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Effect of *Pisum sativum* fractions on the mortality and progeny production of nine stored-grain beetles

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Abstract

Yellow field pea (*Pisum sativum* L.) fractions that were mainly protein (50%), fibre (90%) or starch (85%) were obtained from a commercial pea mill and mixed with wheat kernels or wheat flour. Based on the mortality and the number of offspring produced, protein-rich pea flour was more toxic than fibre, which was more toxic than starch. For the protein-rich pea flour mixed with wheat kernels, the most sensitive insects were *Sitophilus oryzae* (L.), *Sitophilus zeamais* Motschulsky and *Sitophilus granarius* (L.), followed by *Cryptolestes ferrugineus* (Stephens) which was more sensitive than *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). For the protein-rich pea flour mixed with wheat flour, *Cryptolestes pusillus* (Schönherr) was most sensitive, followed by *C. turcicus* (Grouvelle) and *T. confusum* (Jacquelin du Val), with *T. castaneum* being the most resistant. Although protein-rich pea flour did not kill adults to a great extent when mixed with flour, it reduced offspring production significantly. Again *C. pusillus* was the most sensitive, followed by *T. confusum,* with *T. castaneum* offspring being the most resistant. The insecticidal activity of pea fractions decreased after treated wheat kernels were held at 30 °C, 70% r.h. for 8 months. The potential of using pea fractions to control stored-product insects is discussed.

Keywords: Pea; Legume; Toxicity; *Sitophilus*; *Tribolium*; *Cryptolestes*; *Rhyzopertha*

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1. Introduction

Insects are a problem in stored grain throughout the world because they reduce the quantity and quality of grain (Madrid et al., 1990; Sinha and Watters, 1985). Many countries, such as Australia, Canada and France, have zero tolerance for live insects in grain. Synthetic insecticides, such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, pyrethrum, deltamethrin, methoprene and the fumigant phosphine, are currently the main products used to protect stored grain from insects (Bond, 1984; Snelson, 1987). However, increased concern by consumers over insecticide residues in processed cereal products, the occurrence of insecticide-resistant insect strains (Subramanyam and Hagstrum, 1995), and the precautions necessary to work with traditional chemical insecticides, call for new approaches to control stored-product insect pests.

Higher plants are a rich source of novel insecticides (Prakash and Rao, 1997). The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Golob et al., 1999; Jilani and Su, 1983; Malik and Mujtaba Naqvi, 1984; Regnault-Roger and Hamraoui, 1993; Weaver and Subramanyam, 2000). Azadirachtin from the Indian neem tree (*Azadirachta indica* A. Juss., Meliaceae) (Jilani and Saxena, 1990; Saxena et al., 1988) and pyrethrum from chrysanthemums (Prakash and Rao, 1997) have received the most attention. However, the structural complexity of azadirachtin makes it difficult to synthesise, which limits the source of azadirachtin solely to natural extracts. The neem tree and chrysanthemum are restricted to tropical areas. Although the neem tree is fast growing, it takes several years before azadirachtin can be extracted. It is 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, they would be economically attractive.

Legume seeds contain a wide range of allelochemicals with toxic and deterrent effects against insect pests (Bell, 1978; Harborne et al., 1971). An admixture of yellow split-peas (*Pisum sativum* L.) with wheat resulted in a marked reduction of survival and the reproduction rate of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Coombs et al., 1977; Holloway, 1986). Recently, concentrations as low as 0.01% protein-rich pea flour (*P. sativum*) have been shown to cause adult mortality and reduce reproduction in several stored-product insect pests (Bodnaryk et al., 1999; Fields et al., 2001; Hou and Fields, 2004). There are a number of compounds in protein-rich pea flour (polypeptides, soyasaponins, lysolecithins) that are toxic to *S. oryzae* (Taylor et al., 2004a,b,c). Three polypeptides have been isolated from peas that are toxic to stored-product insects (Delobel et al., 1999). These polypeptides are related to pea albumins of the PA1b type (Higgins et al., 1986). One of the PA1b-like polypeptides belongs to the cystine-knot family of toxins (Jouvensal et al., 2003). This polypeptide binds with high affinity to the microsomal fraction of susceptible, but not resistant, *Sitophilus* spp. (Gressent et al., 2003). Hou (2003) showed that pea extracts cause damage to the insect gut.

The objective of this study was to examine the effects of commercially available protein, fibre and starch fractions of pea on the survival of stored-product insects.

2. Materials and methods

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 (Prestige Protein) contained 50% protein, 18% simple sugars, 17% starch, 4% lipids and 4% ash; the fibre fraction (Exlite Fibre) contained 85% fibre, 6% protein and 2% ash; and the starch fraction (Starlite Starch) contained 83% starch, 5% protein, 4% simple sugars and 1% ash (analyses were done at the Department of Applied Microbiology and Food Science, University of Saskatchewan). All fractions had 7% moisture content (m.c., wet basis). Material was stored at −15 °C until needed.

Fractions were mixed with Canada Western Red Spring wheat (*Triticum aestivium* L.) with 16% m.c. at concentrations of 0.001, 0.01, 0.1, 1 and 10% (wt:wt) or white wheat flour (no bran or wheat germ added) at concentrations of 0.01, 0.1, 1, 10 and 50% (wt:wt). Pea fractions and food were mixed by rolling the mixture in a 4-L glass jar for 30 min at 250 rpm (Norton, Arkron, OH, USA). For both wheat kernels and flour there was an untreated control. For the species tested with the flour there was also a treatment with 100% of the pea fraction. Approximately 20 g of treated kernels or flour were placed into glass vials (7 cm high, 2.7 cm diameter). Two sets of five vials were prepared for each concentration. Ten unsexed adults (7–14 days old) were introduced into each vial for the first set of five vials. Six species of stored-product insects, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), lesser grain borer; *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), granary weevil; *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), maize weevil; *S. oryzae,* rice weevil; *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), red flour beetle; and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), rusty grain beetle) were placed on the treated grain. Four species (*Cryptolestes pusillus* (Schönherr) (Coleoptera: Laemophloeidae), flat grain beetle; *Cryptolestes turcicus* (Grouvelle), (Coleoptera: Laemophloeidae), flour mill beetle; *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), confused flour beetle; and *T. castaneum* were placed on treated flour. After 2 weeks at 30 °C and 70% r.h., adult insects and media were placed in a pan, the adults were placed in the second set of vials with the same concentration of fractions. The second set of vials had also been held at 30 °C, 70% r.h. for 2 weeks. The medium, food and pea fractions, was returned to the vial and held at 30 °C, 70% r.h. for 5 weeks before the emerged adults were counted. Mortality of adults was determined after 2, 4 and 6 weeks.

A parallel study was conducted to investigate the stability of the insecticidal properties of the protein-rich pea flour. Wheat kernels with various concentrations of the protein-rich pea flour were aged for 8 months at 30 °C and 70% r.h., and tests were conducted as previously described with *S. oryzae.*

2.1. Data analysis

Probit analysis (Polo-PC) was used to compare sensitivities of the species and the effectiveness of the pea fractions. The concentrations required to reduce offspring production by 50% or 95% were estimated using probit analysis. The control (0% pea fraction) population was used as the initial number of offspring. In some cases the data did not fit the probit model and the lethal doses and their confidence intervals could not be calculated. In these cases the 50% and 95% mortality or offspring reduction were estimated from Figs. 1–3. Differences between the fresh and aged protein-rich pea flour adult mortality and offspring production (Fig. 3) were tested using Mann–Whitney Rank Sum Test (SigmaStat).

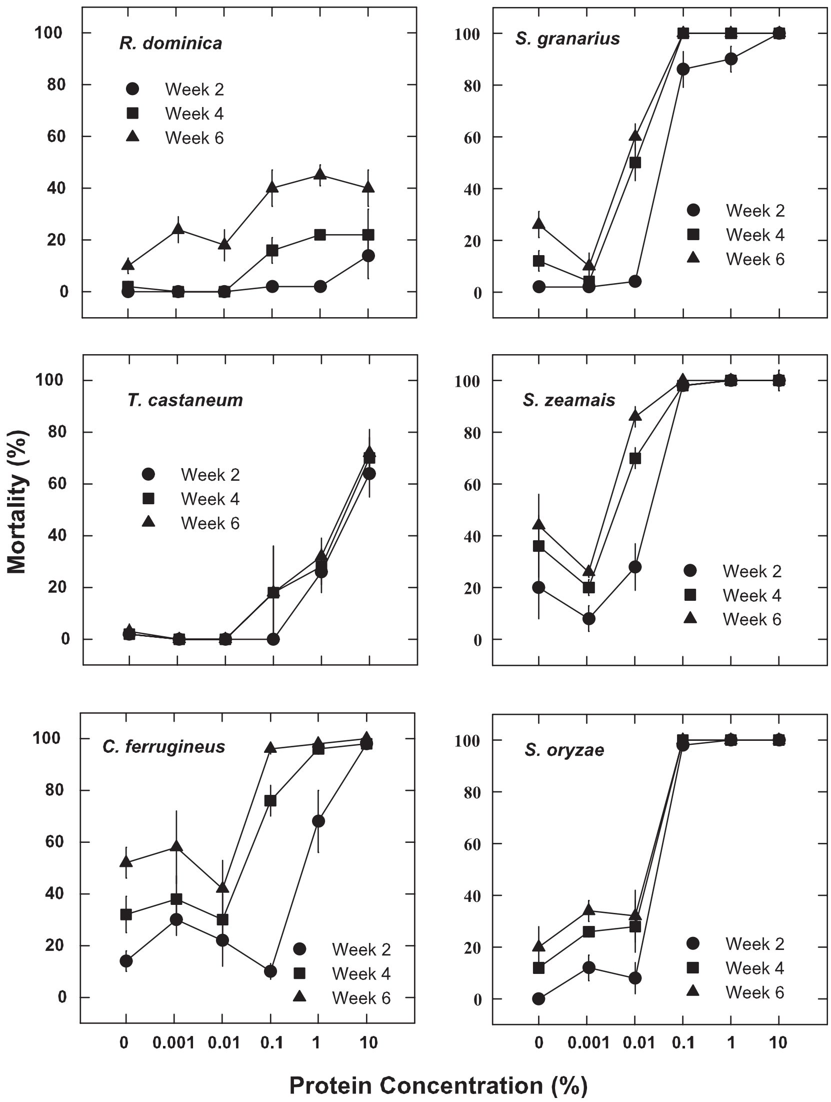

Fig. 1. Mortality (±SEM) of adult *Rhyzopertha dominica, Sitophilus granarius, Tribolium castaneum, Sitophilus zeamais, Sitophilus oryzae* and *Cryptolestes ferrugineus* held on wheat kernels treated with various concentrations of protein-rich pea flour fraction (5 vials/concentration, 10 insects/vial).

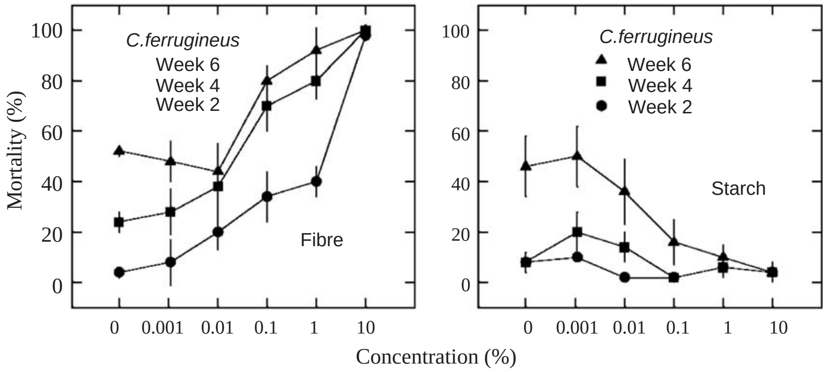

Fig. 2. Mortality (±SEM) of adult *Cryptolestes ferrugineus* held on wheat kernels treated with various concentrations of pea fibre or starch fractions (5 vials/concentration, 10 insects/vial).

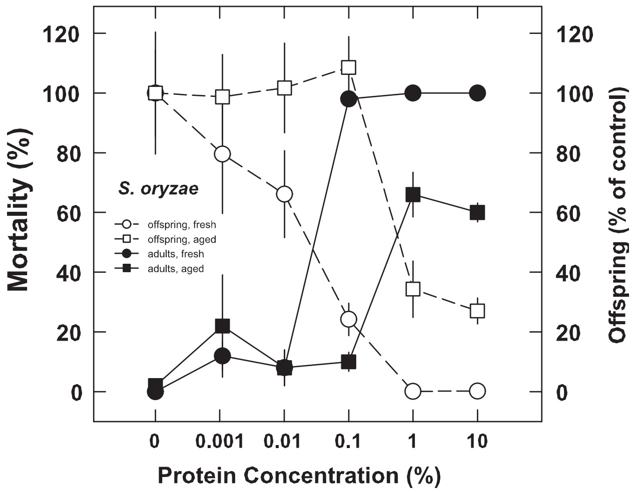

Fig. 3. Mortality (± SEM) of adult *S. oryzae* held for 2 weeks on wheat kernels treated with various concentrations of protein-rich pea flour (5 vials/concentration, 10 insects/vial), and the resulting offspring (expressed as a percentage of control, fresh protein-rich pea flour experiment had 186±38, aged protein-rich pea flour experiment had 233±34 offspring in controls). There were differences between fresh and aged protein-rich pea flour for 0.1, 1 and 10% for both adult mortality and offspring production (Mann–Whitney Rank Sum Test, $P<0.01$).

3. Results

3.1. Kernels

Protein-rich pea flour applied to wheat kernels was toxic to all insects tested (Fig. 1; Table 1). The *Sitophilus* spp. were the most sensitive of the insects tested, followed by *C. ferrugineus, T. castaneum* and *R. dominica*. Protein-rich pea flour also reduced the number of offspring of all insects treated (Table 1). The most sensitive insects, in terms of reduced offspring, were *S. oryzae*

Table 1
The mortality and offspring production of different stored-product insects held on protein-rich pea flour, fibre, or starch fractions mixed with wheat kernels or wheat flour

Food	Fraction	Insect species	Adult mortality (after 2 weeks)				Offspring production			
			LC_{50}* (%)	95% Confidence interval	LC_{95} (%)	95% Confidence interval	LC_{50} (%)	95% Confidence interval	LC_{95} (%)	95% Confidence interval
Wheat kernels	Protein	*S. oryzae*	0.05		0.1		0.02	(0, 0.05)	0.7	(0.2, 35)
		S. zeamais	0.02	(0.01, 0.03)	0.08	(0.05, 0.2)	0.05		0.1	
		S. granarius	0.06		0.7		0.01	(0, 0.04)	0.1	(0.04, 6)
		R. dominica	—		—		0.2	(0.02, 3)	100	(7, —)
		T. castaneum	3	(0.7, 33)	93	(3, —)	1.1	(0.3, 5)	14	(3, —)
		C. ferrugineus	0.9		5.1		0.04	(0.02, 0.1)	2	(0.6, 26)
	Fibre	*C. ferrugineus*	0.8		23		0.08	(0.02, 0.3)	5	(0.8, —)
	Starch	*C. ferrugineus*	—		—		5		—	
Wheat flour	Protein	*C. pusillus*	12	(10, 16)	37	(27, 69)	0.2	(0.1, 0.4)	3	(1, 20)
		C. turcicus	23	(16, 30)	48	(37, 69)	—		—	
		T. castaneum	75		95		10		50	
		T. confusum	32	(26, 39)	88	(70, —)	1.2	(0.4, 3)	42	(12, —)
	Fibre	*T. confusum*	75		100		11	(9, 14)	53	(39, 83)
	Starch	*T. confusum*	—		—		20	(6, 55)	100	(66, —)

*The LC_{50} and LC_{95} were estimated using probit analysis, confidence intervals provided or from Figs. 1–3 confidence intervals not provided; analyses based upon 6 to 7 concentrations, 5 vials/concentration, 10 adults/vial.

and *S. granarius,* followed by *C. ferrugineus* and *S. zeamais.* Offspring production of *T. castaneum* and *R. dominica* were the least affected by protein-rich pea flour. Holding insects on treated wheat kernels beyond 2 weeks did not increase mortality more than the mortality seen in the untreated grain in most species (Figs. 1 and 2).

3.2. Flour

Protein-rich pea flour mixed with wheat flour also increased the mortality of insects (Table 1), but mortalities were generally lower in flour treated with protein-rich pea flour than in similarly treated wheat kernels. For example, *T. castaneum* held on wheat kernels treated with 10% protein-rich pea flour had over 60% mortality, whereas a similar treatment with flour produced no mortality. *Cryptolestes pusillus* was the most sensitive insect, followed by *C. turcicus* and *T. confusum,* with *T. castaneum* being the least affected. As with the wheat kernels, insect mortality attributed to the protein-rich pea flour did not increase after 2 weeks.

Although protein-rich pea flour in flour did not kill adults to a great extent, it reduced offspring significantly (Table 1). Again *C. pusillus* was the most sensitive, followed by *T. confusum* with *T. castaneum* offspring production being the least affected (Table 1). As *C. turcicus* did not produce any offspring in the untreated flour, we do not know if the protein-rich pea flour affected offspring production.

3.3. Comparison of pea fractions

The pea starch had no toxic effects on adult *T. confusum*; after 6 weeks in 100% starch, the mortality was less than 20%. After 6 weeks, pea starch increased the survival of *C. ferrugineus* adults (Fig. 2). However, the starch did reduce the number of offspring of *C. ferrugineus* and *T. confusum* (Table 1).

For *C. ferrugineus* adults and offspring production, pea fibre had similar toxic effects compared to protein-rich pea flour (Figs. 1 and 2; Table 1). For *T. confusum,* fibre was less toxic than protein (Table 1).

3.4. Stability of pea fractions

The insecticidal activity of pea fractions was reduced after treated wheat kernels were held at 30 °C, 70% r.h. for 8 months (Fig. 3). For instance, *S. oryzae* held for 2 weeks on wheat kernels treated with 0.1% fresh protein-rich pea flour had 90% mortality. Under similar conditions, aged protein-rich pea flour only caused 10% mortality (Fig. 3).

4. Discussion

Protein-rich pea flour on wheat kernels had similar toxicity to *Sitophilus* spp. as some diatomaceous earths (Korunic, 1998). It was more toxic than several other plant-derived insecticides, which require 2–5% of plant material mixed with grain to control stored-product insects (Golob et al., 1999; Prakash and Rao, 1997; Weaver and Subramanyam, 2000). Pyrethrum is the only natural product that is used extensively in commercial grain stores, and it is much more toxic than protein-rich pea flour. Pyrethrum is mixed with the synergist piperonyl butoxide at a ratio of 1 part pyrethrum to 10–20 parts piperonyl butoxide, which increases the toxicity by 2- to 10-fold (Lloyd, 1973; Silcox and Roth, 1995). This mixture is commonly applied as a fog to control flying insects in warehouses and food processing facilities. The pyrethrum with piperonyl butoxide has been used less often as a grain protectant (Gillenwater and Burden, 1973) and requires 0.00015% pyrethrum with 0.003% piperonyl butoxide to control stored-product insects (Greening, 1983).

Three insecticidally active compounds have been isolated from peas. Polypeptides related to PA1b are toxic to *Sitophilus* spp (Delobel et al., 1999; Taylor et al., 2004c). Dehydrosoyasaponin I, a minor triterpenoid saponin isolated from protein-rich pea flour, acts as an antifeedant and is toxic to *S. oryzae* (Taylor et al., 2004a). Lysolecithins, although not toxic by themselves, enhance the activity of dehydrosoyasaponin I (Taylor et al., 2004a).

Protein-enriched fractions were more toxic than fiber- and starch-rich fractions. However, with *C. ferrugineus*, toxicity of the protein-rich pea flour and pea fibre fractions were similar, even though the protein-rich pea flour fraction was 50% protein and the fibre fraction was 3% protein. Seed coats, the major source of the fibre fraction, have a number of compounds toxic to insects (Janzen, 1977; Seifelnasr, 1991; Thiery et al., 1994), and the active ingredient in the protein-rich pea flour may not be the same as the one in pea fibre.

It is common for stored-product insects to have large differences in susceptibility to synthetic insecticides (Samson and Parker, 1989; Snelson, 1987). For example *R. dominica* is particularly tolerant to malathion ($LC_{50} = 7$ ppm), whereas *S. oryzae* has a LC_{50} of 2 ppm (Strong et al., 1967). On the other hand, *R. dominica* is more susceptible to the pyrethroid deltamethrin ($LC_{50} = 0.01$ ppm) than *S. oryzae* ($LC_{50} = 0.4$ ppm, Samson and Parker, 1989). To lower the total amount of insecticide needed to control all stored-grain insects, and to delay insecticide resistance, organophosphates and pyrethroids have been applied as a mixture in Australia (Snelson, 1987). There has been little research on the causes of these differences in susceptibilities, but they are probably due to differences in absorption of the insecticide, degradation within the insect and mode of action. For diatomaceous earth, the two-fold difference in susceptibility between two *T. castaneum* strains is related to behaviour (Rigaux et al., 2001). Therefore, it is not surprising that there were large differences in susceptibilities to the protein-rich pea flour between the different stored-grain pests.

There are a number of issues that must be addressed before pea fractions or extracts of peas can be used as a grain protectant. How quickly does it become ineffective under field conditions? A successful grain protectant must have residual activity to protect grain from infestation for several months. High temperature or high grain moisture content increase the degradation of grain protectants. For example, malathion concentrations decreased from 12 ppm to almost 0 ppm after 3 months at 30 °C on 16.8% m.c. wheat, conditions similar to our experiments (Watters and Mensah, 1979). Natural insecticides usually break down faster than synthetic insecticides (Prakash and Rao, 1997). The major drawback of pyrethrum as a grain protectant is its rapid breakdown on grain (Snelson, 1987). As with other grain protectants, the protein-rich pea flour had reduced activity after being held on wheat, but some activity remained after 8 months at 30 °C, 70% r.h. Hou and Fields (2003) found that at lower temperatures and relative humidities there was little decrease in the effectiveness after 9 months at room temperature. Further work is needed to determine the effects of temperature and moisture content on efficacy during storage.

Delobel et al. (1999) tried rearing 90 strains of *S. oryzae, S. granarius* and *S. zeamais* on peas. Four *S. oryzae* strains were able to complete their life cycle on peas. However, none of the strains of *S. granarius* or *S. zeamais* tested could develop on peas. *Sitophilus oryzae* strains resistant to peas have been collected from tropical areas: China, Jamaica, Lesotho, Peru, Singapore, Tanzania and Trinidad (Coombs et al., 1977; Grenier et al., 1997; Holloway and Smith, 1985; Thind and Muggleton, 1981). The resistance to peas is controlled by a single recessive autosomal gene (Grenier et al., 1997; Holloway and Smith, 1985; Thind and Muggleton, 1981). Grenier et al. (1997) screened their laboratory *S. oryzae* populations for this gene and estimated that 0.13% of the population was homozygous and resistant to peas. This type of screening should be done on pest populations found in grain stores before protein-rich pea flour is used commercially to control stored-product insects. To prevent resistance from developing in commercial stores, the protein-rich pea flour could be combined with another insecticide, or an alternative control method used occasionally. Hou et al. (2004a) combined protein-rich pea flour with a number of grain protectants; diatomaceous earth, neem, malathion and pyrethrum. However, none of the these combinations increased efficacy against all insects.

A pea-based stored-grain protectant would have to be able to control species such as *T. castaneum* and *R. dominica* that are not susceptibile to protein-rich pea flour. Protein-rich pea flour is also repellent to stored-product insects, including *T. castaneum* and *R. dominica*

(Fields et al., 2001). In granaries, the immigration of pest insects could be less in grain treated with protein-rich pea flour. Field tests with 11 t bulks of barley have shown that there is increased emigration of *C. ferrugineus* and *T. castaneum* from granaries holding barley treated with protein-rich pea flour at 0.1%. The populations of these two insects were lower than would be predicted from mortality alone (Hou and Fields, 2004). However, repellency may not provide enough population reduction to satisfy commercial standards. An additional insecticide may be needed to provide sufficient control of species other than *Sitophilus* spp.

The selectivity of the pea fractions makes it more difficult to use as a commercial stored-grain protectant. However, the advantage of this selectivity is that it has little effect on two parasitoids of stored-grain insects, *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), that parasitizes *S. oryzae*, or *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae), a parasitoid of *C. ferrugineus* (Hou et al., 2004b). Most of the stored-grain insecticides are lethal to parasitoids (Baker et al., 1995; Perez-Mendoza et al., 1999). However, adding a second insecticide to broaden the spectrum of activity to control *R. dominica* would probably be detrimental to parasitoids.

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