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Rapid method for the detection of storage mites in cereals: feasibility of an ELISA based approach

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Abstract

This paper describes the development of rapid immunodiagnostic tests for the detection of storage mite infestations in cereals and cereal products. The study's first phase (proof of concept) involved the production of a species-specific enzyme-linked immunoassay (ELISA) for the flour mite, *Acarus siro* (L.), a major pest of stored commodities. The specificity of this new assay was assessed against key stored product contaminants (13 species of mites of which three were predatory, five species of insects and five species of fungi) in the presence and absence of grain. The assay was species-specific (no cross-reactivity to other storage contaminants) and was unaffected by the presence of cereal antigens in the extract. In the study's second phase, species- and genera-specific ELISAs were developed for a range of key storage mite pests: the cosmopolitan food mite (*Lepidoglyphus destructor*), the grocers' itch mite (*Glycyphagus domesticus*), the grainstack mite (*Tyrophagus longior*), mites of the *Tyrophagus* and *Glycyphagus* generas, and all storage mites. All tests were demonstrably specific to target species or genera, with no cross-reactions observed to other storage pest contaminants or cereals. The final, validation phase, involved a comparative assessment of the species-specific *A. siro* and the genus-specific *Tyrophagus* ELISAs with the flotation technique using laboratory and field samples. Both ELISAs were quantitative (0–30 mites per 10 g wheat) and produced good comparative data with the flotation technique (*A. siro* $r^2 = 0.91$, *Tyrophagus spp.* $r^2 = 0.99$).

Keywords: ELISA, immunodiagnostic, Mab, detection, storage mites, cereal, *Tyrophagus*, *Lepidoglyphus*, *Glycyphagus*, *Acarus siro*, quality measurement

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Introduction

Storage mites are a primary cause of loss of quality in cereals and cereal-based products in temperate climates (Anon, 2003). In addition, they contaminate the commodities with potent allergens, which can be hazardous to both operators and consumers, causing a range of medical conditions that include farmer's lung, rhinitis, urticaria, asthma and dermatitis (Cuthbert *et al*., 1979; Van Hage-Hamsten *et al*., 1985; Stengard Hansen *et al*., 1996). There have also been several reported cases of anaphylaxis and anaphylactoid reactions following the ingestion of mite contaminated food (Guerra Bernd *et al*., 2001; Matsumoto *et al*., 1996; Sanchez-Borges *et al*., 1997).

The key to successful integrated pest management (IPM) for storage mites is early detection and identification, as it allows for timely implementation of appropriate control strategies before threshold levels are exceeded. Thus, IPM can reduce costs, damage and pesticide usage. Moreover, in so doing, occupational exposure to both pesticides and to mite allergens can be reduced.

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To date, the most commonly used method to detect mites in the field is sieving followed by visual inspection. Other detection methods include flotation (Thind, 2000), the BT Mite Trap (Thind, 2005) and measurement of guanine content (Bischoff *et al*., 1989; Ransom *et al*., 1991). Sieving is unreliable (Lynch & Thind, 1985), subjective and potentially hazardous through exposure to airborne allergens; the flotation technique is time-consuming and technically exacting because it requires an expert acarologist to complete identification; the guanine assay is relatively non-specific as substances other than mites can confound the interpretation of results (Hallas *et al*., 1993); and the BT Mite Traps, although easy to use, are unable to provide rapid results, as they require four days to lure mites into the traps. The absence of a rapid, robust and sensitive detection system by which to help avoid the build up of infestation may be contributing to the apparent increase in the contamination by mites of cereal-based food products in retail outlets (Anon, 1996; Thind & Clarke, 2001).

Immunoassays are a well-established detection technique based on the detection of specific antigens by antibodies. They are reliable, rapid, do not require expert knowledge and can be developed into user-friendly, low-cost field kits, such as lateral flow devices (Danks & Barker, 2000). Species-specific immunoassays have been developed for the detection of storage pests, such as the grain weevil, *Sitophilus granarius* (L.), in wheat (Chen & Kitto, 1993), the khapra beetle, *Trogoderma granarium* (Everts) (Stuart *et al*., 1994), and a wide variety of common insect pests in both grain and flour (Brader *et al*., 2002). For mites, there is an ELISA method for the detection of the house dust mite, *Dermatophagoides pteronyssinus* (Trouessart) (Luczynska *et al*., 1989), but there has been little investigation for such an approach for the detection of storage mites. However, Härfast *et al*. (1996) used a monoclonal antibody to detect and quantify storage mite allergens from *Lepidoglyphus destructor* (Schrank) in dust samples collected from barns; and recently, Kudlíková *et al* (2004) raised polyclonal antibodies to detect *Acarus siro* (L.). Härfast's monoclonal, although proved specific to two Glycyphagid species of mite, but cross-reacted with *Aleuroglyphus ovatus* (Troupeau, 1878), it lacked sensitivity in the presence of grain (Chambers *et al*., 1999a,b; Dunn *et al*., 2002) and Kudlíková's polyclonals have yet to be validated using grain samples.

This paper reports the development and preliminary validation of species and genera-specific ELISAs for the detection of stored product mites: the flour mite (*A. siro*), the cosmopolitan food mite (*L. destructor*), the grocers' itch mite (*Glycyphagus domesticus* (DeGeer)), the grainstack mite (*Tyrophagus longior* (Gervais)), mites of the *Tyrophagus* genus, mites of the *Glycyphagus* genus, and all storage mites. Tests were performed using different qualities of grain and various grain storage pests.

Materials and methods

Mites

The mite species used were: three strains of *A. siro* and one strain of *Acarus farris* (Oudemans), *Acarus immobilis* (Griffiths) and *Acarus gracilis* (Hughes); two strains of *T. longior and Tyrophagus putrescentiae* (Schrank) and one strain of *Tyrophagus palmarum* (Oudemans), *Tyrophagus brevicrinatus* (Robertson), *Tyrophagus neiswanderi* (Johnston and Bruce) and *Tyrophagus perniciosus* (Zachvatkin); and two strains of *L. destructor* and *G. domesticus*, and one strain of *Lepidoglyphus michaeli* (Oudemans), *Aleuroglyphus ovatus* (Troupeau), *Caloglyphus berlesei* (Michael), *Carpoglyphus lactis* (L.), *Tyrolichus casei* (Oudemans), *Cheyletus eruditus* (Schrank), *C. malaccensis* (Oudemans) and *Ctenoglyphus plumiger* (Koch).

Cultures of these mite strains were reared in the dark at 20°C and 80% RH in 50 ml conical flasks, on a finely ground, sterilised and conditioned mite diet consisting of flour and dried yeast (1 : 3 w/w) with the exception of *C. eruditus*, *C. malaccensis* and *C. plumiger*. The predatory mites (*C. eruditus* and *C. malaccensis*) were bred in tubs (14 cm × 14 cm × 14 cm) at 25°C and 75% RH and fed a mixture of storage mite species (*A. siro*, *T. putrescentiae* and *T. longior*). *C. plumiger* was bred in 50 ml flasks, at 20°C and 80% RH on a diet of mouldy yeast and flour, supplemented with tropical fish flakes (Aquarian).

Insects

Insect species tested were *Sitophilus granarius* (L.), *Ahasverus advena* (Watl), *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens) and *Liposcelis bostrychopila* (Badonnel).

Insect species were reared in the dark at 25°C and 70% RH in 0.75-l Kilner jars. *O. surinamensis* was maintained on a diet of wheatfeed, rolled oats and yeast (5 : 5 : 1 w/w), *S. granarius* was reared 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.) and *Cladosporium cladosporioides* ((Fresen.) G.A. de Vries). All fungal species were cultured at 25°C on czapek yeast agar in the dark.

Antigen extraction

As a preliminary to extracting antigen for immunisation purposes, mites were thoroughly washed with phosphate buffered saline (PBS) to remove any adhering materials. The mites were then soaked in 0.15 M sodium chloride overnight at 33°C, and the suspension was then centrifuged at 18,000 gX force for 5 min. The resultant supernatant was then used as mite antigen.

Mite antigen was extracted from infested grain samples by shaking grain in 0.15 M sodium chloride solution (1 ml g^{-1} of grain) in a 30-ml plastic container containing five stainless steel ball bearings (5 mm dia.) for 1 min. The protein concentrations of the extracts were determined, where applicable, using standard Biorad methodology (Bradford, 1976) and bovine serum albumin (Sigma-Aldrich, derivatization grade) as the standard protein. Solutions were diluted to the appropriate concentrations using PBS.

Antibodies

Monoclonal antibodies (Mab) were raised against *A. siro*, *L. destructor*, *G. domesticus*, *T. longior* and *T. putrescentiae* according to standard procedures (Harlow & Lane, 1988).

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For initial antibody screening of supernatants from fusions, 96-well micro titre plates (Nunc Immunoplate, maxi-sorp) were coated overnight with 100 µl per well of 5 µg ml^{-1} 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 difference of at least 3 : 1 in the recognition of the target antigen compared to control wells (blanks) was chosen as a discriminatory threshold for potential diagnostic antibodies. Cell lines were cloned twice by limiting dilution.

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

Indirect, TAS and competitive ELISAs

Indirect PTA, triple antibody sandwich (TAS) and competitive ELISAs were performed in accordance with standard procedures (Wilson & Goulding, 1986; Harlow & Lane, 1988). Optimised TAS ELISAs were used with the *A. siro* Mab, and optimised competitive ELISAs with all the other antibodies raised.

Comparison of antigen obtained from two strains of each of the four primary pest species by Multiphor electrophoresis

Mite antigen supernatants of two strains of *L. destructor*, *G. domesticus*, *T. longior* and *T. putrescentiae* were each diluted in PBS to a concentration of 0.1 mg ml^{-1}. These antigens plus two markers (BioRad High and Low) were then denatured before running the samples on an ExcelGel (SDS Homogenous 12.5) according to the manufacturer's instructions (Multiphor II Electrophoresis Systems, Pharmacia Biotech). The gel was stained using a freshly prepared sensitive silver stain according to Multiphor II manufacturer's instructions.

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

To determine the cross-reactivity of the potential diagnostic antibodies with other potential contaminants, two-fold serial dilutions were made of each respective antigen. These were then used to assess the specificity of the ELISAs described above.

Reactivity with laboratory infested and 'clean' grain samples

Ten 10 g samples of wheat were seeded with mites (0, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 adult *A. siro*). ELISAs were then used to compare seeded grain samples with controls (10 g of unseeded, 'clean' grain) and mites only (no grain).

Evaluation of assay by comparison with flotation technique using both laboratory and field samples

Two batches of wheat samples, each weighing 25 g, were seeded with equal numbers of adult mites (0, 10, 20, 40, 60, 80, 100, 150 and 250 mites) using a light microscope and a single-haired paint brush. One batch was analysed using the ELISA and the other batch by flotation technique (Thind, 2000) with a minor modification to its 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 increased the recovery of mites from grain and speeded-up the extraction process. Previous experiments (unpublished) have demonstrated that this additional step enhances the extraction of mites from within grain kernels.

For the ELISA, a standard curve was constructed from a different set of samples seeded with a known number of mites. This standard curve was then used to deduce 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.

A further batch of grain samples, each weighing 10 g, was seeded in the laboratory with numbers of mites unknown to the operator. Each sample was tested in triplicate. The calibration curves described above were then used to assess the mite numbers. This procedure was replicated nine times with the *A. siro* ELISA and five times with the *Tyrophagus* genus ELISA.

Five field samples of wheat with unknown levels of mite contamination were also tested. Using a sample divider, three 50 g sub-samples were taken from each sample and analysed by flotation analysis. Similarly, three 10 g sub-samples were taken from each field sample and tested in triplicate using the *A. siro* and Tyrophagus genus ELISAs.

Optimisation of A. siro *ELISA by confirming applicability to a wide variety of sample types*

Ten gram samples of two cultivars of wheat, Hereward and Consort; two cultivars of oilseed rape Royal (low erucic acid var.), HEAR with high erucic content; one cultivar of barley (Fighter); and one cultivar of oats (Gerald) were seeded with 0, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 adult *A. siro* mites. Each sample was tested five times in a TAS ELISA.

The ELISA's validation was completed using batches of 10 g grain samples. Each batch contained either 10% of broken grains, insect damaged grain, sprouted grain, wheat straw, wheat dust or extraneous matter (fungal contamination with *P. verrucosum*). Each batch ($n = 5$) was then seeded with 50 mites and tested in a TAS ELISA.

Results

Comparison of antigen obtained from two strains of each of the four primary pest species (*L. destructor, G. domesticus, T. longior* and *T. putrescentiae*) by Multiphor electrophoresis showed species-specific but not strain-specific banding.

Reactivity of A. siro *Mab to other species of mite including predatory mites, insects, wheat and fungi*

A. siro *assay*

Specificity tests were conducted with adults of nine species of storage mite, five species of storage insects, two species of predatory mite, a detritus feeding mite and five species of fungi. The results (table 1) show that the *A. siro* assay was species specific ($P < 0.001$).

Tests were undertaken to confirm that the selected antibodies did not cross-react with wheat when analysing

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Table 1. Specificity for *A. siro* with the *A. siro* Mab against nine other species of storage mite, five species of storage insect, two species of predatory mite, one fungivorous mite and five species of fungi ($n = 5$).

Antigen (tested at 5 μg ml^{-1})	Optical density (405 nm)
Mites	
A. siro	**1.074****
A. farris	0.129*
A. immobilis	0.225*
T. putrescentiae	0.136*
T. longior	0.144*
T. palmarum	0.111*
L. destructor	0.197*
G. domesticus	0.148*
A. ovatus	0.122*
C. berlesei	0.172*
Insects, predatory mites & fungivorous mite	
S. granaries	0.125*
A. advena	0.114*
O. surinamensis	0.097*
C. ferrugineus	0.098*
Psocids	0.132*
C. mallaccensis	0.139*
C. eruditus	0.259*
C. plumiger	0.118*
Fungi	
P. verrocosum	0.111*
A. allternata	0.100*
E. amstolodami (ascospores)	0.118*
E. amstolodami (conidia)	0.111*
A. ochraceus	0.146*
C. cladosporoides	0.117*

*Reading not significantly different from background.
**Reading significantly different from background ($P < 0.001$).

mite-infested grain samples. Figure 1 shows that the selected antibodies fulfilled our test criteria and that the assay's performance was not confounded by the presence of wheat co-extractants.

Panel of antibodies

The results (table 2) show that the antibodies produced for *T. longior*, *L. destructor*, *G. domesticus* the *Tyrophagus* and *Glycyphagus* genera and for all storage mites were specific to their target antigen(s).

Reactivity of A. siro *Mab with laboratory infested and 'clean' grain samples*

Tests, with larger quantities of grain (25 g and 50 g) showed the antigen extraction process and the assay were still able to detect and quantify *A. siro* in these volumes without any detrimental affect on performance (fig. 2).

Evaluation of A. siro *and* Tyrophagus *genera assays*

The results from the laboratory-seeded samples tested using both the ELISA and flotation methods correlated well with a linear regression of $r^2 = 0.9091$ ($y = 0.9563 \times + 5.0059$) for *A. siro* and $r^2 = 0.9895$ ($y = 1.0145 \times - 7.1755$) for the *Tyrophagus* genera.

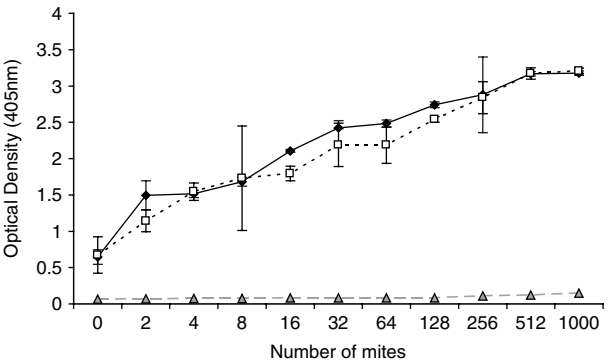

Fig. 1. Quantitative detection of *A. siro* with *A. siro* Mab with and without the presence of 10 g samples of wheat ($n = 5$). (—◆—, mites + wheat; - -□- -, mites only; — ▲ —, wheat only.)

Calibration curves were then set up (using standards) for the ELISA. Results from the 'blind' laboratory samples (tables 3 and 4) and samples received from the field (tables 5 and 6) show that when these samples were run alongside the standards, mite levels were successfully determined, and these results were comparable to those produced by flotation.

Optimisation of A. siro *ELISA by confirming applicability to a wide variety of sample types*

The results show that with the *A. siro* ELISA there was a general and regular increase in response with increase in infestation level and this was similar for all the different types of cereal and oilseed tested (fig 3). Furthermore, despite a high background reading with zero mites due to contaminants within the samples, there was a significant difference (*t*-test, $P < 0.001$) in response with the samples seeded with mites (table 7).

Discussion

Effective control of mite pests requires an efficient detection method that provides early warning of an infestation. Results from this study show that ELISAs can feasibly fulfil this need. For example, the Mabs could detect extremely low levels of mites and measure their increase in numbers in a wide variety of cereal types and qualities (fig 4, table 7) and were specific to their target antigen(s) with no cross-reactivity to other storage pests, thus minimising the potential for false positives (tables 1 and 2).

The immunoassay's success was aided by the development of an innovative antigen extraction procedure. For this, antigen was extracted using a novel 'rinsing' method with an extraction buffer that had sufficient molarity to remove sufficient proteins from the mites. This method avoided the physical homogenisation of the wheat and its associated problems (Chambers *et al.*, 1999a) and will be rapid and easy for the user.

The successful development of the *A. siro* ELISA provided the basis for the study's second phase – the raising of further Mabs that could detect other important and prevalent UK storage mite pests. Prior to the raising of these Mabs, the antigens were assessed to determine whether there were differences in the protein profiles between strains

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Table 2. Reactivity of the six monoclonal antibodies against different mite species, mite diet (yeast and flour), wheat, predatory mites and insects ($n = 3$).

Mite species	Monoclonal antibodies (OD 405 nm)					
	T. longior specific	*L. destructor* specific	*G. domesticus* specific	Tyrophaginae	Glycyphaginae	All storage mites
T. longior	**0.718****	0.098*	0.240*	**1.423****	0.359*	**1.274****
T. putrescentiae	0.09*	0.101*	0.187*	**1.200****	0.269*	**0.816****
T. palmarum	0.132*	0.096*	0.193*	**1.198****	0.324*	**1.089****
T. brevicrinatus	0.096*	0.189*	–	**1.689****	–	–
T. neiswanderi	0.125*	0.190*	–	**1.601****	–	–
G. domesticus	0.116*	0.132*	**1.932****	0.160*	**3.145****	**1.313****
L. destructor	0.115*	**0.620****	0.500	0.160*	**2.796****	**1.020****
T. casei	0.087*	0.188*	0.383*	0.163*	0.313*	**0.906****
C. berlesei	0.096*	0.153*	0.370*	0.163*	–	–
A. ovatus	0.114*	0.145*	0.366*	0.189*	–	**1.119****
A. siro	0.148*	0.096*	0.180*	0.183*	0.400*	**1.103****
A. gracilis	0.170*	0.167*	–	–	–	–
A. farris	0.092*	0.093*	0.150*	0.181*	0.305*	**1.111****
Yeast + Flour	0.076*	0.098*	0.096*	0.155*	0.073*	0.101*
Wheat	0.081*	0.030*	0.098*	O.150*	0.117*	0.099*
C. malaccensis	–	–	0.153*	–	0.178*	0.096*
C. eruditus	–	–	0.155*	–	0.201*	–
O. surinamensis				0.174*	0.065*	0.084*
L. bostrychopila				0.190*	–	–

* Reading not significantly different from background.
** Reading significantly different from background ($P < 0.001$).

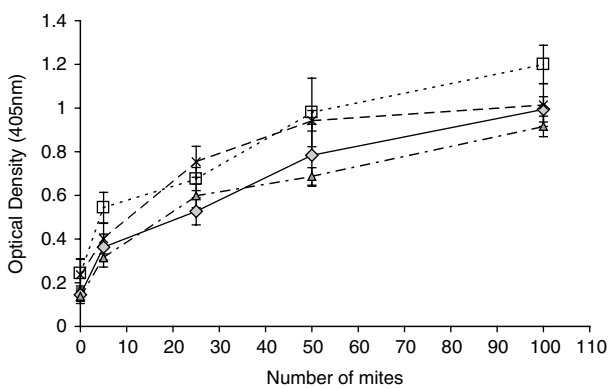

Fig. 2. Detection of *A. siro* with *A. siro* Mab seeded in 0 g, 10 g, 25 g and 50 g of wheat ($n = 5$). (—◇—, 0 g; - -□- -, 10 g; —·△·—, 25 g; - - × - -, 50 g.)

of the same species, as this could potentially affect the sensitivity of the ELISA with field populations. No differences in the banding patterns were found using Multiphor electrophoresis between strains, but distinct differences were observed between species.

The species-specific Mabs produced for the detection of *T. longior*, *L. destructor* and *G. domesticus*, showed high specificity with no cross-reactivity to other species of mites or insects tested, nor to mite food or wheat (table 2). Similarly, the *Tyrophagus* and *Glycyphagus* genus-specific Mabs reacted strongly with only those mites belonging to their respective genus, and the all storage mite Mab reacted with all the storage mite species tested with the exception of predatory mites and storage insects (table 2). The successful development of an all storage mite assay has many benefits.

Table 3. Mean number of *A. siro* mites detected from samples seeded 'blind' in 10 g wheat (nine replicates at each mite density and each replicate tested in triplicate on ELISA plate and LFD).

Number of mites seeded	Mean number and standard deviation of *A. siro* detected by ELISA
0	0 ± 0
7	8.57 ± 4.03
15	15.67 ± 4.27
22	23.11 ± 4.17
30	28.11 ± 3.02

Table 4. Mean number of *Tyrophagus* mites seeded 'blind' (five replicates at each mite density) detected in 10 g of wheat.

Number of mites seeded	Mean number and standard deviation of mites detected by ELISA
0	0.5 ± 0.8
7	7.2 ± 2.0
15	13.8 ± 3.8
22	19.7 ± 5.2
30	25.2 ± 4.4

For example, it would provide the user with a cost-effective means to confirm freedom from mites throughout the food supply chain.

Once the Mabs were raised, ELISA methodology was developed. However, preliminary validation was performed with just two, namely the *A. siro* and the *Tyrophagus* genera assays. These two Mabs were selected as mites of these genera are considered as being the most important mite pests within the UK cereal and allied industry (Thind & Ford, 2004; Thind, 2005). Both the *A. siro* and *Tyrophagus*

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Table 5. Mean number ($n = 3$) of *A. siro* mites detected in five field samples (wheat) by flotation and ELISA.

Sample	Flotation (50 g samples)			ELISA (10 g samples)
	A. siro	*Tyrophagus* genera	*Glycyphagus* genera	
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	113	18	53	27
5	0	3	0	0

Table 6. Mean number ($n = 3$) of *Tyrophagus* mites detected in five field samples (wheat) by flotation and ELISA.

Sample	Flotation (50 g samples)			ELISA (10 g samples)
	A. siro	*Tyrophagus* genera	*Glycyphagus* genera	
1	0	0	0	0
2	0	0	0	0
3	0	0	0	1
4	0	9	0	5
5	0	3	0	4

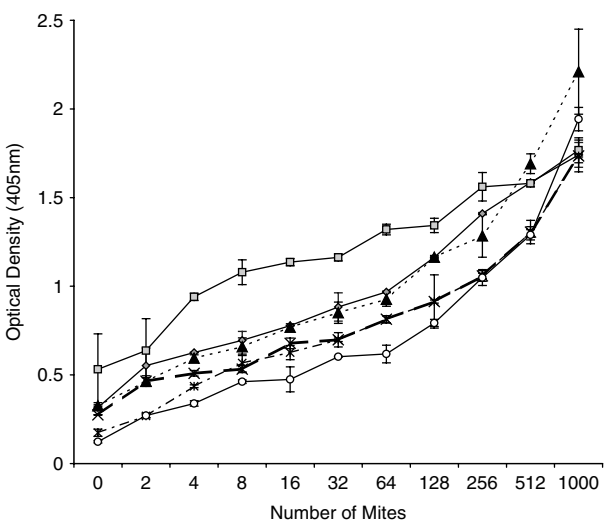

Fig. 3. Detection of *A. siro* with *A. siro* Mab seeded in two cultivars of OSR (Royal and HEAR), three cultivars of wheat (Hereward, Consort and Mercia) and *A. siro* alone ($n = 5$). (—◇—, OSR – Royal; —□—, OSR-HEAR; - - -▲- - -, Hereward; —×—, consort; – ✳ –, Mercia; —○—, mites only.)

genera ELISAs showed good correlation with the flotation technique (correlation coefficient of 0.91 for *A. siro* and 0.99 for *Tyrophagus*) and were able to accurately indicate levels of mites infesting grain in both laboratory and field samples (tables 3–6). Furthermore, in one sample, the *Tyrophagus* genera ELISA was able to detect mites where the flotation technique failed (table 6, sample 3). In addition, the ELISA

Table 7. Optical density values (405 nm) for admixture and screening.

Grain type	0 mites	50 mites
Broken grain	0.419	0.925
Insect damaged grain	0.526	1.305
Sprouted grain	0.410	1.096
Extraneous matter	0.595	1.564
Wheat straw	0.532	1.072
Wheat dust	0.396	1.198

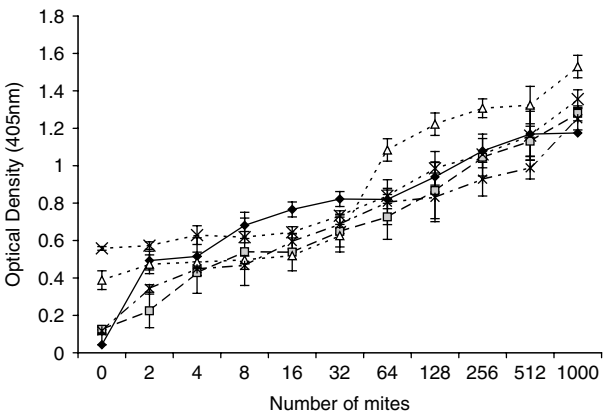

Fig. 4. Detection of *A. siro* with *A. siro* Mab seeded in four different types of grain ($n = 5$). (—◆—, mites + wheat; – –□– –, mites + barley; - - -△- - -, mites + oats; - - -×- - -, mites + OSR; – ✳ –, mites only.)

accurately indicated the presence or absence of mites in all the sub-samples and provided quantitative data.

The development and application of immunoassays for the detection of storage mites in cereals is a major advance from previous works and has wide implications for farmers, grain store keepers, millers and the whole food supply chain management, in as much that the assays can provide a non-subjective, unambiguous result on which to base contractual agreements. Secondly, once fully developed and validated, immunoassays can provide a cost-effective means for quality assurance by establishing its storage-mite free status; and, thirdly, if a mite infestation were to develop, the assays would provide accurate data, allowing for the most appropriate pest control strategy(s) to be applied.

The success of this ELISA-based approach for the detection of mites in grain means that it is now feasible to extend the application of this laboratory-based immunoassay into a storage mite diagnostic kit suitable for use in the field by adapting the assays into lateral flow devices (LFDs). LFDS have several advantages, of which its main attributes are that it can be used on site by end users without the need of any specific expertise to provide almost instantaneous results. Preliminary investigations (unpublished) with prototype LFDs showed promising results with quality assurance personnel detecting low levels of mites in a variety of cereals at their respective premises. In addition, our preliminary results (unpublished) indicate that these prototype LFDs can also detect mites in processed cereals (e.g. flour, dry pet food and animal feed).

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