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Development of Polyclonal Antibodies for the Detection of *Tribolium castaneum* Contamination in Wheat Grain

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Two polyclonal antibodies (Pab) were developed for the detection of *Tribolium castaneum*, which is a stored product pest of medical and economical importance. Selected Pab anti-*T. castaneum*K51 showed low cross-reactivity to other stored product arthropods but revealed high reactivity to *T. destructor*, *T. confusum*, and partly to *Tenebrio molitor*. PTA-ELISA was used to detect adults, larva, and feces of *T. castaneum* in artificially contaminated grain samples. Calibration methods were applied to determine detection limits for each type of contaminants. Anti-*T.castaneum*K51 enabled detection of *T. castaneum* in grain samples; detection limits reached 60 and 640 individuals/kg of grain for larvae and adults, respectively, and 4 mg of feces/kg of grain. After recalculation, the detection limit for feces enables detection of 30 larvae after 5 days of feeding in optimal conditions. The main advantage of the developed assay is traceability of *T. castaneum* contamination, especially when the adults and larvae are removed from contaminated material, based on the detection of feces that persist in the grain.

KEYWORDS: Detection; grain; *Tribolium castaneum*; storage; pest; ELISA

INTRODUCTION

Cereals are a major source of human nutrition. The production of cereals in the EU amounts to 271.3 million tons (*3*). Preventing, or at least slowing, the loss of stored grain is important in maintaining its quality and marketable volume. Grain storage losses are caused by both insect damage and microbial growth (*35*). Insect pests contaminate stored cereals and cause damage by their feeding, production of toxic compounds, and creation of allergens (*2, 21, 27, 28*). Their metabolic products change the smell and taste of the contaminated baking products (*36*). There is an urgent need to eliminate insect infestation in cereals in stores, during transport, and during processing, which would ensure a supply of wholesome food to the consumers (*39*).

Rapid detection and adequate sampling systems are necessary for the detection and elimination of early infestations of pests (*11, 26, 31*). There is a wide range of newly developed methods, such as various trapping systems (*4, 14*), native fluorescence (*1*), filth-flotation (*29*), near-infrared spectroscopy (*23,40*), and microwave detection (*24*), that provide real detection and estimation of insect density. In addition, conventional detection methods including sieving on shakers also provide a quick estimation of insect contamination in grain (*11, 15*).

These currently used methods for the detection of insect contamination in cereals are not focused on the detection of metabolic products, which are potentially hazardous compounds. In arthropods, feces are known to be a serious source of allergens, which is well-documented for house dust mites and cockroaches (*41*).

Immunochemical assays such as ELISA were developed for the detection of wide spectrum food contaminants; while these methods are rarely developed for the detection of insects or mites in stored food, commercial kits are available for the monitoring of certain allergens in house dust and human environments (*17*). Efforts were made to identify the contamination on species or specific proteins levels especially by monoclonal (Mab) antibodies and above species level by developing of polyclonal (Pab) antibodies. It is illustrated on Mabs against *Trogoderma granarium* that were developed to determine individuals caught in a trap to the species level. This assay could rapidly and accurately distinguish *Trogoderma granarium* adults, pupae, and larvae from six other *Trogoderma* species (*38*). To detect and measure a wide range of insect contamination, the antibody against the insect muscle myosin was prepared (*30*). The ELISA sensitivity was about 20 μg of purified insect myosin, which means that the test was able to detect 1 adult of *Sitophilus granarius* in 50 g of spiked samples of grain (*10, 30*). Additional studies showed that this test was not able to detect eggs or stored product mites. A commercial ELISA test (Biotec, Austin, TX) detecting insect myosin was successfully applied for the detection of contaminated flour prepared from grain infested by *Rhyzopertha dominica* (*7, 8*). Recently, the Pabs for detection of stored product mite *Acarus siro* were developed and characterized (*19*). These Pabs were highly reactive to unknown compounds in the feces and enabled the feces detection at an

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extremely low infestation level (*16*). The detection of feces allows for improved tracing of pest contamination in the grain, even if the individual pests were not found, e.g. after grain cleaning and removing of adult or juvenile insect contaminants.

In this study, we focused on a frequent and abundant pest of stored grain, *Tribolium castaneum* (Herbst) (*5, 32*). Pabs against a protein fraction from larvae of *Tribolium castaneum* were prepared and characterized, and immunochemic assay based on ELISA was optimized. The assay was calibrated for the detection of *T. castaneum* contaminants, including adults, larva, and their feces in the wheat grain.

MATERIALS AND METHODS

Antigen Preparation. *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae) originated from laboratory cultures that were reared at Crop Research Institute, Prague. The insects were mass-reared on the diet consisting of wheat germ and oat flakes in 1:1 (wt:wt) at 65% RH, 25 °C, and darkness. For preparation of purified antigen, the larvae were collected from rearing chambers to a fresh mass of about 10 g and homogenized (2 min, 17 500 rpm) with an UltraTurax T25 basic homogenizer (IKA Works, Inc., Wilmington, NC) in 70 mL of PBS (pH 7.4). The homogenate was centrifuged on JOUAN Mr23i cooled centrifuge (Thermo Fisher Scientific, Inc., Waltham, MA) (5 min, 4020*g*, 4 °C), and proteins from the supernatant were isolated by precipitation with a saturated solution of ammonium sulfate $(NH_4)_2SO_4$ (pH 7.4) to 50% saturation.

For characterization of prepared Pabs, antigens in a form of unpurified total extracts were prepared by the same way as above, except that the isolation of proteins was omitted.

Preparation of Polyclonal Antibodies. Purified protein antigen was used as an immunogen. Laboratory rabbits (Giant Chinchilla breed, approximately 6 months old) were immunized by five subcutaneous injections of immunization dose with increasing content of insect proteins (100, 200, 400, 400, and 500 μg of proteins per dose) administered in three week intervals. All doses included AL-SPAN-OIL adjuvant (Sevac, Prague, CR), except the booster, which was administered 2 weeks after the last dose. Eight days after the booster, blood was collected to test tubes with separation clot activator. After collection, blood was allowed to clot for 1 h at 37 °C and was left overnight at 5 °C. The clot and another remaining insoluble material were removed by centrifugation (10 min, 1780*g*, 4 °C, JOUAN Mr23i). The immunization was performed in compliance with the Act of Czech Republic No. 77/2004 Sb. and with the approval of CRI Committee for Animal Protection against Mistreat.

IgGs from the blood plasma were isolated by precipitation with ammonium sulfate and subsequent affinity chromatography on protein A. IgGs in blood plasma (diluted with PBS 1:10) were precipitated by a saturated solution of ammonium sulfate (75 g $(NH_4)_2SO_4$ in 100 mL of H_2O, pH 7.1 adjusted with 1 M NaOH) in an ice bath and left overnight at 4 °C. The precipitate was separated by centrifugation (10 min, 5000*g*, 4 °C, EBA 12R (Andreas Hettich, Tuttlingen, Germany)), resuspended in a binding buffer (20 mM $NaH_2PO_4 \cdot 2H_2O$, 150 mM NaCl, pH 8.0), dialyzed against 50% binding buffer, and applied on the HiTrap protein A column (GE Healthcare, Vienna, Austria) filled with protein A coupled to Sepharose beads. After excessive washing with the binding buffer (10 column volumes), IgGs were eluted by 5 column volumes of elution buffer (0.1 M citric acid, 0.2 mM Na_2HPO_4, pH 3.0) into tubes filled with 100 μL of 1 M Tris-HCl pH 9.0 as prevention against acid hydrolysis of IgGs. IgG solution was dialyzed against 50% PBS and concentrated on centrifugal filter devices Amincon (Millipore, Cork, Ireland) (approximately 60 min, 4020*g*, 4 °C, Hettich EBA 12R). Its protein concentration was determined using DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). The resulting antibody solution was stabilized with 150 mM NaCl and 0.5% BSA, diluted to concentration 1 mg of IgG/mL, and stored at −18 °C until use.

Basic characteristics, specificity, and sensitivity of the obtained antibodies were evaluated by PTA-ELISA at a working concentration 0.1 μg of IgG/mL. The cross-reactivity of the prepared antibodies was tested against unpurified antigens of members of the family Tenebrionidae *Tribolium confusum* (Jacquelin Du Duval, 1868), *Tribolium destructor* (Uyttenboogaart, 1933), *Tenebrium molitor* (Linnaeus, 1758), stored-product mites *Acarus gracilis* (Hughes 1957), *Aleurogylphus ovatus* (Troupeau 1878), *Caloglyphus redickorzevi* (Zachvatkin, 1941) [syn. *Caloglyphus hughesi* (Samsinak, 1966)], *Tyrophagus putrescentiae* (Schrank 1781) and other insect species (*Cryptolestes ferrugineus* (Stephens, 1871), *Trogoderma granarium* (Everst 1898), *Sitophilus granaries* (Linnaeus 1758), *S. zeamais* (Motschulsky 1855), and *Ephestia kuehniella* (Zeller 1879) frequently found and suggested as important insect pests in grain and flour stores (*5, 32, 37*) in grain and flour stores. All these species originated from laboratory cultures maintained at Crop Research Institute, Prague. Calibration curves against the homologous antigen in the concentration range 1−10 μg of proteins/mL were constructed.

Preparation of Contaminated Grain Samples. Common wheat grain (*Triticum aestivum*, variety Nella) was cleaned by bleach to eliminate possible background infestation with arthropods from previous storage, which may have confounded the highly sensitive immunochemical assay. The grain was washed in the bleach (5%) for 30 min, rinsed with water on sieves (2-mm mesh), dried at 70 °C for 6 h, subsequently remoistened to moisture levels from 14.8 to 15%, and used for analyses.

For artificial contamination, adults at 14 day after emergence, 21 day old larvae, and eggs of *Tribolium castaneum* were collected from the rearing boxes. For the feces production, 100 specimens of the 21 day old larvae and adults were reared on 0.5 g oat flakes as a rearing diet in a Petri dish at 65% RH, 25 °C, darkness. The rearing diet was sieved (Retsch sieve 0.15 mm, Retsch, Haan, Germany) daily until the desired amount of sifting was extracted. The sifting was used as the feces fraction.

The contaminants including adults, larvae, and the feces fraction were counted or weighted and directly transferred into 25 g grain samples in plastic containers (Alichem, s.r.o., Chropne, Czech R). The numbers of contaminants recalculated per 1 kg of substratum was as follows: feces of fresh mass 0.08, 0.4, 0.8, and 2 g/kg; larvae 40, 120, 200, 320, 400, and 1000 individuals/kg; adults 330, 400, 1000, 1660, and 3330 individuals/kg. Three hours after contamination, the samples were frozen at −18 °C for 24 h and then processed for the immunochemical assay. For analyses of feces production, 25 g grain samples in plastic containers were infested with *Tribolium castaneum* larvae and adults in densities 3, 5, 8, and 10 individuals per grain sample in three replicates per density and life stage of contaminant. Plastic containers were kept at 25 ± 0.5 °C for 5 days. Subsequently, individuals were removed and samples processed by the same procedure as described for the detection of the feces in the grain (see below).

Immunochemical Detection of Contaminants in the Grain. The contaminated and control samples were homogenized by Ultra-Turrax T25 basic homogenizer in 25 mL of PBS (2 min, 17 500 rpm). After homogenization, the samples were extracted for 2 h at 4 °C, and the crude extracts were drained off and centrifuged (10 min, 4020*g*, 0 °C).

Based on preliminary data, the procedure to extract proteins from feces fraction was carried out as described above, except that the homogenization step was omitted. This procedure did not decrease the amount of proteins eluted from feces into the extraction buffer in comparison with homogenization.

The extracts from adults and larvae were applied in ELISA without dilution, while extracts from samples containing feces were diluted 1:19 in PBS. For evaluation of reliability and recovery of the detection of feces in grain, samples of feces fraction were extracted without grain in the extraction buffer only.

Data Analyses. The analyses were done in QC Expert 2.5 Trilobite statistical software (Trilobite, Pardubice, CZ) and XLStat2007 (Addinsoft Inc., Brooklyn, NY). Calibration methods (*20*) were used to calibrate the extraction procedure and determine its detection limits. These methods work with linear or quadratic regression and include an absolute coefficient even if it is not significant. The initial numbers of specimens or fresh mass of feces constituted the independent variable of the model, absorbance constituted the dependent variable.

The calibration methods provided the back estimation of x values for limits: (i) Instrument detection limit (IDL) is the threshold to which measured value is noise; (ii) limit of detection (LOD) is the lower bound

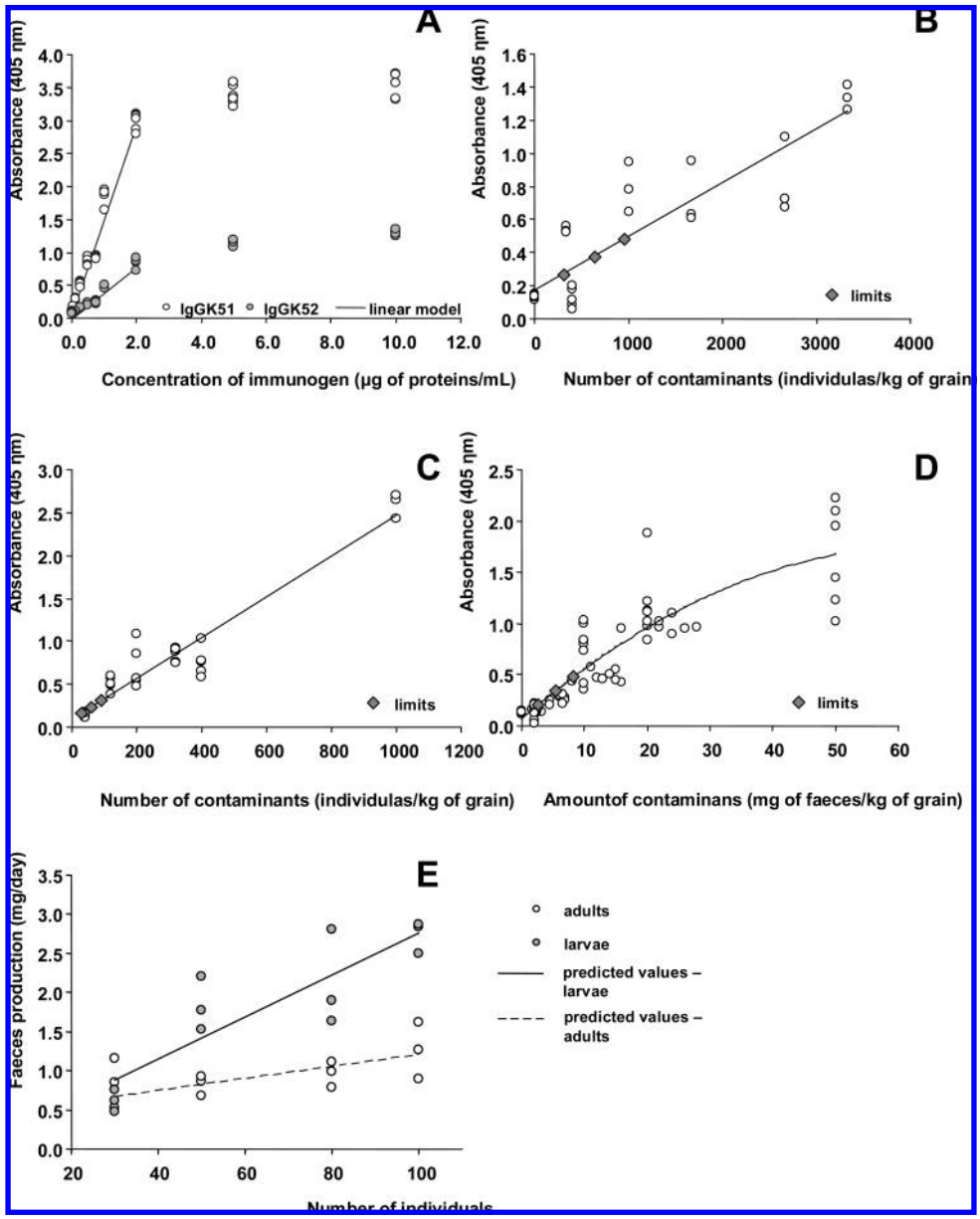

Figure 1. A. Sensitivity of PTA-ELISA with anti-*T. castaneum*K51 and anti-*T. castaneum*K52 to the protein antigen from *T. castaneum* larva; BCD, calibration models of PTA-ELISA with anti-*T. castaneum*K51 for the detection of *Tribolium castaneum* life stages in grain. Black lines indicate predicted values based on the calibration models. B, adults; C, larva; D, feces fraction. The limits of detection are in increasing order: instrument limit; detection limit; quantification limit. E, the application of PTA-ELISA with anti-*T. castaneum*K51 for quantification of feces production by larvae and adults of *T. castaneum* in grain after 5 days of feeding. Lines indicate predicted values based on linear regression.

from which positive samples are detected; (iii) limit of quantification (LOQ) is the lower bound from which reliable quantification of contamination is possible (*25*). These limits were estimated using K, σ from regression methods on a 5% probability level (*20*).

RESULTS

Characterization of Pabs. Two polyclonal antibodies (anti-*T. castaneum*K51 and anti-*T. castaneum*K52) were raised in rabbits against a purified protein antigen from larvae of *T. castaneum*. These antibodies were characterized by PTA-ELISA. At the working concentration of 0.1 μg of IgG/mL, PTA-ELISA response demonstrated that the assay can detect at least 100 ηg of proteins/mL (see **Figure 1**A). In the range of immunogen concentrations between 0 and 10 μg of insect proteins/mL, hyperbolic response curves were obtained for both antibodies. The linear response ($y = kx + q$) was observed in the range from 0 to 2 μg of insect proteins/mL (**Figure 1**). The linear model showed the following parameters and standard deviations; y = absorbance (405 ηm), x = immunogen concentration (μg protein/mL); $k = 1.448 \pm 0.034$; $q = 0.115 \pm 0.022$ and $k = 0.358 \pm 0.015$; $q = 0.064 \pm 0.007$ for anti-*T. castaneum*K51 and anti-*T. castaneum*K52, respectively. Based on calibration models, the detection limits for anti-*T. castaneum*K51 and anti-*T. castaneum*K52 were determined as 0.10 and 0.23 μg of immunogen proteins/mL, respectively. The more sensitive anti-*T. castaneum*K51 was more thoroughly characterized for the detection of the insect contamination.

The ability of the anti-*T. castaneum*K51 assay to detect larvae, adults, and feces of *Tribolium castaneum* was confirmed. The *in vitro* detection limit was estimated as 20−30 μg of fresh mass of larvae or adults/mL of the extraction buffer, which means that there is a potential for a positive detection of even one larva or adult. For feces detection, the PTA-ELISA showed a linear response in the range of concentration 0−30 μg of feces

Table 1. Specificity of PTA-ELISA with Pab Anti-*T. castaneum*K51 to Unpurified Antigens from to Members of Family Tenebrionidae and Other Arthropods[a,b]

species		concentration of IgG: 1 μg/mL; 1 μg/mL antigen	concentration of IgG: 1 μg/mL; 10 μg/mL antigen	concentration of IgG: 0.1 μg/mL; 1 μg/mL antigen	concentration of IgG: 0.1 μg/mL; 10 μg/mL antigen
Tribolium castaneum	immunogen	100	100	100	100
Tribolium castaneum	adults	32	41	19	30
Tribolium castaneum	feces	67	52	35	24
Tenebrium molitor	larva	17	42	8	23
Tenebrium molitor	adults	18	30	6	16
Tenebrium molitor	feces	32	44	15	14
Tribolium confusum	larvae	4	13	2	6
Tribolium confusum	adults	6	23	3	9
Tribolium confusum	feces	68	78	16	12
Tribolium destructor	larvae	39	63	17	47
Tribolium destructor	adults	65	72	38	60
Tribolium destructor	feces	57	59	32	22
Ephestia elutela	feces	nt	nt	0	4
Ephestia kuehniella	larvae	nt	nt	1	2
Plodia interpunctella	feces	nt	nt	1	4
Cryptolestes ferrugineus	larvae	nt	nt	3	9
Sitophilus granarius	adults	nt	nt	1	5
Sitophilus zeamais	adults	nt	nt	1	6
Trogoderma granarius	adults	nt	nt	1	4
Acarus siro	larvae	nt	nt	2	3
Aleuroglyphus ovatus	adults	nt	nt	0	1
Aleuroglyphus ovatus	feces	nt	nt	3	1
Calogylphus redickoverzi	adults	nt	nt	1	1
Caloglyphus redikoverzi	feces	nt	nt	2	1
Tyrophagus putrescentiae	adults	nt	nt	3	1

[a] Cross-reactivities were expressed as a percentage of the immunogen reactivity. Anti-*T. castaneum*K51 was used in the concentration 1 and 0.1 μg IgG/mL. [b] nt = not tested.

Table 2. Calibration Models of PTA-ELISA for Different Types of Contaminants of *T. castaneum*[a]

contaminant	coefficient	estimation	SD	lower limit	upper limit	R2	IDL	LOD	LOQ
adults	k	0.1557	0.0382	0.0776	0.2337	0.90	320	640	960
	q	0.003	3.07*10−5	0.0003	0.0004				
larvae	k	0.0899	0.0550	−0.0231	0.2028	0.96	64	128	192
	q	0.0024	0.001	0.0022	0.0027				
feces	a	−0.004	0.0330	−0.0007	−0.0001	0.90	1.96	3.99	6.09
	b	0.0520	0.0060	0.0410	0.0638				
	c	0.0710	0.0002	0.0045	0.1383				

[a] The coefficients of calibration models are $y = kx + q$ and $y = ax^2 + bx + c$ for linear and quadratic models, respectively. All coefficients were significant (P= 0.05) except for the absolute coefficient for adults. The back estimations for detection limits were calculated by K, σ from calibration models using linear regression and are the following: IDL, instrument detection limit; LOD, limit of detection; LOQ, limit of quantification.

fraction/mL; the detection limit equaled to 3.75 μg of feces fraction/mL. anti-*T. castaneum*K51 did not show significant cross-reactivity to antigens obtained from stored-product mite species, moths, and stored-product beetles out of the family Tenebrionidae (i.e., *Cryptolestes ferrugineus, Trogoderma granarium, Sitophilus granarius*, and *S. zeamais* (**Table 1**)). This Pab showed a wide range of cross-reactivity to the antigens prepared from the members of family Tenebrionidae (i.e., *Tribolium confusum*, *T. destructor*, and *Tenebrion molitor*), including adults, larvae, and feces fraction. For the feces fraction, cross-reactivity ranged from 12 to 32% with the similar values for *T. castaneum* and *T. destructor* in the both working concentrations of IgG tested.

Calibration of Assay for the Detection of Contamination in Grain. The anti-*T. castaneum*K51 enabled detecting *T. castaneum* adult, larvae, and feces in grain samples with various ranges and efficacy. The linear calibration models ($y = kx + q$) were obtained for detection of adults (**Figure 1**B) and larvae (**Figure 1**C), when y = absorbance (405 ηm), x = number of contaminants (individuals/kg of grain). The parameters and standard deviations of the linear model were summarized in **Table 2**. The detection limits were 60 and 640 individuals/kg for larvae and adults, respectively (see **Table 2**). The quadratic calibration model ($y = ax^2 + bx + c$) was obtained for feces detection (**Figure 1**D), when y = absorbance (405 ηm), x = number of contaminants (mg of feces fraction/kg of grain). The parameters and standard deviations were stated in **Table 2**. For detection of feces fraction in grain samples, the calibration model showed that there was a detection limit of 4 μg of feces fraction /mL in the grain samples that corresponded to 4 mg/kg of grain.

Application of Calibrated Assay for Detection of Feces Production. The ability to quantify feces contamination was confirmed in the grain infested by larvae or adults. After 5 days of their feeding in grain, the feces contamination was detected in all tested population densities. The feces production increased with increasing numbers of insect (**Figure 1**E), and the linear regression models were significant ($R^2 = 0.772$, $F = 33.8$, d.f. 1, 11, $P < 0.001$ and $R^2 = 0.317$, $F = 4.7$, d.f. 1, 11, $P = 0.05$ for larvae and adults, respectively). On the basis of calibration model for feces, the production of feces by adults and larvae was determined as 17.2 ± 8.3 and 27.7 ± 7.8 μg of feces/specimen/day, respectively.

DISCUSSION

Two polyclonal antibodies obtained against purified antigen from *Tribolium castaneum* larvae were assessed as highly sensitive, displaying a detection limit that demonstrated the potential for use in cereal insect pest detection, even when they occur in low abundance. The applicability of the immunotest for insect pest detection was confirmed, similar to previously described immunochemical methods (*7–9, 30, 33, 38*). For the assay with anti-*T. castaneum*K51, we did not observe cross-reactivity higher than 10% with stored-product insects out of family Tenebrionidae. Also nonspecific cross-reactivity to sample matrix was negligible as indicated from the values of the detection limit for feces *in vitro* and in artificially contaminated samples. These cross-reactivity parameters allow performing a quantitative assay without significant overestimation of target antigens for Tenebrionid pests (*13*).

The presence of live insects, their fragments, and their products in food is not acceptable in most countries of the world. In the USA, the Food and Drug Administration (FDA) has set the defect action level as the regulatory standard for quality control. For insect contamination, the defect action level is insect-damaged kernels per kg of wheat (*12*). GIPSA sets limits for contamination of grain which is 310 insect-infested kernels/kg of wheat. The detection limit for the assay with anti-*T.castaneum*K51 enables detection of larvae of *T. castaneum* according to both restrictions. In addition, this limit is comparable to a previously published study on immunochemical detections. Quinn et al. (*30*) described an immunochemical assay with antimyosin Pabs that allowed the measurement of insect contamination at the level corresponded to 28 ng of myosin/mL. Atui et al. (*6*) adopted this assay to detect the internal-feeding species *Rhyzopertha dominica*. They reliably detected 10 insects per kg. The detection limit of PTA-ELISA with anti-*T. castaneum*K51 to larvae reaching 60 individuals/kg is comparable to the results on *R. dominica*. The sensitivity of the myosin assay was sufficient to detect the presence of 20 individuals of *Sitophilus granarius* per kg (*30*).

In the comparison to the sieving, the obtained detection limit for *T. castaneum* adults (640 individuals/kg of grain) is not sensitive enough for practical applications. The artificially contaminated grain samples processed on a Retsch AS 240 digit shaker showed the detection limits about 1 individual/kg of grain for both adults and larvae (*15*).

The main advantage of anti-*T. castaneum*K51 is the ability to detect the feces at a level similar to the detection method for the mite *Acarus siro* in the grain (*16, 19*). The Pabs for detection of stored product mite *Acarus siro* were highly reactive to unknown compounds in the feces and enabled its detection at an extremely low infestation level (*16*). Similarly, anti-*T. castaneum*K51 recognizes antigens released from the *T. castaneum* feces. The detection limit of PTA-ELISA with anti-*T. castaneum*K51 for *T. castaneum* feces was determined as 4 mg/kg of grain. The production of feces per individual and day was weighed for one set of 100 individuals in ten replicates, and after recalculation, was 0.032 ± 0.005 and 0.017 ± 0.003 mg for 21 days old larvae and adults at 25 °C, respectively (unpublished observation). After recalculation, the detection limit of the ELISA assay for feces represented approximately 30 metabolically active larvae feeding 5 days in optimal conditions. The feces production of adults and larvae in grain samples determined by ELISA (**Figure 1**E) corresponded with results obtained by direct weighing.

The unwanted but often common practice for farmers is the cleaning of grain by sieving and removing pests with the dust fraction and or mixing contaminated grain with uncontaminated grain. In our laboratory simulation, we proved that optimized immunotechnique enables to detect the contamination after removing of adults and juvenile stadia of *T. castaneum*. In addition, the proteins presented in the feces are of high stability and resistance to decomposition by microbial or endogenous proteases (*18*). *Tribolium* species produce carcinogenic quinoles (*21, 28*) as well as other compounds, which change the taste and smell of food (*36*) and cause allergenic diseases (*2, 34*). Concerns are being raised because the feces are the main sources of allergens in synanthropic mites (*41*) and cockroaches (*22*). These compounds persist in substrate even after the removal of the arthropods. Therefore, the application of anti-*T.castaneum*K51 enables the traceability of *T. castaneum* contamination, especially as the metabolites of adults and larvae may persist in the grain even after the insects/larvae are removed. The described method is suitable for the traceability of *T. castaneum* in grain and should be improved for other storage commodities.

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