Effectiveness of protein-rich pea flour for the control of stored-product beetles

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Accepted: 4 June 2003

Key words: peas, protein, *Sitophilus oryzae*, *Cryptolestes ferrugineus*, *Tribolium castaneum*, toxicity, particle size, temperature, moisture, Coleoptera, Curculionidae, Laemophloeidae, Tenebrionidae

Abstract

Protein-rich pea flour is toxic to many stored-product insects. We investigated several factors that may affect the efficacy of protein-rich pea flour: insect species, insect population densities, grain species, temperature, and moisture. Adult *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) was more susceptible to protein-rich pea flour than *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae). Protein-rich pea flour did not increase the mortality of adult *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). However, it reduced the number of offspring of all three species. The toxicity of protein-rich pea flour was not reduced after 9 months when stored at –15 °C, or at room temperature as flour or mixed with grain. Its toxicity in wheat increased with higher temperature and at lower grain moisture contents. Protein-rich pea flour was more toxic in wheat and barley than in maize. This difference among grain species was not due to the kernel size of the grain, as ground wheat or maize with the same particle size still had different LD_{50}s.

Introduction

Insect pests cause extensive damage to stored grains, both qualitatively and quantitatively (Hall, 1970; Semple et al., 1992). Many grain storage managers use synthetic insecticides to reduce losses of stored grain to insect pests (Arthur, 1996). However, there are several reasons to search for alternatives to synthetic insecticides: consumer preference for food without insecticide residues, worker safety concerns, resistant insect populations, and deregistration of current synthetic insecticides. Higher plants are a good source of novel insecticides (Prakash & Rao, 1997). Thousands of plants have been investigated for their ability to control insect pests (Jacobson, 1989; Golob et al., 1999; Weaver & Subramanyam, 2000). Legume seeds contain a wide range of allelochemicals with toxic and deterrent effects against insect pests (Harborne et al., 1971; Bell, 1977).

Yellow split pea (*Pisum sativum* L. [Leguminosae]) mixed with wheat reduces the survival and reproduction rate of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Coombs et al., 1977; Holloway, 1986). As an animal feed and human food, protein-rich pea flour made from yellow split pea has the potential to be a good grain protectant (Bodnaryk et al., 1997; Fields et al., 2001; Hou & Fields, 2003). It is repellent (Fields et al., 2001) and toxic, and reduces the reproduction of many stored-product insect pests (Bodnaryk et al., 1997). Bodnaryk et al. (1997) produced an extract from protein-rich pea flour that is 20–100 times more toxic than the protein-rich pea flour itself. Delobel et al. (1998) isolated a polypeptide from peas that is toxic to stored-product insects.

There are a number of factors that may affect the toxicity of stored-grain insecticides. The temperature and moisture content of stored-grain are the two most important factors (Ioradanou & Watters, 1969; Snelson, 1987; Samson et al., 1988). Grain is stored under a wide range of temperature and moisture conditions because of differences in geography, seasons, and storage practises. It is important to understand how temperature and moisture influence the efficacy of insecticides. Malathion and pirimiphos-methyl have positive temperature coefficients for stored-product insects (Ioradanou & Watters, 1969; Snelson, 1987). However, some pyrethroids show a negative relationship with temperature for *S. oryzae* (Longstaff & Desmarchelier, 1983). Both positive and negative coefficients to temperature have been reported for lindane (De Vries & Georghiou, 1979) and carbamates (Reichenbach & Collins, 1984). The degradation of malathion and other

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insecticides increases with high temperature (Strong & Sbur, 1960). The toxicity of dichlorvos, chlorpyrifos, and carbaryl increases with high moisture content, whereas the toxicity of malathion (Barson, 1983) and diatomaceous earth (Fields & Korunic, 2000) decreases with high moisture content. Pyrethrins, malathion, and pirimiphos-methyl break down faster in grain with a higher moisture content (Quinlan et al., 1980; Snelson, 1987). Other factors also can affect efficacy. The toxicity of methoprene and fenoxycarb (Samson & Parker, 1989; Samson et al., 1990) varies with grain species.

The purpose of this study was to determine how temperature and moisture content, as well as insect density, grain species, size of grain kernel, and relative humidity (r.h.) affect the efficacy of protein-rich pea flour as a grain storage insecticide.

Materials and methods

Three species of beetles, *S. oryzae*, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) had been cultured in the laboratory at 30 °C, 70% r.h. for over 5 years. *Sitophilus oryzae* was reared on whole kernels of wheat. *Cryptolestes ferrugineus* was reared on wheat kernels with 5% wheat germ and 5% brewer's yeast by weight. *Tribolium castaneum* was reared on wheat flour mixed with 5% brewer's yeast.

Protein-rich pea flour (Progress Protein; 60% protein, 30% starch, and 7% moisture content, Parrheim Food, Saskatoon, SK) was used in this study. It is produced commercially by grinding peas and isolating a protein-rich fraction by air classification. Except where specified, the LD_{50} of protein-rich pea flour was tested by the following procedures. Grain with a 14% moisture content (based on wet weight) was treated with protein-rich pea flour, previously stored at –15 °C, at concentrations of 0, 0.001, 0.01, 0.1, 1.0, and 10.0% (wt/wt). Wheat, barley, or maize was mixed with protein-rich pea flour in a half-litre jar by shaking by hand for 2 min. Twenty grams of treated grain and 20 1- to 2-week-old insect adults were placed in vials (29 mm diameter, 88 mm height) with screening caps. The vials and insects were held at 30 °C, 70% r.h. for 2 weeks, and the number of live and dead insects at each concentration was counted to estimate the LD_{50}. Each treatment had five replications. To examine the effect of protein-rich pea flour on the reproduction of insects in grain kernels, after the adults had been removed, the grain in the vials was incubated for another 5 weeks at 30 °C, 70% r.h., and the number of emerged adults of *S. oryzae*, *C. ferrugineus*, and *T. castaneum* in wheat and barley and *S. oryzae* in maize was counted.

Canadian hard red spring wheat, maize, and barley were adjusted to the moisture content required for the test before being mixed with protein-rich pea flour. To produce the different particle sizes, wheat and maize were ground with a Stein laboratory mill (model M-2, Fred Stein Laboratory, Inc., Achison, KS). The ground particles were sieved with no. 10-, 14-, and 20-mesh sieves (2.0, 1.4, and 0.85 mm openings, respectively). Grain particles in the 2.0–1.4 mm and 1.4–0.85 mm range were collected. The grain kernels or particles were mixed with protein-rich pea flour at different concentrations. The LD_{50}s of the protein-rich pea flour against *S. oryzae* were compared among particle sizes with the same grain species or between and wheat and maize at the same size.

To determine if population density affected the efficacy of protein-rich pea flour against *S. oryzae*, two population densities were prepared by putting 20 adult insects in 20 g wheat kernel vials (equivalent to 1000 insects per kg) and by putting 25 adult insects in 2500 g wheat kernels in 4-l jars (equivalent to 10 insects per kg), the latter density being closer to that found in commercial grain stores (Sinha & Watters, 1985). For each density, wheat kernels were treated with protein-rich pea flour at various concentrations as described above, and there were five replicates per concentration.

To test for stability, the toxicity of protein-rich pea flour against *S. oryzae* was tested immediately upon delivery from the manufacturer (fresh), or after 9 months under the following conditions: held at –15 °C, held in a room where temperature ranged from 20 to 30 °C, 25 to 70% r.h., or held in the same room but on wheat, barley, and maize at various concentrations.

Toxicity of protein-rich pea against *S. oryzae* at temperatures of 20, 25, 30, and 35 ± 1 °C maintained in growth cabinets was tested on wheat. Vials containing wheat and adult *S. oryzae* were placed in desiccators with a constant relative humidity of 75% maintained by a saturated NaCl solution (Loveridge, 1980), and verified with a psychrometer (Cole-Parmer Instruments, Chicago, IL).

To study the effect of moisture, wheat was conditioned to the moisture contents of 18, 16, 14, and 10%, and was placed, respectively, in sealed containers with 300 ml saturated salt solution of KCl, NaCl, $NaNO_2$, and K_2CO_3 (Loveridge, 1980) at 30 °C in a cabinet. The relative humidities were approximately 85, 75, 65, and 45%, and the moisture content of the wheat after 3 weeks was 17.4, 16.7, 13.0, and 10.2%, respectively. The wheat was then treated with protein-rich pea flour at different concentrations. Vials containing wheat and adult *S. oryzae* were immediately placed in their respective containers sealed with a cotton plug to maintain the grain moisture content, yet allowing some air movement. Mortality was noted as above.

POLO-PC probit and logit analysis (LeOra Software, 1994) was used to calculate the LD_{50}s, LD_{90}s, and slopes. LD_{50} and the slopes of the regression lines were compared to determine the equality or parallelism of the regression lines by using the likelihood ratio test ($\alpha = 0.05$). Numbers of offspring in grain treated at different concentrations of protein-rich pea flour were compared with SAS GLM procedure with CONTRAST at a linear function ($\alpha = 0.05$) (SAS Institute Inc., 2000).

Results

Grain species and kernel size

Sitophilus oryzae was less sensitive to fresh protein-rich pea flour when held on maize (LD_{50} = 0.17%) than on wheat and barley (LD_{50} = 0.04%) ($P < 0.05$, Table 1). When the wheat and maize were ground and the particle size standardized between grains, the response of *S. oryzae* to protein-rich pea flour did not change (Figure 1). The LD_{50}s of *S. oryzae* held on whole kernels of wheat and 2.0–1.4 mm granules were 0.03% (0.02–0.05) and 0.04% (0.03–0.05), respectively. The LD_{50}s of *S. oryzae* held on whole kernels of maize and granules of 2.0–1.4 and 1.4–0.85 mm were 0.13% (0.10–0.16), 0.15% (0.11 ± 0.19), and 0.11% (0.09–0.14), respectively. No difference was found within the same grain ($P >$ 0.05).

Stability

For wheat, barley, and maize, no difference in LD_{50} was detected among fresh protein-rich pea flour, flour stored for 9 months at –15 °C, flour stored at room temperature, or flour stored at room temperature on grain for 9 months ($P > 0.05$) (Table 1).

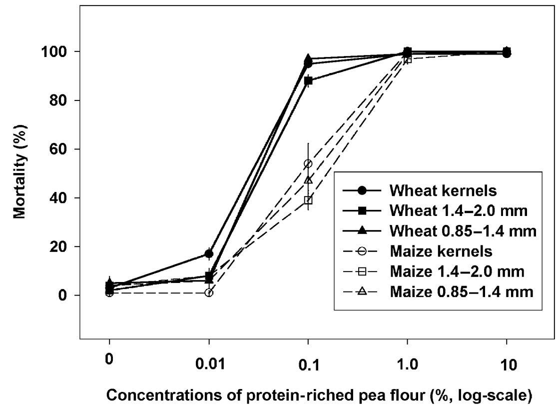

Figure 1 The mortality of *Sitophilus oryzae* (mean ± SE) in whole and ground grain at various particle sizes and treated with various concentrations of protein-rich pea flour. Insects and grain were held at 30 °C, 70% r.h. for 2 weeks.

Insect species and density

Sitophilus oryzae (LD_{50} = 0.03% [0.02–0.04]) was more sensitive to protein-rich pea flour stored at –15 °C than *C. ferrugineus* (LD_{50} = 6.0% [3.2–12.6]) at a population density of 1000 insects per kg wheat. The mortality of *T. castaneum* was less than 20% at all concentrations. No significant difference in the LD_{50} of *S. oryzae* was detected between a population density of 1000 insects per kg and 10 insects per kg ($P > 0.05$). At 10 insects per kg, *S. oryzae* had a LD_{50} = 0.04% (0.03–0.06), g = 0.072, and b = 2.83 ± 0.39. Data for the test using 1000 insects per kg are given in Table 1.

Offspring

Protein-rich pea flour reduced the number of offspring produced by *S. oryzae, C. ferrugineus*, and *T. castaneum* in

Table 1 LD_{50} of protein-rich pea flour to *Sitophilus oryzae* held on wheat, barley, or maize for 2 weeks, treated with protein-rich pea flour that was stored by various methods

	Wheat			Barley			Maize		
Protein-rich pea flour storage conditions	LD_{50} (%) (95% C.L)	Slope	g-value	LD_{50} (%) (95% C.L)	Slope	g-value	LD_{50} (%) (95% C.L)	Slope	g-value
Fresh	0.04 (0.03–0.05)	3.3 ± 0.3	0.034	0.04 (0.03–0.05)	3.4 ± 0.4	0.048	0.17 (0.12–0.23)	1.9 ± 0.2	0.064
Flour at –15 °C for 9 months	0.03 (0.02–0.04)	3.0 ± 0.4	0.059	0.05 (0.02–0.06)	3.4 ± 0.8	0.028	0.21 (0.14–0.28)	1.6 ± 0.2	0.062
Flour in a room for 9 months	0.03 (0.02–0.04)	3.0 ± 0.06	0.137	0.04 (0.03–0.05)	3.0 ± 0.3	0.034	0.17 (0.12–0.23)	1.8 ± 0.2	0.057
Treated grain in a room for 9 months	0.03 (0.02–0.04)	3.2 ± 0.5	0.098	0.03 (0.02–0.04)	3.6 ± 0.6	0.107	0.11 (0.08–0.15)	1.6 ± 0.2	0.043

There was no difference among LD_{50}s and slopes of various storage conditions. There was a difference between LD_{50}s in maize compared to wheat and barley (POLO-PC, $P < 0.05$). There was no difference between LD_{50} in wheat and barley (POLO-PC, $P > 0.05$).

Table 2 Mean number (± SE) of live adult offspring emerged from grain treated with various concentrations of protein-rich pea flour at 30 °C, 70% r.h. after 7-week incubation. Twenty parent adults were removed 2 weeks after being released

Concentration of protein-rich pea flour (%)	Number of insects (mean ± SE)[a]						
	Wheat			Barley			Maize
	S. oryzae	*C. ferrugineus*	*T. castaneum*	*S. oryzae*	*C. ferrugineus*	*T. castaneum*	*S. oryzae*
0	357 ± 46 a	10.8 ± 4.9 a	20.4 ± 2.3 a	234 ± 53 a	20.2 ± 3.7 a	40.4 ± 6.3 a	7.2 ± 1.7 a
0.01	247 ± 33 b	7.6 ± 3.4 a	23.8 ± 2.4 a	205 ± 10 a	26.6 ± 5.2 a	39.4 ± 1.0 a	6.0 ± 1.1 a
0.1	146 ± 25 b	8.6 ± 2.3 a	25.4 ± 2.2 a	77 ± 9 b	24.6 ± 10.4 a	32.4 ± 2.9 a	6.0 ± 1.1 a
1	0.8 ± 0.6 c	2.8 ± 1.4 b	9.6 ± 2.2 b	0.8 ± 0.4 b	3.0 ± 1.8 b	10.6 ± 2.0 b	2.6 ± 0.6 b
10	0 ± 0 c	0.2 ± 0.2 b	9.8 ± 2.5 b	0.2 ± 0.2 b	3.8 ± 3.1 b	3.2 ± 1.5 b	0 ± 0 c

[a]Different letters indicate that the number of insects in a column were significantly different (SAS GLM procedure, $P < 0.05$).

Table 3 The LD_{50} and LD_{90} of protein-rich pea flour (%) for *Sitophilus oryzae* in wheat at various temperatures at 70% r.h. after 2 weeks

Temperature (°C)	LD_{50} (%)[a] (95% C.L)	LD_{90} (%) (95% C.L)	Slope[a]	g-value
20	0.043 (0.034–0.052) a	0.247 (0.175–0.415)	1.68 ± 0.14 a	0.054
25	0.038 (0.033–0.042) a	0.099 (0.086–0.12)	3.07 ± 0.20 b	0.025
30	0.028 (0.024–0.031) b	0.067 (0.067–0.077)	3.32 ± 0.27 b	0.025

[a]Different letters in a column indicate that the LD_{50}s or slopes were significantly different (POLO-PC, $P < 0.05$).

Table 4 The LD_{50} and LD_{90} of protein-rich pea flour (%) for *Sitophilus oryzae* in wheat at three relative humidities and at 30 °C for 2 weeks

Relative humidity (%)	Moisture content (%)	LD_{50} (%)[a] (95% C.L)	LD_{90} (%) (95% C.L)	Slope[a]	g-value
85	17.4	0.085 (0.073–0.098) a	0.224 (0.176–0.33)	3.027 ± 0.289 a	0.072
75	16.7	0.055 (0.046–0.063) b	0.119 (0.10–0.158)	3.776 ± 0.325 a	0.085
65	13.0	0.035 (0.025–0.044) c	0.117 (0.09–0.175)	2.417 ± 0.191 b	0.083

[a]Different letters indicate that the number of insects that the LD_{50}s or slopes were significantly different (POLO-PC, $P < 0.05$).

wheat, barley, and maize (Table 2). The higher the dose of protein-rich pea flour, the greater the reduction in the number of offspring. Treated with protein-rich pea flour at 1%, the populations of *S. oryzae* in wheat, barley, and maize were reduced by 99.8, 99.7, and 63.8%, respectively. Populations of *C. ferrugineus* and *T. castaneum* were reduced by 74% and 52% in wheat, and 85% and 74% in barley, respectively. At 0.1%, protein-rich pea flour reduced the offspring production of *S. oryzae* by 60% in wheat, and 67% in barley. Grain with 0.1% of protein-rich pea flour did not reduce the number of offspring of *T. castaneum* and *C. ferrugineus.*

Temperature and moisture

Temperature affected the toxicity of protein-rich pea flour. LD_{50} decreased with higher temperatures (Table 3). All the *S. oryzae* died at 35 °C, 70% r.h. in all treatments including the controls, and this treatment was excluded. The LD_{50} of protein-rich pea flour decreased with increased relative humidity (Table 4). The mortality of *S. oryzae* exceeded 80% in the controls when the relative humidity was 45% (moisture content of wheat was 10.2%).

Discussion

Protein-rich pea flour is toxic to many stored-product insects (Bodnaryk et al., 1997). We found that the toxicity of protein-rich pea flour was insect specific. A variation in susceptibility by stored-product insect species is known for other insecticides. For deltamethrin, *T. castaneum* is approximately 10-fold, and *Rhyzopertha dominica* (F) is approximately 100-fold more susceptible than *S. oryzae* (Snelson, 1987). For malathion, *S. oryzae* is twofold more susceptible than *T. castaneum* (Snelson, 1987). Diatomaceous earth against *Sitophilus granarius* (L.) is approximately threefold more effective than against *Tribolium confusum* Duv. (Aldryhim, 1990). For protein-rich pea flour, *S. oryzae* adults were approximately 20-fold more susceptible than *C. ferrugineus* adults, and it reduced the production of *S. oryzae* offspring more than *C. ferrugineus* offspring. It reduced production of *T. castaneum* offspring, but did not kill *T. castaneum* adults in the 2-week test. Therefore, it is important to identify which insect pests are present before applying protein-rich pea flour to control infestations in commercial grain stores.

Grain species affected the toxicity of protein-rich pea flour. To achieve the same level of mortality, a higher dose was required in maize than in wheat and barley. The difference in efficacy was not due to the differences in kernel size, because ground particles of the same sizes still showed a difference between maize and wheat. Grain species also affects the toxicity of insect growth regulators and other grain protectants (Samson & Parker, 1989; Samson et al., 1990). For example, methoprene and fenoxycarb are more effective against *R. dominia* in wheat and paddy than in maize (Samson et al., 1990). Diatomaceous earth used against *S. oryzae* is 3–4-fold more effective in wheat than in maize (P. Fields, unpubl.). However, with barley and wheat, grain species did not affect the toxicity of either malathion or fenitrothion against *C. ferrugineus* (Tyler & Green, 1968). Baker (1988) found that *S. oryzae* produces more progeny, and develops faster in barley and wheat than in maize. Therefore, the lower effectiveness of protein-rich pea flour in maize was not likely to be due to the differences in fitness of insects on different grains. Insects may feed less in maize, which may result in a lower uptake of protein-rich pea flour, thereby making it less toxic in maize. More studies are needed to determine the reasons for the difference observed between grain species.

The toxicity of protein-rich pea flour was not affected by storing it at low and room temperatures or on treated grain for 9 months. This property enables protein-rich pea flour to be used as a grain protectant, especially for long-term grain storage. However, warmer and moister conditions should be avoided for the storage of protein-rich pea flour, because there is a reduction of toxicity after storing protein-rich pea flour at 30 °C, 70% r.h. for 8 months (Bodnaryk et al., 1997).

The effect of temperature and moisture on the toxicity of stored-grain insecticides has been extensively studied (Snelson, 1987; Samson et al., 1988, 1990). Usually, increasing the moisture of the grain decreases the effectiveness of organophosphorus insecticides (Samson et al., 1988). Protein-rich pea flour was more toxic when the grain temperature was high or moisture content was low. The effect of environmental factors on insecticidal efficacy can be subdivided into: (i) effects on insect biology, such as development of insects, metabolism of insecticides, and storage and excretion of insecticides; (ii) effects on insecticide toxicity, such as the penetration, movement within the insects, and decomposition; and (iii) effects on the persistence of the insecticides, such as rate of degradation (Snelson, 1987). Each factor has its own relationship to temperature and moisture. Protein-rich pea flour was stable under normal storage conditions. The phenomenon of lower toxicity of protein-rich pea flour at higher moistures might be related to detoxification of pea protein inside *S. oryzae*. The water content of a resistant strain of *S. oryzae* is higher than in a susceptible strain (X. Hou and P. Fields, unpubl.). The stress of low moisture content may make the insects more susceptible to a second stress, such as a toxin. Low moisture content increases the toxicity of synthetic insecticides, such as fenitrothion and pirimiphos-methyl (Samson et al., 1988).

Granaries in Western Canada with high grain temperature immediately after harvest have the most insects after a 2-month storage period (Loschiavo, 1985). Therefore, it would be best to treat grain immediately after harvest when it is warm and protein-rich pea flour would be more effective in reducing the risk of infestation.

The development of resistance to insecticides has been observed in many insects (Cochran, 1995; Subramanyam & Hagstrum, 1995; Ware, 2000). Several tropical strains of *S. oryzae* are capable of breeding on yellow-split peas (Coombs et al., 1977; Grenier et al., 1997). This resistance to yellow-split peas is controlled by a single recessive, autosomal gene (Thind & Muggleton, 1981; Holloway, 1986; Grenier et al., 1997). The possibility of an adaptation of insects to peas should be seriously considered in the development of protein-enriched pea flour for the control of stored-product insects, as well as the effect of the grain species, insect species, temperature, and moisture content of the grain.

Acknowledgements

We thank Tannis Mayert for technical assistance, Bob Lamb and Ian Wise for comments on an earlier version of this paper, the Agri-Food Research and Development Initiative and the University of Manitoba for financial support, and Parrheim Foods for providing the protein-enriched pea flour. This is contribution 1852 from the Cereal Research Centre, Agriculture and Agri-Food Canada.

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