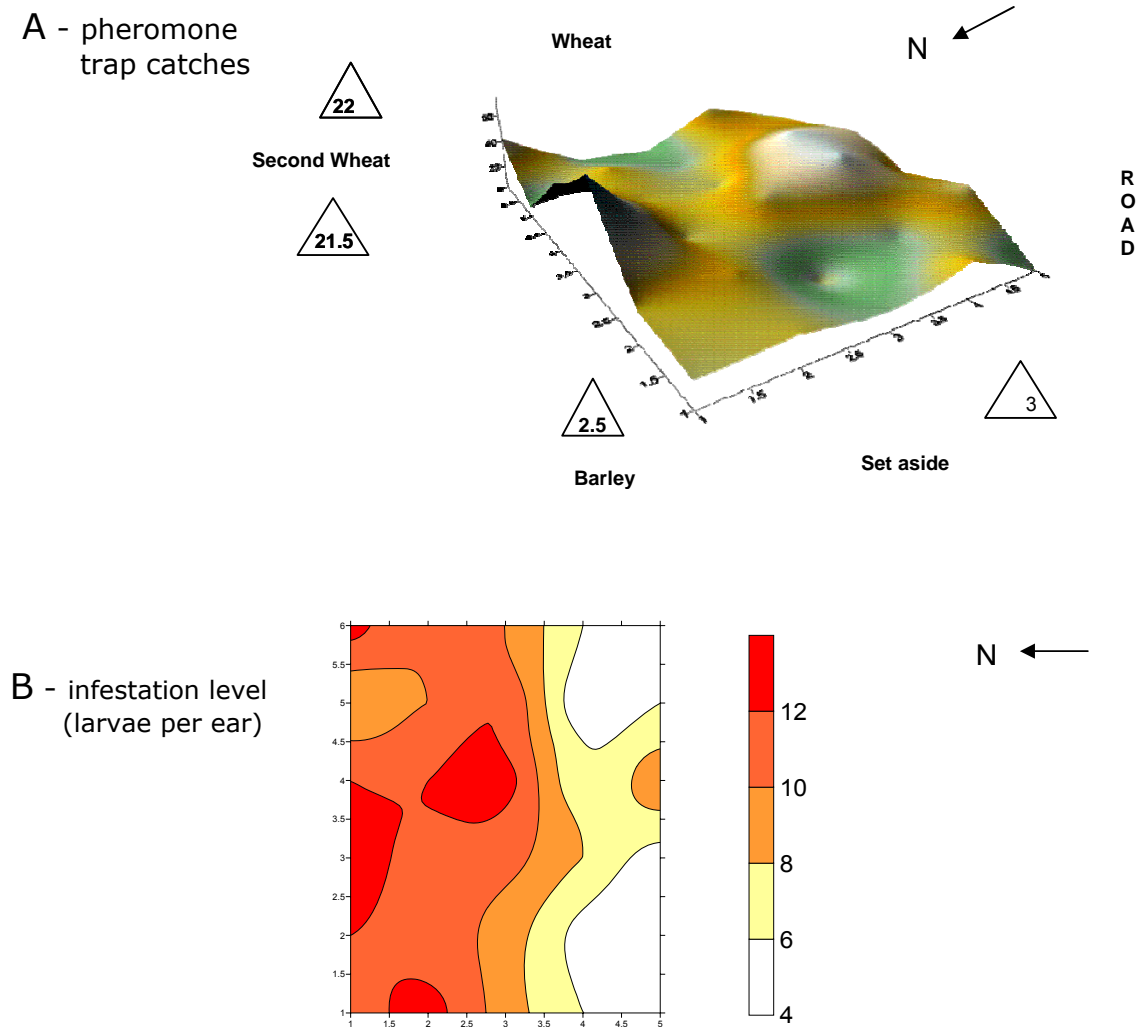
Figure 1. Relationship between (A) Pheromone trap catches within and around a wheat field during the susceptible growth period (catches in adjacent fields shown in triangles), and (B) Infestation level at the end of the season

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).

**Are you growing a
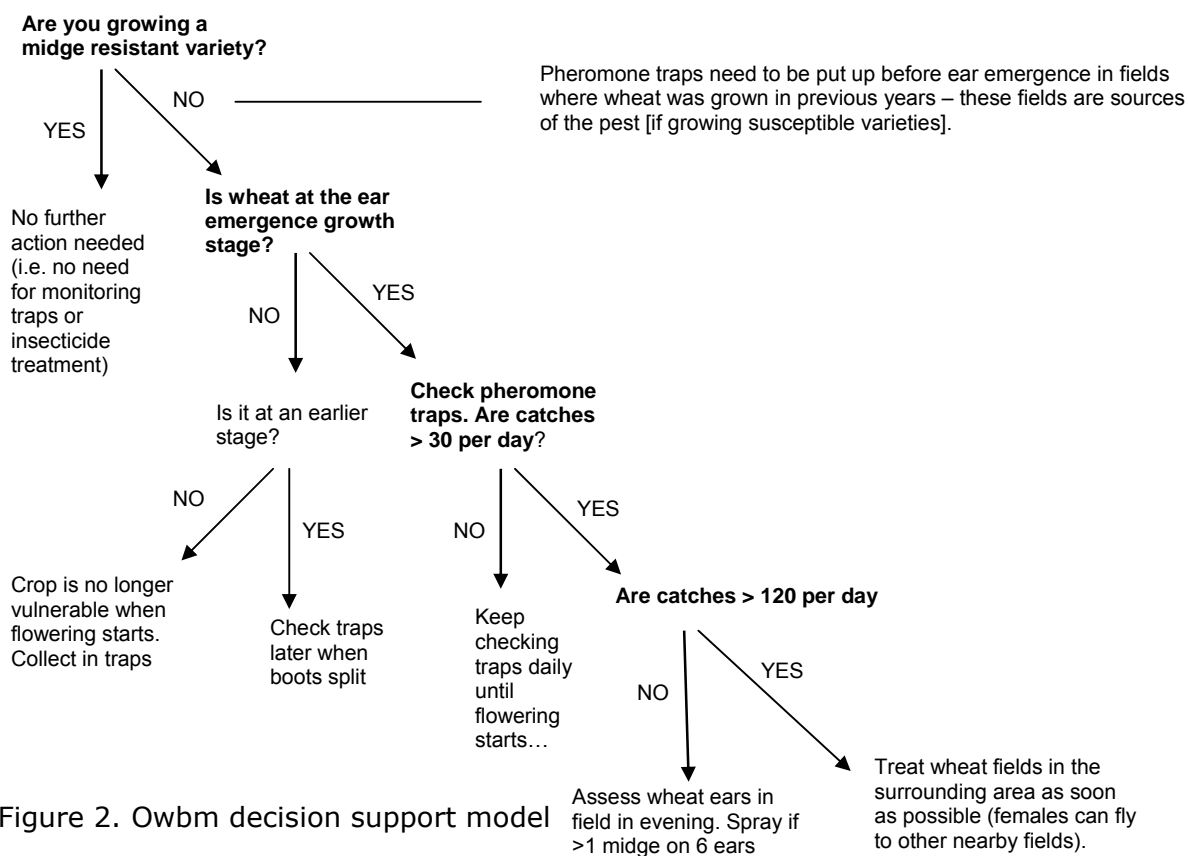midge resistant variety?**

Pheromone traps need to be put up before ear emergence in fields
where wheat was grown in previous years – these fields are sources
of the pest [if growing susceptible varieties].

NO

YES

**Is wheat at the ear
emergence growth
stage?**

No further
action needed
(i.e. no need
for monitoring
traps or
insecticide
treatment)

YES

NO

Is it at an earlier
stage?

**Check pheromone
traps. Are catches
> 30 per day**?

NO

YES

NO

YES

Are catches > 120 per day

Crop is no longer
vulnerable when
flowering starts.
Collect in traps

Check traps
later when
boots split

Keep
checking
traps daily
until
flowering
starts…

NO

YES

Figure 2. Owbm decision support model

Assess wheat ears in
field in evening. Spray if
>1 midge on 6 ears

Treat wheat fields in the
surrounding area as soon
as possible (females can fly
to other nearby fields).

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