SUPPRESSIVE EFFECT OF ALLYL ISOTHIOCYANATE ON POPULATIONS OF STORED GRAIN INSECT PESTS

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ABSTRACT

The efficiency of the WasaOuro® system, based on an extract derived from Cruciferiae plants, was investigated as an insect suppressing agent. The active ingredient was allyl isothiocyanate (AIT). Lasioderma serricorne *and* Tribolium confusum *were exposed to AIT vapor in vented containers. The containers were maintained in a temperature- and humidity-controlled room. The WasaOuro system does not repel or attract* L. serricorne *but does reduce tunneling activity of* T. confusum. *AIT disrupts normal reproductive cycles of both* L. serricorne *and* T. confusum *resulting in an insect population reduction in grain foods.*

INTRODUCTION

Insect pests of the order *Coleoptera* play a major role in the vast destruction of grain foods intended for human consumption. Many entomologists consider this group of insects as the "number one" insect pest of stored meal and flour in the United States. These insects are noted for their prodigious population growth. For example, a single female confused flour beetle, *Tribolium confusum,* may lay up to 400 eggs that may reach reproductive maturity in 20 days. With a survival rate of 60-70% after a four-month storage period at optimum relative humidity and temperature, a population of over 2,000,000 beetles may result (Baur 1984).

Insect pests have a long history of infesting perishable foods, especially milled grains, which often results in aesthetically degraded and poor quality foods, such as infested cereals and noodles, usually resulting in their disposal. Infestation of packaged perishable foods basically originates through three different routes. One of these is the infestation from the outside environment. This is a common means of infestation in poorly packaged products in fiberboard or paper containers and usually occurs when a population of insect pests already exists in a warehouse or

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during transportation on an infested carrier. Another route of infestation is the development from within or on the stored product. This type usually occurs when eggs survive processing, such as milling, and develop during storage and transportation. The third, in or on a packaged product, is a combination of the first and second types of infestation; this route includes the development of a new colony, which then exits out of the original package and enters another one. There is a clear need for a system to control or suppress insect infestation by interrupting one or more of these routes.

The maintenance of quality of most perishable and semi-perishable foods is achieved by refrigerated storage, together with adequate rotation of products and frequent inspections. Because of the low moisture content of meal, flour and milled products, they are seldom placed in refrigerated storage. Although refrigeration generally will not eliminate an infestation of adult flour beetles, it will delay hatching periods, thus extending the product's shelf life. Flour is often fumigated with chemical agents during storage once it has been identified as being insect-infested. Several methods to control insect pests in grain foods have been used, including conventional treatments such as methyl bromide fumigation, and more innovative treatments such as irradiation and microwaves (Gorham 1991). Unfortunately, there are instances when insect infestation is too great for fumigation and the product must be destroyed. In such cases, if fumigation is attempted, less than 100% kill is achieved, thus leaving a population of insects and/or eggs for future infestations. Also, these treatments are often limited to treating only adult beetles and are commercially unacceptable because of high energy costs and consumer's environmental concerns.

Recently, preparations derived from Cruciferiae plants have become commercially available in Japan and are known as the WasaOuro® system (The Green Cross Corp., Osaka, Japan). WasaOuro is the collective name of the preparations derived from specific Cruciferiae plants that contain substances called glycosides. One specific glycoside, called sinigrin, exists in wasabi (*Wasabia japanica Matsumura)*, a Cruciferiae root plant common in Japan. Sinigrin is also found, but at lower levels, in the roots of the mustard and horseradish plant. Sinigrin is hydrolyzed by the catalysis of the coexisting enzyme "myrosin", thus generating allyl isothiocyanate (AIT) (CH^2=CH-CH^2-N=C=S). AIT is highly volatile and has been shown to have antibacterial and antifungal properties (Tokuoka and Issiki 1994; Sekiyama *et al.* 1994; Delaquis and Mazza 1995) and it has been approved in Japan for use as a food additive. A proposal for the Food and Drug Administration's acceptance as a nonsynthetic food additive in the U.S. has been initiated. According to the WasaOuro manufacturers, AIT may have an insect suppression effect. The present study explores the efficiency of the WasaOuro system in controlling insect infestations of grain and the effect on sensory attributes and physical properties of the bread prepared from AIT treated flour samples.

MATERIALS AND METHODS

WasaOuro Pouch

WasaOuro pouches (MN10E) were obtained from the Green Cross Corp. (Osaka, Japan). The pouch contains 10 mg of WasaOuro powder (1 part AIT, 9 part surfactant and sugars) impregnated into an acetate mat which is enclosed in a perforated oriented polypropylene (OPP) or polyethylene (PE) film (Fig. 1). The pouch will release 95% of its AIT within 3 h at 25C and completely release after 6 h.

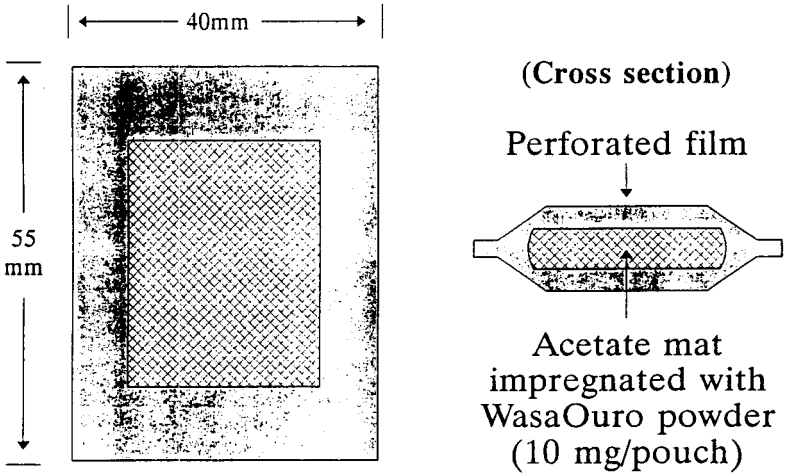


FIG. 1. WASAOURO POUCH

Effect of AIT on Adult Insect Migration

The cigarette beetle, *Lasioderma serricorne,* was selected primarily because of its normal short life span of 21-43 days (Gorham 1991). Rearing was in accordance to the recommended practices prescribed by the United States Department of Agriculture (Anon. 1993). The original test population consisted of adults that had emerged from the pupal chamber 7-10 days prior to the test. A sterile medium consisting of 10 parts whole wheat flour, 10 parts white cornmeal and 1.5 parts of brewers' yeast was used as the insect food. A setup as illustrated in Fig. 2 was used. The setup was designed to determine whether the volatile AIT released from container B discouraged the entry of beetles from source release container A. Seven hundred and fifty cigarette beetles were released into source container A, an empty, unventilated one-pint container with two exits. Each exit had one meter of clear,

1 cm diameter, rubber hose lead to the glass containers B and C. Each glass container was vented through a 1 cm diameter port with #1 filter paper and glass wool. In container B, a WasoOuro pouch was placed in the bottom of the container and 20 g of medium was placed over it. Container C had 20 g of the medium placed into it but no WasaOuro pouch. The medium was lightly tamped in each jar before sealing. The setup was maintained in a temperature/humidity controlled room (26.7± 3.0C, RH 60±5%). Artificial light was provided for 10-12 continuous hours per day. A dissecting scope (25X) was used for visual examination of contents in each container. At every observation, 3 g of samples were isolated from random spots for study.

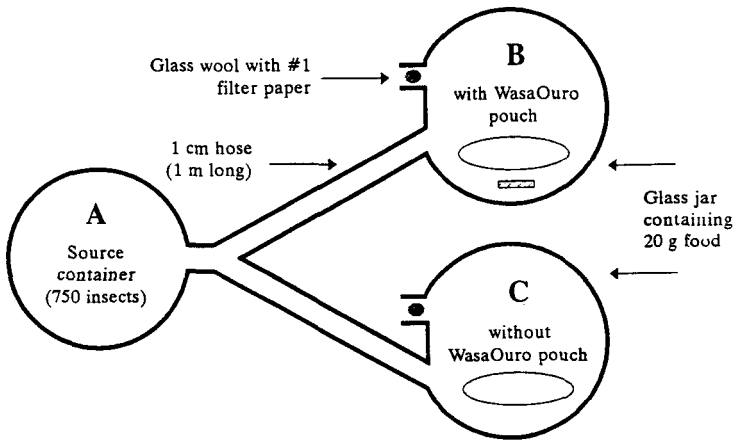


FIG. 2. SETUP FOR CIGARETTE FLOUR BEETLE STUDY

Effect of AIT on Insect Egg Hatching

The confused flour beetle, *Tribolium confusum,* a commonly occurring pest in both adult and larval stages in flour, cornmeal, cereals, and similar materials was used. A sterile medium containing 10 parts whole wheat flour, 10 parts white cornmeal and 1 part brewers' yeast was used (Anon. 1993) for growth. The control glass container (container A) consisted of 400 g medium (Fig. 3). A second glass container (container B) consisted of 400 g medium and two WasaOuro pouches that were placed horizontally aligned in the container. A third glass container (container C) consisted of 400 g of medium and four WasaOura pouches placed in the container. Five hundred adult confused flour beetles (mixed sexes) were released into each glass container. All three containers were placed in the same temperature- and humidity-controlled room as previously described. After 90 days,

the samples were examined for the number of live adults, larvae and pupae at various stages by sifting through a #20 mesh sieve.

Both experiments were repeated three times and each time a new generation was used.

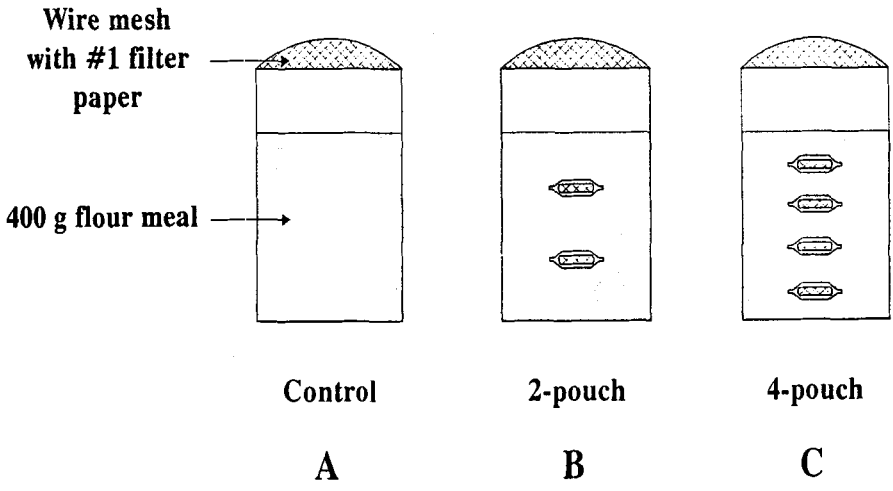


FIG. 3. SETUP FOR CONFUSED FLOUR BEETLE STUDY

Effect of AIT on Aroma and Specific Volume of Bread

WasaOuro pouches (0, 1, 2, and 3) were packed with 200 g of all-purpose flour in PE bags which were then sealed and stored at 25C for 48 h. White bread samples were made from these flour, with pouches removed, in an Auto Bakery ®(Model FAB-100-1; DAK Industries Inc., Canoga Park, CA). Sensory evaluation was conducted to detect AIT odor in bread by a nine member trained panel. Specific volume of the bread was obtained by dividing the loaf volume determined by rape seed displacement by the weight of the loaf. The experiment was repeated three times.

Data Analyses

The data were studied by the analyses of variance. $P < 0.05$ was considered significant. AIT odor was considered nondetectable when less than 1/3 of the panelists could detect (i.e.,"-"); detectable when between 1/3 and 2/3 of the panelists can detect (i.e., "+"); and highly detectable when more than 2/3 of the panelists could detect the odor (i.e., "++").

RESULTS AND DISCUSSION

Results obtained from all three generation cycles showed a similar effect of AIT on insect suppression. Therefore, the results of the third repetition were used for discussion.

Effect of AIT on Adult Insect Migration

The adult cigarette beetle did not seem to be deterred by the AIT vapor as the end of the 28 days over 70% of the insect migrated to either container B or C (Table 1). No significant difference ($p=0.5$) existed between the number of cigarette beetles entering container B and those entering container C. However, after 28 days the control container C was filled with numerous larvae and pupae and the container containing WasaOuro (i.e., B) appeared to have very few larvae and no pupa (Fig. 4 and 5). Three g samples were randomly selected from containers B and C. Container B contained one larva and 36 eggs per sample while container C contained 34 larvae and 11 eggs per sample. After 48 days, both containers showed increased numbers of larvae and pupae; however, the total number of adults, larvae and pupae was 30 times greater in container C than in container B. It appears that AIT has more suppressing effect on larvae and eggs than on adult insects.

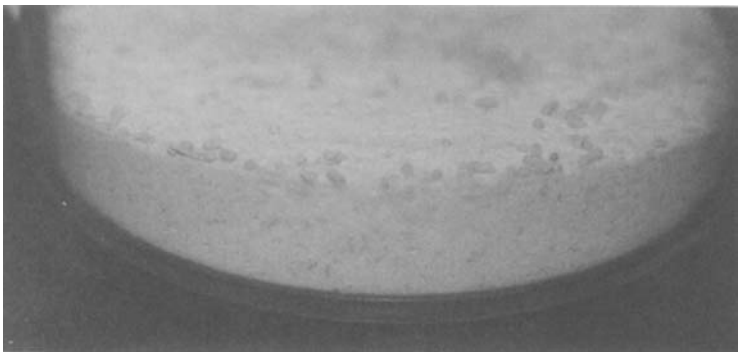
Effect of AIT on Insect Egg Hatching

In this experiment, the adult confused flour beetles immediately began to tunnel into the media following their release into the containers (Fig. 6). Tunneling was observed along the sides of each glass container. The beetles in container A were more active than beetles in containers B and C. The addition of the WasaOuro pouches appeared to suppress tunneling activity along the sides of the containers throughout the experiment's 90-day time period. After 90 days, the adult and immature beetles were separated and then enumerated. The control container had more adults than the other containers but fewer immatures (Table 2). Container B contained 47% fewer adults and container C contained 72% fewer adults than container A. Containers B and C had 58% and 80% more larvae, respectively, than container A. Container A did not contain any pupa while container B had a total of 13 and container C had a total of 720. The larvae in containers A and B were very similar in growth stages while those in container C greatly varied.

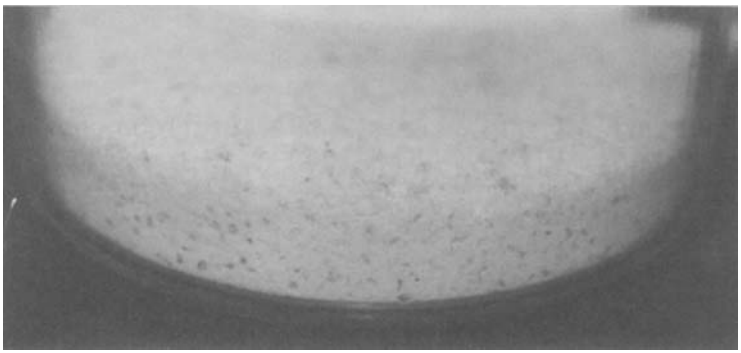
According to the manufacturers, the WasaOuro system has a suppressive effect in that it is lethal to some insects at high concentrations. Studies have been done to determine the lethal concentration (LC_{50}) of AIT on *Sitophilus zeamais* adults and *Plodia interpunctella* adults and larvae (Table 3). Lethal doses were achieved at high concentrations within a controlled environment and with no ventilation. In our experiments, only an estimated 1% of the adults in each container was dead and no

dead larva or pupa was identified after 90 days. The containers in these studies were vented, thus allowing volatile AIT to gradually dissipate into the surrounding environment.

The presence of AIT does appear to disrupt normal reproductive cycles resulting in lower populations of both *Lasoderma serricorne* and *Tribolium confusium.* WasaOuro retarded the hatching of eggs for at least four weeks. Since the active agent, AIT, counts for only 10% of the WasaOuro powder that is impregnated in the pouch, the effect of 1 mg AIT to retard egg hatching in 20 grams of media, under favorable conditions, is considered highly sufficient.

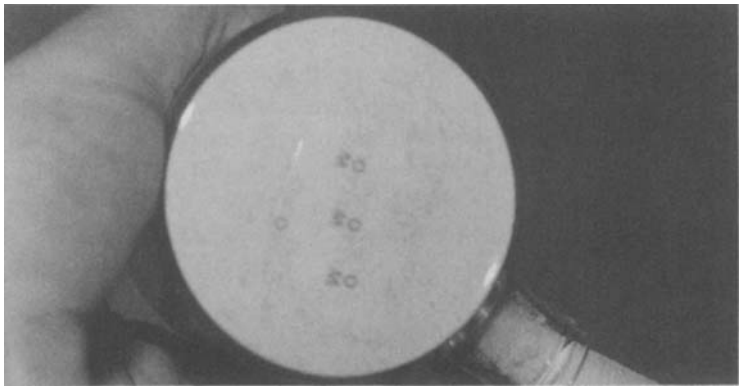


(A) With WasaOuro Pouch

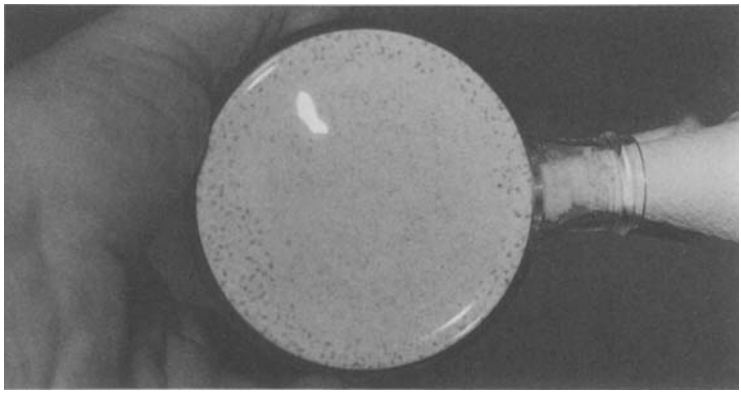


(B) Without WasaOuro Pouch

FIG. 4. CIGARETTE BEETLE LARVE INFESTATION IN INSECT FOOD AFTER 28 DAYS (SIDE VIEW) (A) white pellets are unconsumed brewer's yeasts; (B) dark specks are larve.



With WasaOuro Pouch

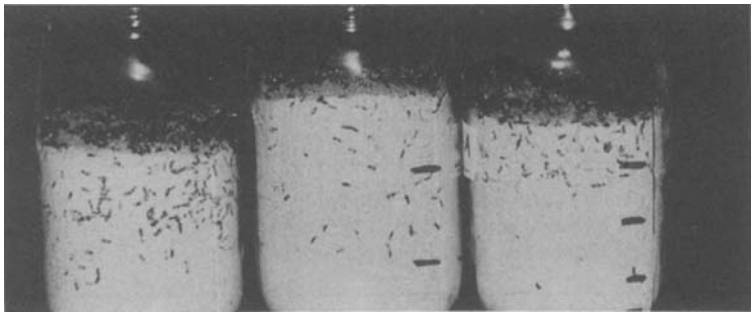


Without WasaOuro Pouch

FIG. 5. CIGARETTE BEETLE LARVE INFESTATION IN INSECT FOOD AFTER 28 DAYS (BOTTOM VIEW)

Effect of AIT on Aroma and Specific Volume of Bread

Results are summarized in Table 4. According to a sensory test conducted by the WasaOuro manufacturer, the minimum concentration of mustard oil that can be detected by the human sense of smell is approximately 10 ppm (Isshiki *et al.* 1992). In this study, the maximum possible retention of AIT by the flour sample was 15 ppm. Flour seemed to trap some of the AIT odor which increased with the number of pouches. However, AIT odor was nondetectable after baking and the bread aroma was normal. AIT did not alter the specific volume of bread samples.



Control 2 Pouches 4 Pouches

FIG. 6. ADULT CONFUSED FLOUR BEETLE SUPPRESSION EFFECT OF WASAOURO POUCHES

TABLE 1.
MIGRATION OF ADULT CIGARETTE BEETLES[1]

Day	Container A (Source)	Container B (with WasaOuro Pouch)	Container C (no WasaOuro Pouch)
1	680	38	32
2	653	49	48
3	549	110	91
4	425	153	162
5	344	198	208
28	212	271	267

[1]Measure of migration based on the number of beetles counted

TABLE 2.
PROGENY FOR POPULATIONS OF *TRIBOLIUM CONFUSIUM* EXPOSED TO WASAOURO

Container	# Pouches	Adults	Larvae	Pupae	Total
A	0	8,882	442	0	9,324
B	2	4,744	1,040	13	5,797
C	4	2,506	2,160	720	5,386

TABLE 3.
LC_{50} OF AIT AGAINST STORED INSECT PESTS [1,2]

Insect pests	LC_{50} (95% Confidence limit)	
	% (w/w)	Estimated ppm[3]
Sitophilus zeamais Motsuchulsky, Adults	0.170 (0.158-0.183)	21.5 (19.9-23.1)
Plodia interpunctella Hubner, Adults	0.0162 (0.0141-0.0186)	2.04 (1.78-2.35)
Plodia interpunctella Hubner, Larvae	0.641 (0.578-0.710)	80.9 (72.9-89.6)

[1] LC_{50}: lethal concentration to eliminate 50% of the original insect population
[2] Data obtained through the courtesy of the Green Cross Corp., Osaka, Japan
[3] Estimated from calibration curve

TABLE 4.
EFFECT OF AIT ON AROMA AND SPECIFIC VOLUME OF BREAD SAMPLES PREPARED FROM WASAOURO TREATED FLOUR

No. Pouches	Detection of AIT odor [1]		Specific volume (cc/g)
	Flour	Bread	
0	-	-	4.48 ± .1
1	+	-	4.48 ± .1
2	+	-	4.38 ± .1
3	++	-	4.47 ± .1

[1] -: non-detectable; +: detectable; ++: highly detectable

CONCLUSIONS

WasaOuro pouch with its associated AIT had suppressive effect on both cigarette beetles and confused flour beetles, especially on their larvae and eggs. Flour treated with WasaOuro pouch will have longer shelf life against insect infestation and can be made into bread products without adverse quality loss.

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