

Granary Trial of Protein-Enriched Pea Flour for the Control of Three Stored-Product Insects in Barley

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ABSTRACT A granary trial was conducted to evaluate the efficacy of protein-enriched pea flour against three common stored-grain insects, *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), and *Cryptolestes ferrugineus* (Stephens). Six 30-t farm granaries were filled with ≈11 t of barley. The barley was either not treated, treated with protein-enriched pea flour at 0.1% throughout the entire grain mass, or treated at 0.5% throughout the top half of the grain mass. Adult insects were released in screened boxes (two insects per kilogram barley for *S. oryzae* and *T. castaneum*; 1.4 insects per kilogram barley for *C. ferrugineus*). Barley was sampled four times during the 70-d trial. The number and mortality of adults and emerged adults in the samples were noted. Four kinds of traps, flight, surface-pitfall, probe-pitfall, and sticky-bar, were placed at different locations in the granaries to estimate the movement of insects. The 0.1% protein-enriched pea flour treatment reduced adult numbers of *S. oryzae* by 93%, *T. castaneum* by 66%, and *C. ferrugineus* by 58%, and reduced the emerged adults by 87, 77, and 77%, respectively. Treating the top half of the barley with 0.5% protein-enriched pea flour had similar effects as treating the entire grain mass with 0.1% pea-protein flour. However, the top-half treatment failed to prevent insects from penetrating into the untreated lower layer. Differences between traps are discussed.

KEY WORDS *Sitophilus*, *Cryptolestes*, *Tribolium*, botanical insecticides, peas, traps

STORED-PRODUCT INSECT PESTS cause losses by directly reducing dry weight, germination, nutritional value, or the grade of harvested grain. The Food and Agricultural Organization of The United Nations estimates that 5–10% of harvested grain is lost in storage, with losses being higher in some developing countries (Hall 1970).

Synthetic insecticides, such as deltamethrin, malathion, chlorpyrifos-methyl, phosphine, and methyl bromide, are the main means to control stored-product insects (Harein and Davis 1992, Arthur 1996). However, chemical residues, insect resistance, and worker safety concerns have increased the interest in alternative control methods. Resistance to malathion, phosphine, and deltamethrin has been reported in several stored-product insects (Zettler and Cuperus 1990, Subramanyam and Hagstrum 1995). In the United States, the use of chlorpyrifos-methyl may be discontinued because of a voluntary cancellation caused by the high cost of updating its registration data (Anonymous 2000). Methyl bromide depletes the ozone and may be banned after 2005 in most indus-

trialized countries (Fields and White 2002). Hence, there is a pressing need to develop new insecticides to protect stored products that are effective and safe for humans and the environment.

Most stored-product insects are unable to develop on legumes (Singh and Wilbur 1966, Sinha and Watters 1985). In Africa, mixing legumes with the grain is used to protect maize from stored-product insect attack. Admixing yellow-split peas with grain was suggested by Coombs et al. (1977) to control *Sitophilus oryzae* (L.). Delobel et al. (1999) reported that a small polypeptide from peas is insecticidal. Protein-enriched pea flour is toxic (Bodnaryk et al. 1999) and repellent (Fields et al. 2001) to many stored-product insects. *Sitophilus* spp. are the most sensitive insect pests, followed by the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) and the red flour beetle, *Tribolium castaneum* (Herbst).

As a human food and as a protein additive for animal feed, pea protein is well suited as a natural stored-grain protectant (Fields et al. 2001). The goal of this study was to determine if the repellent and toxic properties of protein-enriched pea flour are sufficient to reduce the populations of *S. oryzae*, *C. ferrugineus*, and *T. castaneum* in barley under farm storage conditions.

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Materials and Methods

Granaries

Trials were conducted at Agriculture and Agri-Food Canada's experimental farm at Glenlea, Manitoba (48° 38'N, 97° 09'W) from 17 August to 2 November 1999. Each of six 30-t capacity galvanized bolted-steel farm granaries, with fully perforated floors and a 25-cm-high subfloor plenum, were filled with ≈ 11 t of barley (two truck-loads) with $12.9 \pm 0.02\%$ moisture content, 629.2 ± 0.8 kg/m³ bulk density, and $0.69 \pm 0.07\%$ dockage on 17–19 August 1999. The barley (six-row) was harvested on 15 August and temporarily stored in other granaries. Protein-enriched pea flour (Progress Protein; 60% protein, 30% starch, 7% moisture content; Parrheim Food, Saskatoon, SK) was manually shaken into the barley at the base of an 18-cm-diameter auger running at a rate of 0.5 t/min. There were three treatments: untreated barley (control), all barley in a granary treated with 0.1% (wt:wt) protein-enriched pea flour, and only the top half of the barley in the granary treated with 0.5% (wt:wt) protein-enriched pea flour with the bottom half untreated. There were two granaries for each treatment.

Insects

Three insects, *S. oryzae*, *T. castaneum*, and *C. ferrugineus*, were reared in the laboratory at 30°C, 70% RH. All three insects had been cultured in the laboratory for over 5 yr. *S. oryzae* was reared on whole kernels of wheat, *T. castaneum* on wheat flour mixed with 5% brewer's yeast, and *C. ferrugineus* on wheat kernels with 5% wheat germ and 5% brewer's yeast. The *C. ferrugineus* population was a mixture of laboratory strain and a population collected in April 1999 at Glenlea, Manitoba.

Sitophilus oryzae and *T. castaneum* were released at the rate of ≈ 2 insects per species per kilogram of barley (22,000 insects per species per granary), and *C. ferrugineus* was released at ≈ 1.4 insects per kilogram (15,000 insects per granary). Before their release, all insects were sieved out of their rearing medium and 4,400 *S. oryzae*, 4,400 *T. castaneum*, and 3,000 *C. ferrugineus* were placed together in 4-liter jars containing 2.5 kg of untreated barley taken from the granaries. In each granary, insects were released into five boxes (30 × 30 × 30 cm) with a screened floor (2-mm² openings) and an open top. These five boxes were buried in the top layer of grain at the sampling points (Fig. 1) so that the top edge of the box was level with the top surface of the leveled grain mass. The screened bottom and open top allowed insects to move freely between the release boxes and the grain mass. Insects were released into the boxes on 23 August 1999. After emptying one jar with insects, the box was filled with untreated barley to the top edge of the box. The boxes were removed 10 d after insects had been released. The mortality and number of insects remaining in the boxes was assessed after the grain was sieved with a Carter dockage tester (Simon-Day, Winnipeg, Manitoba, Canada) with a No. 8 sieve.

Temperature

A data logger (CR10; Campbell Scientific, Edmonton, Alberta, Canada) was used to record temperature. In each granary, 12 thermocouples were placed in the grain mass at three depths and four locations per depth (Fig. 1). A thermocouple was located 5 cm above the center of the grain surface to measure the air temperature of the head space. Temperatures were checked every 12 min, and the hourly means were recorded. Average of daily temperature was used for the data analysis.

Grain Sampling

Grain samples of ≈ 1 kg per sampling location were taken from each of 10 points (Fig. 1) in each granary, five at the surface and five at 1 m below the surface, on 9 September and 23 September and 7 October and 21 October 1999 (17, 31, 45, and 59 d after insect release). A 0.3-liter torpedo probe sampler was used for the 1-m samples, and a 1-liter cup was used for the surface layer samples. In the laboratory, adult insects were removed from each sample with a sieve (2-mm² openings). Species and number of live and dead insects were recorded. After adult insects were removed, grain samples and sieved dust and broken kernels were placed in a 4-liter jar and held at 30°C, 70% RH for 5 wk. The grain was sieved a second time, and the number of live and dead emerged adults was counted to estimate the mortality of second-generation adults.

Physical Characteristics

Moisture content, dockage, and bulk density of barley samples were measured three times during trials. The first sampling was from truckloads before treatment with protein-enriched pea flour and augering into granaries. Four 1-kg barley samples were randomly taken from each truckload. The second sampling was on 19 August 1999 in granaries after treatment with protein-enriched pea flour but before the release of insects. Finally, the third sampling was on 2 November 1999 in granaries at the end of the trial. In the granaries, sampling was done at 10 locations (Fig. 1).

Traps

Insect movement was monitored with surface-pitfall, flight, probe-pitfall, and sticky-bar traps that were placed around the grain mass (Fig. 1). Flight traps were made with 25-cm-diameter paper plates covered with TangleFoot (The Tanglefoot Company, Grand Rapids, MI) and vertically placed 20 cm above the grain surface. Surface-pitfall traps (Storgard Flit-Trak M²; Trécé, Salinas, CA) were filled with 10 ml corn oil and placed on the surface of the grain. The flight traps and surface-pitfall traps were used to estimate the movement of insects in the head space and on the surface of the grain. Probe-pitfall traps (Storgard WB

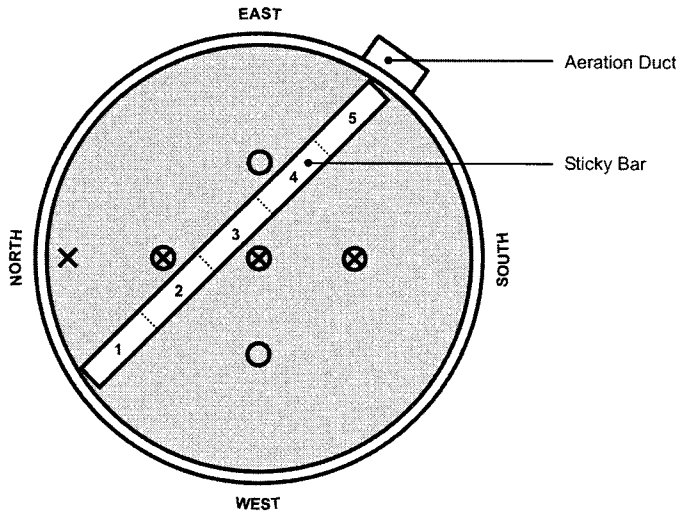
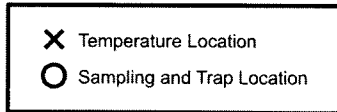
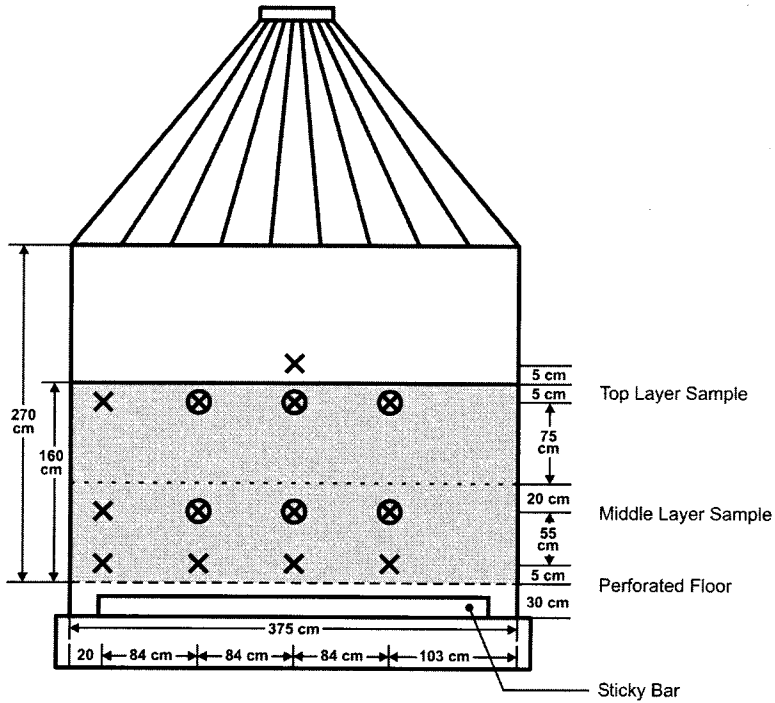


Fig. 1. Diagrams of the arrangement of thermcouple wires, sampling points, and traps in the granaries. Insects were released at the five sampling points in the top layer of grain.

Probe II; Trécé) were inserted into the grain just below the top surface of the grain and to depth of 1 m from the top surface at each sampling point to measure

the movement of insects within the grain mass. To measure the tendency of insects to leave granaries through the bottom, a wooden bar (9 cm width × 330

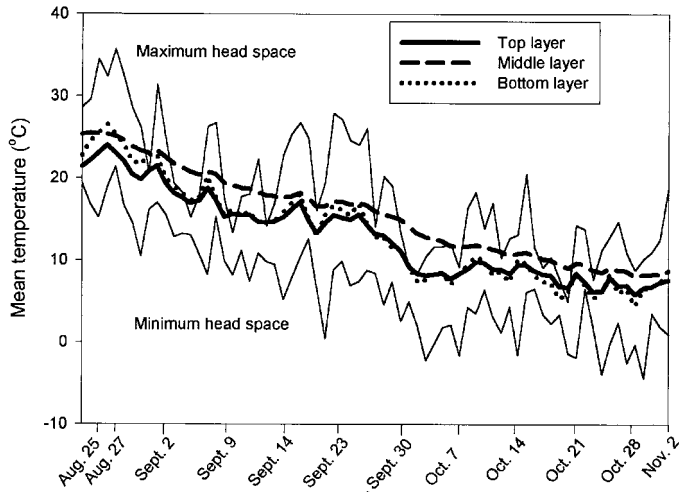


Fig. 2. Grain temperatures in the top layer (5 cm below grain surface), middle layer (100 cm below grain surface), and bottom layer (5 cm above the granary floor), and maximum and minimum air temperatures (5 cm above grain surface) in the granaries filled with 11 t barley.

cm length) covered with TangleFoot was placed underneath the perforated floor of the granaries through the aeration duct. The length of wood was marked into five equal sections, each with an area of 594 cm² (Fig. 1). Insects in all traps were recorded and emptied once a week. For probe-pitfall traps, the number of live and dead insects was recorded.

Data Analysis

Data were analyzed using the PROC GLM procedure (SAS Institute 2000). We used mean daily temperatures to compare layers and treatments. All data for the number of insects in grain samples, release boxes, and traps were expressed as number of insect per kilogram or per trap before being transformed with logarithm of $x + 0.0001$, except for the number of *S. oryzae* emerged adults, which was transformed with the square root of $x + 1$. Mortality data were transformed using arcsine square root. A two-way analysis of variance (ANOVA) was used to test for variation in bulk density, moisture content, and dockage among treatments. The means of treatments were linearly tested with SAS CONTRAST ($\alpha = 0.05$).

Results

Grain Temperature

Grain temperature was the same in all granaries ($F = 0.19$; $df = 5, 432$; $P = 0.9664$) and the same for all treatments ($F = 0.04$; $df = 2, 216$; $P = 0.9603$). Therefore, the grain temperatures of the six granaries were pooled. Middle layer temperatures ($15.9 \pm 0.7^\circ\text{C}$) were significantly different from top layer ($13.3 \pm 0.6^\circ\text{C}$) and bottom layer temperatures ($13.6 \pm 0.7^\circ\text{C}$; $P < 0.05$). There was no difference between top layer and bottom layer ($P > 0.05$; Fig. 2). Grain temperatures declined during the trials from ≈ 25 to 5°C . Av-

erage grain temperature of all granaries was $14.3 \pm 0.7^\circ\text{C}$ during the trial, ranging from 34.8 to -1.1°C . Before 30 September, the average head-space daily maximum temperature was $23.6 \pm 1.0^\circ\text{C}$, ranging from 35.7 to 13.4°C , and the minimum temperature was $11.5 \pm 0.7^\circ\text{C}$, ranging from 22.3 to 0.5°C . After 30 September, the average daily head-space maximum temperature was $12.1 \pm 0.6^\circ\text{C}$, and the minimum temperature was $1.7 \pm 0.5^\circ\text{C}$ (Fig. 2).

Grain Samples

Number of Live Insects. Number of live *S. oryzae* in the grain samples treated with 0.1% protein-enriched pea flour (0.12 ± 0.07 insects/kg) or with the top-half treated 0.5% protein-enriched pea flour (0.17 ± 0.07 insects/kg) was significantly lower than in untreated granaries (1.67 ± 0.36 insects/kg; $F = 17.24$; $df = 2, 30$; $P < 0.0001$), but there was no difference between the two pea flour treatments ($F = 0.71$; $df = 1, 30$; $P = 0.4068$; Fig. 3A). Similar differences were seen in the number of live emerged adults of *S. oryzae* ($F = 27.79$; $df = 2, 30$; $P < 0.0001$; Fig. 4A).

Number of live adult *T. castaneum* in the 0.1% pea flour treated granaries (0.28 ± 0.08 insects/kg) was significantly lower than in untreated granaries (0.83 ± 0.17 insects/kg; $F = 8.52$; $df = 1, 30$; $P = 0.0066$) but was not different from those in the 0.5% pea flour top-half treated granaries (0.42 ± 0.13 insects/kg; $F = 0.95$; $df = 1, 30$; $P = 0.3363$; Fig. 3B). This difference occurred because more insects were found in the middle layer in treated granaries than in the untreated controls ($F = 6.47$; $df = 2, 12$; $P = 0.0124$). Number of live *T. castaneum* emerged adults in 0.1% pea flour treatment (0.87 ± 0.6 insects/kg) and 0.5% pea flour top-half treatment (1.24 ± 0.48 insects/kg) was lower than in the untreated granaries (3.71 ± 2.09 insects/kg; $P < 0.05$), but no difference was seen between the

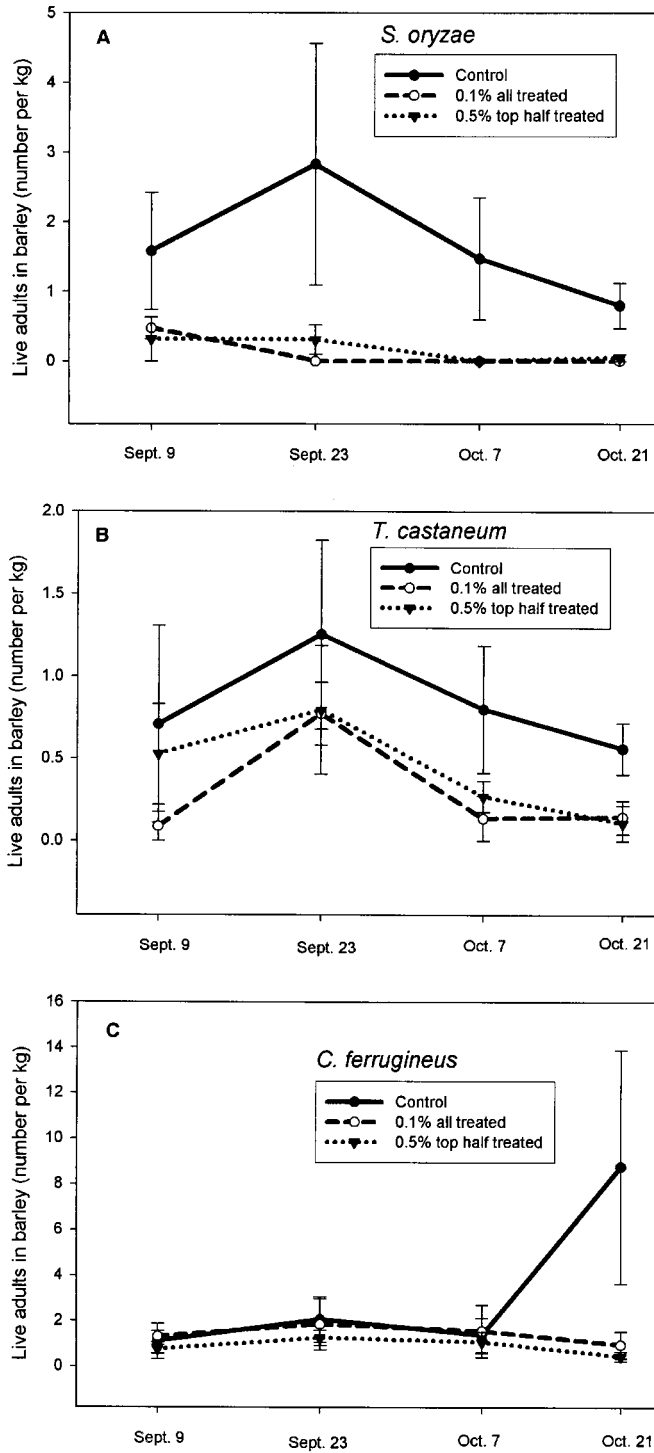


Fig. 3. The number of live adults in barley on different sampling dates from granaries untreated or treated with protein-enriched pea flour at 0.1% with all the grain treated or 0.5% with the top half of the bulk treated. (A) *S. oryzae*; (B) *T. castaneum*; and (C) *C. ferrugineus*.

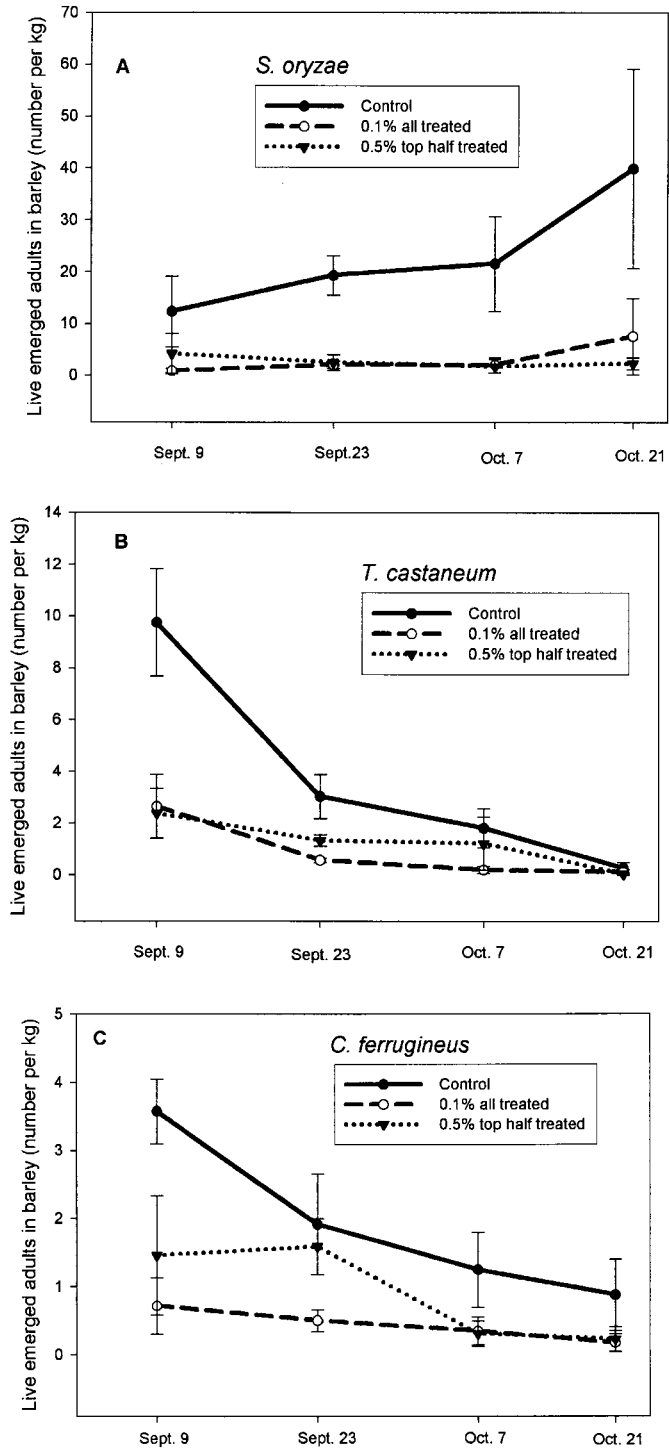


Fig. 4. The number of live emerged adults in barley samples taken from granaries untreated or treated with protein-enriched pea flour at 0.1% with all the grain treated or 0.5% with the top half of the bulk treated at different sampling dates. Grain samples with adults removed were cultured 5 wk at 30°C, 70% RH. (A) *S. oryzae*; (B) *T. castaneum*; and (C) *C. ferrugineus*.

Table 1. The number (mean \pm SEM) of live emerged adults of three insect species found in the middle layer within granaries filled with 11 t barley treated with protein-enriched pea flour at 0.1 or 0.5% (only top half of the bulk treated) or untreated as controls

Insect	Number of emerged adults (mean \pm SEM) ^a			F	P
	Untreated	0.1% All treated	0.5% Top-half treated		
<i>S. oryzae</i>	11.0 \pm 3.2a	0.5 \pm 0.3b	4.8 \pm 1.8b	9.1	0.004
<i>T. castaneum</i>	3.2 \pm 1.5a	0.5 \pm 0.2a	0.7 \pm 0.3a	23.2	0.1701
<i>C. ferrugineus</i>	2.5 \pm 0.4a	0.7 \pm 0.2a	1.4 \pm 0.5a	2.04	0.1731

^a Different letters in the same row indicate significant differences between treatments ($P < 0.05$).

Each treatment had two replicates. The number of emerged adults was recorded when barley samples had been cultured for 5 wk at 30°C, 70% RH, after parent adults were removed (df = 2).

two protein-enriched pea flour treatments ($F = 0.13$, df = 1, 30; $P = 0.7193$; Fig. 4B). Neither protein-enriched pea flour treatments significantly reduced the number of live *C. ferrugineus* adults ($P > 0.05$), but the 0.1% treatment reduced emerged adults of *C. ferrugineus* ($F = 4.87$; df = 1, 30; $P = 0.0351$; Figs. 3C and 4C).

Protein-enriched pea flour changed the distribution of *S. oryzae* and *C. ferrugineus* in the granaries. More live adult *S. oryzae* were found in the middle layer (2.9 ± 0.8 insects/kg) than in the top layer in untreated granaries (0.4 ± 0.2 insects/kg; $P < 0.05$). However, there was no difference between layers in both protein-enriched pea flour treated granaries ($P > 0.05$). There was no difference in the number of adult *C. ferrugineus* between the top layer and the middle layer in the untreated granaries ($P > 0.05$). However, more *C. ferrugineus* were found in the 0.1% protein-enriched pea flour treated top layer (2.5 ± 0.6 insects/kg) than in the middle layer (0.4 ± 0.1 insects/kg; $P < 0.05$). The emerged adults of all three species were found in the middle layer in both protein-enriched pea flour treated granaries (Table 1).

Mortality. Mortality of adult *S. oryzae*, *T. castaneum*, and emerged adults of *S. oryzae* in grain samples treated with 0.1% or 0.5% protein-enriched pea flour was significantly higher than in the untreated control. There was no difference between the two protein-

enriched pea flour treatments (Table 2). Treatments of protein-enriched pea flour did not increase the mortality of adult *C. ferrugineus* ($P > 0.05$) and the emerged adults of *T. castaneum*.

Mortality of emerged adults of *S. oryzae* was high in both the top ($97 \pm 9\%$) and middle layers ($95 \pm 7\%$) in the 0.1% pea flour treatment. However, the mortality of emerged adults of *S. oryzae* was higher in the top layer ($93 \pm 16\%$) than in the middle layers ($6 \pm 5\%$) in the 0.5% pea flour treatment ($F = 23.41$; df = 1, 6; $P = 0.0029$). Mortalities of adults and emerged adults of *T. castaneum* and *C. ferrugineus* in the top and middle layers were not different in any treatment ($P > 0.05$).

Physical Characteristics

There was no difference in the bulk density of the barley throughout the trial before treatment (629 ± 0.8 kg/m³), after the treatment (632 ± 1.1 kg/m³), and at the end of the trial (630 ± 1.5 kg/m³; $F = 2.63$; df = 2, 160; $P = 0.0754$). There was no difference in the bulk density between untreated, 0.1% all grain treated, and 0.5% half treated before treatment ($F = 1.61$; df = 2, 54; $P = 0.2128$), after treatment ($F = 0.66$; df = 2, 54; $P = 0.5194$), and at the end of trial ($F = 0.25$; df = 2, 54; $P = 0.7824$). No difference in the moisture content was found among all treatments within each of the three periods of the trial ($P > 0.05$). For three sampling dates, the dockage was higher in the 0.5% top-half treatment ($0.87 \pm 0.07\%$) than in untreated ($0.55 \pm 0.03\%$) and 0.1% treatment ($0.53 \pm 0.03\%$; $P < 0.05$).

Insects Remaining in Release Boxes

For all three insect species, more insects remained in the boxes in both pea flour treated granaries than in the controls (Table 3). Mortality of *S. oryzae* ($27 \pm 4\%$) in boxes in granaries top-half treated with 0.5% pea flour was significantly higher than in the untreated granaries ($11 \pm 2\%$; $F = 14.38$; df = 1, 27; $P = 0.0008$). However, mortality of *T. castaneum* and *C. ferrugineus* between the two pea flour treatments did not differ ($P > 0.05$).

Table 2. The mortality (mean \pm SEM) of three insect species found in samples from granaries filled with 11 t barley treated with protein-enriched-pea flour at 0.1% or 0.5% (only top half of the bulk treated) or untreated as controls

Stage	Insect	Mortality (%) ^a			F	P
		Untreated	0.1% All treated	0.5% Top-half treated		
Adult	<i>S. oryzae</i>	44 \pm 10a	96 \pm 3b	91 \pm 5c	161.56	<0.0001
	<i>T. castaneum</i>	26 \pm 8a	63 \pm 15b	48 \pm 10b	11.68	0.009
	<i>C. ferrugineus</i>	12 \pm 5a	14 \pm 12a	3 \pm 2a	0.5	0.63
Emerged adults	<i>S. oryzae</i>	2 \pm 1a	67 \pm 10b	50 \pm 16b	52.29	<0.0001
	<i>T. castaneum</i>	4 \pm 4a	8 \pm 4a	15 \pm 9a	1.43	0.3113
	<i>C. ferrugineus</i>	2 \pm 1a	0 \pm 0a	14 \pm 8a	1.58	0.281

^a Different letters in the same row indicate significant differences between treatments ($P < 0.05$).

Each treatment had two replicates. The mortality of emerged adults was recorded when barley samples were cultured 5 wk at 30°C, 70% RH, after parent adults were removed (df = 2).

Table 3. The number (mean \pm SEM) of insects (live and dead) remaining in release boxes 10 days after being placed in granaries filled with 11 t barley treated with protein-enriched pea flour at 0.1 or 0.5% (only top half of the bulk treated) or untreated as controls

Traps	Insects	Number of insects ^a			F	P
		Untreated	0.1% All treated	0.5% Top-half treated		
Insect remaining in release boxes (per box)	<i>S. oryzae</i>	177 \pm 32a	419 \pm 40b	350 \pm 66b	9.05	0.001
	<i>T. castaneum</i>	30 \pm 8a	53 \pm 4b	59 \pm 11b	7.59	0.002
	<i>C. ferrugineus</i>	21 \pm 7a	254 \pm 74b	116 \pm 32b	23.57	<0.0001
Surface-pitfall traps (per trap per week)	<i>S. oryzae</i>	0.8 \pm 0.3a	1.6 \pm 0.3b	3.8 \pm 0.9c	11.12	<0.0001
	<i>T. castaneum</i>	1.5 \pm 0.4a	2.5 \pm 0.8b	3.4 \pm 0.7c	23.86	<0.0001
	<i>C. ferrugineus</i>	0.1 \pm 0.0a	0.2 \pm 0.1b	0.3 \pm 0.1b	5.57	0.004
Flight traps (per trap per week)	<i>S. oryzae</i>	0.04 \pm 0.03a	0.2 \pm 0.1a	1.3 \pm 0.4b	10.4	<0.0001
	<i>T. castaneum</i>	5.8 \pm 1.8a	6.2 \pm 1.8a	4.0 \pm 1.1a	0.24	0.7889
	<i>C. ferrugineus</i>	0.1 \pm 0.1a	0.1 \pm 0.1a	0.04 \pm 0.02a	0.37	0.6882
Probe-pitfall traps (per trap per week)	<i>S. oryzae</i>	13.9 \pm 1.2a	19.2 \pm 2.3b	26.2 \pm 3.8c	116.2	<0.0001
	<i>T. castaneum</i>	29.5 \pm 3.8a	35.9 \pm 4.5a	35.6 \pm 4.7a	0.17	0.8401
	<i>C. ferrugineus</i>	29.4 \pm 6.1a	35.2 \pm 5.2a	24.2 \pm 3.6a	4.1	0.17
Floor sticky traps (per section per week)	<i>S. oryzae</i>	0.02 \pm 0.01a	0.2 \pm 0.1b	0.1 \pm 0.04b	7.57	0
	<i>T. castaneum</i>	0 \pm 0a	0.1 \pm 0.03b	0.1 \pm 0.03b	5.73	0.004
	<i>C. ferrugineus</i>	2.9 \pm 0.3a	5.0 \pm 0.5b	4.3 \pm 0.5ab	3.35	0.0363

^a Different letters in the same row indicate significant differences between treatments ($P < 0.05$).

Each treatment had two replicates. The number of insects caught in various traps were counted every week.

Traps

Surface-Pitfall Traps. Surface-pitfall traps in both pea flour treated granaries caught more *S. oryzae*, *T. castaneum*, and *C. ferrugineus* than in untreated controls (Table 3). No *S. oryzae* were trapped in the surface-pitfall traps after 45 d in the 0.1% pea flour treated granaries, but a few were found in traps placed in the untreated granaries. Most *T. castaneum* were caught during the first 10 d. *Cryptolestes ferrugineus* was found in the traps during the entire trial period, even when grain temperatures were below 10°C.

Flight Traps. Flight traps caught more *T. castaneum* than *S. oryzae* and *C. ferrugineus* (Table 3). Over 90% of all trapped *T. castaneum* were caught during the first 4 d (each trap caught >30 adult *T. castaneum*) when grain temperatures were over 25°C in the top layer. Few were caught after 2 September, when the top layer was below 20°C. Traps in the granaries with the top-half treated with 0.5% pea flour caught few but significantly more *S. oryzae* than in the 0.1% pea flour treatment or in the untreated granaries ($P < 0.05$). There were no differences in the number of *C. ferrugineus* on the traps among all treatments.

Probe-Pitfall Traps. Probe-pitfall traps caught more insects than other traps combined (Table 3). The traps in the granaries treated with 0.5% pea flour in the top half of the barley and treated with 0.1% pea flour in all the barley caught more *S. oryzae* than in the untreated controls. Traps in the 0.1% and the 0.5% pea flour treatment caught more *S. oryzae* at the beginning of the trial than in the untreated controls, and then caught less than in the controls (Fig. 5). There were no differences in the number of *T. castaneum* and *C. ferrugineus* caught in the traps among treatments. More insects were caught in the top layer than in the middle layer for *S. oryzae* (3.7 ± 0.2 , 1.9 ± 0.4), *T. castaneum* (7.6 ± 0.2 , 2.0 ± 0.7), and *C. ferrugineus* (6.8 ± 0.2 , 1.6 ± 0.8 ; $P < 0.05$). The mortality of *S.*

oryzae in traps of the 0.1% and 0.5% top-half pea flour treatments was $74 \pm 9\%$ and $62 \pm 11\%$, respectively, which was higher than in the untreated granaries ($38 \pm 8\%$; $P < 0.05$).

Floor Sticky Traps. Traps underneath the perforated floor caught more *C. ferrugineus* than *S. oryzae* and *T. castaneum* (Table 3). More *S. oryzae* were caught in the 0.1% pea flour and 0.5% pea flour treated granaries than in the untreated granaries. *Sitophilus oryzae* moved down quickly in 0.1% pea flour treated granaries; the sticky trap caught 0.9 insects per section per day during the first 2 d of the trial in 0.1% pea flour treatment while none were found in the 0.5% protein-enriched pea flour treatment and the controls. When grain temperatures dropped below 10°C in the last 2 wk of the trial, no *S. oryzae* were caught on the sticky traps. Traps in both protein-enriched pea flour treated granaries caught more *T. castaneum* and *C. ferrugineus* than in untreated granaries ($P < 0.05$). No *T. castaneum* were found on the sticky traps in untreated granaries during the trials. *Cryptolestes ferrugineus* were found on the traps during the entire trial in all treatments. Middle sections of the sticky trap (sections 3 and 4) caught more *C. ferrugineus* than outer sections ($F = 8.23$; $df = 4, 320$; $P < 0.001$).

Discussion

Laboratory tests (Bodnaryk et al. 1999, Fields et al. 2001) demonstrated that protein-enriched pea flour is repellent and toxic to *S. oryzae*, *T. castaneum*, and *C. ferrugineus*. Our goal was to determine if these properties of protein-enriched pea flour are sufficient to reduce stored-grain pests in commercial granaries. Compared with the number of live insects found in the untreated barley, 0.1% protein-enriched pea flour treatment reduced the parent population of *S. oryzae* by 93%, *T. castaneum* by 66%, and *C. ferrugineus* by

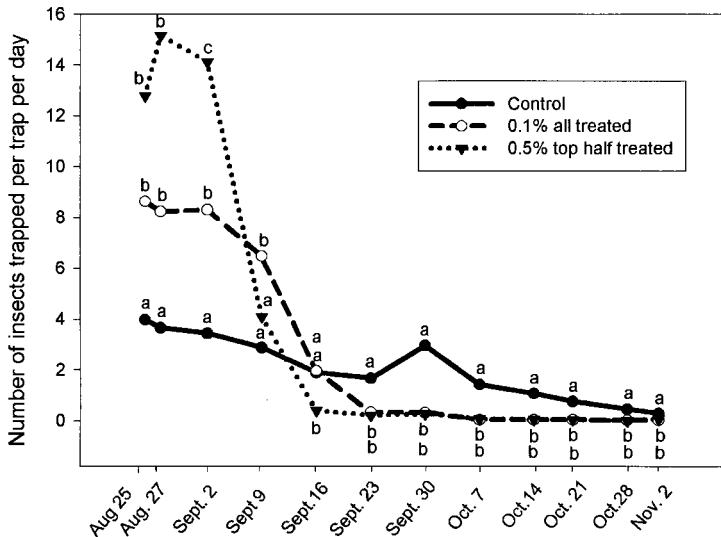


Fig. 5. The number of *S. oryzae* adults caught in probe pitfall traps inserted in granaries untreated or treated with protein-enriched pea flour at 0.1% with all the grain treated or 0.5% with the top half of the bulk treated on different sampling dates. (Different letters vertically on the same day indicate significant difference; $P < 0.05$).

58%. Similar reductions were shown in 0.5% top-half treatment. The effectiveness of protein-enriched pea flour at 0.1% against *S. oryzae* in this granary trial was similar to the 95% mortality reported in a laboratory test (Bodnaryk et al. 1999). However, the reduction in the populations of *T. castaneum* and *C. ferrugineus* in the granary trial was higher than the laboratory tests, in which there was ≈ 20 and 15% mortality, respectively (Bodnaryk et al. 1999). In their laboratory trials, insects were not allowed to leave the test vials. The higher efficacy in the granary trial could be caused by an additional effect of fewer insects moving into the grain mass and more insects leaving the granary because of the repellency of the protein-enriched pea flour.

We believe that the reduction of *S. oryzae* in treated granaries was primarily caused by increased mortality. Few *S. oryzae* were found in traps above or below the granaries, and many dead and few live *S. oryzae* were found within treated granaries. However, we believe that the reduction of *T. castaneum* populations was caused by a combination of repellency and toxicity of the protein-enriched pea flour. More *T. castaneum* were caught on traps and suffered higher mortality in the treated granaries.

Many *C. ferrugineus* left the barley bulks through the perforated floor. By using the number of *C. ferrugineus* caught on the floor sticky trap and extrapolating for the entire floor area, we estimated that 6,475, 11,045, and 9,501 adults (untreated, 0.1% and 0.5% pea flour treated granaries, respectively) left the granaries through the perforated floor. This is significant considering that only 15,000 *C. ferrugineus* were released into each granary. Therefore, protein-enriched pea flour may only be effective against *C. ferrugineus* if they can easily leave the grain as in granaries with

perforated floors and do not return. We believe that there were very few insects immigrating into our granaries other than the ones released. White et al. (1995) showed that at our test location, a Johnson-Taylor insect suction trap operated from 1987 to 1993 caught 0 *S. oryzae*, 6 *T. castaneum*, and 14 *C. ferrugineus* during the entire period of operation, whereas hundreds of stored-grain fungus feeding species were caught.

The higher number of insects remaining in the release boxes and the higher mortality of returned insects in the boxes in treated granaries indicate that protein-enriched pea flour has the potential of reducing the initial population of insects that immigrate into stored grain. Perforated floors may serve as a harborage from which insects can reinfest grain in the following year. The repellency of protein-enriched pea flour could reduce this type of immigration. Mohan (1997) developed an insect removal granary by using a screened wall instead of a solid wall. This granary, used in conjunction with protein-enriched pea flour, could protect grain from stored-product insects (Mohan and Fields 2002).

The live emerged adults in the 0.1% protein-enriched pea flour treatment were reduced by 87% for *S. oryzae*, 77% for *T. castaneum*, and 77% for *C. ferrugineus* compared with the untreated granaries. For *S. oryzae*, the reduction is due in part to the toxicity of protein-enriched pea flour to emerged adults. Also, the reduction of emerged adults could be caused by fewer parent insects in treated grain. The reduction in the parent populations could be caused by reduced immigration, increased mortality, or increased emigration. Other possible factors are the reduction of adult fecundity and reduced larval survival in treated barley. Further tests are needed to determine the relative importance of each factor.

Protein-enriched pea flour shows repellency in the laboratory (Fields et al. 2001). We hypothesized that control could be possible by treating only the top layer of the grain, reducing the number of insects immigrating into the grain. This method would reduce labor costs and the amount of pea protein required in large commercial granaries. Top-half treatment with protein-enriched pea flour had a similar effect as treating the entire grain mass on the reduction of insect populations in this trial. However, the top-half treatment failed to prevent insects from moving into the untreated barley. Unlike the entire treatment, in which *S. oryzae* offspring in the middle layer were killed by protein-enriched pea flour, offspring of *S. oryzae* survived in the middle layer in the top-half treatment. The *S. oryzae* population could apparently increase unhindered in the untreated middle layer of the top-half treatment bins. Therefore, we would not recommend the method of top-half treatment application.

Many factors such as grain temperature, the behavior of insects, and population size affect the number of insects collected in traps (White and Loschiavo 1986, Buchelos and Athanassiou 1999, Wakefield and Cogan 1999). Buchelos and Athanassiou (1999) found the ranking of species collected in probe-pitfall traps differs from that of insects in the grain samples taken from the same granary. In our trial, protein-enriched pea flour increased the movement of insects, which may be caused by the repellency of protein-enriched pea flour (Fields et al. 2001). We assume all granaries had similar densities of insects 2 d after releasing insects. Our laboratory tests have shown that there is no mortality after 3 d in 100% protein-enriched pea flour. However, the number of insects collected in probe-pitfall traps was significantly different among treatments. Thus, the use of traps to evaluate insecticides that affect insect behavior should be analyzed with caution.

Some tropical strains of *S. oryzae* can reproduce on yellow-split pea (Coombs et al. 1977). The inheritance of this ability is controlled by a single recessive, autosomal gene (Thind and Muggleton 1981, Holloway 1986, Grenier et al. 1997) or a few alleles (Holloway and Smith 1985). Symbionts, glutathione-S-transferases, and oxygenases in insects may be involved in the detoxification of allelochemicals from peas (Holloway and Smith 1985; Holloway and Mackness 1988, Grenier et al. 1997). The adaptation of insects to peas should be seriously considered in the development of pea extract for the control of stored-product insects.

Protein-enriched pea flour shows potential as a grain protectant with both toxic and repellent properties (Bodnaryk et al. 1999). Stored-product insects are affected by many plant extracts (Jacobson 1989). Most of them are medicinal plants or spices (Golob et al. 1999) with limited availability, because they are either wild or grown on a small scale. In addition, some extracts are toxic to mammals (Ware 1983, Golob et al. 1999). Peas are consumed by humans and grown around the world, with an annual production of ≈ 12 million tons (Skrypetz 2001). Pea protein flour is also available to many farmers who may be too poor to

afford chemical insecticides. Unlike diatomaceous earth (Korunic et al. 1998, Fields and Korunic 2000), pea protein flour did not reduce bulk density. Also, most grain protectants harm parasitoids (Perez-Mendoza et al. 1999). Protein-enriched pea flour is not toxic to *Anisopteromalus calandrae* (Howard), an ectoparasitoid of several stored-product insects, and *Cephalonomia waterstoni* (Gahan), an ectoparasitoid of *C. ferrugineus* (Hou et al. 2002). Powdered grain protectants are difficult to apply. Further research is required to determine if protein-enriched pea flour or purified extract (Bodnaryk et al. 1999) can be applied in a liquid form.

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