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# Integrated control of wheat blossom midge: Variety choice, use of pheromone traps and treatment thresholds

by

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**Contents**

**Abstract**

The orange wheat blossom midge (wbm) has continued to increase in importance due to our warming climate. Chemical control is often not effective due to difficulties in determining the risk of attack in time to use the very short window for treatment, and may be unpopular due to perceived environmental risk. This project aimed to develop a system of integrated control based on an improved knowledge of the vulnerability and tolerance of commercial varieties to the pest, and a trapping system designed to assess risk at an early stage.

Some varieties have been shown to be resistant to wbm, yielding well even when heavily challenged by the pest and giving a yield advantage of 2 t/ha or more in such situations. Welford and Brompton are now included on the Recommended List as resistant varieties and more will follow.

Other varieties, such as Option, have been shown to be more vulnerable to the pest than most others, with some varieties, like Einstein, less vulnerable to damage. Susceptibility to damage by the pest has been shown to depend on several genes in a separate study conducted by the John Innes Centre (JIC). A mechanism for testing these traits was developed, and now needs to be applied to all varieties in the Recommended List. Until these tests have been done, the main priority for treatment should be group 1 and 2 wheat and seed crops.

Pheromone traps have been developed through to a marketable product. Tests have identified the optimum release rate and mechanism and trap design for UK conditions. The traps give a reliable indication of male wbm activity and an early warning of when crops may be at risk. The numbers of males caught were correlated to the level of egg-laying at an individual site by females. When the male midges are caught, this typically indicates a 2-day window before eggs are laid in the crop by female midges.

However, the risk of migration from adjoining fields and the suitability of the weather for egg laying caused considerable variation between sites, so a system for assessing these risks is needed as part of a decision making process based on the trap catches. A follow-on project was agreed to develop such a system. Varieties were shown to differ in the levels of the volatile chemicals used by female wbm to identify a suitable host and this may be a cause of varying vulnerability. The volatile chemicals were not successful as baits for traps to monitor numbers of egg laying female midges.

**Summary**

The orange wheat blossom midge, *Sitodiplosis mosellana*, is a common and increasingly important pest of wheat in the Northern Hemisphere, causing severe yield losses in years of high infestation. In an outbreak in the UK in 2004, crop losses were estimated to have exceeded £60 million. Larval feeding on the developing seeds causes shriveling and pre-sprouting damage and also facilitates secondary fungal attack by *Fusarium graminearum* and *Septoria nodorum*. This affects both the yield and quality of grain harvested. Due to difficulties in detection of *S. mosellana* the actual degree of damage to crops is often underestimated.

The impact of damage has been shown to vary considerably between varieties and recently a gene conferring resistance against the pest has been identified in Canada. We have tested for differences in **vulnerability** in terms of numbers of larvae produced by a given infestation of female orange wheat blossom midge (wbm) and the degree of **tolerance** of feeding by the larvae exhibited. The degree of development of surviving larvae was also assessed to determine whether the variety was **resistant** to attack.

WBM has a very patchy spatial distribution and also varies 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 midge outbreaks. The egg-laying 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 cryptic position, as well as 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. Detection problems mean that it is hard to predict when infestations that would warrant insecticide treatment have built up. There is considerable grower demand for a reliable monitoring system which would reduce the unnecessary use of pesticides against lower levels of midge infestation and allow populations of beneficial insects to increase providing a greater level of natural control. To address this we have carried out 3 years of field trials of semiochemical baited traps.

**Varietal testing (Technical reports 1 and 2)**

A range of winter wheat varieties selected to cover the main groups and traits in the UK was tested at five sites over three years to establish whether the exhibited differences in vulnerability, tolerance and resistance were related to wbm damage and how these traits could be most effectively assessed.

A critical factor influencing the number of larvae developing on each variety is the degree of coincidence between the vulnerable ear emergence growth stages (GS 53-59) with the egg-laying activity of female wbm at each site. All the varieties grown were assessed for the timing of ear emergence, which ranged between 9

and 14 days after Soissons for the other varieties on the current Recommended List tested. In 2002, wbm activity was biased towards later emerging varieties at all sites. In 2004, the main bias was towards early emerging varieties and sites. The 2003 year differed in this respect with some having an early bias and some a late bias. Over the three years of experiments the bias evened out allowing an assessment of any underlying differences in vulnerability; however, the results highlight the danger of drawing conclusions about this trait from the results from single sites or years. An examination of the results for those varieties grown over all three cropping years showed that there were significant differences between varieties in terms of vulnerability (Table 1), with the most vulnerable variety twice as vulnerable as the least.

Table 1. Mean numbers of larvae found compared to mean of controls.

| Variety | Larval score | Significance * |
|---|---|---|
| ECO 22 | 139.3 | d |
| Option (C) | 121.9 | cd |
| Xi 19 | 108.3 | bc |
| Tanker (C) | 105.3 | bc |
| Solstice | 99.6 | bc |
| Malacca (C) | 94.7 | abc |
| Consort (C) | 89.7 | ab |
| Claire (C) | 88.5 | ab |
| Einstein | 73.3 | a |
| Pennant | 73.0 | a |

Nb. Values followed by the same letter do differ at the 5% significance level.

**Tolerance**

The intention to measure tolerance by introducing plots treated with chlorpyrifos was confounded by very variable control on different varieties and by the incidental control of aphid infestations which decreased yields on untreated plots at some sites. The discovery of resistant varieties with their yields unaffected by the degree of midge infestation at each site allowed an alternative method of assessing yield loss against numbers of larvae found. The results were still subject to a large variation between sites, which may have masked any differences between varieties due to tolerance of damage.

The relative ranking of the different causes in variation between sites was: coincidence > vulnerability > tolerance.

**Resistance**

Antibiotic resistance can be defined as a failure of larvae to grow through to fully sized second or third instar larvae within 21 days of the eggs being laid. The varieties, Brompton, Carlton, Welford, Welland, Kipling and ELS 03-34 were all shown to meet this criterion, with Pennant showing some variation in the expression of the trait. Yields of these varieties were unaffected by the degree of wheat blossom midge challenge with numbers of larvae found greatly reduced compared to other varieties and those found failing to develop properly. Studies by the LINK consortium have so far established that this resistance is not due to the *Sm1* gene and that two or three other resistant genes with a similar mechanism may be involved. Testing of various chromosome substitution, photoperiod insensitive isogenic and Spark x Rialto recombinant doubled haploid lines suggested that the inheritance of susceptibility to wbm is complex, with several genes involved.

**Development of pheromone traps (Technical report 3)**

The female wbm sex pheromone, 2,7-nonanediyl dibutyrate, was synthesised both as racemic material and as pure enantiomers (Fig 5). Formulations were tested in both laboratory and field studies. In 2002, the sex pheromone was shown to be active under both laboratory (Fig 1) and field conditions (Fig 2) in attracting male wbm. From 2003, more detailed field evaluations using different vial loadings and pheromone purity were initiated to optimise the formulation. In 2003, it was shown that cost of pheromone could be reduced by using half the amount of material in a vial (5mg instead of 10mg) without affecting trap performance and also that the final purification step in synthetic pheromone production is economically justifiable as the activity of the material is substantially enhanced by it. In 2004, a range of field formulations were evaluated. Polyethylene vial and rubber septum formulations were compared; pure enantiomers were compared with racemic material, and different trap designs were compared. These showed that:

1. Racemic material was more effective than enantiomerically pure material.
2. Release rates higher than 0.5 µg/day did not increase trap captures enough to improve information on timing of midge flight activity.
3. Good season-long monitoring was obtained using either a 5mg loading in a polyethylene vial or a 1mg loading in a rubber septum. As rubber septa are easier to mass produce and a fifth of the amount of material can be used this formulation is clearly better suited for commercial production.
4. Delta traps with a larger opening (Agrisense) caught more *S. mosellana* than traps with smaller opening (Phero Tech).

Thus, the optimum pheromone release rate and methodology for use in the UK was determined.

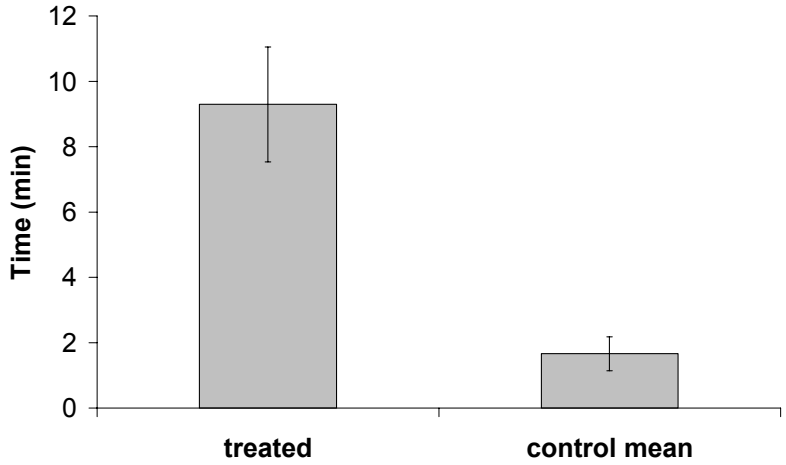


**Fig. 1 Male orange wheat blossom midge response to sex pheromone in an olfactometer**

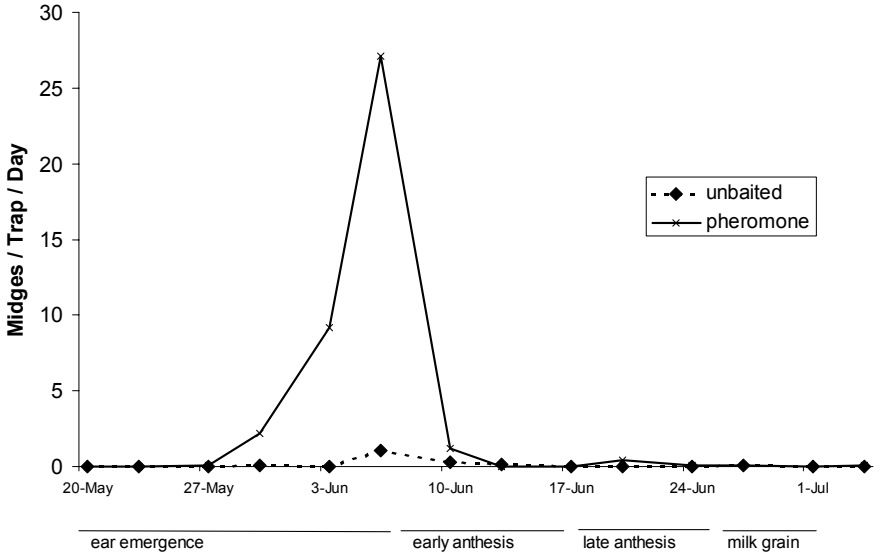


**Fig. 2 Orange wheat blossom midge pheromone trap catches at Rothamsted, 2002**

An investigation to correlate midge infestation in the crop and pheromone and sticky trap catches was carried out in 2004. Two pheromone traps were placed at the edge each of five fields tested (Fig. 3) at Rothamsted, together with a line of 5 yellow sticky traps placed at 5m intervals. Large variations in numbers of midges caught in the pheromone traps (approximately five-fold) and in timing of peak catches were found between fields, even though the distances between neighbouring sites were less than 100m. Catches over the season are shown in Fig. 3. Peak midge catches were highest at Hoosfield and Little Hoos and lowest at Great Knott. Catches peaked a week earlier at Hoosfield than at the other sites.

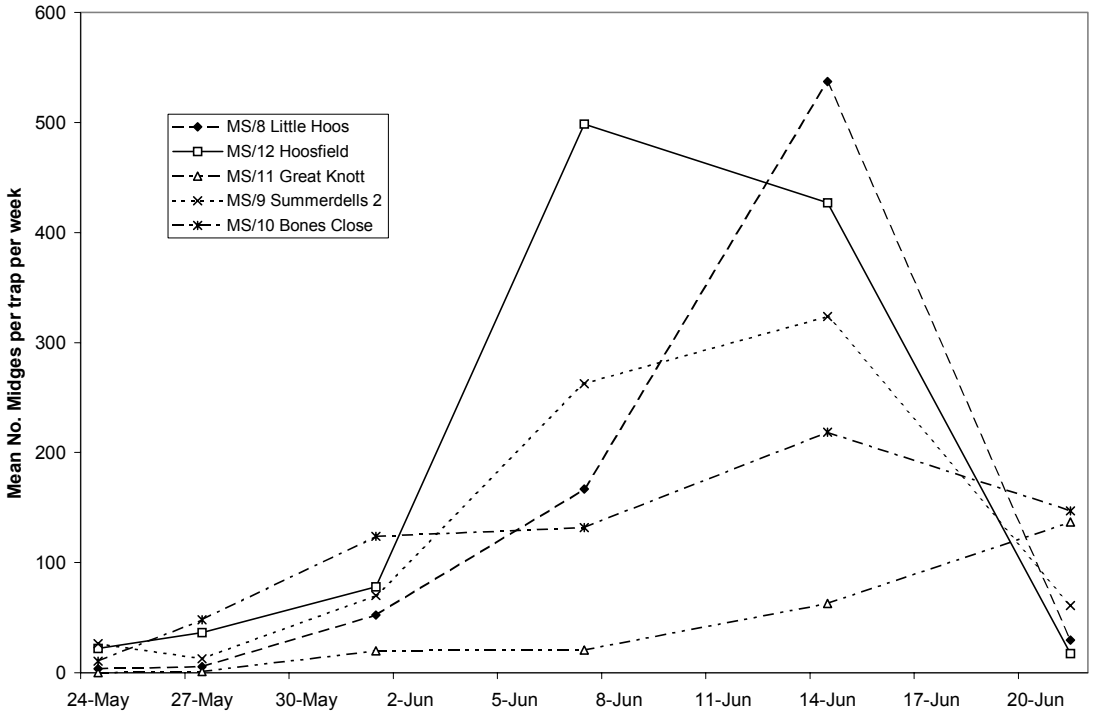


**Fig. 3 Pheromone trap catches at Rothamsted farm, 2004**

In these preliminary trials there was a good correlation between pheromone trap catch and subsequent midge infestation. In contrast to the yellow sticky traps, pheromone traps were highly sensitive and selective, catching at least ten times more *S. mosellana* than the yellow sticky traps and hardly any other non-target insects.

**Development of a female midge attractant**

In an attempt to develop selective traps that would catch female *S. mosellana*, experiments were carried out to investigate attraction to their host plant. Air entrainment samples of wheat volatiles were collected from seven different wheat varieties. Electrophysiological recordings from female midge antennae enabled identification of plant-derived semiochemicals mediating host location. Activity of synthetic wheat volatile blends (based on volatiles from wheat cultivars 'Lynx' and ECO22) was demonstrated in laboratory behavioural studies (Fig 4). Synthetic blends presented in the correct ratio were as attractive as the intact wheat panicles and the air entrainment samples. However, it was essential to release the volatiles in the correct ratio. Use of incorrect ratios resulted in complete loss of activity. The plant volatiles were also evaluated in field trapping experiments. However, considerable difficulty was encountered in developing field formulations that maintained season-long ratio integrity. These problems have so far precluded development of a selective trap for female midges.

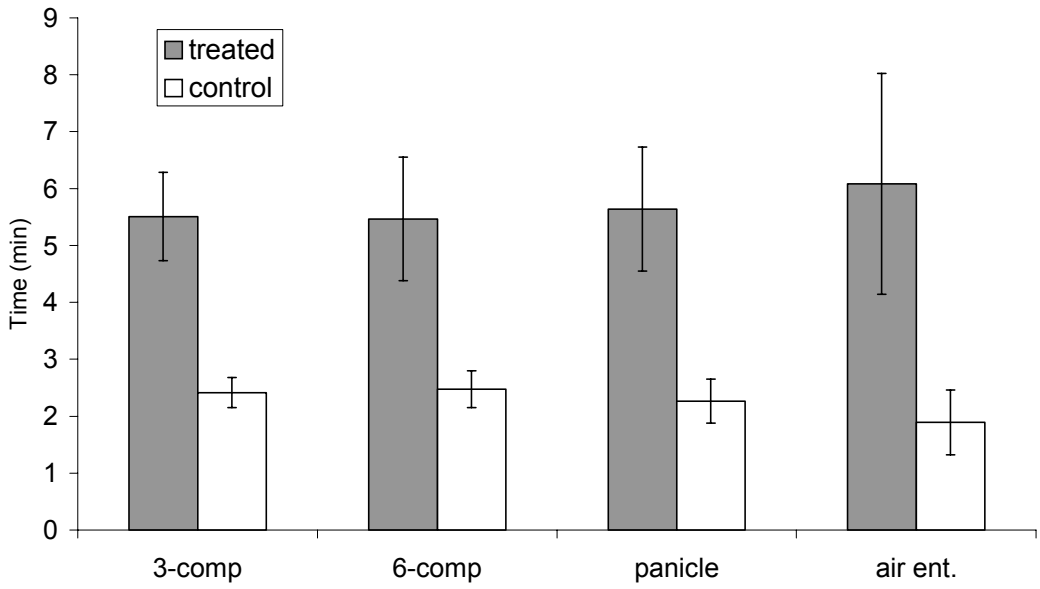


**Fig. 4 Olfactometer experiments with wheat volatiles: time spent by female WBM in treated and control arms**

**Conclusions**

- Resistant varieties, such as Welford and Brompton, retain their yield levels without the need for chemical control, even at heavily infested sites.
- There are significant differences in vulnerability between varieties, but at the farm-scale greater differences in infestation are likely to arise from coincidence factors. The most important of these is the timing of the vulnerable ear emergence stage in relation to wbm activity in the vicinity.
- Greater differences in the responses to control may result from the accuracy of timing than any differences in tolerance between varieties. If daily mean temperatures exceed 20°C, efficacy of control will be reduced and the effective treatment window shortened. When timed correctly for the individual variety, a chlorpyrifos application offered high levels of control.
- WBM pheromone was shown to be attractive to male wbm in both laboratory and field studies. Commercially viable synthesis was achieved that is also amenable to large-scale production.
- A correlation between trap catches and the numbers of eggs laid was found at individual sites, but the relationship differed markedly between sites so that further interpretation is needed to deduce the risk to crops. Given suitable temperatures, catches of around 20 wbm in a trap indicate a risk to crops in the area.

**Implications for integrated control**

1. Resistant varieties have a valuable role in reducing the area of crop at risk and, over time, the number of larvae dormant in the soil.
2. Pheromone traps may be used to monitor for the hatch of wbm and identify which crops are most exposed to egg-laying due to coincidence with their ear emergence period.
3. Vulnerable group 1 and 2 wheat and seed crops should be treated with chlorpyrifos as the highest priority.
4. Should time permit, the most vulnerable group 3 and 4 wheat varieties should be treated as a second priority.
5. Temperatures should be monitored during the period of treatment to calculate when eggs are likely to hatch. Treatment should cease at this stage and when crops reach GS 59, after which their natural resistance to wbm increases.

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**Technical detail 1**

## Field testing of varieties of wheat to establish their degree of vulnerability, tolerance and resistance to the orange wheat blossom midge

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### INTRODUCTION

The objective of the experiments reported here was to establish whether UK varieties differed in the vulnerability, tolerance and/or resistance to wbm and if so whether the differences were sufficiently large that variations in the strategy for control of the pest were appropriate for individual varieties.

A large range of factors, both genetic and environmental, interact with each other to determine both the **vulnerability** of a variety in terms of the numbers of larvae produced by a given infestation of female orange wheat blossom midge (wbm) and the degree of **tolerance** of feeding by the larvae exhibited. Genetic factors affect the suitability of the plant as a host, its attraction to female midges searching for an egg-laying site and the degree of coincidence between the susceptible later ear emergence growth stages (GS55-59) and the flights of the midges. The genetic factors identified to date are shown in Table 1. Antibiotic resistance is defined as a failure of larvae to develop to fully grown second or third instar stages within 21 days of the eggs being laid. Further antibiotic factors relating to mortality of eggs and pubescence slowing larval migration have been proposed, but not yet proven (Berzonsky *et al.*, 2003).

The expression of these characteristics is affected by a range of environmental factors influencing the coincidence between the susceptible stage and the flight of female wbm, the number of wbm hatching in the area and the ability of wbm to find and move to the crop. These environmental factors are listed in Table 2. Whilst some of the factors relating to attraction to the crop may be enhanced within a small plot trial, offering greater choice of variety, they also apply at a field scale as wbm can fly in to attractive fields from a radius of 1 mile from the field boundary.

**Table 1. Genotype : wbm interactions**

| Name | Mechanism | Impact | References |
|---|---|---|---|
| **Resistance 1** | Higher initial level and/ or faster response in producing *p*-coumaric acid in response to attack. | Larvae fail to grow through to second instar. Level tends to increase in all CVs after GS59 protecting against later larvae. | McKenzie *et al.* (2002); Berzonsky *et al.*, 2003), Ding *et al.* (2000). |
| **Resistance 2.** | Higher initial level and/ or faster response in producing ferulic acid in response to attack. | Larvae fail to grow through to second instar. Level tends to increase in all CVs after GS59 protecting against later larvae. | Ding *et al.* (2000). |
| **Tolerance 1** | Attacked grain less affected by larva | Grain size less reduced, retained over sieves into harvested sample | Kurppa (1989). |
| **Escape 1** | Early flowering variety tending to come into ear before the main emergence of wbm. | If achieves GS59 before the start of oviposition may escape entirely. | Barnes (1956); Kurppa (1989) |
| **Escape 2** | Good flowering biology reduces the time spent in GS55-59. | Reduces the period of exposure, but may still coincide with a major oviposition event. | Barnes (1956). |
| **Escape 3** | Close flowering reduces the ability of wbm to lay eggs within the floret forcing them to lay in more exposed positions. | Increases natural mortality of eggs and young larvae migrating to feeding sites. | Wise *et al.* (2001). |
| **Escape 4** | Plant exudes low levels or less attractive blend of acetophenone, (Z)-3-hexenyl acetate and 3-carene during ear emergence. | Attracts fewer midges to fields at susceptible growth stage. | Birkett *et al* 2004. |

**Table 2. Environment : wbm interactions**

| Name | Mechanism | Impact | References |
|---|---|---|---|
| **Agronomy 1** | Crop structure mainly main shoots and primary tillers shortening overall susceptible period | Reduces exposure to ovipositing midges compared to a thinner crop with many secondary tillers | Barnes (1956) |
| **Agronomy 2** | Less wheat dependent rotation reducing number of wheat crops in area in previous five years | Reduced population of hibernating larvae in cropped and surrounding fields | Barnes (1956) |
| **Agronomy 3** | Depth of cultivation greater after wheat and shallower after other crops | Primary cultivation tends to bury midge larvae deeper in the soil and reduce the probability that they will hatch until returned nearer to the surface | Barnes (1956) |
| **Site 1** | Shelter and location of field | Shelter tends to result in an environment more suitable for midge flight within the field increasing oviposition from resident midges | Oakley (1994). |
| **Site 2** | Shelter and location of area | Shelter and topography influence the build up of crop scent and the drift of a plume of scent to adjoining fields, enabling more effective migration to susceptible crops. | Oakley (1994) |
| **Weather 1** | Soil temperature and moisture levels in May and early June | Influences extent and timing of pupation and hatching of adult midges | Oakley *et al.* (1998). |
| **Weather 2** | Suitability of evening weather for flight following hatch of adult midges | Influences ability to fly up to ears to lay eggs within field and to migrate between fields to susceptible crops. | Pivnick & Labbé. (1993). |

## MATERIALS AND METHODS

### Varieties

Experiments were conducted at five sites each year (Table 3). In 2001/02 a selection of varieties grown in 1993 variety trials (Oakley 1994) were included to represent the range of susceptibility found at that time. This was done to assist prediction of field performance of similar varieties against the assessments of the Cereal Quality survey results from 1993 and 1994 (Oakley 1994 and 1995). The mean value of visible grain damage found relative to the 1993 control varieties is shown in parentheses:

Beaver (120), Cadenza (92), Hereward (93), Hussar (70), Lynx (165), Rialto (142), Riband (88), Soissons (129) and Spark (75).

In each year the RL control varieties were grown plus the industrial partners' recommended varieties including other lines showing potential resistance to wbm (Table 4).

In 2001/2 two replicates of each variety were sown in a randomised block design. In the succeeding years four replicates were sown, two of which were treated with chlorpyrifos at 450 g ai/ha when the majority of varieties were between GS 55 and 59.

**Table 3. Experimental sites - location, sowing date and attack on control varieties**

| Location | 2001/2 | 2002/3 | 2003/4 |
|---|---|---|---|
| ADAS Boxworth, Cambridge | 2 November | 15 October | 29 October |
| Advanta, Wolferton, Norfolk | 9 October | 11 October | 1 October |
| Elsoms, Weston, Lincolnshire | 18 October | 5 November | 28 September |
| TAG, Morley, Norfolk | 19 October | 9 October | 20 October |
| Nickersons, Maldon, Essex | 6 October | | |

### Assessment of wheat blossom midge attack

### Sampling and storage

- Samples consisted of 10 ears per plot taken in two groups of five. One ear was chosen at random at two points and the four nearest ears included in the sample.

- Samples were collected in weeks of 24 June or 1 July when the larvae were large enough to see under a low power microscope.
- Samples were stored in individual polythene bags. Samples were kept for up to a week in a refrigerator prior to examination, samples from some replicates were frozen for later examination.

**Examination**

- Each ear was examined under a low power microscope.
- Working from the bottom of the ear the glume and lema were pulled back to reveal each grain which was checked on both sides for the presence of wheat blossom midge larvae.
- Records were made of viable grain sites as clear (C) or the numbers of larvae found. Numbers found per ear were totalled to give
    1. Total grain
    2. Damaged grain
    3. Total larvae
    4. Total larvae per 100 grain sites

Observations were made of the start of ear emergence (GS51) and the beginning of flowering (GS61). Two yellow sticky traps in 2002, or pheromone traps in 2003 and 2004, were located at each site prior to ear emergence and observed weekly to monitor wbm activity.

**Yield assessments**

The plots were harvested in 2003 and 2004 and yields and various quality parameters assessed. Relative yield figures were calculated as a percentage of three resistant varieties grown in both years, Welford, Brompton and Welland, to give an estimate of the yield potential at each site in the absence of wbm damage.

Table 4. Varieties tested in each harvest year and lateness of ear emergence as days after Soissons.

| | 2002 | 2003 | 2004 | Lateness |
|---|---|---|---|---|
| Arran | y | Y | | 13 |
| Beaver | y | | | 14 |
| Cadenza | y | | | 9 |
| Carlton | y | | | 13 |
| Chardonnay | y | | | 13 |
| Charger | y | | | 6 |
| Claire | y | Y | y | 13 |
| Consort | y | Y | y | 14 |
| Deben | y | | | 12 |
| ECO 22 | y | Y | y | 14 |
| Einstein | y | Y | y | 9 |
| Exsept | y | | | 14 |
| Hereward | y | | | 12 |
| Hussar | y | | | 11 |
| Lynx | y | | | 14 |
| Malacca | y | Y | y | 10 |
| Napier | y | | | 11 |
| Option | y | Y | y | 10 |
| Pennant | y | Y | y | 11 |
| Rialto | y | | | 10 |
| Riband | y | | | 11 |
| Savannah | y | Y | | 13 |
| Scorpion 25 | y | Y | | 12 |
| Soissons | y | | | 0 |
| Solstice | y | Y | y | 11 |
| Spark | y | | | 14 |
| Tanker | y | Y | y | 13 |
| Warlock 24 | y | | | 11 |
| Welford | y | Y | y | 13 |
| Xi19 | y | Y | y | 12 |
| Brompton | | Y | y | 13 |
| Istabraq | | Y | y | 12 |
| Nijinsky | | Y | y | 13 |
| Smuggler | | Y | y | 13 |
| Vector | | Y | y | 15 |
| Welland | | Y | y | 12 |
| A42-02 | | | y | 11 |
| A45-02 | | | y | 12 |
| ELS 03-34 | | | y | 13 |
| FD00039 | | | y | 13 |
| Exeter | | | y | 9 |
| NSL WW60 | | | y | 14 |
| NSL WW61 | | | y | 12 |
| NSL WW65 | | | y | 11 |

**RESULTS**

Ear emergence timing relative to the earliest variety Soissons is shown in Table 4, meaned across all the years in which the variety was tested. All the current recommended and candidate varieties started ear emergence between 9 and 14 days after Soissons. Ear emergence was 7 days earlier in 2003 than in 2004.

The pattern of wheat blossom midge activity in relation to ear emergence varied between sites. The degree of bias at each site can be seen from the number of larvae on the earlier control varieties Option and Malacca compared to the mean for these and the later varieties Consort and Tanker at each site (Table 5). In these results the lower the ratio the greater is the bias towards higher infestation of late heading varieties. There was a general bias towards coincidence with later emerging varieties in 2002 and earlier emerging varieties in 2004, with a more mixed spread of activity in 2003. There were insufficient larvae present at the Elsoms site in 2002 for a viable comparison to be made.

Table 5. Ratio of numbers on early emerging control varieties to the mean of early and later emerging control varieties.

| **Location** | **2001/2** | **2002/3** | **2003/4** |
|---|---|---|---|
| ADAS Boxworth, Cambridge | 0.92 | 1.08 | 1.30 |
| Advanta, Wolferton, Norfolk | 0.67 | 0.80 | 1.08 |
| Elsoms, Weston, Lincolnshire | | 0.78 | 1.18 |
| TAG, Norfolk | 0.89 | 1.08 | 0.95 |
| Nickersons, Maldon, Essex | 0.60 | 1.14 | 1.28 |
| Mean | 0.82 | 1.04 | 1.18 |

The numbers of larvae present per hundred viable grain sites on untreated plots from the four sites examined in 2002 are shown in Table 6.. The larvae found on Carlton and Welford were small first instar and appeared to be either dead, or failing to grow. This trait is generally associated with antibiotic resistance. There was some tendency towards this in Pennant but the trait was not fully expressed.

The numbers found on the untreated plots in 2002/03 are shown in Table 7 and those from 2003/04 in Table 8. A general failure of larvae to develop properly was confirmed in Welford and also noted in Bromton, Welland, Kipling and ELS 03-34 and all are classified as possessing antibiotic resistance.

The numbers of larvae in the chlorpyrifos treated plots were assessed on the two HGCA funded sites at Boxworth and Morley in 2002/03 (Table 9) and 2003/04 (Table 10) and at the Elsoms site and on some varieties at the Nickersons site in 2003/04 (Table 11). The degree of control achieved by treatment showed considerable variations between varieties at some sites with a tendency for better control on the later heading

varieties. For example at Boxworth 2003/04 high temperatures prevailed during the air emergence period, shortening the egg hatch period to under 4 days, so that no single spray application could cover all of the risk period for the range of varieties grown.

The yields taken from the untreated plots in 2002/03 are shown in Table 12 and those from 2003/04 in Table 15. The untreated yields were also affected by aphid infestation at the Advanta site in 2002/03 and at Boxworth, Morley and Nickersons in 2003/04. To give an estimate of the yield potential at each site in the absence of wbm infestation the relative yields for 2002/03 (Table13) and 2003/04 (Table 16) have been calculated as a percentage of the mean yield of the three resistant varieties, Welford, Brompton and Welland rather than the conventional control varieties. The yields from treated plots in 2002/03 (Table 14) and 2003/04 (Table 17) are shown separately. Due to variations in control between different varieties and the interference of aphids at some sites, no estimate of the yield response to control is given

**Table 6. Larvae per 100 grain in 2001/2 experiments**

| | ADAS Boxworth | Advanta Wolferton | TAG Morley | Nickersons Maldon | Mean |
|---|---|---|---|---|---|
| Arran | 25 | 5 | 67 | 37 | 33 |
| Beaver | 26 | 7 | 70 | 25 | 32 |
| Cadenza | 31 | 1 | 43 | 14 | 22 |
| Carlton | 9 | 1 | 8 | 1 | 5 |
| Chardonnay | 29 | 2 | 106 | 30 | 42 |
| Charger | 15 | 1 | 32 | 28 | 19 |
| Claire | 36 | 1 | 42 | 14 | 23 |
| Consort | 31 | 5 | 56 | 37 | 32 |
| Deben | 18 | 1 | 30 | 12 | 15 |
| ECO 22 | 50 | 14 | 106 | 60 | 58 |
| Einstein | 15 | 0 | 36 | 10 | 15 |
| Exsept | 31 | 3 | 77 | 51 | 41 |
| Hereward | 34 | 1 | 52 | 25 | 28 |
| Hussar | 14 | 4 | 44 | * | 20 |
| Lynx | 44 | 7 | 75 | 27 | 38 |
| Malacca | 26 | 2 | 45 | 13 | 22 |
| Napier | 21 | 2 | 78 | 16 | 29 |
| Option | 26 | 2 | 39 | 21 | 22 |
| Pennant | 2 | 1 | 27 | 8 | 9 |
| Rialto | 25 | 4 | 45 | 47 | 31 |
| Riband | 30 | 2 | 36 | 22 | 23 |
| Savannah | 20 | 3 | 44 | 40 | 27 |
| Scorpion 25 | 26 | 1 | 42 | 28 | 24 |
| Soissons | 6 | 0 | 1 | 2 | 2 |
| Solstice | 30 | 1 | 61 | 33 | 31 |
| Spark | 25 | 5 | 34 | 8 | 18 |
| Tanker | 30 | 3 | 49 | 43 | 31 |
| Warlock 24 | 36 | 1 | 52 | 34 | 31 |
| Welford | 9 | 0 | 1 | 0 | 3 |
| Xi19 | 48 | 5 | 67 | 39 | 40 |
| SEM (85df) | | | | | 5.919 |
| *P* | | | | | <0.001 |
| CV% | | | | | 46.5 |

Table 7. Larvae per 100 grains on untreated plots in 2002/03 experiments.

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon | Mean |
|---|---|---|---|---|---|---|
| Claire | 15 | 2 | 20 | 40 | 24 | 20 |
| Consort | 12 | 2 | 22 | 23 | 31 | 18 |
| Malacca | 21 | 0 | 13 | 22 | 15 | 14 |
| Option | 25 | 2 | 16 | 18 | 53 | 23 |
| Tanker | 27 | 1 | 23 | 11 | 20 | 16 |
| Savannah | 16 | 3 | 18 | 6 | 25 | 14 |
| Eco 22 | 17 | 1 | 26 | 7 | 52 | 20 |
| Xi-19 | 28 | 1 | 20 | 12 | 19 | 16 |
| Solstice | 24 | 0 | 16 | 6 | 36 | 17 |
| Scorpion | 24 | 1 | 21 | 19 | 21 | 17 |
| Smuggler | 13 | 1 | 17 | 12 | 33 | 15 |
| Vector | 8 | 2 | 23 | 5 | 20 | 12 |
| Einstein | 14 | 0 | 14 | 24 | 16 | 13 |
| Arran | 19 | 0 | 14 | 22 | 18 | 15 |
| Nijinsky | 17 | 0 | 13 | 8 | 23 | 12 |
| Istabraq | 14 | 2 | 14 | 12 | 13 | 11 |
| Welford | 0 | 0 | 0 | 10 | 0 | 2 |
| Pennant | 21 | 1 | 10 | 13 | 33 | 16 |
| Brompton | 0 | 0 | 0 | 20 | 1 | 4 |
| Welland | 0 | 0 | 0 | 10 | 1 | 2 |

**Table 8. Larvae per 100 grains on untreated plots in 2003/04 experiments.**

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon | Mean |
|---|---|---|---|---|---|---|
| Claire | 25 | 17 | 35 | 18 | 31 | 25 |
| Consort | 7 | 23 | 23 | 18 | 29 | 20 |
| Malacca | 28 | 27 | 45 | 24 | 63 | 37 |
| Option | 45 | 35 | 48 | 17 | 71 | 43 |
| Tanker | 32 | 30 | 42 | 27 | 46 | 36 |
| Smuggler | 19 | 27 | 39 | 31 | 40 | 31 |
| Eco | 21 | 39 | 53 | 26 | 57 | 39 |
| Solstice | 31 | 17 | 32 | 36 | 54 | 34 |
| Vector | 7 | 21 | 26 | 7 | 26 | 17 |
| Xi19 | 35 | 23 | 63 | 28 | 34 | 37 |
| A42-02 | 39 | 28 | 61 | 38 | 77 | 49 |
| A45-02 | 33 | 25 | 34 | 2 | 36 | 26 |
| Welford | 0 | 3 | 0 | 0 | 1 | 1 |
| Brompton | 0 | 2 | 0 | 0 | 1 | 0 |
| Welland | 0 | 1 | 0 | 0 | 9 | 2 |
| Kipling | 0 | 1 | 0 | 0 | 0 | 0 |
| ELS03-34 | 0 | 0 | 0 | 1 | 1 | 0 |
| Einstein | 41 | 16 | 24 | 20 | 37 | 28 |
| Nijinsky | 12 | 20 | 42 | 31 | 30 | 27 |
| Istabraq | 15 | 16 | 47 | 19 | 30 | 26 |
| Exeter | 57 | 24 | 32 | 25 | 51 | 38 |
| NSL WW60 | 4 | 15 | 28 | 14 | 24 | 17 |
| NSL WW61 | 18 | 19 | 34 | 21 | 38 | 26 |
| NSL WW65 | 42 | 15 | 24 | 19 | 35 | 27 |
| Pennant | 29 | 22 | 38 | 19 | 41 | 30 |

**Table 9. Numbers of larvae on chlorpyrifos treated plots at Boxworth and Morley in 2002/03 and the percentage reduction in numbers compared to untreated plots.**

| Variety | ADAS Boxworth | | TAGMorley | |
|---|---|---|---|---|
| | larvae/100 grain | % reduction from untreated | larvae/100 grain | % reduction from untreated |
| Claire | 5.4 | 62.8 | 5.6 | 86.0 |
| Consort | 10.3 | 14.5 | 7.4 | 68.3 |
| Malacca | 11.7 | 44.5 | 5.5 | 74.9 |
| Option | 11.4 | 53.5 | 4.7 | 73.4 |
| Tanker | 11.9 | 55.7 | 4.4 | 61.6 |
| Savannah | 5.8 | 64.5 | 4.1 | 27.2 |
| Eco 22 | 5.3 | 68.1 | 8.6 | -28.9 |
| Xi-19 | 11.8 | 57.6 | 6.7 | 44.6 |
| Solstice | 17.7 | 27.6 | 9.1 | -41.1 |
| Scorpion | 8.2 | 65.5 | 2.0 | 89.6 |
| Smuggler | 7.4 | 44.1 | 1.4 | 88.5 |
| Vector | 2.4 | 68.0 | 8.0 | -49.6 |
| Einstein | 13.2 | 2.6 | 7.0 | 70.5 |
| Arran | 10.7 | 43.2 | 7.4 | 66.8 |
| Nijinsky | 6.9 | 60.5 | 5.2 | 30.7 |
| Istabraq | 4.2 | 70.0 | 3.8 | 68.0 |
| Welford | 0.0 | 100.0 | 2.9 | 70.1 |
| Pennant | 9.2 | 55.1 | 9.8 | 24.3 |
| Brompton | 0.5 | 100.0 | 1.3 | 93.5 |
| Welland | 0.2 | 40.0 | 1.9 | 80.4 |
| Mean | 7.7 | 54.9 | 5.3 | 49.9 |

**Table 10. Numbers of larvae on chlorpyrifos treated plots at Boxworth and Morley in 2003/04 and the percentage reduction in numbers compared to untreated plots.**

| Variety | ADAS Boxworth | | TAG Morley | |
|---|---|---|---|---|
| | larvae/100 grain | % reduction from untreated | larvae/100 grain | % reduction from untreated |
| Claire | 23.6 | 16.8 | 7 | 61.1 |
| Consort | 10.7 | -15.8 | 3 | 83.3 |
| Malacca | 25.7 | 22.9 | 3 | 87.5 |
| Option | 39.7 | 14.7 | 5 | 70.6 |
| Tanker | 19.4 | 47.6 | 5 | 81.5 |
| Smuggler | 70.9 | -172.0 | 2 | 93.5 |
| Eco | 12.7 | 47.3 | 6 | 76.9 |
| Solstice | 28.4 | 25.5 | 5 | 86.1 |
| Vector | 2.3 | 63.4 | 1 | 85.7 |
| Xi19 | 23.2 | 47.5 | 3 | 89.3 |
| A42-02 | 37.6 | 0.9 | 7 | 81.6 |
| A45-02 | 21.1 | 26.4 | 3 | 85.0 |
| Welford | 0.1 | | 1 | |
| Brompton | 2.0 | | 0 | |
| Welland | 0.0 | | 0 | |
| Kipling | 0.3 | | 0 | |
| ELS03-34 | 0.0 | | 1 | |
| Einstein | 47.1 | -24.3 | 5 | 75.0 |
| Nijinsky | 9.1 | 37.2 | 3 | 90.3 |
| Istabraq | 27.2 | -53.2 | 5 | 73.7 |
| Exeter | 51.1 | 31.9 | 4 | 84.0 |
| NSL WW60 | 6.2 | 16.0 | 3 | 78.6 |
| NSL WW61 | 19.9 | 4.9 | 7 | 66.7 |
| NSL WW65 | 35.5 | 36.2 | 9 | 52.6 |
| Pennant | 20.2 | 46.2 | 4 | 78.9 |
| Mean | 21.4 | 14.8 | 3.7 | 79.9 |

**Table 11. Numbers of larvae on chlorpyrifos treated plots at Elsoms and Nickersons in 2003/04 and the percentage reduction in numbers compared to untreated plots.**

| Variety | Elsoms, Lincs | | Nickersons, Maldon | |
|---|---|---|---|---|
| | larvae/100 grain | % reduction from untreated | larvae/100 grain | % reduction from untreated |
| Claire | 25.4 | 24.3 | 20.6 | 34.2 |
| Consort | 28.2 | 10.8 | 5.2 | 81.7 |
| Malacca | 36.1 | 27.7 | 44.6 | 28.9 |
| Option | 31.7 | 47.6 | 77.2 | -9.3 |
| Tanker | 30.8 | 40.7 | 10.4 | 77.3 |
| Smuggler | 27.9 | 32.5 | | |
| Eco | 43.2 | 31.2 | | |
| Solstice | 35.1 | 13.3 | | |
| Vector | 19.0 | 42.0 | | |
| Xi19 | 36.0 | 46.0 | | |
| A42-02 | 48.3 | 11.8 | | |
| A45-02 | 21.4 | 48.7 | | |
| Welford | 0.0 | | | |
| Brompton | 0.0 | | | |
| Welland | 0.0 | | | |
| Kipling | 0.0 | | | |
| ELS03-34 | 0.0 | | | |
| Einstein | 20.5 | 25.8 | 15.2 | 59.5 |
| Nijinsky | 34.1 | 26.0 | 13.5 | 54.5 |
| Istabraq | 22.7 | 40.6 | 19.9 | 32.6 |
| Exeter | 31.2 | 2.1 | 33.0 | 35.4 |
| NSL WW60 | 14.2 | 44.2 | | |
| NSL WW61 | 22.6 | 40.0 | | |
| NSL WW65 | 16.7 | 37.9 | | |
| Pennant | 21.6 | 47.9 | | |
| Mean | 22.7 | 32.8 | 26.6 | 38.1 |

**Table 12. Untreated yields (t/ha) in 2002/03.**

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon |
|---|---|---|---|---|---|
| Claire | 7.96 | 10.68 | 6.64 | 9.36 | 10.69 |
| Consort | 7.61 | 10.06 | 6.92 | 8.72 | 9.29 |
| Malacca | 7.00 | 9.54 | 6.90 | 8.95 | 8.78 |
| Option | 8.24 | 10.85 | 5.55 | 8.88 | 8.49 |
| Tanker | 8.08 | 10.60 | 4.87 | 8.75 | 9.88 |
| Savannah | 7.60 | 10.48 | 7.37 | 8.63 | 10.24 |
| Eco 22 | 7.75 | 10.14 | 4.50 | 8.09 | 8.85 |
| Xi-19 | 8.44 | 10.95 | 7.09 | 9.32 | 9.72 |
| Solstice | 8.06 | 10.52 | 6.07 | 8.94 | 9.33 |
| Scorpion | 8.06 | 11.51 | 6.11 | 9.28 | 9.67 |
| Smuggler | 8.21 | 10.87 | 6.87 | 9.15 | 10.17 |
| Vector | 8.25 | 10.88 | 6.88 | 9.59 | 10.29 |
| Einstein | 8.35 | 10.56 | 7.09 | 9.86 | 9.85 |
| Arran | 7.89 | 10.52 | 6.94 | 9.04 | 10.00 |
| Nijinsky | 8.28 | 11.11 | 7.28 | 9.50 | 10.08 |
| Istabraq | 8.35 | 11.15 | 7.46 | 9.59 | 10.68 |
| Welford | 8.09 | 10.61 | 8.36 | 9.15 | 10.29 |
| Pennant | 7.79 | 10.40 | 7.95 | 8.77 | 9.72 |
| Brompton | 8.98 | 10.76 | 8.43 | 8.77 | 10.99 |
| Welland | 8.90 | 10.71 | 8.44 | 9.04 | 10.95 |
| Mean | 8.09 | 10.65 | 6.89 | 9.07 | 9.90 |
| CV% | 2.51 | 0.96 | 3.12 | | 1.86 |

Table 13 Untreated yields in 2002/03 as a percentage of the means of Welford, Brompton and Welland.

| **Variety** | **ADAS Boxworth** | **Advanta Wolferton** | **Elsoms Lincs** | **TAG Morley** | **Nickersons Maldon** |
|---|---|---|---|---|---|
| Claire | 91.9 | 99.9 | 78.9 | 104.1 | 99.5 |
| Consort | 87.9 | 94.1 | 82.3 | 97.0 | 86.5 |
| Malacca | 80.8 | 89.2 | 82.1 | 99.6 | 81.8 |
| Option | 95.2 | 101.5 | 65.9 | 98.8 | 79.1 |
| Tanker | 93.3 | 99.1 | 57.9 | 97.3 | 92.0 |
| Savannah | 87.8 | 98.0 | 87.7 | 96.0 | 95.3 |
| Eco 22 | 89.5 | 94.8 | 53.5 | 90.0 | 82.4 |
| Xi-19 | 97.5 | 102.4 | 84.3 | 103.7 | 90.4 |
| Solstice | 93.1 | 98.4 | 72.1 | 99.4 | 86.8 |
| Scorpion | 93.1 | 107.7 | 72.6 | 103.2 | 90.0 |
| Smuggler | 94.8 | 101.7 | 81.7 | 101.8 | 94.7 |
| Vector | 95.3 | 101.7 | 81.8 | 106.7 | 95.8 |
| Einstein | 96.4 | 98.7 | 84.3 | 109.7 | 91.7 |
| Arran | 91.1 | 98.4 | 82.5 | 100.6 | 93.1 |
| Nijinsky | 95.6 | 103.9 | 86.5 | 105.7 | 93.8 |
| Istabraq | 96.4 | 104.3 | 88.7 | 106.7 | 99.4 |
| Welford | 93.4 | 99.2 | 99.4 | 101.8 | 95.8 |
| Pennant | 90.0 | 97.3 | 94.5 | 97.6 | 90.4 |
| Brompton | 103.7 | 100.6 | 100.2 | 97.6 | 102.3 |
| Welland | 102.8 | 100.2 | 100.3 | 100.6 | 101.9 |
| Control yield t/ha | 8.66 | 16.45 | 8.41 | 8.99 | 13.70 |

Table 14. Chlorpyrifos treated yield (t/ha) in 2002/03.

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon |
|---|---|---|---|---|---|
| Claire | 7.94 | 11.45 | 7.78 | 9.29 | 10.75 |
| Consort | 8.14 | 10.28 | 7.57 | 10.01 | 9.73 |
| Malacca | 7.27 | 10.39 | 6.95 | 9.62 | 9.55 |
| Option | 8.48 | 11.04 | 7.39 | 9.25 | 10.25 |
| Tanker | 8.57 | 10.70 | 7.76 | 9.63 | 10.71 |
| Savannah | 8.02 | 10.86 | 8.04 | 9.45 | 11.06 |
| Eco 22 | 7.82 | 10.36 | 6.82 | 9.15 | 10.12 |
| Xi-19 | 8.41 | 11.99 | 7.88 | 10.79 | 10.88 |
| Solstice | 8.06 | 11.32 | 7.18 | 9.72 | 10.36 |
| Scorpion | 8.36 | 12.21 | 7.39 | 10.42 | 10.53 |
| Smuggler | 8.22 | 11.35 | 7.35 | 10.17 | 11.35 |
| Vector | 8.17 | 11.78 | 7.91 | 10.2 | 10.77 |
| Einstein | 7.90 | 11.62 | 7.60 | 10.22 | 10.06 |
| Arran | 8.38 | 11.48 | 7.14 | 9.79 | 10.32 |
| Nijinsky | 8.32 | 11.35 | 8.05 | 10.02 | 10.49 |
| Istabraq | 8.73 | 12.10 | 8.55 | 10.34 | 11.27 |
| Welford | 8.24 | 11.47 | 8.65 | 9.36 | 10.66 |
| Pennant | 8.92 | 10.86 | 7.88 | 9.04 | 9.97 |
| Brompton | 8.71 | 11.00 | 8.36 | 9.04 | 11.47 |
| Welland | 8.99 | 11.22 | 8.48 | 9.66 | 10.81 |
| Mean | 8.28 | 11.25 | 7.74 | 9.76 | 10.56 |
| CV% | 2.45 | 2.07 | 3.20 | | 1.42 |

**Table 15. Untreated yields (t/ha) in 2003/04.**

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon |
|---|---|---|---|---|---|
| Claire | 8.23 | 10.10 | 9.73 | 6.43 | 7.40 |
| Consort | 8.54 | 8.59 | 10.67 | 7.64 | 7.44 |
| Malacca | 7.48 | 8.87 | 9.79 | 5.87 | 5.80 |
| Option | 7.10 | 9.82 | 11.10 | 6.51 | 5.73 |
| Tanker | 7.85 | 9.67 | 11.25 | 5.89 | 7.04 |
| Smuggler | 8.47 | 10.18 | 11.93 | 6.69 | 8.13 |
| ECO 22 | 8.17 | 7.54 | 10.75 | 7.45 | 6.91 |
| Solstice | 7.47 | 10.04 | 11.66 | 6.12 | 6.67 |
| Vector | 8.22 | 10.74 | 11.31 | 7.54 | 7.00 |
| Xi19 | 8.40 | 9.65 | 11.14 | 7.53 | 7.46 |
| A42-02 | 7.99 | 10.09 | 8.33 | 6.79 | 6.77 |
| A45-02 | 7.66 | 10.52 | 11.45 | 7.33 | 7.74 |
| Welford | 8.55 | 10.73 | 12.10 | 7.01 | 8.53 |
| Brompton | 9.05 | 11.36 | 13.33 | 7.74 | 9.29 |
| Welland | 8.12 | 9.43 | 11.73 | 7.59 | 9.55 |
| Kipling | 8.10 | 10.43 | 11.75 | 7.72 | 9.21 |
| ELS03-34 | 8.29 | 10.36 | 13.02 | 7.46 | 8.71 |
| Einstein | 7.85 | 10.62 | 9.86 | 6.72 | 7.28 |
| Nijinsky | 7.96 | 9.78 | 11.03 | 6.64 | 7.92 |
| Istabraq | 8.40 | 10.04 | 9.64 | 8.37 | 8.39 |
| Exeter | 7.34 | 10.08 | 10.49 | 6.31 | 5.80 |
| NSL WW60 | 9.62 | 10.88 | 12.47 | 7.1 | 8.94 |
| NSL WW61 | 8.13 | 10.71 | 11.35 | 6.96 | 8.87 |
| NSL WW65 | 7.38 | 10.89 | 9.60 | 6.42 | 7.73 |
| Pennant | 8.46 | 10.28 | 11.14 | 6.83 | 6.87 |
| Mean | 8.11 | 9.89 | 11.06 | 6.99 | 7.65 |
| CV% | | 2.34 | 4.0 | 5.26 | 4.46 |

Table 16 Untreated yields in 2003/04 as a percentage of the means of Welford, Brompton and Welland.

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon |
|---|---|---|---|---|---|
| Claire | 96.0 | 96.2 | 78.5 | 86.3 | 81.1 |
| Consort | 99.6 | 81.8 | 86.2 | 102.6 | 81.5 |
| Malacca | 87.3 | 84.5 | 79.0 | 78.8 | 63.5 |
| Option | 82.8 | 93.5 | 89.6 | 87.4 | 62.8 |
| Tanker | 91.6 | 92.1 | 90.8 | 79.1 | 77.2 |
| Smuggler | 98.8 | 96.9 | 96.3 | 89.8 | 89.1 |
| ECO 22 | 95.3 | 71.8 | 86.8 | 100.0 | 75.8 |
| Solstice | 87.1 | 95.6 | 94.1 | 82.2 | 73.1 |
| Vector | 95.9 | 102.3 | 91.3 | 101.2 | 76.7 |
| Xi19 | 98.0 | 91.9 | 89.9 | 101.1 | 81.8 |
| A42-02 | 93.2 | 96.1 | 67.3 | 91.2 | 74.2 |
| A45-02 | 89.4 | 100.1 | 92.4 | 98.4 | 84.9 |
| Welford | 99.7 | 102.1 | 97.7 | 94.1 | 93.5 |
| Brompton | 105.6 | 108.1 | 107.6 | 103.9 | 101.8 |
| Welland | 94.7 | 89.7 | 94.7 | 101.9 | 104.6 |
| Kipling | 94.5 | 99.3 | 94.9 | 103.7 | 100.9 |
| ELS03-34 | 96.7 | 98.6 | 105.1 | 100.2 | 95.4 |
| Einstein | 91.6 | 101.1 | 79.6 | 90.2 | 79.8 |
| Nijinsky | 92.8 | 93.1 | 89.0 | 89.2 | 86.8 |
| Istabraq | 98.0 | 95.6 | 77.8 | 112.4 | 92.0 |
| Exeter | 85.6 | 95.9 | 84.7 | 84.7 | 63.6 |
| NSL WW60 | 112.2 | 103.6 | 100.6 | 95.3 | 98.0 |
| NSL WW61 | 94.8 | 102.0 | 91.6 | 93.5 | 97.2 |
| NSL WW65 | 86.1 | 103.7 | 77.5 | 86.2 | 84.8 |
| Pennant | 98.7 | 97.9 | 89.9 | 91.7 | 75.3 |
| Control yield t/ha | 8.57 | 16.2 | 12.39 | 7.45 | 11.63 |

Table 14. Chlorpyrifos treated yield (t/ha) in 2003/04.

| Variety | ADAS Boxworth | Advanta Wolferton | Elsoms Lincs | TAG Morley | Nickersons Maldon |
|---|---|---|---|---|---|
| Claire | 8.41 | 10.40 | 10.53 | 7.73 | 11.11 |
| Consort | 9.01 | 9.82 | 11.26 | 8.46 | 11.21 |
| Malacca | 8.10 | 9.13 | 10.14 | 7.87 | 9.18 |
| Option | 8.30 | 9.80 | 11.99 | 8.09 | 9.76 |
| Tanker | 8.82 | 10.43 | 12.93 | 7.96 | 11.26 |
| Smuggler | 9.31 | 10.84 | 13.16 | 8.80 | 11.46 |
| ECO 22 | 8.67 | 7.84 | 11.12 | 8.72 | 10.59 |
| Solstice | 8.64 | 10.78 | 11.94 | 8.35 | 10.73 |
| Vector | 9.95 | 11.06 | 11.76 | 8.63 | 11.71 |
| Xi19 | 9.13 | 9.40 | 12.92 | 9.16 | 11.55 |
| A42-02 | 8.55 | 10.72 | 10.49 | 8.77 | 11.07 |
| A45-02 | 9.20 | 10.97 | 12.34 | 8.70 | 11.25 |
| Welford | 8.91 | 10.80 | 12.24 | 8.01 | 11.73 |
| Brompton | 9.12 | 11.08 | 13.47 | 8.87 | 12.24 |
| Welland | 8.99 | 9.85 | 11.79 | 8.20 | 10.67 |
| Kipling | 9.45 | 9.98 | 11.86 | 8.60 | 10.71 |
| ELS03-34 | 9.51 | 10.27 | 12.96 | 8.29 | 11.03 |
| Einstein | 8.19 | 11.05 | 11.58 | 8.07 | 10.34 |
| Nijinsky | 8.56 | 9.96 | 11.78 | 8.42 | 11.58 |
| Istabraq | 8.61 | 10.11 | 11.43 | 8.55 | 11.29 |
| Exeter | 8.17 | 11.23 | 10.84 | 8.57 | 10.08 |
| NSL WW60 | 9.51 | 11.25 | 12.94 | 9.81 | 11.56 |
| NSL WW61 | 9.05 | 11.24 | 12.63 | 8.08 | 11.72 |
| NSL WW65 | 8.08 | 11.56 | 11.67 | 7.99 | 9.85 |
| Pennant | 9.07 | 11.12 | 11.41 | 8.15 | 10.79 |
| Mean | 8.85 | 9.95 | 11.89 | 8.43 | 10.98 |
| CV% | | 1.89 | 3.3 | 5.26 | 2.84 |

## DISCUSSION

### Coincidence

Variations in the degree of coincidence between wbm egg laying activity and the susceptible growth stages of different varieties (Barnes, 1956; Kurppa, 1989) at different sites has been shown to have a large impact on the incidence of wbm in this study. The classification of varietal responses to wbm needs to take this factor into account, preferably through the use of a balanced range of sites producing an even exposure to attack. Alternatively, by measuring the incidence on control varieties with different heading dates, but similar vulnerability, a correction factor could be applied to reduce the variable coincidence effect. In this study a comparison of the numbers on the early heading varieties Malacca and Option to those found on the late heading varieties Consort and Tanker was used to assess the relative coincidence of exposure to wbm. This factor allows the production of a balanced cross-site mean and checking and correcting for any bias. The mean ratio for this factor across the sites and years reported here is 1.01, suggesting that any bias should have been evened out for varieties assessed in all three years of the study, the ratio averages 1.11 for the last two years, when yields were assessed.

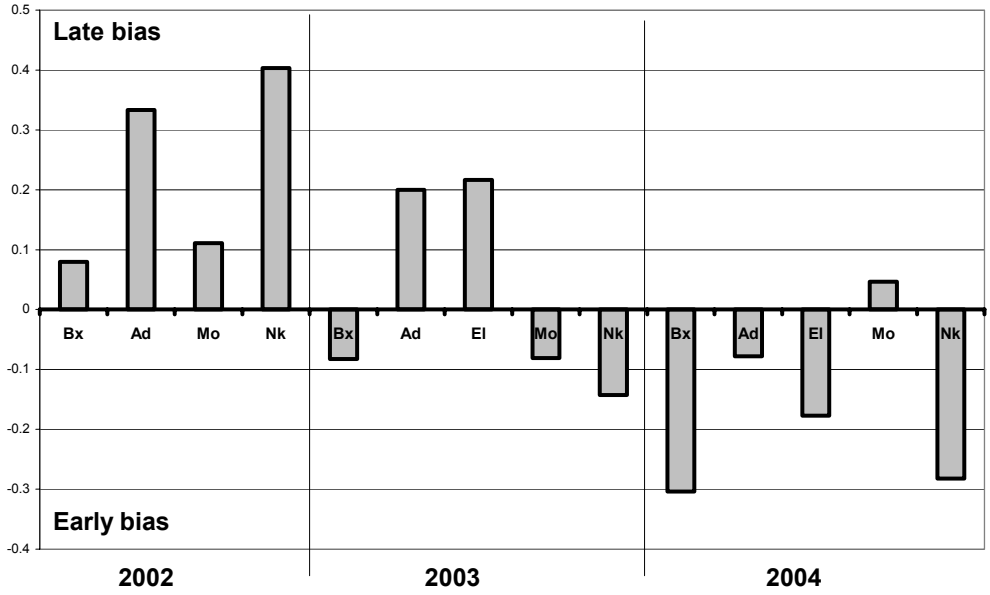



Fig. 1 Bias toward greater infestation of earlier or later emerging varieties at each site

### Vulnerability

The hypothesis that varieties do differ in their inherent vulnerability to attack has been tested using the results for the susceptible varieties grown in all three cropping years of the study, excluding the Advanta sites from 2001/02 and 2002/03 where low numbers resulted in a much higher variation. The results were

analysed by ANOVA and by a Friedman's test of the ranks at each site. Both methods gave similar results with a greater degree of separation when analysed by ANOVA (Table 18).

| ANOVA | | | Friedman's test | | |
|---|---|---|---|---|---|
| Variety | Larvae/100 grain | | Variety | Sum of ranks | |
| ECO 22 | 139.3 | d | ECO 22 | 108.0 | c |
| Option | 121.9 | cd | Option | 97.0 | bc |
| Xi 19 | 108.3 | bc | Xi 19 | 91.5 | abc |
| Tanker | 105.3 | bc | Tanker | 94.0 | abc |
| Solstice | 99.6 | bc | Solstice | 72.5 | abc |
| Malacca | 94.7 | abc | Malacca | 73.5 | abc |
| Consort | 89.7 | ab | Consort | 68.5 | abc |
| Claire | 88.5 | ab | Claire | 64.0 | abc |
| Einstein | 73.3 | a | Pennant | 51.0 | ab |
| Pennant | 73.0 | a | Einstein | 50.0 | a |

It may therefore be concluded that the varieties do differ in their inherent vulnerability to attack. The range of variation has increased from that found in 1993 (Oakley, 1994) with resistant varieties appearing as well as the more susceptible type ECO22.

**Tolerance**

Attempts to assess the degree of tolerance to damage by the use of chlorpyrifos treatment were confounded by the variations in control between varieties and the appearance of significant aphid infestations at some sites. The aphids were controlled very well by chlorpyrifos, aphid damage has been shown to be increased by the presence of wbm larvae that may have already removed any surplus carbohydrate from the plant and induced a degree of stress (Oakley, 2000). As both pyrethroid and pirimicarb insecticides can also effect wbm (Oakley 1994), no selective aphicide is available for protection of the untreated plots from aphid infestation during the ear emergence period in this type of study.

The discovery of resistant varieties where yield is maintained at close to uninfested levels in the presence of wbm provided an alternative means of assessing tolerance (Tables 13 and 16). By using the resistant varieties as controls a relative yield for susceptible varieties can be estimated and compared to the larval infestation present at each site. The data varied considerably between sites due to other factors. In a regression analysis in which the slopes for each variety were constrained to be the same, the yield loss was

estimated as 37% of a grain's weight for each larva feeding on it (VR 84.85, 168 d.f., $p$ >0.001*)*. However, this regression accounted for only 38.3% of the variation in the data. The fit was not improved by allowing the slopes to vary between varieties, as would be the case if there were large differences in tolerance. It must therefore be concluded that the between site variations were too large for any differences in tolerance to be demonstrated from this data.

**Resistance**

Antibiotic resistance, as given by the *Sm1* gene identified in Canada (McKenzie, *et al.*, 2002), can be defined as a failure of larvae to grow through to fully sized second or third instar larvae within 21 days of the eggs being laid. The varieties, Carlton, Welford, Welland, Kipling and ELS 03-34 were all shown to meet this criterion, with Pennant showing some variation in the expression of the trait. Other studies within the LINK consortium have shown that this resistance is not due to the presence of the *Sm1* gene, and the marker developed in Canada does not identify resistant varieties in UK material. However the effect seems to be very similar and would seem to be probably due to a polypropenoid mechanism similar to that due to increased levels of *para*-coumaric acid in response to attack in wheat carrying the *Sm1* gene. Further work is needed to identify the biochemical mechanism and its genetic basis within these varieties to allow for its effective and safe exploitation. This work should now be a high priority.

**Implications for control strategies**

The problems with variable control encountered in these studies highlights the shortening of the effective window for treatment when temperatures are high. Wbm eggs take 10 days to hatch at a mean temperature of 15°C, but only 4 days if the mean temperature rises to 20°C (Oakley, 1994). At Boxworth in 2004 temperatures peaked at 31.5°C during the egg laying period, shortening the time to hatch of larvae to less than four days. Dow AgroSciences do not recommend the use of Dursban at temperatures above 25°C, due to a rapid breakdown of the active ingredient. Whilst at a farm scale treatments may be timed appropriately for each variety taking account of temperatures, the spread of emergence dates across a variety trial makes the choice of timing a compromise that can not cover the 14 days risk period represented by the current UK recommended list. At a farm, even where pheromone traps are used to detect a hatch of wbm the timescale for action may be too short to enable all crops to be treated. It is therefore necessary to prioritise those crops most likely to benefit from treatment and to understand when the window for effective control has passed to avoid ineffective, and possibly environmentally damaging, treatment of lower priority crops. Given the relative prices of milling and feed crops at present protection of quality of milling crops should take precedence in this decision making over any differences in vulnerability of feed varieties.

To this end we would recommend:

1. Using resistant varieties to reduce the area of crop at risk and over time, the number of larvae dormant in the soil.
2. Using pheromone traps to monitor for the hatch of wbm and identify which crops are most exposed to egg laying due to coincidence with their ear emergence period.
3. More valuable group 1 and 2 wheat and seed crops should be treated with chlorpyrifos as the highest priority.
4. Should time permit treat the most vulnerable and least tolerant group 3 and 4 wheats as a second priority.
5. Check for temperatures during the period of treatment and calculate when eggs are likely to hatch. Cease treatment at this stage and when the majority of tillers begin to flower (GS 61) after which their natural resistance to wbm increases.

**REFERENCES**


Barnes, H. F. (1956). *Gall midges of economic importance. Volume VII Gall midges of cereal crops*. London: Crosby Lockwood & Sons, Ltd. pp. 29-82.

Berzonsky, W A; Ding, H; Haley, S D; Harris, M O; Lamb, R J; McKenzie, R I H; Ohm, H W; Patterson F L; Pearis, F B; Porter, D R; Ratcliffe, R K; Shanower, T G (2003). *Breeding wheat for resistance to insects.* In *Plant Breeding Reviews*, Vol. 22. Edited by Jules Janick, John Wiley & Sons Inc., Hoboken, New Jersey, 221-296.

Birkett M A, Bruce T J A, Martin J M, Smart L E, Oakley J N, Wadhams L J (2004). Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. *Journal of Chemical Ecology*, **30**, 1319-1328.

Ding H., Lamb R. J., Ames N. (2000). Inducible production of phenolic acids in wheat and antibiotic resistance to *Sitodiplosis mosellana*. *Journal of Chemical Ecology*, **26**, 969-985.

Kurppa, S (1989). Susceptibility and reaction of wheat and barley varieties grown in Finland to damage by the orange wheat blossom midge, *Sitodiplosis mosellana* (Gehin). *Annales Agriculturale Fenniae*, **28**, 371-383.

McKenzie, R I H; Lamb, R J; Aung, T; Wise, I L; Barker, P; Olfert, O O (2002). Inheritance of resistance to wheat midge, *Sitodiplosis mosellana*, in spring wheat. *Plant Breeding*, **121**, 1-6.

Oakley J. N. (1994) *Orange wheat blossom midge: a literature review and survey of the 1993 outbreak*. Research Review No. 28. 51 pp., Home-Grown Cereals Authority, London.

Oakley J N (2000). *Appropriate aphicide doses for summer aphid control on wheat*. Project report 202E. Home-Grown Cereals Authority, London. Oakley J. N., Cumbleton P. C., Corbett, S. J., Saunders P., Green D. I., Young J. E. B., Rogers R. (1998). Prediction of wheat blossom midge activity and risk of damage. *Crop Protection*, **17**, 145 - 149.

Pivnick, K. A., Labbé, E. (1993). Daily patterns of activity of females of the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae). *Canadian Entomologist*, **125**, 725-736.

Wise, L; Lamb, R J; Smith, M A H (2001). Domestication of wheats (Graminae) and their susceptibility to herbivory by *Sitodiplosis mosellana* (Diptera: Cecidomyiidae). *The Canadian Entomologist*, **133**, 255-267.

**Technical detail 2.**

**Genetic Analysis of Orange Blossom Midge Resistance in Wheat**

**Introduction**

As part of the integrated project 'The Integrated control of wheat blossom midge ' the John Innes Centre undertook a series of experiments to evaluate the genetic basis of variation in the responses of different UK and foreign varieties to orange blossom midge infestation. Much of this work involved the testing of precise genetic stocks of wheat so that any tolerance/resistance observed could be attributed to specific chromosomes or genes.

**Materials and Methods:**

**Materials**

Four different groups of materials were examined:

1. A series of varieties that represent key current and historic germplasm used in the UK winter wheat gene pool as well as lines that are parents of precise genetic stocks or mapping populations. The varieties evaluated are shown in Figure 1.

2. A set of single chromosome substitution lines where chromosomes of a donor variety, the Russian variety Bezostaya, have systematically replaced their homologous chromosome in the recipient variety Cappelle-Desprez. These were developed at the John Innes Centre using the methods described by Law and Worland (1967). Because marker analysis had shown that certain of these were not genetically correct, not all of the possible set of 21 possible substitution lines (1A to 7D) were evaluated, and lines 4B and 5B were absent. All available lines were grown in 2001/02, 2002/03, but only substitution lines not differing significantly in flowering time but showing significant differences from the recurrent parent were grown in 2003/04, namely lines 1D, 3A and 7B, and Bezostaya was also not represented in this trial.

3. A set of isogenic lines differing for alleles at the loci controlling the photoperiod response, and hence the flowering time of wheat, in the genetic background of the winter wheat cultivars Cappelle-Desprez and Mercia. These consisted of lines of Cappelle-Desprez carrying an allele at the locus *Ppd1 (Ppd-D1)* on chromosome 2D conferring photoperiod insensitivity from the Italian variety Mara, and an allele conferring

photoperiod insensitivity at the *Ppd2 (Ppd-B1)* locus on chromosome 2B from the variety Chinese Spring. The Mercia lines carried an allele at the locus *Ppd1 (Ppd-D1)* on chromosome 2D conferring photoperiod insensitivity from the Mexican variety Ciano 67, an allele conferring photoperiod insensitivity at the *Ppd2 (Ppd-B1)* locus on chromosome 2B from the variety Chinese Spring, and an allele for photoperiod insensitivity from the Indian variety C591 at the *Ppd3 (Ppd-A1)* locus on chromosome 2A.

4. A mapping population of 144 recombinant doubled haploid lines developed from the $F_1$ of the cross between the varieties Spark and Rialto, plus the parents.

**Field Trials**

Three years of trials were carried over the growing seasons 2001-2002, 2002-2003, 2003-2004. Not all materials were represented in all years (see Results). All experiments were sown at the Morley Research Station and were treated with standardized programmes of herbicides and fungicides, except no insecticides. The 2001-02 trial was grown at Hackett's field, Deopham Road, Morley; the 2002-03 grown at Eleven Acres field Deopham; and the 2003-04 trial at Corner field, Deopham.

Each experiments used a randomised plot design with 2 replications, drilled using a 110g sample of dressed seed in a Hege 80 drill to produce a plot of 6m x 1m.
All individual plots were scored for ear emergence, and then anthesis tine (growth stage 61). Three weeks after anthesis 10 random ears were collected, a group of 5 ears from 2 different locations within the plot. These were taken back to the laboratory and frozen until they could be assessed for midge infestation.

At maturity, the plots were scored for final height before harvest, and all plots harvested using a plot combine and yields recorded.

**Midge assessments**

Each ear was looked at individually and scored for the presence and number of wheat orange blossom midge by examining each individual floret separately and noting the number of midge larvae in each floret. The location of the floret was noted by counting the spikelet number from the base of the ear and the position of the floret on the ear, either on the right or left of central floret, with 1 being on the outside and 2 as the next floret towards the centre.

The total number of florets containing viable grain were also counted and recorded, as were the number of sterile grains. Sterile grains were grains that had failed to mature or were just full of water. Data were recorded as the number of larvae per 100 grain and as % of grains infested.

**Marker Analysis**

A molecular genetic marker has been reported by a Group in Winnipeg, Canada, as being diagnostic for a major resistance gene, *Sm1*, in Canadian varieties (Thomas et al. 2004). To evaluate whether the Elsoms Seeds resistant varieties Welford and Charton carry this gene, and whether this molecular test is diagnostic for resistance in European wheats, molecular marker analysis was carried out by PCR using primers supplied by the Canadian Group (data and methods provided in confidence).

**Results**

**1. Varieties**

Levels of infestation increased significantly over the three years of trials, being lowest in 2002 and greatest in 2004. Nevertheless, in all years, there were significant differences between the varieties and the relative rankings were maintained as infestation levels increased. In 2002 and 2003 there was a clear trend for earlier flowering, photoperiod insensitive varieties, such as Bezostaya, Poros and Soissons to escape infestation. However, in addition, there were clear genetical differences in infestation levels, and hence genetic resistance/tolerance between the varieties independent of flowering time. Most noticeable were the negligible levels on Welford and Carlton, varieties known to carry major resistance genes.

An evaluation of exotic, old varieties and landraces, such as Squarehead Masters, Chinese Spring, Atlas 66, and *Triticum macha* did not indicate sources of resistance as good as currently know resistance sources already in cultivation. Perhaps it will require a screen of a much larger number of varieties to find new sources. However, it is clear that there are promising sources of resistance in addition to those in Welford and Charlton, in varieties such as Herward and Spark, if the genes involved can be identified by genetic analysis and manipulated by plant breeders.

**2. Chromosome Substitution lines**

Figure 2 shows the levels of infestation in the substitution lines over the three years of trial. Levels of infestation increased significantly over the three years of trials, being lowest in 2002 and greatest in 2004. As levels of infestation increased, differences between lines also decreased. In both years of joint testing, Bezostaya was infested significantly less than Cappelle-Desprez, but also had a significantly earlier flowering time, so this is probably due to escape, rather than resistance/tolerance *per se*.

The substitution lines also showed a similar pattern of increasing infestation over years, and infestation levels increased from between 5-25 larvae per 100 grain in 2002, to 16-55 larvae per 100 grain in 2003. However, within all years, there was a significant difference between the lines, indicating significant genetical variation in levels of infestation. The relative differences between lines were also generally maintained between 2002 and 2003, indicating that they are true genetical effects.
The most significant effect, however, in both 2002 and 2003 was the correlation between levels of infestation and flowering time. This was particularly the case for Cap (Bez 2D) which showed negligible infestation in 2002 due to its earlier flowering time, which is due to it carrying the *Ppd1* (*Ppd-D1)* gene for photoperiod insensitivity (which also accounts for the earlier flowering time of Bezostaya).

In 2002, one line, Cap (Bez 1A) was infested to a significantly greater extent than Cappelle-Desprez, indicating genes for greater attractiveness to the pest, although this was not repeated in 2003, where only Cap (Bez 1B) showed greater infestation levels. However, in 2002 there were four lines that were less infested than Cappelle-Desprez, indicating chromosomes from Bezostaya carrying genes for resistance/tolerance, namely 7B, 3A, 1D (both duplicates) and 2D. All of these also showed lower infestation levels in 2003, although only significant for 7B. In addition, chromosomes 3B, 6A and 7A showed lower 2003 levels, although not significantly different in 2002. 7B also showed lower infestation levels in 2004 and although also earlier flowering than Cappelle-Desprez in all years, this could not account for the effect alone, since in 2004 there was an early flight of midges which coincided with this line reaching anthesis, Nevertheless, it showed less % grain attacked compared to the larvae found, and this could be an indication of resistance or tolerance of this line.

**3. Photoperiod insensitive isogenic lines**

Figure 3 shows the infestation levels on the photoperiod insensitive, and hence early flowering lines, grown in 2002 and 2003, compared to their control parents, Cappelle-Desprez and Mercia. In 2002, Mercia had an infestation level much lower than Cappelle-Desprez, but the isogenic lines of the two varieties behaved similarly in having greatly reduced, and in the case of Mercia (*Ppd1*), a negligible infestation level. In these years, earlier flowering before temperature were suitable for midge flight was clearly an efficient mechanism of escape. Even in 2003, when infestation levels were much higher, earlier flowering was a mechanism for reducing damage.

The *Ppd* alleles differ in the potency of inducing earlier flowering where *Ppd1* is earlier than *Ppd2*, which in turn, is earlier than *Ppd3*. In 2002, this also reflects the differential response to midge infestation where both the *Ppd1* and *Ppd 2* lines have low infestation levels, but *Ppd1* is still less than *Ppd2*. However, in 2003, this trend is not consistent, and whereas the Cappelle *Ppd1* has a level lower than the *Ppd2* line, for the Mercia isogenics the trend is reversed. This clearly indicates that although earlier flowering is a mechanism of

escape, it is not independent of other variables, such as temperature, which can induce midge flight and hence infestation at times other than within the normal range of UK varieties. However, it would generally be expected that possessing photoperiod insensitivity and hence, earlier flowering, is a mechanism that plant breeders can apply to reduce midge damage.

**4. Spark x Rialto recombinant doubled haploid lines**

The difference between the parents, Spark and Rialto is not large (see Figure 1), but was statistically significant in all years of testing, where Spark was infested significantly less than Rialto. This indicates that there are genes for resistance/tolerance segregating between these varieties, and that this cross is amenable for genetic analysis. Although due to time constraints only one rep of the recombinant lines has yet been fully assessed, the results for each line, Figure 4, shows that there is clearly genetic variation between the lines with transgressive segregation relative to the parental levels of Rialto (mean of 27.0 larvae/100 grain) and Spark (mean of 9.6 learvea/100 grain). Certain lines, for example, 51, 116, had lower levels and several, 4, 23, 38, 57,76, 96, had significantly higher levels. This indicates that the mode of inheritance is undoubtedly complex with more than one, and possibly several, genes involved.

The number of genes involved can be resolved by QTL analysis, since a genetic map of this cross is available.

**5. Marker Analysis**

Using primer information, and the optimised protocol obtained from the Canadian Group, marker analysis was carried out on a range of varieties which the trials in the project had shown to differ in levels of infestation over the different years of trial. These including the Elsoms Seeds resistant varieties Welford, Els 24 and Els 28, and the known quite susceptible varieties Tanker and Pennant. In general, these results - see Figure 5 - are disappointing on the potential of this test in UK and European varieties. There appears to be no correlation between possessing the *Sm1* diagnostic band and resistance. Varieties contrasting widely in susceptible, such as Tanker and Welford, both possess the band, and few varieties, only Charger, Rialto and Savannah in this sample, lack the band. This may indicate that although this band is diagnostic in a restricted gene pool where the specific cross is known to segregate both for *Sm1* and the band, it is not of general use in European germplasm.

**References**

Law C N, Snape J W and Worland A J (1987). Aneuploidy in wheat and its use in genetic analysis. In: Wheat breeding: Its scientific basis, Ed. F G H Lupton. Chapman and Hall, London, pp 71-108

Thomas J, Riedel E, Penner G. (2001) An efficient method for assigning traits to chromosomes. Euphytica 119: 217-221

**Figure 1 : Levels of range blossom midge infestation measured as the number of live larvae/100 grain sampled on the varieties tested, for three years of trials.**

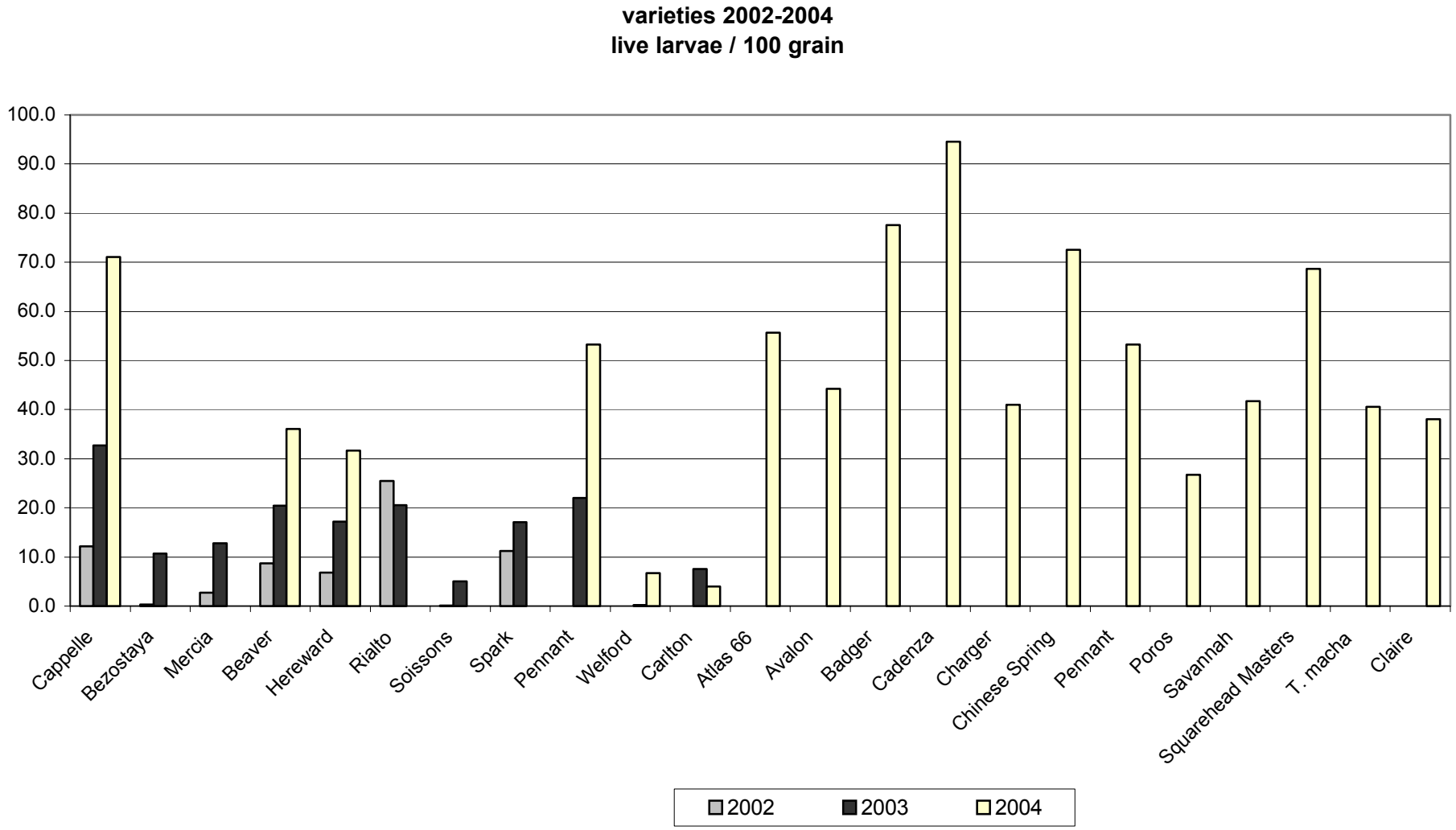


Figure 2 : Levels of orange blossom midge infestation measured as the number of live larvae/100 grain sampled on the Cappelle-Desprez (Cap) Bezostaya (Bez) single chromosome substitution lines, for three years of trials.

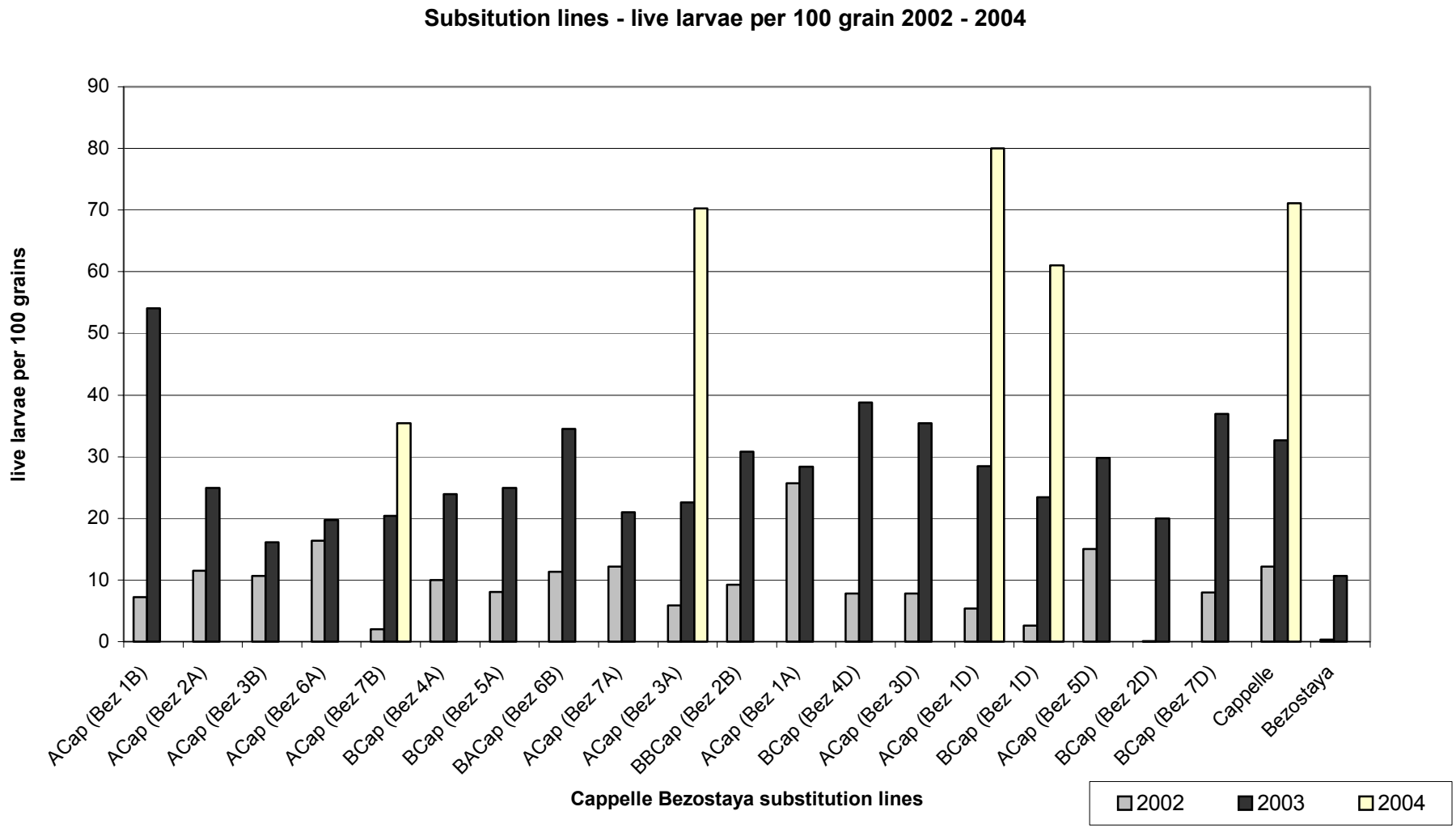


Figure 3 : Levels of orange blossom midge infestation on the Cappelle-Desprez and Mercia isogenic lines for the photoperiod insensitivity genes, *Ppd1 (Ppd-D1)*, *Ppd2 (Ppd-B1)*, and *Ppd3 (Ppd-A1)*, measured as number of live larvae per 100 grain sampled, for two years of trials.

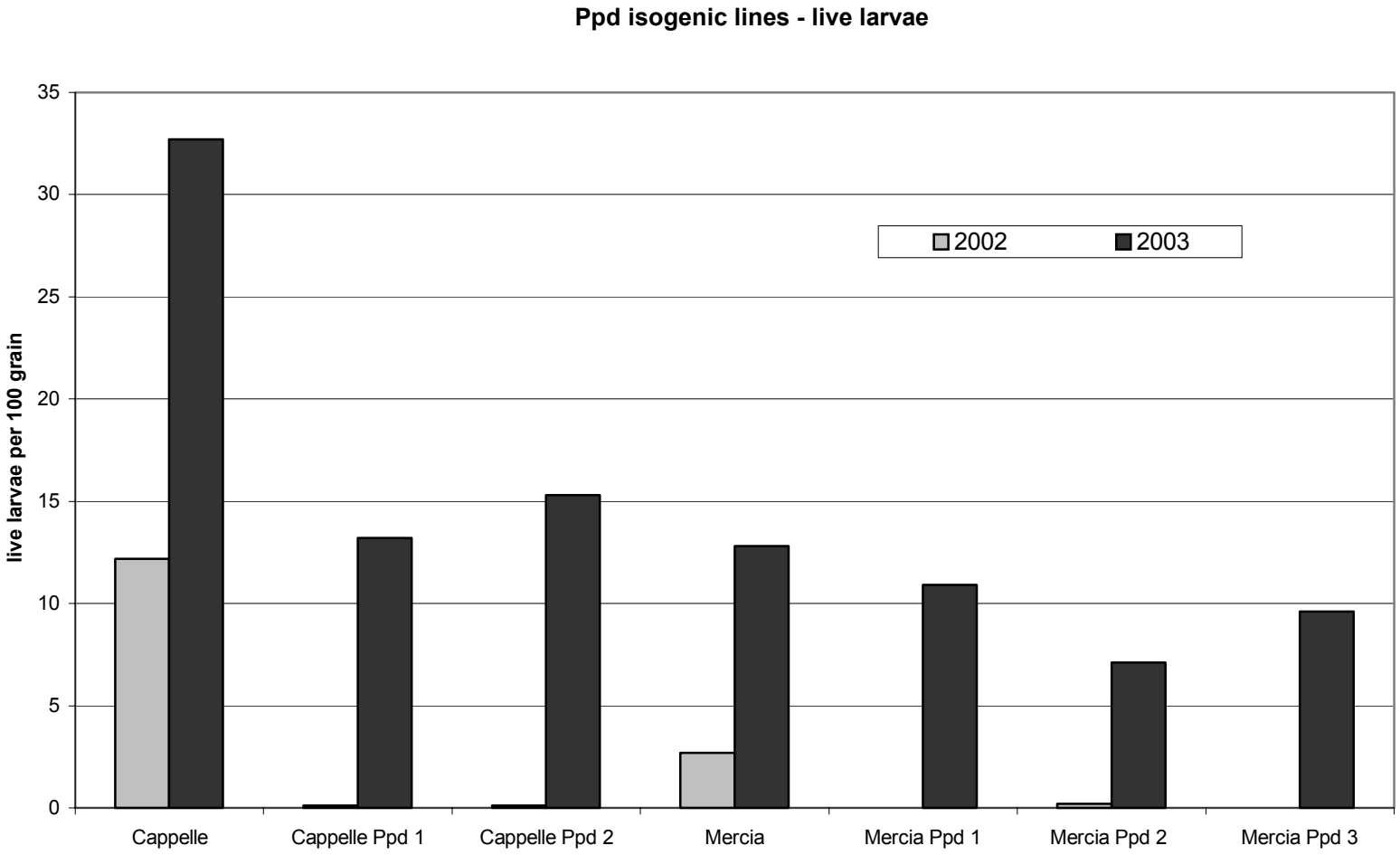


Figure 4 : Levels of orange blossom midge infestation measured as the number of live larvae/100 grain sampled on the individual Spark x Railro recombinant doubled haploid lines evaluated in 2003.

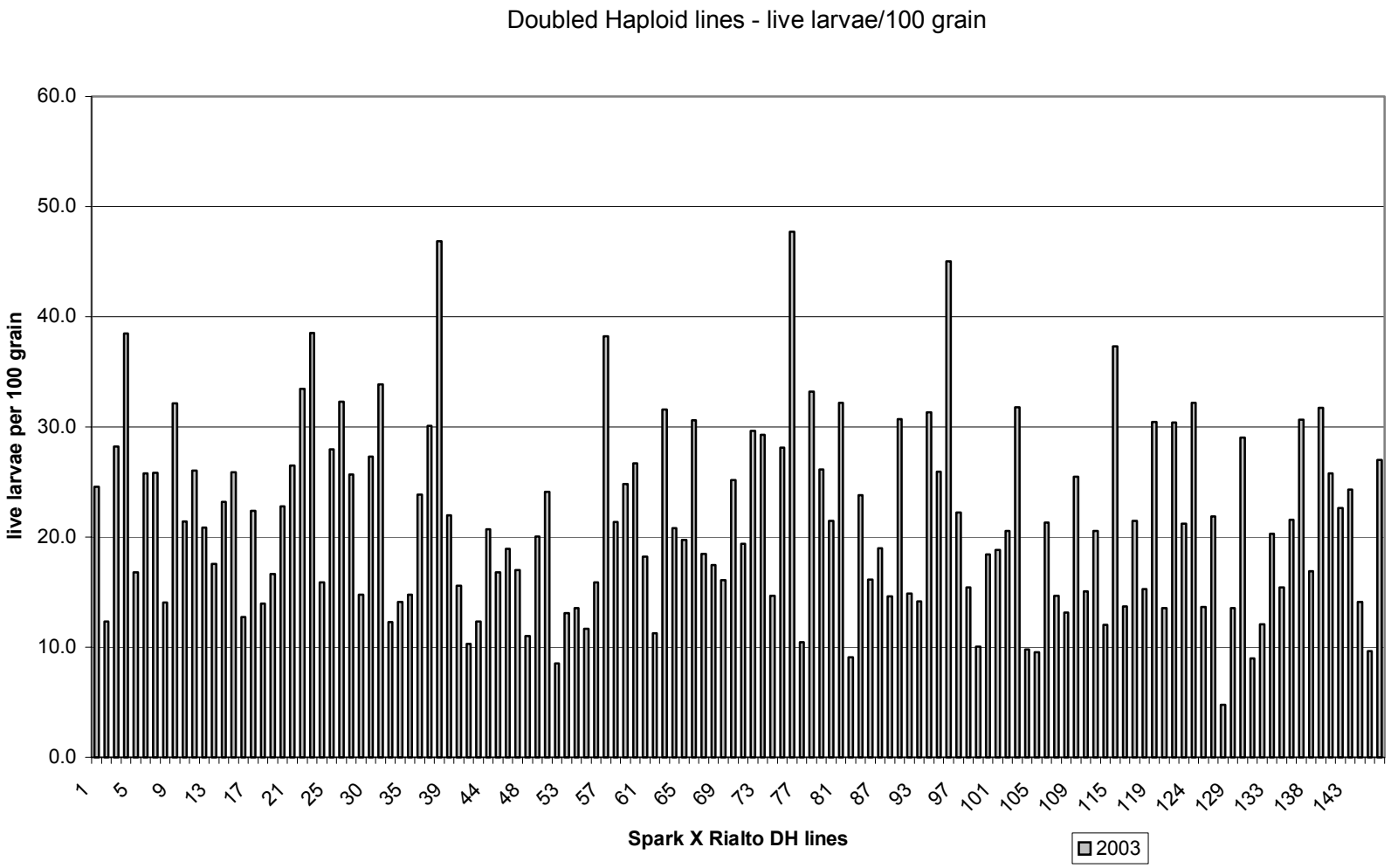
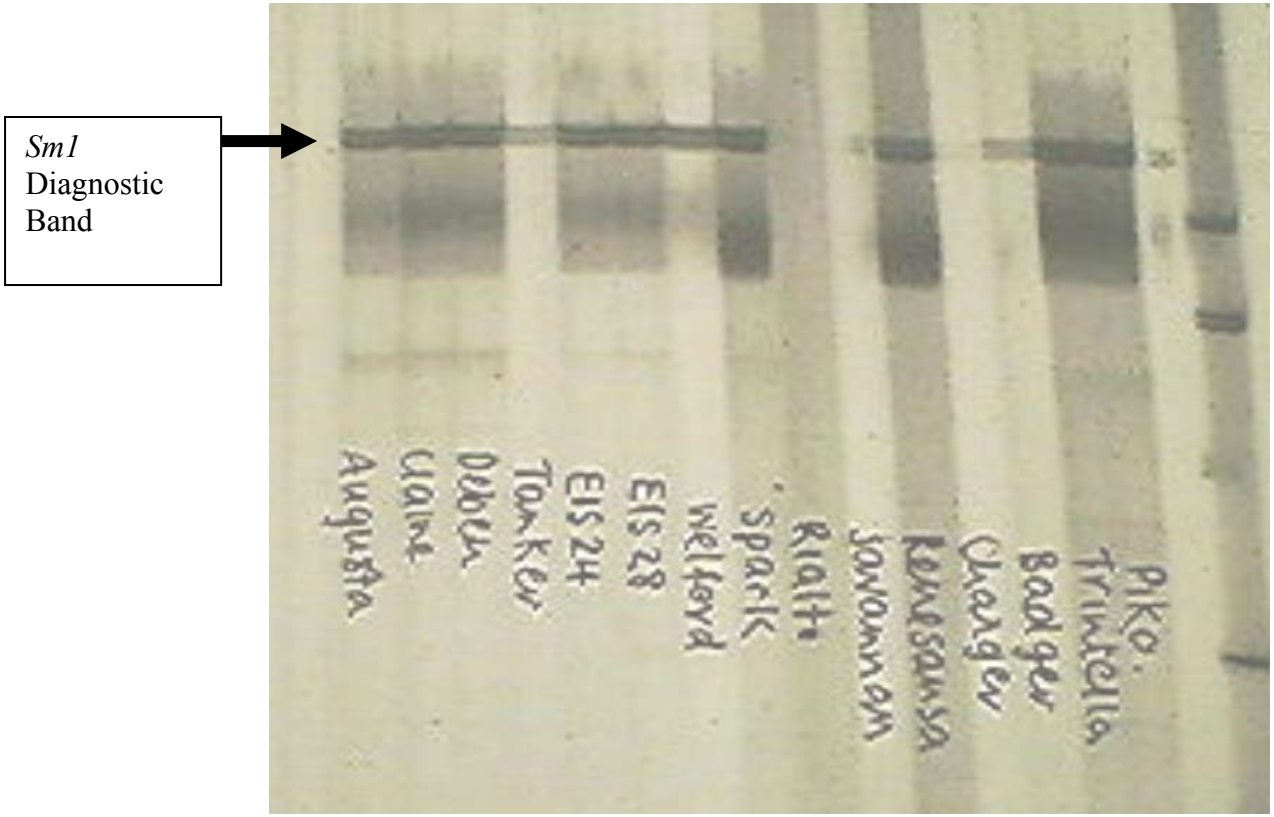


Figure 5: Marker analysis of a sample of varieties using primers diagnostic for the *Sm1* gene giving resistance to orange blossom midge in Canadian varieties



**Technical report 3.**

**Development of pheromone traps**

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Note: a refereed paper has been published relating to laboratory identification of wheat volatiles attractive to female *S. mosellana* (Birkett *et al.* 2004; *J. Chem. Ecol.* **30**, 1319-1328).

## 1. INTRODUCTION

The orange wheat blossom midge, *Sitodiplosis mosellana*, is a common and increasingly important pest of wheat in the Northern Hemisphere, causing severe yield losses in years of high infestation. In an outbreak in the UK in 1993 crop losses were estimated to have exceeded £30 million (Oakley, 1994). 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 *S. mosellana* the actual degree of damage to crops is often underestimated.

*S. mosellana* has a very patchy spatial distribution and infestations vary from year to year depending on climatic conditions. Midge 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 unfavorable. Adult midges 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.* 1988). 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 midge 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 1 week to hatch. 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 else it will not give proper control. These detection problems mean that it is hard to predict when infestations that would warrant insecticide treatment have built up and there is considerable grower demand for a reliable based monitoring system.

A semiochemical based trapping system would reduce the unnecessary use of pesticides against lower levels of midge infestation and allow populations of parasitoids to increase and provide a greater level of natural control. To address the need for an improved monitoring system we have carried out extensive laboratory laboratory studies and field trials of semiochemical baited traps. The sex pheromone, 2,7-nonanediyl dibutyrate, (Gries et al. 2000), was synthesised and different formulations of it have been tested. In addition, wheat volatiles from susceptible growth stages were identified from air entrainment samples, and a number were evaluated in laboratory and field trapping experiments.

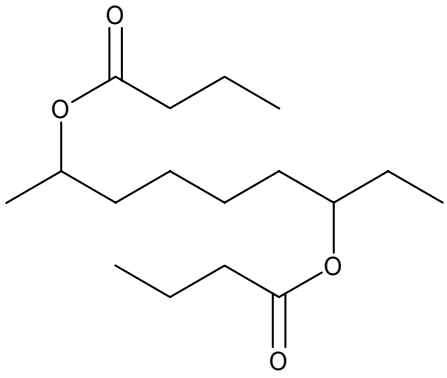
## 2. MATERIALS & METHODS

### 2.1. Synthesis of Pheromone

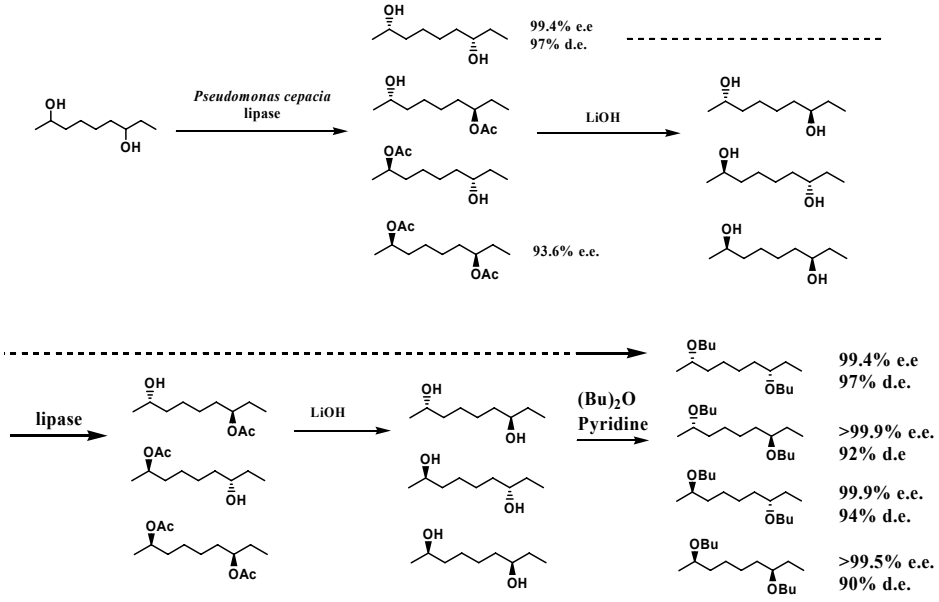
2,7-nonanediyl dibutyrate (Fig 1, **1**) was made racemically using the strategy described by Gries *et al.* (2000). For laboratory behavioural and field trapping studies, samples (0.1 g) of the individual enantiomers of **1** were prepared using a biotechnological approach (Fig 5 Scheme 1). Both carbinol centres can be resolved using a lipase enzyme and the resolution can be repeated to improve the enantiomeric excess of the product. However, this route is not suitable for the provision of the large scale quantities of pheromone required for field studies. The known synthesis (Gries et al., 2000) is costly. Modification of this route has provided a pure mixture of the four diastereoisomers of **1** on a gram scale for field experiments.

### 2.2. Insect Rearing

Soil samples were taken from sites with severe *S. mosellana* damage in the Autumn after crop harvest. These contained *S. mosellana* larval cocoons. The samples were transferred to shallow seed trays and stored at 5°C. After at least 3 months vernalisation, trays were moved to a cabinet (22°C, 75%RH, 16:8 L:D) and watered, to bring adult midges 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.



**1**




**Scheme 1**

**Fig. 1 Synthesis of the wheat blossom midge pheromone, 2,7-nonanediyl dibutyrate, (1) and its enantiomers (Scheme 1)**

### 2.3. Electrophysiology

Electroantennogram (EAG) recordings from whole antennae of *S. mosellana* were made using Ag-AgCl glass electrodes filled with saline solution (composition as in Maddrell, 1969, but without glucose). The abdomen was inserted into the glass capillary of the reference electrode. Both antennae were then directed into the recording electrode so that the distal end was immersed in saline and held in place by surface tension. Signals were passed through a high impedance amplifier (UN-06, Syntech, The Netherlands) and analysed using a software package (Syntech). Coupled GC-EAG was used to locate physiologically active components in complex mixtures. The coupled GC-electrophysiology system, in which the effluent from the

GC column is simultaneously directed to the antennal preparation and the GC detector, has been described previously (Wadhams, 1990).

### 2.4. Olfactometer Bioassay

A 4-arm olfactometer was used (Petterson, 1970). It was made of Perspex and had a lining of filter paper on the bottom. Diffuse uniform lighting was provided from a lamp above. The treated arm contained either 1ng synthetic 2,7 nonanediyl dibutyrate in 1µl redistilled hexane, which was tested with males, or a 10µl aliquot of air entrained wheat sample, which was tested with females. These were applied to a piece of filter paper using a micro-pipette (Drummond "microcaps"). Control arms contained the same volume of solvent on filter paper. Air was drawn through the apparatus at 350 ml / min. Individual *S. mosellana* were introduced into the central chamber and the time spent and number of entries into each arm were recorded using "Olfa" software over a 16 min bioassay period. The olfactometer was rotated 90$^{o}$ every 2 min to avoid directional effects.

### 2.5. Formulations used for field experiments

#### 2.5.1. Pheromone

**2002**

Field formulations used racemic 2,7-nonanediyl dibutyrate. Racemic material is less expensive than enantiomerically pure material and thus more suitable for commercial use. Different formulations of *S. mosellana* sex pheromone, 2,7-nonanediyl dibutyrate, were used for field trials at Rothamsted:

***Sachets*** Polyethylene sheet (100 gauge) was used to make 2cm x 3cm dispensers. These contained a 10mm x 6mm piece of sponge impregnated with 24mg 2,7-nonanediyl dibutyrate. The sides of the dispenser were sealed with a heat sealing device so that they were completely air-tight. Release rate, as measured by weight loss in a wind tunnel at 19$^{o}$C, 0.20m/s windspeed, was 137µg/day.

***Polyethylene vial*** Two different loadings of 2,7-nonanediyl dibutyrate in polyethylene vials (Fisons: WP/5) were tested. These were 10mg and 10µg which provided high and low release rates respectively. Release rate from the 10mg loading vial was 100µg/day

***Chromacol vial*** A 10mg loading of 2,7-nonanediyl dibutyrate in a Chromacol vial (08-CPV(A)) was used. Approximate release rate was 0.8µg/day.

**2003**

Field formulations used racemic 2,7-nonanediyl dibutyrate. The polyethylene vial (Fisons: WP/5) formulation was used because it was the most successful formulation in 2002. Different loadings of pheromone in the vial were compared (0.1mg, 1mg, 5mg and 10mg). Pheromone which had not been through the final purification step of the synthesis was also tested to investigate whether it could be used as a cheaper source for commercial production.

**2004**

The polyethylene vial (Fisons: WP/5) with a loading of 5mg racemic 2,7-nonanediyl dibutyrate was used as the standard formulation. Rubber septa loaded with 1mg and 5mg were also tested as an alternative formulation more amenable to commercial production. Release rate from the 5mg loading rubber septum was determined as 26ng/day. Pure enantiomers and the standard formulation were compared. Lures and traps obtained from PheroTech in Canada were also tested and compared with the standard lure and Agrisense Delta traps.

### 2.5.2. Plant volatiles

These were tested in 2002 and 2003 and were formulated in polyethylene vials with a small piece of sponge inside on which the neat chemical was placed (Expts. 1,3,4,5,6) and in both vials and sachets, the latter to provide high release rates (Expt. 4). For the sachets, polyethylene sheet (1000 gauge) was used to make 4000 gauge 3cm x 3cm dispensers i.e. a bag within a bag etc. The sides of the bags were sealed with a heat sealing device so that they were completely air-tight. The inner bag contained a 25mm x 20mm piece of sponge impregnated with neat compound. Release rates, were measured by weight loss in a wind tunnel at 20$^{o}$C, 0.20m/s windspeed. Details were as follows:

1. *3-carene*
    - 0.3ml on thick sponge in small polyethylene vial released 4.7mg/day
    - 0.5ml on thin sponge in sachet released 17.3mg/day
2. *(Z)-3-hexenyl acetate*
    - 0.5ml on thick sponge in large polyethylene vial released 2.3mg/day
    - 0.5ml on thin sponge in sachet released 6.4mg/day
3. *Acetophenone*
    - 0.5ml on thick sponge in small polyethylene vial released 0.7mg/day
    - 0.5ml on thin sponge in sachet released 3.2mg/day

## 2.6. Field Trapping Experiments

In 2002 there were 2 separate lines of traps and a Latin square at Rothamsted farm and one line of traps at ADAS Boxworth. Three different formulations of *S. mosellana* sex pheromone were used in field trials at Rothamsted (described above in section 2.4.1). One formulation was used at Boxworth (high release polyethylene vial). Lines of traps with an unbaited trap and one of each of 4 pheromone formulations were tested in set-aside in Del Harding field, Rothamsted, and in wheat (cv. 'Hereward') at Broadbalk, Rothamsted. The 4 x 4 Latin square was set up on Del Harding. It had 4 treatments: unbaited trap and 3 pheromone formulations (low release vial, high release vial and sachet). Trap spacing was 10m. At Boxworth there was a line of traps on Sykes field with 5 replicates of pheromone-baited traps and 5 unbaited control traps.

In 2003 field experiments were carried out at two sites on Rothamsted farm. These were ‘West Barn Field’ (6 Latin square arrays of traps ) and ‘Meadow’ (3 Latin squares). Different vial loadings and purity of pheromone were evaluated as well as plant-derived semiochemicals as attractants for female *S. mosellana* in trapping experiments. In addition to this a comparison was made at two locations between lines of 10 yellow sticky traps placed at 5m intervals with two pheromone traps (expt. 2). In this experiment wheat ears were sampled and infestation level in the crop was recorded. Details of treatments are given in Table 1.

In 2004 further field trials were conducted with the pheromone. Polyethylene vial and rubber septum formulations were compared and the pure enantiomers were compared with racemic material, and different trap designs were compared. Release rates from standard formulations were determined. Another field trapping experiment with plant-derived volatiles was conducted. An investigation of correlation between midge infestation in the crop and pheromone and sticky trap catches was also performed on five different sites. Details of treatments are given in Table 2.

**Table 1 Field Trapping Experiments 2003**

| |
|---|
| **Experiment 1 – pheromone loading in vial**<br>4 x 4 Latin square, 2 replicate squares. Treatments:<br>• blank control<br>• 1mg 2,7-nonanediyl dibutyrate<br>• 5mg 2,7-nonanediyl dibutyrate<br>• 10mg 2,7-nonanediyl dibutyrate |
| **Experiment 2 – calibration of pheromone catch with damage**<br>2 pheromone traps (10mg 2,7-nonanediyl dibutyrate) and sticky yellow cards at 3 sites to compare catches with infestation levels monitored in adjacent wheat |
| **Experiment 3 – wheat volatiles alone and in combination**<br>5 x 5 Latin square. Treatments:<br>• blank control<br>• acetophenone<br>• 3-carene<br>• (*Z*)-3-hexenyl acetate<br>• acetophenone & 3-carene & (*Z*)-3-hexenyl acetate |
| **Experiment 4 – wheat volatile release rate**<br>3 x 3 Latin square, 2 squares. Treatments:<br>• blank control<br>• [acetophenone & 3-carene & (*Z*)-3-hexenyl acetate] in vial (low release rate)<br>• [acetophenone & 3-carene & (*Z*)-3-hexenyl acetate] in sachet (high release rate) |
| **Experiment 5 – wheat volatiles in pairs**<br>5 x 5 Latin square. Treatments:<br>• blank control<br>• acetophenone & 3-carene<br>• acetophenone & (*Z*)-3-hexenyl acetate<br>• 3-carene & (*Z*)-3-hexenyl acetate<br>• acetophenone & 3-carene & (*Z*)-3-hexenyl acetate |
| **Experiment 6 – pheromone and wheat volatiles**<br>4 x 4 Latin square. Treatments:<br>• blank control<br>• acetophenone & 3-carene & (*Z*)-3-hexenyl acetate<br>• 10mg 2,7-nonanediyl dibutyrate<br>• [acetophenone & 3-carene & (*Z*)-3-hexenyl acetate] & [10mg 2,7-nonanediyl dibutyrate] |
| **Experiment 7 – pheromone**<br>4 x 4 Latin square. Treatments:<br>• blank control<br>• 0.1mg 2,7-nonanediyl dibutyrate<br>• 10mg 2,7-nonanediyl dibutyrate - low purity<br>• 10mg 2,7-nonanediyl dibutyrate - high purity |

**Table 2 Field Trapping Experiments 2004**

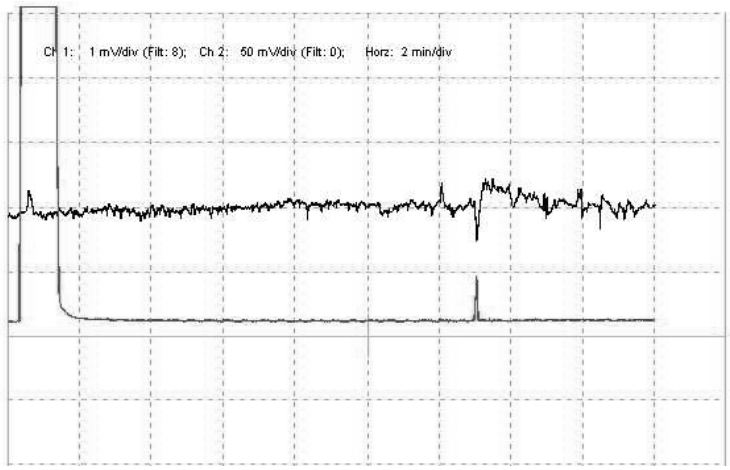
| |
|---|
| **Experiment 1 – calibration of pheromone catch with damage**<br>2 pheromone traps (5mg 2,7-nonanediyl dibutyrate in polyethylene vial) and 5 sticky yellow cards at 5 sites to compare catches with infestation levels monitored in adjacent wheat. |
| **Experiment 2 – pheromone formulation: septum vs. vial**<br>4 x 4 Latin square, 2 replicate squares. Treatments:<br>• blank control<br>• 5mg 2,7-nonanediyl dibutyrate in rubber septum<br>• 5mg 2,7-nonanediyl dibutyrate in polyethylene vial<br>• 1mg 2,7-nonanediyl dibutyrate in rubber septum |
| **Experiment 3 – pheromone enantiomers**<br>6 x 6 Latin square. Treatments:<br>• blank control<br>• 5mg RR 2,7-nonanediyl dibutyrate<br>• 5mg RS 2,7-nonanediyl dibutyrate<br>• 5mg SR 2,7-nonanediyl dibutyrate<br>• 5mg SS 2,7-nonanediyl dibutyrate<br>• 5mg racemic 2,7-nonanediyl dibutyrate |
| **Experiment 4 – wheat volatiles alone and in combination**<br>5 x 5 Latin square, 2 replicate squares. Treatments:<br>• blank control<br>• acetophenone<br>• 3-carene<br>• (*Z*)-3-hexenyl acetate<br>• acetophenone & 3-carene & (*Z*)-3-hexenyl acetate |
| **Experiment 5 – wheat volatile release rate**<br>5 x 5 Latin square, 2 replicate squares. Treatments:<br>• blank control<br>• [acetophenone & 3-carene & (*Z*)-3-hexenyl acetate] – vial / low release<br>• [acetophenone & 3-carene & (*Z*)-3-hexenyl acetate] – sachet / high release<br>• 5mg 2,7-nonanediyl dibutyrate in polyethylene vial<br>• plant volatiles plus pheromone (treatments 2 and 4 combined) |
| **Experiment 6 – Canadian lure/trap**<br>5 x 5 Latin square. Treatments:<br>• blank control<br>• 5mg 2,7-nonanediyl dibutyrate in polyethylene vial in Agrisense delta trap<br>• 5mg 2,7-nonanediyl dibutyrate in polyethylene vial in Pherocon trap<br>• Canadian lure in Agrisense delta trap<br>• Canadian lure in Pherocon trap |

## 3.1. RESULTS – PART 1 SEX PHEROMONE

### 3.1.1 Electrophysiology

Electrophysiological studies with the synthetic sample of 2,7-nonanediyl dibutyrate, indicated that the material was highly active (Fig 2).

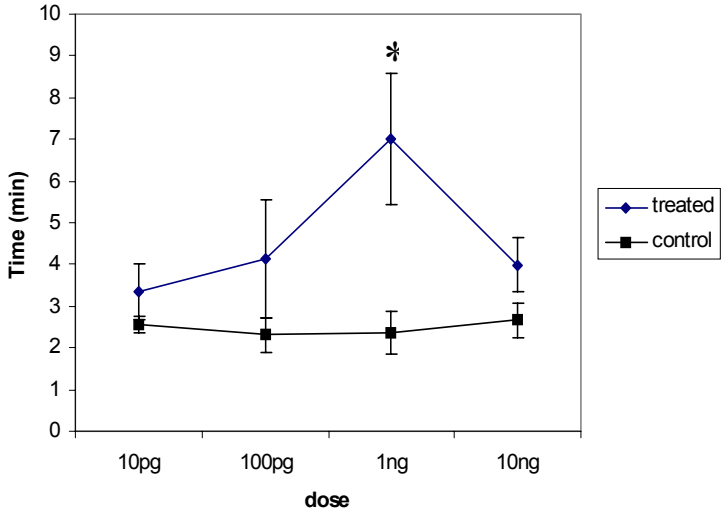
**Fig. 2 GC-EAG response of male *S. mosellana* to a 20ng dose of sex pheromone, 2,7-nonanediyl dibutyrate**




### 3.1.2. Olfactometer

In the olfactometer there was a significant attraction of males to 1ng of the pheromone ($P < 0.005$, paired *t*-test) although, surprisingly, the response to higher or lower stimulus concentrations was reduced (Fig 3).

**Fig. 3 Response of male *S. mosellana* to sex pheromone (2,7-nonanediyl dibutyrate on filter paper) in the olfactometer: time spent in treated and control arms (n = 9)**



### 3.1.3. Field Trapping Experiments

#### 3.1.3.1. 2002 Pheromone Trap Experiments

In the field, significantly more male midges were caught with the high release polyethylene vial and the sachet formulations than with unbaited control traps (Fig 4). Thus, the reduction in response at a higher dose that was observed in the lab was not observed in the field. Indeed, during the week of the midge flight peak some of the sachet and high release vial traps caught in excess of 100 male *S. mosellana.* In the lines of traps at Rothamsted catches in traps baited with chromacol vials were intermediate between high release and low release polyethylene vials.

**Fig. 4. *S. mosellana* Pheromone Trap Catches at Del Harding, 20 May - 4 July 2002**

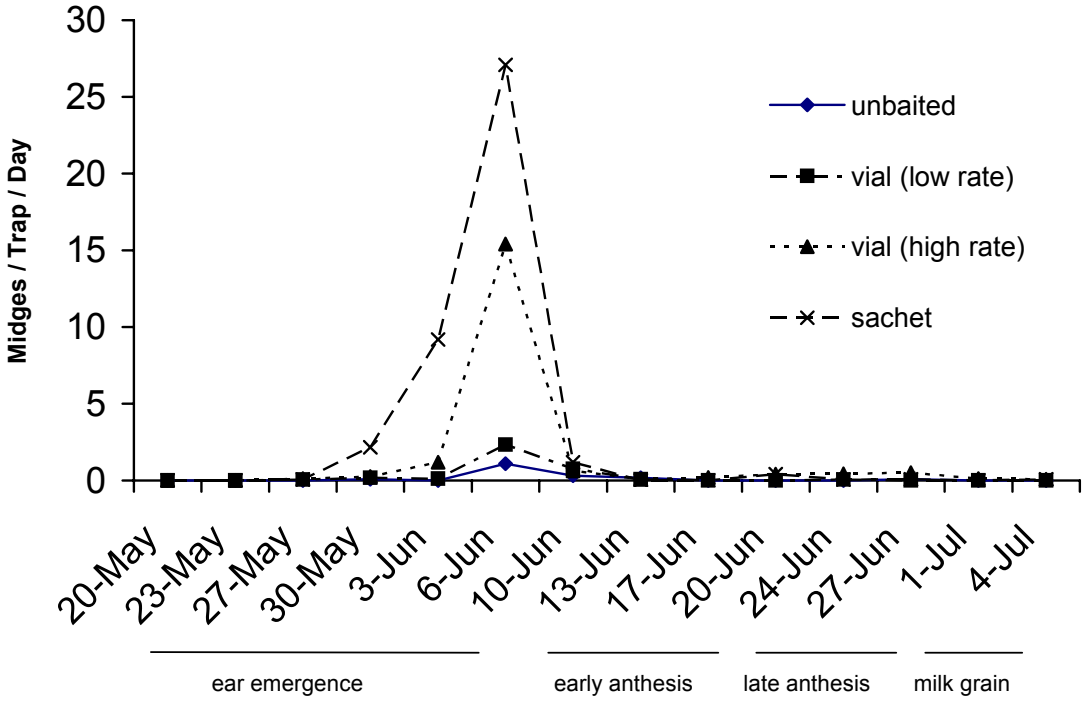
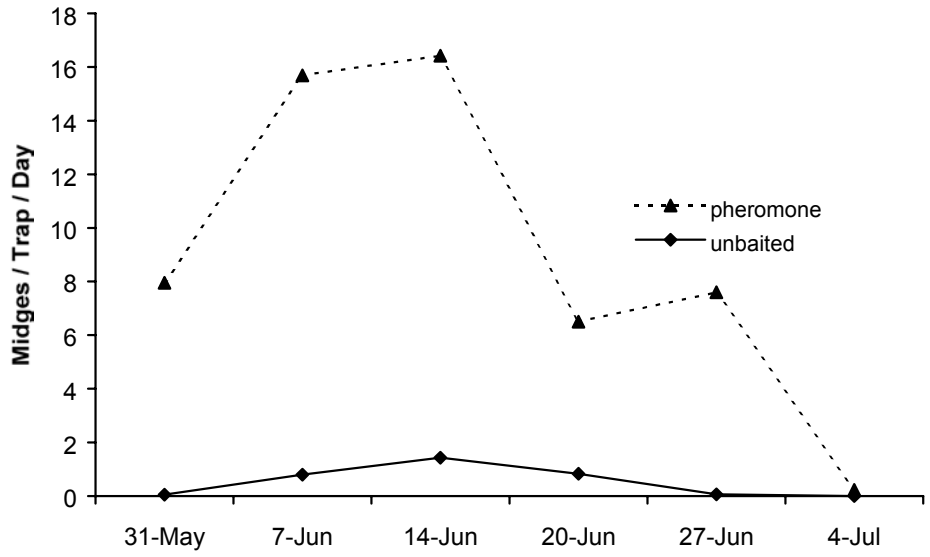


**Fig. 5 *S. mosellana* pheromone trap catches at Sykes field, Boxworth, 31 May - 4 July 2002**




There was a good correlation between timing of midge flight and when wheat was at the susceptible growth stage at Rothamsted (Fig 4). At Boxworth (Fig 5) there appeared to be a more prolonged period of midge flight activity continuing into the late anthesis growth stage when wheat would no longer be suitable for larval survival. At both sites the high release vial captured approximately 15 male *S. mosellana* per trap per day during the peak of midge flight.

### 3.1.3.2. 2003 Pheromone Trap Experiments

The *S. mosellana* sex pheromone, 2,7-nonanediyl dibutyrate, performed well, catching up to 50 male midges per trap per night during the midge flight peak. Although a dose-response relationship was observed in terms of more male midges being caught with the 5mg loading than the 1mg loading, there was little difference between the 5mg and 10mg loadings (Expts. 1A and 1B; Fig 6 & 7). In fact, in Expt 1A slightly more midges were caught with the 5mg loading than the 10mg loading. In Expt 1B fewer males were caught with the 5mg loading but prediction of midge flight activity was not compromised. This means that cost could be reduced by using half the amount of material without affecting trap performance

**Fig. 6. Expt 1A 2003: pheromone dose in polyethylene vial**

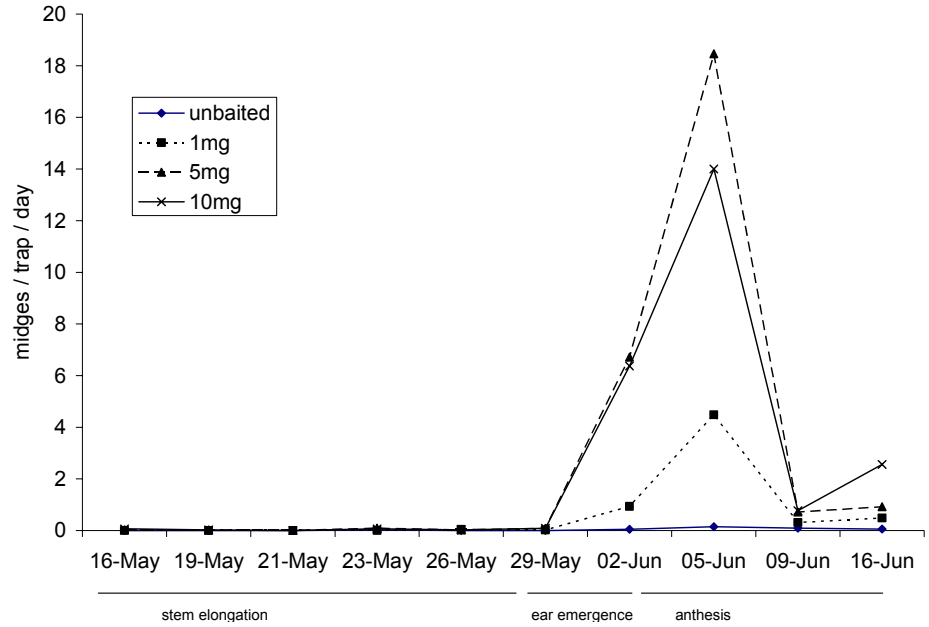
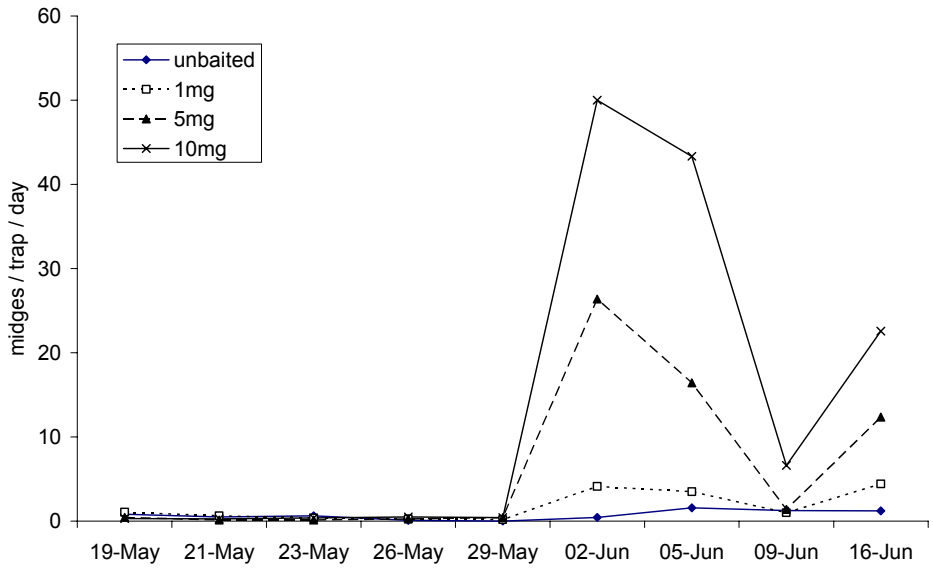



**Fig. 7. Expt 1B 2003: pheromone dose in polyethylene vial**




Experiments 7A and 7B (Fig 8 & 9) showed that low purity pheromone (material without the final purification step) had reduced activity: catches were similar to those with vials with 100 times less pheromone and prediction of midge flight was less accurate. Thus, it would seem that the final purification step is economically justifiable as the activity of the material is substantially enhanced by it.

**Fig 8. Expt 7A 2003: pheromone dose and purity**

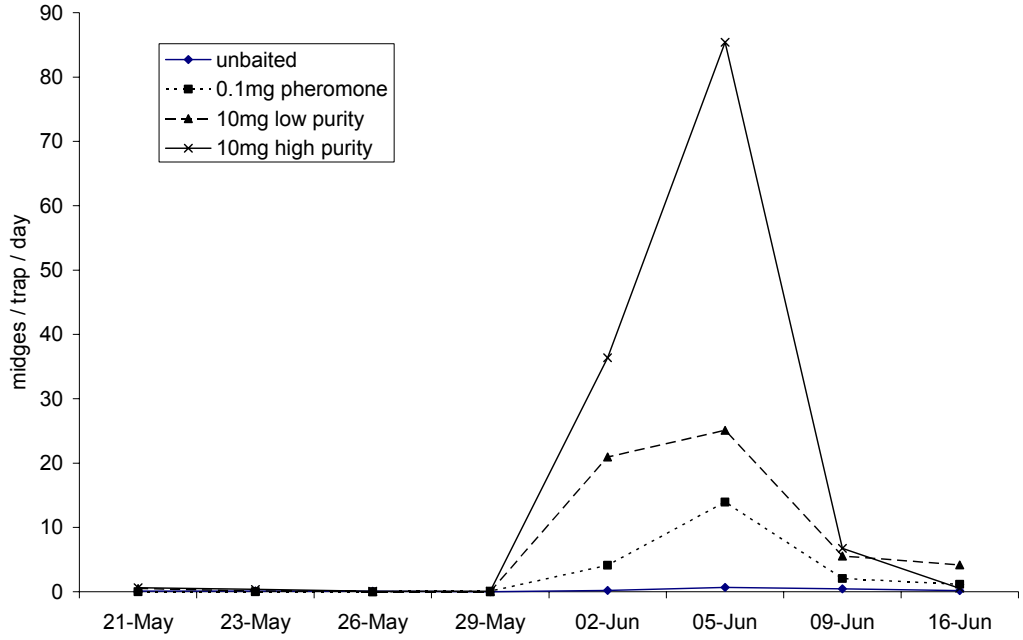
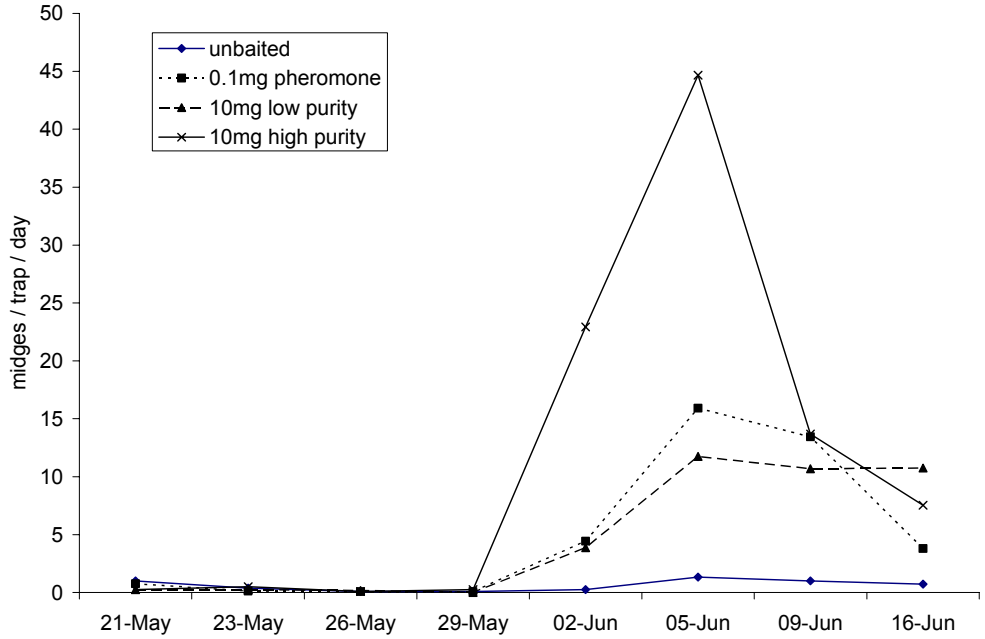



**Fig. 9. Expt 7B 2003: pheromone dose and purity**



### 3.1.3.3. 2004 Pheromone Trap Experiments

In 2004 following discussion with Agrisense BCS the more economically viable rubber septa formulations were evaluated. These trials were highly successful in demonstrating the efficacy of these formulations because similar numbers of midges were caught compared with the previous best formulation the 5mg loading polyethylene vial. (Fig 10). Indeed, a 1mg loading was as effective in predicting midge flight as a 5mg loading thus reducing cost of pheromone fivefold.

**Fig. 10. 2004 Experiment 2: *S. mosellana* pheromone trap catches – comparison of polyethylene vial and rubber septum formulations (Long Hoos, Rothamsted Farm)**

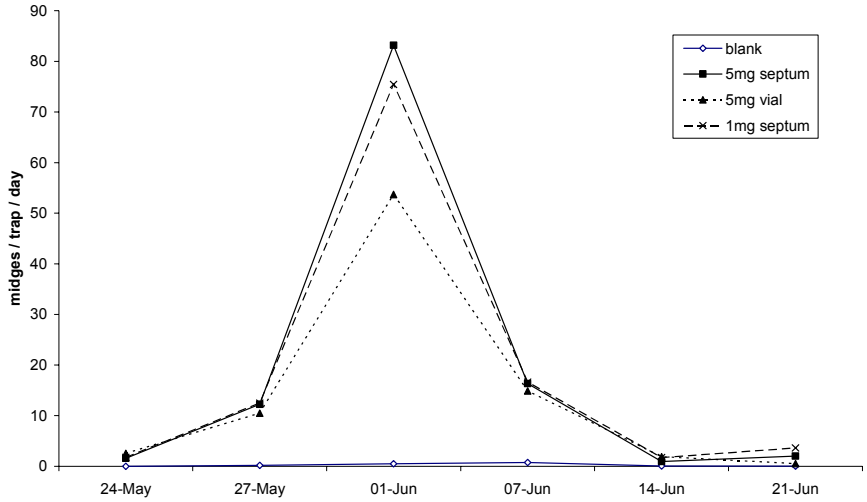
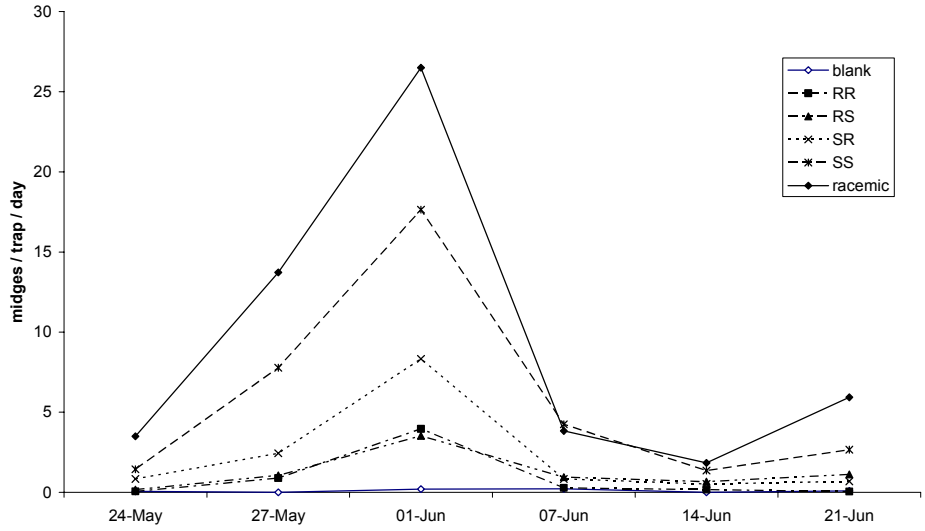


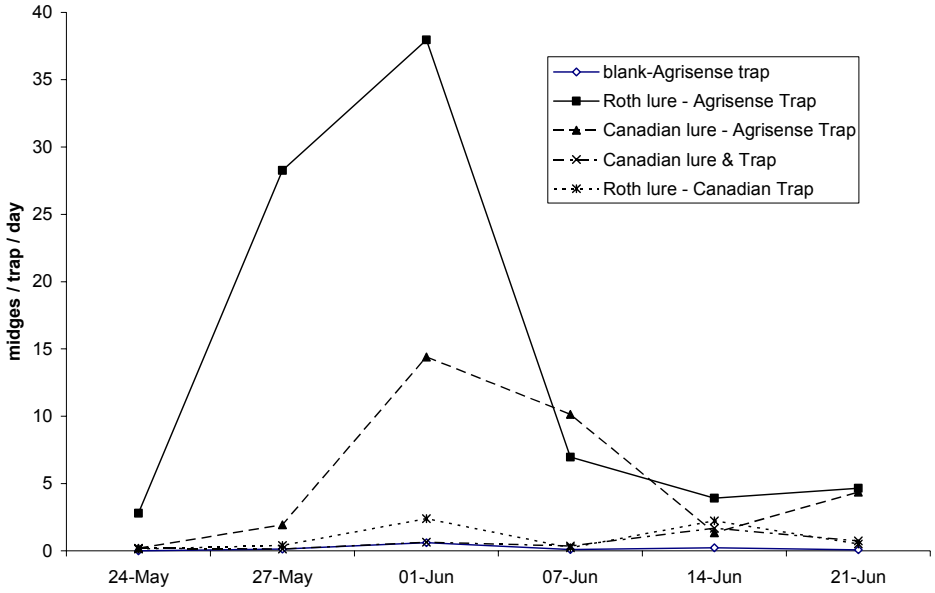

**Fig. 11 2004 Experiment 3: comparison of different enantiomers and racemic material (all with 5mg dose in polyethylene vial) (Gees Croft, Rothamsted Farm)**




Enantiomerically pure pheromone tested in Expt 3 did not provide any enhancement of trap catch (Fig. 15). The best pure enantiomer was the *SS*-stereoisomer of 2,7-nonanediyl dibutyrate. However, the racemic lure caught more male *S. mosellana* even than this suggesting that there may be a difference in pheromone

between Canadian and UK midge populations. Further studies to investigate this are planned. Comparison with Phero Tech lures and traps (Expt 6, Fig. 12) indicated that the Agrisense Delta trap was more than the Phero Tech trap. In addition, comparison of the 5mg loading polyethylene vial with the Phero Tech lure showed the latter to be less effective

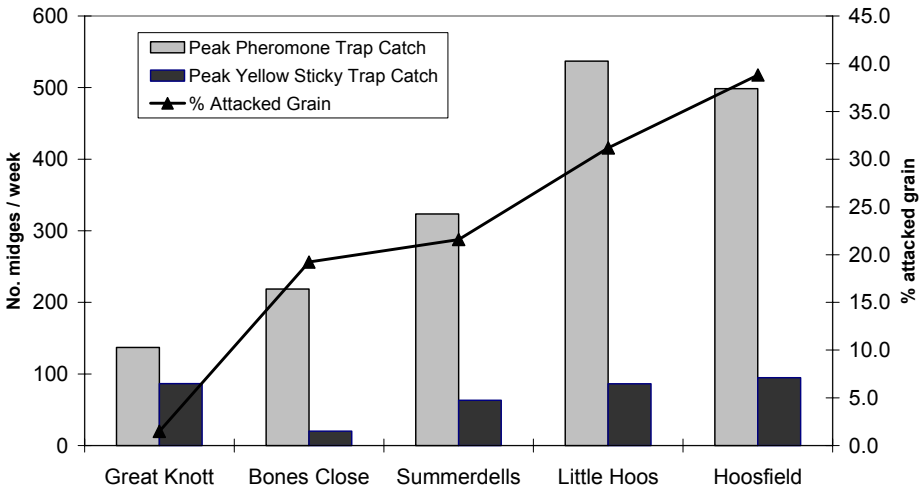
**Fig. 12. Experiment 6: comparison with Phero Tech traps and lures (Long Hoos, Rothamsted Farm)**




### 3.1.3.4. Comparision of pheromone trap catches, yellow sticky trap catches and infestation levels in adjacent wheat crops

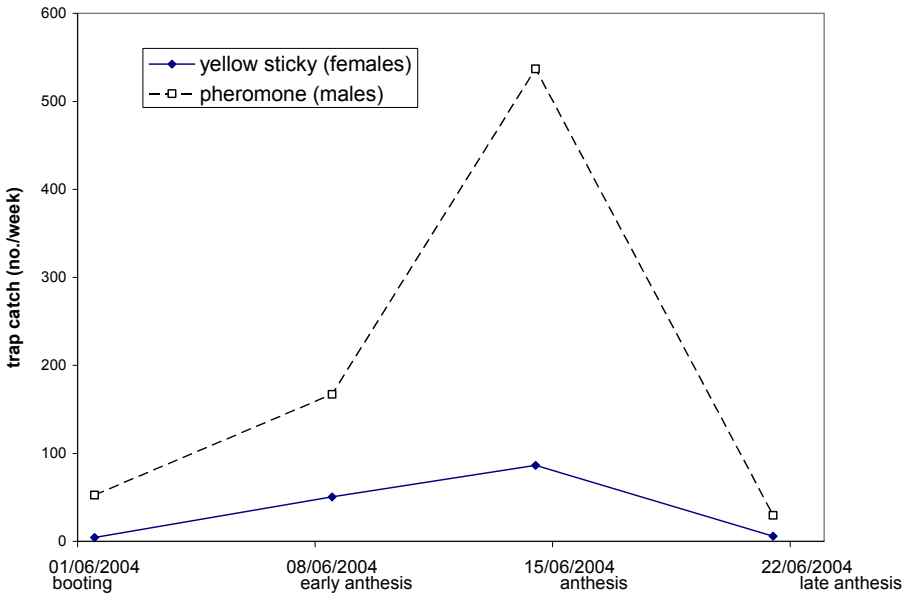
*S. mosellana* catches in pheromone traps with standard lures were compared with catches on yellow sticky traps at 2 fields in 2003 and 5 fields in 2004. *S. mosellana* larval infestation levels were also determined in adjacent wheat fields. In the more extensive 2004 trials (Fig. 13) there was a good correlation with pheromone trap catches and infestation levels with the exception of Great Knott which was sown with the resistant variety Robigus and thus had hardly any attacked grain in spite of midges having been caught in pheromone traps albeit at lower levels than at the other fields.

**Fig. 13. Summary of Expt 2 2004**



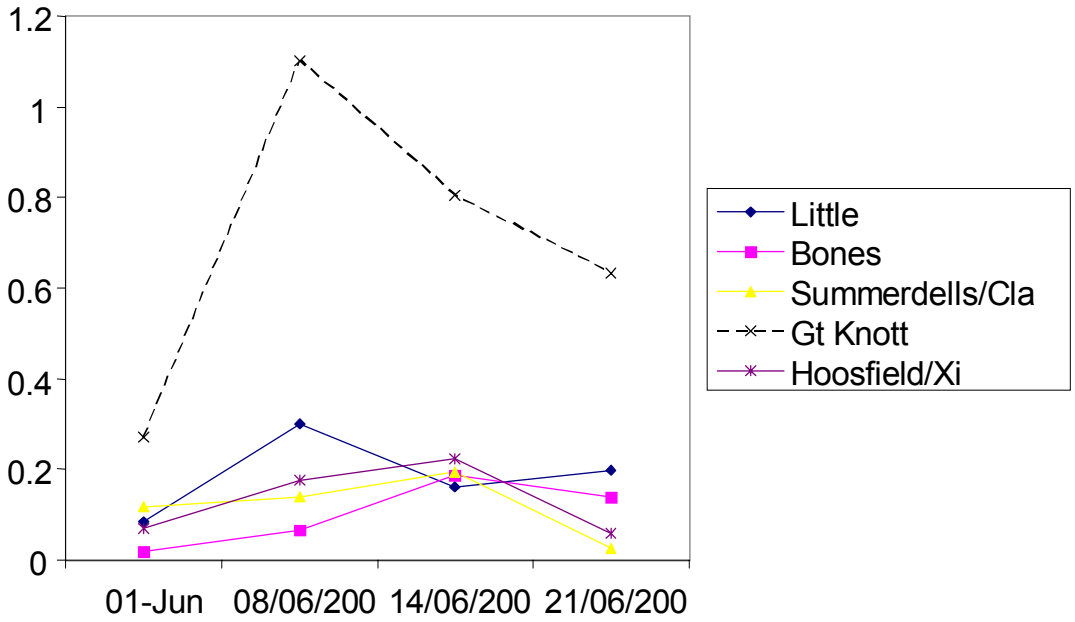

It should be noted that the yellow sticky traps were very unselective and caught many other non-target insects as well as *S. mosellana* making scoring of trap catches difficult whereas the pheromone traps caught at least five times more *S. mosellana* and few other insects (Fig 14).

**Fig. 14 Yellow sticky and pheromone trap catches at one site in Expt 2 2004**




The site at Great Knott field was atypical in that the ratio of females caught on yellow sticky traps to males caught on pheromone traps was higher than at the other sites (Fig. 15) suggesting immigration of females from emergence sites to this field.

**Fig. 15 Ratio of females on yellow sticky: males on pheromone trap**



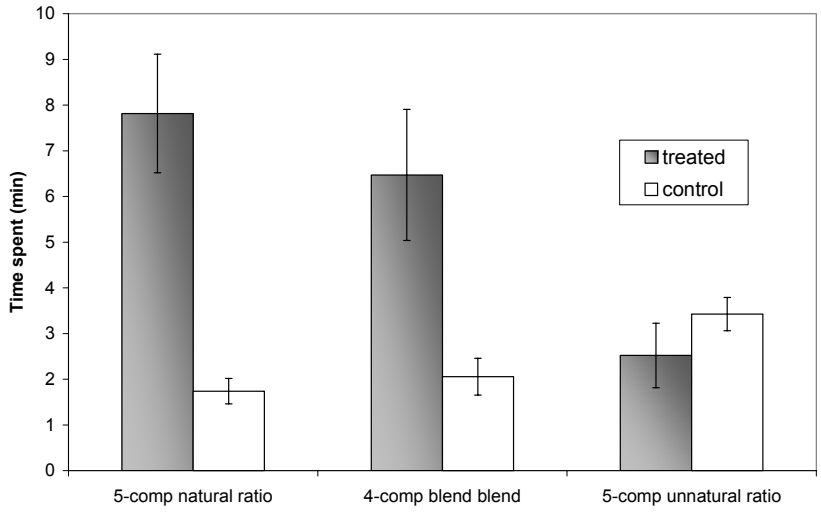

### 3.2 RESULTS : PART 2 – PLANT VOLATILES

Initial laboratory experiments were carried out in 2002 and are reported in Birkett *et al.* (2004). Air entrainment samples of volatiles from panicles of intact wheat, *Triticum aestivum*, cultivar 'Lynx' were collected at the ear emergence/early anthesis growth stage. In the olfactometer bioassay, both freshly cut panicles and an air entrainment sample were found to attract female orange wheat blossom midge adults. Coupled gas chromatography electroantennography (GC-EAG) analyses of panicle volatiles located six electrophysiologically active components. These were identified by coupled gas chromatography-mass spectrometry and co-injection with authentic standards, on polar and non-polar GC columns, as acetophenone, (*Z*)-3-hexenyl acetate, 3-carene, 2-tridecanone, 2-ethyl-1-hexanol and 1-octen-3-ol. Although none of these were active when presented individually at the levels present in the entrainment sample, acetophenone, (*Z*)-3-hexenyl acetate and 3-carene were active in the olfactometer when presented at a higher dose of 100ng on filter paper. However, the six-component blend and a blend of acetophenone, (*Z*)-3-hexenyl acetate and 3-carene, in the same ratio and concentration as in a natural sample was as attractive to female *S. mosellana* as the whole air entrainment sample.

In 2003 further analyses and bioassays were carried out to investigate electrophysiological and behavioural effects of volatiles from the susceptible wheat variety ECO22 (Advanta seeds). This variety was identified by plant breeders as particularly susceptible to *S. mosellana*. Active volatiles in air entrainment samples were located by GC-EAG and identified by GC-MS as α-pinene, 6-methyl-5-hepten-2-one, 3-carene, acetophenone and 2-dodecanone. The natural sample (ECO22-1) was significantly attractive in olfactometer studies ($P = 0.034$, $n = 8$). A five-component blend comprising α-pinene, 6-methyl-5-hepten-2-one, 3-carene, acetophenone and 2-dodecanone in the same ratio as the natural sample (ECO22-1) was

attractive (Fig. 16). In addition, a four-component blend, in which 6-methyl-5-hepten-2-one was not included, was also attractive. However, when 6-methyl-5-hepten-2-one was included at a slightly elevated level (15ng instead of 5ng on filter paper) all activity was lost. The difference was only small - 15ng/µl 6-methyl-5-hepten-2-one was used instead of 5ng/µl - but this was enough to completely switch off attraction. This finding highlighted the importance of the correct ratios in eliciting attraction of female midges and probably contributed to the lack of efficacy of plant volatile traps in the field. Although field formulations were developed that provided stable release under laboratory conditions they were not stable in the field as evidenced by recovery and analysis of lures from the field.

**Fig. 16. Olfactometer Bioassay of female *S. mosellana* response to different blends of ECO22 volatiles**



## 4. CONCLUSIONS

- The project has made considerable progress towards the development of a monitoring system for male *S. mosellana* and identified the optimum pheromone release rate and methodology for use in pheromone traps in the UK.
- The pheromone, 2,7-nonanediyl dibutyrate, (Gries *et al.*, 2000) performed well for indicating the onset of *S. mosellana* flight activity in the vicinity of wheat fields.
- It appears that there is a relationship between numbers of midges caught in the traps and the likelihood of crop damage even though the pheromone traps catch male rather than female insects.
- The threshold level at which an insecticide application to protect the crop is warranted is probably around the level of 20 midges per trap per day but further work on threshold levels for pheromone traps i.e. the numbers of insects that indicate economically damaging levels is necessary.
- It is likely that the threshold will vary according to the level of susceptibility of the wheat cultivar being grown and the market for which it is intended. There would be a lower threshold for bread-making wheat than feed wheat.
- There are possibly situations in which immigration of female *S. mosellana* would mean that pheromone traps underestimate the threat to the crop and this aspect requires future investigation.
- Wheat volatiles attractive to female *S. mosellana* were identified as acetophenone, (*Z*)-3-hexenyl acetate, 3-carene, 2-tridecanone, 2-ethyl-1-hexanol and 1-octen-3-ol from wheat cultivar 'Lynx' and α-pinene, 6-methyl-5-hepten-2-one, and 2-dodecanone from wheat cultivar ECO22. 3-Carene and acetophenone were identified as active components of both varieties.
- These showed a level of attraction similar to the pheromone in laboratory olfactometers.
- Ratio of plant volatiles was crucial because use of an incorrect ratio made the mixture unattractive.
- Development of a field stable formulation of plant volatiles maintaining ratio integrity throughout the season has proved difficult.

## 5. REFERENCES


Birkett, M. A., Bruce, T. J A., Martin, J. A., Smart, L. E., Oakley, J., Wadhams, L. J. (2004). Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. *Journal of Chemical Ecology*, **30**, 1319-1328.

Gries, R., Gries, G., Khaskin, G., King, S., Olfert, O., Kaminski, L-A., Lamb, R. and Bennett, R. (2000) Sex pheromone of orange wheat blossom midge, *Sitodiplosis mosellana*. *Naturwissenschaften*, **87**: 450-454

Lamb RJ, Wise IL, Olfert OO, Gavloski J, Barker PS (1999) Distribution and seasonal abundance of Sitodiplosis mosellana (Diptera : Cecidomyiidae) in spring wheat. Canadian Entomologist **131**:387-397.

Lamb RJ, Tucker JR, Wise IL, Smith MAH (2000) Trophic interaction between Sitodiplosis mosellana (Diptera : Cecidomyiidae) and spring wheat: Implications for yield and seed quality. Canadian Entomologist **132**:607-625.

Lamb RL, Wise IL, Gavloski JE, Kaminski LA, Olfert OO (2002) Making control decisions for *Sitodiplosis mosellana* (Diptera : Cecidomyiidae) in wheat (Gramineae) using sticky traps. Canadian Entomologist **134**:851-854.

Maddrell, S.H.P. (1969). *J. Exp. Biol.* 51: 71-97.

Oakley, J.N. 1994. Orange Wheat Blossom Midge: A Literature Review and Survey of the 1993 Outbreak. HGCA Research Review No. 28.

Oakley JN, Cumbleton PC, Corbett SJ, Saunders P, Green DI, Young JEB, Rodgers R (1998) Prediction of orange wheat blossom midge activity and risk of damage. Crop Protection **17**:145-149.

Petterson, J. (1970) An aphid sex attractant. 1. Biological studies. *Entomol. Scand.* 1: 63-73

Pivnick, K.A. & Labbe, E (1993) Daily patterns of activity of the Orange Wheat Blossom Midge, Sitodiplosis-Mosellana (Gehin) (Diptera, Cecidomyiidae). *Canadian Entomologist* **125**: 725-736

Smith, M.A.H., Wise, I.L, Lamb, R.J. (2004) Sex ratios of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae): implications for pest management in wheat (Poaceae). *Bull. Entomol. Res.* **94**: 569-575

Wadhams, L.J. (1990). The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones, pp. 289-298, *in* A.R. McCaffery and I.D. Wilson (eds.). Chromatography and Isolation of Insect Hormones and Pheromones. Plenum Press, New York and London.