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Suitability of Different Soybean Substrates and a Laboratory Diet on the Development of Indian Meal Moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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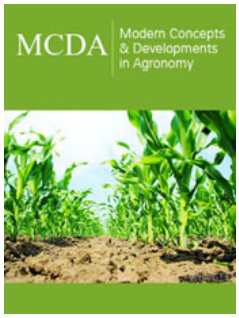

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Abstract

The Indian meal moth, *Plodia interpunctella* (Hübner), is an economically important pest that infests various food commodities. There have been anecdotal reports of infestation by *P. interpunctella* of US soybeans shipped overseas in containers. However, limited information is available on the development of *P. interpunctella* on soybean substrates. Two experiments were conducted to examine the development of *P. interpunctella* on whole soybeans, ground soybeans, and soybean meal, in comparison to a standard laboratory diet. The results from the first experiment showed that the laboratory diet was optimal for survival and development of *P. interpunctella*, while survival and development on whole soybeans was less optimal. The developmental time of *P. interpunctella* among the food substrates from first instars to adult emergence was completed within 25 to 40 days. In the first test with different food substrates, the number of larvae, pupae, and adults was highest on laboratory diet followed by soybean meal, and ground soybean, while no adult emergence was observed on whole soybeans. In the second experiment, infesting whole soybeans or laboratory diet with 100 *P. interpunctella* eggs showed that there was 7.3% adult emergence on whole soybeans compared to 25.8% adult emergence on laboratory diet. These experiments confirmed that soybean substrates are a suitable host for *P. interpunctella* infestation. The findings raise concerns regarding the potential risk of infestation of whole soybeans by *P. interpunctella*, which can lead to quality deterioration and economic impacts to the US soybean exports.

Keywords: Indian meal moth; Food substrate suitability; Survival of insects

Introduction

Soybeans, *Glycine max (L.)* Merril, occupy a prominent position as the primary globally cultivated legume crop that is used as human and animal food [1]. Between 2022 and 2023 period, the worldwide soybean production reached 370.4 million tons, with the United States contributing nearly 116.4 million tons [2]. However, several factors contribute to the deterioration of soybean quality during postharvest handling and storage, especially due to insects and microorganisms [3].

The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is an economically important insect pest that infests a wide range of food commodities, including cereal grains, legumes, nuts, fruits, candies, and grain-processing facilities [4]. The larvae are the destructive stage of this pest, which cause significant losses by feeding on commodities and leaving silken threads, rendering them unsuitable for consumption [5]. The larvae of *P. interpunctella* have been associated with over 177 commodities worldwide [4], and this pest has the ability to infest commodities in commercial shipping containers [6]. Anecdotal reports have indicated that overseas shipments of US soybeans in containers are being rejected due

to *P. interpunctella* infestations, as this insect pest is reported to be associated with soybeans [4].

Despite reports of *P. interpunctella* infestation on soybeans, there is limited information regarding the suitability of soybeans for the development of *P. interpunctella*. The larvae of *P. interpunctella* thrived well on soybean meal and cracked soybeans compared to whole soybeans [7]. The development of *P. interpunctella* on soybean meal and cracked soybeans was comparable from to that in corn meal, cracked corn, black-eyed peas, and rolled oats [7]. In their study, the actual number of eggs/larvae on different food substrates was not given. Additionally, a quotient was calculated based on log10 number of moths divided by minimum number of days for egg to adult development. The moth yield and developmental time were not provided making it difficult to interpret the quotient. The preference and development of *P. interpunctella* on 11 different hosts was evaluated in the laboratory by offering ground diets to laboratory and field collected moths [8]. This study revealed that ground soybeans were notably more conducive for the development of field moths of *P. interpunctella* compared to laboratory moths. In this study, development of *P. interpunctella* on different diets was conducted using individual first instars without competition from cohorts. The findings have raised concerns about potential *P. interpunctella* infestation issues on soybeans, highlighting the need for additional research to confirm these observations. In the present study, two separate experiments were performed. In the first experiment, we studied the suitability of whole soybeans, ground soybeans, and soybean meal compared with a standard laboratory diet for the survival and development of *P. interpunctella*. The purpose of the second experiment was to ascertain whether whole soybeans are a suitable diet for the development of *P. interpunctella*.

Materials and Methods

Insect rearing

Cultures of *P. interpunctella* utilized in the experiments originated from laboratory cultures that have been maintained since 1999 in the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA. These cultures were established on a laboratory diet comprising of 1 kg of poultry mash (16% layered crumbles, Orscheln Farm & Home, Missouri, USA), 150 ml of honey, 150 ml of glycerol, and 75 ml of distilled water [9,10]. Approximately 250 g of the laboratory diet was taken in 0.94 L glass mason jars and was seeded with *P. interpunctella* eggs after which the jars were closed with metal lids fitted with wire mesh screens (250 µm) and 9 cm diameter filter papers. The cultures were kept inside growth chambers (Model I-36 VL; Percival Scientific, Perry, Iowa, USA) maintained at a temperature of 28 °C, 65% relative humidity (r.h.), and a 14:10 light-dark photoperiod.

Collection of eggs

Adults (≤ 24-h-old) from the laboratory cultures were collected after anaesthetizing them with carbon dioxide, and gently transferred to 0.94 L glass mason jars secured with 925 µm metal mesh and lids. The jars with adults were inverted over 9 cm glass Petri dishes to collect the eggs.

Hatchability tests

The hatchability of the *P. interpunctella* eggs was determined by placing 100 freshly laid eggs in 9 cm glass Petri dishes with lids. Three such Petri dishes were maintained for the test, and all dishes were held inside the growth chamber at 28 °C and 65% r.h. and observed for egg hatching under a stereo microscope (Model SMZ 1000, Nikon Inc., New York, USA) daily for five days. The data on the number of eggs hatched was calculated and expressed as a percentage.

Suitability of food substrates

To investigate the impact of soybean substrates on the development of *P. interpunctella*, two separate experiments were undertaken. In the first experiment, we evaluated the suitability of three soybean substrates (cleaned whole soybeans, ground soybeans, and soybean meal) on the development of *P. interpunctella* compared to the standard laboratory diet. The second experiment was conducted as a follow-up to the first experiment because no adult emergence was observed on whole soybeans. In the first experiment, each diet (whole soybeans, soybean meal, ground soybeans, and laboratory diet) was tested separately to assess its suitability for the development of *P. interpunctella*. The soybean meal used in the study was obtained from the O.H. Kruse Feed Mill at Kansas State University. Whole soybeans were obtained from shipping containers located at Kansas State University's North campus in Manhattan, Kansas, USA, during 2022, specifically for the purpose of this study. Ground soybeans were prepared by grinding 300 g of cleaned whole soybeans using an Oster blender (Service number BLSTMB, assembled in Mexico), for a duration of 5 minutes. The moisture contents of whole soybeans, soybean meal, ground soybeans, and the laboratory diet were determined by the Association of Official Agricultural Chemists method number 930.15 [11]. A 10 g sample of each food substrate was dried at 135 °C for 2 h inside a hot air oven. Moisture contents were determined in triplicates for each food substrate. The moisture loss was then calculated on a wet basis and expressed as a percentage. The mean ± SE (n = 3) moisture contents of whole soybeans, soybean meal, ground soybeans, and the laboratory diet were 9.0 ± 0.0%, 11.2 ± 0.43%, 9.1 ± 0.4%, and 13.5 ± 0.9%, respectively.

Each food substrate (2 g) was taken in 30 ml clear plastic condiment cups with a top diameter of 4.19 cm, a bottom diameter of 2.92 cm, with a height of 2.9 cm. Twenty freshly laid (≤ 24-h-old) *P. interpunctella* eggs were added to each cup and covered with a plastic lid. Each lid was punctured with a small pin to make five holes to facilitate air diffusion. All cups were placed inside the growth chamber at 28 °C and 65% r.h. Since all eggs hatched on day 5 (time 0), condiment cups were first sampled at 5 day intervals for 45 days from time 0. Each food substrate and observation time was replicated four times. At each observation time, data were collected on the number of live larvae, pupae, and adults.

In the second experiment, *P. interpunctella* development was determined on whole soybeans and compared to development on laboratory diet. A total of 100 *P. interpunctella* eggs (≤ 24-h-old) were added to 0.47 L glass mason jars containing either 30 g

of cleaned whole soybeans or laboratory diet. There were 10 replications (jars) each for whole soybeans and the laboratory diet. Jars were closed with metal lids fitted with filter papers (7 cm diameter) and wire mesh screens (250 µm). All the glass jars after infestation with eggs were placed inside the growth chamber maintained at 28 °C and 65% r.h. After 42 days, data on the number of live larvae, pupae, and adults in each jar were counted.

Statistical Analysis

The data on insect counts on different soybean substrates in the first experiment were transformed to log10 (x+1) scale because some counts had zeros [12]. The data on number of larvae, pupae, or adult counts were subjected to two-way analysis of variance (ANOVA) to determine differences in insect counts among food substrates and among observation times. To determine differences among food substrates, insect count data by stage at each observation time were subjected to one-way ANOVA and means among substrates were separated using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test [13]. Although data for statistical analysis was performed on transformed scale, means ± SE presented in Table 1 are based on untransformed scale. Data (untransformed scale) on number of larvae, pupae, or adult counts on whole soybean and laboratory diet from the second experiment were subjected to two-sample t-tests to determine significant differences in larval, pupal, and adult numbers on whole soybeans and the laboratory diet. Statistical differences were considered significant at $\alpha = 0.05$.

Table 1: Mean ± SE number of larvae, pupae, and adults of *P. interpunctella* on four food substrates.

[a]Means for each stage and observation time followed by different letters are significantly different ($P < 0.05$; REGWQ test).

[b]No pupae or adults were observed at 5, 10, and 15 observation times.

[c]No live larvae were observed at 25, 30, 35, 40, and 45 observation times.

[d]No live larva or pupae were observed at 40 and 45 observation times.

Observation Time (Days)	Food Substrates	Mean ± SE (n = 4) number of [a]:		
		Larvae	Pupae	Adults
5[b]	Laboratory diet	8.75 ± 1.3 a		
	Whole soybeans	0.8 ± 0.5 b		
	Soybean meal	3 ± 1.5 ab		
	Ground soybeans	1.5 ± 1.0 b		
10[b]	Laboratory diet	13.75 ± 2.7 a		
	Whole soybeans	0 ± 0 c		
	Soybean meal	3.75 ± 1.3 b		
	Ground soybeans	1.5 ± 1.0 bc		
15[b]	Laboratory diet	11.75 ± 1.8 a		
	Whole soybeans	0 ± 0 c		
	Soybean meal	2.25 ± 0.9 b		
	Ground soybeans	1.25 ± 0.48 bc		
20	Laboratory diet	7.75 ± 1.0 a	5.25 ± 0.6	0 ± 0
	Whole soybeans	0 ± 0 b	0 ± 0	0 ± 0
	Soybean meal	1.75 ± 0.6 b	0 ± 0	1.8 ± 1.0
	Ground soybeans	1.5 ± 1.0 b	0 ± 0	0 ± 0
25[c]	Laboratory diet		5.0 ± 2.0	10.8 ± 3.6 a
	Whole soybeans		0 ± 0	0 ± 0 b
	Soybean meal		0 ± 0	3.3 ± 1.1 ab
	Ground soybeans		0 ± 0	1.3 ± 0.8 b
30[c]	Laboratory diet		7.8 ± 1.4 a	7.5 ± 2.7 a
	Whole soybeans		0.5 ± 0.5 b	0 ± 0 b
	Soybean meal		1.3 ± 0.5 b	3.3 ± 1.1 ab
	Ground soybeans		1.8 ± 0.6 b	0 ± 0 b
35[c]	Laboratory diet		1.5 ± 0.6	12.3 ± 1.1 a
	Whole soybeans		0 ± 0	0 ± 0 c
	Soybean meal		1.5 ± 1.0	4.8 ± 0.6 b
	Ground soybeans		0.8 ± 0.8	3.0 ± 1.1 b

40[c,d]	Laboratory diet			14.5 ± 0.9 a
	Whole soybeans			0 ± 0 c
	Soybean meal			4.8 ± 0.5 b
	Ground soybeans			2.5 ± 0.9 b
45[c,d]	Laboratory diet			13.8 ± 1.1 a
	Whole soybeans			0 ± 0 c
	Soybean meal			5.8 ± 2.3 ab
	Ground soybeans			1.5 ± 1.0 bc

Results

The egg hatchability tests showed that no eggs hatched after day 1. All eggs hatched on day 5. The cumulative mean ± SE percent hatchability on days 2, 3, 4, and 5 were 43.0 ± 4.5%, 67.7 ± 3.5%, 81.0 ± 3.6%, and 91.3 ± 5.3%, respectively.

In the first experiment, the laboratory diet was found to be more suitable for development of *P. interpunctella* with highest number of larvae surviving followed by soybean meal, ground soybeans, and whole soybeans (Table 1). Larvae were observed on all food substrates in condiment cups on day 5. Larvae were not observed on whole soybeans at 10, 15 and 20 day observation times. No larvae were observed in any of the food substrates between 25 and 45 day observation times. Two-way ANOVA showed that the number of larvae varied among the four diets ($F = 43.43$; df = 3, 108; $P < 0.0001$) and among observation times ($F = 33.71$; df = 8, 108; $P < 0.0001$). The interaction effect of diet and observation time was also significant ($F = 7.10$; df = 24, 108; $P < 0.0001$), suggesting that the number of larvae on the diets over time were not consistent. One-way ANOVA consistently showed that the number of larvae was significantly higher ($P < 0.05$) on the laboratory diet compared to soybean food substrates at all observation times.

The first pupae were formed only on the laboratory diet at the 20 day observation time (Table 1). Pupae were observed on all food substrates at 30 and 35 day observation times, except for whole soybeans where no pupae were observed at the 35 day observation time. Two-way ANOVA showed that both the main effects of diet ($F = 41.29$; df = 3, 108; $P < 0.0001$) and observation time ($F = 24.34$; df = 8, 108; $P < 0.0001$), and the interaction effects of diet and observation time ($F = 8.69$; df = 24, 108; $P < 0.0001$) for number of pupae were all significant. One-way ANOVA showed that the number of pupae were significantly higher ($P < 0.05$) on laboratory diet at 20, 25, and 30 day observation times compared to the soybean food substrates, except at 35 day observation time, where the pupal numbers among substrates were not significantly different from one another ($F = 1.37$; df = 3, 12; $P = 0.301$).

After 20 days, adult emergence was only noticed on soybean meal diet (Table 1). Adult emergence was observed on all food substrates at 25, 30, 35, 40, and 45 day observation times, except for whole soybeans where no adult emergence was noticed. Two-way ANOVA showed that both the main effects of diet ($F = 50.05$; df = 3, 108; $P < 0.0001$), observation time ($F = 25.31$; df = 8, 108; $P < 0.0001$), and the interaction effect of diet and observation time ($F = 5.31$; df = 24, 108; $P < 0.0001$) for number of adults emerged were all significant. One-way ANOVA showed that the adult emergence was significantly higher ($P < 0.05$) on the laboratory diet at 25, 30, 35, 40, and 45 observation times compared to the soybean food substrates.

In the second experiment, the laboratory diet was found to be more suitable for development of *P. interpunctella* with the highest number of larvae surviving compared to whole soybeans (Table 2). The two-sample t-tests showed that the larval or pupal numbers on whole soybeans and the laboratory diet were not significantly different from one another ($P > 0.05$). The mean number of adults emerging from the laboratory diet was significantly higher ($P < 0.05$) compared to whole soybeans.

Table 2: Mean ± SE number of larvae, pupae, and adults of *P. interpunctella* on whole soybeans and the laboratory diet.

[a]Variances for larval numbers on whole soybeans and the laboratory diet were equal ($F = 1.03$; df = 9, 9; $P = 0.9697$).

[b]Variances for pupal numbers on whole soybeans and the laboratory diet were equal ($F = 3.39$; df = 9, 9; $P = 0.0832$).

[c]Variances for adult numbers on whole soybeans and the laboratory diet were unequal ($F = 6.07$; df = 9, 9; $P = 0.0130$).

*Significant ($P < 0.05$; two-sample t-test)

Insect Stage	Whole Soybeans	Laboratory Diet	t-value (df)	P-value
Larvae[a]	7.0 ± 1.2	5.4 ± 1.2	0.91 (18.0)	0.3733
Pupae[b]	5.8 ± 1.6	3.5 ± 0.9	1.28 (18.0)	0.2179
Adults[c]	7.3 ± 2.8	25.8 ± 2.1	8.00 (11.9)	< 0.0001*

Discussion

The mean cumulative hatchability of *P. interpunctella* eggs in our study was 91.3% after 5 days. The egg hatchability of *P. interpunctella* ranged between 90 and 100% after 4 to 5 days [10, 14]. It is obvious from the present study results that the laboratory diet demonstrated a high level of suitability for the survival and development of *P. interpunctella*, as evidenced by higher larval survival compared to the soybean substrates in both

the experiments. This result is expected because the laboratory cultures of *P. interpunctella* have been reared on a poultry mash-based diet for many years, leading to their adaptation and better performance on the laboratory diet. In the first experiment, no larvae were observed at 25, 30, 35, 40, and 45 observation times on any of the food substrates. These results suggested that the hatched larvae either developed into pupae or successfully transformed into adults, or they did not survive on food substrates due to their poor suitability for larval feeding and development, which was mostly observed on whole soybeans. Similar findings were reported by LeCato [7], who observed that whole soybeans were the least suitable diet for *P. interpunctella* development compared to soybean meal and cracked soybeans. Sambaraju and Phillips [8] observed 100% larval survival on a laboratory diet consisting of cornmeal, chick starter, egg crumbles and glycerol (4:2:2:1 ratio) compared to 80% survival observed on ground soybeans. They also found that the laboratory strains survived and developed better on ground soybeans than the field populations. It is important to note that the survival rate of *P. interpunctella* larvae on ground soybeans in their study cannot be directly compared with the current investigation. This disparity arises from the fact that they individually infested food substrates with first instars, without any competition among larvae. In contrast, our study involved infesting all food substrates with 20 *P. interpunctella* eggs, possibly leading to increased competition and subsequently lower survival rates on ground soybeans.

In our study, adult emergence was observed on soybean meal after 20 days, while on other diets, adult emergence occurred after 25 days, except for whole soybeans, where no adult emergence was observed. This was in agreement with earlier reports indicating the development period of first instars to adult emergence was around 25 to 26 days [14, 15]. However, the number of adults that emerged on the soybean meal and ground soybean diets was lower than that on the laboratory diet. Infesting whole soybeans with 100 eggs of *P. interpunctella* in the second experiment showed a reasonable number of adult emergence from hatched eggs after 42 days. However, the number of *P. interpunctella* adults that emerged from eggs was significantly lower compared to emergence on the laboratory diet.

In summary, the laboratory diet was most suitable for survival and development of *P. interpunctella*. LeCato [7] suggested that fine particulate foods may be preferable for the growth and development of this moth species, especially for first instars. However, our study revealed low survival and development of *P. interpunctella* on whole soybeans. Therefore, the risk of quality deterioration and economic impacts to the US soybean export cannot be overlooked due to *P. interpunctella* infestations. Future research should focus on investigating the preference of field populations of *P. interpunctella* to whole soybeans and draw conclusive insights into soybean suitability as a substrate for *P. interpunctella* growth and development.

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