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The effect of repellents on penetration into packaging by stored-product insects

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Abstract

Two known repellents of stored-product insects, DEET and neem, were compared to protein-enriched pea flour, defatted protein-enriched pea flour, and pea protein extract for their efficacy at reducing penetration and invasion by several common stored-product insects: *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens), and *Oryzaephilus surinamensis* (L.). The methods of preparation of pea extract affected the penetration of *S. oryzae*. Protein-enriched pea flour, DEET and neem reduced the penetration of *S. oryzae,* but defatted protein-enriched pea flour and pea protein extract did not. The number of *S. oryzae, T. castaneum, C. ferrugineus* and *O. surinamensis* entering pierced paper envelopes that contained wheat and were treated with DEET was reduced by 99%, 86%, 97% and 91%, respectively. Neem was less effective than DEET in reducing penetration and invasion of insects. Protein-enriched pea flour did not prevent insects entering pierced envelopes.

Keywords: DEET; Neem; Pea extracts; Repellency; Invasion

1. Introduction

Although finished products can be shipped from production facilities uninfested, stored-product insects can enter packaged goods during transportation, storage in the warehouse, or in retail stores. Ultimately, the consumer of the products holds the manufacturer responsible for any insect infestation, even if the cause of the problem is poor storage by a third party. The packaging of products is the last line of defense for processors against insect infestation of their finished

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products. There are two types of insects that attack packaged products: ‘‘penetrators’’, which are insects that can bore holes through packaging materials; and ‘‘invaders’’, which are insects that enter packages through existing holes, such as folds and seams and air vents (Highland, 1984; Newton, 1988). *Sitophilus* spp., *Rhyzopertha dominica* (F.), *Plodia interpunctella* (Hübner), *Lasioderma serricorne* (F.), and *Stegobium paniceum* (L.) are some of the stored-product insects that are capable of penetrating food packaging. However, *Tribolium* spp., *Cryptolestes ferrugineus* (Stephens), and *Oryzaephilus* spp. cannot penetrate intact packages and must enter through existing holes in the package (Highland, 1991).

In addition to improving the packaging material and design, insect repellents are used to prevent insects from entering packages by modifying the behavior of insects (Highland, 1984; Mullen, 1994; Watson and Barson, 1996; Mullen and Mowery, 2000). Pyrethrins synergized with piperonyl butoxide were approved for use as a treatment for insect-resistant packaging on the outer layer of packages or with adhesive in the USA (Highland, 1991). The repellency of pyrethrins was the primary mode of action against insect penetration and invasion (Laudani and Davis, 1955). Methyl salicylate, an insect repellent, has been registered to be used in food packaging to control stored-product insects in the USA (Radwan and Allin, 1997). DEET, neem, and protein-enriched pea flour are repellent to many stored-product insects when tested by exposure on filter paper or in preference chambers (Khan and Wohlgemuth, 1980; Xie et al., 1995; Fields et al., 2001). The purpose of this work was to explore the possibility of using these insect repellents to prevent insects from penetrating or invading food packages.

2. Materials and methods

2.1. Insects

Four insect species, *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *C. ferrugineus,* and *Oryzaephilus surinamensis* (L.) were reared in the laboratory at 30°C, 70% relative humidity (r.h.). All species had been cultured in the laboratory for over five years. One- to two-week-old adults were used in all the experiments. *Sitophilus oryzae* was reared on whole kernels of wheat, and *T. castaneum* was reared on wheat flour mixed with 5% brewer’s yeast. *Cryptolestes ferrugineus* and *O. surinamensis* were reared on wheat kernels, with 5% wheat germ and 5% brewer’s yeast, by weight.

2.2. Compounds

Protein-enriched pea (*Pisum sativum* L.) flour, defatted protein-enriched pea flour, pea protein extract, DEET (diethyl-*m*-toluamide, content >95%, Record 100, Recochem Inc., Vancouver, Canada) and neem (Amazin™ with 3% azadirachtin, AMVAC Inc., Los Angeles, USA) were used to estimate their effect on the penetrating ability of *S. oryzae.* Protein-enriched pea flour (60% protein, 30% starch) was provided by Parrheim Foods, Saskatoon, Canada. Defatted protein-enriched pea flour was prepared from protein-enriched pea flour by defatting with chloroform for 1 h at room temperature. Pea protein extract was prepared from defatted protein-enriched pea flour by a batch process with a copolymeric resin of styrene and divinylbenzene (Bodnaryk et al., 1999). One hundred milligram of protein-enriched pea flour produced about 1 mg of pea protein extract. Test materials were dissolved in water, except pea protein extract and DEET, which were dissolved in 70% ethanol.

2.3. Paper selection

To select suitable paper for the penetration test, the following papers were tested: filter paper (Whatman number 1, 4, and 5), waxed paper, rough tissue paper, napkin paper, and coffee filter paper. The weights by area and the thickness of paper (measured with a Manostat 15–100–500 calliper) are listed in Table 1. The test apparatus was similar to that described by Newton (1988) (Fig. 1). Test paper was clamped between two steel plates (0.5 cm thick). Each plate had 10 holes (1.0 cm diameter). The top plate had a small notch close to the paper to help the insect penetrate the paper. One adult *S. oryzae* was placed in each hole, and confined with a metal screen and covered with another metal plate on the top. The assembled blocks were placed at 30°C, 70% r.h. The number of insects penetrating the paper was noted after 12, 24, 48, 72, and 96 h. The tests were repeated three times.

2.4. Penetration test

The apparatus for penetration tests consisted of four metal plates the same as those used in the paper selection test (Fig. 1). Napkin paper was clamped between two steel blocks. Neem, protein-enriched pea flour, and defatted protein-enriched pea flour were mixed with water. DEET and pea protein extract, were mixed with 70% ethanol. The doses used in this test were: 31.25 g/m^2 for protein-enriched pea flour and defatted protein-enriched pea flour, 12.5 g/m^2 for pea protein extract and neem, and 0.02 g/m^2 for DEET. Fifty microliters of each mixture was placed on the napkin paper within each well. The paper clamped in the plates was allowed to dry at room temperature in a fume hood for 24 h. A similar set of plates with napkin paper was treated with either 50 µl of water to serve as the control for neem, protein-enriched pea flour, and defatted protein-enriched pea flour, or 50 µl of 70% ethanol to serve as the control for DEET and pea

Table 1
Percentage of *Sitophilus oryzae* that penetrated through various types of paper ($n = 3$)

Paper	Paper weight (mg/cm^2 ±SEM)	Thickness (µm ±SEM).	Number of insects penetrating paper (% ±SEM). Time after insect release (h)				
			12	24	48	72	96
Tissue paper	1.6±0.1	6.7±0.1	77±12	90±6	100±0	100±0	100±0
Coffee filter paper	3.2±0.1	10.1±0.1	57±3	93±3	100±0	100±0	100±0
Napkin paper	3.5±0.02	15.9±0.2	83±3	83±3	90±3	93±3	100±0
Waxed paper	6.8±0.1	3.3±0.1	0±0	43±12	47±3	63±3	83±3
Whatman no. 1	8.5±0.3	17.3±0.1	0±0	0±0	0±0	0±0	0±0
Whatman no. 4	9.8±0.1	21.7±0.1	0±0	0±0	3±3	10±6	10±6
Whatman no. 5	9.9±0.1	19.7±0.1	0±0	0±0	3±3	13±3	13±3

Fig. 1. The unstacked apparatus for penetration tests. One adult *S. oryzae* was confined in the hole in the second plate.

protein extract. One adult *S. oryzae* was placed in each hole. Plates with the control paper were placed on the top and the two sets of plates were screwed together. The plate with a small notch was placed close to the treated paper. The clamped plates were shaken gently and placed vertically in incubators at 30°C, 70% r.h. The number of insects penetrating the treated paper after 24 h was noted. The test was repeated four times.

2.5. Invasion test

Protein-enriched pea flour (50 mg protein-enriched pea flour, mixed with 200 μl water), neem (200 μl of Amazin), or DEET (50 μl DEET mixed with 150 μl 70% ethanol) were pipetted on to each envelope ($9 \times 15\,cm^2$). The suspensions or solutions containing repellent were evenly pipetted on a 1 cm wide strip near the bottom of the envelopes. Envelopes were allowed to dry overnight at room temperature, and then within the treated strip, six holes (2 mm diameter) on each side of the envelope were punched. The holes simulated the stitching, damaged packaging, or poor sealing of packages. Water was used as a control for neem and protein-enriched pea flour. Seventy percent ethanol was used as the control for DEET. The envelope was filled with 80 g hard red spring wheat [15% moisture content (m.c.), wet weight-based] and placed in a $30 \times 30 \times 30\,cm^3$ screened box. Six treated envelopes were split into two groups of three and placed on the bottom of the box at opposite corners. Three control envelopes were placed at each of two remaining corners. The box was placed at 30°C, 70% r.h. Two hundred adults each of *S. oryzae*, *T. castaneum*, *C. ferrugineus*, and *O. surinamensis* were placed at the middle of the bottom of the box, and confined for 1 h with a 2-l jar to allow them to acclimate before being released into the cage. The number and species of insects inside each envelope were noted after 1 week. Tests were repeated three times.

2.6. Data analysis

SAS CORR (SAS Institute, 2000) procedure was used to measure the correlation between the paper and the number of insects that penetrated the paper. Data from penetration tests were analyzed using the SAS GENMOD procedure by comparing the number of each insect species that penetrated through the treated paper. For envelope tests, the sum of insects caught in six treated and untreated envelopes was compared by the GENMOD procedure with the expectation that insects were evenly distributed between treated and untreated bags. To compare the effectiveness of three repellents, the proportion of the total number of insects in treated envelopes out of all insects found in the screen boxes was transformed with the arcsine function and compared pairwise with GT2 in the GLM procedure (SAS Institute, 2000).

3. Results and discussion

The heavier the paper, the fewer *S. oryzae* that penetrated the paper (Table 1). The correlation coefficients of paper weight to the penetration of *S. oryzae* at 12, 24, 48, 76, and 96 h were −0.81, −0.91, −0.92, −0.85, and −0.82, respectively ($P<0.0001$). Most of the *S. oryzae* penetrated tissue paper and coffee filter paper within 12 h, and all insects escaped after 48 h. Whatman filter papers were more resistant than all other papers. Most of the *S. oryzae* penetrated waxed paper after 96 h. The correlation coefficients of the thickness of the paper to the penetration at 12, 24, 48, 76, and 96 h were −0.40, −0.70, −0.68, −0.66, and −0.63, respectively ($P<0.1$). We chose napkin paper for further experiments for two reasons. First, most of the *S. oryzae* could penetrate it within 24 h thus reducing the adverse effects of starvation. Second, it absorbed the test solutions well.

DEET and neem prevented *S. oryzae* penetration of treated paper (Table 2). No insects penetrated through napkin paper treated with DEET at 0.02 g/m^2. Neem has antifeedant effects (Saxena et al., 1989). The reduction of the penetration by neem may be due to its repellent and antifeedant properties. The original protein-enriched pea flour significantly reduced the number of insects penetrating the treated paper. However, defatted protein-enriched pea flour and pea protein extract did not reduce the penetration of *S. oryzae*. This suggested that repellent compounds had been removed by chloroform, or the defatting procedure destroyed the repellent compounds in the protein-enriched pea flour.

DEET reduced the number of insects entering the envelopes (Table 3). Based on the total number of insects found in both treated and untreated envelopes, DEET repelled *S. oryzae, T. castaneum, C. ferrugineus,* and *O. surinamensis* by 99%, 86%, 97%, and 90%, respectively. The total number of all insect species in the DEET-treated envelopes was only 6% of insects found in all envelopes. Neem was also repellent, but it was less effective than DEET (Table 4) ($P<0.05$, GT2). The number of *S. oryzae, T. castaneum, C. ferrugineus,* and *O. surinamensis* in neem-treated envelopes was 37%, 21%, 44%, and 39% of the same insects caught in both treated and untreated envelopes, respectively. The total number of all insect species in neem-treated envelopes was reduced by 38% of all insects found in envelopes. Protein-enriched pea flour did not stop insects from entering the envelopes (Table 5). Its effectiveness was significantly lower than DEET and neem ($P<0.05$, GT2). Protein-enriched pea flour treated envelopes attracted more *S. oryzae,*

Table 2
Number of *Sitophilus oryzae* (±SEM) that penetrated through napkin paper treated with different materials after 24 h. Ten insects were used in each treatment ($n = 4$)

Materials	Dose (g/m^2)	Number of insects that penetrated through treated paper[a]
Water (control)	625	7.5±0.5 a
70% ethanol (control)	625	7.5±0.6 a
Pea protein extract	12.5	7.3±0.5 a
Defatted protein-enriched pea flour	31.25	7.3±0.6 a
Protein-enriched pea flour	31.25	3.5±0.3 b
Neem	12.5	4.3±0.3 b
DEET	0.02	0±0 c

[a] Different letters indicate that the materials were significantly different (SAS PROC GENMOD, $P<0.05$).

Table 3
Number of insects (±SEM) in envelopes treated with DEET at 50 μl/envelope, 1 week after insects were released ($n = 4$)

Insect	Number of insects		χ^2	P
	Treated	Untreated		
Sitophilus oryzae	2±0.3	189±3	735.82	<0.0001
Tribolium castaneum	17±3	101±8	197.88	<0.0001
Cryptolestes ferrugineus	4±0.7	117±28	354.1	<0.0001
Oryzaephilus surinamensis	11±3	100±8	246.77	<0.0001
All insects	34±5	507±31	1481.56	<0.0001

T. castaneum, and *O. surinamensis* than untreated envelopes. The total number of insects in pea flour-treated envelopes was 60% of all insects found in both treated and untreated envelopes. As a natural product, protein-enriched pea flour showed a repellent effect to many insects when it was tested on grain in chambers (Fields et al., 2001). It repelled *S. oryzae* in penetration tests but not in the invasion test. This suggested that the repellency of pea flour was weak, or there might be an interaction between the chemicals in pea flour and wheat.

Mullen and Mowery (2000) state that most insects enter into finished products through openings caused by sewing, folding, or damage, not by chewing through packaging. Some adult insects can pass through holes less than 1 mm in diameter, and their larvae can enter through smaller holes (Cline and Highland, 1981). Therefore, the ability of chemical barriers to prevent insects from invading is more important than the prevention of penetration. Although protein-enriched pea flour showed repellency in the penetration test, it attracted insects into packages so it would not provide added protection to packaging. Neem is repellent to many insects (Xie et al., 1995). Our data showed that neem was repellent enough to reduce insect immigration into packages. Further work is needed to determine if this has commercial potential.

Hundreds of materials have been investigated for use in insect-resistant packaging, such as synthetic pyrethroids, natural botanical antifeedants, and silica gel (Bloszyk et al., 1990; Highland et al.,1984; Laudani and Davis, 1955; Watters, 1966). Highland et al. (1984) showed that insects

Table 4
Number of insects (±SEM) in envelopes treated with neem at 200 μl/envelope, 1 week after insects were released ($n = 4$)

Insect	Number of insects		χ^2	P
	Treated	Untreated		
Sitophilus oryzae	70±9	121±12	41.63	<0.0001
Tribolium castaneum	20±4	74±10	95.26	<0.0001
Cryptolestes ferrugineus	60±10	76±1	7.06	0.0079
Oryzaephilus surinamensis	57±4	89±8	20.24	<0.0001
All insects	220±16	361±12	104.3	<0.0001

Table 5
Number of insects (±SEM) in envelopes treated with protein-enriched pea flour in at 50 mg/envelope, 1 week after insects were released ($n = 4$)

Insect	Number of insects		χ^2	P
	Treated	Untreated		
Sitophilus oryzae	116±2	67±3	40.19	<0.0001
Tribolium castaneum	70±4	59±9	3.4	0.0653
Cryptolestes ferrugineus	73±16	69±12	0.13	0.7178
Oryzaephilus surinamensis	75±10	42±2	26.81	<0.0001
All insects	337±24	229±12	61.54	<0.0001

did not infest cereal–grain packed bags treated with permethrin. Included in the construction of the multiple-wall bags was a barrier layer that prevented the migration of permethrin into the cereals. Both the penetration and the envelope tests in this study suggested that DEET has a great potential for preventing the infestation of packaged goods. This repellent is mainly used for protecting humans from biting flies. Its acute oral toxicity to rats is 2.0 g/kg (Ware, 1980). There is no evidence showing DEET to be acutely toxic, carcinogenic, developmentally toxic, or mutagenic (Anonymous, 1998). DEET is also used on clothing and mosquito netting. Our data suggest it might also be effective on jute bags, which are commonly used in many developing countries for grain storage. However, DEET has a plasticizing action and is not compatible with wax paper and plastic sheet, which are currently used in many food packages. The packaging would have to be modified to prevent the contamination of the finished product by DEET, and the contamination of neighboring products by the volatile action of DEET. Barriers developed for preventing the migration of pyrethrins into packaging (Highland, 1975), or similar barriers may prevent the contamination of food by DEET.

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