



PERGAMON

Journal of Stored Products Research 37 (2001) 359–370

Journal of
**STORED
PRODUCTS
RESEARCH**

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Repellent effect of pea (*Pisum sativum*) fractions against stored-product insects

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Accepted 19 September 2000

Abstract

Peas (*Pisum sativum*) are toxic to some stored-product insects. The repellent effect of fractions of pea seed to stored-product insects was evaluated in multiple-choice tests in which wheat kernels were dusted with fractions rich in either protein, fibre or starch at 0.001 to 10% (wt:wt). There was a negative correlation between pea protein concentration and the number of adults found in grain for *Cryptolestes ferrugineus* and *Sitophilus oryzae*, but not for *Tribolium castaneum*. Pea fibre repelled *C. ferrugineus* adults but not *S. oryzae* and *T. castaneum*. Pea starch did not repel any of the insects. One-week old and 6-week old *C. ferrugineus* were equally repelled by pea protein. Repellency was detectable 1 h after exposure. *Cryptolestes ferrugineus* and *S. oryzae* did not become habituated to the repellent action of pea protein even after 4 weeks of exposure. Habituation was observed, however, when *C. ferrugineus* was exposed to pea fibre for 4 weeks. In a two-choice bioassay (0 vs. 0.1% and 0 vs. 1% pea protein), the pea-protein-treated grain had significantly fewer insects (*C. ferrugineus*, *S. oryzae*, *Sitophilus zeamais*, *T. castaneum*, and *Tribolium confusum*) than untreated grain. The properties of the pea protein fractions seem well suited for developing a natural stored grain protectant. Crown Copyright © 2001 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Sitophilus* spp.; *Tribolium* spp.; *Cryptolestes ferrugineus*; Behaviour; Pea

1. Introduction

Insects are a problem in stored grain throughout the world because they reduce the quantity and quality of grain (Sinha and Watters, 1985; Madrid et al., 1990). Synthetic residual insecticides and fumigation are the main methods of grain protection and pest control. However, increased

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public concern over the residual toxicity of insecticides applied to stored grain, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides calls for new approaches to control stored-product insect pests.

Higher plants are a rich source of novel insecticides (Arnason et al., 1989). The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Su et al., 1972; Su, 1977; Jilani and Su, 1983; Malik and Mujtabe-Naqvi, 1984; Weaver et al., 1991; Saxena et al., 1992; Regnault-Roger and Hamraoui, 1993; Golob et al., 1999). Extracts from the Indian neem tree (*Azadirachta indica* A. Juss. Meliaceae), such as azadirachtin, have received the most attention (Saxena et al., 1988; Jilani and Saxena, 1990). However, the extreme structural complexity of azadirachtin makes it very difficult to synthesize, which limits the source of azadirachtin solely to natural extracts. The neem tree is restricted to tropical areas and although the tree is fast growing it takes several years before azadirachtin can be extracted. It would be highly desirable to find alternative plant sources with simpler phytochemicals making them more amenable to synthesis, and that can be grown in temperate climates. If these phytochemicals could be extracted from a high-yielding annual crop, it would be economically attractive.

Legume seeds contain a wide range of allelochemicals with toxic and deterrent effects against insect pests (Harborne et al., 1971; Bell, 1978). An admixture of yellow split-peas (*Pisum sativum* L.) with wheat resulted in a marked reduction of survival and reproduction rate of *Sitophilus oryzae* (L.) (Coombs et al., 1977; Holloway, 1986). Recently, concentrations as low as 0.01% pea protein were shown to cause adult mortality and reduced reproduction for several stored-product insect pests (Bodnaryk et al., 1999; Delobel et al., 1999).

Dethier et al. (1960) defined insect repellents as chemical substances which cause the insect to make oriented movements away from the source of the substance. Repellents have potential for the exclusion of stored-product pests from grain, and have been used to prevent insect feeding and oviposition. There is a need for repellents which are effective, persistent and practical to use. Given that peas are toxic to most stored-product insects (Coombs et al., 1977; Holloway, 1986; Bodnaryk et al., 1999; Delobel et al., 1999), we investigated the repellency of pea fractions against some stored-product insects.

2. Materials and methods

2.1. Insects and test materials

Cryptolestes ferrugineus (Stephens) (rusty grain beetle) were reared on Canada Western Hard Red Spring wheat (cultivar Columbus) at 16% moisture content (m.c.) mixed with 10% cracked wheat (wt:wt). *Sitophilus oryzae* (L.) (rice weevil) and *S. zeamais* Motschulsky (maize weevil), were reared on wheat at 14% m.c. *Tribolium confusum* Jacquelin du Val (confused flour beetle) and *T. castaneum* (Herbst) (red flour beetle) were reared on unbleached wheat flour with 5% (wt:wt) brewer's yeast. All species had been kept in laboratory culture for over 3 years and were maintained at $30 \pm 1^\circ\text{C}$, $70 \pm 5\%$ r.h.

The three pea fractions (protein, fibre and starch) used in this study were provided by Parrheim Foods, Saskatoon, Saskatchewan, Canada. The pea seeds were dehulled to remove the fibre, milled, and the protein and starch granules were separated by air classification (Tyler et al., 1981).

The protein fraction contained 60% protein and 30% starch, the fibre fraction contained 90% fibre and 3% protein and the starch fraction contained 85% starch and 5% protein (analyses were done at the Department of Applied Microbiology and Food Science, University of Saskatchewan). All fractions had 7% m.c.

2.2. Multiple-choice bioassay

Pea fractions (protein, fibre and starch) were mixed thoroughly with Canada Western Hard Red Spring wheat at concentrations of 0, 0.001, 0.01, 0.1, 1.0 and 10.0% (wt:wt). Food preference chambers (Loschiavo, 1952) were used to conduct multiple-choice bioassays. The cylindrical brass chamber was 6 cm high, 30 cm diameter, with a raised platform (2 cm high, 10 cm diameter) in the centre of the chamber. The chamber was divided into six equal sections by brass partitions. Sections were randomly selected and filled with 200 g treated wheat grain. Two hundred unsexed adult beetles (1–2 week-old adult *T. castaneum*, *C. ferrugineus* and *S. oryzae*; species were tested separately) were introduced into the centre of the platform, confined by a brass ring (2.5 cm high, 5 cm diameter) and a circular black-painted plate was placed on the top of the chamber. After 1 h confinement, to allow beetles time to return to normal activity (Loschiavo, 1952), beetles were released by raising the brass ring without interrupting their activity. The chamber was held at $30 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ r.h. for 48 h. The content of each section was then gently vacuumed and the number of beetles in each was determined. The experiment was repeated four times.

2.3. Two-choice bioassay

The same general procedures used for the multiple-choice experiment were used with the two-choice bioassay. Wheat was mixed at either 0.1 or 1.0% (by weight) pea protein. The six sections were alternatively filled with treated or untreated wheat. Two hundred mixed-age, unsexed adults of each species (*T. castaneum*, *T. confusum*, *C. ferrugineus*, *Rhyzopertha dominica* (F.), *S. oryzae* and *S. zeamais*) were placed in the chamber as before and held for 24 h, before the distribution of the insects was assessed. The experiment was repeated twice. Each species was tested separately.

2.4. Insect age and test duration

To test the repellent effect of pea fractions (0, 0.001, 0.01, 0.1, 1.0 and 10.0% (wt:wt)) with respect to insect age and exposure time, two separate multiple-choice tests, based on insect age and exposure time, were conducted with pea protein and *C. ferrugineus*. Four age classes of insects, <1 week-old, 1–2 week-old, 3–4 week-old, and 5–6 week-old, were used. All test procedures were identical to those previously described. To test the rapidity of response, 200 unsexed adult beetles (1–2 week-old) were introduced into the test chamber, and counted after 0.5, 1, 2, and 4 h. Four replicates were used for each experiment.

2.5. Habituation test

Approximately 1000 adult *C. ferrugineus* (1–2 week-old) and *S. oryzae* (1–2 week-old) were placed on 3 kg of wheat kernels treated with 0.01% protein, or 0.01% fibre (for *C. ferrugineus*

only) for 4 weeks at $30 \pm 1^\circ\text{C}$, $70 \pm 5\%$ r.h. This concentration was selected because it caused approximately 20% mortality for both species (Bodnaryk et al., 1999), and also was repellent. After 4 weeks the insects were sifted out and used for a multiple-choice test as mentioned above.

2.6. Statistical analysis

Linear regression analysis was applied to define all dose–response relationships when correlation was found to be significant (Anonymous, 1991). A $\log_{10}(x + 0.0001)$ transformation was performed before the regression analysis (where x = pea fraction concentration (%)). Analysis of variance (ANOVA) was applied to the repellency data which were not significantly correlated with concentrations (SAS Institute Inc., 1990). Original data were transformed into arcsine square root percentage values before ANOVA tests. Analysis of covariance (ANCOVA) was used to test differences between regression coefficients (slope and intercept) (SAS Institute Inc., 1990).

3. Results and discussion

3.1. Multiple-choice bioassay

Wheat treated with pea protein repelled *C. ferrugineus* and *S. oryzae*. The number of insects in each section was negatively correlated with pea protein concentration (Fig. 1A and B). The correlation between protein concentration and number of insects in the grain was not significant for *T. castaneum* (Fig. 1C). However, if the data from 10% were deleted from the analysis, there is a significant negative correlation between protein concentration and number of insects ($r^2 = 0.93$, $P = 0.008$).

Pea fibre also repelled *C. ferrugineus*. The number of insects per section was significantly ($P = 0.004$) and negatively correlated with pea fibre concentration (Fig. 2A). In contrast, it did not repel *S. oryzae* and *T. castaneum* (Fig. 2B and C). Pea starch did not show any repellent effect on the three insect species tested (Fig. 3). Furthermore, *T. castaneum* was actually attracted, having significantly more insects in the sections treated with either 10% fibre, or 10% starch than at lower concentrations (ANOVA, $P < 0.05$) (Figs. 2C and 3C). *Tribolium castaneum* was reared on wheat flour and may be attracted to the 10% pea flour concentration because it is physically similar to wheat flour.

The strong repellency of pea protein fractions in this study was reflected by reduced numbers of insects on treated wheat. We presumed that this reduction was caused by chemosensory effects of the fractions, either olfactory or gustatory. Peas (Coombs et al., 1977; Holloway, 1986; Grenier et al., 1997), pea fractions (Bodnaryk et al., 1999) and proteins purified from peas (Delobel et al., 1999) can be toxic to stored-product insects. For the three pea fractions, the rank order was the same for toxicity and repellency, with protein greater than fibre, fibre greater than starch. For a given species, the repellency of the fractions observed in this study was directly related to the mortality they caused. There were significant correlations between toxic effect (mortality) (Bodnaryk et al., 1999) and repellent effect (Fig. 1A and B; Fig. 2A) for protein with *C. ferrugineus* ($r^2 = 0.80$, $P = 0.04$), and for protein with *S. oryzae* ($r^2 = 0.84$, $P = 0.01$), as well

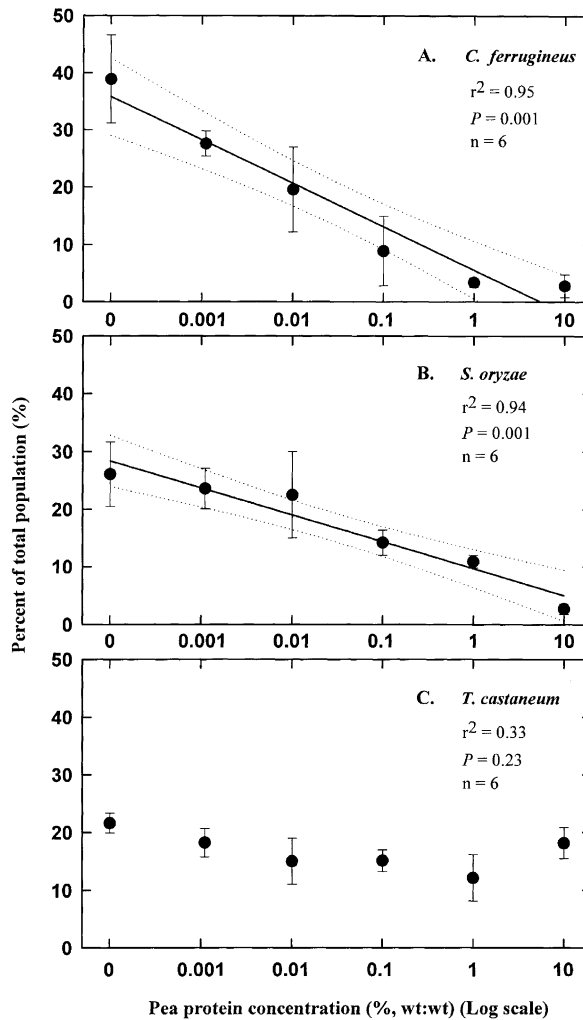


Fig. 1. Number of adult *C. ferrugineus* (A), *S. oryzae* (B) and *T. castaneum* (C) in wheat treated with various concentrations of pea protein under multiple-choice condition. Dotted lines represent 95% confidence intervals. Bars indicate standard deviation (SD) of observations.

as for fibre with *C. ferrugineus* ($r^2 = 0.70$, $P = 0.04$). However, this was not the case for protein with *T. castaneum*; protein (10%) caused over 60% mortality for *T. castaneum* after 2 weeks (Bodnaryk et al., 1999), even though it was not repellent.

Under an identical test procedure, repellency and toxicity of extracts from the neem tree (*Azadirachta indica*) against three stored-product beetles (*C. ferrugineus*, *S. oryzae* and *T. castaneum*) were correlated (Xie et al., 1994). Some pyrethroids are both toxic and repellent to the two-spotted spider mite, *Tetranychus urticae* Koch (Penman et al., 1986). However, a close relation between repellency and toxicity is not always the case, even for natural plant products. Talukder and Howse (1993) found that pithraj (*Aphanamixis polystachya* Wall and Parker) seed

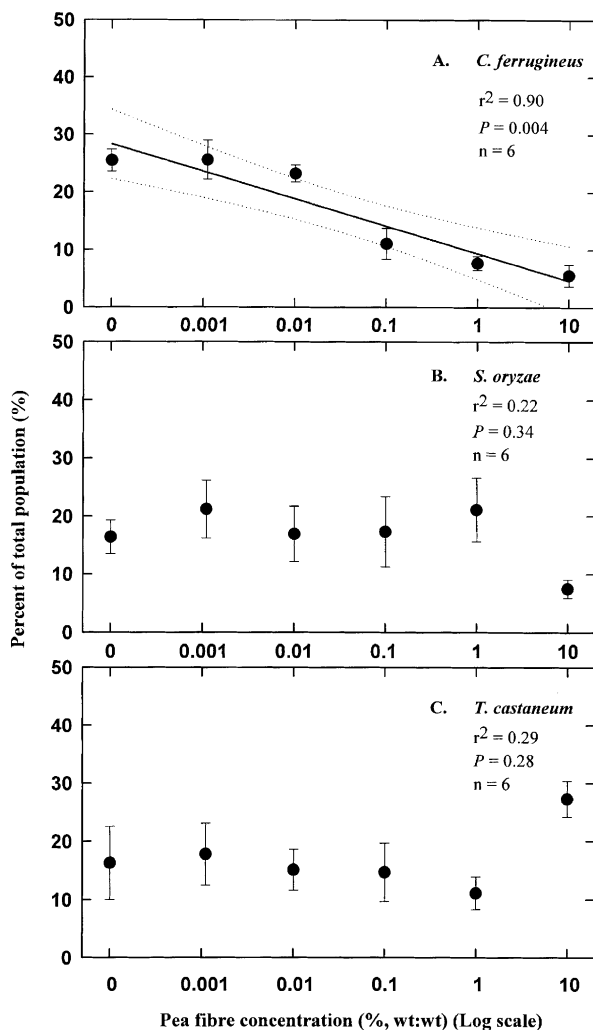


Fig. 2. Number of adult *C. ferrugineus* (A), *S. oryzae* (B) and *T. castaneum* (C) in wheat treated with various concentrations of pea fibre under multiple-choice conditions. Dotted lines represent 95% confidence intervals. Bars indicate standard deviation (SD) of observations.

extract was highly toxic to the pulse beetle, *Callosobruchus chinensis* (L.), but was a weak repellent for this insect. Both the toxicity and repellency of pea fractions make these materials good candidates as stored-grain protectants.

3.2. Two-choice bioassay

As in the multiple-choice tests, *C. ferrugineus* and *S. oryzae* were repelled by the pea protein when given a choice between treated or untreated grain (Table 1). The other insects tested were

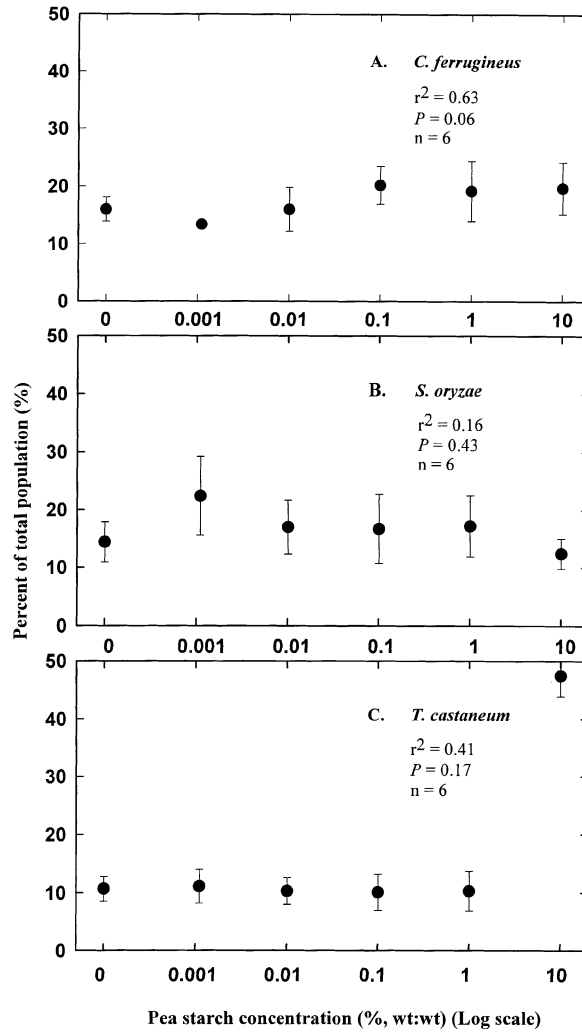


Fig. 3. Number of adult *C. ferrugineus* (A), *S. oryzae* (B) and *T. castaneum* (C) in wheat treated with various concentrations of pea starch under multiple-choice condition. Bars indicate standard deviation (SD) of observations.

also repelled by pea protein, except for *R. dominica* at 1.0%. Only *S. oryzae* was repelled more by the higher concentration.

The two-choice bioassay is the more common method of testing repellency (Jilani and Su, 1983; Jilani and Saxena, 1990; Malik and Mujtabe-Naqvi, 1984), but the multiple-choice test allows for testing several concentrations at a time. Both methods would mimic typical storage conditions. In bulk storage, grain treated with a product during augering has a wide variation in concentrations (White et al., 1986) and would be closer to the conditions in the multiple-choice experiment. With package goods, whether it is 50 kg sacks of grain or 500 g breakfast cereal boxes, there would be situations that are closer to a two-choice test, when treated goods or packages are placed beside untreated ones.

Table 1

Repellency of 0.1 or 1.0% pea protein to adults of various insect species after 24 h. The two concentrations were run independently

Species	Insects (%) found in sections ^a						0.1 vs. 1.0 ^b
	0.1% protein			1.0% protein			
	Untreated	Treated	<i>p</i>	Untreated	Treated	<i>p</i>	
<i>C. ferrugineus</i>	61	39	***	67	33	***	n.s.
<i>S. oryzae</i>	61	39	***	69	31	***	*
<i>S. zeamais</i>	56	44	*	62	38	***	n.s.
<i>T. castaneum</i>	68	32	***	72	28	***	n.s.
<i>T. confusum</i>	65	35	***	64	36	***	n.s.
<i>R. dominica</i>	56	44	*	50	50	n.s.	n.s.

^aSignificantly different from 50% in untreated and treated (chi-squared test; $p > 0.05 = \text{ns}$, $p \leq 0.05 = *$, $p < 0.001 = \text{***}$).

^b0.1 significantly different from 1.0% (z -test; $p > 0.05 = \text{ns}$, $p \leq 0.05 = *$).

Table 2

Parameters of the regression of age of adult *C. ferrugineus* versus repellency of pea protein^a

Insect age (week)	Intercept ^b	Slope ^b	R^2	P
<1	7.46 a	-6.16 a	0.96	0.0006
1–2	5.58 a	-7.57 a	0.95	0.0011
3–4	8.52 a	-5.44 a	0.86	0.0076
5–6	7.81 a	-5.34 a	0.90	0.0035

^aLinear regression performed after a logarithmic transformation [$\log_{10}(x + 0.0001)$, where x = pea protein concentration].

^bFigures followed by the same letters within a column indicate no significant difference ($P > 0.05$) in the ANCOVA test.

3.3. Insect age and test duration

The age of *C. ferrugineus* (from <1 to 5–6 weeks old) did not affect the repellency of pea protein. ANCOVA indicated that all intercepts and slopes were not significantly different ($P > 0.05$), therefore *C. ferrugineus* of different ages had a similar response to the repellency of pea protein (Table 2).

Sensitivity of insects to insecticidal activity of chemical substances, either repellency, deterrence, or toxicity, frequently appears inversely related to insect age or development stage (Isman et al., 1989). However, this was not the case with pea protein acting on *C. ferrugineus*. An equal repellent activity of pea protein was observed against *C. ferrugineus* of all ages (Table 2).

After 0.5 h exposure to pea protein-treated wheat kernels, adult *C. ferrugineus* was almost equally distributed in all sections (Fig. 4A). However, after 1 h, *C. ferrugineus* was significantly repelled by pea protein-treated wheat (Fig. 4B). As exposure duration increased, the linear

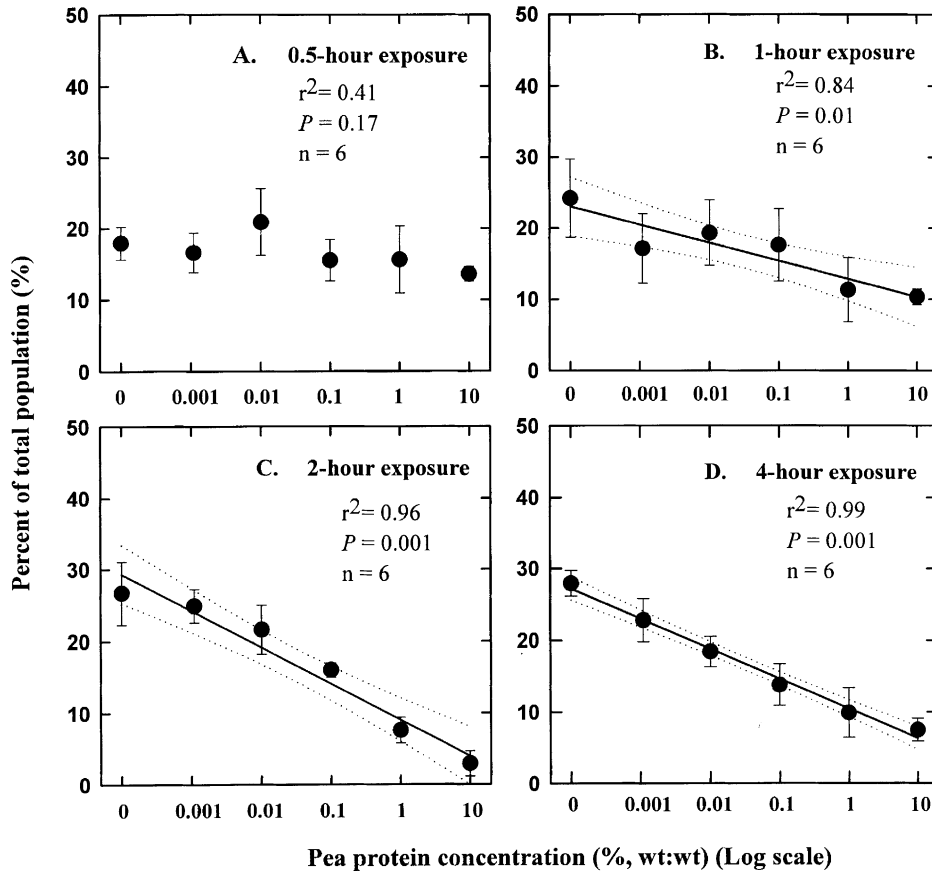


Fig. 4. Number of adult *C. ferrugineus* in wheat treated with various concentrations of pea protein after 0.5 h (A), 1 h (B), 2 h (C), and 4 h (D) exposure under multiple-choice conditions. Dotted lines represent 95% confidence intervals. Bars indicate standard deviation (SD) of observations.

relationship between repellency and concentration was increased (coefficient of determination from 0.41 to 0.99) (Fig. 4), although ANCOVA indicated that slopes for 1, 2, and 4 h exposure were not significantly different ($P > 0.05$). In contrast, Talukder and Howse (1993) found there was a rapid drop in repellency of pithraj plant extract against *T. castaneum* as exposure increased (from 1 to 5 h). Jilani and Saxena (1990) believe that repellency of compounds with low molecular weights and high volatility decreases rapidly over time. The fact that the repellent action of pea protein occurred after insect exposure as short as 1 h (Fig. 4), as well as the fact that the repellency of pea protein was not related to insect age (Table 2), suggest that pea protein would be a useful grain protectant.

3.4. Habituation test

After 4 weeks feeding on wheat treated with pea protein (0.01%), *C. ferrugineus* and *S. oryzae* were still repelled by pea protein. The responses were the same as those of insects that had never

been exposed to pea fractions (Fig. 5A and B). In contrast, *C. ferrugineus* adults lost sensitivity to the repellent action of pea fibre after being held on fibre-treated wheat (0.01%) for 4 weeks (Fig. 5C).

Habituation of insects to repellents and/or antifeedants is one of the important problems concerning their practical application (Schoonhoven, 1982; Jermy, 1990). This is not the case with pea protein after 4 weeks exposure (Fig. 4A and B). However, more extensive tests would have to be conducted to determine if habituation did occur under longer-term field conditions. In contrast, habituation of *C. ferrugineus* would be likely to occur with pea fibre.

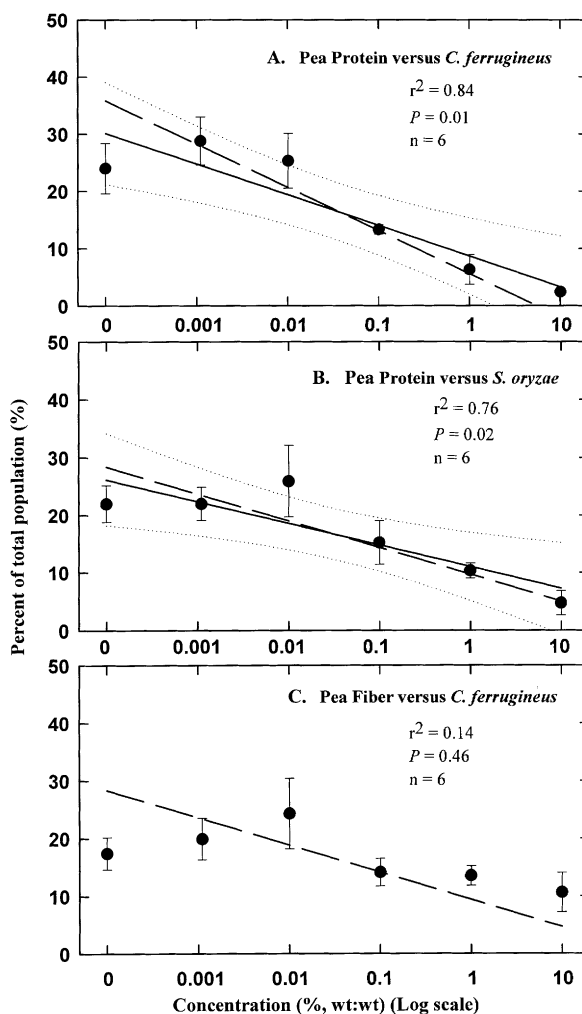


Fig. 5. Number of adult *C. ferrugineus* and *S. oryzae* in wheat treated with various concentrations of pea protein or pea fibre. Adults were held on wheat treated with 0.01% pea protein or pea fibre for 4 weeks before conducting multiple-choice test of corresponding pea fraction. Dashed lines represent regression lines for insects without adaptation (data from Figs. 1 and 2). Dotted lines represent 95% confidence intervals. Bars indicate standard deviation (SD) of observations.

The diverse behavioural and physiological effects of the pea fractions, such as repellency shown in this study, and toxicity, oviposition deterrence, and reproduction inhibition (Bodnaryk et al., 1999) suggest the active principles may constitute a complex of chemicals. Delobel et al. (1999) have isolated a 37 amino acid protein from pea seed that is toxic to *Sitophilus* spp. However, they have not determined if it is also repellent to stored-product insects. The isolation and identification of the active substances from the pea fractions are currently under investigation in our laboratory.

Acknowledgements

We thank Roy Jenkins and Tannis Mayert (Agriculture and Agri-Food Canada) for technical assistance, and R.P. Bodnaryk and P. Palaniswamy (Agriculture and Agri-Food Canada) for reviewing the manuscript. This project would not have been possible without Ken Fulcher of Parrheim Foods, Saskatchewan, Canada, whose initial observations on the insecticidal nature of pea fractions led to this work.

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