# MODELING HYDROPRENE EFFECTS ON EGGS AND 5$^{th}$ INSTAR WANDERING PHASE LARVAE OF THE INDIANMEAL MOTH, *PLODIA INTERPUNCTELLA* (LEPIDOPTERA: PYRALIDAE)

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## ABSTRACT

The control of Indianmeal moth [*Plodia interpunctella* (Hübner)], a commonly found serious stored product pest around the world, relies mainly upon chemical control methods. Because of recent changes in the laws and regulations governing pesticide usage in the United States, there is an increasing need for finding safer chemicals to control insect pests. Hydroprene, an insect growth regulator, is considered to be a safe alternative. In this study, I quantified the effects of hydroprene on two critical life stages of Indianmeal moth, the eggs and 5$^{th}$ instar wandering phase larvae. Maximum development time in the untreated controls was 13.6 ± 0.6 d at 16°C and minimum development time was 2.3 ± 0.4 d at 32°C. At 20°C and 24°C, the effect of hydroprene on egg development became more evident; development time generally increased with exposure interval, with some variability in the data. The mean egg mortality among all temperatures was 7.3 ± 4.6%. Among the treatments, mortality of eggs increased as the exposure periods increased within any given temperature, with a dramatic increase in mortality with increase in temperature. Egg mortality was lowest at 16°C when exposed for 1 h (0 ± 3%), but mortality gradually increased up to 32 ± 3% when exposed for 18 h. Within each exposure interval, there was a direct increase in mortality as the temperatures increased. For the 5$^{th}$ instar wandering phase larvae, the longest development time among the treatments of 47.2 ± 1.3 d occurred at 16ºC when the larvae were exposed for 30 h, whereas the shortest development time of 7.0 ± 0.5 d occurred when the larvae were exposed for 1 h at 32ºC. Among treatments, the greatest larval mortality (82.0 ± 0.1%) occurred when larvae were exposed for 30 h at 28ºC, while the minimum mortality of 0.0 ± 0.5% occurred at 16ºC when larvae were exposed for 1 h. Response-surface models derived from this study can be used in simulation models to estimate the potential consequences of hydroprene on Indianmeal moth population dynamics.

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## DEDICATION

To My Grand Father Raghava Iyengar, A Gentle Man Who Along With Many Others Stood Up

Against The Colonial British For India's freedom;

Taught Me The Values of Honesty, Truthfulness And Dignity.

And to,

My School Teacher, Mr. Amalraj Who Taught Me English.

# CHAPTER 1

# INTRODUCTION AND RESEARCH OBJECTIVES

## Introduction

The Indianmeal moth, *Plodia interpunctella* (Hübner), is an insect pest with a worldwide distribution that infests stored-products (Hinton, 1943). Richards and Thomson (1932) and Deso (1976) reported that there are 83 or more kinds of food that Indianmeal moth can feed upon. They include raw grains, grain products, cornmeal, oatmeal, dried fruits, dried nuts, seeds, cocoa, chocolate, powdered milk, processed foods, pasta, cookies, crackers, biscuits, bread meal, breakfast food, pet foods, bird seed, materials of vegetable origin, and dried insects (Strumpel, 1969; Stehr, 1987; Nansen and Philips, 2003; Welch, 2004). Indianmeal moth is an external feeder whose larvae continuously spin a silken web both inside and on top of the food surface, and feed within the web. The webbing consists of larval frass material and cast skin, and gives an unpleasant odor to the infested commodity. The infested commodity is often covered on the surface with a thick mat of silken webbing, making it difficult to effectively apply insecticide treatments (Smith, 2000). There is no direct economic assessment of the damage caused by Indianmeal moth but it is commonly believed that losses due to this pest could be in millions of dollars every year in the United States alone. Loss due to food quality degradation, in addition to the weight loss of a commodity, is also a serious concern with Indianmeal moth damage.

Indianmeal moth has been known as a serious pest for more than a century. Holland (1903) in The Moth Book, A Guide to North American Moths, described Indianmeal moth in the following words, “There is nothing which seems to come amiss to its appetite, and it is, when established in a house or a store-room, a veritable nuisance, …, and the reader of these lines will do well to remember that if the thing has established itself under his roof, it will require industry, patience, and great regard to cleanliness and order to get rid of it”.

Today, Indianmeal moth continues to be a pest of the same status, if not worse, in many parts of the world. A re-evaluation program for traditional pesticides, initiated by the United

States Environmental Protection Agency (USEPA), has triggered a search for alternative control methods for many insect pests, including Indianmeal moth. In addition, a general demand for reduced pesticide use and higher food quality is prevalent among the general public, increasing the demand for testing newer management strategies in post-harvest pest control (Phillips et al., 2000)

Many of the management strategies mostly rely upon the use of conventional chemical sprays and/or fumigation. Such management practices have been shown effective in many situations however, due to an increasing awareness of risk to human health and environmental safety, a greater scope for evaluation of safer management methods for all stored product pests including Indianmeal moth is prevalent. The use of insect growth regulators as an alternative to conventional pesticides has long been tried for managing stored product pests.

One such insect growth regulator hydroprene, a juvenoid hormone analog recommended for control against cockroach (Bennet *et al.,* 1986; King & Bennett 1988) was tested on the last instar larvae of red flour beetle, *Tribolium castaneum* Jacquelin duVal and confused flour beetle, *Tribololium confusum* (Herbst) (Arthur 2000). Demonstrations of the feasibility of hydroprene as a control option for various lepidopteran pests are widely available. Morphological disorders (Mathai & Nair 1990; Vijayalakshmi & Ramaraj 1991), prolongation of stage specific developmental times (Bell & Edwards 1999; Chakravorty *et al.,* 1990), adult sterility (Bell & Edwards 1999; Nair & Muraleedharan 1998) and reduced fecundity (Gelbic & Matolin 1984; Mathai & Nair 1990) as a result of hydroprene application are commonly reported in these studies. Ever since hydroprene was registered for the management of stored products pests in the 1990's, it has commonly been used as a surface spray insecticide in storage facilities mostly as part of a combined management strategy towards control of multiple stored grain pests. However, there are no published reports on effects Indianmeal moth populations as a result of

hydroprene application. In addition, a critical evaluation of the effects of hydroprene on the life stages of any of the stored products pests except for red flour and confused flour beetles (Arthur 2000) has not been done before.

Studying the effects of hydroprene on the critical life stages of Indianmeal moth will help in their proper management. Since hydroprene is commonly used as a surface treatment insecticide, the critical life stages of Indianmeal moth that are at risk due to spraying of hydroprene are the eggs, wandering phase fifth instar larvae, pupae and the adults. In this study, I quantified the effects of hydroprene on the hatch of Indianmeal moth eggs, and the development rate and mortality of wandering phase fifth instar larvae.

**Research Objectives and Hypotheses**

Research Objectives:

1. To determine the effects (development time and mortality) applying labeled rate of hydroprene to the eggs of Indianmeal moth exposed to various temperature and exposure period combinations
2. To statistically derive models for Indianmeal moth egg development time and mortality when exposed to the labeled rate of hydroprene
3. To determine the effects (development time and mortality) of applying labeled rate of hydroprene to the $5^{th}$ instar wandering phase larvae of Indianmeal moth exposed to various exposure period combinations
4. to statistically derive models for the $5^{th}$ instar indianmela moth development time and mortality when exposed tot the labeled rate of hydroprene

Research Hypothesis:

$H_{01}$: Hydroprene has no effect on the development time of Indianmeal moth eggs exposed to various temperature and exposure period combinations

$H_{a1}$: Hydroprene causes significant changes to the egg development time when exposed at various temperature and exposure period combinations.

$H_{02}$: Hydroprene has no effect on the mortality of Indianmeal moth eggs exposed to various temperature and exposure period combinations.

$H_{a2}$: Hydroprene causes significant mortality to $5^{th}$ instar Indianmeal moth larvae when exposed to various temperature and exposure period combinations.

$H_{01}$: Hydroprene has no effect on the development time of $5^{th}$ instar Indianmeal moth larvae exposed to various temperature and exposure period combinations.

$H_{a1}$: Hydroprene causes significant changes to the $5^{th}$ instar Indianmeal moth larval development time when exposed at various temperature and exposure period combinations.

$H_{02}$: Hydroprene has no effect on the mortality of $5^{th}$ instar Indianmeal moth larvae exposed to various temperature and exposure period combinations.

$H_{a2}$: Hydroprene causes significant mortality to $5^{th}$ instar Indianmeal moth larvave when exposed to various temperature and exposure period combinations.

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# CHAPTER 2

# HYDROPRENE PROLONGS DEVELOPMENT TIME AND INCREASES MORTALITY OF INDIANMEAL MOTH (LEPIDOPTERA: PYRALIDAE) EGGS

**Abstract**

Eggs of the Indianmeal moth, *Plodia interpunctella* (Hübner), were exposed to the labeled rate of hydroprene ($1.9 \times 10^{-3}$ mg[AI]/ $cm^2$) sprayed on concreted petri dishes. These eggs were exposed for 1, 3, 6, 12, and 18 h and until hatching (continuous exposure) at temperatures of 16, 20, 24, 28, and 32ºC and 57% relative humidity until the emergence of 1st instars. The development time and egg mortality were significantly influenced by temperature and exposure periods. At 16ºC, hydroprene did not cause differences in development time when eggs were exposed for different periods. At temperatures above 16ºC, both exposure period and temperature influenced development time. The maximum development time ($15.0 \pm 0.2$ d) occurred at 16ºC, and the minimum development time ($3.2 \pm 0.3$ d) occurred at 32ºC. Mortality increased when eggs were exposed to hydroprene for longer periods of time at all of the five tested temperatures. The greatest mortality ($81.6 \pm 2.1$%) occurred when eggs were continuously exposed on treated surfaces at 32ºC. I used development time instead of rate (1/development time) to fit simple linear or polynomial regression models to the development data. Appropriate models for development time and mortality were chosen based upon lack-of-fit tests. The regression models can be used in predictive simulation models for the population dynamics of Indianmeal moth to aid in optimizing use of hydroprene for insect management.

**KEY WORDS** *Plodia interpunctella*, insect growth regulator, surface treatment, population dynamics

The Indianmeal moth, *Plodia interpunctella* (Hübner), is a cosmopolitan pest of raw stored commodities and of packaged and processed food (Cox and Bell 1991). Currently, management of Indianmeal moth often depends upon the use of conventional insecticides. Hydroprene, a juvenile hormone analogue, is considered to be an alternative to conventional insecticides because of its specific activity against immature insect stages, low persistence in the environment, and non-toxic effects on mammals. Most early tests with hydroprene were conducted against stored-product beetles (Loschiavo 1975, McGregor and Kramer 1975, Amos et al. 1977, Rup and Chopra 1984, Shanthy et al. 1995). Hydroprene completely suppressed adult emergence of almond moth, *Cadra cautella* (Walker), formerly *Ephestia cautella* (Walker), when inshell peanuts were sprayed at 5 ppm concentration (Nickle 1979). More recently, Arbogast et al. (2000) showed reduction of insect populations of several stored-product insects, including the Indianmeal moth, when hydroprene was applied as a surface spray treatment in a retail store.

There are several reports of the effects of hydroprene on the eggs of different lepidopteran field crop pests. However, most of these studies describe indirect effects of hydroprene on eggs developing within the adult female. Inhibition of egg maturation, variations in the compound egg chambers in the ovarioles, and sterility have been reported for castor semilooper, *Achaea janata* (L.) exposed to hydroprene (Nair and Muraleedharan 1992, 1998). Hydroprene caused oocyte resorption in *Leptocoris coimbatorensis* (Kaur et al. 1987). Chakravorty et al. (1989) showed abnormalities in the growth and differentiation of gonads and reduction in egg and sperm maturation in three lepidopteran species: rice moth, *Corcyra cephalonica* (Stainton); angled gem, *Anomis sabulifera* (Guenée); and crimson-speckled moth, *Utetheisa pulchella* (L.), when hydroprene was applied to the food of immature stages.

There are few published studies regarding the direct effects of hydroprene on the eggs of stored-product insects that are developing externally during the post-oviposition period. In addition, most studies were conducted by administering hydroprene into the diet of insects or by topical application. One study, Bhargava and Urs (1993), reported reduction in hatching percentage of eggs of rice moth, belonging to different age groups, as a result of hydroprene application. Under natural storage circumstances in warehouses and retail environments, hydroprene is applied on the surface as either an aerosol or spray treatment, and there are no data regarding direct effects when the eggs are exposed on a treated surface. The objective of this study was to quantify the effect of hydroprene as a surface treatment on the eggs of Indianmeal moth, including the effects of temperature and exposure time on egg development time and mortality.

## Materials and Methods

**Experimental Design.** The experiment was designed as a split-plot structure (Kuehl 2000), with incubators as the whole plot and concreted petri dishes as subplot experimental units. Five temperatures (16, 20, 24, 28, and 32ºC) were randomly assigned to whole plots and six exposure intervals (1, 3, 6, 12, and 18 h and a continuous exposure period) randomly assigned to the subplots (30 treatment combinations of temperature and exposure intervals). The continuous exposure mimicked conditions that would normally occur when eggs were laid on a treated surface, and the timed exposure intervals were selected to quantify the effects of time as a dosage factor. Five incubators (ThermoForma®, Marietta, OH), one for each temperature, were used in the study. Humidity chambers were created inside plastic containers (26 by 36.5 by 15 cm) with a waffle-type plastic grid in the bottom. A saturated NaBr solution was used to maintain 57% relative humidity (RH) inside each plastic container (Greenspan 1977), and two containers were

used for each incubator. I used 57% RH, which is approximately the same humidity condition found in stored product environments. Daily temperature and humidity inside the individual incubators were monitored by placing a HOBO® (Onset Computer Corporation, Bourne, MA) inside the humidity containers. Humidity was uniform across all of the whole and subplot treatments and therefore was not considered as part of the treatment design.

**Insects.** Indianmeal moth eggs were obtained from insecticide-susceptible laboratory cultures reared on a laboratory standard diet mixture of cracked wheat, wheat bran, wheat germ, honey, glycerin, yeast, sorbic acid, benzoic acid and water. The laboratory strain is a mixture of several field-collected strains and has been maintained for approximately 5-7 yr at the Grain Marketing and Production Research Center, Manhattan, Kansas. All cultures are held inside incubators set at 27ºC and 60% RH. Indianmeal moth eggs were collected by placing fifty adult mating pairs in a 0.95 liter glass jar with a screened lid, and inverting the jar on a piece of black filter paper set inside a 62 $cm^2$ petri dish. The dish was placed inside an incubator set at 27ºC and 60% RH. The following day, eggs that were 18-24 h old were collected and were transferred to a new piece of black filter paper. A camel's hair paint brush was used to transfer 60-70 eggs to each randomly chosen treated and untreated concrete arena. The final number of eggs per dish was 50; some of the initial 60-70 eggs were lost during transfer and the remainder was removed by using a camel's hair brush. Eggs were then exposed for the required interval, removed and held until the eggs hatched. Randomization for subplot treatments was done by randomly selecting a concrete arena for each exposure period. The treated arenas along with eggs were placed inside a humidity container inside individual temperature incubators, and the untreated controls were held inside the second container. Upon completion of the exposure interval, the eggs were removed and placed on top of sterilized filter paper inside individual, pesticide-free petri dishes, and placed back in the same humidity chambers. Every eight hours, each arena was placed under a

dissecting microscope to count the number of emerging larvae. The larvae were usually found within the food media. Eggs in the treatments, including continuous exposure period were recorded as dead if they had not hatched after forty days.

**Experimental Arenas and Hydroprene Formulation.** Hydroprene is registered as an aerosol fog, surface spray, or as an impregnated disc (Arthur 2003). In our study, hydroprene was applied as a surface treatment on concrete because this is a common floor surface in warehouses, some retail stores, and other storage facilities throughout much of North America. Concrete (Rockite®, Hartline Products Co., Cleveland, OH) was mixed in an approximate ratio of 3,200 g of concrete in 1,600 ml of water to a thick running consistency (Arthur 1999). The liquid slurry was then poured into individual petri dishes (62 $cm^2$), to approximately half the capacity of the dish. A total of 90 dishes were created in this manner, and then dried for about 48 h at room temperature (27ºC).

The hydroprene formulation used in the study was made with Gentrol® (9.0% active ingredient [AI], approximately 90 mg[AI]/ml). Label directions specify application by mixing 1 oz in 1 gallon of water to cover 1500 $ft^2$ (29.57 ml in 3.79 liters of water to cover 134.8 $m^2$), which is 1.9 X $10^{-3}$ mg[AI]/$cm^2$. The area of the concrete petri dish was 62 $cm^2$, so the volume of spray needed for this area was 0.17 ml. However, this amount was too small to formulate individual concentrations. Therefore, I prepared the hydroprene concentrations by mixing 0.38 ml of Gentrol® in 50 ml of distilled water, thoroughly shaking the solution, and removing individual 0.17 ml aliquots for each petri dish; these individual solutions were sprayed on the concrete arenas using an artist's airbrush (Badger® No.100 LG., Franklin Park, IL). The liquid was sprayed by holding the airbrush approximately 5 to 10 cm above the treatment arenas and by slowly releasing pressure until all of the material was dispensed. Twenty five dishes were treated in this manner. In order to avoid cannibalism among the emerging larvae after transferring them

to new dishes upon completion of required exposure periods, about 5 ml of standard larval media was provided in each new dish. Eggs on the continuous exposure period were not transferred to new petri dishes but left on the same dish. Therefore, for the five concreted petri dishes that were used for the continuous exposure period, I sprayed by blocking the 1 $cm^2$ central area of the dish. The food in these petri dishes was put in the center that was blocked while spraying to avoid contamination by hydroprene. These 30 dishes comprised a replicate, and another 30 companion concreted dishes were sprayed with same volume of distilled water for the untreated control treatment. In subsequent trials, two treated replicates were created as described above, along with an untreated control, for a total of five replications.

**Data Analysis.** Kramer et al. (1991) showed that erroneous predictions could occur in least square estimations when model parameters are estimated by using some modified form of data, such as rate (1/development time). Minimizing the squared error for development rate is not the same as minimizing the squared error for development time, especially in the longer development time range. Therefore, in this study, I used time instead of rate to fit all our regression models for egg development time. The effects of hydroprene on the egg development time and mortality were modeled by fitting individual, three dimensional (3D) response surface models by using temperature and exposure intervals as predictor variables. These models showed no significant cross-product (or) interaction effect between temperature and exposure period for both development time and mortality (Table 1). Such 3D models, especially when they are static and presented in black and white colors are difficult to interpret (Merwin et al. 1994), and offer less quantitative information to a scientific reader than two dimensional (2D) graphs. Wickens et al. (1994; 1996) found no significant difference in the perception of information by human subjects when presented with 2D and 3D displays of biological data. Therefore, for our data, two kinds of plots showing comparisons for mortality and development time were plotted; the effect

of exposure interval within a given temperature, and the effect of temperature within a given exposure interval. Simple linear and polynomial model fitting were done using the Linear Modeling procedure (Chambers 1992) in S-Plus® (Version 5.1 for Sun SPARC, SunOS 5.5, Insightful Corporation, Seattle, WA).

The regression models for development time and mortality were chosen based upon lack-of-fit-tests, but not $R^2$ or adjusted $R^2$ values, which are traditionally considered to be standards for model selection. As this is a designed experiment and the observations are derived from replicated units, it was possible to conduct lack-of-fit tests by partitioning the residual sum of squares into lack of fit and pure error components (Weisberg 1985). This involved determining the part of the residual sum of squares that can be predicted by including additional terms for the predictor variables in the model, like higher order polynomial terms, and the part of the residual sum of squares that cannot be predicted by any additional terms, i.e., the sum of squares for pure error. A test of lack-of-fit for the model without the additional terms was then performed, using the mean square pure error as the error term. This provided a sensitive test of model fit because the effects of the additional higher order terms were removed from the error. Care was taken to fit models that not only described the data adequately but also that were more biologically reasonable (Throne 1994, Faraway 1999).

Thus, I selected appropriate models for individual data sets by computing comparisons made between the desired and saturated models with higher order polynomial terms by the way of F-testing methodology (Faraway 1999). Influential observations in the dataset were checked for by using Cooks distance plots. Non-constant variance (heteroscedascity), and nonlinearity were checked by plotting residuals for the selected models (Faraway 1994). The strengths of the regression relationships were measured by their adjusted $R^2$ values (Seber 1977), and 95%

confidence intervals on the mean and prediction intervals were plotted for individual equations (Becker et al. 1988, Murrell 1999).

## Results

**Development Time.** The number of days required for eggs to hatch in the untreated controls and in the treatments generally decreased as the temperature increased (Fig. 1, Table 2). Maximum development time in the untreated controls was 13.6 ± 0.6 d at 16°C and minimum development time was 2.3 ± 0.4 d at 32°C. At 16°C, there was no relationship between egg developmental time and exposure interval ($F$ = 0.87; df = 1, 23; $P$ = 0.35) (Fig. 2A), and the average development time was 14.35 ± 0.3 d. At 20°C and 24°C, the effect of hydroprene on egg development became more evident; development time generally increased with exposure interval, with some variability in the data. (Figs. 2B, C). Linear models fit the data at 20°C and 24°C (Table 2). The development time increased at 28 and 32°C as exposure intervals increased; however, this increase was more gradual than at the lower temperatures and tended to level at the highest exposure intervals (Fig. 2D, E). Thus, quadratic models were fit to the data (Table 2).

At each exposure interval, the development time decreased as the temperatures increased (Fig. 3, Table 2). At 1, 3 and 6 h, the development time ranged from 3 to 15 d, and quadratic models were fit to the data (Figs. 3A, B, C, respectively). At 12 and 18 h exposures and continuous exposure, development time ranged from 5 to 15 d, but the effects of temperature were more gradual, and quadratic models fit the data. (Figs 3D, E, F. Table 2).

**Mortality**. Although mortality appeared to increase as the temperatures increased, there was no significant relationship between egg mortality and temperature in the untreated controls ($F$ = 2.95; df = 1, 73; $P$ = 0.08) (Fig. 4). The mean mortality among all temperatures was 7.3 ± 4.6%. Among the treatments, mortality of eggs increased as the exposure periods increased

within any given temperature, with a dramatic increase in mortality with increase in temperature (Fig. 5A-E, Table 3). The mortality was lowest at 16°C when exposed for 1 h (0 ± 3%), but mortality gradually increased up to 32 ± 3% when exposed for 18 h. A quadratic model was fit to the data at 16 and 20ºC. Linear equations were fit to the data for 24, 28, and 32°C.

Within each exposure interval, there was a direct increase in mortality as the temperatures increased (Figs. 6A-E). Quadratic regressions were fit to the data at 1, 3, 6, and 12 h (Table 3). At the 18 h exposure interval, there was a sharper increase in mortality with each successive increase in temperature, to a maximum of 78 ± 7.5% at 32ºC (Fig. 4E), and the data were described by a linear equation. When the eggs were continuously exposed to hydroprene, mortality also increased with increase in temperature (Fig. 6F). A cubic model fit the data.

**Discussion**

The lower development threshold temperature for two strains of Indianmeal moth eggs, one wild-type and another laboratory-reared strain, were reported as 14.8°C (Johnson et al. 1995). Indianmeal moths reared below 14.8°C diapause in the pupal stage. Therefore, the lowest temperature used in our study was 16°C to avoid inducing diapause. Hydroprene did not have a significant effect on the development time of eggs at 16°C; however, hydroprene caused significant mortality (up to 33 ± 3%) at this temperature. As the temperature increased, there was a steady increase in the delaying effect of hydroprene, suggestive of increased hydroprene activity at increasing temperatures above 16°C. Low volatility, binding to the concrete surface, and/or low penetration rate across the eggshell at low temperatures could have contributed to less development effect and mortality at 16°C. The results suggest that hydroprene is most effective against the eggs of Indianmeal moth at temperatures above 20°C.

Bhargava and Urs (1993) reported mortality effects on the eggs of rice moth exposed to various doses of hydroprene and other growth regulators. Among the three age groups of eggs that they exposed to hydroprene, the hatching percentage was highly reduced in the freshly laid eggs (0-12 h old) compared to older eggs. Their tests were conducted on eggs that were kept on laboratory media. In the USA, hydroprene is not labeled for direct use on stored food and, therefore, there is less chance for the eggs that are developing inside the food material to contact hydroprene, unless hydroprene is applied as an aerosol fog. The results described by Bhargava and Urs (1993) might not be directly applicable to the storage conditions in the USA.

Under storage conditions used in the USA, it is possible that the eggs may be laid on surfaces treated with hydroprene. Adult Indianmeal moths lay their eggs on the food or near the vicinity of food sources. Mullen and Arbogast (1977) showed that oviposition was stimulated by the presence of food materials in two stored-product moths, Indianmeal moth and the almond moth. Several other studies that followed this described the oviposition behavior of Indianmeal moth by conducting choice tests of egg laying preferences of female moths on various food media (Mbata 1990, Phillips and Strand 1994, Nansen and Phillips 2003). Phillips and Strand (1994) found that female Indianmeal moths orient towards food odor, and laid more eggs on dishes containing food media and those contaminated with larval secretions. The proportion of eggs laid on non-food surfaces, when food was inaccessible, is not evident from these studies. Recently, in a study conducted under a simulated warehouse set up with controlled environmental conditions, adult Indianmeal moths walked on the warehouse floors and laid their eggs on the surfaces or inside the containers provided with different stored-product commodities (Silhacek et al. 2003). In that study, fewer eggs were laid directly on commodities and more were laid on the surfaces of containers. In a simulated food product environment, when eggs of the three species of stored-products pests, *Tribolium castaneum*, *T. confusum*, and *C. cautella*,

were placed in open and closed food media and hydroprene was applied as a surface spray, the insects showed lengthened larval development and sterility in adults (Bell and Edwards 1999). In the absence of food or when the food is inaccessible due to packaging or due to other barriers, the female Indianmeal moths may lay their eggs within the vicinity of food sources so emerging larvae can crawl towards the commodity. Therefore, there is a possibility for eggs to contact surfaces sprayed with hydroprene, although the actual proportion is not clearly known.

Insect resistance to juvenile hormone analogs was once thought to be impossible (Williams 1967). Today, reports of resistance to compounds that are similar to hydroprene are not uncommon. Mosquito resistance to methoprene is widely known (Dame et al. 1998; Cornel et al. 2000, 2002). Cornel et al. (2002) reported a several thousand fold increase in $LC_{50}$ and $LC_{90}$ tolerance levels for the Fresno strain of the mosquito, *Ochlerotatus nigromaculis* (Ludlow), and control strategies involving methoprene were discontinued to prevent further resistance development. Whitefly species have developed resistance to pyriproxifen, another insect growth regulator (Horowitz and Ishaaya 1994, Horowitz et al. 2002). One possible method to delay the development of resistance by Indianmeal moth towards hydroprene is by timing to target the application towards the vulnerable life stages of Indianmeal moth, including the egg stage.

Hydroprene is the only insect growth regulator that is registered for spot and crack-and-crevice treatment for stored-product pest management in the USA. To our knowledge, this is the first published report regarding the effect of hydroprene on the eggs of Indianmeal moth developing on a concrete surface. The results of this study show that hydroprene can be used to control the egg stage of Indianmeal moth when applied as a surface treatment. Other insect growth regulators may also possess similar properties and should be tested for Indianmeal moth management as well. The long-term influence of hydroprene on German cockroaches has been studied before (Reid and Bennet 1994). Similar studies on the populations of stored-product

pests, including that of Indianmeal moth, would help in proper use of hydroprene in food handling environments that are sensitive to chemical applications like in packaging and retail facilities. Simulation models can be used to predict pest occurrence and evaluate a management strategy such as hydroprene application. Models for development and mortality derived from this study can be incorporated into a simulation model for the population dynamics of Indianmeal moth to optimize management strategies.

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**Footnotes**

Web addresses provided in the reference section of the manuscript, and their contents were current at the time of writing this manuscript.

**Table 1. Equations describing 1) relationship between temperature, exposure interval and development time and 2) relationship between temperature, exposure interval and mortality for Indianmeal moth eggs exposed to hydroprene**

| | Estimate | *P* | *t* | Adjusted $R^2$ |
|---|---|---|---|---|
| 1) Development time | | | | 0.95 |
| a | 25.43 ± 1.55 | < 0.01 | 16.32 | |
| b | -0.68 ± 0.10 | < 0.01 | -6.52 | |
| c | -0.44 ± 0.32 | 0.04 | -1.38 | |
| d | 0.01 ± 0.00 | 0.20 | 2.20 | |
| 2) Mortality | | | | 0.70 |
| a | -19.37 ± 18.64 | 0.30 | -1.03 | |
| b | 2.11 ± 1.26 | 0.01 | 1.67 | |
| c | 4.69 ± 3.87 | 0.02 | 1.20 | |
| d | -0.13 ± 0.08 | 0.11 | -1.57 | |

*a* = intercept, *b* = temperature, *c* = exposure interval, *d* = temperature x exposure interval. All models were computed with df = 4, 121 and are of the form *y* (development time (or) mortality) = $a + b + c + d$.

**Table 2. Equations describing relationships between temperature or exposure interval and development time for Indianmeal moth eggs exposed to hydroprene. Untreated controls (UTC) averaged across all temperatures. For continuous exposure (CONT), eggs were not removed from the treated surface**

| | Simple linear regression model | | | | Polynomial Model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *a* ± SE | *b* ± SE | Adj. $R^2$ | Lack-of-fit *P* | *a* ± SE | *b* ± SE | *c* ± SE | *d* ± SE | Adj. $R^2$ | Lack-of-fit *P* |
| Temp. | | | | | | | | | | |
| UTC | 24.93 ± 0.30 | -0.72 ± 0.012 | 0.97 | < 0.01 | 33.95 ± 0.99 | -1.53 ± 0.096 | 0.016 ± 0.0017 | -- | 0.98 | 0.05 |
| 20ºC | 10.39 ± 0.10 | 0.077 ± 0.010 | 0.70 | 0.06 | | | | | | |
| 24ºC | 7.92 ± 0.097 | 0.09 ± 0.0095 | 0.81 | 0.45 | | | | | | |
| 28ºC | 5.14 ± 0.013 | 0.12 ± 0.013 | 0.76 | < 0.01 | 4.74 ± 0.17 | 0.27 ± 0.050 | 0.0080 ± 0.0025 | -- | 0.83 | 0.05 |
| 32ºC | 3.56 ± 0.015 | 0.12 ± 0.015 | 0.70 | < 0.01 | 3.043 ± 0.18 | 0.32 ± 0.054 | 0.010 ± 0.0027 | -- | 0.81 | 0.39 |
| Exposure | | | | | | | | | | |
| 1 h | 24.30 ± 0.59 | -0.66 ± 0.024 | 0.96 | < 0.01 | 34.16 ± 1.98 | -1.53 ± 0.17 | 0.018 ± 0.0035 | -- | 0.98 | 0.06 |

**Table 2 cont.**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 h | 24.28 ± 0.57 | -0.67 ± 0.023 | 0.97 | < 0.01 | 35.72 ± 1.44 | -1.64 ± 0.12 | 0.020 ± 0.0025 | -- | 0.99 | 0.06 |
| 6 h | 23.54 ± 0.44 | -0.06 ± 0.018 | 0.97 | < 0.01 | 31.60 ± 1.26 | -1.32 ± 0.10 | 0.014 ± 0.0022 | -- | 0.99 | 0.10 |
| 12 h | 12.70 ± 0.47 | 2.31 ± 0.019 | 0.97 | < 0.01 | 29.94 ± 1.65 | -1.19 ± 0.14 | 0.013 ± 0.0029 | -- | 0.90 | 0.03[a] |
| 18 h | 22.88 ± 0.27 | 3.00 ± 0.011 | 0.99 | 0.01 | 25.73 ± 1.19 | -0.79 ± 0.10 | 0.0049 ± 0.0021 | -- | 0.99 | 0.04[a] |
| CONT | 21.71 ± 0.52 | -0.47 ± 0.021 | 0.95 | < 0.01 | 28.15 ± 2.13 | -1.046 ± 0.18 | 0.011 ± 0.0038 | -- | 0.96 | < 0.01 |
| | | | | | 68.08 ± 8.54 | -6.38 ± 1.13 | 0.24 ± 0.048 | | | |
| | | | | | | | -0.0031 ± 0.00067 | | | |
| | | | | | | | | | 0.98 | 0.18 |

*a, b, c, d* = $\hat{\beta}_0$, $\hat{\beta}_1 e$, $\hat{\beta}_2 e^2$, $\hat{\beta}_3 e^3$, respectively, for development time models within temperatures and $\hat{\beta}_0$, $\hat{\beta}_1 t$, $\hat{\beta}_2 t^2$, $\hat{\beta}_3 t^3$, respectively, for development time models within exposure intervals. All models are of the form *y* (development time) = $a + bx + cx^2 + dx^3$, where *x* is either temperature or exposure interval. All simple linear regression models were computed with df = 1, 23. All polynomial models were computed with df = 2, 22 except for the cubic model for untreated control with 2, 72.

[a] Although the lack-of-fit test for the quadratic models yielded significant results, cubic models that fit the data more closely are less likely to be biologically reasonable for this data.

**Table 3. Equations describing relationships between temperature or exposure interval and mortality for Indianmeal moth eggs exposed to hydroprene. For continuous exposure (CONT), eggs were not removed from the treated surfaces**

| | Simple Linear Model | | | | Polynomial Model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *a* ± S.E | *b* ± S.E | Adj. $R^2$ | Lack-of-fit *P* | *a* ± S.E | *b* ± S.E | *c* ± S.E | *d* ± S.E | Adj. $R^2$ | Lack-of-fit *P* |
| Temp. | | | | | | | | | | |
| 16ºC | 7.39 ± 1.22 | 1.26 ± 0.12 | 0.81 | < 0.01 | 2.85 ± 1.28 | 3.010 ± 0.37 | -0.091 ± 0.019 | -- | 0.90 | 0.08 |
| 20ºC | 20.16 ± 1.029 | 0.89 ± 1.10 | 0.76 | 0.03 | 17.34 ± 1.30 | 1.98 ± 0.37 | -0.057 ± 0.019 | -- | 0.82 | 0.44 |
| 24ºC | 33.13 ± 0.94 | 0.93 ± 0.92 | 0.80 | 0.57 | | | | | | |
| 28ºC | 38.83 ± 0.99 | 1.37 ± 0.97 | 0.89 | 0.55 | | | | | | |
| 32ºC | 39.79 ± 1.43 | 1.71 ± 0.14 | 0.85 | 0.36 | | | | | | |
| Exposure | | | | | | | | | | |
| 1 h | 28.64 ± 4.53 | 2.32 ± 0.18 | 0.86 | < 0.01 | -110.24 ± 13.13 | 9.52 ± 1.13 | -0.15 ± 0.023 | -- | 0.95 | 0.30 |
| 3 h | 16.80 ± 3.66 | 2.05 ± 0.14 | 0.88 | < 0.01 | -78.00 ± 11.86 | 7.45 ± 1.028 | -0.11 ± 0.021 | -- | 0.94 | 0.13 |
| 6 h | 18.64 ± 2.71 | 2.32 ± 0.11 | 0.94 | < 0.01 | -53.61 ± 10.81 | 5.40 ± 0.93 | -0.064 ± 0.019 | -- | 0.96 | 0.11 |

**Table 3 cont.**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 h | 12.72 ± 3.18 | 2.31 ± 0.12 | 0.93 | 0.03 | -40.89 ± 14.23 | 4.030 ± 1.26 | -0.037 ± 0.026 | -- | 0.93 | 0.07 |
| 18 h | 22.88 ± 3.88 | 3.00 ± 0.15 | 0.93 | 0.06 | | | | | | |
| CONT | -20.16 ± 2.95 | 3.16 ± 0.12 | 0.96 | < 0.01 | 7.041 ± 13.14 | 1.76 ± 1.13 | -0.042 ± 0.025 | -- | 0.97 | < 0.01 |
| | | | | | 263.31 ± 57.62 | 33.72 ± 7.63 | 1.54 ± 0.32 | | | |
| | | | | | | | | -0.020 ± 0.0045 | | |
| | | | | | | | | | 0.98 | 0.08 |

*a, b, c, d* = $\hat{\beta}_0$, $\hat{\beta}_1 e$, $\hat{\beta}_2 e^2$, $\hat{\beta}_3 e^3$ respectively, for mortality models within individual temperatures and $\hat{\beta}_0$, $\hat{\beta}_1 t$, $\hat{\beta}_2 t^2$, $\hat{\beta}_3 t^3$ respectively, for mortality models within individual exposure intervals. All models are of the form *y* (mortality) = $a + bx + cx^2 + dx^3$, where *x* is either temperature or exposure interval. All simple linear models were computed with df = 1, 23, and all polynomial models with df = 2, 22 except for the cubic model for continuous exposure with df = 3, 21.

**Figure Captions**

**Fig. 1.** Duration of development of Indianmeal moth eggs in untreated control at different temperatures. Fitted regression model (solid line), confidence intervals, 95% c.i. at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from three replications.

**Fig. 2A-E.** Duration of development of Indianmeal moth eggs when exposed to hydroprene at various temperatures for different exposure periods. Fitted regression model (solid line), confidence intervals, 95% c.i. at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 3A-E.** Duration of development of Indianmeal moth eggs when exposed to various exposure periods in different temperatures. **F.** Duration of development of Indianmeal moth eggs when exposed continuously on treated surface at different temperatures. Fitted regression model (solid line), confidence intervals, 95% c.i. at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 4.** Percentage mortality of Indianmeal moth eggs in untreated control at various temperatures. Open circles indicate independent observations from three replications.

**Fig. 5A-E.** Percentage mortality of Indianmeal moth eggs when exposed to hydroprene at various temperatures for different exposure periods. Fitted regression model (solid line), confidence intervals, 95% c.i. at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 6A-E.** Percentage mortality of Indianmeal moth eggs exposed to hydroprene for different exposure intervals at different temperatures. **F.** Percentage mortality of Indianmeal moth eggs when exposed continuously on the treated surface at different temperatures. Fitted regression model (solid line) and confidence intervals, 95% c.i. at mean (dotted line) and prediction intervals. Open circles are independent observations from five replications.

**Fig. 1.**

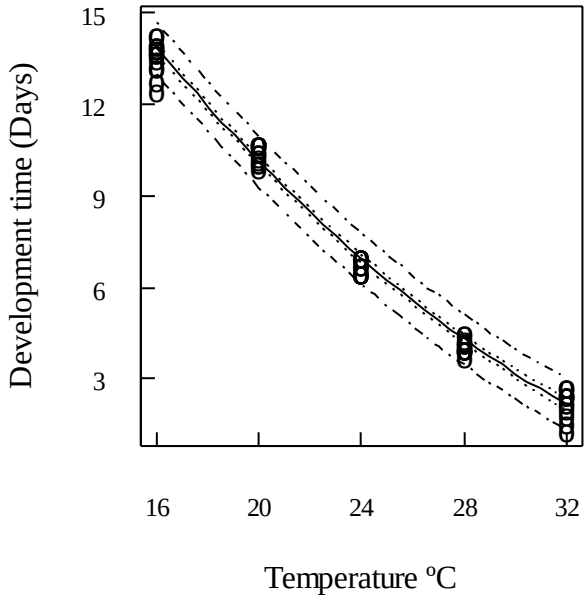


**Fig. 2.**

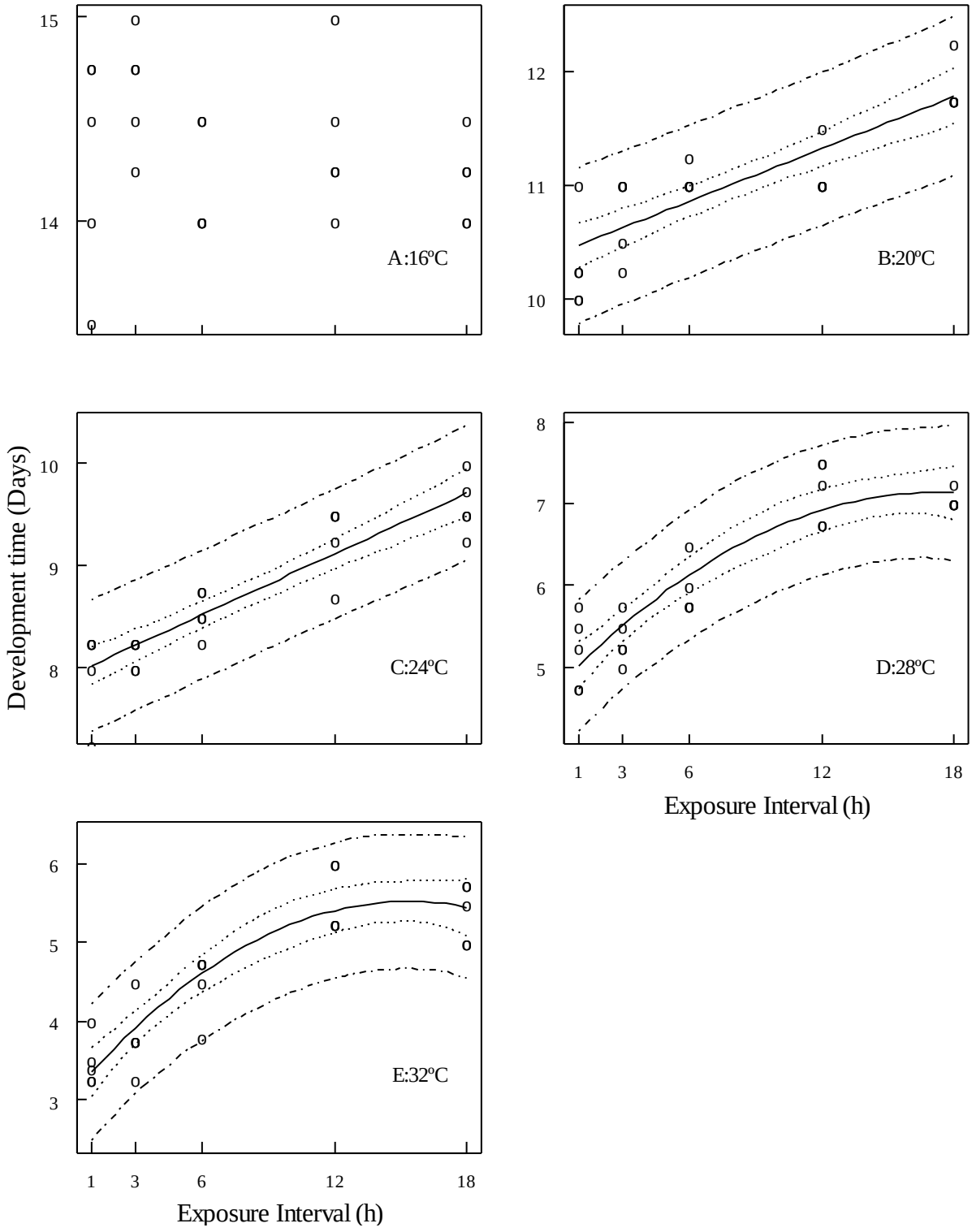

A:16ºC
B:20ºC
C:24ºC
D:28ºC
E:32ºC
Development time (Days)
Exposure Interval (h)
Exposure Interval (h)

**Fig. 3.**

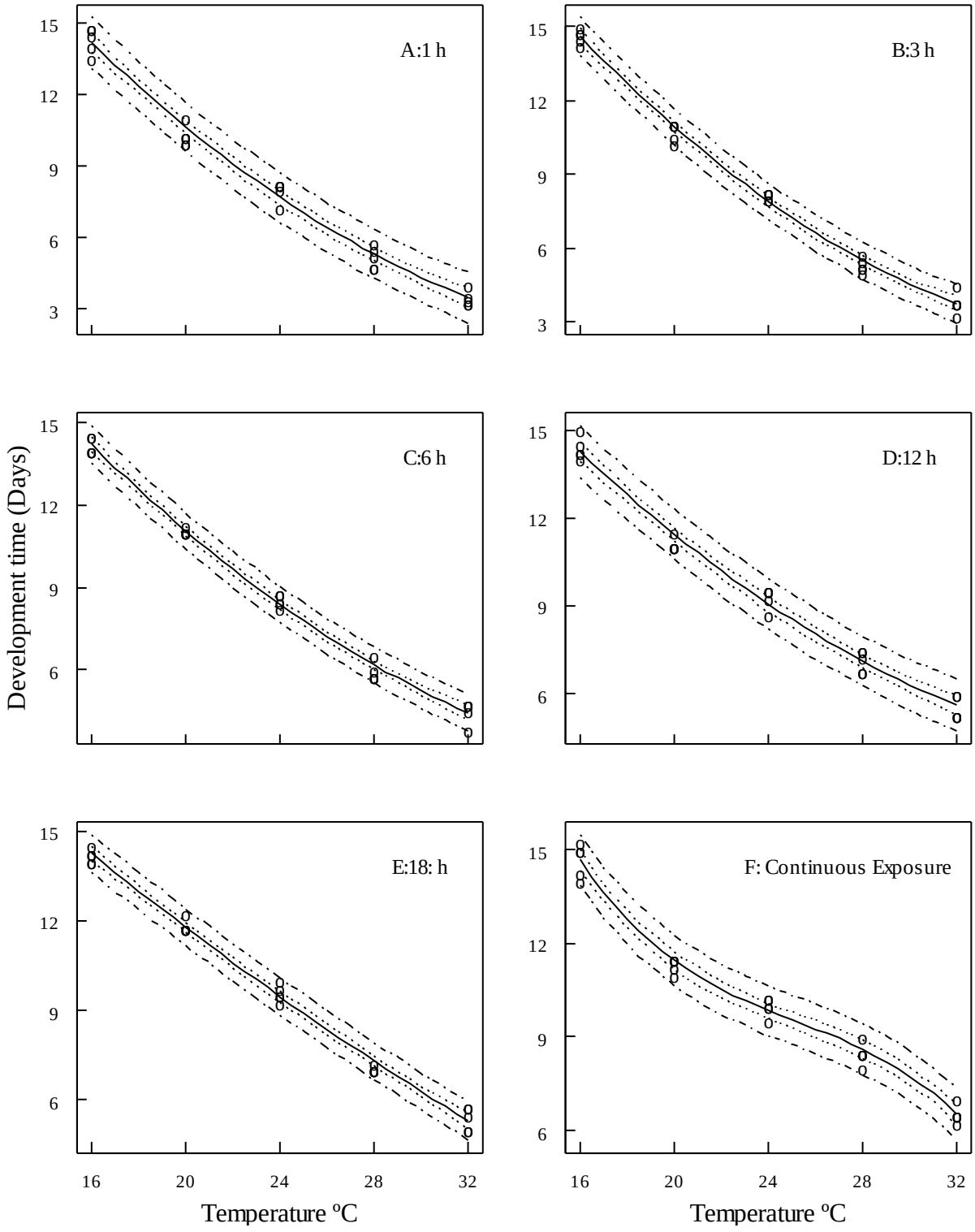

A:1 h
B:3 h
C:6 h
D:12 h
E:18: h
F: Continuous Exposure
Development time (Days)
Temperature ºC
Temperature ºC
15
12
9
6
3
16
20
24
28
32

**Fig. 4.**

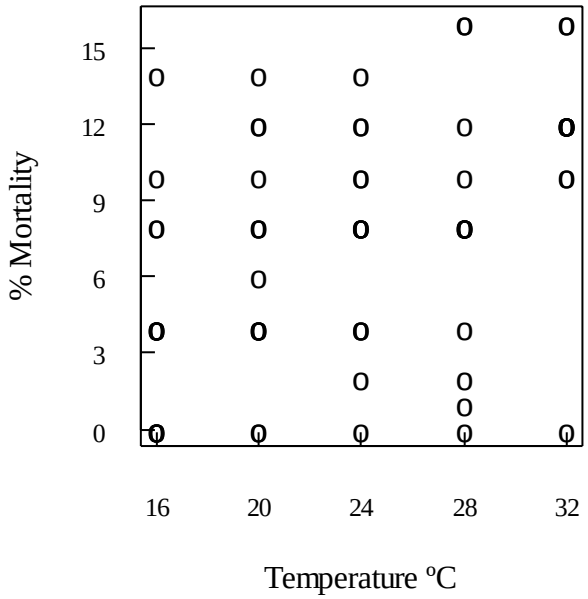


**Fig. 5.**

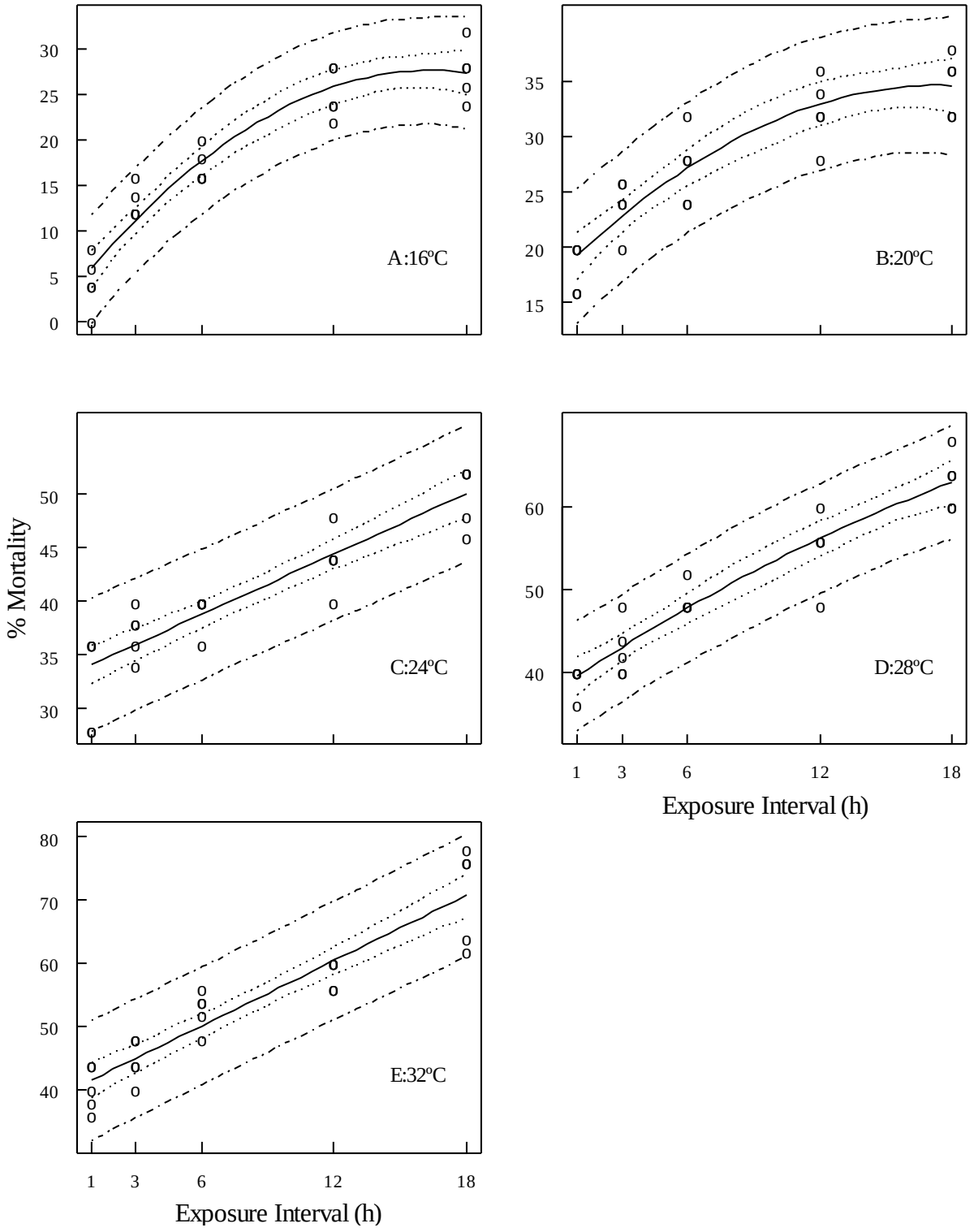
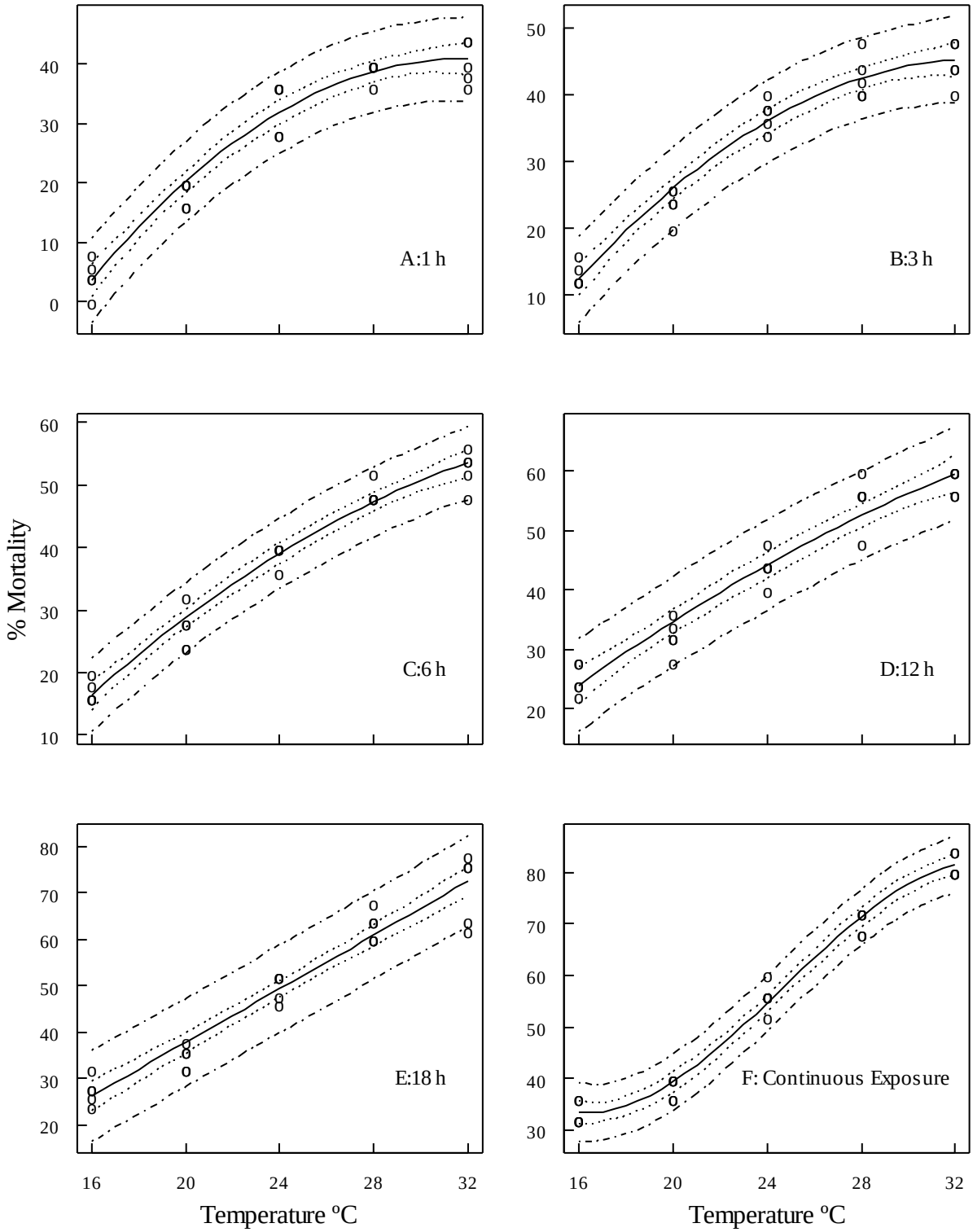

A:16ºC
B:20ºC
C:24ºC
D:28ºC
E:32ºC
% Mortality
Exposure Interval (h)
Exposure Interval (h)

**Fig. 6.**


A:1 h
B:3 h
C:6 h
D:12 h
E:18 h
F: Continuous Exposure
% Mortality
Temperature ºC
Temperature ºC

# CHAPTER 3

# HYDROPRENE PROLONGS DEVELOPMENT TIME AND INCREASES MORTALITY IN WANDERING-PHASE INDIANMEAL MOTH (LEPIDOPTERA: PYRALIDAE) LARVAE

**Abstract**

Wandering-phase Indianmeal moth larvae, *Plodia interpunctella* (Hübner), were exposed to the labeled rate of hydroprene (1.9 X $10^{-3}$ mg[AI]/ $cm^2$) sprayed on concreted petri dishes. Larvae were exposed for 1, 3, 6, 12, 18, 24, and 30 h and maintained at 16, 20, 24, 28, and 32ºC and 57% relative humidity until adult emergence. Larval development time and mortality were significantly influenced by temperature and exposure intervals. Maximum development time (47.2 ± 1.3 d) occurred at 16ºC, and the minimum development time (7.0 ± 0.5) occurred at 32ºC. Larval mortality generally increased at all of the five tested temperatures as exposure period increased. The greatest mortality (82.0 ± 0.1%) occurred when larvae were exposed for 30 h at 28ºC, and minimum mortality (0.0 ± 0.5%) occurred at 16ºC when larvae were exposed for 1 h. The relationships between temperature, exposure period, and development time were described by polynomial models, based on lack-of-fit tests. Hydroprene has potential to be an effective alternative to conventional insecticides in surface treatments for Indianmeal moth management. Response-surface models derived from this study can be used in simulation models to estimate the potential consequences of hydroprene on Indianmeal moth population dynamics.

**KEY WORDS** *Plodia interpunctella*, development, mortality, population dynamics, insect growth regulator

## Introduction

The Indianmeal moth, *Plodia interpunctella* (Hübner), is a serious cosmopolitan pest of stored commodities such as raw, packaged, and animal foods (Cox and Bell 1991, Campbell et al. 2002), and it has been recorded infesting over 83 kinds of stored-food (Richards and Thomson 1932, Deso 1976). Indianmeal moth larvae continuously spin silken webbing and feed from within the webbing. The webbing contains larval cast skins and frass, which impart an unpleasant odor to the infested commodity. Larval infestation found in packaged food leads to immediate rejection and erodes consumer confidence in the product. In grain bins, the surface layer can be covered with a thick mat of webbing, which renders chemical treatments difficult or at times impossible to apply. All five larval stages of Indianmeal moth move and feed within the infested commodity. However, the fifth instars, called the wandering-phase larvae, are especially important economically because they often leave the infested commodity and wander long distances in search of a suitable pupation site. The distance traveled by these larvae is so great that when the adults emerge well away from their feeding sites, they are sometimes confused with clothes moths, such as the webbing clothes moth, *Tineola bisselliella* (Hummel), the casemaking clothes moth, *Tinea pellionella* L., or the carpet moth, *Trichophaga tapetzella* (L.) (Smith 2000). Due to this wandering nature, fifth instar larvae are suitable candidates for management using surface insecticidal applications.

Hydroprene is a juvenile hormone analog registered for stored-product pest management in the USA, and it can be used for surface treatments. Surface treatment with hydroprene is a common management practice in food processing and storage facilities such as retail stores, warehouses, and flour mills. Hydroprene can also be used in aerosol and impregnated disc applications. Hydroprene is an alternative to conventional insecticides, many of which are being re-evaluated or facing threat of removal because of new regulatory laws and interpretation of

existing laws. Hydroprene is classified as a biopesticide because of its virtually non-toxic nature towards vertebrates, rapid biodegradability, and specific activity against insects. There are several reports of the effects of hydroprene on household pests, especially cockroaches (Bennett et al. 1986; King and Bennett 1988, 1989, 1990; Reid and Bennett 1994; Edwards et al. 1995; Kaakeh et al. 1997; Stoltzman and Stay 1997; Bell et al. 1999), and some of these studies involved spraying hydroprene on floors to control cockroach populations. Many of the earlier studies on hydroprene with stored-product insect pests were conducted with beetles (Loschiavo 1975, 1976; McGregor and Kramer 1975; Amos and Williams 1977; Rup and Chopra 1984), and relatively fewer studies were conducted with lepidopteran larvae (Nickle 1979; Stockel and Edwards 1981).

Arbogast et al. (2002) showed reduction in population of several stored-product pests, including the Indianmeal moth, when hydroprene was applied as a spot-treatment in a botanical warehouse. In the laboratory, exposing Indianmeal moth eggs to hydroprene sprayed on cemented petri dishes prolonged the egg development time and caused mortality in a dose-dependent manner (unpublished data). Timing of hydroprene application and assessment of its likely population consequences can be improved with the aid of simulation models of population dynamics (Hagstrum and Flinn 1990, Flinn and Hagstrum 1990, Flinn et al. 1997). Insecticide effectiveness is related to temperature (Scott 1995) and the length of exposure period (Arthur 2001). The objective of this study was to quantify the effects of hydroprene sprayed on concreted petri dishes on the development and mortality of wandering-phase Indianmeal moth larvae. Equations derived from this and other studies will ultimately be incorporated into a population dynamics model for use in the management of Indianmeal moth.

## Materials and Methods

**Experimental Design.** This experiment was set up as a split-plot design (Kuehl 2000), with five levels of temperature (16, 20, 24, 28, and 32ºC) as the whole-plot factors and seven levels of exposure period to hydroprene (1, 3, 6, 12, 18, 24, and 30 h) as sub-plot factors. There were 35 treatment combinations in total. Six different incubators (ThermoForma®, Marrietta, OH) were used as whole-plot experimental units, one each for each temperature, and cemented petri dishes were used as sub-plot units for individual exposure periods. Two response variables, Indianmeal moth development time and mortality, were quantified. In order to maintain 57% relative humidity (RH) throughout the experiment, humidity chambers were created using plastic containers (26 by 36.5 by 15 cm) with a waffle-type plastic grid in the bottom. A saturated NaBr solution maintained humidity inside each plastic container (Greenspan 1977). Two containers, one each for treatments and controls, were placed in each incubator. Humidity was uniform across all of the whole and subplot treatments and therefore was not considered as part of the treatment design. Daily temperature and humidity inside the individual incubators were monitored by placing a HOBO® (Onset Computer Corporation, Bourne, MA) inside each humidity container.

**Experimental Arenas and Hydroprene Formulation.** I applied hydroprene as a surface treatment on concrete because this is a common floor surface in warehouses, some retail stores, and other storage facilities throughout much of North America. Concrete (Rockite®, Hartline Products Co., Cleveland, OH) was mixed in an approximate ratio of 3,200 g of concrete in 1,600 ml of water to a thick running consistency (Arthur 1999). The liquid slurry was then poured into individual petri dishes (62 $cm^2$). For 90 petri dishes, concrete was poured to approximately half the capacity of the dish, and for another 90 petri dishes, the dish was filled with concrete. A total

of 180 dishes were created in this manner, and then dried for about 48 h at room temperature (27ºC).

The hydroprene formulation used in the study was made with Gentrol® (9.0% active ingredient [AI], approximately 90 mg[AI]/ml). Label directions specify application by mixing 1 oz in 1 gallon of water to cover 1500 $ft^2$ (29.57 ml in 3.79 liters of water to cover 134.8 $m^2$), which is 1.9 X $10^{-3}$ mg[AI]/$cm^2$. The area of the concrete petri dish was 62 $cm^2$, so the volume of spray needed for this area was 0.17 ml. This amount was too small to formulate individual concentrations. I prepared the hydroprene concentrations by mixing 0.38 ml of Gentrol® in 50 ml of distilled water, thoroughly shaking the solution, and removing individual 0.17 ml aliquots for each petri dish; these individual solutions were sprayed on the concrete arenas using an artist's airbrush (Badger® No. 100 LG., Franklin Park, IL). The liquid was sprayed by holding the airbrush approximately 5 to 10 cm above the treatment arenas and by slowly releasing pressure until all of the material was dispensed.

The experiment was completed in two stages. For the first stage, thirty dishes were treated as described above. These 30 dishes comprised a replicate for 6, 12, 18, 24, and 30 h exposure periods, and another 30 companion concreted dishes were sprayed with the same volume of distilled water for the untreated control. In subsequent trials, two treated replicates were created as described above, along with an untreated control, for a total of five replications. In the second stage of the experiment, two additional exposure periods (1 and 3 h) were included, and all procedures were followed as previously described.

**Insects.** Fifth instar Indianmeal moth larvae were obtained from an insecticide-susceptible laboratory strain, which is a mixture of several field-collected strains maintained at the USDA Grain Marketing and Production Research Center, Manhattan, KS. This laboratory culture is reared on a standard diet mixture of cracked wheat, wheat bran, wheat germ, honey, glycerin,

yeast, sorbic acid, benzoic acid, and water. All cultures are reared inside incubators set at 27ºC and 60% RH. Prior to setting up the experiment, Indianmeal moth larvae reared inside a 3.8 L jar were placed on a tray by carefully transferring them, along with the laboratory media, by using a spatula. Ten actively wandering $5^{th}$ instars were then transferred to each of the treated and untreated petri dishes by using a featherweight forceps (Bioquip®, Rancho Dominguez, CA). Larvae were held in between a sandwich of two cemented petri dishes placed on top of each other with opposing sides facing each other. The bottom dish was completely filled with concrete and the top dish was only half-filled. This set-up gave maximum hydroprene exposure to the larvae while they moved within the available space. Randomization for subplot treatments was done by randomly selecting a pair of concrete arenas for each exposure period. The arenas, along with the larvae were sealed by using scotch tape and placed inside a humidity container inside individual temperature incubators. The untreated controls were held inside the second container. Upon completion of the exposure interval, the larvae were removed randomly from each treatment combination and placed on top of sterile filter paper inside individual, pesticide-free petri dishes, and then the dishes containing larvae were sealed and placed back in the same humidity chambers. Every twenty four hours for forty days, the number of emerging adults inside each petri dish was recorded. Larvae that did not emerge as adults after fifty days were considered as dead.

**Data Analysis.** Regression models were used to evaluate impacts of temperature and hydroprene exposure period on development time. Kramer et al. (1991) showed that erroneous predictions could occur in least-square estimations when model parameters are estimated by using some modified form of data, such as rate (1/development time). Minimizing the squared error for development rate is not the same as minimizing the squared error for development time, especially in the longer development time range. I used time instead of rate to fit all our

regression models for larval development time. The regression models for development time were chosen based upon lack-of-fit-tests, but not $R^2$ or adjusted $R^2$ values, which are traditionally considered to be standards for model selection. As this is a designed experiment and the observations are derived from replicated units, it was possible to conduct lack-of-fit tests by partitioning the residual sum of squares into lack-of-fit and pure error components (Weisberg 1985). This involved determining the part of the residual sum of squares that can be predicted by including additional terms for the predictor variables in the model, like higher order polynomial terms, and the part of the residual sum of squares that cannot be predicted by any additional terms, i.e., the sum of squares for pure error. A test of lack-of-fit for the model without the additional terms was then performed, using the mean square pure error as the error term. This provided a sensitive test of model fit because the effects of the additional higher order terms were removed from the error. Care was taken to fit models that were biologically reasonable and described data adequately (Throne 1994, Faraway 1999).

Appropriate models for individual data sets were selected by computing comparisons made between the desired and saturated models with higher order polynomial terms by way of F-testing methodology (Faraway 1999). Influential observations in the dataset were checked by using Cook's distance plots. Non-constant variance (heteroscedascity) and nonlinearity were checked by plotting residuals for the selected models (Faraway 1994). The strengths of the regression relationships were measured by their adjusted $R^2$ values (Seber 1977), and 95% confidence intervals on the mean and prediction intervals were plotted for individual equations (Becker et al. 1988, Murrell 1999).

Analysis of variance computation (Chambers et al. 1992) in R© (Version 1.9.0 for Windows®, Vienna, Austria) (Ihaka and Gentleman 1996; R - Development Core Team 2004) showed no significant interaction effect between temperature and exposure period on

development time, but there was a significant interaction on percentage mortality (Table 1). The effects of hydroprene on larval development time and mortality were modeled by fitting three-dimensional (3D) response surface models by using temperature and exposure periods as predictor variables in TableCurve 3D (SYSTAT Software Inc., Point Richmond, CA) (Figs. 1, 2). Such 3D models, especially when they are static and presented in black and white, are difficult to interpret (Merwin et al. 1994) and offer less quantitative information to a scientific reader than two-dimensional (2D) graphs. Therefore, for our data, the percentage larval mortality and development time were plotted and regressed individually within different temperatures and exposure periods using R©.

## Results

**Development Time.** Within each temperature, the number of days taken for wandering-phase larvae to emerge as adults generally increased with increase in exposure period to hydroprene (Fig. 3), and within each exposure period, the development time decreased as temperature increased (Fig. 4). A cubic model was fit to the data for each temperature, and a simple linear model was fit to the data for each exposure period (Table 2). The longest development time among the treatments of 47.2 ± 1.3 d occurred at 16ºC when the larvae were exposed for 30 h, while the shortest development time of 7.0 ± 0.5 d occurred when the larvae were exposed for 1 h at 32ºC. Longest development time in the untreated controls was 32.2 ± 1.0 d at 16ºC, and the shortest development time was 7.0 ± 1.0 d at 32ºC.

**Mortality.** Among treatments, the greatest mortality of 82.0 ± 0.1% occurred when larvae were exposed for 30 h at 28ºC, while the minimum mortality of 0.0 ± 0.5% occurred at 16ºC when larvae were exposed for 1 h. Quadratic equations adequately fit the data at 16, 20, 28 and 32ºC when percentage mortality was regressed on exposure interval (Fig. 5, Table 3). When

percentage mortality was regressed on temperature (Fig. 6, Table 3), simple linear equations fit the data at 1, 3, 6, 12, and 24 h and a quadratic model fit the data at 30 h. However, at 18 h, higher order polynomial models, including a cubic model, showed significant lack-of-fit. Among the untreated controls, there was no significant effect of temperature or exposure period on mortality ($F = 0.5$; df = 3, 101; $P = 0.6$). The mortality in untreated controls averaged 16.8 ± 11.2% and ranged between 0-40% (Fig. 6).

I did not expect a non-linear trend for mortality at the 18 h exposure period because simple linear models adequately fit the data at most of the other exposure periods below 30ºC. Adding a cubic term to the quadratic model did not change the parameter estimates (Table 3). Therefore, I checked for the presence of influential data points within the 18-h-exposure dataset (Faraway 1994, 1999). Cook's distance plot for this data set revealed that observation 23 (60% mortality at 32ºC) was considerably farther away from the rest of the data points with a Cook's distance of 0.27, suggesting that the influence of this data point alone on the simple linear model was higher than all the other data points put together (Cook's distance = < 0.10). I removed observation 23 and re-fit the model. However, the lack-of-fit was still significant. On plotting Cook's distance plot for the modified data again, observation 24 (70% mortality at 32ºC) stood out separate from other observations with a Cook's distance = 0.19, while the Cook's distance for all other data points were < 0.10. I removed observation 24 and re-fit a simple linear model, and this time there was no significant lack-of-fit (Fig. 7).

**Discussion**

Larval mortality was as high as 40% in the untreated controls. However, the mean control mortality across all temperatures was only 13.6 ± 10.4%, and a majority of the observations ranged between 10-20% (Fig. 8). Highest mortality (40%) occurred only twice in temperatures

20 and 32ºC. Therefore, the higher percentage mortality I recorded in the untreated controls is unlikely to have been due to the influence of an additional external factor.

Percentage larval mortality data reported in this study can be analyzed using probit analysis (Throne et al. 1995) and can be used to estimate the toxicity and/or relative potency of hydroprene as compared with other conventional insecticides used for surface spray. Detailed procedure for this kind of analysis can be found in Hubert (1992). However, I fit regression equations to the data so that they can be used in a population dynamics simulation model for Indianmeal moth that is currently being developed in our lab. The regression equations for development time and mortality derived in this and other bioassays can be directly used in a simulation program, which will help a pest manager determine the timing of hydroprene application and to estimate the effectiveness of hydroprene on Indianmeal moth populations.

I fit simple linear equations to the data for development time within each exposure period. On looking at the scatter plot of the data, all observations except for the ones at 20ºC aligned in a linear fashion, while the observations at 20ºC were consistently below the linear trend. One possible explanation for this could be a sudden increase in volatility of hydroprene (Atkins et al. 1998) at 20ºC, and a steady increase in volatility at temperatures above this range. However, I could not find a reasonable hypothesis for why this change in volatility would result in the pattern observed. Given the lack of reasoning, for the non-linear trend, I fit simple linear equations to development time within each exposure period despite the lack-of-fit.

I previously quantified the effects of hydroprene-treated concrete surfaces on the development and mortality of Indianmeal moth eggs (unpublished data). These studies show that hydroprene sprayed as a surface treatment significantly delays development time of eggs and larvae, and decreases egg hatch and emergence of adults when exposed as wandering-phase larvae. Treating concrete floor surfaces with hydroprene in food-storage facilities may be an

effective alternative to conventional insecticides. In the future, stored-product pest management will consist more of a combined approach rather than dependence on a few chemical insecticides (White 1992, Arthur and Phillips 2003). Management strategies that combine tactics have been shown to be effective for control of other insect pests (Johnson et al. 1998, 2002). One or more of the hydroprene application methods, such as a surface spray application, crack-and-crevice treatment, application as a fog, or as impregnated discs, may be used in combination management strategies for Indianmeal moth control.

I tested only one mode of hydroprene application, surface application, and on one specific floor surface, concrete. Tests with other insect growth regulators have shown varied effects of hydroprene on different flooring surfaces. Atkins et al. (1998) showed that absorbent surfaces such as unfinished plywood, fiberboard, and vinyl tile had more activity on the mortality of German cockroach nymphs than their non-absorbent counterparts such as glass, stainless steel, ceramic tile, and formica. Hydroprene-treated stainless steel surfaces had less residual activity than masonite and unpainted plywood (Kaakeh et al. 1997). Differences in the persistence or residual activity of hydroprene may differ from one type of surface to another. Nevertheless, these studies and our own study indicate that hydroprene can be used for surface treatments in facilities having concrete flooring surfaces.

Resistance by insects to other insect growth regulators has been cited in the literature (Dame et al. 1998; Cornel et al. 2000, 2002), therefore necessary steps to slow resistance development by insects to hydroprene should be devised and followed. Insects may evade a lethal dose of hydroprene when applied only as a surface treatment; therefore, alternating the use of several of the hydroprene application methods could slow insect resistance development. Another possible method to slow resistance development is by timing and targeting of

hydroprene application towards specific life-stages of Indianmeal moth, at least in storage facilities where overlapping generations do not occur.

Other insect growth regulators may possess toxicity to Indianmeal moth and should be evaluated against different life-stages. Methoprene and pyriproxifen recently have been labeled as aerosol treatments and for some surface applications, and they should also be tested for their effects on Indianmeal moth. More studies are needed to find alternative chemicals that can be used in rotation with hydroprene in stored-product environments.

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**Footnotes**

Web addresses provided in the reference section of the manuscript, and their contents, were current at the time of writing this manuscript.

**Table 1. Equations describing 1) relationship between temperature, exposure interval, and development time; and 2) relationship between temperature, exposure interval, and mortality for wandering-phase Indianmeal moth larvae exposed to hydroprene**

| | Estimate | *t* | *P* | Adjusted $R^2$ |
|---|---|---|---|---|
| 1) Development time (days) | | | | 0.95 |
| a | 61.04 ± 1.14 | 53.24 | < 0.01 | |
| b | -1.66 ± 0.046 | -35.87 | < 0.01 | |
| c | -0.32 ± 0.067 | 4.76 | < 0.01 | |
| d | 0.0019 ± 0.0027 | 0.69 | 0.48 | |
| 2) Mortality (%) | | | | 0.87 |
| a | -1.040 ± 0.42 | -2.45 | 0.01 | |
| b | 0.11 ± 0.017 | 6.47 | < 0.01 | |
| c | 0.057 ± 0.025 | 2.28 | 0.02 | |
| d | 0.0050 ± 0.0010 | 4.90 | < 0.01 | |

*a* = intercept, *b* = temperature (ºC), *c* = exposure interval (h), and *d* = *b(c)*. All models were computed with df = 3, 171 and are of the form *y* (development time (or) mortality) = *a* + *b* + *c* + *d*.

**Table 2. Equations describing relationships between temperature or exposure interval and development time for wandering-phase Indianmeal moth larvae exposed to hydroprene.**

| | *a* ± SE | *b* ± SE | *c* ± SE | *d* ± SE | Adj. $R^2$ | Lack-of-fit *P* |
|---|---|---|---|---|---|---|
| Temp. (ºC) | | | | | | |
| 16 | 33.29 ± 0.49 | 0.49 ± 0.037 | -- | -- | 0.75 | < 0.01 |
| | 32.38 ± 0.46 | 1.020 ± 0.11 | -0.010 ± 0.0042 | -- | 0.82 | < 0.01 |
| | 31.86 ± 0.44 | 1.79 ± 0.24 | -0.096 ± 0.022 | 0.0017 ± 0.00050 | 0.85 | 0.06 |
| 20 | 23.27 ± 0.30 | 0.43 ± 0.023 | -- | -- | 0.86 | < 0.01 |
| | 22.58 ± 0.25 | 0.83 ± 0.064 | -0.015 ± 0.0025 | -- | 0.95 | 0.04 |
| | 22.41 ± 0.26 | 1.085 ± 0.14 | -0.039 ± 0.013 | 0.00056 ± 0.00029 | 0.92 | 0.10 |
| 24 | 17.70 ± 0.44 | 0.56 ± 0.033 | -- | -- | 0.83 | < 0.01 |
| | 16.61 ± 0.34 | 1.20 ± 0.086 | -0.023 ± 0.003 | -- | 0.92 | < 0.01 |
| | 16.27 ± 0.33 | 1.70 ± 0.18 | -0.073 ± 0.016 | 0.0011 ± 0.00037 | 0.93 | 0.05 |
| 28 | 12.37 ± 0.32 | 0.49 ± 0.024 | -- | -- | 0.88 | < 0.01 |
| | 11.79 ± 0.30 | 0.82 ± 0.076 | -0.012 ± 0.0027 | -- | 0.91 | < 0.01 |
| | 11.42 ± 0.28 | 1.38 ± 0.15 | -0.067 ± 0.014 | 0.0012 ± 0.00032 | 0.93 | 0.25 |
| 32 | 7.67 ± 0.18 | 0.38 ± 0.013 | -- | -- | 0.93 | < 0.01 |
| | 7.33 ± 0.16 | 0.58 ± 0.042 | -0.0075 ± 0.0015 | -- | 0.95 | < 0.01 |
| | 7.20 ± 0.17 | 0.78 ± 0.092 | -0.026 ± 0.0084 | 0.00044 ± 0.00019 | 0.95 | 0.07 |
| Exposure (h) | | | | | | |

**Table 2. Cont.**

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 51.51 ± 1.15 | -1.44 ± 0.046 | -- | -- | 0.90 | < 0.01[a] |
| 1 | 58.64 ± 1.74 | -1.61 ± 0.070 | -- | -- | 0.95 | < 0.01[a] |
| 3 | 62.90 ± 1.70 | -1.69 ± 0.069 | -- | -- | 0.96 | < 0.01[a] |
| 6 | 63.69 ± 1.59 | -1.65 ± 0.064 | -- | -- | 0.96 | < 0.01[a] |
| 12 | 65.83 ± 0.47 | -1.64 ± 0.068 | -- | -- | 0.96 | < 0.01[a] |
| 18 | 70.09 ± 1.23 | -1.72 ± 0.053 | -- | -- | 0.98 | < 0.01[a] |
| 24 | 68.21 ± 1.82 | -1.60 ± 0.071 | -- | -- | 0.95 | < 0.01[a] |
| 30 | 69.65 ± 1.89 | -1.59 ± 0.077 | -- | -- | 0.94 | < 0.01[a] |

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*a, b, c, d* = $\hat{\beta}_0$, $\hat{\beta}_1 e$, $\hat{\beta}_2 e^2$, $\hat{\beta}_3 e^3$, respectively, for development time models within temperatures and $\hat{\beta}_0$, $\hat{\beta}_1 t$, $\hat{\beta}_2 t^2$, $\hat{\beta}_3 t^3$, respectively, for development time models within exposure intervals. All models are of the form *y* (development time) = $a + bx + cx^2 + dx^3$, where *x* is either temperature or exposure period. All simple linear regression models within temperatures were computed with df = 1, 23, all quadratic models with df = 2, 22, and all cubic models with df = 3, 21. All simple linear models within exposure periods were computed with df = 1, 54.

[a] Although lack-of-fit for simple linear models was significant, higher order models that fit the data more closely were less biologically reasonable for these data.

**Table 3. Equations describing relationships between temperature or exposure interval and % mortality for wandering-phase Indianmeal moth larvae exposed to hydroprene.**

| | *a* ± SE | *b* ± SE | *c* ± SE | *d* ± SE | Adj. $R^2$ | Lack-of-fit *P* |
|---|---|---|---|---|---|---|
| Temp. (ºC) | | | | | | |
| 16 | 7.92 ± 1.27 | 1.27 ± 0.095 | -- | -- | 0.76 | 0.01 |
| | 6.42 ± 1.32 | 2.15 ± 0.33 | -0.033 ± 0.012 | -- | 0.78 | 0.08 |
| 20 | 13.92 ± 1.76 | 1.43 ± 0.13 | -- | -- | 0.68 | 0.01 |
| | 12.35 ± 1.88 | 2.35 ± 0.47 | -0.034 ± 0.017 | -- | 0.69 | 0.24 |
| 24 | 13.53 ± 1.50 | 2.00 ± 0.11 | -- | -- | 0.85 | < 0.01 |
| | 11.31 ± 1.49 | 3.30 ± 0.37 | -0.048 ± 0.013 | -- | 0.87 | 0.17 |
| 28 | 17.13 ± 1.25 | 2.33 ± 0.094 | -- | -- | 0.91 | < 0.01 |
| | 14.32 ± 1.04 | 3.97 ± 0.26 | -0.061 ± 0.0095 | -- | 0.95 | 0.88 |
| 32 | 21.98 ± 1.46 | 2.12 ± 0.11 | -- | -- | 0.87 | < 0.01 |
| | 19.43 ± 1.39 | 3.61 ± 0.35 | -0.056 ± 0.012 | -- | 0.90 | 0.80 |
| Exposure (h) | | | | | | |
| 1 | -14.88 ± 4.51 | 1.08 ± 0.18 | -- | -- | 0.58 | 0.15 |
| 3 | -9.80 ± 5.62 | 1.23 ± 0.22 | -- | -- | 0.53 | 0.20 |
| 6 | -3.44 ± 3.24 | 1.40 ± 0.13 | -- | -- | 0.82 | 0.09 |
| 12 | -4.72 ± 5.55 | 1.97 ± 0.22 | -- | -- | 0.76 | 0.57 |
| 18 | 9.04 ± 4.37 | 1.88 ± 0.17 | -- | -- | 0.82 | 0.03 |
| | -3.10 ± 21.18 | 2.95 ± 1.83 | -0.02 ± 0.0038 | -- | 0.92 | 0.02[a] |

**Table 3 cont.**

| | | | | | | |
|---|---|---|---|---|---|---|
| | 206 ± 113.10 | -24.93 ± 14.98 | 1.17 ± 0.64 | -0.16 ± 0.00031 | 0.92 | 0.03[a] |
| | 6.91 ± 4.19 | 1.99 ± 0.17 | -- | -- | 0.85 | 0.05[b] |
| 24 | 3.24 ± 5.59 | 2.27 ± 0.22 | -- | -- | 0.80 | 0.24 |
| 30 | 1.64 ± 7.16 | 2.60 ± 0.29 | -- | -- | 0.76 | < 0.01 |
| | -95.50 ± 27.76 | 11.17 ± 2.40 | -0.17 ± 0.049 | -- | 0.84 | 0.11 |

*a, b, c, d* = $\hat{\beta}_0$, $\hat{\beta}_1 e$, $\hat{\beta}_2 e^2$, $\hat{\beta}_3 e^3$, respectively, for mortality models within temperatures and $\hat{\beta}_0$, $\hat{\beta}_1 t$, $\hat{\beta}_2 t^2$, $\hat{\beta}_3 t^3$, respectively, for mortality models within exposure periods. All models are of the form *y* (development time) = $a + bx + cx^2 + dx^3$, where *x* is either temperature or exposure period. All simple linear regression models within temperatures were computed with df = 1, 22, and all quadratic models with df = 2, 21. All simple linear models within exposure periods were computed with df = 1, 54, all quadratic models with df = 2, 53 and all cubic models with df = 3, 52.

[a] Although the lack-of-fit test for the quadratic and cubic models yielded significant results, higher order models that fit the data more closely were not biologically reasonable for these data.

[b] Parameter estimates for regression equation without data points 23 and 24. These estimates were derived with df = 1, 20.

**Figure captions**

**Fig. 1.** Response-surface model for wandering-phase Indianmeal moth larval development time. This model was chosen based on highest *F*-value (2999.1; df = 3, 172) and is in the form $lnz = a + blnx + cy^{0.5}$, where $a = 138.19 \pm 1.73$, $b = -38.19 \pm 0.54$, $c = 2.65 \pm 0.08$, $x$ = temperature (ºC), and $y$ = exposure period (h), and $z$ = larval development time (days). Adjusted $R^2 = 0.97$.

**Fig. 2.** Response-surface model for wandering-phase Indianmeal moth larval mortality. This model was chosen based on highest *F*-value (975; df = 3, 172) and is in the form $lnz = a + blnx/x^2 + clny$, where $a = 3.22 \pm 0.05$, $b = 91.8 \pm 5.0$, $c = 0.44 \pm 0.01$, $x$ = temperature (ºC), $y$ = exposure period (h), and $z$ = % larval mortality. Adjusted $R^2 = 0.97$.

**Fig. 3.** Duration of development of wandering-phase Indianmeal moth larvae exposed to hydroprene at various temperatures for different exposure periods. Regression model (solid line), 95% confidence intervals at mean (dotted line), and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 4.** Duration of development of wandering-phase Indianmeal moth larvae exposed to hydroprene for various periods at different temperatures. Regression model (solid line), 95% confidence intervals at mean (dotted line), and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 5.** Percentage mortality of wandering-phase Indianmeal moth larvae when exposed to hydroprene at various temperatures for different exposure periods. Regression model (solid line),

95% confidence intervals at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 6.** Percentage mortality of wandering-phase Indianmeal moth larvae when exposed to various periods at different temperatures. Regression model (solid line), 95% confidence intervals at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 7.** Regression equation for % mortality at 18 h exposure without observations 23 (* at 70% mortality) and 24 (* at 60% mortality). Regression model (solid line), 95% confidence intervals at mean (dotted line) and prediction intervals (dashed line). Open circles are independent observations from five replications.

**Fig. 8.** Box plot showing distribution of larval mortality observations recorded in untreated controls.

**Fig 1.**

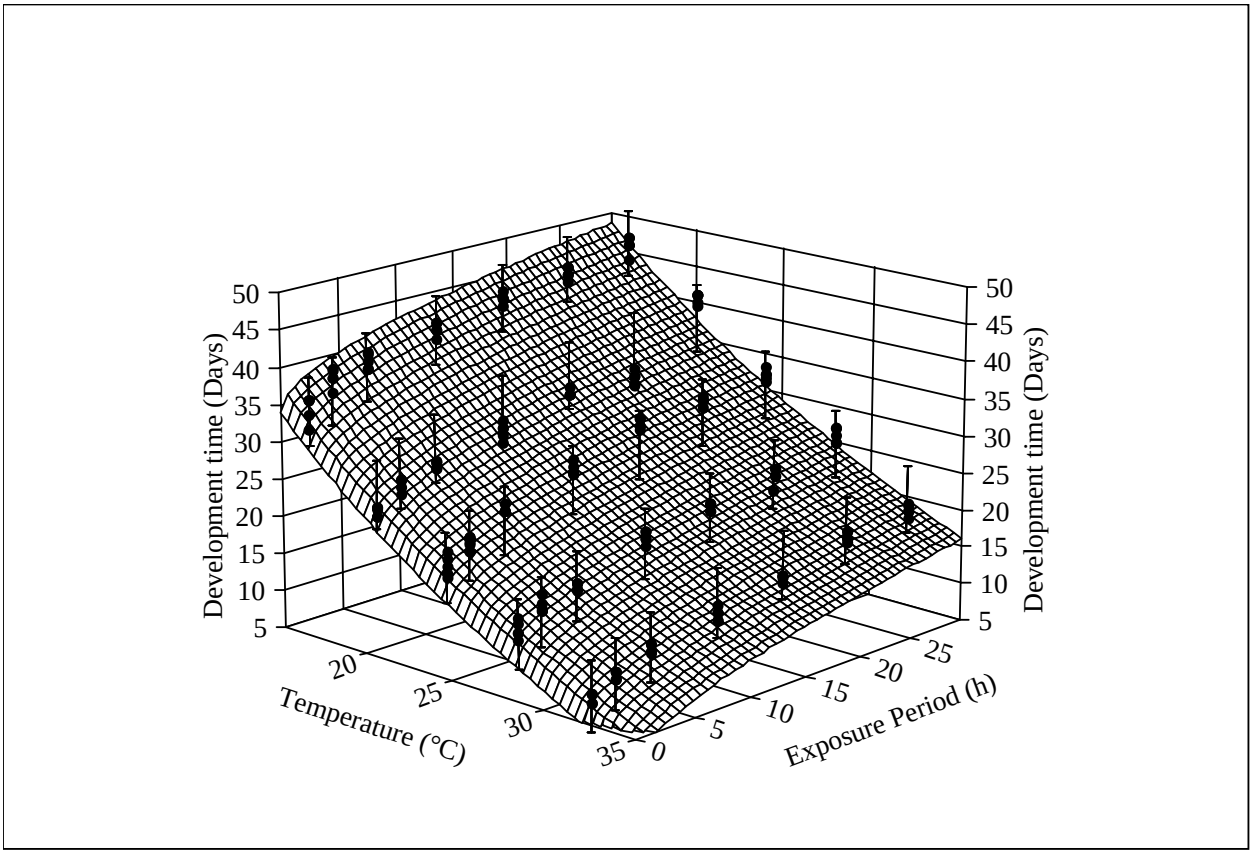

Development time (Days)
50
45
40
35
30
25
20
15
10
5
Temperature (°C)
20
25
30
35
Exposure Period (h)
0
5
10
15
20
25
Development time (Days)

**Fig. 2.**

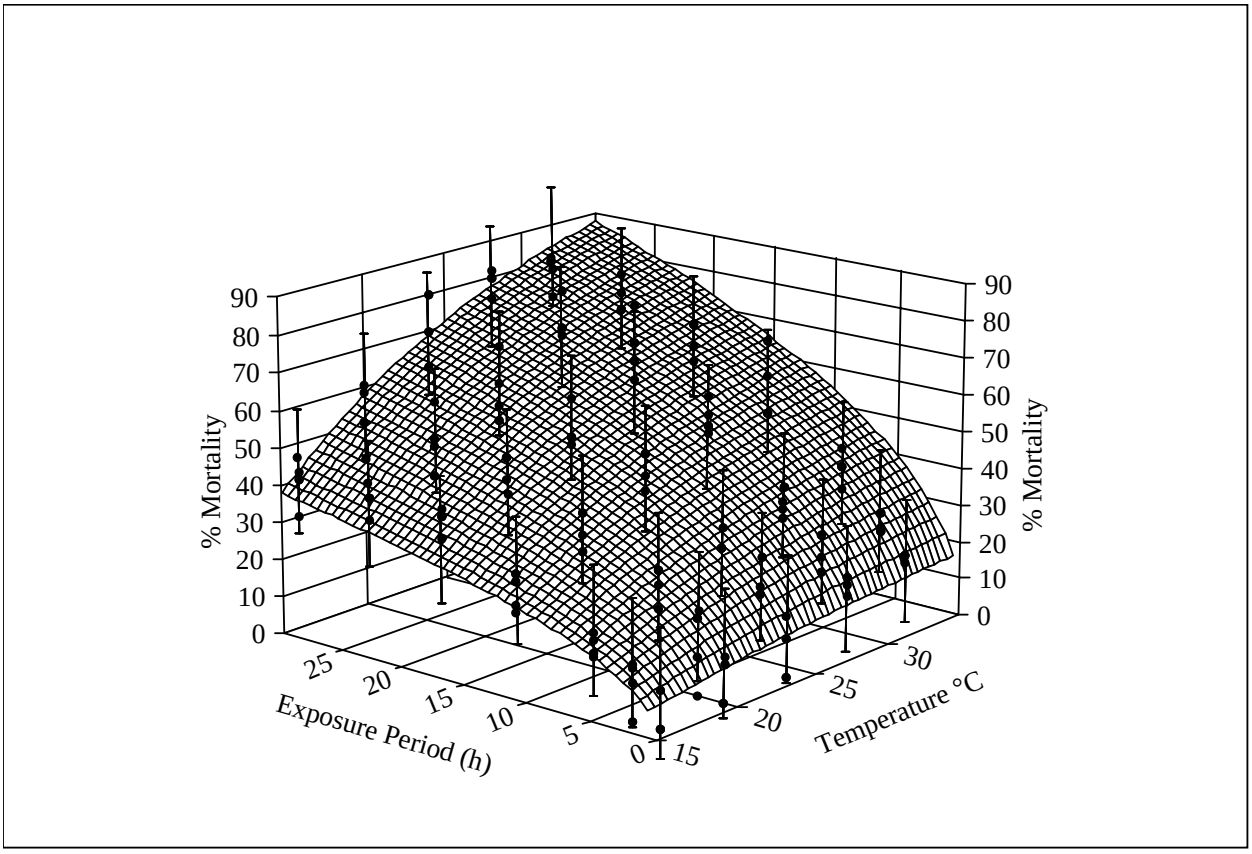

% Mortality
90
80
70
60
50
40
30
20
10
0
25
20
15
10
5
0
Exposure Period (h)
15
20
25
30
Temperature °C
% Mortality
90
80
70
60
50
40
30
20
10
0

**Fig. 3.**

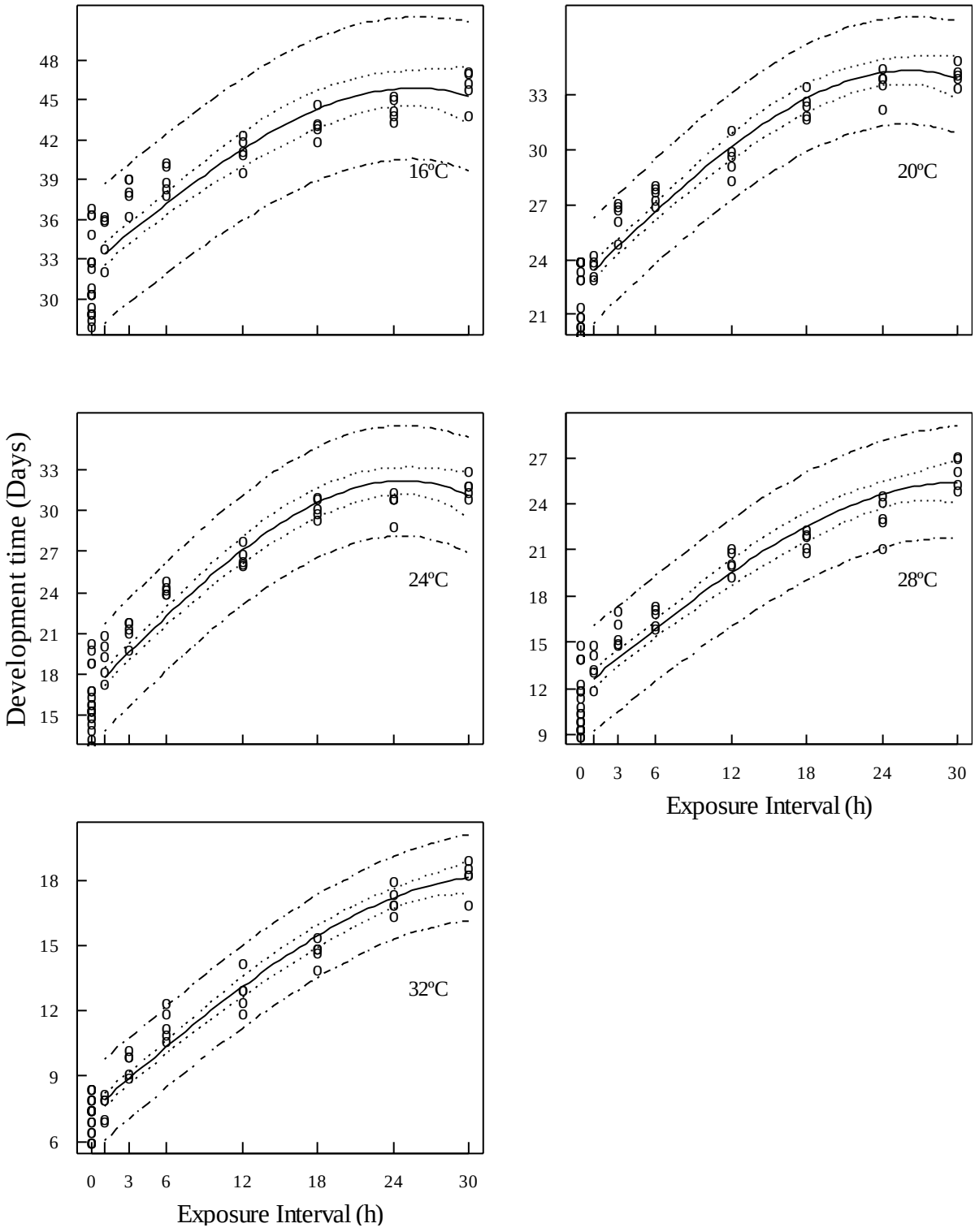

Development time (Days)
16ºC
20ºC
24ºC
28ºC
32ºC
Exposure Interval (h)
Exposure Interval (h)

**Fig. 4.**

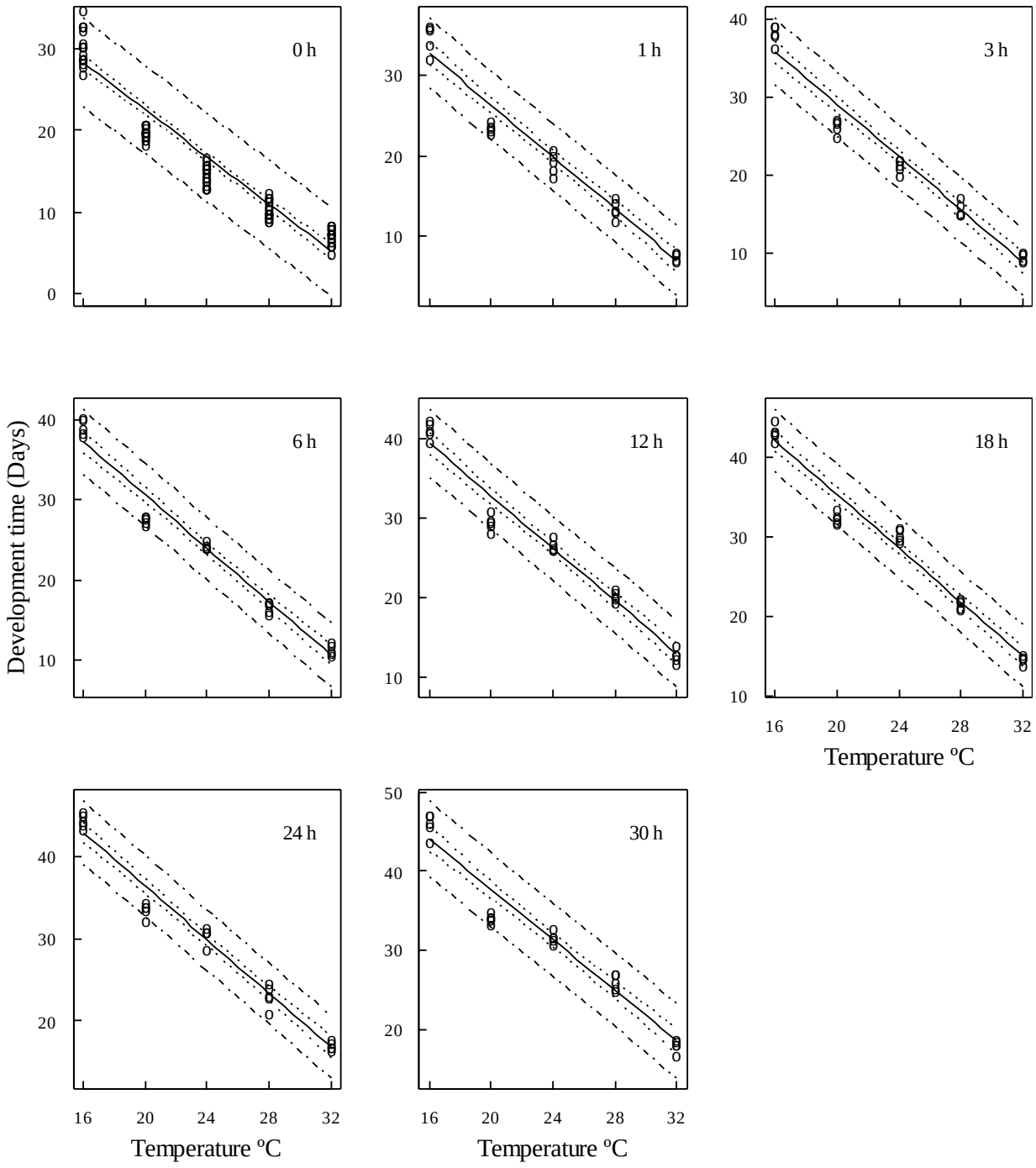

0 h
1 h
3 h
6 h
12 h
18 h
24 h
30 h
Development time (Days)
Temperature ºC
Temperature ºC

**Fig. 5.**

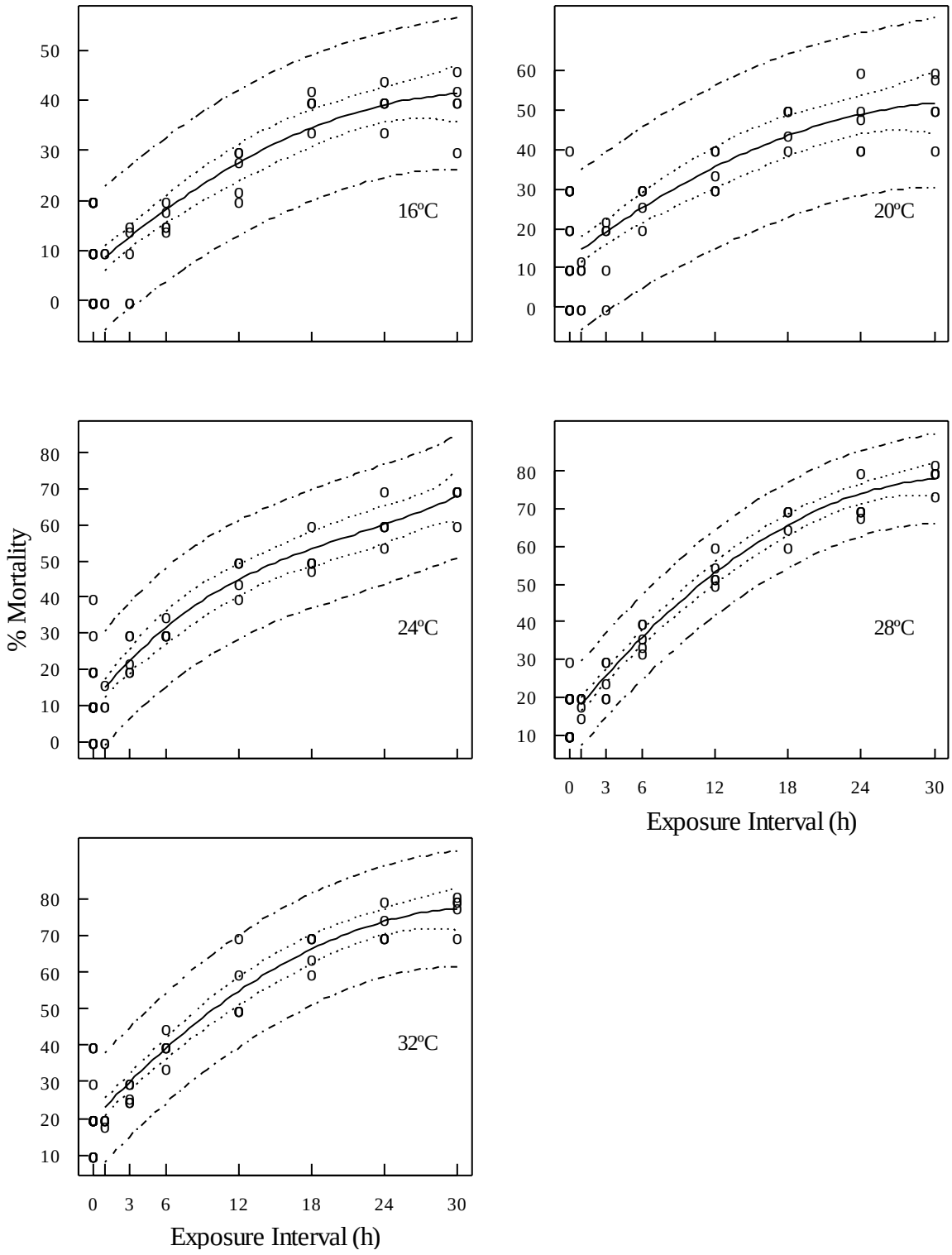

16ºC
20ºC
24ºC
28ºC
32ºC
% Mortality
Exposure Interval (h)
Exposure Interval (h)

**Fig. 6.**

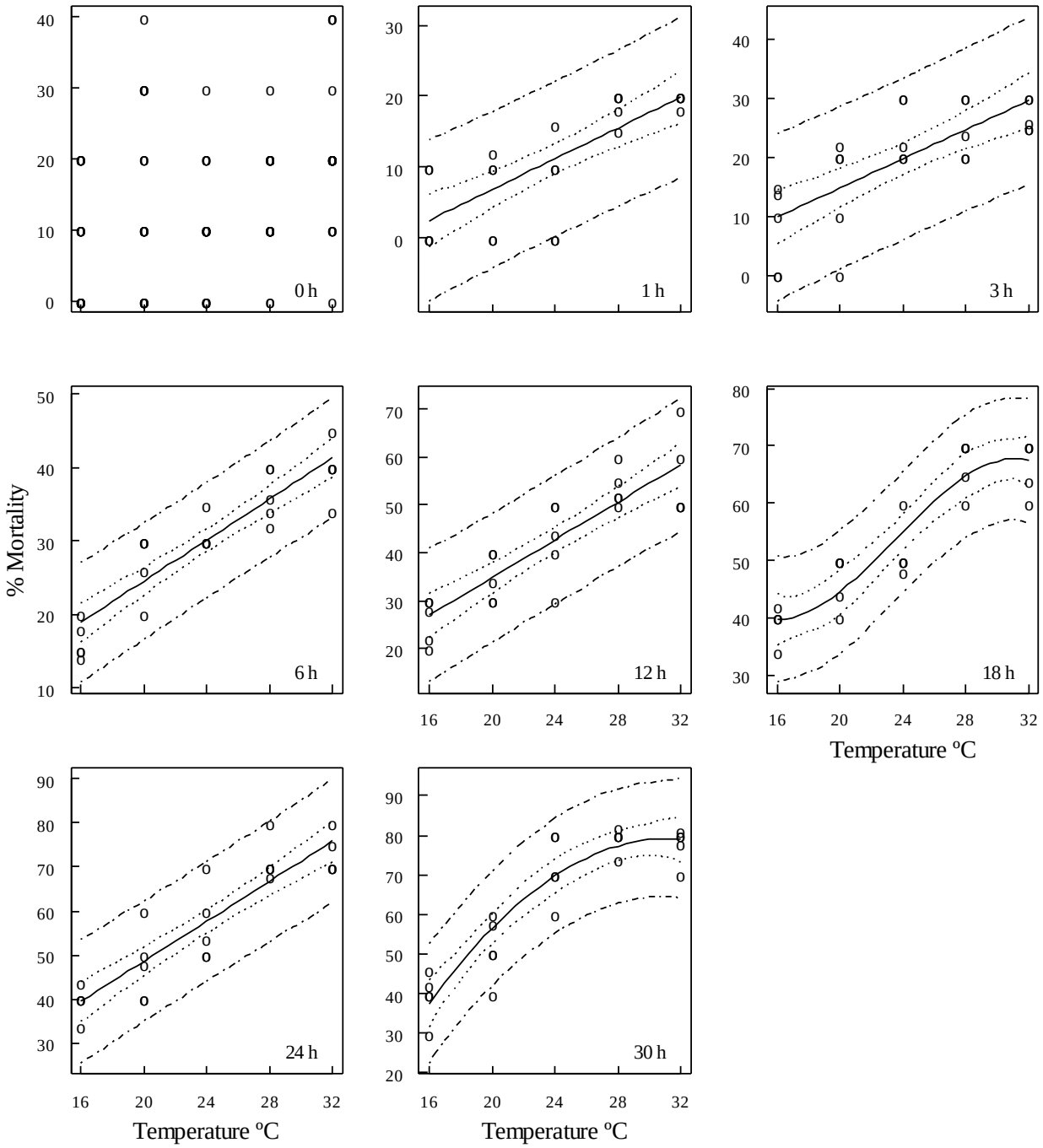

0 h
1 h
3 h
6 h
12 h
18 h
24 h
30 h
% Mortality
Temperature ºC
Temperature ºC

**Fig. 7.**

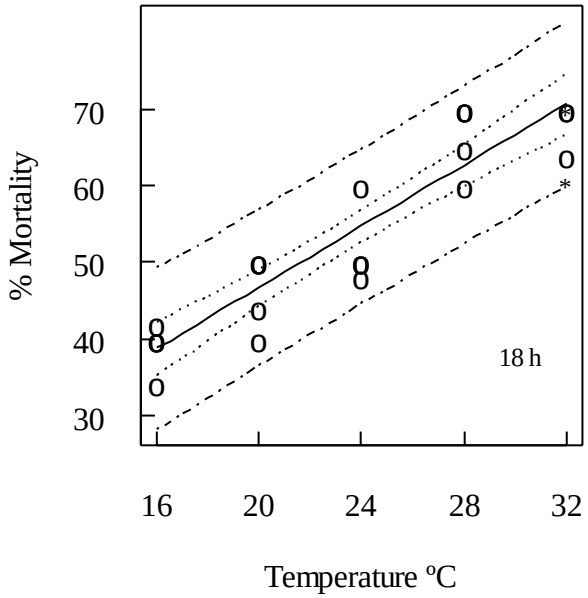
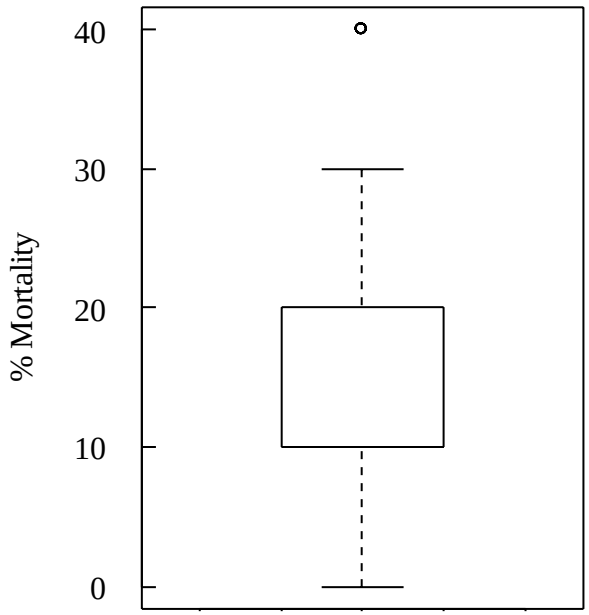


**Fig. 8.**



# CONCLUSIONS

The results of this study show that hydroprene can be used to control the egg and larval stages of Indianmeal moth when applied as a surface treatment. Other insect growth regulators may also possess similar properties and should be tested for Indianmeal moth management as well. The long-term influence of hydroprene on German cockroaches has been studied before (Reid and Bennet 1994). Similar studies on the populations of stored-product pests, including that of Indianmeal moth, would help in proper use of hydroprene in food handling environments that are sensitive to chemical applications like in packaging and retail facilities. Simulation models can be used to predict pest occurrence and evaluate a management strategy such as hydroprene application. Models for development and mortality derived from this study can be incorporated into a simulation model for the population dynamics of Indianmeal moth to optimize management strategies.

Resistance by insects to other insect growth regulators has been cited in the literature (Dame et al. 1998; Cornel et al. 2000, 2002). Therefore, necessary steps to slow resistance development by insects to hydroprene should be devised and followed. Insects may evade a lethal dose of hydroprene when applied only as a surface treatment; therefore, alternating the use of several of the hydroprene application methods could slow insect resistance development. Another possible method to slow resistance development is by timing and targeting of hydroprene application towards specific life-stages of Indianmeal moth, at least in storage facilities where overlapping generations do not occur.

I tested only one mode of hydroprene application, surface application, and on one specific floor surface, concrete. Tests with other insect growth regulators have shown varied effects of hydroprene on different flooring surfaces. Differences in the persistence or residual

activity of hydroprene may differ from one type of surface to another. Nevertheless, these studies and our own study indicate that hydroprene can be used for surface treatments in facilities having concrete flooring surfaces. Other insect growth regulators may possess toxicity to Indianmeal moth and should be evaluated against different life-stages. Methoprene and pyriproxifen recently have been labeled as aerosol treatments and for some surface applications, and they should also be tested for their effects on Indianmeal moth. More studies are needed to find alternative chemicals that can be used in rotation with hydroprene in stored-product environments.

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