THE EFFECTS OF VARIOUS TEMPERATURE REGIMES AND COOLING RATES ON THE MORTALITY AND REPRODUCTIVE ABILITIES OF TWO STORED GRAIN INSECT SPECIES

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the degree of
MASTER OF SCIENCE
July 1990

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ACKNOWLEDGMENTS

I wish to express sincere appreciation to Dr. W.S. Fargo for his interest, encouragement, friendship and advice throughout my graduate program. My thanks also go to Drs. G.W. Cuperus and B. Clary for serving on my graduate committee and for their suggestions and support.

I would like to extend my special thanks to Mr. E. Bonjour for his help in statistical analysis. My wife, Shama and sons Salman and Imran, encouraged and supported me all the way. Thanks go to them for keeping my goal in sight as well as keeping up my moral. To my parents Mr. M. Azam Khan and Mehrun-Nisa Begum and my Father in-law Mr. Nazir Ahmed Khan, Mother in-law Akbari Begum who gave me moral support and helped me in every way I extend many many thanks. To my brother Khan Zafar Iqbal and his family who gave me their best advice whenever I needed and for their support and help, I extend many thanks.

To USAID and the Government of Pakistan who provided finances for my studies I extend my most sincere thanks. I extend special thanks to my brothers and sisters and friends who supported me throughout the studies.

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ABBREVIATIONS USED

MM	Mean mortality
MCM	Mean cumulative mortality
MAE	Mean adult emergence
MCAE	Mean cumulative adult emergence
Trt.A	Treatment A
Trt.B	Treatment B
Trt.C	Treatment C
Trt.D	Treatment D
Trt.E	Treatment E

CHAPTER I

INTRODUCTION

INTRODUCTION

Insecticides have long been used for control of stored grain insect pests due to their availability, low cost, and effectiveness. However, the continuous use of these chemicals has caused resistance in stored grain insects. Several studies have shown that stored grain insects have developed resistance to malathion and phosphine in some parts of the world (Taylor & Elder 1986, Dyte & Hallisay 1985, Pasalu & Bhatia 1985, Wallbank 1984). Consumers are also becoming increasingly aware of chemical residue in food grains (Zettler & Cuperus 1990). This situation has forced wheat producing countries to explore the possibilities of using non-chemical measures to control stored grain pests.

Grain temperature, moisture content, and relative humidity are key factors affecting the growth and reproduction of stored grain insect pests. For each insect species there is a particular zone of temperature and moisture within which the capacity for population increase is highest. Grain temperatures of 15-20°C are considered minimal for the development of most stored grain pests. Grain damage by insects in the 25-35°C temperature range may be severe. At grain temperatures above and below this level serious damage is unlikely (Cotton 1963, Bishop 1959, Howe 1965, Surtees 1963, Surtees 1965).

Without temperature and moisture stress, species such as Rhyzopertha dominica (F.) and Cryptolestes ferrugineus (Stephens) are long lived. Mean life spans may range from

4-5 months to >1 year (Back & Cotton 1926, Birch 1953, Bishop 1959, Park et al. 1961). R. dominica, a primary grain pest, is among the most abundant and widespread grain insects in Oklahoma (Coppock & Pitts 1985, Cuperus et al. 1986). The Cryptolestes spp. are the most abundant secondary grain insect in Oklahoma (Cuperus et al. 1986).

Studies have shown that rapidly dropping the insect's environmental temperature can be more detrimental to the insect than gradually lowering the temperature (Ushatinskaya 1948, Somme 1968, Evans 1983). To kill insects within 24 h, temperatures of -20°C are required, and Malthein (1968) suggested that -30°C was needed within 24 h for complete eradication.

The objectives of this study were to investigate differential mortalities and reproductive abilities in the lesser grain borer, R. dominica, and the rusty grain beetle C. ferrugineus, under various temperature regimes and cooling rates. Both intraspecific and interspecific comparisons were made.

CHAPTER II

EFFECT OF INSTANTANEOUS DECREASE IN ENVIRONMENTAL TEMPERATURE ON MORTALITY AND REPRODUCTIVE ABILITIES IN *R. dominica* AND *C. ferrugineus*

INTRODUCTION

The effect of low temperature on stored product insects has been documented since the early 1900's. In California, DeOng (1921) reported control or prevention of insect infestations on dried fruit by use of low temperatures. Later, Cotton & Frankenfeld (1942) recommended freezing as a control measure for mill and warehouse insects, employing the cold ambient conditions in the northern parts of United States.

Knowing the effect of temperature on stored grain insects, entomologists have long been experimenting with the use of aeration in the colder months to cool grain masses into the temperature ranges undesirable to insects (Armitage & Stables 1984, Armitage & Llewellin 1987, Burgess & Burell 1964, Burell 1967, Bloom & Cuperus 1984, Southerland et al. 1971, Southerland et al. 1986, Navarro 1973, 1969, Ghaly 1984, Hunter & Tayler 1980, Taylor et al. 1982). Because grain masses exhibit good insulating properties, their temperatures can remain in the undesirable range for several months (Oxley 1948, Bloom & Cuperus 1984). Cuperus et al. (1986) reported that in Oklahoma insect numbers remain low during the summer months and build to their highest level during October and November, they then decline through the remainder of the winter. This winter decline is more pronounced in small bins where natural cooling is more effective. Cooling, using aeration during the fall, causes very rapid declines in live insect counts.

The rate at which the temperature gradient decreases also has a significant effect on stored grain insects (Evans 1983). Studies have shown that rapidly reducing the insect's environmental temperature can be more detrimental to insects than gradually lowering the temperature (Ushatinskay 1948, Somme 1968, Evans 1983). In another study, Evans (1987) found that the immature grain beetles under study did not survive after exposure to 9°C for longer than 39 weeks.

High winter mortalities in stored grain insect under natural conditions in the North Central States were reported by Cotton et al. (1960). The stored grain insect population remained low in North Dakota bins but a resurgence of populations occurred in the Kansas bins each summer. Grain temperatures were cited as the primary cause for the differences in infestation levels at the two localities. Mean bin temperature reached 21.1°C for 5 months in Kansas, whereas only surface temperatures reached this level for two months in North Dakota.

The southern half of United States is considered a high risk region to stored grain insects primarily because of temperature (Storey et al. 1979) and time of harvest (Hagstrum 1988). Historically, most entomologists did not emphasize aeration as a major management tool in developing an integerated management system. Due to warm temperatures, length from harvest, and exponential growth curves, aeration strategies need to be examined. These methods includ wheat temperature reduction is needed to induce mortality and

prevent progeny production. The objectives of the present study were to:

i. Document impact of instantaneous decrease in environmental temperature on mortality in R. dominica and C. ferrugineus.

ii. To see the effect of instantaneous decrese in environmental temperaure on population development in R. dominica and C. ferrugineus.

Both interspecific and intraspecific comparisons were made.

MATERIALS AND METHODS

R. dominica and C. ferrugineus cultures were maintained in the laboratory at 30±1°C and 70±3% RH. Ten 2-4 week old unsexed adults of each species were placed in each of separate 9 cm diameter plastic petri dishes (10 replications), containing a thin layer of an appropriate diet (Miller et al. 1969). The petri dishes were placed in 75.7 liter glass aquaria. These were covered with a plastic sheet equiped with an access door. A stand covered with metal screening was placed inside each chamber to hold the petri dishes. A plastic container with a saturated $NaCl_2$ solution was placed under each stand to maintain 70% RH (Young 1967).

These glass chambers were then placed inside dark incubation chambers. Incubation Chambers were maintained at

30±1, 25±1, 20±1, 15±1, 10±1, 5±1, and 0±1°C, for 42 days. Adult mortality in each petri dish was recorded every seven days. To determine mortality, suspected dead adults were placed in a chamber at 30±1°C for five minutes and observed under a dissecting microscope. If there was no sign of mobility, they were assumed dead and discarded.

At the end of 42 days, petri dishes were removed from the chambers. After removing dead and surviving adults, the dishes were placed at 30±1°C at 70±3% RH for progeny emergence. Newly emerged adults were recorded at 4-week intervals for 12 weeks. After counting, adults were removed and discarded.

Data collected included mean weekly and mean cumulative mortality, mean monthly and mean cumulative progeny emergence. These data were collected for each species at each temperature. Data were analyzed with a general linear model procedure (SAS Institute 1985, 433-506). The variable means with significant differences were separated using Duncan's multiple range test ($P<0.05$ {SAS Institute 1985}).

RESULTS

Rhyzopertha dominica

Mean cumulative mortality (MCM) was significantly greater at 0,5 and 10°C then at other temperatures (Table 1). Mean cumulative mortality was lowest in the intermediate temperatures (15 and 20°C) and somewhat higher at 25 and 30°C.

Table 2 shows mean mortality (MM) at different

temperature and time (days) levels. At 0°C, most (99%) insects died after 21 days and no difference was noted for MM at 0°C after day 7, 14 or 21. Complete mortality at 10°C was observed after day 42. At 5°C, significant differences were seen for MM after day 7, 14, and 21. Yet MM after day 7 and 42 were not different.

Days 14 and 28, days 21 and 28, and days 14 and 35 were not different for MM when compared with each other. At 10°C, MM after day 7, 14 and 21 was significantly different. In contrast, the MM was not different after day 7, 28 and 35, and day 7, 35 and 42.

At intermediate temperatures, 15° and 20°C, no difference was seen for MM between time intervals. No differences were seen for MM at 25°C after 7, 14, 21, 35, and 42 days, but MM after day 28 was different from that of day 7, 21, and 35. MM after day 14, 28 and 42 were not different. No difference was seen for MM at 30°C after day 7, 14 and 42. Similarly, MM was not different after day 14, 21, 28, 35 and 42. Additionally, MM after day 7 was different from day 21, 28 and 35.

Progeny production

Table 3 shows mean adult emergence (MAE) after weeks 4, 8 and 12 for R. dominica after exposure to different temperature levels. Petri dishes which were exposed to cold temperatures, 0, 5 and 10°C, had no progeny production. Adult emergence was not seen in petri dishes exposed to intermediate

temperatures, 15 and 20°C, after 4 weeks. Adult emergence was seen following week 8. The mean number of adults emerged after both 8 and 12 weeks, were not different from each other. Progeny production was seen in petri dishes exposed to 25 and 30°C after each 4-week interval. Mean number of adults emerged in petri dishes exposed to 25°C after weeks 4 and 12, and after week 4 and 8 were not different. But MAE after weeks' 8 and 12 was significantly different. No difference in MAE at 30°C was seen between time intervals (after week 4, 8 or 12).

Mean adult emergence after 4 weeks was significantly different and was highest at 30°C, however, after 8 weeks, MAE was not different after 15 and 20°C, and after 25 and 30°C. After 12 weeks MAE was significantly higher between 20°C, but no difference was seen at 15, 25 and 30°C.

Mean cumulative adult emergence (MCAE)at 30° and 20°C (Table 4) were different compared to other temperature levels. However MCAE was not different at 15 and 25°C. MCAE was maximum at 20°C.

Cryptolestes ferrugineus

Table 1 compares different temperature levels for MCM in C. ferrugineus. Mean cumulative mortality was not different at 0, 5 and 10°C, but this group of temperature levels were different from the group containing 15, 20, 25 and 30°C. MCM at 15 and 20°C were not different. Similarly, the MCM at 25 and 30°C were also not different.

Table 5 shows MM at different temperature and time (days) levels. 99% MCM at 0°c was seen on day 35. 95% and 96% MCM was seen on day 42 at 5 and 10°C, respectively. At 0°C, differences were seen in MM after day 7 compared to the MM after day 14, 21, 28, 35 or 42.

At 5°C, MM was not different after day 7, 14 or 28. Similarly, no difference was seen for the MM after day 7, 28, 35 or 42. However, the MM after day 21 was significantly different from all time intervals but day 14. Mean mortality at 10°C was not different from that of day 7, but for these two intervals days 7 & 14 however, MM was different from rest of the time intervals.

At 15°, 20° and, 30°C, MM was not different for all time intervals. At 25°C, MM after day 42 was different from the rest of the time intervals.

Table 1 shows the interspecific comparison for MCM at all temperature levels. No significant differences were seen at any temperature between R. dominica and C. ferrugineus.

Progeny production

Table 6 shows MAE in C. ferrugineus after exposure to different temperature levels. No adult emergence was seen in petri dishes exposed to cold temperatures (0, 5, and 10°C). Adult emergence was seen after 4 weeks in petri dishes exposed to 15, 20, 25 and 30°C. Mean adult emergence in petri dishes exposed to 15°C was higher after 4 weeks compared to 8 and 12 weeks. Similar results were observed in petri dishes

Table 1. Comparison of mean cumulative mortality among different temperatures between R. dominica and C. ferrugineus in instantaneous decrease in environmental temperature

	R. dominica	C. ferrugineus	F	P
Temperature	MCM	MCM		
0°C	10.0 a	9.9 a	1.00	0.3306
5°C	9.8 a	9.5 a	0.80	0.3823
10°C	10.0 a	9.6 a	3.27	0.0872
15°C	0.2 b	0.1 b	0.36	0.5560
20°C	0.2 b	0.2 b	0.00	1.00
25°C	0.6 cb	1.7 c	3.35	0.0838
30°C	1.0 c	1.9 c	2.09	0.1656
F	1217.18	184.54		
P	0.0001	0.0001		

Means within columns followed by the same letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]). For each column, df=6, 63 and each row, df=1,18.

Table 2. Comparison of mean mortality at a temperature between days in R. dominica in instantaneous decrease in environmental temperature

	Temperature						
	0°C	5°C	10°C	15°C	20°C	25°C	30°C
Days	Mean	Mean	Mean	Mean	Mean	Mean	Mean
7	3.20a	0.20d	0.70cd	0.00a	0.10a	0.00b	0.50a
14	3.60a	2.00bc	2.80b	0.00a	0.10a	0.10ab	0.20ab
21	3.10a	3.30a	4.00a	0.10a	0.00a	0.00b	0.10b
28	0.10b	2.70ab	1.50c	0.10a	0.00a	0.30a	0.00b
35	0.00b	1.00cd	0.90cd	0.00a	0.00a	0.00b	0.00b
42	0.00b	0.60d	0.10d	0.00a	0.00a	0.20ab	0.20ab
F	29.17	8.57	14.14	0.80	0.80	1.88	2.18
P	0.0001	0.0001	0.0001	0.5546	0.5546	0.1134	0.0702

Means within same columns followed by same letter are not significantly different (P >0.05 Duncan's multiple range test [SAS Institute 1985, 448]).

Table 3. Comparison of mean number of weekly adult emergence between temperatures in R. dominica in instantaneous decrease in environmental temperature

	(Mean adult emergence)				
Temperature	4-weeks	8-weeks	12-weeks	F	P
0°C	0.0aC	0.0aD	0.0aC	0.00	0.00
5°C	0.0aC	0.0aD	0.0aC	0.00	0.00
10°C	0.0aC	0.0aD	0.0aC	0.00	0.00
15°C	0.0bC	66.4aAB	61.1aB	10.22	0.0007
20°C	0.0bC	86.3aA	119.5aA	22.4	0.0001
25oC	45.3abB	31.5bC	57.8aB	4.69	0.0196
30°C	63.2aA	62.0aB	63.9aB	0.03	0.968
F	54.29	23.01	28.68		
P	0.0001	0.0001	0.0001		

Means within rows followed by the same lowercase letter or means within columns followed by same uppercase letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]). For each row df=2,27, and for each column df=6,63.

Table 4. Comparison of mean cumulative adult emergence among temperatures between R. dominica and C. ferrugineus in instantaneous decrease in environmental temperature

	R. dominica	C. ferrugineus		
Temperature	MCAE	MCAE	F	P
0°C	0.0 c A	0.0 b A	0.0	0.0
5°C	0.0 c A	0.0 b A	0.0	0.0
10°C	0.0 c A	0.0 b A	0.0	0.0
15°C	127.5 b A	45.7 b B	7.56	0.0132
20°C	205.8 a A	17.3 b B	48.75	0.0001
25°C	134.6 b A	17.8 b B	109.56	0.0001
30°C	189.1 a A	144.6 a A	0.97	0.3376
F	37.91	8.62		
P	0.0001	0.0001		

Means within columns followed by the same lowercase letter or means within rows followed by same uppercase letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]). For each column, df=6, 63 and for each row df=1,18.

Table 5. Comparison of mean mortality at a temperature between days in instantaneous decrease in environmental temperature in C. ferrugineus

	Temperature						
	0°C	5°C	10°C	15°C	20°C	25°C	30°C
Days	Mean	Mean	Mean	Mean	Mean	Mean	Mean
7	6.40a	1.60bc	3.30a	0.00a	0.10a	0.10b	0.30a
14	1.60b	2.50ab	3.80a	0.10a	0.00a	0.10b	0.50a
21	0.60b	3.00a	1.60b	0.00a	0.00a	0.20b	0.40a
28	0.60b	1.30bc	0.30b	0.00a	0.00a	0.10b	0.10a
35	0.70b	0.90c	0.40b	0.00a	0.00a	0.00b	0.20a
42	0.00b	0.20c	0.20b	0.00a	0.00a	1.20ab	0.40a
F	16.88	4.88	8.75	1.00	0.80	3.28	0.59
P	0.0001	0.0009	0.0001	0.4267	0.5546	0.0118	0.7091

Means within same columns followed by same letter are not significantly different (P >0.05 Duncan's multiple range test [SAS Institute 1985, 448]).

Table 6. Comparison of mean number of weekly adult emergence between temperatures in C. ferrugineus in instantaneous decrease in environmental temperature

	(Mean adult emergence)				
Temperature	4-weeks	8-weeks	12-weeks	F	P
0°C	0.0aB	0.0aB	0.0aB	0.0	0.0
5°C	0.0aB	0.0aB	0.0aB	0.0	0.0
10°C	0.0aB	0.0aB	0.0aB	0.0	0.0
15°C	32.9aB	11.3bB	1.5bB	6.23	0.0069
20°C	13.69aB	3.1bB	0.6bB	6.71	0.0051
25°C	13.0aB	3.7bB	1.1bB	6.11	0.0075
30°C	93.6aA	44.8abA	4.4bA	4.66	0.02
F	8.08	6.34	7.91		
P	0.0001	0.0001	0.0001		

Means within rows followed by the same lowercase letter or means within columns followed by same uppercase letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]). For each row df=2,27, and column df=6,63.

exposed to 20 and 25°C. No difference in MAE in petri dishes kept at 30°C was seen after 4 and 8 weeks, likewise no difference in MAE was seen after 8 and 12 weeks.

After 4, 8 and 12 weeks MCAE was significantly higher at 30°C compared to other temperature levels.

MCAE for each species at 30°C was significantly different from all other temperatures (Table 4).

Interspecific comparison showed significantly higher MCAE for R. dominica at 15, 20, and 25°C, however no difference was seen at 30°C (Table 4).

DISCUSSION

Under natural conditions, the only mortality directly attributable to temperature is from freezing, insect stage related natural causes and excessively high temperatures. Stored grain insects die even at temperatures well above freezing (Wigglesworth 1972). The actual cause of death is not understood. According to the criteria of Solomon and Adomson (1955), C. ferrugineus is cold hardy while R. dominica is only moderately cold hardy. The present study showed that both C. ferrugineus and R. dominica could not survive at temperatures $\leq$ 10°C for a period of 6 weeks. In contrast, temperatures $\geq$ 15°C did not effect mortality in either species. Earlier, Smith (1970) noted considerable variability in response to cold among populations of C. ferrugineus. He speculated that genetic heterogeneity was the cause. However, since the present study did not include

this aspect, we are not clear about the deviation in the response of C. ferrugineus to cold.

Cotton et al. (1960) caged adult R. dominica in the center of a 2,740 bushel wheat bin. Ambient temperature reached 10°C 138 days latter. They reported complete insect mortality after 153 days. In contrast, we observed complete mortality at 10°C after 42 days. The difference in time for complete mortilty may be attributed to conditions used in each study. Cotton et al (1960) conditions were of gradually dropping temperature and no humidity control. This study used instantaneous envvironmental temperature change under controlled humidity. Evans (1983) reported complete mortality of R. dominica at 9°C and 45% R.H. after weeks 2-4, and week 7 at 70% R.H. However, this difference may be due to biological variation in populations.

No effect of temperatures at 15, 20, 25 and 30°C on MM in R. dominica was observed. However, 15 and 20°C did delay the emergence of adults compared to 25 and 30°C. This observation is consistent with Khare & Agrawal (1970) who reported adult emergence in R. dominica at 75% R.H. at 18, 25, and 30°C as 56, 50, and 32 days, respectively. Mean number of emerged adults after 8 and 12 weeks did not vary much among temperature regimes.

Temperatures $\leq$10°C also affected the progeny in C. ferrugineus. No adult emmergence was seen in petri dishes exposed to temperatures of 0, 5, and 10°C. This may be due to fact that most of the adults died at these temperatures by

day 42. The delay in adult emergence of R. dominica at 15 and 20°C is due to day degrees requirements for development. Adult emergence after 4 weeks at 25 and 30°C shows the preference of high temperatures by R. dominica and C. ferrugineus for reproduction

The interspecific comparison for progeny production shows significantly higher reproductive potential of R. dominica at all temperatures levels except at 30°C.

Although R. dominica died earlier at 0°C, the MCM after week 6 was not different compared with that of 5 and 10°C. Similar results were also seen for C. ferrugineus. This suggests that quickly dropping the grain temperature below 10°C and keeping it for about 6 weeks can not only be lethal to the adults of C. ferrugineus and R. dominica, but also prevent further progeny production in both R. dominica and C. ferrugineus.

CHAPTER III

EFFECT OF GRADUAL DECREASE IN ENVIRONMENTAL TEMPERATURE ON MORTALITY AND REPRODUCTIVE ABILITIES IN *R. dominica* AND *C. ferrugineus*

INTRODUCTION

Different species of insects react differently to temperature. Stored grain insects will acclimate to low temperatures when cooled gradually or held at an intermediate temperature before being exposed to low temperatures (Robinson 1926, Edwards 1958, Earnst & Mutchmor 1969, Smith 1970, David et al. 1977, Evans 1981, Evans 1983, Granovsky & Mills 1984). Due to thermal resistance (Oxley 1948), grain mass temperature tends to decrease gradually even though outside temperatures are much lower than grain bulk temperatures. Smith (1970) and Hagstrum (1987) reported 1-2°C drop per week in wheat bins in winter. Under natural conditions, the insects become acclimated to seasonal temperature changes (David et.al. 1977). The rate at which the temperature decreases also an effect on stored grain insects (Evans 1983).

Evans (1981) studied cold acclimation at 15°C in adults of C. ferrugineus, Oryziphilus surinamensis (L.), R. dominica, and Tribolium castaneum (Herbst). He found that exposure to the 15°C acclimation temperature lowered the chill-coma temperature and oxygen consumption in all species. The greatest change in mean chill coma temperature, i.e. the temperature at which 50% of exposed insects were unable to survive (ET_{50}) occurred with O. surinamensis, which dropped from 9.97 to 5.64°C. Evans (1983) focused experiments on several stored grain insects. He found that, depending on species, insects which were cooled gradually to

9°C survived for an average of 5 to 8 weeks, but insects transfered directly to 9°C lived for only 2 to 4 weeks. In a later study, Evans (1987) found that immature grain beetles did not survive exposure to 9°C for longer than 39 weeks.

The actual effects of exposure to gradually decreasing temperature on stored grain insects have had limited study. Reviewing diapause in stored grain insects, Howe (1962) listed 20 species which demonstrated diapause in storage, including C. ferrugineus. Atwal (1960) studied survival of 0.5-4.5-day-old pupae of Anagasta kuhniella (Zeller) after a 4-hour exposure to -15°C. Before exposure, pupae were "conditioned" at 5, 10, 15, 20, or 25°C for various periods up to 16 hours. He found that as conditioning temperatures were lowered from 25 to 10°C, sub-zero exposure mortality gradually decreased, but if "conditioned" at 5°C, mortality increased, indicating that the best physiological adjustments occurred at temperatures of 10°C. Short duration (1-4 h) conditioning was more beneficial than longer periods (1-6 days) which increased mortality. Furthermore, adult age also influenced tolerance to sub-zero temperatures.

Similarly, Somme (1968) studied the effect of low temperature on Tribolium confusum (Jacquelin du Val). Adult beetles were maintained at 27°±1°C or 12±1°C and then exposed to 0°C to compare survival. Time required to kill 50% (LT_{50}) exposed to 27°C and 12°C were 2.9 days and 5.6 days, respectively. If beetles were acclimated for varying periods (0 h to 8 days) at 12°C, the mortality after 4 days at 0°C

was greatly altered. A 3 h acclimation at 12°C reduced mortality from 85% to 47%, whereas with 6 to 8 days of acclimation only 10% mortality occured.

Smith (1970) studied the effects of cold acclimation at 15°C for periods up to 28 days on survival of 1-to-3 week old adults of C. ferrugineus at -6 and -12°C. Several weeks of acclimation were needed to produce maximum effect on adult survival at sub-zero temperatures. LT_{50} of adults acclimated at 15°C and tested at -6°C increased from 9.4 to 31.4 days as acclimation time increased from 7 to 14 days. At -12°C, maximum survival occurred when beetles were maintained at 15°C for at least 20 days.

Building on this past work, the objectives of present study were;

i. To investigated the effect of rate of temperature drop of temperature on the mortality of adults of R. dominica and C. ferrugineus.

ii. To see the effect of rate of temperature drop on population development in R. dominica and C. ferrugineus.

Both interspecific and intraspecific comparisons were made.

MATERIALS AND METHODS

R. dominica and C. ferrugineus were taken from laboratory cultures kept under standard conditions of 30±1°C and 70±3 % RH. Ten 2-4 week-old unsexed adults of each of

the species were placed in each of 10 replications, 9 cm petri dishes containing a thin layer of appropriate diet (Miller et al., 1969). Ten such petri dishes for each species were placed in small 75.7 l aquaria. These were covered with a plastic sheet equiped with an access door. A stand covered with metal screening was placed inside each chamber to hold petri dishes. A plastic container with saturated $NaCl_2$ solution was placed under the stand for maintaining 70% RH (Young 1967). One glass chamber was then placed inside each dark incubation chamber.

Treatment A. After 1 week at 30°C, the insects were exposed in succession for 1 week each at 20° and 10°C, and then held at 0°C.

Treatment B. After 1 week at 30°C, the insects were exposed in succession for 1 week each at 25, 20, 15, 10, 5°C and then held at 0°C.

Treatment C. After 1 week at 30°C, the insects were exposed for 1 week at 15°C and then held at 0°C.

Treatment D. After 1 week at 30°C, the insects were exposed to 15°C for 2 weeks and then held at 0°C.

Treatment E. After 1 week at 30°C, the insects were exposed to 15°C for 3 weeks and then held at 0°C.

Observations for adult mortality were taken at 7 d intervals at the end of each temperature exposure. Those adults presumed dead were placed at 30±1°C for five minutes and then observed under the microscope. If there was no sign of mobility, they were considered dead and discarded.

Following the mortality experiment and removal of survivors, the petri plates were kept at 30±1°C at 70±3%RH. Adult emergence was recorded after weeks 4, 8 and 12. Data collected included mean weekly and mean cumulative mortality, mean monthly and mean cumulative adult progeny emergence. These data were collected for each temperature treatment. Data were analyzed with a general linear model procedure (SAS Institute 1985, 433-506). Variable means with significant differences were separated using Duncan's multiple range test ($P<0.05$ {SAS Institute 1985}).

RESULTS

The results on mortality and progeny production for each species under study are presented separately.

R. dominica

Table 7 shows a comparison of mean cumulative mortality (MCM) within treatments. No significant difference was seen between Trt.A, Trt.B, and Trt.C. However, Trt.D and Trt.E were significantly lower than the other treatments.

Table 8 shows the comparison of mean mortality (MM) among different time intervals (days) for different treatments. In Trt.A, no difference was seen for MM after day 7, 14, 21 or 28. In contrast, differences were seen for MM after day 35 and 42.

For Trt.B no differences were seen for MM after day 7, 14, 21, 28, 35 or 42 (group 1). Similar results were seen

after day 49, 56 and 63 (group 2). However, when compared for MM, these groups were significantly different from each other.

For Trt.C, no difference for MM was seen after day 7, 14, or 21, but differences were seen after day 28 and 35.

For Trt.D, no difference for MM was seen after day 7, 14, 21 or 28 but differences were seen after day 35 and 42.

No difference for MM was seen at Trt.E, after day 7, 14, 21, 28, or 35 (group 1). Additionally, MM after day 35 and 42 (group 2) were not different. However, after day 49 MM was significantly different when compared with groups 1 and 2.

Table 7 shows %MCM for R. dominica. Mean cumulative mortality was 100%, 87%, 88%, 68% and 56% for Trt.A, Trt.B, Trt.C, Trt.D, and Trt.E, respectively. MCM was significantly higher for Trt.A, Trt.B, and Trt.C.

Table 7 shows interspecific comparison where MCM was significantly higher for Trt.A in R. dominica. No difference was seen in MCM in Trt.B or Trt.C. However, MCM for Trt.D and Trt.E were significantly higher in C. ferrugineus.

Progeny production

Table 9 shows mean adult emergence (MAE) in R. dominica. No adult emergence was observed in any treatment except Trt.E. Adult emergence was delayed until the 8th week, but MAE was not different between week 8 and 12.Mean adult emergence after 4, 8 and 12 weeks was significantly

different in Trt.E compared to other temperature treatments.

C. ferrugineus

Table 7 shows a comparison of MCM among treatments. No statisticaly ($P<0.05$) significant differences were seen among treatments.

Table 10 shows the comparison of MM among days at different temperature treatments. For Trt.A, MM after day 28 was different from the rest of time. Similar results were seen in Trt.C.

For Trt.B, MM after day 49 was different from the rest of the time intervals.

For Trt.D, MM after day 35 was different from the rest of the time intervals. At Trt.E, MM was different after day 35 or 42; however, no difference were seen for MM in the rest of the time intervals.

Figure 2 shows %MCM in each treatment. MCM was 73%, 70%, 65%, 100%, 100%, at Trt.A, Trt.B, Trt.C, Trt.D, and Trt.E, respectively.

Progeny production

Table 11 shows no difference in MAE in Trt.A after weeks 4, 8, or 12. In Trt.B, MAE was significantly different after week 4, but MAE was not different after week 8 and 12. No adult emergence was seen for Trt.D or Trt.E. After 4 weeks, MAE was higher at Trt.B compared to other treatments, however, after 8 and 12 weeks no difference was seen among

Table 7. Comparison of mean cumulative mortality in and between R. dominica and C. ferrugineus at different temperatures following a gradual decrease in environmental temperature

	R. dominica	C. ferrugineus			
Treatment	Mean	Mean	F	P	df
A	10.0aA	7.3aB	8.62	0.008	1,18
B	8.7aA	6.4aA	3.04	0.098	1,18
C	8.8aA	6.3aA	3.44	0.080	1,18
D	6.8bA	10.0aB	13.84	0.0059	1,8
E	4.6cA	10.0aB	112.15	0.0001	1,8
F	17.09	2.18			
P	0.0001	0.0914			

Means within columns followed by the same lowercase letter or means within each row followed by same uppercase letter are not significantly different ($P > 0.05$: Duncan's multiple range test [SAS institute 1985, 448]). For each column, df=4, 35.

Table 8. Comparison of mean mortality at a temperature between days following a gradual decrease in environmental temperature in R. dominica

	Temperature Treatments				
	Trt.A	Trt.B	Trt.C	Trt.D	Trt.E
Days	Mean	Mean	Mean	Mean	Mean
7	0.2 c	0.10 b	0.10 b	0.4 c	0.0 c
14	0.0 c	0.20 b	0.10 b	0.0 c	0.0 c
21	0.0 c	0.00 b	0.10 b	0.0 c	0.0 c
28	0.8 c	0.00 b	4.20 a	0.0 c	0.0 c
35	6.2 a	0.00 b	4.30 a	2.2 b	0.4 bc
42	2.8 b	0.00 b		4.2 a	1.0 b
49		2.70 a			3.2 a
56		2.90 a			
63		2.80 a			
F	58.01	9.09	17.05	10.30	19.55
df	5, 54	8, 81	4, 45	5, 24	6, 28
P	0.0001	0.0001	0.0001	0.0001	0.0001

Means within same columns followed by same letter are not significantly different (P >0.05 Duncan's multiple range test [SAS Institute 1985, 448]).

Table 9. Comparison of mean number of weekly adult emergence at different temperatures in R. dominica following a gradual decrease in environmental temperature

(Mean adult emergence)					
Temperature	4-weeks	8-weeks	12-weeks	F	P
Trt.A	0.0aA	0.0aB	0.0aB	0.0	0.000
Trt.B	0.0aA	0.0aB	0.0aB	0.0	0.000
Trt.C	0.0aA	0.0aB	0.0aB	0.0	0.000
Trt.D	0.0aA	0.0aB	0.0aB	0.0	0.000
Trt.E	0.0bA	43.8aA	46.2aA	23.82	0.0001
F	0.0	51.2	135.37		
P	1.0	0.0001	0.0001		

Means within rows followed by the same lowercase letter or means within columns followed by same uppercase letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]). For each row df=2, 27, and column df=4, 30.

Table 10. Comparison of mean mortality at a temperature between days in C. ferrugineus following a gradual decrease in environmental temperature

	Temperature Treatments				
	Trt.A	Trt.B	Trt.C	Trt.D	Trt.E
Days	Mean	Mean	Mean	Mean	Mean
7	0.5 b	0.60 b	0.40 b	1.0 b	0.6 c
14	0.1 b	0.30 b	0.10 b	0.0 bc	0.0 c
21	0.3 b	0.50 b	0.10 b	0.4 c	0.2 c
28	4.1 a	0.10 b	4.10 a	1.2 b	0.0 c
35	1.2 b	0.10 b	0.90 b	7.4 a	3.8 b
42	0.7 b	0.50 b	0.50 b	0.0 c	5.4 a
49	0.3 b	3.50 a	0.40 b	0.0 c	0.0 c
56	0.1 b	0.20 b		0.0 c	0.0 c
63		0.00 b			0.0 c
70		0.40 b			
77		0.80 b			
F	6.06	5.98	6.54	53.40	33.64
df	7, 67	10, 84	6, 58	7, 32	8, 36
P	0.0001	0.0001	0.0001	0.0001	0.0001

Means within same columns followed by same letter are not significantly different (P >0.05 Duncan's multiple range test [SAS Institute 1985, 448]).

Table 11. Comparison of mean number of weekly adult emergence at different temperatures in C. ferrugineus following a gradual decrease in environmental temperature

	(Mean adult emergence)				
Temperature	4-weeks	8-weeks	12-weeks	F	P
Trt.A	0.3 aB	1.80 aA	0.3 aA	2.26	0.1243
Trt.B	2.6 aA	1.1 abA	0.1 bA	3.28	0.0529
Trt.C	0.2 aB	1.50 aA	0.0 bA	1.64	0.2127
Trt.D	0.0 bB	0.00 bA	0.0 bA	0.00	0.000
Trt.E	0.0 bB	0.00 bA	0.0 bA	0.00	0.000
F	4.36	0.96	1.09		
P	0.0058	0.4400	0.3782		

Means within rows followed by the same lowercase letter or means within columns followed by same uppercase letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]). For each row, df=2, 27, and column df=4, 35.

Table 12. Comparison of mean cumulative adult emergence among different temperatures between R. dominica and C. ferrugineus following a gradual decrease in environmental temperature

	R. dominica	C. ferrugineus			
Treatment	MCAE	MCAE	F	P	df
A	0.0 aA	2.4 abA	1.68	0.217	1,18
B	0.0 aA	8.7 aB	8.07	0.010	1,18
C	0.0 aA	4.9 abB	5.53	0.030	1,18
D	0.0 aA	0.0 bA	0.00	1.000	1,8
E	90.0 bA	0.0 bB	54.62	0.0001	1,8
F	87.78	2.62			
P	0.0001	0.0513			
df	4, 30	4, 35			

Means within columns followed by the same lowercaseletter or means within rows followed by same uppercas letter are not significantly different (P >0.05: Duncan's multiple range test [SAS institute 1985, 448]).

all treatments.

Table 12 shows the comparison of MCAE within temperature treatments both in R. dominica and C. ferrugineus. In C. ferrugineus, no differences were seen among Trt.A, Trt.B or Trt.C. Treatment B was significantly higher than Trts.D and E, as no adult emergence was seen in the latter treaments. However MCAE between Trt.A, Trt.C, Trt.D, and Trt.E were not significantly different. Mean cumulative adult emergence was significantly high in R. dominica at Trt.E compared to other temperatures.

Table 12 shows interspecific comparison for MCAE at each treatment. No difference for MCAE was seen among either species in Trt.A and Trt.D. Mean cumulative adult emergence was significantly higher in C. ferrugineus at Trt.B and Trt.C. However, in Trt.E the MCAE was higher in R. dominica.

DISCUSSION

Several researchers (Edwards 1958, Malthein 1968, David et al. 1977, Evans 1981, and Evans 1983), have shown that prior acclimation to cold conditions lowered the chill-coma temperature and increased the survival of stored grain insects exposed to unfavourably low temperatures. David et al. (1977) reported that acclimated R. dominica survived after exposure to 4.4°C for six weeks. The present results are consistent with these previous studies. Acclimation increased the survival of adult R. dominca with increases in acclimation time. The MCM of adult R. dominica decreased

from 88% to 68% and 56% when acclimation time was increased from 7 days to 14 and 21 days, respectively.

Smith (1970) reported that cold acclimation enhances C. ferrugineus adult survival at freezing temperatures. Smith (1965) also reported that cold acclimation appears to occur initially at a temperature slightly lower than 20°C. This indicates that those temperatures at which physiological development is inhibited sufficiently to arrest growth, enhances the abilities of insects to survive at freezing temperatures.

We also observed that acclimation of adults of C. ferrugineus in Trt.A, Trt.B and Trt.C increased their survial as MCM at Trt.A was decreased by 27%, and in Trt.B MCM was decreased by 30%. In Trt.C, MCM was reduced by 35%. However, for Trt.D and Trt.E, where C. ferrgineus had a longer exposure at 15°C i.e. 14 and 21 days, MCM was 100%. Atwal (1961) and Salt (1961) theorized that longer exposure of adult C. ferrugineus at 15°C (>3-4 weeks) produced the accumulation metabolic by-products which became increasingly toxic. This accmulation may have diminished the benifits of acclimation. In our study, this phenomenon may have occurred as early as the 2nd week of acclimation at 15°C, thereby causing mortality in all the adults of C. ferrugineus.

All temperature treatments, except Trt.E, supressed reproductive abilities of R. dominica. This is suggested by cold hardiness developed by increase in production of fructose and glyserol in R. dominica when transfered from

30°C and then kept at intermediate temperature (15°C) for 3 weeks (unpublished data).

Acclimation for shorter period of times at intermediate temperatures increased survival and reproductive abilities in C. ferrugineus but acclimation for slightly longer period (2-3 weeks at 15°C) was not favourable for MCAE. Interspecific comparison for MCAE shows that reproductive abilities of R. dominica was affected in treatments where acclimation at intermediate temperatures (15 or 20°C) but it did not affect C. ferrugineus. However, acclimation at intermediate temperatures for longer periods (2-3 weeks) produced the opposite results. The explanation mentioned earlier, given by Salt (1961) for similar type of results may be the best explanation.

The present study highlights the marked degree of both between- and within-species variation. However, it should be noted that the temperature change in the present study (i.e. 5, 10, and 15°C in one week's time) is faster than the natural situation.

SUMMARY AND CONCLUSIONS

Cold temperatures, 0, 5, and 10°C were lethal after 6 weeks for both R. dominica and C. ferrugineus. Neither species could survive at these temperatures. Progeny production was also not seen at these temperatures. Intermediate (15-20°C) and high (25-30°C) temperatures has little or no effect on the adult survival. Intermediate

temperatures however, did delay adult emergence in both species.

In gradual decrease in environmental temperature Trt.A, Trt.B, and Trt.C, where acclimation at intermediate temperatures was for a shorter period (one week), R. dominica did not survive. In Trt. D, where acclimation at 15°C was for a longer period (2-3 weeks), R. dominca survival was significantly higher than C. ferrugineus. No adult emergence was seen for either species at any temperature treatments except at Trt.E. Even here, only R. dominica had adult emergence.

The present study further supports the evidence that the ability to acclimate to cold conditions in nature is common among stored grain insects. This study provides evidence that adult C. ferrugineus and R. dominica are able to acclimate physiologically to temperatures at least as low as 15°C. As the grain gradually cools during autumn, there is enough time for both C. ferrugineus and R. dominica to acclimate to lower temperatures. Temperatures in the center of the bin are usually higher than outside. Insects in the insulated part of grain mass could survive for a longer period. However quickly reducing grain temperature to less than 10°C and keeping it there for several weeks could still prove useful in controlling R. dominica and C. ferrugineus as well as eliminating the chances of further progeny production in both species.

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