The Toxicity of Bean Flour (*Phaseolus vulgaris*) to Stored-Product Mites (Acari: Acaridida)

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Abstrakt

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Legume proteins were shown to have insecticidal activity against stored-product pests. Grain enriched by bean (*Phaseolus vulgaris*) flour inhibits the growth of stored-product mites. In this study, we tested the toxicity of bean flour to storage mites under optimal conditions for their population growth (i.e. rearing diet, temperature 25°C and humidity optimum 85% RH). Bean flour was added to the diet in one of eight concentrations: 0, 0.01, 0.1, 0.5, 1, 2.5, 5, 10%). The population growth of *Tyrophagus putrescentiae, Acarus siro* and *Aleuroglyphus ovatus* initiating from a density of 50 mites per 0.2 g of diet was recorded for 21 days. The enrichment of grain with bean flour suppressed the population growth of the tested species. These differed in their sensitivity to bean flour. Population growth was decreased to 50% in comparison to the control (rC_{50}) by the bean flour concentration of 0.02% in *T. putrescentiae,* 0.04% in *A. siro,* and by 4.87% in *A. ovatus.* The concentration of 5% bean flour in diets kept populations of *A. siro* and *T. putrescentiae* at the initial level. The results are discussed in the context of applying bean flour in the integrated control of stored-product mites.

Keywords: botanical acaricides; mite; storage; grain; food safety

Stored-product mites are abundant and frequent pests in cereal stores in the Czech Republic (Stejskal *et al.* 2003), UK, Canada and other countries. The main injurious aspect of these mites is the production of allergens that cause hypersensitivity not only in those who work with stored grain such as farm workers, millers and bakers, but they also seriously endanger the health of the general population (Arlian 2002). Mite allergens can cause anaphylaxis and anaphylactoid reactions after the ingestion of mite-infested food contaminated during processing (Matsumoto & Satoh 2004). The increase in the hypersensitivity of consumers to the allergens of stored-product mites requires intensive research focused on their reduction and elimination in grain. On a worldwide basis, the control of food industry and storage pests is still dominated by the use of organophosphate (OP) pesticides (White & Leesch 1996). Since some OP have been banned for food industry pest

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control, alternatives are needed (Collins 2006). Botanical acaricides represent compounds with high potential for suppression of stored-product mites (Czajkowska 1972).

We had found that the admixture of common bean (*Phaseolus vulgaris*) flour with wheat grain suppressed the population growth of five species of stored-product mites (Hubert *et al.* 2007). In this study, we focused on incorporation of various concentrations of bean flour into rearing diets and observed the suppressive effect on three species: *Acarus siro, Aleuroglyphus ovatus* and *Tyrophagus putrescentiae* (Acari: Acaridida).

MATERIAL AND METHODS

Stored-product mites

The species used in this study originated from laboratory cultures at the Research Institute of Crop Production. *Acarus siro* L. and *Tyrophagus putrescentiae* (Schrank) had been collected in a grain store at Buštěhrad, Czech Republic, in April 1996. *Aleuroglyphus ovatus* (Troupeau) was obtained from the Central Science Laboratory, Sand Hutton, York, UK.

The individuals were mass-reared in frit-chambers (S0-frit, Kavalier Sázava, Czech Republic) plugged by rubber pierced by a steel tube (5 mm diameter). Both ends of the tube were covered by a mesh. About 1.5 g of rearing diet was used in each frit. The frits were placed into desiccators boxes (Secador) at 85% RH and 25°C and kept in darkness.

Rearing and experimental diet. The rearing diet (100 g) consisted of 44 g oat flakes, 44 g wheat germs, 10 g lyophilised yeast and 2 g dried *Daphnia*; it was ground to a powder and sifted. The experimental diet contained common bean (*Phaseolus vulgaris*) that had been ground to a powder and added to the rearing diet in concentrations of: 0 (control), 0.01, 0.1, 0.5, 1, 2.5, 5 and 10% (wt/wt).

Experimental design

Glass tubes (20 ml volume, Kavalier® Sázava, Czech Republic) were filled with 3 g of zeolite. The zeolite was moistened by 0.15 ml of 1% solution of AJATIN® (KI and I2 in distilled water) to prevent fungal contamination. Each tube contained 0.2 g of the experimental or control diet and 50 mites were added. The mites were collected by camel hair brush from the surface of the rubber or sides of the rearing frit. The tubes were covered by a textile mesh and placed into a desiccator box (Secador®) at 85% RH and 25°C and kept in darkness during the experiment. Ten replicates per species and every concentration were used. The population growth was determined after 21 days by extraction of living mites in Berlese-Tullgren funnels. The mites were collected in a saturated solution of picric acid and counted under a stereomicroscope (Stemi 2000-C Zeiss).

Data analysis

All the analyses were done using Statistix® software. The correlation between population growth and bean flour concentration was tested by linear regression, when population growth was the dependent variable and bean flour concen-

Table 1. The effect of species and transformed bean flour concentration of final population density of stored-product mites based on linear regression

Data	Predictor variable	Coeficient	SD	Student's *t*-test	VIF	*P*	R^2	*F*	*P*
Unstadardised	constant	–512	83	–6		< 0.001	0.51	122	< 0.001
	LN (concentration)	–80	6	–12	1	< 0.001			
	species	313	34	9	1	< 0.001			
Standardised	constant	–22.5782	5.406	–4.18		< 0.001	0.56	149	< 0.001
	LN (concentration)	–6.75607	0.416	–16.22	1	< 0.001			
	species	12.6419	2.176	5.81	1	< 0.001			

SD – standard error; VIF – variance inflation factors (large VIF's in a multiple regression indicate that collinearity is a problem)

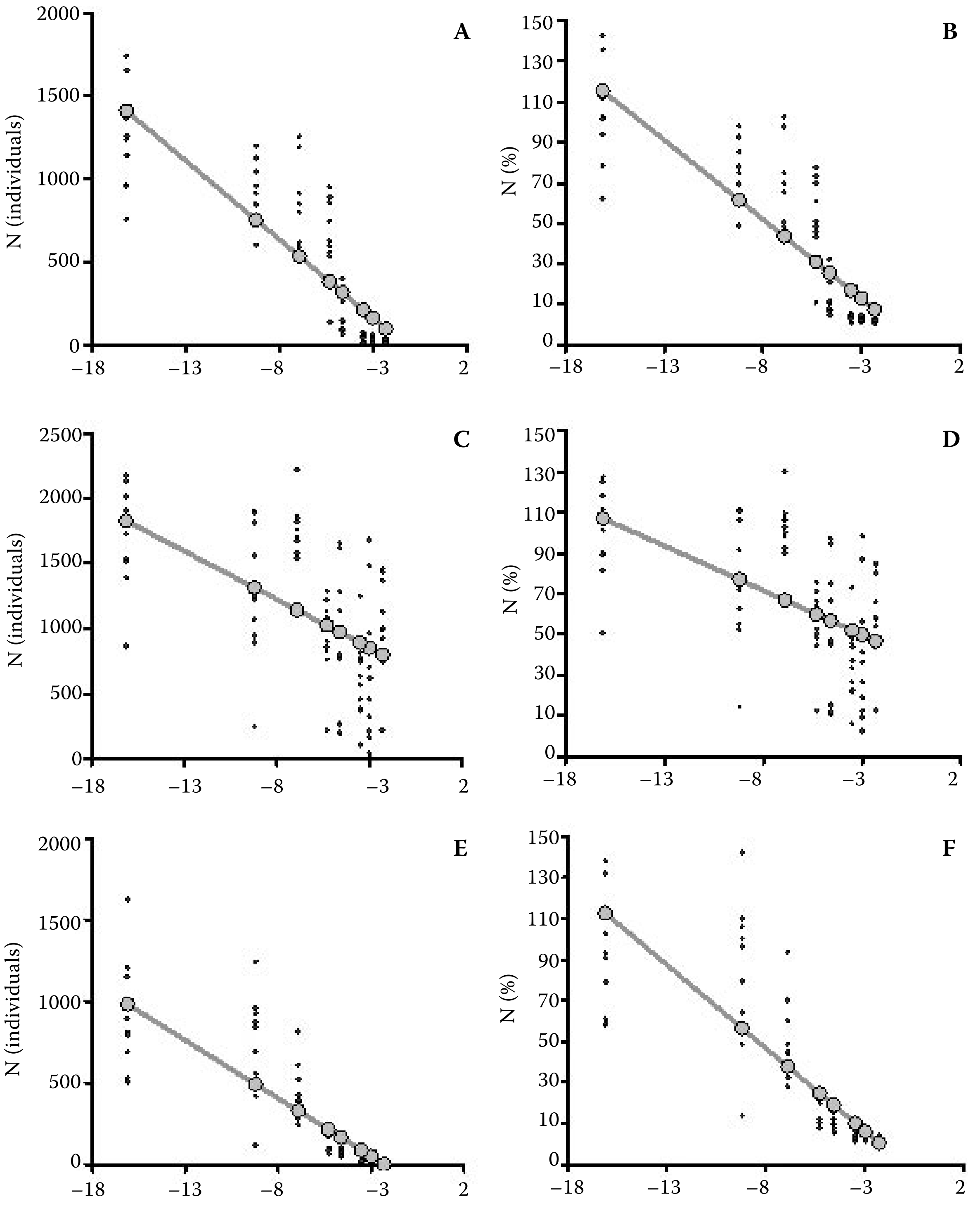

Figure 1. Effect of bean flour concentration on final density of tested mites. Density data are either non-standardised (ACE) or standardised by dividing all the densities within species by the mean density of that species in the control (no flour added) treatment (BDF). Bean flour concentration was log-transformed

AB – *Acarus siro*; CD – *Aleuroglyphus ovatus*; EF – *Tyrophagus putrescentiae*; N – final density; N (%) – relative final density (% of population growth from the control) tration the independent variable. We compared the un-transformed and log-transformed data on concentration in the test. The transformation was LN (bean flour concentration 1×10^{-7}). Furthermore, the following parameters were calculated from regression models testing log-transformed bean flour concentration of each concentration on each species separately: rC_{50} and rC_{90} – concentration for 50% and 90% decrease of population in comparison to control, rC_{100} – concentration without any increase of population, i.e. when the population is 50 individuals.

RESULTS

The addition of bean flour to the diet was toxic for the studied species (*Acarus siro, Aleuroglyphus ovatus* and *Tyrophagus putrescentiae*), as indicated by the suppression of their population growth (Table 1). The linear regression models including final density as dependent variable and transformed or untransformed bean flour concentration indicated a better fit for transformed bean flour concentration than untransformed as shown by the higher F value ($F_{(2, 235)} = 121$; $R^2 = 0.50$ and $F_{(2, 235)} = 59$; $R^2 = 0.33$). The final population was influenced by the effect of species, because their population growth differed. On control diets, the final density of mites decreased from *A. ovatus* to *Acarus siro* and *T. putrescentiae* (Figure 1). The data were standardised by recalculation to percent of control. The effect of species on standardised data was still significant after transformation (Table 1). It indicates the different sensitivity of the species to bean flour. The addition of bean flour in the range from 0.02 and 0.04% reduces population growth of *A. siro* and *T. putrescentiae* to 50% in comparison to the control (Table 2). *Aleuroglyphus ovatus* was the species least sensitive to bean flour, with 50% population growth observed at a concentration of 5% of bean flour.

DISCUSSION

In this study we confirmed the suppressive effect of bean flour on stored-product mites. Similar results have been reported for beetles after massive application of a pea (*Pisum sativum*) protein enriched fraction in stored grain (Fields 2006). Although our experiments were carried out under optimal abiotic (temperature and moisture) and nutritional conditions for population growth (Solomon 1946; Aspaly *et al*. 2005) of the tested mites, a concentration of 5% bean flour is able to suppress any population growth, thereby keeping mite population at the starting density (Table 2). The suppression of mites directly limits allergen production under the sensitisation level since all studied species are known as allergen producers (Arlian 2002). In comparison to chemical pesticides, the main advantage of bean flour is that it is a natural part of human food, and has no negative effect on final consumers. The legume flour proteins, such as lectins and digestive enzyme inhibitors, showed toxic potential to many species of stored-product beetles (Czapla 1997; Chrispeels 1997). Nevertheless, the exact mechanisms of toxicity of bean flour to stored-

Table 2. Effect of bean flour concentration on the final density of tested mite species, the results are based on analysis using transformed bean flour concentration

Species		Final density				Fitted concentration of bean flour (%)		
		coefficient	SD	pval	R^2	rC_{50}	rC_{90}	rC_{start}
Acarus siro	slope	–7.8	0.6	< 0.001	0.71	0.04	7.41	5.77
	constant	–10.3	4.4	0.022				
Aleuroglyphus ovatus	slope	–4.4	0.8	< 0.001	0.29	4.87	not-applicable	
	constant	36.7	5.9	< 0.001				
Tyrophagus putrescentiae	slope	–8.1	0.6	< 0.001	0.69	0.02	3.05	5.19
	constant	–18.4	4.7	< 0.001				

The following parameters were calculated from regression and describe the suppression effect of bean flour concentration; rC_{50} and rC_{90} – concentration for 50% and 90% decrease of population in comparison to control; rC_{start} – concentration without any increase of population; SD – standard error product mites have not yet been elucidated and need further studies.

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