

The Development of ELISAs for the Detection of Storage Mites in Grain

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*The study forms the first part of the development of an immunoassay to detect storage mite infestations in cereals and their derived products. This was achieved by examining the suitability of an existing monoclonal antibody (MAb) raised against the cosmopolitan food mite, *Lepidoglyphus destructor* (Schrunk). The results demonstrate that in the absence of grain the MAb could detect and quantify unknown numbers of *L. destructor*, ranging between 0 and 100 adult mites. However, in the presence of grain the sensitivity of the assay decreased. This was probably because the existing antibody had not been raised for this purpose. To overcome this, an additional polyclonal antibody was used which gave satisfactory detection of 0 to 100 adult mites seeded in 5 g of grain. The work has shown the excellent potential of an immunoassay method for the rapid detection of storage mites in unmilled cereals.*

Keywords: Mites, detection, grain, ELISA

INTRODUCTION

Storage mites are a serious and increasing pest problem. They reduce the quality of grain, oilseeds and their derivatives (Wilkin & Thind, 1984; Prickett, 1997) and can cause occupational and domestic allergies (Van Hage-Hamsten & Johansson, 1992; Cuthbert & Jeffrey, 1993; Armentia *et al.*, 1997). There is an urgent need for rapid measurement technology such as ELISA that can match the scales and speeds of the operations of harvesting, storage and processing. The aim of this study was to establish whether an existing antibody, raised for medical purposes against the storage mite *Lepidoglyphus destructor* (Schrunk), could form the basis of an immunological method for the early detection of mites in samples of unmilled cereals.

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MATERIALS AND METHODS

Mites

Storage mite species used were: *L. destructor*; *Glycyphagus domesticus* (De Geer); and *Acarus siro* (L.).

Antigen Release From Mites in the Absence of Grain

Mites cleansed from contaminants were homogenized in 50 µl phosphate-buffered saline (PBS) using plastic homogenizers (TreffLab, Switzerland). Protein concentrations were determined (Biorad protein assay, Biorad, Munchen, Germany). Solutions were diluted to appropriate protein concentration in PBS and used as antigen in ELISAs.

Antigen Release From Mites in Grain

Five gram samples of wheat and barley were seeded with known numbers of mites. The samples, plus 1 ml PBS, were placed in plastic bags (12 cm × 12.5 cm) containing a gauze mesh inner (9.5 cm × 12 cm) to retain the grain from the antigen supernatant. A roller fitted with ball bearings was used to crush the mites and grain for 1 min while the bags were trapped in position on a flat surface. Protein concentrations were determined.

Antibodies

The monoclonal antibody (MAb) cell line raised against *L. destructor* (Härfast *et al.*, 1992) was donated from the Karolinska Hospital, Sweden. Hybridomas were grown and MAb collected according to established techniques (Köhler & Milstein, 1975; Banks, 1996).

An aliquot of monospecific polyclonal antibody (PAb) raised against *G. domesticus* was donated from the Withington Hospital, Manchester, UK.

Indirect and TAS ELISAs

Indirect ELISA. Microtitre plates (Nunc Immunoplate, maxi-sorp) were coated with 50 µl mite homogenate or mite antigens extracted from the crushed wheat samples, and left to incubate overnight at 4°C. After incubation, excess antigen was removed from the wells and the plates rinsed in three changes of 0.1 M-PBST (PBS plus 0.05% v/v Tween 20, Sigma), for 3 min and blotted dry. Plates were incubated for 30 min on a plate shaker at room temperature with 100 µl per well of PBST plus 5% Marvel milk powder. The plates were then rinsed as before and incubated with either tissue culture supernatant ([1:5] diluted in PBST) or purified MAb ([1:1000] diluted in PBST) for 1 h on a plate shaker at room temperature. After three more rinses, 50 µl of second antibody conjugate (alkaline phosphatase conjugated rabbit anti-mouse IgG, Sigma) at 1:1000 dilution in PBST was added to the wells and the plates were shaken for 45 min at room temperature. Finally after three more rinses with PBST, 100 µl of alkaline phosphatase (Kirkegaard and Perry Laboratories [KPL]) was added to each well and incubated at room temperature in the dark. The plates were measured photometrically at 405 nm using a microtitre plate reader (Molecular Devices plus SOFTmax software).

TAS ELISA. Microtitre plates were coated with 100 µl purified PAb [1:2000] diluted in PBS and left to incubate at 4°C overnight. After washing, the wells were incubated with 200 µl of blocking buffer (PBST plus 5% Marvel milk powder) for 30 min at room temperature on a plate shaker. After further washing, the wells were incubated with 100 µl of mite homogenate or grain extract for 1 h at room temperature on a plate shaker. The plates were then washed again followed by incubation with 100 µl of MAb [1:1000] diluted in PBST, for 1 h at room temperature on a plate shaker. Then, after further washing, the wells were incubated with 100 µl of second antibody conjugate (alkaline phosphatase conjugated rabbit anti-mouse IgG, Sigma) [1:1000] diluted in PBST, and the plates shaken for 45 min at room temperature.

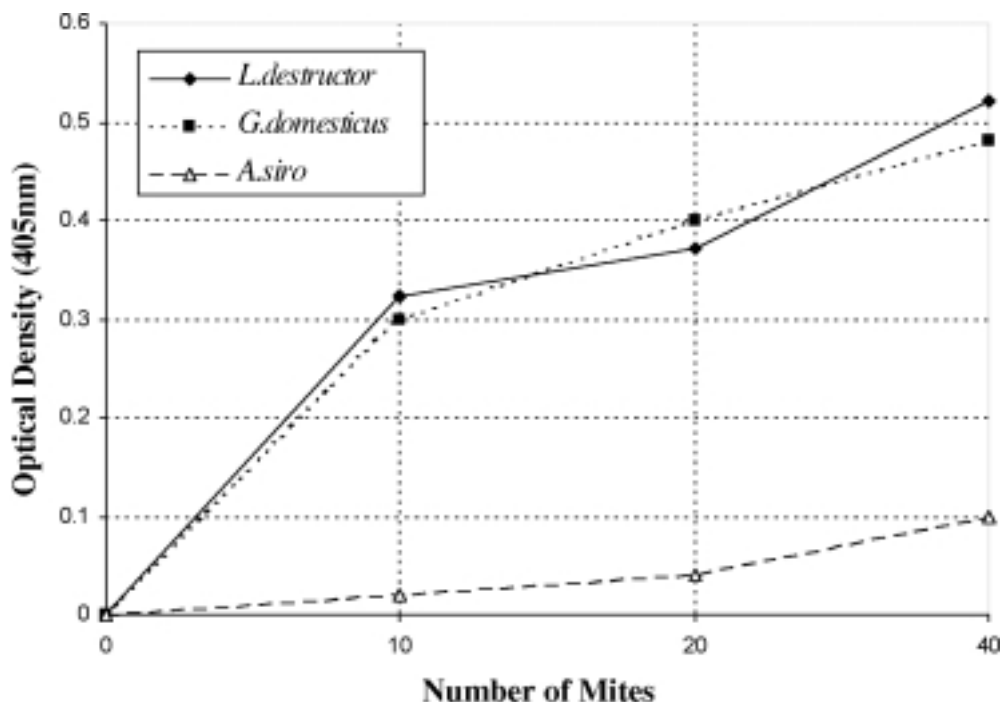


FIG. 1. Sensitivity and selectivity with preparations of *L. destructor*, *G. domesticus* and *A. siro* without the presence of wheat in an indirect ELISA.

Finally, after three more rinses with PBST, 200 μ l of alkaline phosphatase substrate (KPL) was added to each well and incubated at room temperature in the dark. The plates were measured photometrically at 405 nm using a microtitre plate reader (Molecular Devices plus SOFTmax software).

RESULTS AND DISCUSSION

L. destructor is a primary pest of cereals and related products and its allergens are occupational and domestic health hazards (Revsbech & Andersen, 1987; Tee, 1994; Armentia *et al.*, 1997). As a MAb already existed against this species, it was judicious to test it in a prototype immunoassay for the detection of mites in grain. Immunoassays are quick, relatively cheap and easy to use, and can be made into a kit form for use on-site and would therefore be ideal for monitoring mites in the field.

The MAb was used to construct calibration curves with tissue culture serum and mites, in an indirect ELISA. The antibody raised against *L. destructor* cross-reacted with its close relative, *G. domesticus*, but not with species from the Acaridae family such as *A. siro* (Figure 1). The sensitivity of this method suggests that it would be able to detect infestations of *L. destructor* and *G. domesticus* at very low levels.

The sensitivity of the indirect assay was, however, considerably reduced when the mites were crushed with grain. It is known that this MAb detects a 39 kDa allergen in *L. destructor* which is resistant to a variety of treatments. Its resistance to trypsin and protease seems to suggest that the epitope recognized is not proteinaceous but rather of a carbohydrate nature (Van Hage-Hamsten *et al.*, 1992). It is therefore possible that the lack of sensitivity in the presence of grain was caused by the MAb being at least partly bound to cereal carbohydrates and therefore unavailable to detect the antigen.

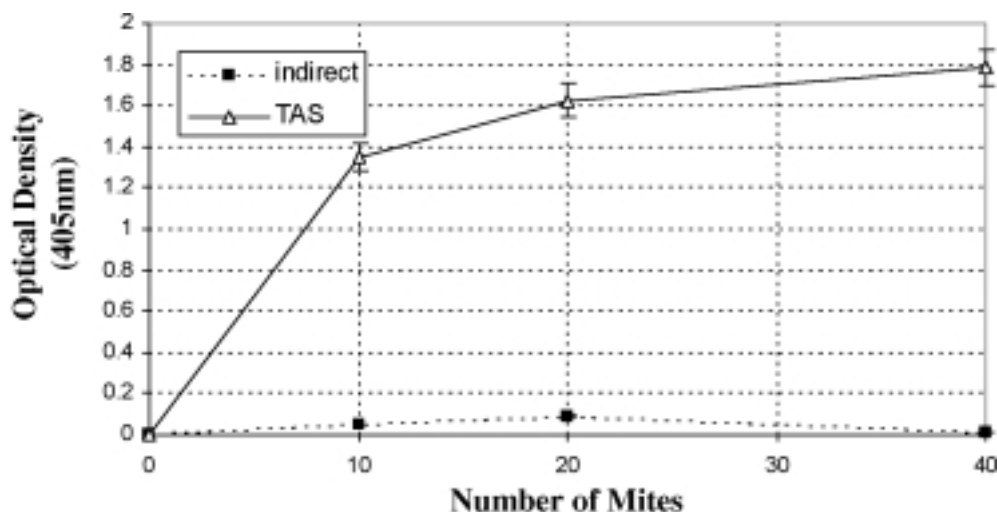


FIG. 2. Sensitivity with preparations of *L. destructor* crushed in wheat using indirect and TAS ELISAs.

To overcome this, an anti-15 kDa PAb raised against *G. domesticus* was used in a TAS ELISA to improve sensitivity. The PAb was able to trap out the antigen from the plant debris in the sample suspension and, with the extra amplification step, standard curves with relatively good optical densities were produced (Figure 2).

These results demonstrate for the first time that antibody-based ELISAs have the potential to quantify and speciate mites in infested grain samples. This is a major advance on previous immunoassays which have focused on detecting mites only in dust (Luczynska *et al.*, 1989; Härfast *et al.*, 1996). However, due to the limitations of these existing antibodies, future work will focus on raising antibodies that are specifically tailored to detect a wider range of mite species and at the same time do not cross-react with grain.

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