
**PROJECT REPORT No. 284**

# A RAPID METHOD FOR DETECTING THE PREDOMINANT STORAGE MITE PEST SPECIES, *ACARUS SIRO*, IN THE PRESENCE OF GRAIN

JULY 2002

Price £4.00

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# A RAPID METHOD FOR DETECTING THE PREDOMINANT STORAGE MITE PEST SPECIES, *ACARUS SIRO*, IN THE PRESENCE OF GRAIN

by

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This is the final report of a two year project which started in April 2000. The work was funded by a grant of £88,887 from the HGCA (Project No. 2183).

The Home-Grown Cereals Authority (HGCA) has provided funding for this project but has not conducted the research or written this report. While the authors have worked on the best information available to them, neither HGCA nor the authors shall in any event be liable for any loss, damage or injury howsoever suffered directly or indirectly in relation to the report or the research on which it is based.

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**Contents**

**Abstract**

The aim of this study was to prove the feasibility of developing a rapid method for detecting storage mite infestations in cereals and their derived products. This was achieved by careful raising of specific antibodies to a model species, the flour mite, *Acarus siro* (L.), for detection by immuno-diagnostics. The specificity of the assay was assessed against 13 species of mite, 5 species of insect pest and 5 species of fungi. Its sensitivity was determined by studying the reaction to different numbers of mites in the presence and absence of grain, and evaluated by comparison with a standard reference method, the flotation test.

The results demonstrate that the assay:-

- was specific to *A. siro* and showed no cross-reactivity to wheat;
- detected both dead and live adult mites of *A. siro* and its faeces;
- detected all life stages of *A. siro* and the different strains of that species tested;
- detected and quantified numbers of *A. siro* with and without wheat, ranging between 0 – 1000 mites;
- gave results which correlated well with those of the flotation test (linear regression, $r^2 = 0.9091$).

The work reported here has proven the potential of a laboratory-based immunoassay as a method for the rapid and sensitive detection of mites in cereal grain. We now propose to develop this method further by the production of lateral flow devices (LFDs) which can be used in the field with minimal training and have been validated for commercial use with, for example, plant viruses, bacteria, fungi and mycotoxins. We also plan to extend the applicability of the field kit to a wider range of mite species. It is envisaged that the LFD could be used throughout the food supply chain to establish the degree of contamination by mites.

**Summary**

Storage mites are a pest problem throughout the UK grain industry. They damage stored grain and cause occupational health problems to farmers and workers in the grain and milling industry. Storage mites are strongly allergenic and in recent years there have been several reported cases of anaphylaxis and anaphylactoid reactions due to ingestion of mites from mite-contaminated food.

Not only is the occurrence of storage mites in various premises in the UK widespread, but the short developmental cycle at optimum conditions, favours rapid population growth and increases the risk of the development of pesticide resistance. The latter applies even to pirimiphos-methyl, the most commonly used insecticide in UK stores for fabric treatments and admixture to grain. With inadequate control strategies, contamination due to storage mites and their products may increase in cereal stores and progress throughout the food supply chain.

To counter this mite problem a method is needed by which mites can be detected at an early stage to help prevent the build up of populations. Immuno-diagnostics have the potential to meet just such requirements as they are reliable, rapid, relatively cheap, user-friendly and can be developed into a kit form for use in the field.

In a previous HGCA project we started investigating the potential of immuno-diagnostics with the only available storage mite antibody. We showed that it could be used to detect the cosmopolitan food mite, *Lepidoglyphus destructor* (Schrank), but not in the presence of grain. Therefore the purpose of this study was to build on the experience gained in that project and to raise antibodies that were specific to a model species of mite, namely the flour mite, *Acarus siro* (L.) but did not cross-react to wheat.

This was successfully achieved by (a) developing a new method for antigen extraction, and (b) careful raising and screening of antibodies. The resultant working assay was shown to satisfy all the necessary criteria. It was sensitive to *A. siro* even in the presence of wheat. It was selective to *A. siro* with no cross-reactivity to other storage mites, not even to species such as *Acarus farris* or *Acarus immobilis* which belong to the same genera. Neither was there any response with any of the five storage insect pest species tested or to the five species of storage fungi. In addition, the assay did not differentiate between different strains of *A. siro* e.g. field and laboratory strains with differing degrees of pesticide resistance and susceptibility. All life stages of *A. siro*, were also detected as were dead adult mites and mite faeces but there was no response to mite food (yeast and flour). These results suggest that the assay would be useful for the detection of contamination by mites or their products.

To test the feasibility of further development of the assay, known numbers of mites were seeded in different quantities of unmilled wheat. The antigen was extracted using a novel rinsing method which avoided the use

of homogenisation and its associated problems such as releasing proteins from the wheat itself. These tests showed that the assay could successfully quantify numbers of mites, ranging between 0-1000, in 0g, 10g, 25g and 50g of grain. To validate the immuno-assay, a replicate set of samples was analysed using a standardised flotation test for grain. The results from these two methods were comparable (linear regression of $r^2 = 0.9091$).

The work reported here has proven the potential of an immuno-diagnostic method as a tool for the rapid and sensitive detection of mites in grain samples and is a significant advance on previous immunoassays which have focussed on detecting mites in dust only.

Given these promising results, we now propose to develop this method further by the production of lateral flow devices which are user friendly, suitable as field kits, and have been fully validated by users in different premises. We also plan to extend the applicability of the kit to a wider range of mite species. We will also construct a model for the distribution of mites in grain bulks in order to guide the use of the kits and to interpret their results. It is envisaged that the kit could be used throughout the food supply chain to establish the degree of contamination by mites.

## INTRODUCTION

Storage mites are a particular quality problem of UK grain, oilseeds and their derivatives. For example, infestations taint the grain making it unpalatable to livestock (Wilkin and Thind, 1984), reduce its suitability for milling (Wilkin and Stables, 1985; Wilkin and Thind, 1984) and can cause occupational health problems to workers in the grain and milling industry e.g. farmer's lung, rhinitis, asthma, dermatitis, etc. (Cuthbert *et al*., 1979; van Hage-Hamsten *et al*., 1985; Stengard Hansen *et al*., 1996). Mites have also been implicated in the transmission of fungi, bacteria and prions (Steinbrink and Boer, 1984; Wisniewski *et al*., 1996). In response to this serious situation, efforts are being made to develop and implement more effective and environmentally sensitive, integrated pest management (IPM) systems.

IPM systems need efficient mite detection techniques to provide early warning of their presence and to monitor their numbers. Such techniques can be used to target control action to problem areas thereby reducing costs, damage, and pesticide usage. Current methods for the detection of storage mites in cereals and associated products include flotation (Thind, 2000) and use of a purpose designed mite trap (Thind, 1997). However, both of these require laboratory-based analysis by experienced workers. Consequently, monitoring for mites in stored commodities is not undertaken with sufficient thoroughness and may be contributing to the demonstrated contamination by mites of cereal-based food products in retail outlets (Anon 1996; Thind and Clarke, 2001). What is needed is a simple method which can be used by cereal workers with minimal training to provide rapid and reliable assessments of mite populations on site.

Such a method could be based on the use of immuno-diagnostic kits using antibodies raised against specific mite species. Since such kits are easy to use, rapid and low cost (approximately £2-4 per kit) they would enable a larger number of samples to be analysed than previous methods. This would provide greater confidence that the samples taken are representative of a bulk. In a previous study we started investigating the potential of immuno-diagnostics with the only existing antibody raised to storage mites. We showed that it could be used to detect the cosmopolitan food mite, *Lepidoglyphus destructor* (Schrank), but not in the presence of grain (Chambers *et al.,* 1999a, 1999b). The study reported here overcame this problem by careful raising and screening for antibodies that were specific to a model species of mite, namely, the flour mite, *Acarus siro* (L.), and the development of a laboratory based Enzyme Linked Immunoassay (ELISA) (Anon., 2001). The results show that immuno-diagnostics have the potential to detect and quantify mites in the presence of grain. The success of this project means that the methods and antibodies can be developed into a kit suitable for use in the field.

## MATERIALS AND METHODS

### Mites

The mite species used in this project were: *Acarus siro* strain 9258 (pesticide resistant laboratory strain), strain 9266/1 (pesticide susceptible laboratory strain) and strain D6 (pesticide resistant field strain); *A. farris* (Oudemans), A17; *A. immobilis* (Griffiths); *Tyrophagus putresentiae* (Schrank), T13; *T. longior* (Gervais), T96; *T. palmarum* (Oudemans) T32; *Lepidoglyphus destructor* (Schrank), G6; *Glycyphagus domesticus* (DeGeer), GAG; *Aleuroglyphus ovatus* (Troupeau), AL2; *Caloglyphus berlesei* (Michael), C3; *Cheyletus eruditus* (Schrank); *C. malaccensis* (Oudemans); *Ctenoglyphus plumiger* (Koch).

Cultures of mite strains, except for *C. eruditus*, *C. malaccensis* and *C. plumiger*, were reared in the dark at 20°C and 80% RH in 600 ml cell growth flasks (115mm long × 165mm wide × 50mm high), on a finely ground, sterilized and conditioned mite diet consisting of flour and dried yeast (1:3 w/w). The predatory mites, *C. eruditus* and *C. malaccensis,* were bred at 25°C and 75% RH on a mixture of storage mite species (*A. siro*, *T. putrescentiae*, and *T. longior*). *C. plumiger* was bred at 20°C and 80% RH on a diet of mouldy yeast and flour, plus tropical fish flakes (Aquarian).

### Insects

Insect species tested were: *Sitophilus granarius* (L.), Windsor*; Ahasverus advena* (Waltl), susc.*; Oryzaephilus surinamensis* (L.), susc.*; Cryptolestes ferrugineus* (Stephens) C124*; Liposcelis bostrychopila* (Badonnel).

Insect species were reared in the dark at 25°C and 70% RH in 0.75 litre Kilner jars except for *C. ferrugineus* which was bred at 30°C and 70% RH. *A. advena, C. ferrugineus* and *O. surinamensis* were fed on a diet of wheatfeed, rolled oats and yeast (5:5:1 w/w). *S. granarius* was fed on wheat only and *L. bostrychopila* on skimmed milk powder, wheatfeed, yeast and wholemeal flour (1:1:1:1 w/w).

### Fungi

The fungal species tested were: *Penicillium verrucosum* (Dierckx)*; Alternaria alternata* ((Fr.) Keissl.); *Aspergillus ochraceus* (G. Wilh.); *Eurotium amstelodami* ((Talice & J.A. Mackinnon) Kozak.); *Cladosporium cladosporioides*

All fungal species were cultured at 25°C on czapek yeast agar in the dark.

**Antigen extraction**

Prior to any antigen extraction process, the mites were washed with phosphate buffered saline (PBS) to remove any adhering particles of food, etc. Three different methods were then investigated to extract the antigen from the mites. It should be noted that all mites and insects were alive at the start of the antigen extraction process unless otherwise stated.

The first method involved homogenising whole mites with plastic homogenisers (TreffLab) in 100µl of PBS for 30 seconds. The second method involved soaking the test organisms in 0.15M sodium chloride overnight at 33°C. The third method used molecular weight sieves (Centrikon) that retained the 15Kda allergen that has been reported to be the major allergenic component of *A.siro* (Johansson *et al.*, 1994) and centrifugation at 30000rpm at 25°C for 5 minutes. However this third method was disregarded as it was impractical to obtain a sufficient quantity of protein for immunisation purposes.

Protein analysis of the antigen extracted from the two chosen methods and from different strains of *A. siro* was undertaken according to standard electrophoretic procedures (Pharmacia). The protein concentration of the extracts were determined (Biorad). Solutions were diluted to the appropriate protein concentration in PBS and used as antigen in ELISA's.

Antigen was extracted from wheat samples (0g, 10g, 25g, or 50g) by soaking the samples with 0.15M sodium chloride (1ml/g of grain) in plastic containers for 1 minute, and then shaking them with five stainless steel ball bearings (diameter 5mm) for 1 minute.

**Antibodies**

The monoclonal (Mab) and polyclonal antibodies (Pab) were raised according to standard procedures (Galfre and Milstein, 1981; Harlow and Lane, 1988; Banks *et al*., 1994).

**Initial Antibody Screening**

For initial screening of supernatants from fusions, 96-well microtitre plates (Nunc Immunoplate, maxi-sorp) were coated overnight with 100µl per well of 5µg/ml antigen in coating buffer. Supernatants were screened by an indirect, plate trapped antigen (PTA) ELISA, using rabbit anti-mouse IgG labelled alkaline phosphatase (Sigma) as the second antibody. An optical density of at least 3:1 in the recognition of *A. siro* was chosen as a discriminatory threshold for potential diagnostic antibodies. Cell lines were cloned twice by limiting dilution.

End-users of *A. siro* immunoassays will require kits which are capable of working in the presence of grain and other storage contaminants. For this reason, the screening of Mabs produced for this project included a screen for Mabs which recognised *A. siro* but no other storage pest or wheat.

The Mabs selected from this screen were then further selected for their compatibility with the Pabs, i.e. we required two antibodies that did not recognise the same antigenic epitope.

**Purification of Antibodies**

Once the chosen cell lines had produced a sufficient volume of tissue culture supernatant (*ca.* 500mls) the antibodies were isotyped (Immune Systems) and then purified according to standard methods using 5ml HiTrap Protein G affinity columns (Pharmacia, Biotech).

**Enzyme-Linked Immunosorbent Assay (ELISA)**

Further investigations of Mabs and antisera were undertaken with an optimised triple antibody sandwich (TAS) ELISA. For this, microtitre plates (Nunc Immunoplate, maxi-sorp) were coated with 100µl purified Pab [1:2000] diluted in coating buffer and left to incubate at 4°C overnight. After washing with PBST (phosphate buffered saline plus 0.05% v/v Tween 20, Sigma), the wells were incubated with 250µl of blocking buffer (PBST plus 5% Marvel milk powder) for 1hour at 33°C. After further washing, the wells were incubated with 100µl of antigen homogenate or grain extract for 1 hour at 33°C. The plates were then washed again followed by incubation with 100µl of purified Mab [1:200] diluted in PBST, for 1 hour at 33°C. Then, after washing, the wells were incubated with 100µl of second antibody conjugate (alkaline phosphatase conjugated rabbit anti-mouse IgG, Sigma) [1:4000] diluted in PBST, and the plates left to incubate for 1hour at 33°C. Finally after three more rinses with PBST, 100µl of alkaline phosphatase substrate (KPL) was added to each well and left to incubate at room temperature for 45-60 minutes. The intensity of the colour reaction was measured photometrically at 405nm using a microtitre plate reader (Molecular Devices plus SOFTmax software).

**Reactivity with other species of mite, wheat, insects, predatory mites and fungi**

To determine the cross-reactivity of the potential diagnostic antibodies with other potential contaminants, two-fold serial dilutions were made of the respective antigen. These were then probed with the antibodies using the protocol described above.

**Reactivity with dead and live adult mites, different life stages of *A. siro* and their faeces**

Adult *A. siro* mites were killed by heat, 0.12% pirimiphos-methyl (organophosphate pesticide) or by 2 formulations of decimite (silicate-oxide based pesticide). The different life stages were separated by sieving the mite cultures through 500, 185, 150 and 75µm aperture sieves. Further sieving with a 25µm aperture

sieve, separated out faecal pellets along with 10-15% spent food. The antigen was then extracted using the rinsing method and tested in an ELISA as described above.

**Reactivity with laboratory infested and uninfested grain samples**

Samples (n=10) were made up in 10 g of wheat each with between 0 and 1000 adult *A. siro*. Each sample was compared with the same number of mites in the absence of wheat and with a sample of 10g of wheat to which no mites had been added. The antigen was extracted using the rinsing method and tested in an ELISA.

**Evaluation of assay by comparison with flotation test**

Two sets of 25g grain samples were carefully seeded with equal numbers of adult mites (0, 10, 20, 40, 60, 80, 100, 150 and 250 mites) using a light microscope and single haired paint brush. One set was analysed using the ELISA method and the other set by a standardised flotation test (Thind, 2000) with a minor modification to the pre-extraction stage. This modification entailed subjecting the wheat grain, when suspended in the aqueous phase, to ultrasonication for ten minutes in an ultrasonic bath. This procedure was carried out to increase the recovery of mites from grain and to speed up the extraction process. Previous experiments (unpublished) have demonstrated that this additional step facilitates the extraction of mites from within grain kernels.

For the ELISA a standard curve was constructed from a different set of samples seeded with known numbers of mites. This standard curve was then used to extrapolate the number of *A. siro* mites in the grain samples analysed using the ELISA method. The results from the flotation test and the ELISA were then correlated using linear regression analysis.

## RESULTS

### Antigen extraction

The antigen, extracted by homogenisation or the rinsing method, was evaluated by electrophoresis. The results (Appendix A) show that both methods released good quality antigen, *i.e*. bands specific to *A. siro* only. Therefore three mice and two rabbits were immunised with NaCl extracted antigen and another three mice were immunised with antigen extracted by homogenisation.

### Antibody and Antigen Screening

Of the 1000 hybridoma lines produced, 24 reacted positively in ELISA to *A. siro*. These cell lines were allowed to grow until a sufficient quantity of antibodies had been produced and then screened again against a wider range of mite species. From this second screen six cell lines were selected for specificity to *A. siro* and no cross-reactivity to wheat and the remaining eighteen frozen. A third screen for antibody compatibility narrowed the selection to one cell line, namely Mab 173, which had been raised using antigen from the rinsing method.

Mab 173 showed essentially no cross-reactivity with other storage mites (Table 1). The Pab did, however, cross react slightly with *A. farris* (Table 1) but as the Mab was so specific to *A. siro* this was considered inconsequential. The important fact was that the Pab did not cross-react with wheat (Table 1) as this antibody was designated to be the trapping antibody and the Mab the detecting antibody.

Table 1. Cross-reactivity of selected Mab and Pab using tissue culture supernatant (TCS)

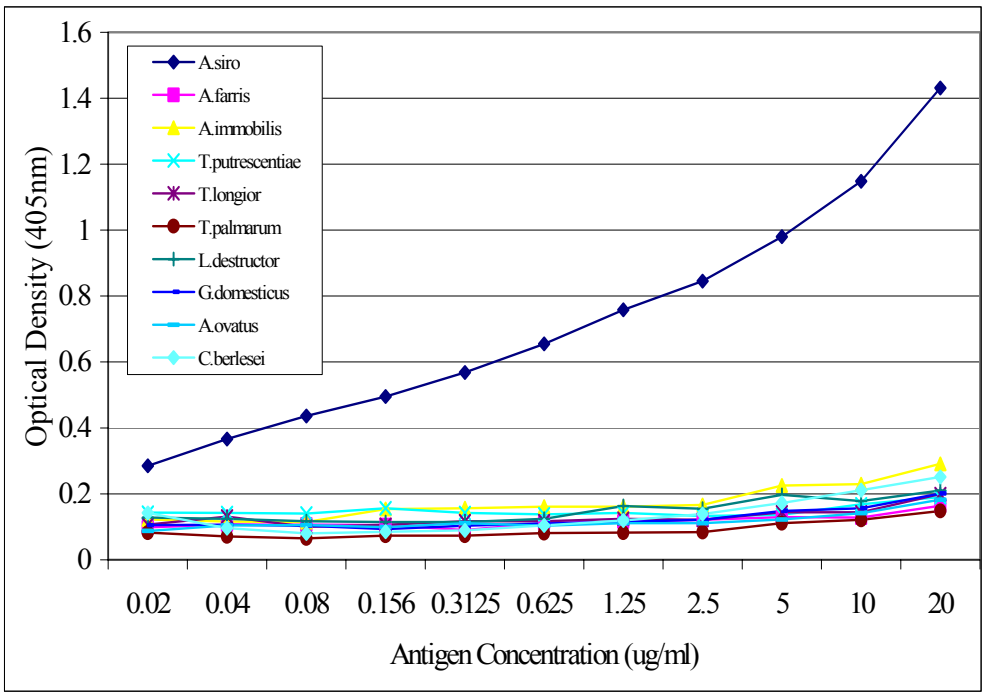
| **Mite Species** | **Optical Density value at 405nm with Mab** | **Optical Density value at 405nm with Pab** |
|---|---|---|
| *A.siro* 9258/2 | 1.990 | 1.119 |
| *A.siro* D6 | 2.054 | 1.058 |
| *A.farris* | 0.446 | 0.739 |
| *T.putrescentiae* | 0.199 | 0.233 |
| *T.longior* | 0.217 | 0.431 |
| *T.palmarum* | 0.227 | 0.480 |
| *L.destructor* | 0.197 | 0.341 |
| *G.domesticus* | 0.285 | 0.332 |
| *A.ovatus* | 0.206 | 0.397 |
| *C.berlesei* | 0.197 | 0.306 |
| Yeast and Flour | 0.096 | 0.173 |
| Wheat | 0.108 | 0.092 |

### Reactivity with other species of mites

Once the antibodies were characterised and purified and the assay optimised to work with the chosen antibodies, tests were carried out for specificity. Tests were conducted with adults of the following species of storage mite: *A. siro*; *A. farris*; *A. immobilis*; *T. longior*; *T. putrescentiae*; *T. palmarum*; *L. destructor*; *G. domesticus*; *A. ovatus*; *C. berlesei*.

The antigen was serially diluted to confirm that cross-reactivity was not a problem at lower or higher concentrations. The results (Figure 1) clearly show that the assay is very specific to *A. siro*

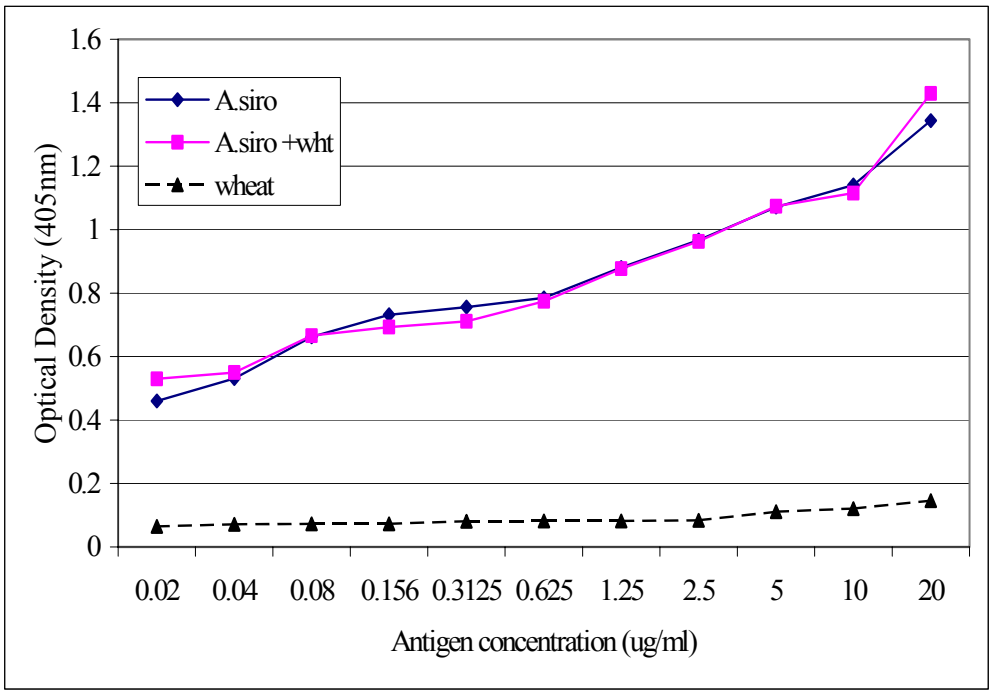
Figure 1. Specificity for *A. siro* versus nine other species of storage mite



**Reactivity with wheat**

It was crucial that the purified antibodies did not cross-react with wheat when analysing mite infested grain samples. Therefore tests were undertaken to confirm that the selected antibodies were suitable. Figure 2 shows that the antibodies selected did fulfil the criteria and that quantitative detection of *A. siro* was unaffected by the presence of wheat.

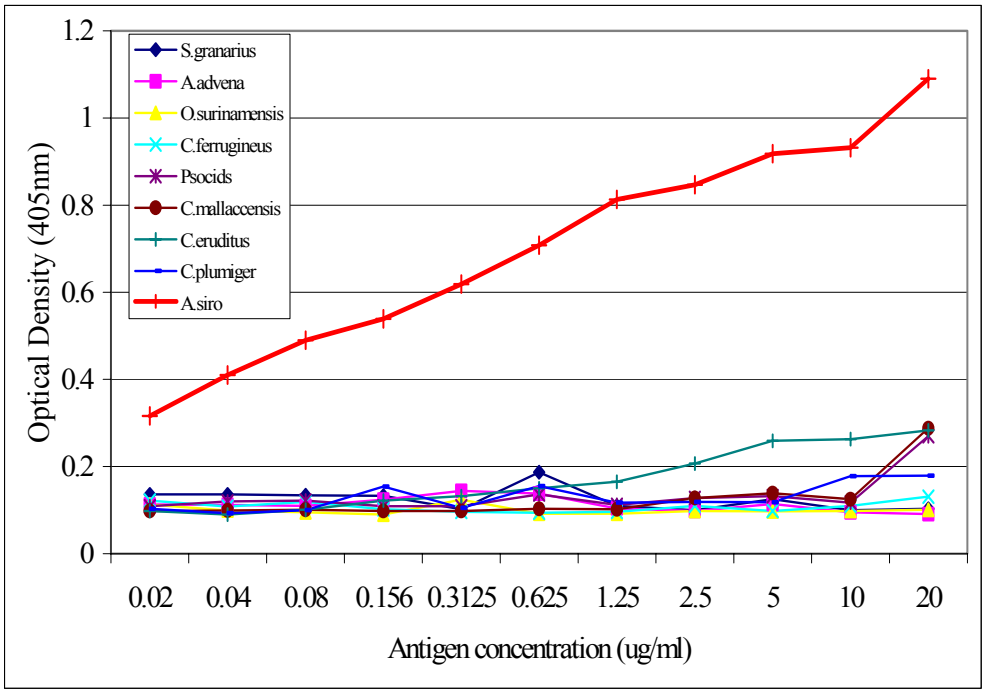
Figure 2. Quantitative detection of *A. siro* antigen with and without the presence of wheat



**Reactivity with insects and predatory mites**

Reactivity was tested with adults of the following storage insects: *S. granarius; A. advena; O. surinamensis; C. ferrugineus* and *L. bostrychopila,* the following predatory mites: *C. eruditus* and *C. malaccensis,* and the following fungivorous mite: *C. plumiger.* Figure 3 shows that there was negligible reactivity with these species.

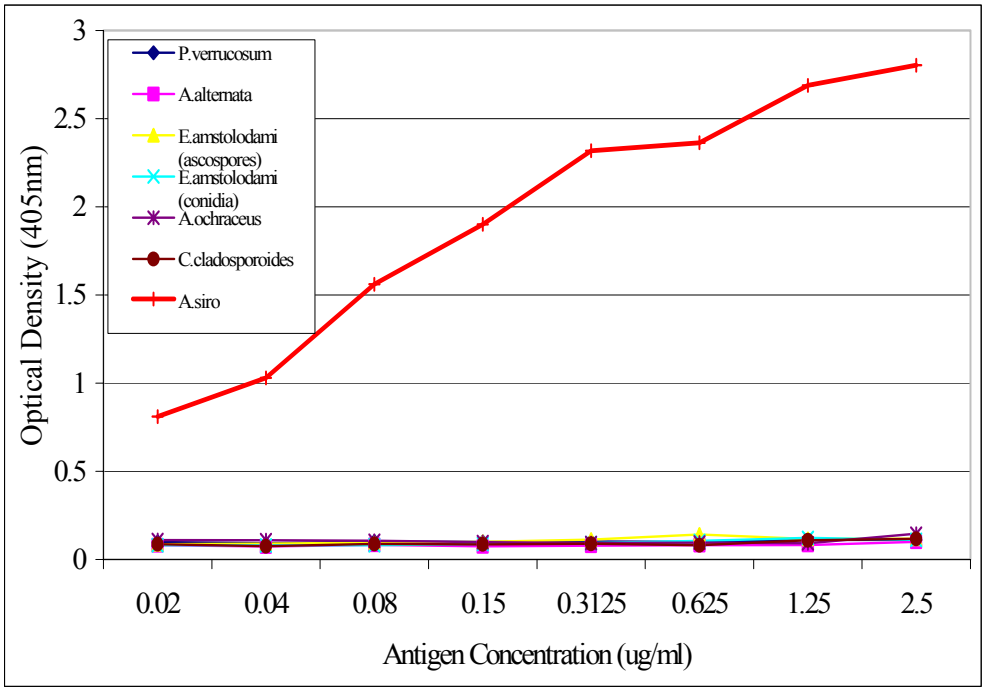
Figure 3. Specificity for *A. siro* versus 5 species of storage insect, 2 species of predatory mite, and 1 fungivorous mite



**Reactivity with fungi**

Reactivity was tested with the following species of fungi: *P. verrucosum*; *A. alternata; A. ochraceus; E. amstelodami* and *C. cladosporioides*. Figure 4 shows that there was no cross-reactivity with any of these species either.

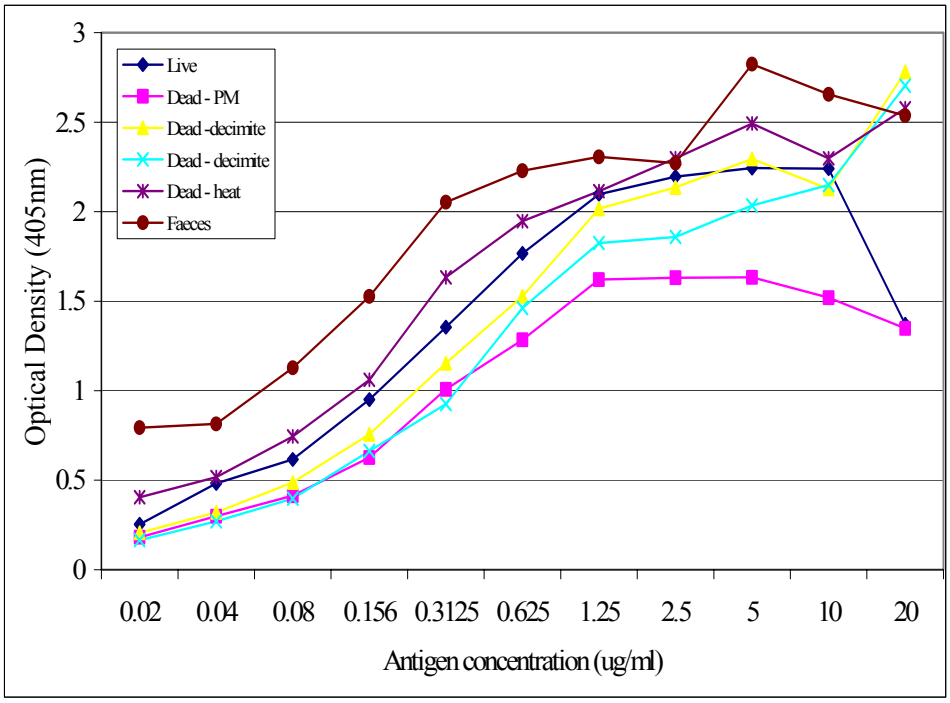
Figure 4. Specificity for *A. siro* versus five species of fungi




**Reactivity with dead and live adult mites, different life stages of *A. siro* and their faeces.**

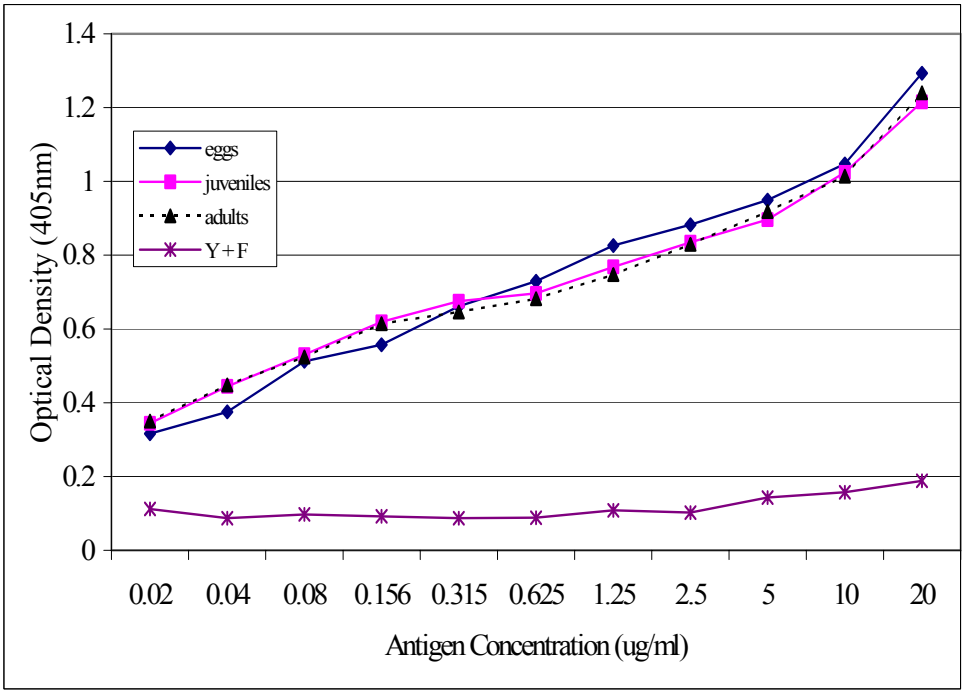
Figure 5 shows that the response with adult mites, killed either by heat or by different pesticides such as pirimiphos methyl (PM) or decimite was similar to that of live adult mites. The response with mite faeces was particularly strong.

Figure 5. Dead and live *A. siro* mites and their faeces




Tests were also undertaken to assess the reactivity of *A. siro* eggs, juveniles and adult mites. Yeast and flour mite food (Y + F) was also tested to act as a negative control. Figure 6 shows that there all three life stages responded but there was no reactivity with the yeast and flour (Y + F) mite food.

Figure 6. Different life stages of *A. siro* including faeces



**Reactivity with laboratory infested and uninfested grain samples**

To standardise the procedure 10g samples of wheat, seeded with and without *A. siro* were used. The results (Figure 7) show that the presence of wheat had no effect on the detection and quantification of *A. siro*. Further tests were then undertaken with larger quantities of grain (25g and 50g). Figure 8 shows that both the antigen extraction process and the assay were able to detect and quantify *A. siro* in these larger quantities without any difficulty.

Figure 7. 10g samples of wheat seeded with and without *A. siro*

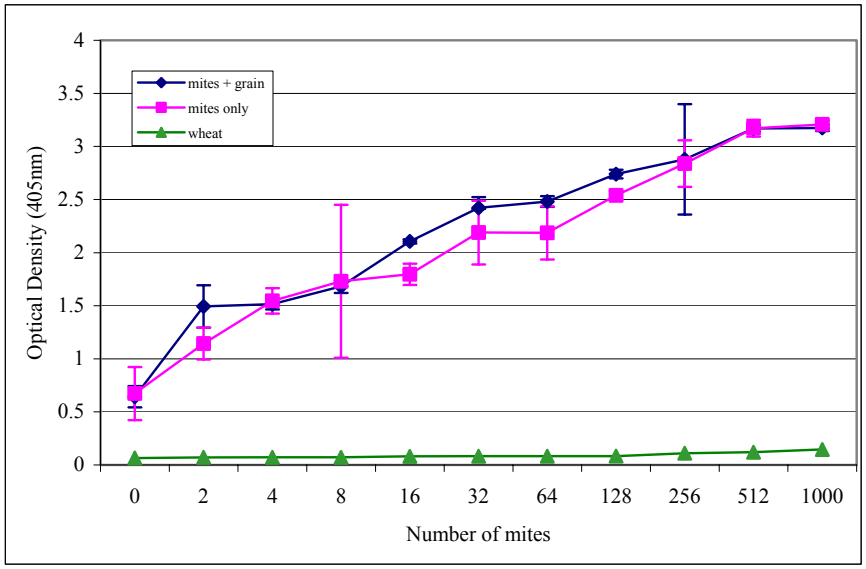
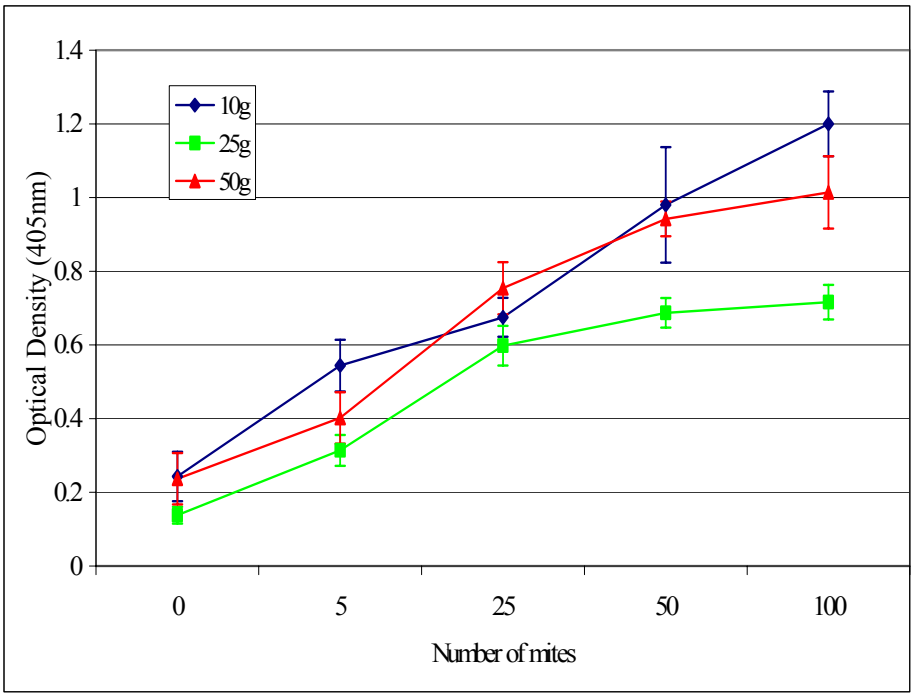


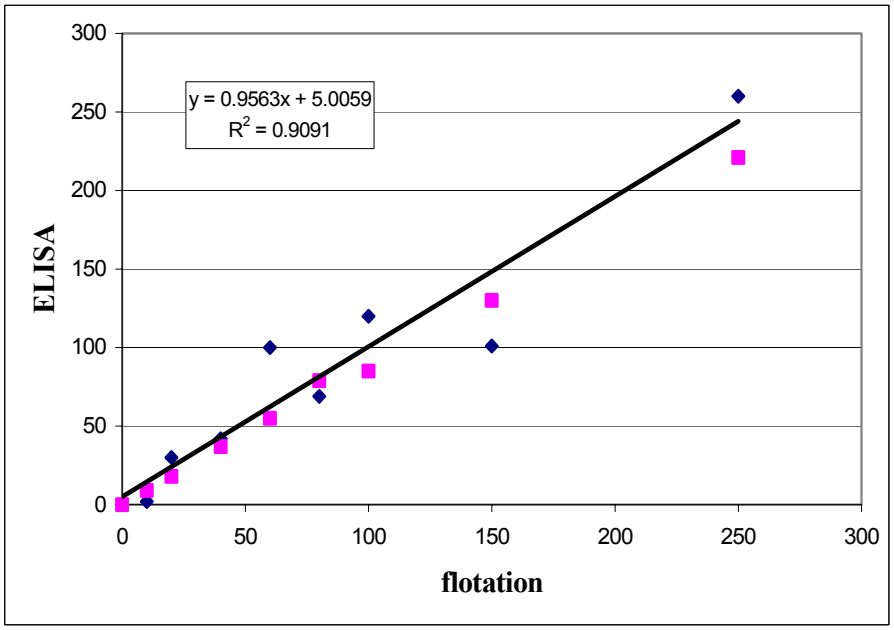
Figure 8. *A. siro* seeded in 10g, 25g and 50g of wheat




**Evaluation of assay by comparison with flotation test**

The results from the flotation test and ELISA correlated well with a linear regression of $r^2 = 0.9091$ (Figure 9).

Figure 9. Linear regression of the ELISA results and flotation counts.



**DISCUSSION**

Good antibody production is based on the quality of the immunising antigen and the selective screening afterwards for the required end purpose. Therefore much time, effort and consideration was put into the antigen extraction process. It is known that physical homogenisation of test organisms can produce a sufficient quantity of antigen (Curtis *et al*., 1998) but this method can also release proteins and/or glyco-proteins from the wheat itself (Chambers *et al*., 1999a) which are then free to compete with the detecting antibodies. Therefore antigen was also extracted by soaking the mites in an extraction buffer which had sufficient molarity to lyse the proteins from the mites. This method avoided the physical homogenisation of the wheat and its associated problems and would also be easier for the end-user to employ. The gels confirmed the validity of both these methods.

Prior to immunisation, protein analysis using electrophoretic techniques was undertaken on antigen extracted by homogenisation and rinsing method on different field and laboratory strains of *A. siro* to establish whether there were any differences. As there was no perceivable difference between the strains (appendix A) mice were immunised with both a field (9258/2) and laboratory strain (D6) of *A. siro* to ensure that any antibodies raised would not be restricted to a particular strain. The initial TCS screen (Table 1) confirmed that the antibodies could be raised by the rinsing method (Mab 173) and that they did have applicability to different strains of A.siro.

Immunisation produced several hundred hybridomas that required screening. Screening is the second crucial step in the production of successful antibodies. Therefore certain criteria were set which had to be met if the antibodies were to be selected. Principally, these were specificity to *A. siro*, no cross-reactivity with wheat, and compatibility with other *A. siro* specific antibodies.

From the screening process two antibodies were chosen – a monoclonal and polyclonal antibody. As can be seen from the results the working assay was very specific to *A. siro,* with no cross-reactivity even to its close cousins, *A. immobilis* or *A. farris*. In addition, there was no response with other grain contaminants such as storage insects and fungi. Even more importantly there was no response with wheat.

It was also important, for the purposes of interpretation, to determine whether the assay could detect dead mites, any of the different life stages, or mite faeces. The results showed that the assay would detect mites whether alive or dead, live mites in all life stages and mite faeces. It therefore has much potential as a measure of contamination by mites and their products throughout the grain production process. The response to all three life stages - eggs, juveniles and adults – suggested that the epitope is not only stable in structure but also present throughout the life cycle of *A. siro*. It should be noted that to standardise the test,

equal amounts of protein for each life stage were used, to achieve this a very large number of eggs were collected, more than would be realistically present in a normal population.

The intended end-point of this project was an immuno-diagnostic assay to quantify mites in wheat samples which could, after further development, form a field kit. Therefore tests were undertaken with mites seeded in different quantities of grain, and the mites extracted using the rinsing method, which would be suitable for field use. The results show that the assay could successfully and accurately quantify mites from 0g, 10g, 25g and 50g of grain and when the method was evaluated against the flotation test the results from the two tests were comparable (linear regression, of $r^2 = 0.9091$). In addition the minimum level of detection for the immunoassay was 0.01 mite/ml which means that the assay could detect down to 20 mites per kg of grain. This is a significant advance on previous immunoassays which have focussed on detecting house dust and storage mites in dust, or were ineffective in detecting mites in grain (Luczynska, *et al*., 1989; Harfast, *et al*., 1996; Chambers *et al*., 1999a).

The success of this project means that it is now possible to develop this laboratory-based assay into a storage mite diagnostic kit suitable for use in the field. Initially this will involve adapting the *A. siro* assay into a field kit format but will also involve extending the applicability of the kit to a range of mite pests. In addition a model will be developed to guide the use of the kit and interpretation of the results.

**Acknowledgements**

The authors would like to thank the Home-Grown Cereals Authority for funding the work.

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## APPENDIX A

**Figure 9. Gel to assess antigen extraction methods - rinsing versus homogenisation**