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A simple technique to assess compounds that are repellent or attractive to stored-product insects

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Abstract

We have developed a simple and rapid technique that mimics storage conditions, and determines if products are repellent or attractive to stored-product insects. The technique determines the response of insects to potential repellents and attractants by measuring their movement from grain. The technique used a device consisting of a perforated cup (2 mm perforations) that holds 200 g of wheat. A Petri dish and cup collected the insects as they left the wheat. Several natural products were tested for repellency: diatomaceous earth (DE), ground peas (*Pisum sativum*), protein-rich pea flour, pea starch, and pea fibre. Adult insects of three species were tested: the rice weevil, *Sitophilus oryzae*, the red flour beetle, *Tribolium castaneum*, and the rusty grain beetle, *Cryptolestes ferrugineus*. DE at 0.01% was repellent to all insects tested. Pea fibre, pea protein, and ground pea at 1% caused increased emigration of *C. ferrugineus* from the wheat. Pea starch did not affect movement out of the grain for all three insects. Only pea fibre and ground pea increased the movement of *T. castaneum* out of the grain. For *S. oryzae*, there were no differences after 1 h, but after 24 h both pea protein and ground pea increased movement out of the grain. Several potential attractants were placed outside the grain and the emigration out of the grain noted. For *R. dominica*, the commercial *R. dominica* pheromone increased the emigration of insects from the grain; *R. dominica* adults on broken grain enclosed in a ventilated vial in the collection jar also increased emigration, but not as much as the synthetic pheromone. The commercial *Tribolium* pheromone did increase movement out of the grain for *T. castaneum*, but the other treatments were no different from the control. None of the potential attractants increased the movement of *S. oryzae* from the grain. The implications of this work are discussed with reference to controlling and sampling stored-product insect pests. Crown Copyright © 2001 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

Annual post-harvest losses resulting from insect damage, microbial deterioration, and other factors are estimated to be 10–25% of production world wide (Matthews, 1993). Synthetic pesticides are currently the method of choice to protect stored grain from insect damage. However, their widespread use has led to the development of pest strains resistant to insecticides (Subramanyam and Hagstrum, 1995). Also, there is a demand for safer insecticides because of concerns about insecticide residues on grain and health hazards to grain handlers. Therefore, there is an interest in finding alternatives to synthetic insecticides. Natural products such as fumes of gazelle dung, spices, waste water from olive pressings and neem, have been used to control stored-product insect pests since the dawn of agriculture (Levinson and Levinson, 1998). In addition to being toxic to stored-grain insects, many natural products are also repellent or attractive. However, many of these products must be used at high doses or they do not provide sufficient control.

Many attractants and repellents have been tested using laboratory bioassays (Burkholder, 1990; Dowdy et al., 1993), however, these tests do not mimic field conditions (White et al., 1990; Morgan et al., 1998) or require large amounts (0.6–1 kg/test) of grain to be treated (Loschiavo, 1952). Therefore, we developed a simple and rapid technique to determine if products are repellent or attractive to stored-product insects. This method exploits the oriented movement of insects away from or towards the natural product and uses grain as a medium instead of filter paper treated with repellents or attractants.

2. Materials and methods

2.1. Cup bioassay

The cup bioassay has a perforated cup that holds 200 g of hard red spring wheat. It is made of plastic screening with 2 mm perforations shaped into a cylinder of 7 cm diameter \times 10 cm high, with a mesh bottom, and is partially placed in a clear plastic cup of 7 cm diameter \times 9 cm high. For testing compounds in the grain, a Petri dish below the cup collects the insects that left through the sides, while the cup collected the insects that left through the bottom (Fig. 1). The insects are released into the centre of the grain mass in the container through a long-stemmed funnel. The funnel is removed, and the number of insects leaving the treated grain compared to the untreated controls give a measure of repellency or attractiveness of the compound. The container has a lid to prevent the escape of flying insects.

When testing volatile compounds for attractiveness, the perforated container (without the cup) was hung inside a sealed 4-l glass jar with the potential attractant on the bottom of the jar. The rate of insects leaving the grain was determined by counting the insects that are collected in the jar. All experiments were run at $25 \pm 1^\circ\text{C}$, $75 \pm 10\%$ relative humidity (r.h.). Tests were run for 1–72 h. All grain used in these tests had 15% moisture content (m.c.), wet mass basis. There were three replicates per treatment.

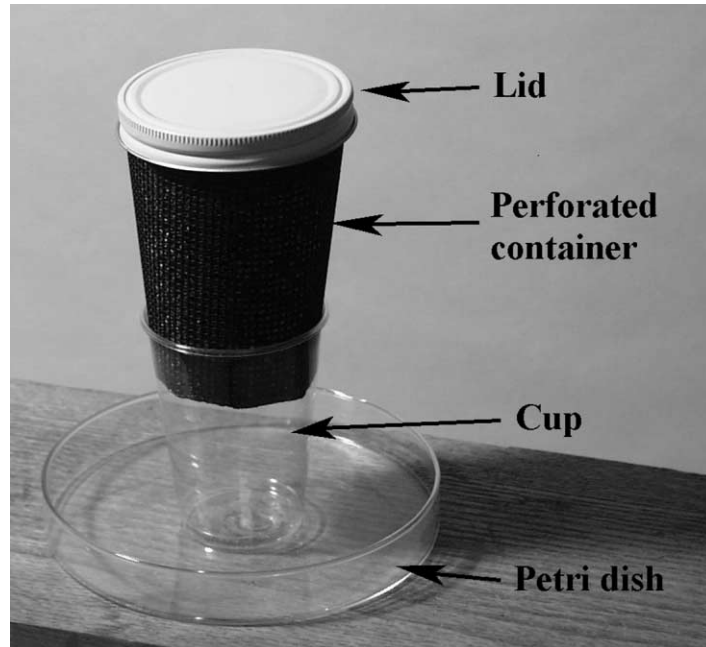


Fig. 1. Apparatus used in the cup bioassay to determine repellency or attractiveness of products to stored-product insects. Grain is placed in the perforated container, insects are introduced by a funnel into the centre of the grain mass. Insects leaving the grain are captured in the cup or Petri dish.

2.2. Test insects and test products

Unsexed adults were used in the study. The insect species studied were: *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), and *Rhyzopertha dominica* (F.). All cultures had been maintained in the laboratory for at least 5 years.

To assess the effectiveness of the cup bioassay, the insects were tested against the following potential repellents (Bodnaryk et al., 1999) or attractants. Three pea (*Pisum sativum* L.) fractions (protein, fibre, and starch) used in this study were provided by Parrheim Foods, Saskatoon, Saskatchewan, Canada. The peas were dehulled to remove the fibre, milled, and the protein and starch granules were separated by air classification (Tyler et al., 1981). The protein fraction contained 60% protein and 30% starch, the fibre fraction contained 90% fibre and 3% protein and the starch fraction contained 85% starch and 5% protein (analyses were done by the Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada). All fractions had 7% m.c. The ground pea was prepared by grinding *P. sativum* (cultivar, AC Tamor) in a UDY mill (UDY Corporation, Ft. Collins, Colorado, USA). The repellency of grain treated with diatomaceous earth (DE) was also tested (Protect-It[®], Hedley Technologies Inc., Mississauga, Ontario, Canada; Fields and Korunic, 2000). For the attractants, we used 20 g of broken wheat kernels (cracked with Model 4-E, Straub Co., Croydon,

Pennsylvania, USA) in a glass vial with a screened lid, 20 g of broken wheat kernels with 30 adults of the species being tested (to naturally produce pheromones): *R. dominica* pheromone, Dominicalure (Storgard Cap[®], Trécé Inc., Salinas, California, USA) for *R. dominica*; *T. castaneum* pheromone (Storgard Cap[®], Trécé Inc., Salinas, California, USA) for *T. castaneum*; and oil and pheromone bait (Pantry Patrol which contains: food oil, pheromones for *Tribolium* spp., *Plodia interpunctella* (Hübner), *Lasioderma serricorne* (F.), and *Trogoderma variabile* (Ballion); Insects Limited Inc., Westfield, Indiana, USA) for *S. oryzae*.

2.3. Insect density test

Cryptolestes ferrugineus, *T. castaneum*, and *S. oryzae* were introduced into the grain at densities of 10, 20, and 30 insects per cup. The number of insects in the collection device was noted after 1 h. The grain was treated with DE at 0.01% or pea protein at 0.1% by mass.

2.4. Pea products

Pea starch, fibre, protein fractions, and whole ground pea were mixed into the wheat at 0, 0.01, 0.1, and 1%. Three species of insect were tested, *C. ferrugineus*, *T. castaneum*, and *S. oryzae* (20 insects/treatment). One species at a time was tested against the various products. The number of insects that had left the grain was noted at 1 h. The test with *S. oryzae* was held for 24 h to allow for additional collection, because of the low number of insect emigrating out of the grain.

2.5. Attractants

The perforated container was hung in a sealed 4-l glass jar. The various attractants were placed on the bottom of the jar. Twenty insects were introduced into the centre of the grain mass and the number of insects that had left the grain was noted at 1, 24, and 72 h. For *R. dominica* and *S. oryzae*, the collected insects and the insects remaining in the grain were sexed at the end of the test (Bousquet, 1990).

2.6. Statistical analyses

All the data were transformed before analysis by taking the square root of the arcsin of the proportion of insects leaving the grain. Two-way ANOVA was used for density and pea products. The dose effects within a product, especially in different pea products, were also studied with one-way ANOVA using Dunnett's test. For attractants, the effects of different treatments were compared for each duration separately using a one-way ANOVA and the Student–Newman–Keuls method. We used a *z*-test to test for differences in the proportions of females exiting the wheat. We used SigmaStat 1.0 (Jandel Corp., San Raphael, California, USA) for all statistical analyses.

3. Results

3.1. Insect density

Cryptolestes ferrugineus and *S. oryzae* in wheat treated with DE at 0.01% or pea protein at 0.1% moved out of the grain faster than untreated wheat (Table 1). For *T. castaneum*, only DE caused increased emigration from the grain. More *T. castaneum* left the grain at 20 adults/200 g than at 10 or 30 adults/200 g. More *S. oryzae* left the grain at 20 and 30 adults/200 g than at 10 adults/200 g. However, given that there is no consistent trend with density, we concluded that density is not an important factor at the levels we tested. Density had no effect on the emigration of *C. ferrugineus*.

3.2. Pea fractions

The pea fibre, pea protein, and ground pea all caused increased emigration of *C. ferrugineus* from the wheat, but only at the highest (1%) dosage level (Table 2). Pea starch did not increase the movement out of the grain for any of the three insects tested. Only pea fibre and ground pea increased the movement of *T. castaneum* out of the grain. None of the products increased emigration of *S. oryzae* compared to the controls after 1 h, but after 24 h both pea protein and ground pea had caused increased movement out of the grain.

Table 1

The percentage (mean \pm S.E.) of stored-products insects leaving 200 g of wheat after 1 h. Grain was treated with diatomaceous earth (DE) or pea protein and tested at different insect densities ($n = 3$)

Species	Insects density (#/cup)	Insect leaving grain (%) ^a				ANOVA					
		Control	DE (0.01%)	Pea protein (0.1%)		Density		Product			
						<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>		
<i>Cryptolestes ferrugineus</i>	10	a	0 \pm 0	b	27 \pm 3	c	20 \pm 0.1	0.81	0.2	<0.001	72
	20	a	5 \pm 3		28 \pm 2		10 \pm 0.1				
	30	a	2 \pm 1		41 \pm 1		4 \pm 3				
<i>Tribolium castaneum</i>	10	a	77 \pm 3	b	100 \pm 0	a	70 \pm 6	0.002	9.5	<0.001	205
	20	b	83 \pm 4		100 \pm 0		98 \pm 2				
	30	a	77 \pm 2		100 \pm 0		71 \pm 1				
<i>Sitophilus oryzae</i>	10	b	5 \pm 1	b	10 \pm 0	c	18 \pm 0	<0.001	15	<0.001	171
	20	a	5 \pm 1		19 \pm 1		19 \pm 1				
	30	a	5 \pm 1		19 \pm 1		20 \pm 1				

^a Products or density that are significantly different have different letters (Student–Newman–Keuls, $P < 0.05$).

Table 2

The percentage (mean \pm S.E.) of stored-products insects leaving 200 g of wheat after 1 h or 24 h. Grain was treated with various pea fractions and tested at different concentrations ($n = 3$)

Species	Duration (h)	Compound	Insects leaving grain (%) ^a					
			Dose (%)				ANOVA	
			0	0.01	0.1	1	<i>P</i>	<i>F</i>
<i>Cryptolestes ferrugineus</i>	1	Pea starch	27 \pm 2	28 \pm 2	32 \pm 2	32 \pm 2	0.16	2.28
		Pea fibre	12 \pm 2	10 \pm 0	12 \pm 2	25 \pm 5*	<0.001	42.3
		Pea protein	12 \pm 2	10 \pm 0	10 \pm 0	33 \pm 7*	<0.001	31.5
		Ground pea	22 \pm 4	25 \pm 5	33 \pm 7	63 \pm 7*	<0.001	10.2
<i>Tribolium castaneum</i>	1	Pea starch	90 \pm 0	90 \pm 3	93 \pm 2	93 \pm 2	0.42	1.07
		Pea fibre	80 \pm 3	85 \pm 0	88 \pm 2*	92 \pm 2*	0.01	7.13
		Pea protein	80 \pm 0	82 \pm 4	82 \pm 3	85 \pm 3	0.72	0.45
		Ground pea	73 \pm 2	75 \pm 3	85 \pm 0*	80 \pm 3	0.02	6.1
<i>Sitophilus oryzae</i>	1	Pea starch	5 \pm 0	3 \pm 2	5 \pm 3	3 \pm 2	0.85	0.27
		Pea fibre	7 \pm 2	3 \pm 2	7 \pm 3	2 \pm 2	0.14	2.43
		Pea protein	3 \pm 3	1 \pm 4	7 \pm 2	1 \pm 3	0.23	1.78
		Ground pea	3 \pm 3	5 \pm 3	3 \pm 3	5 \pm 3	0.96	0.1
<i>Sitophilus oryzae</i>	24	Pea protein	32 \pm 7	58 \pm 7	78 \pm 9*	60 \pm 8*	0.02	6.33
		Ground pea	47 \pm 14	25 \pm 0	57 \pm 2	85 \pm 8*	0.004	10.0

^a Concentrations that are significantly different from the untreated control are marked with an * (Dunnett's test, $P < 0.05$) tested for each product

3.3. Attractants

The *R. dominica* synthetic pheromone placed outside the grain increased the emigration of *R. dominica* from the grain; broken grain and insects also increased emigration but not as much as the synthetic pheromone. Broken grain alone did not attract more insects than the control. After 72 h, 83% of the insects that had left the grain in the synthetic pheromone treatment were females (significantly different from the control, z -test, $P = 0.038$), compared with 47% for the broken grain and insects (not significantly different from the control, z -test, $P = 0.84$), 42% for the broken grain (not significantly different from the control, z -test, $P = 0.96$), and 33% for the control.

For *S. oryzae*, generally the treatments had no effect, although after 24 h the oil and pheromone mixture did cause slightly greater emigration compared to the control and other treatments. Unlike *R. dominica*, there were equal numbers of male and female *S. oryzae* leaving the grain (none of the treatments differed significantly from the control, z -test, $P > 0.80$). For *T. castaneum*, after 1 h all treatments showed increased movement of insects out of the grain compared to the control (Table 3). After 24 h all insects had left the grain.

Table 3

The percentage (mean \pm S.E.) of stored-products insects leaving 200 g of wheat after 1 or 72 h. Attractants were placed outside the grain ($n=3$). The apparatus was enclosed in a sealed jar

Species	Duration (h)	Insects leaving grain (%) ^a				ANOVA	
		Control	Broken grain	Broken grain and insects	Pheromone ^b	<i>P</i>	<i>F</i>
<i>Rhyzopertha dominica</i>	1	2 \pm 2b	2 \pm 2b	12 \pm 2a	2 \pm 2b	0.05	4.2
	24	5 \pm 0a	5 \pm 0a	13 \pm 2a	47 \pm 2b	<0.001	266.8
	48	7 \pm 2a	8 \pm 3a	20 \pm 0b	47 \pm 2c	<0.001	45.2
	72	10 \pm 3a	12 \pm 2a	25 \pm 0b	50 \pm 0c	<0.001	59.5
<i>Sitophilus oryzae</i>	1	5 \pm 3a	7 \pm 2a	8 \pm 2a	10 \pm 0a	0.33	1.33
	24	62 \pm 2a	57 \pm 2a	60 \pm 0a	68 \pm 2b	0.03	11.6
	48	77 \pm 3a	82 \pm 3a	78 \pm 6a	77 \pm 3a	0.878	0.22
	72	98 \pm 2a	97 \pm 2a	97 \pm 2a	100 \pm 0a	0.363	1.22
<i>Tribolium castaneum</i>	1	68 \pm 3a	82 \pm 2b	88 \pm 2bc	93 \pm 2c	<0.001	24.1
	24	100 \pm 0a	100 \pm 0a	100 \pm 0a	100 \pm 0a	1	1

^a Products or density that are significantly different have different letters (Student–Newman–Keuls, $P < 0.05$).

^b For *R. dominica* the attractant is *R. dominica* pheromone; for *S. oryzae* the attractant is food oil mixed with several stored-product insect pheromones; for *T. castaneum* the attractant is *T. castaneum* pheromone.

4. Discussion

There are a few techniques for determining if substances repel or attract stored-product insects. One needs approximately 0.6–1 kg of grain to apply the method of Loschiavo (1952), compared with 200 g required by our method. Others use filter paper or glass test arenas (Burkholder, 1990; Dowdy et al., 1993) that do not allow testing products on the grain. One of our goals in this study was to develop a technique that would more closely mimic grain storage conditions. Unlike other bioassays, this method allows three-dimensional movement of insects in grain. We tested it with some compounds that are known to be repellent or attractive. Increased movement out of DE-treated and pea-protein-treated grain was detected after only 1 h. The method was effective in evaluating repellency or attractiveness of compounds to *C. ferrugineus*, *R. dominica*, and *S. oryzae*. Slow moving species, such as *R. dominica* and *S. oryzae* required longer test periods to detect differences. On the other hand, *T. castaneum* moved so rapidly out of the untreated grain that it was difficult to detect repellency or attractiveness of products. More grain in the test container or lower temperatures may solve these problems for fast-moving insects such as *T. castaneum*. We feel that the cup bioassay would be a good method to rapidly screen products for their repellency or attractiveness.

White et al. (1966) showed that DE is repellent to *S. oryzae*. Our tests also showed that DE is repellent to *S. oryzae* as well as *C. ferrugineus* and *T. castaneum*. We obtained similar results using the cup bioassay as were obtained by Bodnaryk et al. (1999) using the pie-shaped choice chamber (Loschiavo, 1952); pea starch is not repellent, while pea fibre and pea protein are repellent. As in the pitfall (Burkholder, 1990) and Y-tube (Dowdy et al., 1993) bioassays, our tests with the cup bioassay showed that the *R. dominica* pheromone was a strong attractant. However, unlike other

studies (Khorramshahi and Burkholder, 1981; Obeng-ofori and Coaker, 1990) where males and females were equally attracted, our studies showed that more females were attracted than males. This could potentially have the added advantage of removing females from the grain mass and reducing the number of eggs laid on the grain.

Repellency of the DE and the pea fractions would have positive and negative effects on the control of stored-product insects in commercial store houses. One negative effect is that residual control products are never applied evenly, and in the case of DE, it is used as a top dressing or as a layer treatment to mitigate the negative effects on grain bulk density. In these situations the insects could be concentrated into grain that has lower concentrations of the product. Another more subtle effect would be that these products would increase insect movement in the grain mass and bias sampling methods, probe-pitfall traps (White et al., 1990) and pitfall traps (Cogan and Wakefield, 1994; Mullen, 1992) that are correlated with movement.

One positive effect would be that there could be reduced immigration into a grain mass and greater emigration out of a grain mass treated with these products, hence, reducing insect populations. This effect could be used in combination with small insect-removal granaries (Mohan, 1997). These granaries hold 50–500 kg, are doubled walled, the inner wall is made of screening that holds the grain but allows the insects to exit the grain bulk. There is a container at the bottom of the granary to collect the insects and prevent their reentry into the grain. The 7 days it takes to remove 90% of the insects (*T. castaneum*, *S. oryzae*, and *R. dominica*) could be reduced if the grain is treated with a repellent such as DE or ground pea or the collection container had an attractant such as a pheromone.

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