COMBINATION TREATMENTS FOR CONTROLLING STORED PRODUCT INSECTS

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COMBINATION TREATMENTS FOR

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PRODUCT INSECTS

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DEDICATION

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NOMENCLATURE

°C	Temperature degree(s) at Celsius
cm	Centimeter(s)
F_1	First generation
g	Gram(s) (or gm)
Hg	Height of mercury
h	Hour(s) (or H)
kg	Kilogram(s)
L	liter (or l)
L: D	Light: Dark
LD_{99}	Lethal dose for 99% mortality
m	Meter(s)
m^3	Cubic meter(s)
mg	Milligram(s)
ml	Milliliter(s)
mm	Millimeter(s)
N	Sample size
OP	Organophosphate
ppm	Part per million
r.h.	Relative Humidity (or RH)
v	Volume
w	Weight
%	Percent
<	Less than
>	More than
±	Plus or minus

CHAPTER I

GENERAL INTRODUCTION

Background

Ancestors of humans appeared 3 million years ago, which was late about 250 million years compared with insects (Ware and Whitacre, 2004). Human beings have cultivated and stored agricultural products for the past 50-100 centuries (Zohary and Hopf, 1993). Many agricultural insect pests have been known to humans for thousands of years, and mankind has struggled to control insect damage to agricultural products for centuries. Archaeological evidence of some important insect pest species were found in the remains of foods placed in Egyptian tombs, which were built in 2,500 BC (Chaddick and Leek, 1972; Solomon, 1965). Of at least one million insect species worldwide, 10,000 species are crop-feeders. Among them, about 700 species cause damage to agriculture, including stored products (Ware and Whitacre, 2004). Many insects are well adapted to damage human stored products (Phillips et al., 1993), and hundreds of insect species are infesting stored products today (Gorham, 1987).

Stored product insects cause substantial economic and quality losses to these products. Losses due to insects worldwide are estimated to be 5-10% of the products. Even more alarming is that the proportion of stored grain destroyed by insects sometimes exceeds 20% of the volume (Ross, 1981). Heavier losses occurring in the developing tropical countries may reach 30-50%. In the United States the net value of losses in storage are estimated at $200 million up to $5 billion annually (Weaver and Petroff, 2005; Pimentel, 1991).

To manage storage product insect pests, several methods for prevention, suppression, and control are used. Methods include cultural controls, such as sanitation, aeration, drying, and exclusion; physical controls, such as extreme temperatures, inert dusts, controlled atmospheres, irradiation, and impact; biological control, such as insect natural enemy, microbial insecticides; and chemical control, such as residual synthetic insecticides and fumigants.

Recent Problems

Humans have used insecticides to control insect pests for millennia. Three thousand years ago sulfur in fumigation. The Chinese used arsenic to control garden insects 1,000 years ago (Ware, 1983). Compared with other controls measure today, chemical control is highly effective and easily employed. Since the1930s – 1940s, methyl bromide, DDT, and organophosphate insecticides have been discovered (University of Sydney, 2004). Farms used many of them exclusive. These insecticides were wide spectrum and cheap. According to Ware (1983), "pesticides are big business. The United States market is the world's largest representing 34 percent of the total."

There are significant problems associated with application of insecticides. They are hazardous to humans, and the natural environment. On the other hand, control of stored product insects face serious challenges worldwide, not only for food safety, but also for government regulation, and biological pressure because insects become more and more difficult to control (Phillips, 1994). Therefore, people should use integrated pest

management (IPM) to protect stored products. IPM techniques should consider all methods, although not all of them are needed in every situation. However, pesticides still need to be available (Weaver and Petroff, 2005)

Objective

To protect the world's food supply with minimal risk to human health and environment, four "Rs" must be considered: risk, residues, resistance, and resurgence. First of all, risk. Insecticides can harm humans and beneficial insects, and their use can lead to minor pests becoming major pests. The ecology can lose its balance and control can fail. Secondly, resistance. Due to their high reproductive ability many insects become resistant to insecticides. Thirdly, residues. Long-live insecticide residues can be dangerous. If insecticides are not steadily degraded by the natural environment, the soil will become a huge storage reservoir for insecticides. Residues of organo-chloride insecticides have been found not only in soil and water, but also in animal and human tissues. Fourthly, resurgence. Some populations treated with insecticide may quickly recover. This leads to repeated insecticide applications (ReVelle and ReVelle, 1974; Ross, 1981; University of Sydney, 2004).

Many chemicals and non-chemical alternatives to the hazardous insecticides have been proposed and researched for management stored product insects, and are effective. However, little is known about the effects of combining some control measures. The hypothesis of my research is that the combination of two or more insecticides or physical

methods or both can result in better control (mortality) than using individual compounds or physical methods in some cases. The overall goal of my project is to develop technologies for stored product insect management that rely on alternatives to organophosphate insecticides and the fumigants methyl bromide and phosphine. The three research chapters in this thesis are written as separate scientific articles intended for publication.

The first experiment uses a combination of low pressure and ethyl formate as an alternative to methyl bromide fumigation in a laboratory study. Second, I present the effects of combining methoprene and aeration against stored grain insects in a field study. Third is a laboratory study of diatomaceous earth combined with other insecticides to control stored wheat insects. Through the above investigations, I hope to determine the best combinations that can increase efficacy (even synergisms), and decrease risk for people and the environment. I hope to provide evidence or basic data for improved stored product insect management and for industry to develop new products.

Review of Some Stored Product Insect Biology

In Oklahoma, the main stored product insect pests are beetles and weevils (Coleoptera). Also some moth pests (Lepidoptera) damage stored products. The insect pets are divided into two groups according to feeding habits: external grain feeders and internal grain feeders. External grain feeders, such as *Plodia interpunctella, Tribolium castaneum* and *Cryptolestes ferrugineus,* also called secondary pests, can not infest sound grain but feed

on broken kernels debris, high moisture weed seeds, and grain damaged by internal grain feeders. In general, the immature stages of this group are found external to the grain. Moreover, after becoming established, external grain feeders contribute directly to grain spoilage, just as primary pests do. Internal grain feeders, such as *Rhyzopertha dominica* and *Sitophilus oryzae*, also called primary pests, are capable of penetrating and infesting intact kernels of grain, and have larvae that develop within a kernel of grain. However, internal grain feeders are the most important among stored grain pests because they cause insect-damaged-kernels (IDK) and reduce grain grade, as well as facilitating infestation by other insects and fungi (Phillips and Burkholder, in press; Weaver and Petroff, 2005). Following are brief descriptions of the insect species studied in this thesis.

The Indianmeal moth, *Plodia interpunctella*, (Lepidoptera: Pyralidae), is a common worldwide and serious pest of grain and milled products (Cuperus et al., 1990; Doud and Phillips, 2000). It is fully domesticated and adapted to human habitations (Phillips and Strand, 1994). Larvae have strong mandibles that easily penetrate hard seed coats.A female lays more than 200 eggs. The Indianmeal moth causes significant economic impact because of direct quantity and quality losses and indirectly due to the cost of pest control (Phillips et al., 2000). Adults are very active and short-lived, with a life cycle of about 30 days under optimum conditions (30 ^{o}C, and 75% r.h.) (Rees, 2001).

The red flour beetle, *Tribolium castaneum***,** (Coleoptera: Tenebrionidae), attacks stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Via,

1999; Weston and Rattlingourd, 2000). It does not infest sound grain. These beetles may elicit an allergic response (Alanko et al., 2000). They are important pests of stored products in homes, grocery stores, and flour mills. The adults can live more than one year. A female lays hundreds of eggs that can develop in a broad range of conditions (Phillips and Burkholder, in press). Their life cycle is about 20 days at optimum conditions (35 ^{o}C, and 75% r.h.) (Rees, 2001).

The rusty grain beetle, *Cryptolestes ferrugineus,* (Coleoptera: Laemophloeidae), is a common grain insect. Eggs are deposited loosely between kernels and in the cracks or furrows on the grain surface. After hatching, larvae feed in the germ layer of wheat kernels on broken kernels and grain dust. They often proliferate in large numbers, particularly through the winter, in the core of fine material that develops in the center of the grain mass. Adults are hardy and fly well in warm temperatures. The insect prefers high moisture grain or moist, decaying food. Females can lay up to 400 eggs that are often in splits or cracks in grain over a lifespan of up to nine months. The life cycle is 23 days at optimum conditions (33^{o}C, and 70% r.h.) (Rees, 2001).

The lesser grain borer, *Rhyzopertha dominica*, (Coleoptera: Bostrichidae), is a serious pest. Adults can fly very well and chew holes in grain. A female lays eggs in the grain mass outside of kernels and first instar larvae bore into the kernels. Larval development and pupation proceeds inside the kernel and young adults chew an exit hole to emerge (Potter, 1935; Hagstrum, et. al., 1995). It is a major pest of whole cereal grains, and proliferates in hot, dry conditions. The life cycle is 25 days at optimum conditions (34^{o}C,

and 70% r.h.) (Rees, 2001). Because most of the development occurs inside kernels, the lesser grain borer is very difficult to kill with contact insecticides applied directly to stored wheat (Arthur, 2004).

The rice weevil, *Sitophilus oryzae*, (Coleoptera: Curculionidae) adults can fly, and make a tiny feeding puncture in a grain kernel. It is a serious stored grain pest (Phillips et al., 1993). A female deposits an egg in a feeding puncture. The larvae hatch and feed within the grain kernel. Fumigation is recommended for grain that has already been attacked by rice weevils. The life cycle is 25 days at optimum conditions (30 ^{o}C, and 70% r.h.) (Rees, 2001). In Oklahoma the moisture content of stored grain is often less than 11.5%, so the rice weevil is not a serious pest in this state, since it requires more than 12% grain moisture content for development (Cuperus et al., 1995).

The cottonwood borer, *Plectrodera scalator,* (Coleoptera: Cerambycidae), belongs to the long-horned beetle family and is common in North America (Mason, 2005). Adults begin to emerge in late May or early June in Oklahoma (Anonymous, 2005). The adult is about 38mm long (not including antennae), with a black body color that is obscured by patches and cross stripes of fine pure white hairs that surround black, hairless areas. Mated females dig burrows at the base of trees and lay eggs in niches chewed in bark. Development requires 1 year or occasionally 2 years, before larvae pupate within larval galleries. Larvae are legless and oval in cross section, with creamy-white bodies and brown-to-black heads. Cottonwood borers primarily infest cottonwood, but also occur on poplars and willows. Larvae tunnel around the crown and buttress roots, and can cause

significant damage to young trees (Anonymous, 2004). The cottonwood borer was used in this research as a surrogate for quarantined wood-boring insects that could not be obtained for study.

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CHAPTER II

COMBINATION OF LOW PRESSURE AND ETHYL FORMATE AS AN ALTERNATIVE TO METHYL BROMIDE FUMIGATION

Abstract

In January, 2005, use of the fumigant methyl bromide was discontinued in the U.S. because it damaged the ozone layer. Many chemical and non-chemical alternatives are effective, but there has been little research about the potential synergisms of combining different treatments. In this laboratory study, a combination of low-pressure vacuum plus the insecticidal natural product ethyl formate were investigated for controlling three insect species. When eggs of the Indianmeal moth, *Plodia interpunctella*, exposed 4 hours to 50mm Hg vacuum or 50gm/m³ ethyl formate, the mortalities did not exceed 70.00%. However, a combination of 50mm Hg vacuum with doses up to 10gm/m³ ethyl formate was synergistic and resulted in complete mortality of all eggs in 4 hours. Probit analysis showed that the LD_{99} at 50mm Hg vacuum was 8.48gm/m³ ethyl formate for a 4-hour exposure. There were no significant differences in mortality between the untreated controls under a 50mm Hg vacuum alone for lesser grain borer, *Rhyzopertha dominica,* eggs, or cottonwood borer, *Plectrodera scalator,* larvae, However, ethyl formate combined with a 50mm Hg vacuum proved effective at killing both these life stage in four hours, and this effect improved with increased vacuum. Therefore, this combination showed excellent potential as a methyl bromide alternative against stored product and wood-boring insects.

Key Words: *P. interpunctella, R. dominica, P. scalator,* ethyl formate, vacuum

Introduction

Fumigation has been used against stored product insects for millennia. Sulfur was used as fumigant 3,000 years ago; phosphrous was used for pest control from the 1840s; methyl bromide has been used as fumigant since the 1930s (Ware and Whitacre, 2004). In the past, insect control in stored products relied almost entirely on fumigation by using methyl bromide and phosphine (PH_3). Methyl bromide is a toxic, ozone-depleting fumigant for crops, stored products, and quarantine insecticide applications. It was echoed in the decision of the Montreal Protocol. By 2005 and 2015 methyl bromide was or will be discontinued in developed countries and worldwide, respectively (UNEP, 1997). The U.S. ban of this fumigant in 2005 has motivated research on alternative methods to control stored product pests (Fields and White, 2002). Phosphine gas is another fumigant, but it is difficult to get good distribution of pellets in large grain bins, and a large bin needs a recirculation system. Another problem is the risk of development of insecticide resistance in the case of PH_3.

Chemical and non-chemical alternative treatments, such as ethyl formate and vacuum, are necessary and also available. Ethyl formate is an alternative that has been proposed for research. It can have a very rapid action against stored product insects. Therefore, it may be useful for stored product disinfestations (Damcevski and Annis, 1998). Application of vacuum or low pressure to a commodity in a gas-tight vessel results in an insecticidal low oxygen atmosphere at ambient temperatures (Mbata and Phillips, 2001).

Ethyl formate is a versatile volatile orgainic compound (Hilton and Banks, 1997), and has a long worldwide history as a storage fumigant (Neifert et al., 1925; Muthu et al., 1984). It is considered among "Substances Generally Recognized as Safe" (GRAS), and is a biologically-based fumigant and a food-safe insecticidal volatile that occurs naturally in many fruits and vegetables. Ethyl formate occurs in a variety of products including essential oils of grasses, beer, rice, beef, cheese, grapes, and wine (Desmarchelier, 1999). One important advantage of using volatiles for fumigation is that the residues remaining on treated commodities are often in a very little amount. So, the Food and Drug Administration (FDA) has reviewed the use of ethyl formate and has characterized this compound as GRAS. Ethyl formate was previously registered in California for control of several stored product insects, incloding the confused flower beetle, *Tribolium confusum* and the raisin moth, *Ephestia figulilella*. Ethyl formate was recently registered for insect pest control in Australia (Tipping et al., 2002).

Using low pressure for stored product insect control was employed eighty years ago (Back and Cotton, 1925), and has recently been revived (Finkelman et al., 2004). A low oxygen atmosphere achieved by application of a weak vacuum has been demonstrated to be an effective disinfestation method for durable food products. Vacuum treatment with flexible chambers is simple to apply, and is not toxic and inexpensive.

Even though many methods including chemical and physical controls are effective, each chemical has limitations for controlling insects (Bell et al. 1996), or has to be used in high doses. Some physical controls are expensive or damaging to commodities (Mbata

and Phillips, 2001). On the other hand, little has been investigated about the effects of combining one factor with some others. The objective of this study was to determine the effect of using low pressure combined with ethyl formate in a 4-hour control procedure for commodities infested with eggs of *Plodia interpunctella,* or *Rhyzopertha dominica,* or larvae of *Plectrodera scalator*.

The Indianmeal moth and the lesser grain borer represent typical stored product insects in two different orders and different life styles that are targets for methyl bromide fumigation. The cottonwood borer is tested here as a surrogate for exotic wood-boring insects of quarantine significance, such as the Asian long horned beetle. The reason is as following. Wooden crates and pallets are used when importing commodities to the U.S., and are usually associated with solid wood packing material (SWP). Exotic wood-boring insect pests can enter the U.S. in SWP. For example, the introduction of the Asian long horned beetle presumably started in China with beetle-infested SWP. The impending loss and world-wide reduction in the use of methyl bromide for fumigation necessitates alternative quarantine treatments.

Materials and Methods

Insects Rearing. *P. interpunctella* and *R. dominica* were from laboratory colonies at Oklahoma State University. *P. interpunctella* was reared on diet that contained about 37% corn meal, 25% egg crumbles, 25% chick starter, and 13% glycerin (v:v) and

maintained in a growth chamber at 28^{o}C, 65 ± 5% relative humidity, with an hourly photoperiod of 16:8 (L: D); *R. dominica* was reared on 400cm^3 sound wheat with 2.5cm^3 yeast in each jar at 28-30^{o}C, and 65 ± 5% relative humidity. *P. scalator* and an artificial rearing diet for larvae were obtained from the USDA Forest Service, NCRS, East Lansing, MI.

Treatments. Ethyl formate (98% + pure), $C_3H_6O_2$, was obtained from Acros Organics Co., New Jersey, USA. It is a water-white liquid with a fruity odor. Fumigation chambers for all experiments consisted of sealed 4-L glass jars configured with a syringe inserted through an air-tight injection port. The vacuum pump (Welch Scientific Company, Chicago, Illinois, USA) provided a negative pressure of 50mm Hg (-28 inches) to each fumigation jar. Eggs of *P. interpunctella* and *R. dominica* were used within 24 hours oviposition. *P. interpunctella* eggs were collected from jars containing 1-3 day-old moths. *R. dominica* eggs were obtained by placing many 4-5 week old adult beetles in a plastic box with sound wheat kernels and 5% all purpose wheat flour for 24 hours. Adults and food were then sieved to obtain eggs. For the vacuum-only treatment, and the combination treatments, vacuum was applied and checked for stable pressure for 4 hours. Twenty-five eggs of each species were attached to double-sided sticky tape on black paper, and placed in 6.0ml shell vials containing 100mg of whole wheat flour. Last larval instars of *P. scalator* were placed into plastic cups containing 100gm of artificial diet. These were then transferred in groups (4) into a 4-L fumigation jar. Then, low pressure of 50mm Hg was achieved by evacuating each labeled jar, and ethyl formate of a specified dose was introduced to each labeled jar through the airtight rubber septum connected to

its lid, and it rapidly volatilized. Finally, after treated jars were held for 4 hours in a growth chamber maintained at 25^{o}C.

Following fumigation, eggs or larvae were removed from their jars and transferred into a growth chamber maintained at 28^{o}C, 65 ± 5% r.h., with an hourly photoperiod of 16:8 (L: D) for *P. interpunctella* and *R. dominica* eggs, or 25^{o}C, 65 ± 5%, and an hourly photoperiod of 16:8 (L: D) for *P. scalator* larvae to allow for recovery and development. Egg mortality was evaluated on the 4th and 10th day post-fumigation for *P. interpunctella* and *R. dominica*, respectively, by observations of egg hatch. *P. scalator* larval mortality was evaluated after 3 days of recovery by observation for movement when stimulating larvae with a probe.

Statistical Analysis. Data were analyzed separately for egg mortality of *P. interpunctella* and *R. dominica,* and larvae mortality of *P. scalator* by using the General Linear Models (GLM) procedure (SAS Institution, 2002). All data were subjected to analysis of variance (ANOVA) followed by a Student-Neumann-Kohl (SNK) test for comparison of mean to analysis. Proportional data were transformed by the angular transformation prior to ANOVA, while means and standard errors (SE) were untransformed. The significant differences were considered at $P \leq 0.05$ level. Probit procedure (SAS Institution, 2002) was employed to estimate LD_{99} in gm/m^3 for ethyl formate alone, and when combined with 50mm Hg vacuum.

Results

There was a slight but significant difference between control and 50mm Hg vacuum alone for *P. interpunctella* egg mortality (Table 2-1). The egg mortality of *R. dominica* was not significantly different between the vacuum and control (Table 2-2). Ethyl formate elicited substantial mortality at 50gm/m^3 alone, the egg mortality of *P. interpunctella* and *R. dominica* was 70.00% and 90.33% at 50gm/m^3 alone, respectively. However, egg mortality increased to 100% by using the combination of vacuum plus ethyl formate for both species (Table 2-1; 2-2).

There was not significantly different between untreated controls and the 50mm Hg vacuum treatment alone for *P. scalator* larvae (Table 2-3.). Ethyl formate at 50gm/m^3 alone proved highly effective at killing this species in four hours 66.67%, but ethyl formate at 50gm/m^3 combined with vacuum of 50mm Hg showed a synergistic effect with 100% mortality.

Probit analysis was conducted to determine dose-response with the two species of stored product insects. The results showed that the concentration of ethyl formate needed to achieve 99% mortality (LD_{99}) of *P. interpunctella* was reduced from 552.62gm/m^3 for ethyl formate alone to 8.48gm/m^3 ethyl formate when it was combined with vacuum 50mm Hg. For *R. dominica* this difference was from 365.49gm/m^3 ethyl formate alone

to 44.04gm/m^3 ethyl formate, when it was combined with vacuum at 50mm Hg (Table 2-4).

Discussion

Ethyl formate demonstrates potential as an alternative fumigant for methyl bromide against stored product insect pests. People need an effective method to control storage insect after methyl bromide was banned. Results of this study provide evidence that the combination of vacuum plus ethyl formate is an effective method to meet this need.

Controlling *Plectrodera scalator* is more difficult challenge. Earlier work (Liu, unpublished) found that larvae of *P. scalator* could survive more than 14 days when held under low pressure only. Vacuum alone caused no mortality. Ethyl formate at 50gm/m^3 alone proved caused 66.67% mortality. Nevertheless, the combination of vacuum and 50gm/m^3 of ethyl formate over four hours caused 100% mortality of *P. scalator* larvae, and it is showing a potential synergism. Therefore, combination of ethyl formate and vacuum has good potential as a methyl bromide alternative for treating timber or SWP material that may be infested with woodborers, such as *Plectrodera* species. A treatment of 4 hours is practical for quarantine purposes.

Some of the experiments reported here suggest that vacuum and ethyl formate were synergistically to kill insects. The potential synergisms may be improved with vacuum

-- a low oxygen atmosphere. When insets are exposed to a low oxygen environment, oxygen concentration falls below critical levels needed for insect respiration. Moreover, low pressure could cause insects to lose water (Mbata and Phillips, 2001). This may increase susceptibility to ethyl formate in this situation, but different species could have different tolerances to low pressure.

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Table 2-1. Mean mortality of *P. interpunctella* eggs using ethyl formate or vacuum alone, or in combination

Treatment	Mean Mortality % (SE)	SNK Grouping
Control	2.75 (0.25)	D
V50	10.50 (1.55)	C
EF50	70.00 (10.69)	B
V50 + EF50	100.00 (0.00)	A

V50 = Vacuum 50 mm Hg; EF50 = Ethyl formate 50 gm/m^3.

Means followed by a different letter are significantly different.

SNK test, $P \leq 0.05$; N=4.

Table 2-2. Mean mortality of *R. dominica* eggs using ethyl formate or vacuum alone, or in combination

Treatment	Mean Mortality % (SE)	SNK Grouping
Control	6.67 (0.67)	C
V50	8.50 (0.50)	C
EF50	90.33 (1.76)	B
V50 + EF50	100.00 (0.00)	A

V50 = Vacuum 50 mm Hg; EF50 = Ethyl formate 50 gm/m^3.

Means followed by the same letter are not significantly different.

SNK test, $P \leq 0.05$; N = 4.

Table 2-3. Mean mortality of *P. scalator* larvae caused by using ethyl formate or vacuum alone, or in combination

Treatment	Mean Mortality % (SE)	SNK Grouping
Control	0.00 (0.00)	C
V50	0.00 (0.00)	C
EF50	66.67 (8.33)	B
V50 + EF50	100.00 (0.00)	A

V50 = Vacuum 50 mm Hg; EF50 = Ethyl formate 50 gm/m^3.

Means followed by the same letter are not significantly different.

SNK test, $P \leq 0.05$; N = 4.

Table 2-4. Estimated LD_{99} in gm/m^3 for ethyl formate alone, and when combined with 50mm Hg vacuum determined from dose-response studies

Species	EF50 alone	V50 + EF
IMM	552.62	8.48
LGB	365.49	44.04

V50 = Vacuum 50 mm Hg; EF = Ethyl formate

IMM: *Plodia interpunctella,* the Indianmeal moth

LGB: *Rhyzopertha dominica,* the lesser grain borer

CHAPTER III

COMBINATION OF METHOPRENE AND AERATION AGAINST STORED GRAIN INSECTS

Abstract

There are significant risks associated with application of hazardous insecticides against stored grain insects. Integrated pest management can utilize reduced-risk insecticides, such as physical barriers and bio-rational controls. In this study, the insect growth regulator methoprene alone or in combination with grain aeration was evaluated to prevent infestation of wheat in storage bins. Grain temperature monitoring and aeration fan control were automatically operated by the OPI computer system. Results from storage bins and laboratory bioassays showed that methoprene is excellent for controlling externally feeding stored grain insects such as *Plodia interpunctella*, *Tribolium castaneum*, *Cryptolestes ferrugineus* and some internal feeders such as *Rhyzopertha dominica.* Methoprene did not give good control of the more tolerant internal feeders such as *Sitophilus oryzae*. Aeration treatment alone was somewhat effective in controlling insect development. Nevertheless, when methoprene was used alone or in combinations it clearly enhanced control. Grading grain at the end of the study confirmed these results, as there were 9 IDK (insect damaged kernels per 100g wheat) in aeration control bins, compared with 4.25 and 3.33 IDK in top and methoprene alone treatments, respectively. There were only 2 IDK in combined methoprene plus aeration treatments. This study revealed that the combination treatments were effective. This course leads to combined applications in the future where aeration could be combined with some short-lived, unstable reduced-risk insecticides against stored grain insects.

Key Words: *Plodia interpunctella*, *Tribolium castaneum*, *Cryptolestes ferrugineus, Rhyzopertha dominica, Sitophilus oryzae*, methoprene, aeration, stored grain bins, OPI system.

Introduction

Since the 1940s, synthetic insecticides have been used against insect pests. Many of these insecticides are high toxicity neurotoxins. Stored grain insects cause substantial economic and quality losses to grain. Although fumigation treatments are useful, there are significant problems associated with fumigation such as high toxicity, development of resistance in pests, and environmental and economic damage. New insecticide development is not easy. So since the 1980s identification of alternatives to highly toxic insecticides has been became a focus for research worldwide (Dales, 1994). During the past decade, integrated pest management (IPM) has received increasing emphasis for stored grain. IPM involves using reduced-risk insecticides and non-chemical controls for stored grain insect pests, that include bio-rational controls (such as insect growth regulators (IGRs), botanical chemicals, and behavior modifying chemicals), biological controls (parasitoids, predators, and pathogens), physical controls (inert dusts, extreme temperature, aeration, and controlled atmospheres).

IGRs include juvenile hormone analogues (JHAs), which were discovered 1960s. JHAs are considered reduced-risk insecticides. They interfere with insect metabolism in a manner that disrupts normal growth and development. Usually, the insect dies before it

mature. IGRs are selective for insects and exhibit low mammalian toxicity. Insects develop from eggs to adults through immature stages during which the exoskeleton is renewed a number of times by the process of molting. IGRs may either inhibit the synthesis of the chitin required for forming new cuticle at each molt, or disrupt or replace the production of JHAs that controls the molting (Mian et al., 1990; Bengston, 1987; Dales, 1994).

Methoprene is a synthetic JHA that has been evaluated for insect control (Oberlander et al., 1997). During the 1980s in the United States, a methoprene formulation called Diacon was registered as a stored grain protectant. Unfortunately, it reached a deadlock in the market due to its expense as well as the existence of other effective insecticides (Arthur, 2004). In recent years, along with more consideration of the problems in employing insecticides, there is renewed interest in using IGRs in stored grain (Oberlander et al., 1997). A new formula, (S)-methoprene, the active ingredient in trade mark Diacon II, is registered for controlling the following insects: almond moth, Indianmeal moth, cigarette beetle, lesser grain borer, sawtooth grain beetle, merchant grain beetle, red flour beetle, and confused flour beetle.

Aeration is a part of IPM strategies to control insect infestation in stored grain (Phillips et al., 2000). At grain temperatures of less than 15^{o}C, insect activity slows, while higher temperatures allow for increased insect growth and breeding. At temperatures less than 5^{o}C, many insects become dormant, and some die. Insect mortality increases at lower temperatures. Aeration is effective in controlling stored product insect populations

through drying and cooling, and is recommended to control stored grain insects (Phillips and Burkholder, in press). Methoprene or aeration have been proposed and researched, but little is known about the effects of Diacon II alone, or combining it with aeration.

The first objective of this study was to compare and contrast 1.0ppm methoprene used alone as a 50cm top-dressing and complete treatment to grain, or combined with grain aeration to prevent infestation of new hard red winter wheat stored in grain bins. The second objective was to evaluate the stability of methoprene residues on hard red winter wheat stored in field bins for 10 months. Activity against insects through methoprene residual analysis at the beginning and the end of the storage and laboratory bioassays after three storage periods was evaluated. The grade of the grain at the beginning and the end of storage period was also compared.

Materials and Methods

Field Study

Diacon II (EPA registration number is 2724-427; (S)-Methoprene: Isopropyl (2E, 4E, 7S)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) was used in this study. The chemical belongs to the family Terpenoid; formula is C_{19} $H_{34}O_3$. Methoprene was 33.6% in the undiluted formula, with 66.4% other ingredient (petroleum distillates; Wellmark International, Schaumburg, Illinois, USA).

Insect Rearing. *Tribolium castaneum*, the red flour beetle (Coleoptera: Tenebrionidae), *Rhyzopertha dominica*, the lesser grain borer (Coleoptera: Bostrichidae), and *Cryptolestes ferrugineus*, the rusty grain beetle (Coleoptera: Laemophloeidae) were used in this field study. The insects were from laboratory colonies. *T. castaneum* was reared on 400cm^3 whole hard red winter wheat flour plus 2.5cm^3 yeast in a rearing jar. *R. dominica* was reared on 400cm^3 sound wheat with 2.5cm^3 yeast in each jar. *C. ferrugineus* was reared on 400cm^3 oats with 5cm^3 whole hard red winter wheat flour and 2.5cm^3 yeast in each jar. All beetles were placed at 28-30^{o}C and 65 ± 5% r.h. growth chambers.

Treatments. Sixteen, 500-bushel cylindrical steel bins at the Oklahoma State University Stored Product Research and Education Center (SPREC) were used. Bins were equipped with aeration fans and temperature sensors that recorded temperatures of the grain through the grain mass. Sensors are placed on permanent cables suspended from the top of each bin. Grain temperature was monitored and the fans were controlled by the computerized OPI system automatically. Grain used was newly harvested hard red winter wheat, *Triticum aestvium.* The following four treatments were randomly applied to four bins each: aeration only; methoprene only used at the 1.0ppm label rate; methoprene 1.0ppm just to the top 50cm of the grain mass; and a combination of 1.0ppm methoprene plus aeration. Methoprene spray treatments were applied directly to grain as it was loaded into bins by spraying the diluted product onto grain as it was dumped from a truck into the hopper at the bottom of an auger-type conveyor that elevated the grain into the bins.

A radio transmitter station sends temperature data to the computer that controls all aeration fans turning them on automatically to cool the grain when the outside ambient air temperature drops a set interval below the grain temperature. It turns them off when the air temperature exceeds a set grain temperature. Four aeration cycles were applied to the grain: cooling to a set-point of 30°C during the summer; cooling to 25°C in the later summer; cooling to 15°C in the fall and the spring; and a cooling to 7°C in the winter.

After methoprene were treated, one hundred adults each of *T. castaneum*, *R. dominica*, and *C. ferrugineus* were added to each bin once weekly for four weeks. After insects were added one month, probe trap samples and grain trier samples were taken. One WB-II probe trap was inserted into the top 30cm in grain for 7 days. Captured insects were then identified and counted. Grain trier (1.5m-long double brass tube) samples (more than 1.0kg of grain) were collected for weighing and sifting and for species identification and counting. Samples were taken once per month for a total of 6 times.

Laboratory Bioassay

Insects Rearing. The Indianmeal moth, *Plodia interpunctella*, (Lepiodptera: Pyralidae), the red flour beetle, *Tribolium castaneum*, (Coleoptera: Tenebrionidae), the lesser grain borer, *Rhyzopertha dominica*, (Coleoptera: Bostrichidae), and the rice weevil, *Sitophilus oryzae*, (Coleoptera: Curculionidae) were used in the laboratory growth chambers. *P. interpunctella* was reared in glass jars on the diet that contained 37% cornmeal, 25% egg crumbles, 25% chick starter, and 13% glycerin (v:v) at 28^{o}C and 65 ± 5% r.h., with an hourly photoperiod of 16:8 (L:D). *T. castaneum* and *R. dominica* was reared as the

insect rearing of field study. *S. oryzae* was reared on 400cm^3 sound wheat plus 2.5cm^3 of yeast in each jar, at 28-30^{o}C and 65 ± 5% r.h. growth chambers.

Treatments. Grain used for laboratory bioassays was taken directly from the field study grain bins at different times after treatment: immediately after loading (Time 0 month), after 5 months (Time 5 months) and 10 months (Time 10 months) storage. *P. interpunctella* eggs that were 0-24 hours old were collected from jars containing 1-3-day-old moths. Twenty *P. interpunctella* eggs attached to double-sided sticky tape on black paper were placed in a 120ml jar containing 20g sound wheat plus 20g crushed wheat and maintained at 28^{o}C and 65 ± 5% r.h. with an hourly photoperiod of 16:8 (L: D) in a growth chamber. Egg hatch was checked after 1 week, and counts of normal adults were made after 6, 7 and 8 weeks.

Fifty of *T. castaneum*, *R. dominica*, and *S. oryzae* adults were individually placed in 240ml jars containing 100g wheat (5g crush for *T. castaneum*) and maintained at 28^{o}C and 65 ± 5% r.h. and an hourly photoperiod of 16:8 (L: D) in a growth chamber. After 1 week the parent adults were removed and placed in individually clean Petri dishes for a recovery period of 24 hours, and then counted to determine mortality. After 6 weeks F_1 progeny in the grain were sifted and counted.

Grading and methoprene residual analysis were carried out at both the beginning and the end of this study. Samples (each > 1.0Kg) from each bin were sent to a grain inspection

company that was authorized by the Federal Grain Inspection Service to determine of final grade, to Wellmark International for methoprene residue analysis.

Statistical Analysis

Data were analyzed separately for each species or different treatments by using the General Linear Models (GLM) procedure (SAS Institution, 2002). All data were subjected to analysis of variance (ANOVA) followed by a protected least significant difference (LSD) test for comparison of means. Data were transformed by square root of the number plus 0.5 prior to ANOVA, while mean and standard errors (SE) were untransformed. The significant differences were considered at $P \leq 0.05$ level.

Results

Field Study

Tables 3-1 and 3-2 present mean numbers of *T. castaneum* adults collected by probe traps or grain trier samples. Statistical analysis revealed as follows. First, from probe trap samples, in August there were no significant differences among the mean adult numbers in three methoprene treatments, but there were differences between aeration control compared with methoprene combined with aeration (Table 3-1). In October and November there were differences between aeration control and methoprene alone or in combination. In December there were no differences among all treatments. There was no difference between aeration and methoprene top treatment in September and April 2005, however there were significant differences between aeration and methoprene alone or as a combination treatment. To understand this phenomenon, the mean numbers of

treatments at different sampling months were analyzed. In the aeration control treatment mean adult numbers increased from August to September 2004 then declined through April 2005. In the three methoprene treatments (top, methoprene alone, and combination with aeration) there were no significant differences between August and September, then the mean adult numbers decreased through April 2005. Secondly, from grain trier samples in August and September there were no significant differences in adult numbers among the three methoprene treatments, but there were differences between aeration controls compared with methoprene combined with aeration, or methoprene alone (Table 3-2). In October there were no significant differences between aeration control and methoprene top, between methoprene top and methoprene alone or between methoprene alone and in combinations, however methoprene combined with aeration proved an effect. In November, December, or in April 2005 there were no differences between treatments. On the other hand, analysis for each treatment shows that mean adult numbers decreased in aeration control treatment. In the top-dressing methoprene mean adult numbers in September and October were larger than those in other months. In methoprene alone treatments in all months there were no differences across the row. In methoprene combined with aeration treatment the mean adult numbers increased from August to September, then decreased and was not significantly different from October to April 2005.

Tables 3-3 and 3-4 present mean numbers of *R. dominica* collected by probe traps or grain trier samples. Statistical analysis showed as follows. First, from probe samples, in August, September, and November there were no significant differences in mean adult

numbers among control aeration and three methoprene treatments (Table 3-3). In October mean number in control aeration was larger than other treatments. In December mean number in top methoprene was larger than other treatments. In April 2005 mean number in methoprene combined with aeration was larger than other treatments. Analysis for the mean adult numbers of each treatment at different sampling times showed that mean adult numbers were not significantly different in aeration treatment or methoprene alone. In top methoprene the mean adult number in December was larger than those in other months. Mean adult number decreased in methoprene combined with aeration treatment. Secondly, the results from grain trier samples (Table 3-4) showed that there were no significant differences among the three methoprene treatments, but there were significant differences between aeration controls and methoprene top treatment in August or methoprene alone in October and November. Mean adult number from top treatment was larger than those in other treatments in September. There were no differences between aeration control and three methoprene treatments in April, 2005. Analysis for each treatment shows that mean adult numbers from control aeration treatment in October and December, methoprene top treatment in September, or methoprene combined with aeration treatment in August and October were larger than those in other months. Mean adult numbers decreased from methoprene alone treatment.

Tables 3-5 and 3-6 present mean numbers of *C. ferrugineus* adults recovered by probe traps or grain trier samples. Statistical analysis revealed as follows. First, the results from probe samples showed that in August and September there were no significant differences in mean adult numbers among control aeration and three methoprene

treatments (Table 3-5). In October the mean adult number in aeration alone treatment was significantly different and larger than that in methoprene alone treatment. In November and December the mean adult number in aeration alone treatment was significantly different and larger than those in methoprene alone or combined with aeration treatment. In April 2005 the mean adult number in aeration treatment was significantly different and larger than those in all three methoprene treatments. Analysis for each treatment shows that mean numbers in the aeration control treatment decreased from August to December and increased in April 2005. In methoprene top treatment mean numbers were not significantly different. Mean numbers decreased in methoprene alone and methoprene combined with aeration treatment. Secondly, from grain trier samples, in August there were no significant differences in adult numbers among the three methoprene treatments but there were differences between aeration control and methoprene combined with aeration treatment (Table 3-6). In November there were no significant differences in adult numbers among three methoprene treatments but there were differences between aeration control and methoprene alone or methoprene combined with aeration. In September, October, and April 2005 there were no significant differences in adult numbers among three methoprene treatments but there were differences between aeration control and three methoprene treatments. In December methoprene alone and the combination had a big effect. Analysis for each treatment showed that the peaks of mean adult numbers from aeration control and top methoprene treatments appeared in December. The peak from methoprene alone treatment was in August, and those from methoprene combined with aeration treatment were in September and November.

Laboratory Baioassay

None of the methoprene treatments had any significant effect on parent adults of the beetles tested after one week of exposures when compared with untreated grain. Table 3-7 presents the results of F_1 progeny from laboratory bioassays for each beetle species individually. Statistical analysis showed that methoprene was effective to control *T. castaneum*, the red flour beetle, at the three residual storage times including the methoprene top treatment for the three residual times. Methoprene was also effective to control *R. dominica*, the lesser grain borer, at the three residual times even though top methoprene treatment was different from methoprene alone, and in combination. Methoprene was not effective in controlling *S. oryzae*, the rice weevil.

Table 3-8 presents the results for *P. interpunctella* emergence to normal adults following exposure to treated grain at different storage residual times. Methoprene had an excellent effect for controlling this moth even though top methoprene treatment was different from methoprene alone and in combination with aeration after being stored 10 months. No normal adults emerged in methoprene alone and methoprene combined with aeration treatments from the wheat stored 0, 5 or 10 months.

Grain Grading

Grading results showed that the test weight and the grade of stored grain were not decreased after 10 months storage (Table 3-9). There were 9 damaged kernels per 100 gram (IDK) in aeration treatments; there were 4.25 and 3.33 IDK in methoprene top and

methoprene alone treatment, respectively; but there were only 2 IDK in the methoprene combined with aeration treatment.

Residue Analysis

Treated wheat samples for (S)-Methoprene analysis was carried out at the start and end of storage period (Table 3-10). Each sample was in a clear, canning glass jar with typical canning lid and a number written on top. Sample results in w/w% for (S)-Methoprene were converted to ppm. Methoprene residue analysis showed that methoprene had 71.38% and 68.72% remaining from methoprene alone and combination treatment, respectively, after grain was stored for 10 months. Statistical analysis showed that aeration did not significantly affect the residues remaining after 10 months of stored. Methoprene top treatment still had 36.87% remaining after grain was stored for 10 months.

Figure 3-1 shows that grain temperatures were less than 15^{o}C in aerated bins. Aeration treatment had an effect on cooling the internal grain temperature, which can have an effect on insect growth in the fall and the winter season (Table 3-1, 3-3). However, some species of stored grain insect pests tolerate cool temperature, such as *Cryptolestes ferrugineus* (Table 3-5, 3-6).

Discussion

Insects in this study are from two orders and four families and represent common pests in stored grain in Oklahoma. These would be the most challenging to affect with an insect

growth regulator. This study showed that the new methoprene formulation Diacon II was effective for controlling some stored grain insect species, such as *Plodia interpunctella, Tribolium castaneum*, *Rhyzopertha dominica*, and *Cryptolestes ferrugineus*. Unfortunately, it was not effective or it is difficult to control the more tolerant insects such as *Sitophilus oryzae*. Therefore, methoprene was effective against external-feeding, and some internal-feeding stored grain insects such as *R. dominica*, in which the eggs are laid in the grain mass outside of kernels and the larvae bore into the kernel. Methoprene does not give effective control of some internal-feeding stored grain insects such as *Sitophilus* species because the eggs are laid inside the grain kernels and the larvae avoid contact with the residue. On the other hand, this study implied that at different times of the year, results among all treatments are not only dependent on the treatment but are also closely related the seasonal temperature.

Diacon II is not effective against adults, which was expected, but it showed an excellent effect on preventing the development of exposed larvae. So this study did not focus on mortality of exposed adults to methoprene but studies focused the effects on progeny. In general, wheat is stored for 3-9 months in farm bins (Martin et al., 1997). So, application of methoprene to wheat needs to provide protection from insect infestations for up to 9 months. The probe trap samples show insect moving ability, the grain trier samples represent a direct sample of insect density. The two types of sampling methods had similar levels of variation. Diacon II at 1.0ppm could be applied to wheat to provide protection from insect infestations for at least 10 months based on methoprene residual analyses. The results of laboratory bioassay confirmed the results from the field study.

Methoprene is relatively expensive compared with other insecticides, so a low dose is necessary. Upon comparing and contrasting the three methoprene treatments, top-dressing may be a good method to apply. Furthermore, top treatment or combination with other methods may prove economical results if it is effective. Moreover, methoprene does not control all pests. This limits its usefulness as a stored product protectant. Its potential is limited by the need for an integrated pest management method to be effective on the more tolerant insects. In order to overcome this problem, methoprene applied in combination with other insecticides or methods may be a good idea. This combination may enhance population suppression of internal feeders in stored grain. These results show that combinations with aeration can help increase progeny suppression of some grain pests in certain situations.

In this study, aeration did not significantly reduce methoprene degradation or extend the chemical effectiveness. Because methoprene is relatively stable, aeration could not show an effect on reducing chemical degradation. Perhaps aeration combining with other reduced-risk insecticides, especially insecticides that are not very stable, can show more effects by helping to increase progeny suppression of internal grain pests. Regardless, when methoprene was combined with aeration it seemed to have an indirect effect on population suppression of internal feeders in stored grain. The end grading confirmed to these results: there were 9 IDK in control aeration bin,but there are only 2 IDK in methoprene combination with aeration. The combination treatment had an effect. This

study revealed that combinations have potential in the future when aeration combined with some unstable reduced-risk insecticide against stored grain insects.

The integrated pest management approach to stored grain considers the profit through cost-benefit analysis. From temperature records, the fan running hours ranged between 884.43 and 903.10 during this study. It cost $0.0026 / Kg. If there is a severe insect problem, it might be worth cooling the grain to lower temperatures to control more insects. Aeration may be not useful during the summer time in Oklahoma because it spends lots of energy and it is very difficult to get grain below 20°C. Moreover, if temperature remains above 25°C, insects will continue to develop. Seeing that aeration is used to cool and dry grain, it may have more use during fall, winter and spring time. During the late fall and winter aeration prevents moisture accumulating or condensing in bins when outside bin temperatures are below that of the grain temperature. Aeration will slow insect growth and development because most species are very sensitive to the temperatures below 20°C and the moisture contents below 12-13%. According to Hagstrum and Flinn (1990), even if infestation already occurs, cooling has a significant effect on the insect population. Thus, my suggestion is that aeration is used only in the fall and winter in order to save energy and avoid increased insect development in the summer time. In Oklahoma, the stored grain temperatures cooled down close to 0^{o}C is possible in the winter season. If there is a severe insect problem, aeration could be cost-effective.

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Table 3-1. Numbers of *T. castaneum* adults captured by probe traps in wheat bins (7 days)

08/19/2004 – 04/28/2005

Treatment	Mean (± SE) and t Grouping					
	19-Aug	16-Sep	14-Oct	11-Nov	9-Dec	28-Apr
Aeration (Control)	1017.50Ab (227.57)	1974.75Aa (491.75)	41.75 Abc (16.87)	22.00 ABc (11.74)	79.00 Ac (50.49)	2.50 Ac (1.04)
Top 50 cm Methoprene	660.25ABa (242.47)	974.25ABa (473.69)	153.00 Ab (79.31)	46.00 Ab (28.12)	33.75 Ab (30.45)	1.50ABb (0.78)
Methoprene	772.50ABa (383.41)	340.25Bab (243.02)	24.25 Bbc (7.49)	5.00 Bc (1.00)	1.75 Ac (1.44)	0.25 Bc (0.25)
Methoprene + Aeration	250.25 Ba (33.20)	301.00 Ba (73.32)	7.25 Bb (0.95)	5.50 Bb (1.32)	0.00 Ab (0.00)	0.00 Bb (0.00)

Means within the same column with the same capital letters are not significantly different. Means across rows with the same small letters are not significantly different. LSD test, $P \leq 0.05$; N=4.

Table 3-2. Numbers of *T. castaneum* adults by grain trier in wheat bin samples (per Kg)

08/19/2004 – 04/28/2005

Treatment	Mean number (± SE) and t Grouping					
	19-Aug	16-Sep	14-Oct	11-Nov	9-Dec	28-Apr
Aeration	11.73 Aa (0.89)	5.64 Aab (2.16)	5.08 Aab (1.85)	6.36 Aab (4.68)	3.39 Ab (2.20)	0.61 Ab (0.61)
Top 50 cm Methoprene	0.76 Bab (0.44)	2.19 ABa (0.73)	2.97 ABa (1.11)	1.47 Aab (1.47)	1.47 Aab (0.79)	0.0 Ab (0.00)
Methoprene	0.95 Ba (0.48)	0.79 Ba (0.57)	0.93 BCa (0.35)	0.55 Aa (0.55)	0.19 Aa (0.19)	0.0 Aa (0.00)
Methoprene + Aeration	0.58 Bb (0.37)	1.86 Ba (0.49)	0.36 Cb (0.36)	0.55 Ab (0.35)	0.56 Ab (0.35)	0.0 Ab (0.00)

Means within the same column with the same capital letters are not significantly different. Means across rows with the same small letters are not significantly different. LSD test, $P \leq 0.05$; N=4.

Table 3-3. Numbers of *R. dominica* adults captured

by probe traps in wheat bins (7 days)

08/19/2004 – 04/28/2005

Treatment	Mean number (± SE) and t Grouping					
	19-Aug	16-Sep	14-Oct	11-Nov	9-Dec	28-Apr
Aeration	1.25 Aa (0.95)	1.75 Aa (0.63)	3.50 Aa (1.04)	1.25 Aa (0.75)	9.75 ABa (8.76)	0.25 Ba (0.25)
Top 50 cm Methoprene	1.25 Ab (0.48)	1.25 Ab (0.95)	2.75 ABb (1.80)	0.00 Ab (0.00)	17.75 Aa (9.56)	0.00 Bb (0.00)
Methoprene	0.75 Aa (0.48)	0.25 Aa (0.25)	0.50 Ba (0.50)	0.50 Aa (0.29)	1.25 Ba (0.63)	0.75 ABa (0.48)
Methoprene + Aeration	3.50 Aa (1.85)	0.25 Ab (0.25)	0.50 Bab (0.29)	0.50 Aab (0.50)	2.25 ABab (1.44)	2.00 Aab (0.71)

Means within the same column with the same capital letters are not significantly different. Means across rows with the same small letters are not significantly different. LSD test, $P \leq 0.05$; N=4.

Table 3-4. Numbers of *R. dominica* adults by grain trier in wheat bin samples (per Kg)

08/19/2004 – 04/28/2005

Treatment	Mean number (± SE) and t Grouping					
	19-Aug	16-Sep	14-Oct	11-Nov	9-Dec	28-Apr
Aeration	2.71 Aab (0.72)	2.83 ABab (0.86)	4.36 Aa (1.15)	2.42 Aab (0.94)	3.76 Aa (0.31)	1.60 Ab (1.60)
Top 50 cm Methoprene	0.58 Bb (0.19)	4.04 Aa (1.43)	2.25 ABab (1.54)	2.01 ABab (1.13)	1.29 Bab (0.56)	0.37 Ab (0.21)
Methoprene	1.35 ABa (0.37)	0.95 Bab (0.71)	0.74 Bab (0.43)	0.18 Bb (0.18)	0.55 BCab (0.35)	0.00 Ab (0.00)
Methoprene + Aeration	1.70 ABa (0.76)	0.92 Bab (0.46)	1.10 ABa (0.47)	0.37 ABab (0.21)	0.00 Cb (0.00)	0.00 Ab (0.00)

Means within the same column with the same capital letters are not significantly different. Means across rows with the same small letters are not significantly different. LSD test, $P \leq 0.05$; N=4.

Table 3-5. Numbers of *C. ferrugineus* adults captured by probe traps in wheat bins (7 days)

08/19/2004 – 04/28/2005

Treatment	Mean number (± SE) and t Grouping					
	19-Aug	16-Sep	14-Oct	11-Nov	9-Dec	28-Apr
Aeration	40.00 Aa (13.40)	34.00 Aab (17.71)	26.25 Aab (9.58)	24.25 Aab (13.37)	5.25 Ab (1.93)	43.50 Aa (12.28)
Top 50 cm Methoprene	29.00 Aa (10.78)	20.00 Aa (6.49)	14.50 ABa (11.24)	7.00 ABa (1.73)	7.25 Aa (2.50)	11.50 Ba (10.19)
Methoprene	45.74 Aa (8.53)	28.75 Aa (12.58)	2.75 Bb (1.04)	1.50 Bb (0.87)	0.00 Bb (0.00)	0.00 Bb (0.00)
Methoprene + Aeration	43.50 Aa (28.23)	25.25 Aa (7.03)	5.25 ABb (2.56)	3.25 Bb (2.14)	0.75 Bb (0.75)	1.00 Bb (0.41)

Means within the same column with the same capital letters are not significantly different. Means across rows with the same small letters are not significantly different. LSD test, $P \leq 0.05$; N=4.

Table 3-6. Numbers of *C. ferrugineus* adults by grain trier in wheat bin samples (per Kg)

08/19/2004 – 04/28/2005

Treatment	Mean number (± SE) and t Grouping					
	19-Aug	16-Sep	14-Oct	11-Nov	9-Dec	28-Apr
Aeration	6.40 Ab (2.85)	17.66Aab (2.22)	12.04 Aab (3.47)	22.40 Aab (12.95)	31.00 Aa (3.78)	1.04 Ab (2.27)
Top 50 cm Methoprene	1.74ABab (0.86)	1.47 Bab (1.00)	0.55 Bab (0.35)	2.92 ABab (1.82)	3.13 Ba (1.25)	0.00 Bb (0.00)
Methoprene	0.96 ABa (0.19)	0.40 Bb (0.40)	0.00 Bb (0.00)	0.00 Bb (0.00)	0.00 Cb (0.00)	0.00 Bb (0.00)
Methoprene + Aeration	0.96 Bab (0.57)	1.48 Ba (0.79)	0.18 Bab (0.18)	1.47 Ba (0.67)	0.19 Cab (0.19)	0.00 Bb (0.00)

Means within the same column with the same capital letters are not significantly different. Means across rows with the same small letters are not significantly different. LSD test, $P \leq 0.05$; N=4.

Table 3-7. Normal adults of F_1 progeny at different residual times;

Mean (± SE) F_1 adults per 50 parent adults

Trt.	Stored 0 months			Stored 5 months			Stored 10 months		
	RFB	LGB	RW	RFB	LGB	RW	RFB	LGB	RW
A	248.50 A (14.29)	696.75 A (36.95)	680.50 A (30.97)	133.25 A (22.75)	482.00 A (15.54)	598.75 AB (23.68)	278.50 A (26.56)	514.75 A (44.69)	724.25 A (55.90)
T	0.00 B (0.00)	1.50 B (1.19)	825.75 A (20.21)	0.00 B (0.00)	31.25 B (25.63)	430.50 B (53.88)	0.00 B (0.00)	25.25 B (8.39)	517.75 AB (81.62)
M	0.00 B (0.00)	0.00 B (0.00)	1260.00 A (588.58)	0.00 B (0.00)	0.75 C (0.75)	582.25 AB (34.31)	0.25 B (0.25)	0.25 C (0.25)	487.00 B (50.85)
MA	0.00 B (0.00)	0.50 B (0.50)	464.50 A (133.47)	0.00 B (0.00)	0.25 C (0.25)	681.25 A (61.18)	0.25 B (0.25)	0.25 C (0.25)	680.00 AB (100.26)

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. N=4.

Trt: treatment; A: aeration; T: top methoprene 50 cm; M: methoprene alone;

MA: methoprene + aeration.

RFB: *T. castaneum*, the red flour beetle. LGB: *R. dominica*, the lesser grain borer.

RW: *S. oryzae*, the rice weevil.

Table 3-8. Percentage of *P. interpunctella* emerging to normal adults at different storage residual times

Treatment	Mean % normal adults (± SE) and t Grouping		
	Stored 0 months	Stored 5 months	Stored 10 months
Aeration	98.69 A (1.32)	98.75 A (1.25)	98.75 A (1.25)
Top 50 cm	3.88 B (2.51)	8.00 B (5.07)	21.84 B (10.55)
Methoprene	0.00 C (0.00)	0.00 C (0.00)	0.00 C (0.00)
Methoprene + Aeration	0.00 C (0.00)	0.00 C (0.00)	0.00 C (0.00)

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. N=4.

Table 3-9. Grain grading at the start and the end of the storage period

Treatment	Rep. & Mean	The Start			The End		
		Grade	TW	IDK	Grade	TW	IDK
Aeration	1	2	59.60	3	1	60.20	3
	2	2	58.90	0	1	60.30	2
	3	2	59.80	2	1	60.00	10
	4	2	59.50	0	1	59.50	11
	Mean	2	59.45	1.25	1.00	60.00	9.00
	(SE)	0	0.19	0.75	0.00	0.18	4.18
Top 50 cm	1	1	60.70	2	1	63.10	2
	2	1	60.60	1	1	61.20	6
	3	1	60.50	0	1	61.10	2
	4	1	61.40	1	1	62.40	7
	Mean	1	60.80	1.00	1.00	61.95	4.25
	(SE)	0	0.20	0.41	0.00	0.48	1.31
Methoprene	1	2	59.40	0	1	62.10	2
	2	1	60.10	3	1	61.90	2
	3	1	61.00	2	1	62.00	6
	4	1	61.10	1			
	Mean	1.25	60.40	1.50	1.00	62.00	3.33
	(SE)	0.25	0.40	0.65	0.00	0.06	1.33
Methoprene + Aeration	1	1	60.80	3	1	61.70	3
	2	2	59.80	1	1	61.60	1
	3	1	60.50	1			
	4	1	60.60	1			
	Mean	1.25	60.43	1.50	1.00	61.40	2.00
	(SE)	0.25	0.22	0.50	0.00	0.30	1.00

TW: Test weight. IDK: Insect damage kernels per 100g wheat.

Table 3-10. Methoprene residues at the start and the end of the storage period

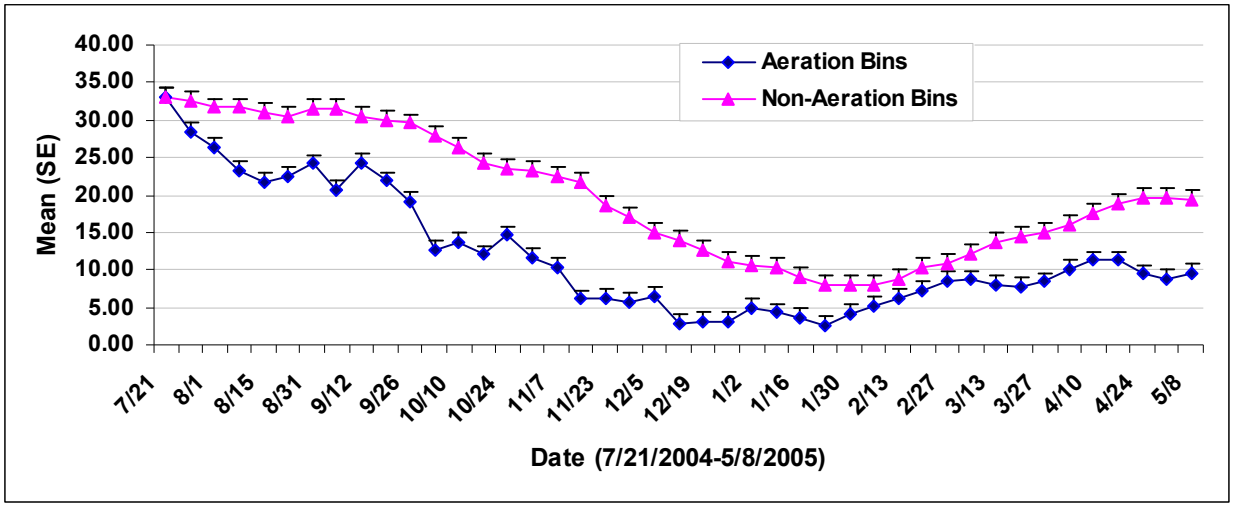
Treatment	Replicate	Concentration at start (ppm)	Concentration at end (ppm)	Individual and Mean % Remain
Top 50 cm	1	0.32	0.11	34.38
	2	0.48	0.13	27.66
	3	0.66	0.30	45.45
	4	0.65	0.26	40.00
				$\bar{X}$=36.87 (3.81) B
Methoprene	1	2.06	1.27	61.65
	2	1.44	1.38	95.83
	3	1.51	1.10	72.85
	4	2.61	1.44	55.17
				$\bar{X}$=71.38 (8.93) A
Methoprene + Aeration	1	1.40	1.24	88.57
	2	1.13	0.63	55.75
	3	3.42	1.56	45.61
	4	1.33	1.13	84.96
				$\bar{X}$=68.72 (10.65) A

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$.

Figure 3-1. Mean temperature (°C) each week in aeration and non-aeration bins

N = 8 each



Four aeration cycles:

Cooling to a set-point of 30°C during the summer.

Cooling to 25°C in the later summer.

Cooling to 15°C in the fall and the spring.

Cooling to 7°C in the winter.

CHAPTER IV

COMBINATIONS OF DIATOMACEOUS EARTH WITH INSECTICIDES TO CONTROL STORED WHEAT INSECTS

Abstract

At present, each stored grain protectant has its advantages and disadvantages. In order to overcome the drawback, there are some strategies such as combining insecticides that may alleviate the problems. In this study, *Tribolium castaneum* and *Rhyzopertha dominica* were exposed to diatomaceous earth (DE) combined with Spinosad, Pyrethrins and Storcide II, respectively. The combinations demonstrated good effects and potential synergisms while DE at 100ppm (1/3 label rate) alone was not, or was very slightly significantly different to control *T. castaneum* (Herbst) and *R. dominica*. Spinosad at 0.1ppm combined with DE at 100ppm was effective and synergistic, and which prevented 42.94% F_1 progeny of *T. castaneum* (Herbst), while Spinosad at 0.1ppm alone was unable to control *T. castaneum* (Herbst). Pyrethrins at 1.0ppm reduced F_1 adults of *T. castaneum* (Herbst) by 65.78% when conbined with DE at 100ppm and enhanced 144.39% to kill parent adults and prevented 98.24% F_1 progeny of *R. dominica*. Storicide II at 0.1 of its label rate reduced F_1 adults of *R. dominica* by 82.07% when combined with DE 100ppm. The combinations showed an excellent effect in this study. Therefore some combinations are ideal methods to control some stored grain insect species, which can either reduce the amount of insecticides needed to control stored grain insects or produce synergistic effects.

Key Words: *Tribolium castaneum, Rhyzopertha dominica*, wheat, diatomaceous earth, Spinosad, Pyrethrins, Storcide II, laboratory bioassay.

Introduction

Ashes were used to control insect pests thousands of years ago (Ebeling, 1971). Diatomaceous earth (DE) has been used as an insecticide for centuries (Quarles, 1992). Inert dusts as stored-grain protectants were researched from the 1920's (Golob, 1997; Fields and Muir 1995; Korunic, 1997, 1998; Ebeling, 1971). Commercial formulations of DE have been used in the USA to control stored grain insects since the 1960s (Korunic, 1998). DE is an inert, nontoxic dust. According to Ross (1981), diatomaceous earth as a traditional insecticide and has many attractive features that should be considered, even though it is not a miracle cure. DE as a protectant is registered for stored grain and structural treatment in the USA, China, Australia, Canada, Germany, and some other countries.

DE is fossilized skeletons of diatoms, which are single-celled aquatic algae. DE is found in freshwater or marine deposits throughout the world. The diatoms ingested dissolved silica that they converted into highly organized shells that contain many inner pores creating a large surface area (Fields and Timlick, 1994).

When insects move through the grain and pick up dust particles on their cuticle, the DE absorbs the cuticle of the insect and causes water loss by desiccation (Korunic, 1998). Once insects lose this waterproof layer, they become vulnerable to desiccation and the insects die by dehydration (Fields and Timlick, 1994).

DE is generally regarded as safe for food (Fields and Timlick, 1994; Ross, 1981). Normally, it only acts on insects. Because its action against insects is wholly mechanical, probably no harmful residues are released to enter the digestive systems of other life forms. Furthermore, insects probably can not develop resistance to DE (Glenn et al., 1999; Abrams, 1954).

If DE is kept dry it can have long-term residual insecticidal activity (Quarles and Winn, 1996). However, DE may experience reduced residual efficacy by continuation with accumulated food material (Arthur, 2000a).

There are four types of inert dusts: powdered clay, DE, silica gels, and non-silica dusts (Fields and Muir 1995). The main advantage of inert dusts is their low toxicity for mammals and effectiveness for long durations. In the USA, DE is registered as an animal feed additive, and silicon dioxide is registered as a human food additive (Desmarchelier and Dines, 1987; Korunic et al., 1996). Recently some new formulations of DE have been registered for controlling stored grain insects (Quarles and Winn, 1996).

DE shows variation in controlling stored grain insects (Arthur, 2000b). The insecticidal properties can vary by twenty times depending on the different origin of the diatomaceous earth. Effective diatomaceous earths should have more than 80% SiO_2 content, a density below 300g per liter, and a pH below 8.5 (Korunic, 1997). Therefore, over the past 3-4 decades, some research indicated that DE is effective against most

stored-grain insects if well distributed among the grain (White et al., 1975), alternatively several references did not show good effects of DE (LaHue, 1967).

DE has some drawbacks. First, there is a chance that workers may develop pneumoconiosis if the diatomaceous earths are inhaled for a long period of time (Abrams, 1954; Cuperus et al., 1990). Second, although new formulations are considerably more effective than older products (Subramanyam et al., 1998), diatomaceous earths can affect the physical properties of grain, like test weight (Korunic et al., 1996, 1998). The grain may become "Sample grade" if rates are too high. Third, treated grain can cause increased wear on machinery. Fourth, some DE products, even the new formulation Protect-It, could adversely affect natural enemies such as parasitoids (Perez-mendoza et al., 1999). Fifth, compared with other insecticides, DE requires relative higher concentrations or longer exposure intervals to control insect infestation in stored grain, even though newer formulations are better than old and can be applied at lower rates (Arthur, 2000a, 2001).

Some current grain protectants (Table 4-1) have problems. For example, from the early 1960s Malathion was registered as a grain protectant. It is not a suitable grain protectant because it breaks down rapidly, and today, key stored-grain insects are resistant to it. Reldan 4E (Chlorpyrifos-methyl) was registered in 1985. Some populations of *Rhyzopertha dominca* are developing resistance to it. Reldan 4E is used on wheat at 6.0ppm until December 2005. Currently 3.0ppm is allowed past 2005 (Arthur, 1994). Storcide (Chlorpyrifos-methyl + cyfluthrin) was registered in 2002 and has been replaced

by Storcide II because cyfluthrin is a problem in international trade. Some grain protectants with a good effect, such as, Spinosad, Pyrethrins, and Storcide II, are described in more detail below.

Table 4-1. Some insecticides labeled for use as stored grain protectants

Active Ingredient (a.i.)	Formulations and Brand Names	
	Liquid	Dust
Malathion	Malathion 5EC	Big 6 Grain Protector®
Chlorpyrifos-methyl	Reldan 4E®	
Chlorpyrifos-methyl + cyfluthrin	Storcide®	
Diatomaceous earth		Protect-It™, Insecto®
Methoprene	Diacon II®	
Pyrethrins	Pyrenone®	
Spinosyn	Spanosad®	
Chlorpyrifos-methyl+deltamethrin	Storcide II®	

(Weaver and Petroff, 2005; USARC, 2005)

Spinosad, a microbial insecticide, is a reduced-risk and broad-spectrum commercial insecticide based on the fermentation products of the bacterium *Saccharopolyspora spinosa* (Mertz and Yao, 1990), and it exerts its toxic to insects by ingestion and contact (Ada´n et al., 1996; Liu et al., 1999; Wanner et al., 2000). Spinosad has a unique mode of action on the insect nervous system at the sites of nicotinic acetylcholine receptor and at

the GABA (gamma-amino-butric acid) receptor (Salgado, 1997, 1998). Spinosad has low toxicity for mammals (Thompson et al., 2000). It is a mixture of spinosyns A and D, and the former is the predominant metabolite (Bret et al., 1997).

Spinosad is registered in 25 countries and labeled for use on at least 100 crops in the USA (Thompson et al., 2000). It provides effective control against insects in 5 orders, i.e, Lepidoptera, Diptera, Thysanoptera, and some species of Coleoptera and Orthoptera (Sparks et al., 1995; Peck and McQuate, 2000; Cloyd and Sadof, 2000). In field crops, Spinosad degrades quickly because when exposed to sunlight (UV radiation), and loses activity after one week (Brunner and Doerr, 1996; Saunders and Bret, 1997; Liu et al., 1999).

In January, 2005, the U.S. Environmental Protection Agency (USEPA) issued a registration for the use of Spinosad, a product developed and manufactured by Dow AgroSciences LLC. It was awarded the 1999 Presidential Green Chemistry Challenge Award in the USA, as a stored grain and seed protectant on commodities including several kinds of grain, such as wheat, corn, oats, rice, sorghum and barley **(**USARC, 2005). Spinosad is highly effective in controlling stored grain insects (Fang et al., 2002a, b; Toews and Subramanyam, 2003). In farm bins, where most of the wheat is not exposed to sunlight, Spinosad degrades very little during one year of storage without appreciable loss of insecticidal activity against *Rhyzopertha dominica* (F.) (the lesser grain borer), and *Tribolium castaneum* (Herbst) (red flour beetle) (Fang et al., 2002b).

Pyrethrum is a botanical insecticide that is neurotoxic and causes insect knock-down. Pyrethrum is an axonic poison like DDT, leading eventually to paralysis (Ware and Whitacre, 2004). Pyrethrum is extracted from the flowers of the pyrethrum daisy, *Chrysanthemum cinaeriofolium*, grown in Kenya and Ecuador, and commercially used for many years. So far, natural pyrethrum is not common due to its expense and instability. Many synthetic pyrethrin-like insecticides are available known as pyrethoids. In 1940, sesame oil was patented as a synergist for pyrethrin insecticides. Synergized pyrethrum maintains a high degree of repellency. In 1858, pyrethrum was first used in the USA (Ware, 1983; La Hue, 1966). In the 1960s more synthetic pyrethroids appeared, which were more stable at high temperatures and their breakdown rates were slow compared with pyrethrin (Elliott *et al.,* 1972). Today we are using the fourth generation pyrethroids, such as acrinathrin (Rufast®), imiprothrin (Pralle®), registered in 1998. Pyrethroids affect both the peripheral and central nervous system of the insect. As a grain protectant pyrethroids may be more effective than Reldan on stored grain insects (Arthur, 1995).

Storcide II is the combination of a synthetic pyrethroid with an organophosphate and is a new product reformulated from Gustafson. It is a liquid grain protectant that is applied to grain at 3.0ppm of chlorpyrifos-methyl (Reldan) and 0.5ppm of deltamethrin and does not contain cyfluthrin like Storcide. Both cyfluthrin and deltamethrin are synthetic pyrethroid insecticides. According to the Gustafson Company, Storcide II was approved in November 2004 by the USEPA. Gustafson will only distribute Storcide II and no longer distributes the original Storcide (Johnson, 2005).

Storcide II is labeled for direct application to stored wheat, barley, rice, sorghum and oats, for use as a top-dressing in a grain bin and warehouse spray. It offers excellent protection against a broad spectrum of insect pests, such as lesser grain borer, weevils, beetles and moths. Storcide II does have established codex MRLs (maximum residue limits), so the grain treated with Storcide II does not have a problem in international trade. Glen Karaffa, the stored grain products manager for Gustafson, said, "Storcide II can play an integral role in an effective integrated pest management program." (Gustafson, 2005).

Even though DE has some disadvantages discussed above, there are still some strategies to resolve it. One possibility for alleviating the problems associated with DE is to reduce the application rate by combination, that is, low rates of conventional chemicals combined with the DE to enhance efficiency (Arthur, 2004), even potential synergisms. So far, there are not many studies of newer DE formulations in combination with other insecticides. Insects exposed to DE combined with other insecticides may either reduce the amount of DE needed to control stored grain insects or produce synergistic effects. My objective is to evaluate the efficacy and examine potential synergistic effects of three insecticides, Spinosad, Pyrethrins, or Storcide II combined with DE, respectively, against two stored product insect species, *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica*.

Materials and Methods

Diatomaceous Earth. The commercial formulation is called Protect-It (Hedley Technologies Inc. Mississauga, Ontario). Its chemical name is amorphous silicon dioxide. Ingredient (w/w %) (with CAS number) is natural DE – fresh water (DE) 90% (61790-53-2), and silica gel 10% (112926-00-8). Crystalline silica from DE is less than 0.9%. Physical state is fine grey powder.

Spinosad. Spinosad (NAF-313. Lot number: MJ28160P31) was obtained from Dow AgroSciences LLC, Indianapolis, IN. The active ingredient is 11.6% (w/w). It was diluted with distilled water, and prepared for 0.005% and 0.001% solution.

Pyrethrins. Product name is X-5914-04 (it is also a product code). Lot number is LPJ72604. Pyrethrins as an organic insecticide concentrate for experimental use only, was obtained from McLaughlin Gormley King Co., Minneapolis, MN. Active ingredient is 45% (w/w). It was diluted with acetone, and prepared for 0.03% and 0.01% solution.

Storcide II. Storcide II (Lot number is TAR109:8-1) was obtained from Gustafson LLC, McKinney, TX, which is as a solution with chlorpyrifos-methyl 3.0ppm and deltamethrin 0.5ppm. It was diluted with distilled water, and prepared for 0.005% and 0.001% solution.

Insects Rearing. Insects used in experiment were *Tribolium castaneum* (Herbst), the red flour beetle (Coleoptera: Tenebrionidae), and *Rhyzopertha dominica* (F), the lesser grain borer (Coleoptera: Bostrichidae). *T. castaneum* was reared on 400cm^3 whole hard red winter wheat flour with 2.5cm^3 yeast in a jar, and *R. dominica* was reared on 400cm^3 sound wheat with 2.5cm^3 yeast in a jar at 28^{o}C and 65 ± 5% r.h. in a walk-in growth chamber.

Wheat treatment and insect setup. New hard red winter wheat was used for the experiment. One hundred gram sound wheat was placed into 240ml jars with metal screen and filter paper cover for *R. dominica* tests, and 95g sound wheat mixed with 5g crushed wheat was placed in 240ml jars with filter paper cover for *T. castaneum* tests. A total of 112 jars for both species were used.

Experimental Protocol. One hundred gram grain was placed into each 240ml jar was treated with 1ml of 0.001% Spinosad solution respectively to obtain rates of 0.1ppm active ingredient Spinosad for each jar (total 16 jars). The grain from each jar was placed in a dockage sieve bottom pan and sprayed jar by jar by using an airbrush. The jars were placed in fume hoods for 24 hours to dry the grain. Then 8 jars of them were treated with 10mg Protect-It respectively to obtain rates of 100ppm plus 0.1ppm Spinosad. Therefore, there are four jars 0.1ppm Spinosad alone for *T. castaneum* and four jars for *R. dominica*. Similarly, there are four 100ppm DE combined with 0.1ppm for *T. castaneum* and four for *R. dominica*. By the same way, there are 14 treatments as follows:

(1). Control (1ml water)

(2). DE100ppm alone (1/3 label rate)

(3). Spinosad 0.1ppm

(4). Spinosad 0.5ppm

(5). DE 100ppm + Spinosad 0.1ppm

(6). DE 100ppm + Spinosad 0.5ppm

(7). Pyrethrins 1.0ppm

(8). Pyrethrins 3.0ppm

(9). DE 100ppm + Pyrethrins 1.0ppm

(10). DE 100ppm + Pyrethrins 3.0ppm

(11). Storcide II 0.1 label rate

(12). Storcide II 0.5 label rate

(13). DE 100ppm + Storcide II 0.1 label rate

(14). DE 100ppm + Storcide II 0.5 label rate.

Fifty adult *T. castaneum* and 50 adult *R. dominica* were separately placed into each 240ml jar with 100g treated wheat (5 g crush for *T. castaneum*) at 28^{o}C, 65 ± 5% r.h., and an hourly photoperiod of 16:8 (L: D) growth chamber. After one week adults were removed to each clean petri dish for recovery 24 hours, and then mortality was counted. After 6 weeks F_1 progeny adults were counted.

Statistical Analysis. Data were analyzed separately for mean percentage mortality of parent adults for each species and for production of F_1 adult numbers by using the

General Linear Models (GLM) procedure (SAS Institution, 2002). All data were subjected to analysis of variance (ANOVA) followed by a protected least significant difference (LSD) test for comparison of means. Data were transformed by square root of the number plus 0.5 prior to ANOVA, while means and standard errors (SE) were untransformed. The significant differences were considered at $P \leq 0.05$ level.

Results

Table 4-2 presents the results of mean percentage mortality of *T. castaneum* parent adults and production of F_1 progeny on wheat treated with Spinosad alone or combined with DE. Statistical analysis showered that DE at100ppm, Spinosad at 0.1ppm and 0.5ppm alone, and both combined with DE at 100ppm, respectively, were not significantly different from untreated control to kill *T. castaneum* adults after one week. The mean F_1 adults of *T. castaneum* at DE 100ppm and Spinosad at 0.1ppm alone were not significantly different from those at untreated control after 6 weeks. The mean F_1 adults of *T. castaneum* at Spinosad 0.1ppm combined with DE 100ppm were significantly different from those at Spinosad 0.1ppm alone after 6 weeks. The mean F_1 adults of *T. castaneum* at Spinosad 0.5ppm combined with DE 100ppm were not significantly different from those at Spinosad 0.5ppm alone after 6 weeks, but they were significantly different from those at control after 6 weeks.

Table 4-3 presents the results of mean percentage mortality of *R. dominica* parent adults and production of F_1 progeny on wheat treated with Spinosad alone or combined with

DE. Statistical analysis showed that Spinosad at 0.1ppm and 0.5ppm alone, and both combined with DE 100ppm, respectively, were not significantly different from each other but they were significantly different from DE at100ppm that was also significantly different from untreated control to kill *R. dominica* adults after one week. The mean F_1 adults of *R. dominica* at DE 100ppm were not significantly different from those at untreated control after 6 weeks. The mean F_1 adults of *R. dominica* at Spinosad 0.1ppm combined with DE 100ppm were significantly different from those at Spinosad 0.1ppm alone, which were as well as significantly different from those at untreated control after 6 weeks. The mean F_1 progeny of *R. dominica* at Spinosad 0.5ppm combined with DE 100ppm was not significantly different from that at Spinosad 0.5ppm alone after 6 weeks, however, both them were significantly different from those at untreated control after 6 weeks.

Table 4-4 presents the results of mean percentage mortality of *T. castaneum* parent adults and production of F_1 progeny on wheat treated with Pyrethrins alone or combined with DE. The results showed that DE at 100ppm, pyrethrum at 1.0ppm and 3.0ppm alone, and both combined with DE 100ppm, respectively, were not significantly different from untreated control to kill *T. castaneum* adults in one week. The mean F_1 adults of *T. castaneum* at DE 100ppm were not significantly different from those at untreated control after 6 weeks. The mean F_1 progeny of *T. castaneum* at Pyrethrins 1.0ppm alone was not significantly different from that at DE 100ppm after 6 weeks. However, the mean F_1 adults of *T. castaneum* at Pyrethrins 1.0ppm combined with DE at 100ppm were significantly different from those at Pyrethrins 1.0ppm alone after 6 weeks. The mean F_1

adults of *T. castaneum* at Pyrethrins 3.0ppm combined with DE at 100ppm were not significantly different from those at pyrethins 3.0ppm alone after 6 weeks, but the mean F_1 adults of *T. castaneum* were significantly different from untreated control after 6 weeks.

Table 4-5 presents the results of mean percentage mortality of *R. dominica* parent adults and production of F_1 progeny on wheat treated with Pyrethrins alone or combined with DE. Pyrethrum at 3.0ppm alone, and Pyrethrins at 1.0ppm, 3.0ppm combined with DE at 100ppm, respectively, were not significantly different each other but they were significantly different from Pyrethrins at 1.0ppm to kill *R. dominica* adults in one week exposed. Pyrethrins 1.0ppm and 3.0ppm were significantly different from DE at 100ppm that was also significantly different from untreated control to kill *R. dominica* adults after one week. The mean mortality of *R. dominica* adults is 13.60% from treatment DE at 100 ppm, and 34.94% from treatment Pyrethrins at 1.0ppm alone after one week exposed. However, when *R. dominica* adults were exposed to pyrethrum at 1.0ppm combined with DE 100ppm after one week, the mean mortality was 85.39%.The mean F_1 adults of *R. dominica* at DE 100ppm were not significantly different from those at untreated control after 6 weeks. The mean F_1 adults of *R .dominica* at Pyrethrins 1.0ppm combined with DE at 100ppm were significantly different from those at Pyrethrins 1.0ppm alone, which were significantly different from those at untreated control after 6 weeks as well. The mean F_1 adults of *R. dominica* at Pyrethrins 3.0ppm combined with DE 100ppm were not significantly different from those at Pyrethrins 3.0ppm alone after 6 weeks, but they were significantly different from those at untreated control after 6 weeks.

The mean percentage mortality of *T. castaneum* parent adults and production of F_1 progeny on wheat treated with Storcide II alone or combined with DE are presented from laboratory bioassays (Table 4-6). The results showed that DE at 1/3 label rate (100ppm), Storcide II at 0.1 label rate, at 0.5 label rate alone or their combinations were not or very slight significantly different from untreated control to kill *T. castaneum* adults after one week. The mean F_1 adults of *T. castaneum* at DE 1/3 label rate were not significantly different from those at untreated control after 6 weeks. The mean F_1 adults of *T. castaneum* at Storcide II at 0.1 label rate combined with DE at 1/3 label rate were significantly different from those at Storcide II at 0.1 label rate alone, which was also significantly different from untreated controls after 6 weeks. The mean F_1 adults of *T. castaneum* from grain treated with Storcide II at 0.5 label rate combined with DE at 1/3 label rate were not significantly different from grain treated with Storcide II at 0.5 label rate alone after 6 weeks, but they were significantly different from untreated controls after 6 weeks.

The mean percentage mortality of *R. dominica* parent adults and production of F_1 progeny on wheat treated with Storcide II alone or combined with DE are presented from laboratory bioassays (Table 4-7). DE at 1/3 label rate and Storcide II at 0.1 label rate alone were not significantly different from untreated control to kill *R. dominica* adults after one week. Storcide II at 0.1 label rate combined with DE at 1/3 label rate was slight significantly different from Storcide at II 0.1 label rate alone to kill *R. dominica* adults after one week. The mean mortality of *R. dominica* adults is 13.60% from DE at1/3

label rate treatment, 28.60% from Storcide II at 0.5 label rate alone one week exposed. However, when *R. dominica* adults exposed to Storcide II at 0.5 label rate combined with DE at 1/3 label rate after one week, the mean mortality was 97.47%. The mean F_1 adults of *R. dominica* at DE 1/3 label rate were not significantly different from those at untreated control after 6 weeks. the mean F_1 adults of *R. dominica* at Storcide II 0.1 label rate combined with DE at 1/3 label rate were significantly different from those at Storcide II at 0.1 label rate alone after 6 weeks, which were significantly different from those at untreated control after 6 weeks as well. The mean F_1 adults of *R. dominica* from grain treated with Storcide II at 0.5 label rate combined with DE at 1/3 label rate were significantly different from grain treated with Storcide II at 0.5 label rate alone, which were also significantly from Storcide II at 0.1 label rate combined with DE at 1/3 label rate after 6 weeks.

Discussion

Integrated pest management strategies are needed in grain storage to replace some organophosphate (OP) grain protectants. None is a miracle cure as an insecticide. Each has its advantages and disadvantages. Combination methods are a good idea to deal with some disadvantages. In this experiment, DE alone at 100ppm was not effective on the parent adults of *T. castaneum* (Herbst), very slight or not effective on the parent adults of *R. dominica,* and not effective on preventing both F_1 adults of *T. castaneum* (Herbst) and *R. dominica*. However, the combinations of DE with low doses of other protectants demonstrated a good effect, and in some cases which were potential synergisms. The

results of this insecticidal laboratory bioassay confirmed the hypothesis that the combinations would demonstrate a potential synergism when some protectants are combined together.

A combination of DE at100ppm and Spinosad at 0.1ppm reduced F_1 progeny of *T. castaneum* (Herbst) by 42.94%; complete control would be between Spinosad 0.1 and 0.5ppm when it combined with DE 100ppm. Spinosad at 0.1ppm combined with DE at 100ppm still showed that the combination reduced F_1 adults of *R. dominica* by 99.62%, even though Spinosad at 0.1ppm reached 99% mortality on the parent adults.

A combination of DE at 100ppm and Pyrethrins at 1.0ppm reduced F_1 adults of *T. castaneum* (Herbst) by 65.78%, which presented a potential synergism. Pyrethrins at 1.0ppm demonstrated a potential synergism on both the parent adults and the F_1 adults of *R. dominica* when it was combined with DE at 100ppm. The combination enhanced Pyrethrins at 1.0ppm 144.39% parent adults' mortality of *R. dominica* and reduced F_1 adults of *R. dominica* by 98.24 %. Pyrethrins 3.0ppm was an overdose on the F_1 adults of both *T. castaneum* (Herbst) and *R. dominica* when it combined with DE at 100ppm.

Both Storcide II 0.1 label rate and 0.5 label rate combined with DE 1/3 label rate, respectively, showed effects and potential synergisms on the mortality of parent adults of *R. dominica* and increased effect by 274.22%, 240.80%, respectively. The combination of Storcide II 0.1 label rate and DE at 1/3 label rate reduced F_1 adults of *R. dominica* by 82.07% compared with Storcide II 0.1 label rate. Storcide II 0.1 label rate was over the

effective dose for *T. castaneum* (Herbst) F_1 progeny when it combined with DE at 1/3 label rate.

In summary, this study presents the results of mean mortality of the parent adults and mean F_1 progeny of *T. castaneum* (Herbst) and *R. dominica* from laboratory bioassays by using Spinosad, pyrethrum, and Storcide II alone at test rates or combination with DE at 100ppm, respectively. Statistical analysis clearly indicated that there were not or very slight effective to kill parent adults of *T. castaneum* (Herbst) among three insecticides alone at test rates or combination with DE at 100ppm, respectively. However, three insecticides act on the F_1 Progeny of *T. castaneum* (Herbst) that showed very good effects and potential synergisms when they were combining with DE at 100ppm, such as Spinosad at 0.1ppm, Pyrethrins at 1.0ppm, and Storcide II at 0.1 label rate even though Storcide II at 0.1 label rate showed overdose when they were combining with DE at 100ppm to prevented F_1 Progeny of *T. castaneum* (Herbst). There were similar levels of variation for the parent adults and the F_1 adults of *R. dominica* from three insecticide combinations. There were potential synergisms from Spinosad at 0.1ppm to prevented F_1 adults , Pyrethrins at 1.0ppm, and Storcide II at both 0.1 label rate and 0.5 label rate to kill parent adults and to prevent F_1 adults of *R. dominica* when they were combined with DE 100ppm while Spinosad 0.5 ppm, Pyrethrins3.0ppmand Storcide II 0.5 label rate showed overdoses.

Some highly toxic chemical insecticides are hazardous to humans, the natural environment and ecology. This study clearly revealed that combinations of DE with low

doses of other protectants demonstrated a very good effect and suggest potential for developing low risk and low residue grain protectants. When insects move through the grain and pick up dust particles on their cuticle, the DE absorbs the waxes and causes water loss by desiccation. Once insects lose this waterproof layer, they will be very sensitive to chemical insecticides, even low dose. Therefore, DE in combination with low dose of insecticide works better than either alone. These combinations have boundless prospects. This conclusion and detail experimented information will offer useful evidence or thinking for insecticide companies to develop improved products, and will also help users directly to remold the way of using available products now by recombining them reasonably to more efficiently control stored product insects.

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Table 4-2. Mean percentage mortality of *T. castaneum* parent adults and production of F_1 progeny on wheat treated with Spinosad alone or combined with DE

Treatment (ppm)	Mean Mortality %	± SE	t Grouping	Mean F_1 adult numbers	± SE	t Grouping
Control	0.50	0.50	A	270.25	42.55	A
DE 100	0.52	0.52	A	236.00	20.91	A
Spin 0.1	0.48	0.48	A	221.25	12.73	A
Spin 0.5	0.00	0.00	A	2.00	0.41	C
DE + Spin 0.1	0.50	0.50	A	126.25	11.75	B
DE + Spin 0.5	0.45	0.45	A	1.25	0.75	C

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. DE: Diatomaceous earth; Spin: Spinosid.

Table 4-3. Mean percentage mortality of *R. dominica* parent adults and production of F_1 progeny on wheat treated with Spinosad alone or combined with DE

Treatment (ppm)	Mean Mortality %	± SE	t Grouping	Mean F_1 adult numbers	± SE	t Grouping
Control	8.41	1.73	C	396.25	17.75	A
DE 100	13.60	2.00	B	427.00	20.20	A
Spin 0.1	99.01	0.57	A	16.00	6.89	B
Spin 0.5	99.51	0.49	A	0.50	0.29	C
DE + Spin 0.1	100.00	0.00	A	1.50	0.87	C
DE + Spin 0.5	100.00	0.00	A	0.00	0.00	C

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. DE: Diatomaceous earth; Spin: Spinosad.

Table 4-4. Mean percentage mortality of *T. castaneum* parent adults and production of F_1 progeny on wheat treated with Pyrethrins alone or combined with DE

Treatment (ppm)	Mean Mortality %	± SE	t Grouping	Mean F_1 adult numbers	± SE	t Grouping
Control	0.50	0.5	A	270.25	42.55	A
DE 100	0.52	0.52	A	236.00	20.91	AB
Py 1ppm	0.00	0.00	A	160.00	28.26	B
Py 3ppm	1.00	0.58	A	13.75	8.14	D
DE+Py 1ppm	1.01	0.58	A	54.75	16.98	C
DE+Py 3ppm	0.51	0.51	A	0.00	0.00	D

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. DE: Diatomaceous earth; Py: Pyrethrins.

Table 4-5. Mean percentage mortality of *R. dominica* parent adults and production of F_1 progeny on wheat treated with Pyrethrins alone or combined with DE

Treatment (ppm)	Mean Mortality %	± SE	t Grouping	Mean F_1 adult numbers	± SE	t Grouping
Control	8.41	1.73	D	396.25	17.75	A
DE 100	13.60	2.00	C	427.00	20.20	A
Py 1ppm	34.94	3.22	B	14.25	2.93	B
Py 3ppm	74.77	3.64	A	1.00	0.58	C
DE+Py 1ppm	85.39	1.82	A	0.25	0.25	C
DE+Py 3ppm	88.06	5.57	A	0.00	0.00	C

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. DE: Diatomaceous earth; Py: Pyrethrins.

Table 4-6. Mean percentage mortality of *T. castaneum* parent adults and production of F_1 progeny on wheat treated with Storcide II alone or combined with DE

Treatment (label rate)	Mean Mortality %	± SE	t Grouping	Mean F_1 adult numbers	± SE	t Grouping
Control	0.50	0.50	BC	270.25	42.55	A
DE 1/3	0.52	0.52	BC	236.00	20.91	A
Stor 0.1	0.00	0.00	C	13.75	9.17	B
Stor 0.5	1.97	0.80	AB	0.00	0.00	C
DE + Stor 0.1	0.00	0.00	C	0.00	0.00	C
DE + Stor 0.5	3.97	1.40	A	0.00	0.00	C

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. DE: Diatomaceous earth; Stor: Storcide II.

Table 4-7. Mean percentage mortality of *R. dominica* parent adults and production of F_1 progeny on wheat treated with Storcide II alone or combined with DE

Treatment (label rate)	Mean Mortality %	± SE	t Grouping	Mean F_1 adult numbers	± SE	t Grouping
Control	8.41	1.73	C	396.25	17.75	A
DE 1/3	13.60	2.00	C	427.00	20.20	A
Stor 0.1	7.68	1.72	C	178.50	24.85	B
Stor 0.5	28.60	8.66	B	4.75	1.18	D
DE + Stor 0.1	28.74	7.04	B	32.00	5.02	C
DE + Stor 0.5	97.47	1.93	A	0.00	0.00	E

Means in the same column with the same letters are not significantly different.

LSD test, $P \leq 0.05$. DE: Diatomaceous earth; Stor: Storcide II.

VITA

SIWEI LIU

Candidate for the Degree of

Master of Science

Thesis: COMBINATION TREATMENTS FOR CONTROLLING STORED PRODUCT INSECTS

Major Field: Entomology

Biographical:

Personal: Born in Henan, China, on December 15, 1959, the son of Chuanhan Liu and Yue Li.

Education: Graduated from Beiye High School, Henan, China in 1979; received Bachelor of Science degree in Plant Protection from Henan Agricultural University, Chain, in 1983. Completed Master of Science degree with a major in Entomology at Oklahoma State University, USA, in December 2005.

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Abstract

Stored product insects cause substantial economic and quality losses to products. This project explored technologies for use in stored product insect management that rely on alternatives to organophosphate insecticides and the fumigants methyl bromide and phosphine. Experiments were carried out in storage bins and in the laboratory. Six major stored product insect species were tested to screen combinations of five protectants and three physical methods individually. (1) The concentration of ethyl formate needed to achieve 99% mortality of two species was reduced by 98.47% and 87.95%, when combined with low pressure that was very slightly or not significantly different from control. (2) Diacon II is excellent for controlling external feeders and some non-completely internal feeding stored grain insects for at least 10 months, but it does not control some internal feeders. Methoprene combined with aeration enhancedthe control effect. (3) The combinations of DE at 100ppm with Spinosad at 0.1ppm, Pyrethrins at 1.0ppm, or Storcide II at 0.1 the label rate presented potential synergisms. DE can either reduce the amount of protactants needed to control stored grain insects or increase effects even synergistic effects.

Key Words: Stored product insects, insecticides, physical controls, combining control methods, storage grain bins, laboratory bioassays.

Advisor's Approval: Dr. Thomas W. Phillips