Control of Stored-Product Beetles with Combinations of Protein-Rich Pea Flour and Parasitoids

XINGWEI HOU,[1] PAUL FIELDS,[2] PAUL FLINN,[3] JOEL PEREZ-MENDOZA,[3] AND JAMES BAKER[3]

Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9, Canada

Environ. Entomol. 33(3): 671–680 (2004)

ABSTRACT Protein-rich pea flour is toxic and repellent to three major stored-grain pests: the rice weevil, *Sitophilus oryzae* L.; the red flour beetle, *Tribolium castaneum* (Herbst); and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). This study found that protein-rich pea flour was not toxic to, and did not reduce the offspring of, *Anisopteromalus calandrae* (Howard), a parasitoid of *S. oryzae*, nor did it reduce offspring of *Cephalonomia waterstoni* (Gahan), a parasitoid of *C. ferrugineus*. Protein-rich pea flour was also not repellent to *A. calandrae.* Small-scale and large-scale tests of a combination of protein-rich pea flour and parasitoids were conducted in 2-liter jars and in barrels containing 330 kg wheat. A larger population of *A. calandrae* was found at a high host infestation rate (24 adults/kg for 25 d), but the parasitoid did not become established at middle and low host infestation rates (2.4; 0.24 adults/kg for 25 d). The combinations of protein-rich pea flour and parasitoids reduced populations of *S. oryzae* in both tests. Additional effects of protein-rich pea flour and parasitoids were found in the large-scale test. Releasing parasitoids alone reduced the populations of *S. oryzae* by 46% and *C. ferrugineus* by 49%. Treating wheat with 0.04 or 0.1% protein-rich pea flour reduced the population of *S. oryzae* by 26 and 79% and *C. ferrugineus* by 27 and 43%, respectively. Combining parasitoids with 0.04 or 0.1% protein-rich pea flour reduced *S. oryzae* populations by 76 and 98% and *C. ferrugineus* populations by 42 and 75%, respectively. At the end of the large-scale experiment, grain treated with protein-rich pea flour alone or in combination with parasitoids had better grain quality than the untreated controls.

KEY WORDS Pea protein, *Anisopteromalus calandrae, Cephalonomia waterstoni, Sitophilus, Cryptolestes*

STORED-GRAIN INSECT PESTS CAUSE damage to grain by reducing its dry weight, nutritional value, and seed viability (Semple et al. 1992). As a result, protecting grain from insect attack during the storage period is of prime importance to the food industry. Botanical compounds are a rich and promising source of new and alternative chemicals that may help replace synthetic insecticides for grain protection (Prakash and Rao 1997). Many plant-derived chemicals are insecticidal to stored-product pests (Jacobson 1989, Golob et al. 1999, Weaver and Subramanyam 2000). Azadirachtin from the Indian neem tree (*Azadirachta indica* A. Juss., Meliaceae) (Saxena et al. 1988, Jilani and Saxena 1990) and pyrethrum from chrysanthemums (Prakash and Rao 1997) have received the most attention. However, because of the structural complexity of azadirachtin and the instability of pyrethrum combined with the restricted availability and the high cost of both, the search for other natural insecticides is ongoing (Arthur 1996).

Legume seeds contain a wide range of allelochemicals with toxic and deterrent effects against insect pests (Harborne et al. 1971, Bell 1977). Yellow split peas (*Pisum sativum* L.) mixed with wheat reduce the survival and reproduction of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) (Coombs et al. 1977, Holloway 1986). Recently, Delobel et al. (1998) isolated a polypeptide from peas that is toxic to stored-product insects. Additional studies have demonstrated that protein-rich pea flour obtained from these peas is repellent (Fields et al. 2001), as well as toxic to many stored-grain insects (Bodnaryk et al. 1997, Hou and Fields 2003a, b). However, nothing is known of the effects of this allelochemical on the interaction of parasitoids with their insect hosts.

The rice weevil, *Sitophilus oryzae;* the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); and the red flour beetle, *Tribolium castaneum* (Herbst), are cosmopolitan stored-product insect pests. Protein-rich pea flour at 0.1% reduces populations of these insects in farm granaries (Hou and Fields 2003a). *Anisopteromalus calandrae* (Howard) (Hymenoptera:

[1] Department of Entomology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

[2] E-mail: pfields@agr.gc.ca.

[3] Grain Marketing and Production Research Center, USDA–ARS, Manhattan, KS 66502.

Pteromalidae) is an ectoparasitoid of the immature stage of *S. oryzae*. *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae) is an ectoparasitoid of late-instar larvae of *C. ferrugineus* (Flinn and Hagstrum 1995). Both parasitoids can suppress the populations of *S. oryzae* or *C. ferrugineus* (Press et al. 1983, Cline et al. 1985, Flinn et al. 1996). Preliminary tests showed that protein-rich pea flour did not affect *A. calandrae* or *C. waterstoni*. Therefore, we hypothesized that these parasitoids could be used in combination with protein-rich pea flour, because the parasitoids attack the immature stages and the protein-rich pea flour is toxic to the adults. This hypothesis was tested by exposing these two parasitoids to protein-rich pea flour and evaluating the efficacy of the combination on the reduction of insect populations in a small-scale and a large-scale experiment.

Materials and Methods

Insects

Sitophilus oryzae were reared on whole kernels of wheat, *T. castaneum* on wheat flour mixed with brewer's yeast (5% by weight), and *C. ferrugineus* on wheat kernels with wheat germ (5% by weight) and brewer's yeast (5% by weight).

Anisopteromalus calandrae and *C. waterstoni* were obtained from the Grain Marketing and Production Research Center, USDA-ARS, Manhattan, KS. *A. calandrae* was maintained on 2- to 3-wk-old cultures of *S. oryzae* in wheat. Adult *A. calandrae* were provided with moistened raisins and honey. *C. waterstoni* was reared on 3- to 4-wk-old *C. ferrugineus* larvae in a diet of whole wheat flour, wheat germ, and yeast. Adult *C. waterstoni* were also provided raisins and honey. All rearing was carried out at 30°C and 70% RH. The parasitoids were reared with a 12-h photophase and 12-h scotophase. The pest species were reared in the dark.

Effect of Protein-Rich Pea Flour on Parasitoids

Protein-rich pea flour (60% protein, 30% starch, and 7% moisture content, Progress Protein; Parrheim Food, Saskatoon, Canada) was used in this study. It is produced commercially by grinding peas and isolating a protein-rich fraction by air classification. Studies were conducted at 30 ± 1°C, 70 ± 5% RH, unless specified otherwise. There were five replicates in each treatment.

Direct Contact Toxicity. Without provision of honey, ≈25 1-d-old *A. calandrae* adults were placed in 15 by 100-mm plastic petri dishes containing 30 mg protein-rich pea flour, 30 mg wheat flour, 5 g wheat kernels infested with 2- to 3-wk-old *S. oryzae* larvae, or as a control, uninfested wheat kernels. The number of dead *A. calandrae* was recorded each day until all adult *A. calandrae* died. In experiments conducted at the same time, honey was provided as food for *A. calandrae* with a filter paper streaked with honey. The paper was taped underneath the cover of each petri dish. Mortality of *C. waterstoni* with protein-rich pea flour or wheat flour was observed using the same methods as for *A. calandrae*.

Choice Test. One section of a divided petri dish was filled with 10 g of untreated wheat, and the other section was filled with wheat treated with 0.1% protein-rich pea flour. The wheat was treated by shaking in a jar with protein-rich pea flour for 2 min. Wheat in both sections contained 2- to 3-wk-old *S. oryzae* larvae. A single 1-d-old *A. calandrae* female was placed in each petri dish and allowed to oviposit for 0, 24, 48, or 96 h. Untreated and treated wheat from the two dish sections were collected separately and placed in vials (29 by 80 mm) with screen lids, and the number of emerged *A. calandrae* adults in each was recorded after 4 wk.

No-Choice Test. Twenty 1-d-old *A. calandrae* females were placed in vials with 20 g of wheat, which contained 2- to 3-wk-old *S. oryzae* larvae, and treated at 0, 0.04, or 0.1% protein-rich pea flour. The parasitoids were removed after 0, 1, 3, 5, or 7 d. The numbers of emerged *A. calandrae* and *S. oryzae* were counted after 4 wk. For testing *C. waterstoni*, 100 3- to 4-wk-old *C. ferrugineus* larvae were placed in 16% moisture content wheat that had been either untreated or treated with 0.1% protein-rich pea flour and mixed with ground wheat germ. Five *C. waterstoni* females were left in the vials for 24, 48, 72, or 96 h. The numbers of emerged *C. waterstoni* and *C. ferrugineus* were counted after 4 wk.

Combination of Protein-Rich Pea Flour and Parasitoids

Small-Scale Test. The small-scale test was conducted at 25 ± 1°C, 60 ± 5% RH. Wheat was disinfested by placing it in a room at −15°C for >3 wk. The wheat was moisturized in open bags at 25 ± 1°C, 60 ± 5% RH for 3 wk. Wheat (2.5 kg) was placed in each of 22 4-liter jars and infested with 60 unsexed 1- to 2-wk-old *S. oryzae* adults for 25 d. Infested wheat from different jars was thoroughly mixed with a modified cement mixer for 30 min at 20 rotations per min. To obtain different densities of *S. oryzae*, infested wheat was diluted with uninfested wheat in the mixer at the following ratios (infested wheat:uninfested wheat): 1:0 (high infestation rate), 1:9 (moderate infestation rate), and 1:99 (low infestation rate). Three 500-g samples were taken from each infestation level as initial untreated controls before treatment of protein-rich pea flour with the mixer. Wheat with the three levels of infestation was divided into two equal samples using a sample divider (Humboldt MFG, Norridge, IL) and mixed with 0 or 0.04% protein-rich pea flour in the mixer for 30 min. The wheat was divided into 2-liter jars with 500 g in each jar.

Twenty-nine days after the initial infestation, 12 newly emerged female *A. calandrae* were added to the jars once (single release), or four parasitoids were added in each of 3 consecutive wk for a total of 12 parasitoids (multiple release). Controls contained no parasitoids and no protein-rich pea flour. Jars were

Table 1. Number of *A. calandrae* (mean ± SEM) in wheat with a high initial *S. oryzae* population in a small-scale test

Weeks after first release of parasitoids	Total number of *A. calandrae* (per jar, mean ± SEM)[a]					
	Parasitoid single release	Parasitoid multiple release	Protein-rich pea flour with parasitoid single release	Protein-rich pea flour with parasitoid multiple release	*F*	*P*
3	24 ± 3	24 ± 3	15 ± 2	16 ± 3	2.80	0.109
6	32 ± 6ab	48 ± 3b	37 ± 2ab	28 ± 4a	4.85	0.033
9	60 ± 32	81 ± 3	55 ± 20	41 ± 6	0.76	0.548
12	728 ± 377	350 ± 215	225 ± 44	449 ± 299	0.65	0.602
15	3413 ± 526	2046 ± 885	1608 ± 271	786 ± 428	3.67	0.063

Treatment with protein-rich pea flour was at 0.04%. Twelve *A. calandrae* were released once (single release) or four parasitoids each week for 3 wk (multiple release).

[a] Means in a row followed by different letters are different (PROC GLM, Tukey's, $P < 0.05$, df = 3,8).

sealed with a metal screen and filter paper to prevent cross-contamination. There were six treatments for each infestation rate: no parasitoids and no pea flour as untreated controls, 0.04% protein-rich pea flour with no parasitoids, parasitoids single release and no pea flour, parasitoids multiple release with no pea flour, parasitoids single release with 0.04% pea flour, and parasitoids multiple release with 0.04% pea flour. Three jars were randomly taken from each treatment every 3 wk. The grain was sifted, and the number of live and dead adults of *A. calandrae* and *S. oryzae* was counted. The number of insects in the initial untreated control was checked at the same time, and all insects were returned to the jars.

Large-Scale Test. Twenty-one steel barrels (58 by 168 cm) (White and Jayas 1991) were used to evaluate the combination effects of parasitoids and protein-rich pea flour. Because parasitoids can easily move between treatments (Flinn et al. 1996), tests were conducted at two separate locations. Nine barrels were set up at the Cereal Research Centre (CRC) with no parasitoids. Twelve barrels were set up nearby at the Department of Biosystem Engineering at the University of Manitoba (UM). The rooms were heated, and the temperature and RH were monitored with HOBO data loggers (Onset Computer, Bourne, MA). Each barrel was filled with 330 kg of hard red spring wheat with 13.5% moisture content. Wheat was treated with protein-rich pea flour at 0, 0.04, or 0.1% by dusting the grain and augured into each barrel on 22–24 May 2001. On 18 June 2001, each of 660 *S. oryzae*, *C. ferrugineus*, and *T. castaneum* were released together on the wheat surface in all barrels. After 29 d, a single release of 660 *C. waterstoni* and 660 *A. calandrae* (1–7 d old and mixed-sex adults) was conducted at the UM site. Fluoropolymer resin (TeFlon; DuPont, Wilmington, DE) was brushed on the top inside of the metal wall to prevent insects from climbing, and the barrels were sealed with fine cloth to prevent insects from escaping by flying and to protect wheat from external contamination. Three grain samples (~1 kg each) were gently taken by a vacuum every 3 wk (7 and 28 August, 18 September, 9 and 29 October) through small holes on the side of the barrel located at the top (20 cm from the grain surface), the middle (68 cm from the top), and the bottom layer (24 cm from the bottom) of the barrel. The samples were weighed, sifted, and the number of live and dead adult insects of each species was counted. A set of samples was taken on 11 July, after release of pest insects but before release of parasitoids, for measuring the efficacy of protein-rich pea flour before parasitoids were released. These samples were cultured separately at 30°C and 70% RH for 4 wk, and the number of emerged *S. oryzae* adults was counted to estimate the number and distribution of immature insects within the wheat available for the parasitoids at the time of sampling. The dockage, moisture, and bulk density of wheat was measured with samples taken on 11 July before the release of insects and on 29 October at the end of the experiment. There

Table 2. Number of live *S. oryzae* (mean ± SEM) in wheat with a high initial *S. oryzae* population in a small-scale test

Weeks after first release of parasitoids	Number of live *S. oryzae* (per jar) (mean ± SEM)[a]							
	Untreated	Parasitoid single release	Parasitoid multiple release	Protein-rich pea flour	Protein-rich pea flour with parasitoid single release	Protein-rich pea flour with parasitoid multiple release	*F*	*P*
3	81 ± 10a	61 ± 5a	76 ± 12a	81 ± 10a	75 ± 5a	82 ± 9a	0.81	0.563
6	199 ± 3a	149 ± 10b	154 ± 4b	146 ± 7b	128 ± 9b	141 ± 6b	12.34	<0.001
9	1083 ± 40a	872 ± 59ab	756 ± 35ab	754 ± 77ab	616 ± 58b	834 ± 134ab	4.37	0.017
12	4799 ± 264a	2833 ± 670a	2752 ± 502a	4564 ± 845a	3734 ± 763a	2630 ± 255a	2.60	0.081
15	12305 ± 802a	8902 ± 1034ab	3522 ± 659c	10777 ± 479a	3721 ± 495c	5189 ± 1731bc	15.20	<0.001
Overall mean	3488 ± 1165a	2563 ± 912bc	1451 ± 404c	3264 ± 1110ab	1655 ± 481c	1775 ± 597c	12.83	<0.001

Treatment with protein-rich pea flour was at 0.04%. Twelve *A. calandrae* were released once (single release) or four parasitoids each week for 3 wk (multiple release).

[a] Means in a row followed by different letters are different (PROC GLM, Tukey's; $P < 0.05$, df = 5,12 for means within weeks; df = 5,60 for overall means).

Table 3. Number of emerged adults (mean ± SEM) in wheat treated with protein-rich pea flour and parasitoids and sampled on 11 July before release of parasitoids in a large-scale test at the CRC and the UM

	Number of total insects (per kg, mean ± SEM)[a]									
	CRC				UM					
	Untreated	0.04% protein-rich pea flour	0.1% protein-rich pea flour	*F*	*P*	Untreated	Parasitoid	Parasitoid + 0.04% protein-rich pea flour	Parasitoids + 0.1% protein-rich pea flour	*F*	*P*
S. oryzae[b]	159 ± 82a	75 ± 38a	23 ± 13b	9.95	0.012	48 ± 27a	78 ± 39a	20 ± 12b	3 ± 2c	16.86	<0.001
C. ferrugineus	16 ± 4a	12 ± 3a	8 ± 2b	5.33	0.047	21 ± 5a	22 ± 5a	14 ± 3b	12 ± 2b	10.23	<0.001
T. castaneum	23 ± 8a	15 ± 4a	7 ± 1a	2.85	0.135	5 ± 2a	5 ± 2a	11 ± 4a	5 ± 2a	3.99	0.052

Wheat was incubated at 30°C, 70% RH for 4 wk.

[a] Means in a row at same location followed by different letters are different (PROC MIXED, Tukey's, $P < 0.05$; df = 2,18 for the CRC, df = 3,24 for the UM).

[b] The mortalities of *S. oryzae* adults in wheat samples in 0.1 and 0.04% were 83 and 24% at CRC and 67 and 10% at UM. All other mortalities were <10%.

were four treatments at the UM site: untreated controls, parasitoids alone, parasitoids with 0.04% protein-rich pea flour, and parasitoids with 0.1% protein-rich pea flour. There were three treatments at the CRC site: untreated controls, 0.04% protein-rich pea flour, and 0.1% protein-rich pea flour. There were three barrels per treatment.

Data analysis

Analysis of variance (ANOVA), either SAS PROC GLM or PROC MIXED (SAS Institute 2000), followed by Tukey's multiple range test was used to determine if there were significant differences among treatments. Where required (Table 2, overall means; Table 3, *S. oryzae;* Table 4; Figs. 1–3), the data were transformed using log (1 + x) to equalize variances. For the direct contact test, the day that the mortality of the parasitoids in the wheat flour control treatment was closest to 50% was chosen to test for differences between treatments. In the small-scale test, treatments without parasitoids were excluded from the analyses to compare the number of parasitoids reproduced among treatments in which parasitoids had been released. A repeated-measures variance analysis, with SAS PROC MIXED, was used for the large-scale test. The mean of the three layers was used for the analysis. Akaike's Information Criterion was used to determine the best covariance structure for the repeated measures test.

Results

Effect of Protein-Rich Pea Flour on Parasitoids

Direct Contact. After 120 h, the mortality of *A. calandrae* in the dishes with wheat kernels infested with *S. oryzae* larvae was greater than that in the dishes with pea flour and wheat flour, but not significantly greater than uninfested wheat kernels (65 ± 4, 46 ± 4, 48 ± 4, and 52 ± 4, respectively; $F = 4.92$; df = 3,32; $P = 0.006$, Tukey's multiple range test). Honey did not significantly reduce the mortality of *A. calandrae* after 120 h (50 ± 3%) compared with wasps that received no honey (55 ± 3%; $F = 1.99$; df = 1,32; $P = 0.167$). After 72 h, the mortality of *C. waterstoni* without access to honey was the same in protein-rich pea flour (72 ± 13%) as it was in wheat flour (60 ± 11%; $F = 0.505$; df = 1,8; $P = 0.497$).

Choice Test. Parasitism of weevils by *A. calandrae* was not affected by the protein-rich pea flour. The more time that *A. calandrae* had access to the weevil-infested wheat, the more *A. calandrae* progeny emerged from the control and treated wheat. ($F = 14.57$; df = 3, 16; $P < 0.001$). The number of *A. calandrae* emerging from the treated wheat (2.8 ± 0.8 insects per section) was not significantly different than the number emerging from untreated wheat (3.7 ± 0.8 insects per section) ($F = 1.62$; df = 1.8; $P = 0.220$).

No-Choice Test. No significant difference in the number of emerged *C. waterstoni* was detected be-

Table 4. Number of emerged adults in wheat sampled at different layers of the barrels on 11 July before the release of parasitoids in a large-scale test at the CRC and the UM

Layers	Number of total insects (per kg)					
	CRC			UM		
	S. oryzae	*C. ferrugineus*	*T. castaneum*	*S. oryzae*	*C. ferrugineus*	*T. castaneum*
Top[a]	257 ± 64a	5 ± 2b	28 ± 8a	111 ± 28a	4 ± 1b	16 ± 3a
Middle	0.7 ± 0.4b	11 ± 2b	12 ± 2b	0.6 ± 0.3b	20 ± 2a	3 ± 1b
Bottom	0 ± 0c	21 ± 2a	5 ± 1c	0.2 ± 0.1b	27 ± 3a	1 ± 1c
F	759.76	13.11	30.87	337.15	72.86	91.02
P	<0.001	0.001	<0.001	<0.001	<0.001	<0.001

Wheat was incubated at 30°C, 70% RH for 4 wk.

[a] Means in a column followed by different letters are different (PROC MIXED, Tukey's, $P < 0.05$; df = 2,18 for CRC and 2,24 for UM).

tween untreated wheat (6.4 ± 1.5 insects/vial) and wheat treated with 0.1% protein-rich pea flour (5.4 ± 1.1 insects/vial; $F = 0.32$; df = 1,35; $P = 0.574$). There was no difference in the number of emerged *C. ferrugineus* adults between untreated wheat (5.2 ± 0.7 insects/vial) and wheat treated with 0.1% protein-rich pea flour (4.3 ± 0.6 insects/vial; $F = 1.03$; df = 1,35; $P = 0.318$). There were no differences in the total number of emerged *A. calandrae* (71 ± 3, 73 ± 4, and 76 ± 3 insects/vial, respectively; $F = 0.52$; df = 2,48; $P = 0.598$) and in the total number of emerged *S. oryzae* (21 ± 1, 22 ± 2, and 21 ± 2 insects/vial, respectively; $F = 0.61$; df = 2, 48; $P = 0.547$) among wheat treated at 0, 0.04, or 0.1% of protein-rich pea flour. However, the mortality of emerged *S. oryzae* adults in the 0.1% treatment (20 ± 2%) was significantly higher than that in the 0.04% treatment (6 ± 1%) and in the untreated control (3 ± 1%; $F = 54.76$, df = 2,48; $P < 0.001$, Tukey's multiple range test). This mortality resulted in fewer live *S. oryzae* adults in the 0.1% treatment (16 ± 1 insects/vial) than in the 0.04% treatment (21 ± 1 insects/vial) or in the control treatment (20 ± 1 insects/vial; $F = 8.99$; df = 2,48; $P = <0.001$, Tukey's multiple range test).

Combination of Protein-Rich Pea Flour and Parasitoids

Small-Scale Test. Three infestation rates of *S. oryzae* were successfully established. In the first sample, the total number of emerged *S. oryzae* adults at the high, moderate, and low infestation rates in the initial untreated controls was 82 ± 11, 7 ± 1, and 1 ± 0.6 insects/jar, respectively; in the untreated control that had been mixed in the mixer for 30 min, it was 95 ± 9, 7 ± 0.6, and 1 ± 0.6 insects/jar, respectively; and in the treatment of 0.4% protein-rich pea flour, it was 81 ± 10, 7 ± 1, and 1 ± 1, respectively. The mixing did not affect the total number of emerged *S. oryzae* ($F = 0.06$; df = 1,42; $P = 0.808$) nor did the treatment with 0.4% protein-rich pea flour ($F = 0.14$; df = 1,42; $P = 0.713$).

Anisopteromalus calandrae did not establish a population (12 ± 0.1 insects/jar) at the low *S. oryzae* infestation rate. At the moderate infestation rate, the total numbers of live and dead *A. calandrae* (14 ± 0.4 insects/jar) increased in the first 3 wk by approximately two parasitoids. However, no live *A. calandrae* were found in later samples. A large population of *A. calandrae* became established in all treatments with high infestation rates of *S. oryzae* (Table 1). There were no significant differences in the overall means of the number of *A. calandrae* among treatments with a single release (851 ± 366), multiple release (510 ± 259), protein-rich pea flour with single release (388 ± 171), or protein-rich pea flour with multiple release (264 ± 121; $F = 0.56$; df = 3,40; $P = 0.647$). Compared with the high number of *S. oryzae* (Table 2), the ratios of *A. calandrae* to *S. oryzae* at the end of the experiment were 0.38 in single release, 0.58 in multiple releases, 0.43 in single release with pea flour, and 0.15 in multiple releases with pea flour. Compared with the untreated control, both the single and multiple releases of parasitoids reduced the number of live *S. oryzae.* The combinations of protein-rich pea flour with the single release or multiple releases reduced more live *S. oryzae* than 0.4% protein-rich pea flour treatment alone.

Large-Scale Test. Although there was no significant difference in the ambient mean daily temperature between the CRC and UM sites ($F = 2.18$, df = 1,166; $P = 0.141$), the relative humidity was significantly different between the two locations ($F = 39.51$, df = 1,166; $P < 0.001$). At CRC, the mean temperature was 24.0 ± 0.04°C, with a range of 12.9–30.7°C, and the mean RH was 50 ± 0.3%, with a range of 24–79%. At UM, the mean temperature was 23.7 ± 0.03°C, with a range of 19.0–27.9°C, and the RH was 37 ± 0.3%, with a range of 23–84%. In addition, the numbers of total emerged *S. oryzae* (86 ± 31 insects/kg, CRC; 37 ± 13 insects/kg, UM; $F = 5.73$; df = 1,12; $P = 0.034$) and *T. castaneum* (15 ± 3 insects/kg, CRC; 6 ± 1 insects/kg, UM; $F = 47.80$; df = 1,12; $P < 0.001$) in the untreated barrels before the release of parasitoids (11 July) were different between CRC and UM but not for *C. ferrugineus* (12 ± 2 insects/kg, CRC; 17 ± 2 insects/kg, UM; $F = 0.43$; df = 1,12; $P = 0.522$). Therefore, data from the two locations were analyzed separately.

The number of adults emerging from samples taken on 11 July, before release of the parasitoids, gives an estimate of the immature stages present at sampling. Before parasitoid release, treatments with 0.1% protein-rich pea flour alone reduced the number of emerged *S. oryzae* and *C. ferrugineus* at both CRC and UM (Table 3). More *S. oryzae* were in the top layer at the CRC and the UM, and more *T. castaneum* were

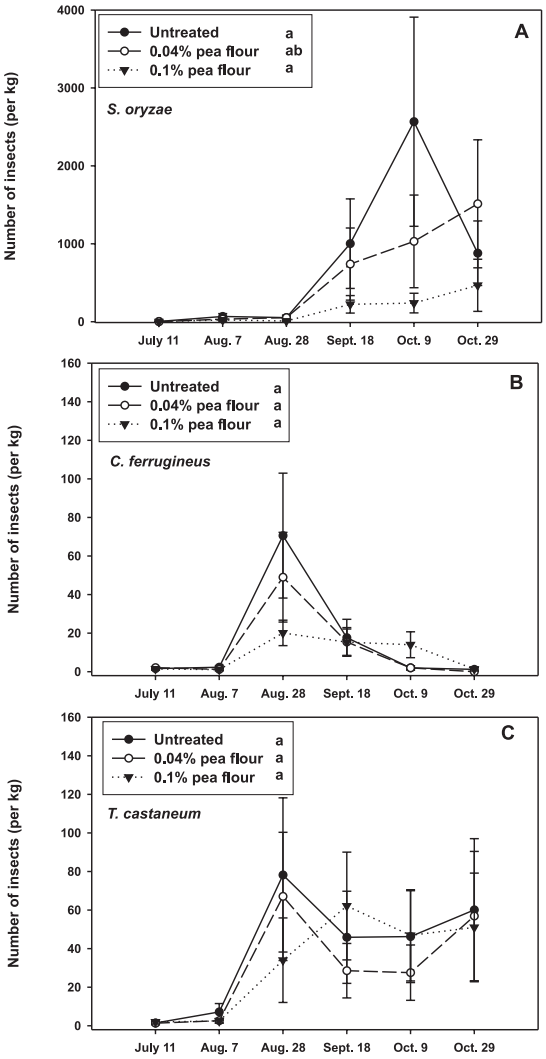

Fig. 1. Number of live pest insects in barrels containing wheat treated with protein-rich pea flour at 0, 0.04, and 0.1% at the Cereal Research Centre ($n = 3$). (A) *S. oryzae*; (B) *C. ferrugineus*; (C) *T. castaneum.* Treatments followed by different letters were significantly different ($P < 0.05$).

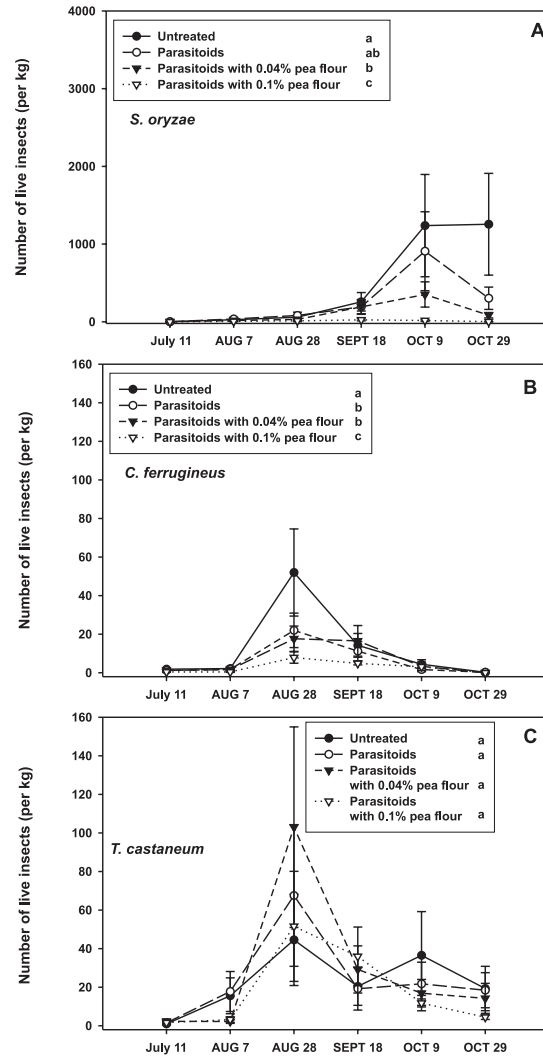

Fig. 2. Number of live pest insects in barrels containing wheat treated with parasitoids alone, parasitoids plus 0.04% protein-rich pea flour, parasitoids plus 0.1% protein-rich pea flour, and no parasitoids and no protein-rich pea flour (control) at the University of Manitoba ($n = 3$). *A. calandrae* and *C. waterstoni* were released at 2 insects/kg of wheat 29 d after *S. oryzae*, *C. ferrugineus*, and *T. castaneum* were released at 2 insects/kg wheat. (A) *S. oryzae*; (B) *C. ferrugineus*; (C) *T. castaneum.* Treatments followed by different letters were significantly different ($P < 0.05$).

also in the top layer at the CRC and the UM. More *C. ferrugineus* were present in the bottom layers than in the top layer at both CRC and the UM (Table 4).

At CRC, compared with the mean number in the untreated control (762 ± 271 insects/kg all dates), the population of live *S. oryzae* in the treatment with 0.04% protein-rich pea flour was reduced by 26% (562 ± 194 insects/kg) and was reduced 79% in the 0.1% treatment (160 ± 64 insects/kg; $F = 16.28$; df = 2,6; $P = 0.004$; Fig. 1A). Protein-rich pea flour did not significantly reduce the population of *C. ferrugineus* ($F = 2.28$; df = 2,6; $P = 0.184$) or *T. castaneum* ($F = 3.74$; df = 2,6; $P = 0.088$; Fig. 1, B and C).

At UM, no *A. calandrae* or *C. waterstoni* were found in the control barrels. Compared with the number in the untreated control (473 ± 167 insects/kg), the number of live *S. oryzae* with only parasitoids (254 ± 95 insects/kg) was not significantly reduced, but the number was reduced by 76% in the combination of parasitoids and 0.04% protein-rich pea flour (113 ± 35 insects/kg) and reduced by 98% in the combination of parasitoids with 0.1% protein-rich pea flour (10 ± 3 insects/kg; $F = 92.25$; df = 3,8; $P < 0.001$; Fig. 2A). The number of live *C. ferrugineus* was significantly lower in all three treatments (6 ± 2 insects/kg with parasitoid alone, 7 ± 2 insects/kg with parasitoid with 0.04% protein-rich pea flour, and 3 ± 0.6 insects/kg with

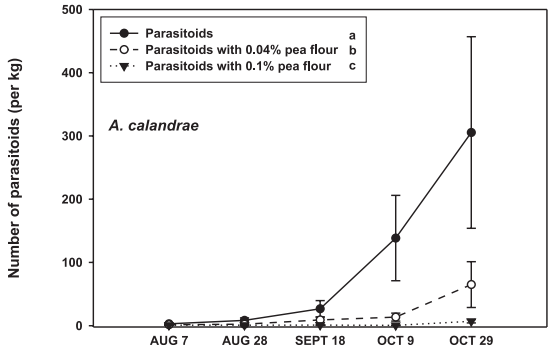

Fig. 3. Number of live *A. calandrae* in barrels wheat treated with parasitoids alone, parasitoids plus 0.04% protein-rich pea flour, parasitoids plus 0.1% protein-rich pea flour, and no parasitoids and no protein-rich pea flour (control) at the University of Manitoba ($n = 3$). *A. calandrae* was released at 2 insects/kg wheat 29 d after *S. oryzae*, *C. ferrugineus*, and *T. castaneum* were released at 2 insects/kg wheat. Treatments followed by different letters were significantly different ($P < 0.05$).

parasitoids plus 0.1% protein-rich pea flour) than in the untreated control (12 ± 4 insects/kg; $F = 816.19$; df = 3,8; $P < 0.001$, Fig. 2B). There were no differences among the treatments of parasitoids alone and the combination of parasitoids with 0.04% protein-rich pea flour ($F = 0.36$; df = 1,8; $P = 0.565$). However, the combination of parasitoids and 0.1% protein-rich pea flour reduced live *C. ferrugineus* more than treatments of parasitoids alone ($F = 5.76$; df = 1,8; $P = 0.043$). There was no difference in the number of *T. castaneum* among all treatments ($F = 0.81$; df = 3,8; $P = 0.525$; Fig. 2C).

The population of *A. calandrae* was larger than that of *C. waterstoni.* The mean numbers of total live and dead *A. calandrae* in the treatment of parasitoids without protein-rich pea flour (96 ± 36 insects/kg), parasitoids plus 0.04% protein-rich pea flour (18 ± 8 insects/kg), and parasitoids plus 0.1% protein-rich pea flour (2 ± 0.7 insects/kg) were significantly different ($F = 36.08$; df = 2,6; $P < 0.001$; Fig. 3). At the end of the test, the ratios of the number of *A. calandrae* to *S. oryzae* adults were 1.0 (parasitoids alone), 0.8 (combination of parasitoids with 0.04% protein-rich pea flour), and 3.3 (combination of parasitoids with 0.1% protein-rich pea flour). There were significantly more *A. calandrae* in the top layer (84 ± 27 insects/kg) than in the middle (3 ± 0.8 insects/kg) and bottom (0.5 ± 0.2 insects/kg; $F = 331.34$; df = 2,12; $P < 0.001$). The total *C. waterstoni* populations were similar in all treatments (parasitoids alone, 0.3 ± 0.1 insects/kg; parasitoids with 0.04% protein-rich pea flour, 0.2 ± 0.1 insects/kg; and parasitoids with 0.1% protein-rich pea flour, 0.5 ± 0.4 insects/kg; $F = 1.72$; df = 2,12; $P = 0.257$). There were no differences in the numbers of *C. waterstoni* in the top (0.5 ± 0.3 insects/kg), middle (0.10 ± 0.04 insects/kg), or bottom layers (0.08 ± 0.05 insects/kg; $F = 2.73$; df = 2,12; $P = 0.105$) of wheat.

At the beginning of the experiment there were no differences in grain quality between the treatments for a given site (Table 5). At the end of the experiment, the grain treated with 0.1% protein-rich pea flour had better quality than the untreated grain, which had greater dockage and a lower bulk density than the grain treated with 0.1% protein-rich pea flour. However, the 0.04% protein-rich pea flour was unable to prevent the decline in quality. When parasitoids were combined with the 0.04% protein-rich pea flour, grain quality was better than the untreated control and no different than that in the 0.1% protein-rich pea flour and parasitoid treatment (Table 5, UM).

Discussion

Parasitoids and insecticides are generally incompatible because beneficial insects are often more susceptible to insecticidal materials than their hosts (Croft 1990, Schöller and Flinn 2000). For example, diatomaceous earth is more toxic to *A. calandrae* than to *S. oryzae* (Perez-Mendoza et al. 1999). By direct contact, diatomaceous earth takes >1 h to kill 50% of *A. calandrae*, but it takes 24 h to kill 50% of its host, *S. oryzae* (Perez-Mendoza et al. 1999). In contrast, our studies demonstrated that protein-rich pea flour and parasitoids were compatible for reducing populations of *S. oryzae* and *C. ferrugineus.* It had no direct contact toxicity, and it did not affect parasitism by *A. calandrae* or *C. waterstoni.* Protein-rich pea flour had no effect on the emergence of *A. calandrae*, *C. waterstoni*, and their hosts. Assuming this was also true in large-scale test, the low number of emerged *A. calandrae* in the protein-rich pea flour treatment in the large-scale test probably was not caused by any adverse effect of protein-rich pea flour but to the low number of available immature weevils resulting from the protein-rich pea flour treatment.

We hypothesized that parasitoids and protein-rich pea flour are compatible for the control of *S. oryzae* because parasitoids target the hidden immature stages, and the protein-rich pea flour is effective against adults only. An additive effect of this combination was observed in the large-scale test. The percentage reduction in the number of live insects in the combination treatment was higher than that in the treatment of protein-rich pea flour alone at 0.04 or 0.1%. In addition, the combination of parasitoids and protein-rich pea flour maintained the grading quality factors of wheat, whereas either of the individual treatments did not. However, this effect was not observed in the small-scale test.

A high ratio of parasitoids to hosts was required to control the pest populations. In the large-scale test, a higher ratio of *A. calandrae* to *S. oryzae* was observed in the protein-rich pea flour treatment than in the control. *C. waterstoni* remained low, and this may explain the small reduction of *C. ferrugineus* populations in the large-scale test. In the small-scale test, the lack of additional control in the combination treatment was likely caused by the low ratio of the number of *A. calandrae* to *S. oryzae* and to the production of

Table 5. Changes in physical characteristics of wheat before release of insects and at the end of a large-scale test at the CRC and at the UM sites

Date	Grain quality	Number of total insects (per kg, mean ± SEM)[a]									
		CRC					UM				
		Untreated	0.04% protein-rich pea flour	0.1% protein-rich pea flour	*F*	*P*	Untreated	Parasitoid	Parasitoid + 0.04% protein-rich pea flour	Parasitoids + 0.1% protein-rich pea flour	*F*	*P*
Start	Dockage (%)	4.7 ± 0.6a	4.8 ± 0.7a	4.6 ± 0.4a	0.02	0.988	7.6 ± 0.9a	6.7 ± 0.4a	6.7 ± 0.5a	5.0 ± 0.7a	1.68	0.247
	Moisture content (%)	13.6 ± 0.01a	13.7 ± 0.1a	13.7 ± 0.1a	2.86	0.083	13.4 ± 0.1a	13.5 ± 0.1a	13.4 ± 0.1a	13.6 ± 0.1a	1.06	0.418
	Bulk density (kg/m^3)	717 ± 2a	718 ± 1a	716 ± 1a	0.41	0.680	716 ± 1a	714 ± 1a	712 ± 2a	715 ± 2a	1.03	0.400
End	Dockage (%)	16.3 ± 5.2a	16.8 ± 5.2a	8.3 ± 0.6b	14.0	0.006	15.2 ± 3.2a	11.9 ± 1.2ab	9.2 ± 0.8bc	7.0 ± 0.7c	14.47	<0.001
	Moisture content (%)	13.0 ± 0.4a	13.1 ± 0.3a	12.9 ± 0.3a	0.38	0.691	12.9 ± 0.5a	12.7 ± 0.5ab	12.4 ± 0.6b	12.5 ± 0.7ab	4.79	0.034
	Bulk density (kg/m^3)	649 ± 36b	654 ± 35b	694 ± 11a	12.61	0.007	687 ± 15c	702 ± 7b	709 ± 2ab	717 ± 2a	22.95	<0.001

[a] Means in a row at same location followed by different letters are different (PROC MIXED, Tukey's, $P < 0.05$; df = 2,18 for the CRC and df = 3,24 for the UM).

high moisture levels in the grain produced by insect metabolic activity. Hou and Fields (2003b) found that protein-rich pea flour is less effective in high-moisture grain.

Sufficient numbers of hosts in the appropriate stages were required to establish the parasitoid population. In the small-scale test, *A. calandrae* became established at the high pest infestation rate but not at the moderate and low infestation rates, apparently because of the low numbers of available hosts. *A. calandrae* adults live ≈10 d, and they prefer parasitizing late-instar larvae or prepupae (Rilett 1949, Burks et al. 1999). Therefore, only certain stages of *S. oryzae* were parasitized by *A. calandrae*, although all host stages of *S. oryzae* were provided by allowing parent adults to lay eggs continuously for 25 d. Based on the number of *S. oryzae* adults emerged in the untreated control during the first 3 wk, we estimated that there were ≈27, 2.7, and 0.27 suitable hosts per week for *A. calandrae* at the three infestation rates, respectively. Parasitoids did not persist, probably because few suitable hosts were available at moderate and low infestation rates. Unsuitable host stages, such as eggs and very young larvae, developed without the impact of the parasitoids, and the treatments failed.

Differences in the distribution of *S. oryzae* and *C. ferrugineus* in the bulk grain may have affected the establishment of *A. calandrae* and *C. waterstoni* in the large-scale test. Most immature *S. oryzae* were in the top layer and would be easily located and parasitized by *A. calandrae.* The few *S. oryzae* in the lower layer that survived exposure to the protein-rich pea flour may have acted as a reservoir to maintain a low density of hosts and allowed some *A. calandrae* to survive. In contrast, most immature stages of *C. ferrugineus* were found in the middle and bottom layer. *C. waterstoni* adults find *C. ferrugineus* by following a kairomone produced by *C. ferrugineus* (Howard and Flinn 1990). It is not known if the low number of *C. waterstoni* is because of its inability to penetrate the grain to the necessary depth in these tests or because of low host density.

Release time has a greater effect than the number of *C. waterstoni* on the dynamics of *C. ferrugineus* populations (Flinn and Hagstrum 1995). Augmentative release of parasitoids was tested in a granary trial (Flinn et al. 1996). However, both the single and multiple releases of *A. calandrae* had equal efficacy on the reduction of populations of *S. oryzae* in the small-scale test. More studies on the optimum release time and the effects of other environmental factors are required to determine the optimum parasitoid to host ratio.

In addition to the failure of the *C. waterstoni* populations to establish, the small reduction of *C. ferrugineus* in the large-scale test may also be because of the protein-rich pea flour being less effective against *C. ferrugineus* (Hou and Fields 2003b). Also the repellency of protein-rich pea flour that was a major factor in reducing *C. ferrugineus* in granary trials (Fields et al. 2001, Hou and Fields 2003a) was excluded in this test.

Populations of *T. castaneum* were not significantly reduced in any of the treatments in the large-scale test. In previous studies, *T. castaneum* was least affected by the protein-rich pea flour of the three insects tested (Bodnaryk et al. 1997, Hou and Fields 2003a, b). Also, *T. castaneum* were unable to leave the grain mass in this experiment. This is different from a granary trial, in which *T. castaneum* were able to leave the grain. Hou and Fields (2003a) observed lower populations in grain treated with protein-rich pea flour compared with untreated grain in such a granary trial.

One limitation of using protein-rich pea flour to control stored-product insects in commercial granaries is that *Rhyzopertha dominica* (F.), the lesser grain borer, one of the most destructive stored-product insects, is not controlled with a 0.1% concentration of protein-rich pea flour (Bodnaryk et al. 1997). However, *A. calandrae* is a generalist parasitoid and parasitizes a number of stored-product Coleoptera and Lepidoptera (Schöller and Flinn 2000), including *R. dominica* (Chatterji 1955, Ahmed 1996). Because *A. calandrae* is not adversely affected by the pea flour treatments, the combination of this parasitoid and protein-rich pea flour may help suppress mixed populations of stored-product insects that include *R. dominica.*

Acknowledgments

We thank T. Mayert, C. Demianyk, A. Heida, A. Bailleux, and J. Canaferina for technical assistance, S. Woods for statistical advice, T. Arbogast and J. Nechols for reviewing the manuscript, the Agri-Food Research and Development Initiative and the University of Manitoba for financial support, and Parrheim Foods for providing the protein-rich pea flour. This is contribution 1866 of the Cereal Research Centre (Agriculture and Agri-Food Canada).

References Cited

Ahmed, K. S. 1996. Studies on the ectoparasitoid, *Anisopteromalus calandrae* How. (Hymenoptera: Pteromalidae) as a biocontrol agent against the lesser grain borer, *Rhyzopertha dominica* (Fab.) in Saudi Arabia. J. Stored Prod. Res. 32: 137–140.

Arthur, F. H. 1996. Grain protectants: current status and prospects for the future. J. Stored Prod. Res. 32: 293–302.

Bell E. A. 1977. Toxins in seeds, pp. 143–161. *In* J. B. Harborne (ed.), Biochemical aspects of plant and animal coevolution. Academic, New York.

Bodnaryk, R. P., P. G. Fields, Y. S. Xie, and K. A. Fulcher. 1997. Insecticidal factors from peas. USA Patent. 5.955: 082.

Burks, C. S., J. E. Baker, and D. W. Hagstrum. 1999. Optimum host age and stage for development of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) reared on the rice weevil (Coleoptera: Curculionidae) in wheat. J. Kan. State Entomol. Soc. 72: 440–447.

Chatterji, S. 1955. Studies on the biology of *Aplastomorpha calandrae* Howard (Insecta: Hymenoptera: Chalcidae) parasitic on some storage pests. Proc. Zool. Soc. Lond. 8: 11–23.

Cline, L. D., J. W. Press, and B. R. Flaherty. 1985. Suppression of rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), inside and outside of burlap, woven polypropylene, and cotton bags by the parasitic wasp, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). J. Econ. Entomol. 78: 835–838.

Coombs, C. W., C. J. Billings, and J. E. Porter. 1977. The effect of yellow split-peas (*Pisum sativum* L.) and other pulses on the productivity of certain strains of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the ability of other strains to breed thereon. J. Stored Prod. Res. 13: 53–58.

Croft, B. A. 1990. Arthropod biological control agents and pesticides. Wiley-Interscience, New York.

Delobel, B., A. Grenier, J. Gueguen, E. Ferrasson, and M. Mbailao. 1998. Use of a polypeptide derived from a PA1b legume albumen as insecticide. Patent WO 99/58695.

Fields, P. G., Y. S. Xie, and X. Hou. 2001. Repellent effect of pea (*Pisum sativum*) fractions against stored-product insects. J. Stored Prod. Res. 37: 359–370.

Flinn, P. W., and D. W. Hagstrum. 1995. Simulation model *of Cephalonomia waterstoni* (Hymenoptera: Bethylidae) parasitizing the rusty grain beetle (Coleoptera: Cucujidae). Environ. Entomol. 24: 1608–1615.

Flinn, P. W., D. W. Hagstrum, and W. H. Mcgaughey. 1996. Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. Environ. Entomol. 25: 505–511.

Golob, P., C. Moss, M. Dales, M. Fidgen, J. Evans, and I. Gudrups. 1999. The use of spices and medicinals as bioactive protectants for grains. Food and Agricultural Organization of the United Nations, Rome, Italy.

Harborne J. B., D. Boulter, and B. L. Turner. 1971. Chemotaxonomy of the Leguminosae. Academic, London, UK.

Holloway, G. J. 1986. The potency and effect of phytotoxins within yellow split-pea (*Pisum sativum*) and adzuki bean (*Vigna angularis*) on survival and reproductive potential of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Bull. Entomol. Res. 76: 287–295.

Hou, X., and P. G. Fields. 2003a. Granary trial of protein-rich pea flour for the control of three stored-product insects in barley. J. Econ. Entomol. 96: 1005–1015.

Hou, X., and P. G. Fields. 2003b. Effectiveness of protein-rich pea flour for the control of stored-product beetles. Entomol. Exp. Appl. 108: 125–131.

Howard, R. W., and P. W. Flinn. 1990. Larvae trails of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) as kairomonal host-finding cues for the parasitoids *Cephalonomia waterstoni* (Hymenoptera: Bethylidae) Ann. Entomol. Soc. Am. 83: 239–245.

Jacobson, M. 1989. Botanical pesticides: past, present, future, pp. 1–10. *In* J. T. Arnason, B.J.R. Philogene, and P. Morand (eds.), Insecticides of plant origin. American Chemical Society, Washington, DC.

Jilani, G., and R. C. Saxena. 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). J. Econ. Entomol. 83: 629–634.

Perez-Mendoza, J., J. E. Baker, F. H. Arthur, and P. W. Flinn. 1999. Effects of Protect-It on efficacy of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitizing rice weevils (Coleoptera: Curculionidae) in wheat. Environ. Entomol. 28: 529–534.

Prakash, A., and J. Rao. 1997. Botanical pesticides in agriculture. CRC, Boca Raton, FL.

Press, J. W., L. D. Cline, and B. R. Flaherty. 1983. Suppression of residual populations of the rice weevil, *Sitophilus oryzae*, by the parasitic wasp, *Anisopteromalus calandrae.* J. Georgia Entomol. Soc. 19: 102–110.

Rilett, R. O. 1949. The biology of *Cephalonomia waterstoni* Gahan. Can. J. Res. Dis. 27: 93–111.

Saxena, R. C., G. Jilani, and A. Abdul-Kareem. 1988. Effects of neem on stored grain insects, pp. 97–111. *In* M. Jacobson (ed.), Focus on phytochemical pesticides, vol. 1, the neem tree. CRC, Boca Raton, FL.

SAS Institute 2000. SAS On-line Doc®, version 8. SAS Institute, Cary, NC.

Schöller M., and P. W. Flinn. 2000. Parasitoids and predators, pp. 229–272. *In* B. Subramanyam and D. W. Hagstrum (eds.), Alternatives to pesticides in stored-product IPM. Academic Publishers, Boston, MA.

Semple, R. L., P. A. Hicks, J. V. Lozare, and A. Castermans. 1992. Towards integrated commodity and pest management in grain storage: a training manual for application in humid tropical storage systems. Food and Agricultural Organization of the United Nations, Rome, Italy.

Weaver, D., and Bh. Subramanyam. 2000. Botanicals, pp. 303–320. *In* Bh. Subramanyam and D. Hagstrum (eds.), Alternatives to pesticides in stored-product IPM. Kluwer Academic Publishers, Dordrecht, Germany.

White, N.D.G., and D. S. Jayas. 1991. Control of insects and mites with carbon dioxide in wheat stored at cool temperatures in non-airtight bins. J. Econ. Entomol. 86: 1933–1942.

Received 26 June 2003; accepted 20 February 2004.