EVALUATION OF FOOD PACKAGE RESISTANCE TO INSECT INVASION

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Abstract - The data presented here addresses a new approach to the package evaluations which differs in all aspects from previous techniques except for the use of destructive sampling. Our evaluations are for a maximum period of a life-cycle of the challenging pest. By keeping the effect for their life stages separate, corrective approaches can be affected to the packages. All challenges are conducted in areas of controlled relative humidity and temperature in glass aquaria large enough to hold the number of packages needed for one replicate. Evaluations have been conducted with challenges from the Indian meal moth, *Plodia interpunctella* (Hübner). The use of insect resistant packages merits continued investigation as the shipping package provides the only protection food and feed processors and manufacturers can be assured of when their product leaves their control and enter the distribution channels.

Key words - Insect resistant packaging, Indian meal moth, *Plodia interpunctella*

INTRODUCTION

Package evaluations for resistance to insect and mite attack on package contents is a relatively new approach in the food and feed distribution arena. After the conclusion of World War II, there was a great need for storing and distributing large quantities of raw and processed food and feed to meet the nutritional needs of many nations whose infrastructure had been destroyed or was in great disarray. To minimize the cost of packaging and distributing, such commodities were often packed and distributed in 50 pound or larger bags. If such large containers became infested a large quantity was destroyed or rendered of low nutritional value. Research initiated at that time involved methods of improving the gluing, pasting, and taping bag closures as well as developing new cartons and new insect resistant multi-walled paper and cotton bags. The use of greaseproof paper, baler bags and plastic moisture barriers were found to assist in retaining food and feed quality for longer storage periods.

In the 1950s and 1960s investigations turned to the use of pesticides on the outer layer or one or more layers of multi-wall paper bags. The approach for a time offered great promise, but ultimately the migration of pesticides through the bag and into the contents caused a problem considered insurmountable. Today, in the United States, only pyrethrins synergized with piperonyl butoxide (alpha -[2-(2-butoxethoxy) ethoxy] - 4, 5(methylenedioxy)-2-propyltoluene) is registered for use as an insect-resistant package treatment. This combination of chemicals must not exceed 6 mg of pyrethrins and 60 mg of piperonyl butoxide per square foot of the outer surface of the multi-walled paper bags (Anonymous, 1966) or 5.5 mg of pyrethrins and 55 mg of piperonyl butoxide per square foot on the outer surface of cotton bags (Anon. 1968). The treated cotton bag must have a waxed paper liner and the product must contain no more than 4% fat. All these pesticide treated bags had to contain at least 50 pounds of dry food.

The U. S. Environmental Protection Agency has also approved (Anon., 1974) the use of a treated cellophane-polyethylene laminate (Yerington, 1974) to protect prunes, raisins and other dried fruits from insect infestation. The pyrethrins and piperonyl butoxide are incorporated into the laminating adhesive at the rate of 10 and 50 mg per square foot, respectively. Residue must not exceed more than 0.31 mg pyrethrins and 3.12 mg piperonyl butoxide per ounce of fruit. The main reasons for caution in using chemical repellents has been the possibility of product contamination by the repellent migrating from the treated package into the product.

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Table 1. Methods of attack by insects seeking to enter packaged food and feed.

Family name and species	Life stage(s) at time of attack[a]	
	Invader	Penetrator
Tenebrionidae		
Tribolium confusum - confused flour beetle	Adult	
T. castaneum - red flour beetle	Adult	
Cucujidae		
Oryzaephilus surinamensis - sawtoothed grain beetle	Adult	
Oryzaephilus mercator - Merchant grain beetle	Adult	
Curculionidae		
Sitophilus granarius - granary weevil	Adult	
S. oryzae - rice weevil	Adult	
Dermestidae		
Trogoderma spp. - warehouse beetles		Adults & Larvae
Anobiidae		
Lasioderma serricorne - cigarette beetle		Adult
Bostrichidae		
Rhyzopertha dominica - lesser grain borer		Adult
Phycitidae		
Plodia interpunctella - Indianmeal moth	Young Larvae	Mature Larvae

[a]The use of this classification is not strict as most insects can be either invaders or penetrators depending upon the conditions under which they are found.

Table 2. Observed egg dimensions for selected stored-product insects (Adapted from LeCato and Flaherty, 1974).

Family name and species		Dimensions (mm)
Dermestidae	- *Dermestes maculatus* DeGeer	1.56 ± 0.03x0.52 ± 0.01
	- *Trogoderma variabile* Ballion	0.71 ± 0.04x0.24 ± 0.02
Anobiidae	- *Lasioderma serricorne* (F.)	0.41 ± 0.02x0.21 ± 0.01
Bostrichidae	- *Rhyzopertha dominica* (F.)	0.52 ± 0.05x0.20 ± 0.01
Ostomidae	- *Tenebroides mauritanicus* (L.)	1.31 ± 0.04x0.27 ± 0.01
Tenebrionidae	- *Cynaeus angustus* (LeConte)	0.97 ± 0.04x0.48 ± 0.02
	- *Tribolium castaneum* (Herbst)	0.60 ± 0.03x0.31 ± 0.02
	- *Tenebrio molitor* L.	1.99 ± 0.10x0.84 ± 0.03
Nitidulidae	- *Carpophilus dimidiatus* (F.)	0.71 ± 0.03x0.23 ± 0.01
Cucujidae	- *Oryzaephilus mercator* (Fauvel)	0.71 ± 0.03x0.24 ± 0.01
	- *Cryptolestus pullus* (Schönherr)	0.58 ± 0.02x0.15 ± 0.01
Bruchidae	- *Callosobruchus maculatus* (F.)	0.50 ± 0.03x0.30 ± 0.02
Anthribidae	- *Araecerus fasciculatus* (DeGeer)	0.57 ± 0.03x0.32 ± 0.01
Curculionidae	- *Sitophilus granarius* (L.)	0.80 ± 0.06x0.32 ± 0.02
	- S. *oryzae* (L.)	0.65 ± 0.04x0.27 ± 0.02
Phycitidae	- *Cadra cautella* (Walker)	0.46 ± 0.02x0.31 ± 0.02
	- *Plodia interpunctella* (Hübner)	0.49 ± 0.02x0.29 ± 0.01

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The development of suitable and regulatory approved insect resistant packaging is also complicated by insect behavior. Insect species attack the contents of food and feed packages by either entering existing holes caused by other insects or by mechanical damage caused by equipment and/or faulty handling (invaders) or by penetrating the packaging by chewing holes (penetrators). Table 1 provides some of our common stored-product insects that are invaders and penetrators. It must be recognized that many insects and mites can infest packages and will from time to time become problems for the manufacturer. Cline and Highland (1981) reported that the adult red flour beetles, *Tribolium castaneum* (Herbst) can enter through openings less than 1.35 mm in diameter. Mites and newly hatched insect larvae can be expected to enter through much smaller holes. One would suspect that the size of opening available for invasion would have a lower limit close to the size of the first larva instar head capsule. Species of Acari could use even smaller entrance holes. Table 2 reports on the diameter of insect eggs and implies the approximate size of openings needed by several common insect species for access in to packaged products. LeCato and Flaherty (1974) reported on the size and appearance of 40 species of Coleoptera and Lepidoptera and Arbogast *et al.,* (1980) reported on the size and appearance of the eggs of 10 species of stored-product moths. Wohlgemuth (1979) reported that newly hatched larvae of the Indian meal moth were able to enter containers through holes of about 0.25 mm diameter.

In the development of insect resistant packaging it is important to recognize that the attraction of food or feed packages is primarily odor. Barrer and Jay (1980) reported that free flying female almond moths, *Ephestia cautella* (Walker) tended to oviposit near the source of a grain odor. Mullen (1994) reported the same response, but to packages of cereal with and without dried fruit and nuts, when challenged by the Indian meal moth. Obviously insects can select packages containing products that would be favorable for the development of their larvae. It is the objective of the studies reported here to provide a rapid evaluation (challenge) in which temperature, relative humidity, and light are easily controlled and the maximum amount of insect pressure can be applied by the researcher. This is accomplished by ignoring the attractiveness of odor and seeding the insect's eggs directly on the package's surface and in the immediate surroundings.

Approximately 2 years ago a large U. S. bakery firm approached Pest Management Consultants & Associates, Inc., with the desire to improve upon the insect resistance of the packaging of a new nutritional fruit filled snack bar. Knowing that this fruit filled snack bar would be very susceptible to insect attack if it was not adequately protected during the time it was in the distribution channels, they wished to investigate the use of a plastic overwrap on the shipping cases. The packaging of the fruit filled snack bar is shown in Figure 1 and is described as follows: Wrapper - 75 ga OPP (oriented polypropylene) / adhesive / 60 ga MET(metallized) OPP / cold Seal; Carton - 18 point clay coated news board (CCN) with full overlapping seal end (FOSE) style and glue spots not specified but basically using two one-inch glue shots; and the packing case is 200 pound B Fluted, regular slotted, corrugated board. In the United States, the commonly occurring pest of bakery products is the Indian meal moth which was selected for this challenge.

One of the major considerations of the manufacturer was that the product was a new release and therefore time necessary for research would be quite valuable. At that time insect resistant packaging research took from 3 to 24 months to conduct depending upon the protection time desired in the distribution channels. After discussions with all concerned parties within the manufacturing firm it was believed that the time could be shortened to 6 to 8 weeks. And if so, it would show that such studies could be conducted more frequently and at a considerable saving of both time and money.

MATERIALS AND METHODS

Insects. The Indian meal moths for this study were reared by the method used at the former United States, Department of Agriculture's Stored-Product Insects Research and Development Laboratory in Savannah, Georgia. The diet had been used for more than 30 years before the Laboratory's closure in

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Figure 1. Packaging of fruit filled snack bars showing the shipping case, the 12 cartons and the individually wrapped bars.

Figure 2. The equipment used to collect the Indianmeal moth eggs. Adult moths are anesthetized and moved to the egg collection jar where eggs are retained on the white paper.

Figure 3. The use of 75-gallon aquaria as chambers for challenging the package resistance.

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1994. The diet and the quantities of constituents per one and ten 1-gallon culture jars are presented in Table 3. Moths were reared in 1-gallon plastic jars with 40 mesh screen lids. The rearing conditions are 80 º± 2 ºF., 65-75% relative humidity and a 12 hr-12 hr light-dark cycle.

Rearing. All adult moths, between 1 and 3 days old, were removed from their rearing containers with the aid of carbon dioxide to anesthetize them to allow for safe placement in their jars for egg laying (Figure 2). The jars for egg laying contained a pleated sheet of black construction paper to provide additional resting surface for the moths and facilitates egg laying. The jar cover had an opening approximately three-quarters the area of the cover and was fitted with a 16 mesh screen. The jar was inverted over a clean sheet of paper and the eggs laid fall through the screen on to the paper. The eggs were collected 24 hours later and appropriately 1,000 eggs are placed in a new gallon culture jar containing newly prepared medium.

Challenge study. This was conducted using 75-gallon glass aquaria that measured 48 inches long, 21 inches high and 18 inches wide (Figure 3). Each aquarium held four cases that measured 14 inches long, 9 inches wide and 6 inches high. The bottom of each aquarium was covered with black paper to provide a surface on which we might observe the Indian meal moth eggs and larvae before they entered the package. It also provided a surface that facilitated the larvae movement.

Sixty four cases of the fruit filled snack bars, each case containing 12 cartons with 6 fruit filled snack bars in each were used. One-half of the cases with shrink wrap and one-half of the cases without shrink wrap had a fruit filled snack bar wrapper intentionally damaged (imperfect wrapper). This was to assure that the study would not be dependent upon any damage that might occur on the packaging line or the handling for presence of a source of an attractive odor. The remaining cases were designated as cases with fruit filled snack bars with perfect wrappers.

The four variables used in this challenge of case overwraps as physical and odor barriers were as follows: (a) all perfect wrappers on the fruit filled snack bars with no shrink wrap over the case, (b) all perfect wrappers with shrink wrap over the case, (c) at least one intentionally damaged wrapper in a case with no shrink wrap on the case and, (d) at least one intentionally damaged wrapper with shrink wrap on the case. One of each type of the 4 different cases in the study was placed right side up on the bottom of each of four aquaria. A different arrangement of the cases was used in each aquarium replication. Four aquaria were established for each of the four weekly challenge breakdowns for a total of 64 cases.

Following the establishment of each series of cases in the aquaria, approximately 20,000 eggs mixed with equal parts of whole-wheat flour and white cornmeal were placed in a salt shaker and sprinkled over the cases. Each aquarium was covered with a sheet of corrugated paperboard to assist in maintaining the relative humidity. Each day the interior glass surface of each aquarium was lightly sprayed with water to further assist in providing a high relative humidity in the aquaria. To make the relative humidity more uniform throughout each day, four small dishes, which contained a mixture of plaster-of-Paris and activated charcoal saturated with water, were placed in the corners of each aquarium. The use of a plaster-of Paris and activated charcoal substrate is a common method for maintaining approximately 90% relatively humidity in small enclosures. A 9:1 ratio of plaster-of-Paris and activated charcoal will provide this relative humidity in an enclosure if the color of the hardened mixture remains black. This study was conducted at ambient laboratory temperatures that ranged between 68° and 72° F. Light was not controlled.

In the breakdown of this challenge study all four replications of 4 cases each were examined after they had been challenged for the four time periods of 7-days, 14-days, 21-days and 28-days by first instar larvae of the Indian meal moth. The numbers of larvae found under the shrink-wrap, inside the cases, inside the individual cartons and inside the wrapper and in the fruit filled snack bars were counted. The study was composed of 64 cases, with one-half of them with shrink-wrap over-covers. Each case contained 12 cartons each with 6 individual fruit filled snack bars. There was a total of 4,608 fruit filled snack bars in the challenge, which were individually examined for wrapper damage and presence of moth larvae.

RESULTS AND DISCUSSION

Very few of the 4,608 fruit filled snack bars examined were found attacked. The summary provided in Table 4 shows that only 73 fruit filled snack bars were found attacked. There was noted a slight increase in the number of attacks with time, particularly with those cases containing an intentionally damaged

Table 3. Rearing medium for Plodia interpunctella (Hubner)- Indianmeal moth (A modification of the USDA, Savannah Laboratory's mixture after Haydak (1936). Davis,R & Bry R. E. (1985) Handbook of Insect Rearing vol.II Singh, P. & Moore, R. F. (Eds.) Elsevier Sci. Pub. Company, Inc.

Ingredients	Liters[a]	Cups[b]
White cornmeal	4	2
Whole wheat flour	4	2
Ground pet food[c]	2	2
Dried Brewer's yeast	1	1/2
Honey	1	1/2
Glycerin	1	1/2
Wheat germ	1/2	1/4
Roll	1	1/2

[a]Preparation using liter quantities will provide enough diet for ten 1-gallon sized culture jars.
[b]Preparation using cup quantities will provide enough diet for two 1-gallon sized culture jars.
[c]any nutritionally balanced dry mammalian pet food.

Table 4. The number of snack bars observed to be infested during each of the breakdowns at 7, 14, 21 and 28 days of the challenge by first instar larvae of the Indianmeal moth from their eggs seeded upon the cases of the snack bars with and without shrink wraps. Total number of snack bars examined was 4,608.

Duration of challenge	Replications	Case with shrink wrap		Cases without shrink wrap	
		Perfect wrapper	Imperfect wrapper	Perfect wrapper	Imperfect wrapper
7 Days	1	0	0	0	0
	2	0	0	0	0
	3	0	0	1	0
	4	0	1	0	1
14 Days	1	2	0	0	1
	2	0	1	0	8
	3	0	6	0	1
	4	0	1	0	1
21 Days	1	0	1	0	3
	2	0	3	0	1
	3	0	4	2	2
	4	0	2	0	1
28 Days	1	0	2	0	3
	2	0	2	0	2
	3	0	4	5	9
	4	0	1	0	1
Totals		2	29	8	34

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wrapper. Of the 64 cases examined for the four week challenge period 31 of the 32 cases with shrink wrap had first and second instar larvae under the wrap. One case in the 7-day examination did not have larvae under the shrink over-wrap. Larvae were found in 13 of the 16 cases opened at the end of the 7-day breakdown illustrating that the larvae moved rapidly into the packaging. In this breakdown only three larvae were found inside the wrappers of the fruit filled snack bars, two in intentionally damage wrappers and one in a perfect wrapper. The perfect wrapper had been damaged, probably on the packaging line as the wrapper had a crack at one side of a seal. It is quite obvious that with the presence of odor the larvae are able to negotiate the packaging and reach the fruit filled snack bars with the damaged wrappers. It was also observed that the cases with the intentionally damaged wrapper also exhibited that cartons with the imperfect wrappers suffered more infested fruit filled cereal snack bars. This effect of odor emitting from packages increases their attraction for many insects has been shown by Mullen (1994).

The increase in attacks over time may have been caused by the effects of moisture within the aquaria providing uniform conditions throughout the package and providing a better environment for survival and/or the moistening of the paperboard portions of the packaging to provide easier access. The presence or absence of the shrink wrap had very little effect, if any, on the efficacy of the package's insect resistance to the early instars of the Indian meal moth. The larger older larvae are active penetrators and must be treated quite differently. Sanitation is the first line of defense against infestation of food and feed packages particularly in preventing attacks by older moth larvae and beetles. Packages must be segregated as to their type of packaging, infestablity, duration of storage and proper storage rotation. This challenge shows the importance of odor in providing insect resistance to the packaging of food and feed. The presence of just one damaged wrapper was most assuredly a potent attractant for the entire case for the moth larvae in this challenge study.

CONCLUSIONS

We have presented only a portion of the problem of insect attack on and the infestation of food and feed packages. The problem of insect invaders has been elucidated by the challenge study presented here. However, we have presented little that will assist the researcher or the food processor and package engineer with the problem of insect penetrators. The use of pesticides was quite effective in repelling penetrators, but are now considered to be too dangerous. Evaluation of packaging materials is an area that has been quite well researched, but still offers some continuing promise (Highland 1984, Newton 1988). We still believe that a solution(s) resides in continued research into the behavior and ecology of these insects that bore into packages. Questions such as why do well-fed insects often bore into packages while starved insects will not? Is there an effect of age or sex that leads to when an insect will attack? What are the possible roles of food odors, pheromones, and insect density? We are continuing to seek answers to these and other questions and to encourage others to also investigate these questions.

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