FINAL REPORT

Studies on Diapause in the Bamboo Borers; *Omphisa fuscidentalis* Hampson, (Lepidoptera; Pyralidae)

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PREFACE

Bamboo borer, *Omphisa fuscidentalis* Hampson (Lepidoptera; Pyralidae), commonly known as a bamboo borer, is a tropical moth found in South, and Southeast Asia. The mature larvae enter the diapause in September and pupae in June. The larval stage lasts for 280-300 days. The pupal stage lasts for 30-40 days. The adult moths can live 8 - 13 days.

Diapause, a sleeping time, is a development resting stage that insects use to avoid hyperthermia (environmental stress - photoperiod and temperature) and other associated physiological stresses (dehydration, starvation). It is also an important mechanism for synchronizing life cycles with the growing season. *O. fuscidentalis* is a univoltine species over its whole distribution range. That's means diapause in bamboo borer lasts more 8-9 months. A long diapause period in bamboo borer hindered the commercialization of this species. If a way is found to terminate diapause of bamboo borer, then farmers could rear the bamboo borer commercially all round the year, and help poverty alleviation of Thai farmers. Therefore, for commercial purpose, it is necessary to reproduce the adult moths twice or thrice a year. Bamboo worms are the main sources of protein to hill tribe people. The villagers will not have malnutrition problems in their family. Bamboo worms are highly demanded in local markets as well as in international markets. Therefore, the villagers will have income source which will help to alleviate poverty. The finding of this research may provide information to villagers for commercializing this species, bamboo borer.

ACKNOWLADGEMENT

I would like to give sincere thanks from the bottom of my heart to the President of Mae Fah Luang University for kindly allowing conducting this research to conservation of this economic species.

I would also like to thanks to all those staffs and students who directly and indirectly give me their helping hands during this research period.

Ratna Thapa

EXECUTIVE SUMMARY

To determinate the clue to terminate diapausing period of bamboo borer larvae and pupae.

Bamboo worms are the main sources of protein to hill tribe people. The villagers will not have malnutrition problems in their family. Bamboo worms are highly demanded in local markets as well as in international markets. Therefore, the villagers will have income source which will help to alleviate poverty

In total, 600 hundreds larvae were kept at 5, 10, 15, 20, 25 and 30°C. Each plate was contained 10 larvae. Each plate was checked every week to determine mortality of larvae.

The results showed that when the temperature was raised above 35°C, all larvae were dead. The optimum temperature for survival was between 10-14°C. The photoperiods did not effect on larvae. The results suggest that photoperiod is not a key to terminate diapauses in bamboo moth. However the moisture played a key role in diapausing. The larvae supplied with moist cotton buds live up to 7 months. The larvae treated with moist cotton balls were terminate diapausing period shorter than natural condition. The nutrients also did not show any effect on terminating of diapausing. The larvae of bamboo borer can survive up to 6-7 moths without food supplied.

In conclusion, at low temperature (5°C), all the larvae were dead and at high temperature above 35°C, all larvae will die. The larvae were not found sensitive to temperature. Temperature, light intensity (photoperiods), nutrient and water are not key factors to terminate diapauses of bamboo borer larvae. However, the results of this research suggested that water could be a secondary key factor that could terminate diapausing of bamboo borer larvae.

ABSTRACT

Diapause is a period of arrested development, which is most frequently a change in day length (photoperiods), rather than in temperature or humidity which may be very variable. Termination of diapause in bamboo bore has never studied. The objective of this research was to determine the clue to terminate diapausing period of bamboo borer larvae and pupae.

In total, 600 hundreds larvae were kept at 5, 10, 15, 20, 25 and 30°C. In total, 324 larvae were exposed to photoperiod 8 hr and 10 hr. In total, 250 larvae were kept in plastic cup with wet cotton buds at room temperature 26-28°C with a photoperiod L:D 10:14. In total, 300 larvae were kept at room temperature in plastic box simply by covered with a tissue paper. In total 788 larvae were used in this experiment. The larvae were divided into two groups. The group-A was treated with food (fresh culm), and the culm were changed once a week. The group-B did not feed. The pupae were sexually (males and females) separated from each other and they were separately hung on chopsticks and kept at room temperature (26-28C°).

The results suggest that the larvae of bamboo borers were unable to tolerate low temperature (5°C) and high temperature (30°C). The optimum temperature for survival of larvae was between 10-14°C. The light intensity (photoperiods LD: 08:16 hr and 10:14hr did not terminate diapause. Subsequently, nutrients did show positive effect. However, as long as the food available, larvae can survive, but in absent of food, larvae use body fat. The water could be a key factor they can terminate diapause. The results suggest that diapausing pupae can be terminate with high temperature. In conclusion, the diapausing period of bamboo borer larvae cannot be terminated; it is probably that this species is a univoltine species.

In further study, it is recommended to focus on water as a key factor to terminate diapausing larvae, temperature effects on diapausing pupae and should also focus on the juvenile hormone (JH). Because, JH governs diapause in almost all insect.

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ABBREVIATION AND SYMBOLS

LD	Light and dark
°C	degree centigrade

CHAPTER – 1
INTRODUCTION

1.1 Background

Bamboo borer, *Omphisa fuscidentalis* Hampson (Lepidoptera; Pyralidae), commonly known as a bamboo borer, is a tropical moth found in South, and Southeast Asia. The *O. fuscidentalis* are larvae which live in internodes of some selective bamboo species. Adult females lay around 200 on an average (personal observation) on young bamboo shoots in the raining season. Larvae are hatched within 5-7 days, and make a hole at the basal stem, and migrate inside the young bamboo shoot. Then, they start to feed inner pulp of the internodes till the pupal stage. *O. fuscidentalis* usually make a characteristic noise, which helps the bamboo borer hunters to locate their position (Yhoungaree and Puwastien, 1997). The mature larvae enter the diapause in September and pupae in June (Singtripop *et al.*, 1999). The diapause is a strategy for survival of larvae in a harsh environment condition (Denlinger, 1985). The larval stage lasts for 280-300 days. The pupal stage lasts for 30-40 days. The adult moths can live 8 - 13 days. In previous research, I have developed a mass rearing technique of adult moths in the laboratory, and release them in the field.

1.2 Statement and significance of this research

The term diapause, in fact, a dramatic example of extending the life span, has been used for developmental delays that allow the organisms to survive during the unfavorable conditions and to synchronize growth and development with favorable conditions and resume as soon as hindrance is removed (Andrewartha, 1952; Beck, 1962). Diapause, a sleeping time, is a development resting stage that insects use to avoid hyperthermia (environmental stress - photoperiod and temperature) and other associated physiological stresses (dehydration, starvation) (Ushatinskaya, 1987). It is also an important mechanism for synchronizing life cycles with the growing season (Beck, 1980). *O. fuscidentalis* is a univoltine species over its whole distribution range. That's means diapause in bamboo borer lasts more 8-9 months. A long diapause period in bamboo borer hindered the commercialization of this species. If a way is

found to terminate diapause of bamboo borer, then farmers could rear the bamboo borer commercially all round the year, and help poverty alleviation of Thai farmers. Therefore, for commercial purpose, it is necessary to reproduce the adult moths twice or thrice a year. To obtain the knowledge of diapause is essential for understanding the seasonal biology of bamboo borer for manipulating commercialized purposes. Termination of diapause in bamboo bore has never studied.

1.3 Objective

Understanding diapause and its regulation has always been central to understanding insect phenology and seasonal patterns of distribution. Therefore, the main objective of this research was to determinate the clue to terminate diapausing period of bamboo borer larvae and pupae.

1.4 Benefits of this research

- Bamboo worms are the main sources of protein to hill tribe people. The villagers will not have malnutrition problems in their family.
- Bamboo worms are highly demanded in local markets as well as in international markets. Therefore, the villagers will have income source which will help to alleviate poverty, and
- Bamboo worms are main source of bamboo forests conservation. Because, the bamboo worms are lived inside the bamboo culm without damaging the bamboo. Therefore, if the villagers want to make extra income by selling b bamboo worms, they have rear in the bamboo which is a natural habitat of bamboo borers. Another reason is that the villagers have to protect the young bamboo shoots, which are heavily harvested from Doi Tung and sell to the can food factories.

CHAPTER - 2
LITERATURE REVIEW

2.1 Diapause

Diapause is a period of arrested development, characterized by low oxygen consumption. The cue to enter diapause is most frequently a change in day length (photoperiods), rather than in temperature or humidity which may be very variable. In the cabbage white butterfly, for example, the shorten day length at the end of summer switch off brain hormone in the final larval stage. The pupa can be formed, but cannot moult, and therefore, goes into diapause. After an extended period of chilling (winter) the brain hormones switches on again, the pupa moult and butterfly emerges.

The term diapause has been used for developmental delays that allow organisms to survive unfavorable conditions and to synchronize growth and development with favorable conditions. It is important to distinguish between true diapause, in which development is arrested in advance of unfavorable conditions and does not respond immediately to amelioration of the external environment, and quiescence, in which development is temporarily inhibited by an unfavorable environment and may be resumed as soon as the hindrance is removed (Andrewartha, 1952). During diapause, there is a refractory phase (Mansingh, 1971), also known as diapause development (Andrewartha, 1952). During diapause, development is inhibited even if environmental conditions have become favorable again. In addition, diapause-stimuli are perceived before the induction of diapause begins, usually during a stage prior to the one that enters diapause.

When the correct stage for diapause has been attained, the insect arrests its development, switches on the new metabolic machinery that will sustain it during metabolic suppression, and then "decides" the correct time to resume development. In this section I first offer a brief overview of the hormonal signaling systems that preside over diapause and then discuss patterns of gene expression associated with the diapause stage.

Among insects that use day-length to program their diapause, the window of sensitivity to photoperiod usually is fairly brief and occurs well in advance of the

actual diapause stage. The basic requirement is a mechanism to distinguish long days from short days and a mechanism to count the number of short days that have occurred (a counter). The clock literature has blossomed over the past few years, culminating in detailed characterizations of several critical clock genes including period, timeless, clock, cycle, doubletime, and vrille (Dunlap, 1999; Schotland and Sehgal, 2001), as well as several forms of cryptochrome, genes that encode a photoreceptor involved in circadian rhythmicity (Cashmore and Jarillo, 1999; Hall, 2000).

The arrest in development, accompanied by suppressed metabolism, has been observed in diverse embryonic stages, different larval instars, pupae, pharate adults, and adults, but for any given species, the potential for diapause is usually restricted to a single stage. In a few cases diapause occurs at a specific stage in each generation, regardless of the prevailing environmental conditions (obligatory diapause), but more commonly, environmental tokens such as day-length are utilized for the programming of diapause (facultative diapause). Most commonly, the short day-lengths of late summer signal the advent of winter to temperate zone species. Winter is thus anticipated long before the onset of low temperatures, allowing the insect to store additional energy reserves and seek a protected site for overwintering.

Insertion of diapause into the life cycle requires mechanisms for monitoring environmental cues, storing this information in the brain until the appropriate developmental stage is attained, and acting upon this information to bring about a halt in development, maintaining the body in a state of suppressed metabolism, and eventually lifting the arrest so that the insect can resume development or reproduction. Extensive research on diapause during the past half century has yielded a fairly comprehensive view of the environmental regulators of diapause (Danks, 1987; Tanaka et. al., 1998; Zaslavski, 1988), the hormonal systems that direct the onset and termination of diapause (Denlinger, 1985), and the theoretical properties of the clock mechanisms involved in insect photo-periodism (Denlinger et al., 2001; Takeda and Skopik, 1997). The conspicuous void that persists in this field is understanding the molecular underpinnings of diapause, and this topic is the focus of this review.

1.2 Endocrine regulators

Several key hormones serve as regulators of diapause, but precisely which hormones are involved depends on the species and the developmental stage in which diapause occurs (Denlinger, 1985). In brief, embryonic diapauses are controlled several ways. The best-known case is the regulation of early embryonic diapause in the silk moth *Bombyx mori* (Yamashita, 1996). In this species embryonic diapause is induced by the action of diapause hormone, a neuropeptide released from the suboesophageal ganglion of the female. In the gypsy moth, diapause intercedes at a later stage of development, after embryonic development has been completed but before the first instar larva breaks through the chorion. This diapause is maintained by elevation of ecdysteroids, and the ecdysteroid titer must drop before development can proceed (Lee and Denlinger, 1997). Yet a different endocrine mechanism operates in the giant silkmoth *Antheraea yamamai* (Suzuki, et al., 1990). In this species a factor from the mesothorax represses the action of a development-promoting factor from the abdomen, but thus far, the identity of neither factor is known.

Larval and pupal diapauses are usually characterized by a shutdown of the brain-prothoracic gland axis. As a result, the prothoracic gland fails to synthesize the ecdysteroids needed to promote development. This may be caused by either the failure of the brain to release the neuropeptide prothoracicotropic hormone needed to stimulate the prothoracic gland (Richard, 1987) or by failure of the prothoracic gland to respond to prothoracicotropic hormone until an adequate period of chilling has been experienced (Meola and Adkisson, 1977). In some species of Lepidoptera that undergo stationary molts during larval diapause, the juvenile hormone (JH) titer remains elevated, thus guaranteeing that any molt will be stationary rather than progressive (Yin and Chippendale, 1977).

Most cases of adult diapause can be attributed to the absence of JH (Denlinger, 1985). A number of reproductive events in insects are regulated by JH, and blocking its production, as occurs in diapause, results in the cessation of egg maturation, atrophy of accessory glands, degeneration of flight muscles, and a halt in mating activity. Activation of the corpus allatum, the gland that secretes JH, terminates diapause: Flight muscles regenerate, mating activity ensues, and egg maturation begins.

CHAPETER – 3
MATERIALS AND METHODS

3.1 Establishing a laboratory colony

The larvae were reared at room temperature and measure the temperature inside the bamboo to determine the optimum temperature of larvae.

3.2 Experiment -I : The effect of temperature

In total, 600 hundreds larvae were kept at 5, 10, 15, 20, 25 and 30°C. Each plate was contained 10 larvae. Each plate was checked every week to determine mortality of larvae. There were 5 replicates in each experiment. In total, 237 larvae in petri dishes were used in this experiment. Each petri dish was contained 10 larvae. Each treatment had 50 larvae **(FIGURE 1)**.

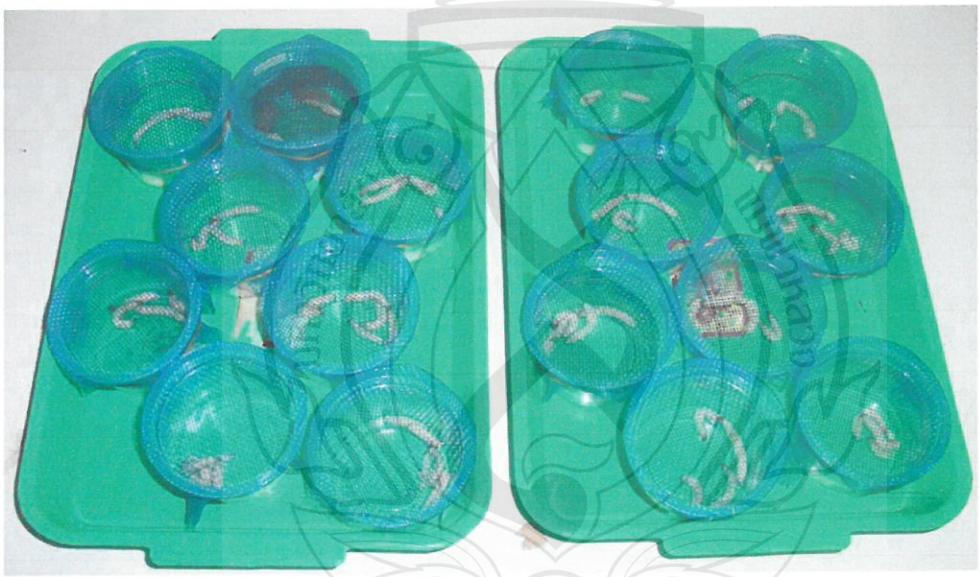

FIGURE 1. Mature larvae in plastic cups

3.3 Experiment -II: The effect of photoperiod

In total, 324 larvae were used in this experiment. The larvae were grouped into two groups; group-A and group-B. In group-A, 181 larvae were used and in group-B, 143. The group-A larvae were exposed 8 hour (LD 8: 16) to the light, whereas group-B larvae were exposed to (LD 10:14) **(FIGURE 2)**.

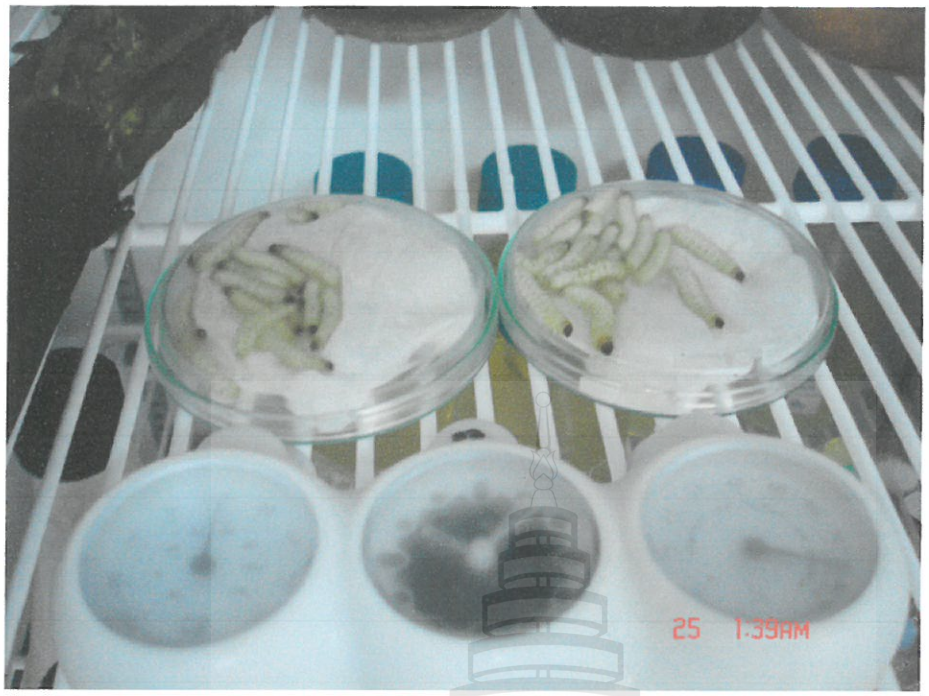

FIGURE 2. Larvae in growth chamber

3.4 Experiment - III: The effect of moisture

3.4.1 Moisture effects

In total, 250 larvae were kept in plastic cup with wet cotton buds at room temperature 26 - 28°C with a photoperiod LD 10:14 hr **(FIGURE 3)**. The cotton buds were weekly changed. There were 5 replicates in each experiment.

FIGURE 3. Larvae on wet cotton buds

3.4.2 Dried effects

In total, 300 larvae were used. The larvae were kept at room temperature 26-28°C in plastic box (size 6 × 8 × 2.5 cm) by simply covered with a tissue paper to protect from the day light **(FIGURE 4)**.

FIGURE 4. Larvae on dried plastic boxes with tissue paper

3.5 Experiment - IV: The effect of nutrient

In total, 788 larvae were used in this experiment. The larvae were divided into 2 groups. The group-A was treated with food (fresh culm), and the culm were changed once a week. The group-B did not feed. The larvae were kept without feeding at 26°C with a photoperiod LD 14:10. After that they were kept in the growth chamber at 18°C with a photoperiod LD = 12:12 for 22 days, and again kept in the growth chamber at 10°C with a photoperiod LD 12:12 for 90 days **(FIGURE 5)**.

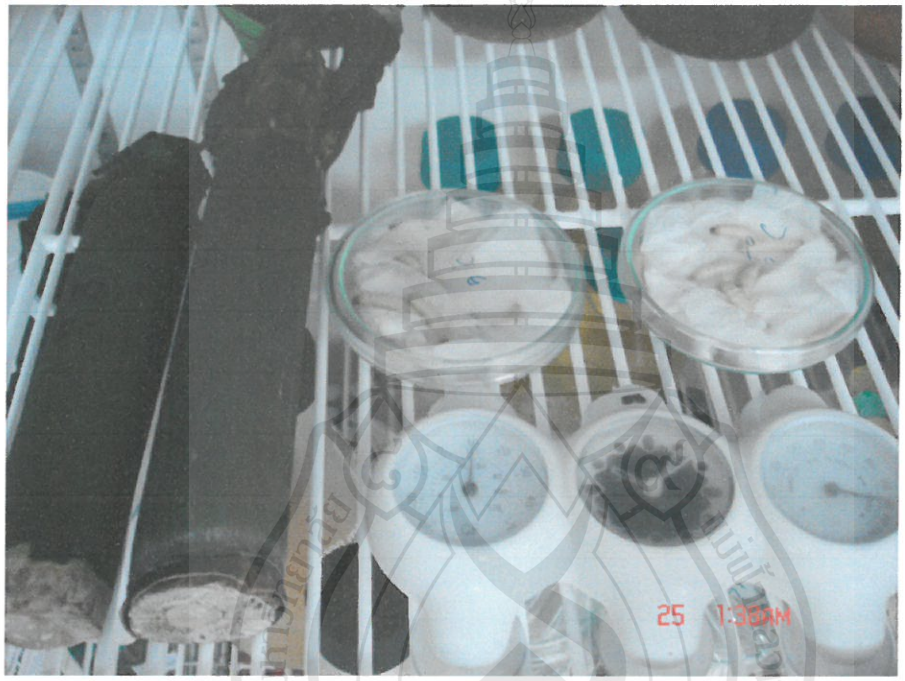

FIGURE 5. Larvae in an incubator

3.6 Diapausing pupae

3.6.1 The effect of temperature

The pupae were sexually (males and females) separated from each other and they were separately hung on chopsticks and kept at room temperature (26-28C°) **(FIGURE 6)**.

FIGURE 6. Female pupae pasted on a chopstick

CHAPTER – 4
RESULTS

4.1 Bamboo temperature

The temperature inside the bamboo with larvae was on an average 21.8+1.5°C (range from 18°C to 9°C, n=18) in the night whereas average 21.9+2.4°C (range from 19°C to 26.5°C, n 23°C) in the day time **(FIGURE 7)**.

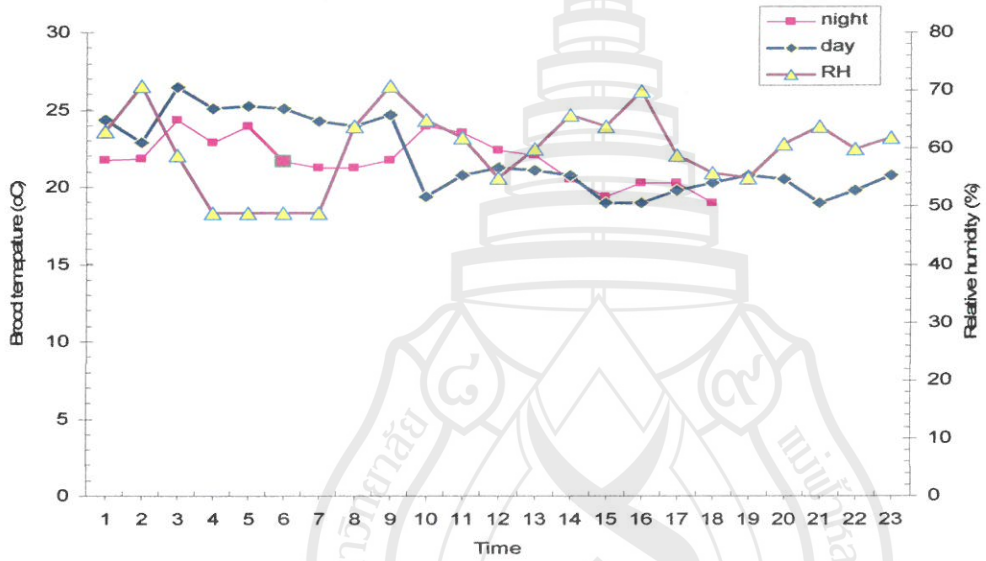

FIGURE 7. Night and day temperature inside the bamboo

4.2 Effect of temperature

At 5°C, 21.1% larvae were death within a week. However, 11.3-11.8% of larvae were death between 10-15°C. As the temperature rose from 20-30°C, 14.3-21.1% of larvae were death. At 35°C, 100% larvae were death **(FIGURE 8)**. The optimum temperature for survival was between 10-14°C **(FIGURE 9)**.

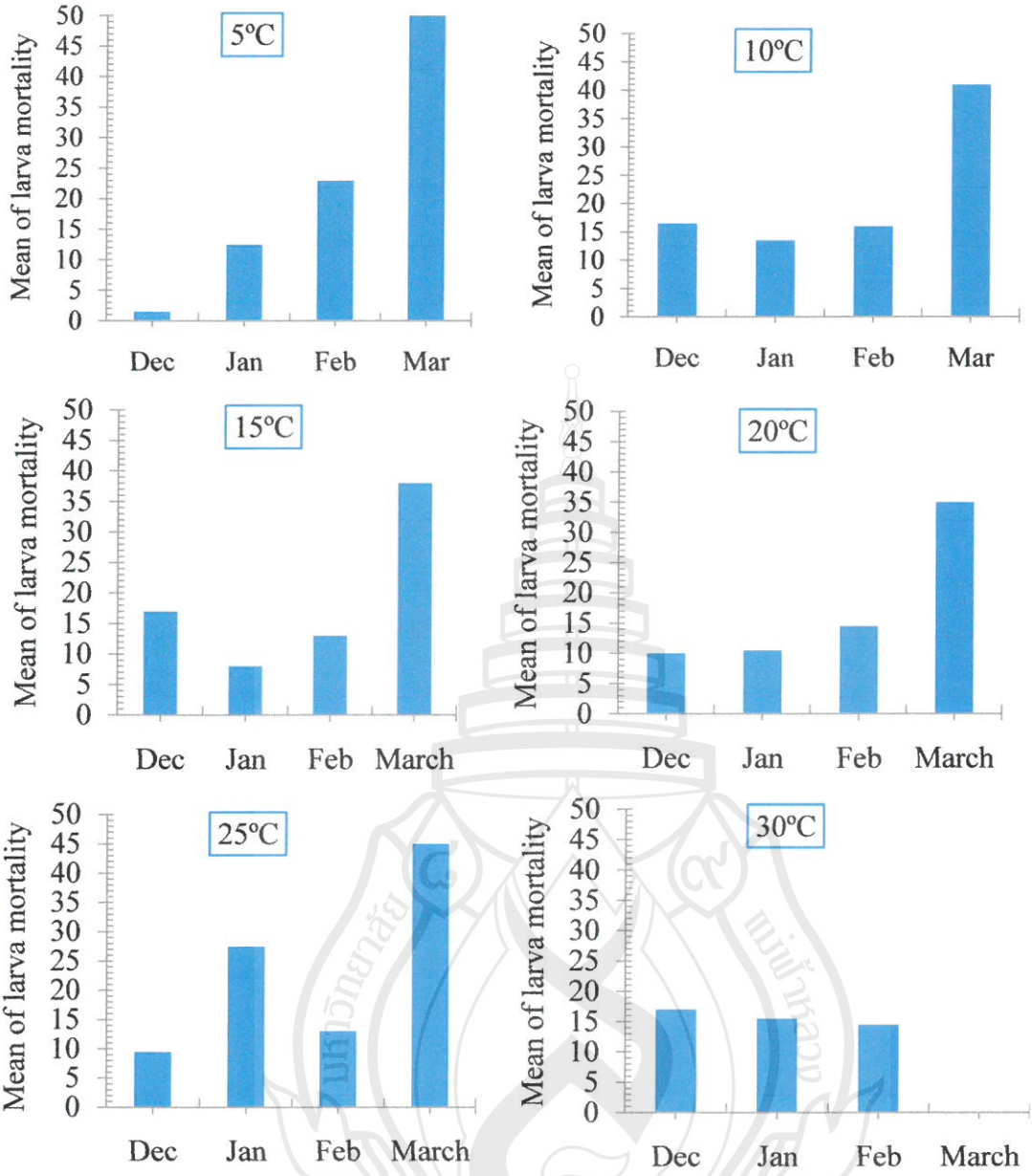

FIGURE 8. The effect of temperature on larvae

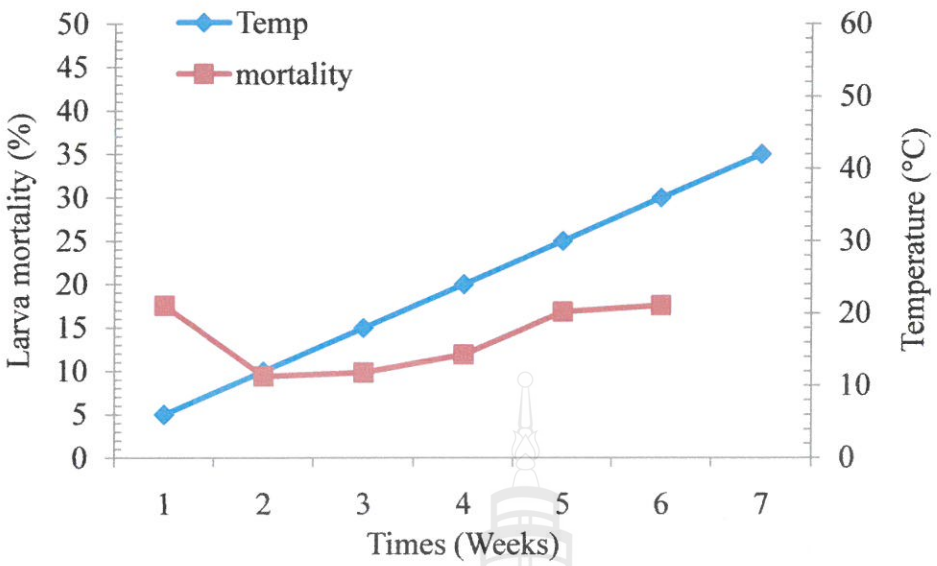

FIGURE 9. Mortality of larvae at different temperatures

4.3 Effect of photoperiods

Ninety-nine-eight percent were dead after 10 weeks exposition to the light 8 hr and 10 hr. First week, 11 % of mortality was observed in 8 hr exposed whereas 4.9% in 10 hr. A highest number of mortality (13.8-17.6%) was observed in week-5. On week-9, 13.8 and 15.5% mortality were observed in 8 and 10 hr exposed respectively **(FIGURE 10)**.

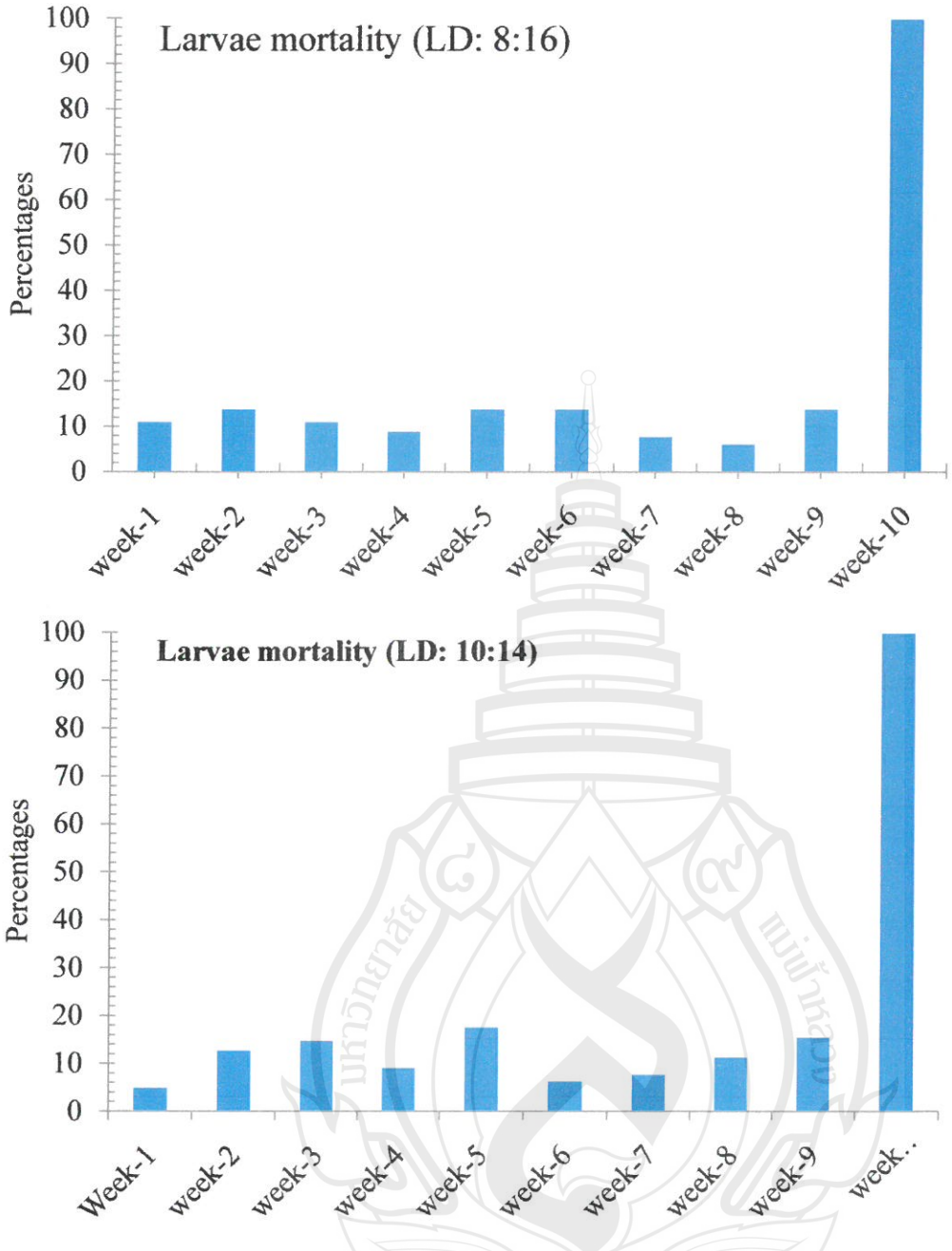

FIGURE 10. Percentages of survival and mortality of larvae with a photoperiods

4.4 Effect of moisture

The larvae supplied with moist cotton buds live up to 7 months **(FIGURE 12)**. The larvae supplied with moist cotton buds pupate on 7th of March, 2010. The larvae did not supply with moist cotton buds were dead **(FIGURE 11)**.

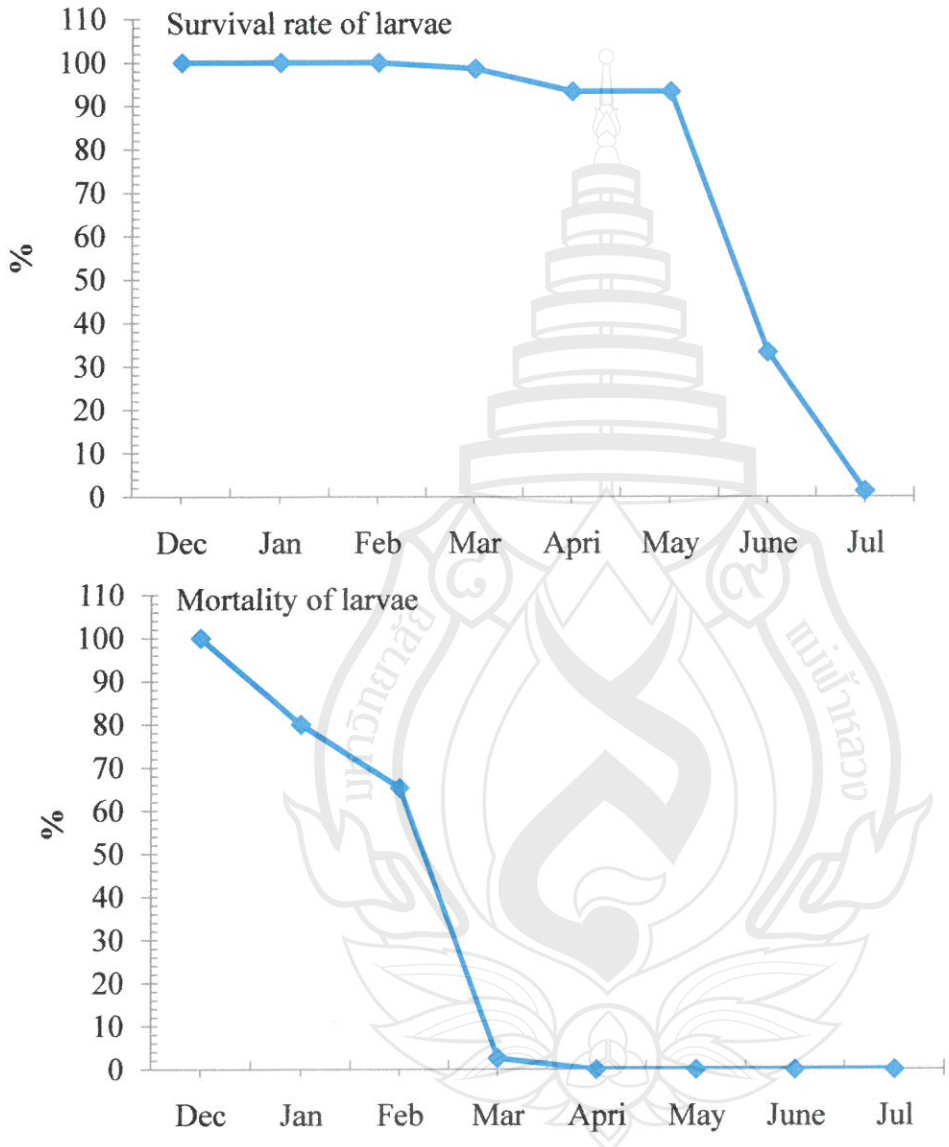

FIGURE 11. Moisture effects on survivality and mortality of larvae

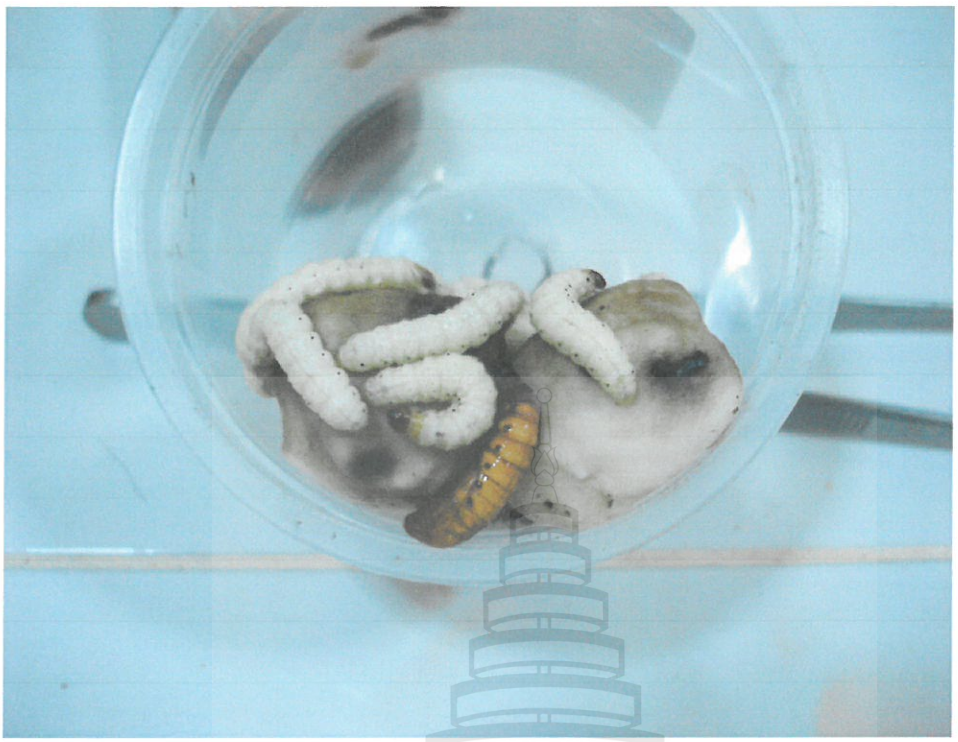

FIGURE 12. Larvae on moist cotton buds pupated on 7th of March 2010.

4.5 Effect of nutrient

In December 2008, 77.4 % larvae were survived in December, 2008. In Feb, 2009, 57.8% of larvae were survived. In the first week of March 2009, not a single larva was survived. In December 2008, 22.6% were dead, 32.2% in January, and 42.1% in February, 2009. All the larvae died after 3 months **(FIGURE 13)**.

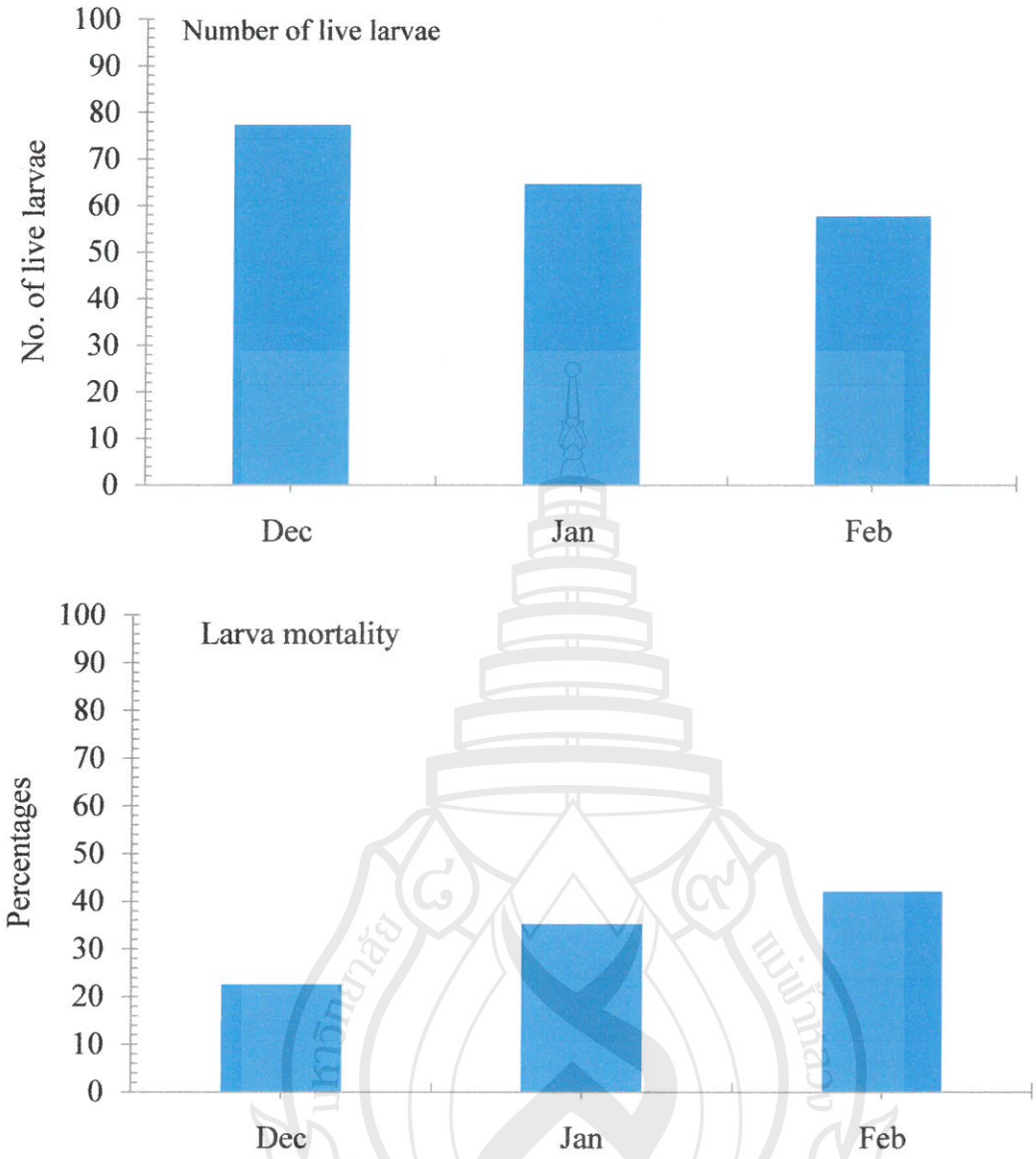

FIGURE 13. The effect of food on larvae

4.6 Diapausing pupae

4.6.1 The effect of temperature

In Week-5, 80% of male pupae were emerged 3 days earlier than the female moths. In week-5, 80% of male moths were emerged whereas 60% of female moths emerged. On week-6, 13.3% males and 22.6% female emerged. 6.7% of male and 17.4 females did not emerge **(FIGURE 14)**.

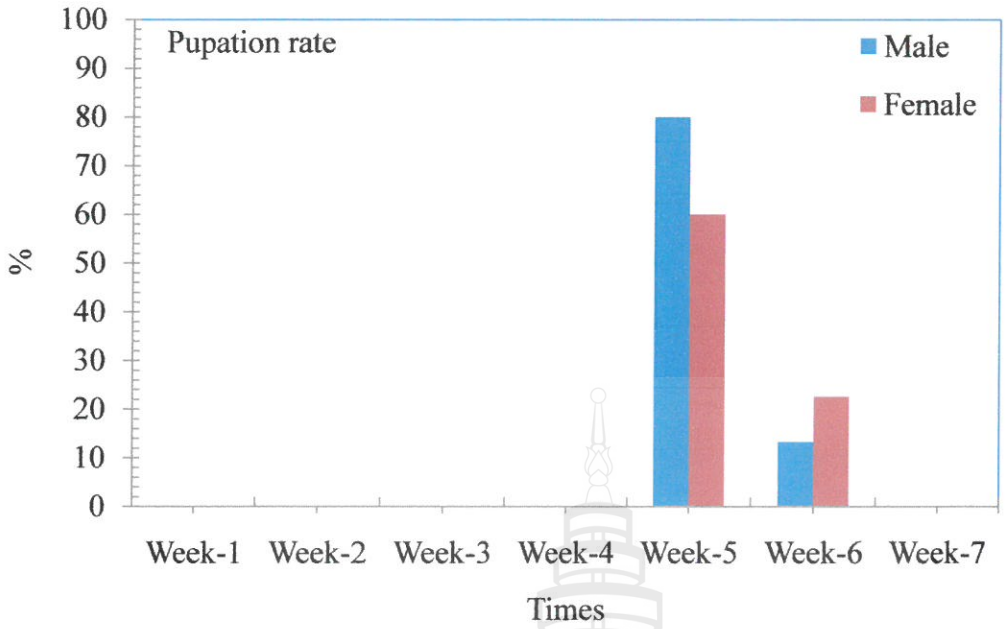

FIGURE 14. Emergence of adult moths from pupal stage

CHAPTER – 5
DISCUSSION

The present study suggested that the larvae of *O. fuscidentalis* were unable to tolerate low temperature (5°C) and high temperature (30°C). Therefore, they are protected by bamboo culm. When the larvae were exposed to the lowest temperature (5°C), all the larvae were dormant stage, and just freeze. However, after later, if they exposed room temperature, they started to move. However, it they are exposed for a long time in lowest temperature, they will die.

The present results indicated that larvae exposed to different intensity of light were not sensitive. The present results suggest that light intensity does not seem a key factor to terminate the diapause. It suggests that larvae of this species were unable to distinguish day and night photoperiod.

The present results suggested that larvae did not supply with food were survival up to 1-2 months on their body fat. After that, they were suffered with black spots, a bacterial disease.

The present results suggested that larvae supplied water (moist) were survived as long as they get water. In this experiment, some larvae were pupating in the first week of March, which is earlier than the seasonal. Usually, the larvae are started to pupate from the week of July to the mid of August. Therefore, this result suggests that water (moisture) could be a (weak key) factor to terminate diapause in bamboo borer larvae.

The results suggested that the first postulate explanation is that in some species of Lepidoptera that undergo stationary molts during larval diapause, the juvenile hormone (JH) titer remains elevated, thus guaranteeing that any molt will be stationary rather than progressive (Yin and Chippendale, 1977). Insect molting and metamorphosis are regulated by 2 classes of hormones: ecdysteroids and juvenile hormones (JH) (Gilbert et al., 2002). The second postulate explanation is that ecdysteroids are a family of polyhydroxylated steroids that are the molting hormones. In larvae of most insect species, the prothoracic glands synthesize and secrete

ecdysone, which is hydroxylated by peripheral tissues to form 20-hydroxyecdysone. 20 -Hydroxyecdysone then acts on target tissues such as the epidermis to elicit hormonal effects (Smith 1985; Gilbert et al. 2002). JH is a sesquiterpene that is synthesized and secreted by the corpora allata (Yin 1994; Kou and Chen 2000). It is generally accepted that during insect postembryonic development, the interplay of ecdysteroids and JH serves to orchestrate the progression from one developmental stage to the next, with ecdysteroids initiating the molting process and JH regulating the quality of the molt. Numerous studies have been conducted to clarify the developmental regulation of these 2 important hormones (Smith 1985, Gu and Chow, 2003). The third postulate explanation is that Diapause Hormone (DH), the regulator of the embryonic diapause in *B. mori*, is a 24–amino acid peptide released from the suboesophageal ganglion of the female to elicit embryonic diapause in her progeny (Yamashita, 1996). The cDNA encodes a polyprotein precursor from which DH, pheromone biosynthesis- activating neuropeptide, and three other neuropeptides are cleaved (Sato et. al., 1993; Xu, et al., 1995). The DH–pheromone biosynthesis–activating neuropeptide gene consists of 6 exons and 5 introns, and expression can be noted in 12 neuro-secretory cells within the subesophageal ganglion. This gene is expressed at the time of diapause induction, and recent evidence suggests that the expression of DH mRNA is promoted by high levels of dopamine present in the central nervous system (CNS) and hemolymph of females that produce diapausing progeny (Noguchi, and Hayakawa, 2001). Only *B. mori* regulates its diapause by production of a diapause-inducing hormone. Attempts to elicit diapause in other species with DH have consistently failed. However, extracts with DH activity from other species, including non-Lepidoptera, are capable of inducing diapause in *B. mori*, thus suggesting that DH activity may be widely distributed, but only in *B. mori* has this peptide been captured as a regulator of diapause. Quite possibly, its original role in the Lepidoptera was as a regulator of carbohydrate metabolism, but in the silk moth the peptide has been subverted for diapause induction. The third postulate explanation is that under natural overwintering conditions insects are usually capable of initiating development long before development is actually initiated in the spring. For example, diapausing pupae of the flesh fly *S. bullata* are in a fixed period of latency during autumn and early winter and fail to break diapause in response to high temperatures at that time (Denlinger, 2001). However, by early January they are fully capable of responding to high temperatures, but the low temperatures that prevail at that time of

year prevent this from happening. Only when the soil temperatures rise in the spring is development observed in the field. This seasonal change in responsiveness is to distinguish between diapause (the period of fixed latency occurring before January in the flesh fly example) and post-diapause (the stage that is fully capable of initiating development when favorable conditions are present, after January in the flesh fly example). In most ways, insects in post-diapause appear physiologically identical to those in diapause: Developmentally, they are indistinguishable, and their metabolic rates remain suppressed. At the molecular level, it is quite likely that these two phases could be distinguished, but this question has not yet been adequately addressed. Possibly, expression of some of the late diapause genes (e.g., upregulation of *usp* in flesh flies), or the downregulation of certain genes noted late in diapause (e.g., down regulation of the 55-kD a gut protein in the gypsy moth) could be associated with this transition. When diapause is terminated one would expect to see major shifts in the patterns of gene expression. The insect rapidly increases its metabolic rate and promptly initiates development. Thus, one would predict that genes involved in the mechanisms that suppress development would be switched off and new sets of genes involved in initiating development would be switched on. Of greatest interest are the signals that initiates this switch, but one can imagine that the regulatory genes may be obscured by a multitude of downstream genes that are expressed as the insect makes this transition. In most species it is not possible to know precisely when the transition occurs because it may occur over a period of several days. However, it is possible to circumvent this problem in the flesh fly and stage events at precise intervals after diapause termination. This can be achieved by topically applying hexane, a treatment that prompts the immediate termination of diapause (Denlinger et. al., 1980). Though the mechanism whereby hexane exerts its effect remains unknown, it offers a powerful method for generating precisely timed flies. Using hexane as a tool for breaking diapause, the fate of select genes in flesh flies was monitored at hourly (or shorter) intervals after diapause termination. The fourth postulate explanation is that the two diapause upregulated heat shock protein genes, *hsp23* and *hsp70*, are both down regulated 6 hours after hexane treatment (Rinehart, et. al., 2000; Yocum, et. al., 1998). *hsp90*, the diapause own regulated gene, is again expressed at higher levels 12 hours after diapause is terminated (Rinehart and Delinger, 2000). Expression of the cell cycle regulator, *pcna*, a gene that is down regulated during diapause, increases 15-fold within 12 hours after hexane application, and shortly thereafter the arrested

cells of the brain can be observed reentering the cell cycle (Tammariello and Denlinger, 1998). The mRNA levels for the genes encoding both EcR and USP, the two proteins that comprise the functional ecdysone receptor, increase in flesh flies after hexane application, but the dynamics are slightly different: The mRNA for EcR is elevated within 1 hour after hexane application, whereas the mRNA for USP increases 9 hours after hexane is applied (Rinehart et. al., 2001). This increase in expression of ECR and USP mRNAs correlates nicely with the elevation in the ecdysteroid titer that follows hexane application. Thus far, the increase in EcR mRNA that is observed within 1 hour after hexane application is the earliest gene response noted.

The results suggested that diapausing pupal stage was terminated within 35 days. However, Singtripop, et al., (1999) reported that pupal stage was last 45 days. In pupae of the sweet potato hornworm, *Agrius convolvuli*, a transcript encoding a cytochrome c oxidase subunit is upregulated in the brain 24 hours after the pupae are transferred from their chilling temperature (10.5±.4°C) to 28.0±.3°C for diapause termination. Presumably, the increase in abundance of this electron transfer protein is associated with the boost in oxygen demand that accompanies the termination of diapause and resumption of development. Cytochrome content in fat body mitochondria of *Heliocoverpa zea* during diapause is approximately 10% of that observed in nondiapausing pupae. To meet the needs of the pharate adult and adult moth, the cytochromes are resynthesized at diapause termination, but the elevation is a late event, occurring quite a few days after diapause has been terminated (Gruetzmacher and Keeley, 1982).

From the results of present study, it was concluded that diapausing larval stage of bamboo borer cannot be terminated. However, the diapausing stage of pupae was terminated from 45 days to 35 days.

CHAPTER – 6
CONCLUSION

If the temperature drops below the 5°C, all the larvae will die and if the temperature rises above 25°C, than all larvae will die. The larvae were not found sensitive to temperature.

Temperature, light intensity (photoperiods), nutrient and water are not key factors to terminate diapause of bamboo borer larvae. However, the results of this research suggested that water could be a secondary key factor that could terminate diapausing of bamboo borer larvae.

The programming of diapause channels development along a specific pathway characterized by behavioral, morphological, and physiological design features that uniquely prepare the diapause-destined insect for a period of developmental arrest, and all of these preparative steps can be expected to be associated with gene expression patterns. Therefore, it is recommended that next research should focus on JH, which is the main factor to determine diapausing in many Lepidopterian insects.

REFERENCES

Andrewartha, H. G. (1952). Diapause in relation to the ecology of insects, *Biological Reviews of the Cambridge Philosophical Society* 27, 50-107.

Beck, S. D. (1962). Photoperiodic induction of diapause in an insect. *The Biological Bulletin*, vol 122 #1, 13 pp

Beck, S. D. (1980). Insect photoperiodism (2nd eds) Academic press, London 82 pp

Cashmore A. R., Jarillo J. A. and Wu Y.J., Liu D. (1999). Cryptochromes: blue light receptors for plants and animals. *Science* 284:760–65

Danks, H. V. (1987). *Insect Dormancy: An Ecological Perspective*. Ottawa: Biol. Survey Can. 315 pp.

Denlinger, D. L. (1985). Hormonal control of diapause. In Kerkul *et al.,* (*eds*) Comprehensive insect physiology. *Biochem. Pharma.* vol. 31. *Annual Review Oxford*. 353-421.

Denlinger D. L, Giebultowicz J. M. and Saunders D. S. (2001). *Insect Timing: Circadian Rhythmicity to Seasonality*. Amsterdam: Elsevier

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell* 96:271–90

Gilbert, L. I., Rybczynski, R. and Warren, J. T. (2002). Control and biochemical nature of the ecdysteroidogenic pathway. Annu. Rev. Entomol. 47: 883-916.

Gruetzmacher M. C. and Keeley L. L. (1982). Cytochrome degradation and synthesis in fat body mitochondria during diapause, diapause termination and metamorphosis of *Heliothis zea*. *Insect Biochem.* 12:49–54

Gu S. H. and Chow, Y. S. (2003). Stage-dependent effects of 20-hydroxyecdysone on DNA synthesis of corpus allata cells in the silkworm, *Bombyx mori*. J. Exp. Zool. 297A: 138-146.

Hall J. C. (2000). Cryptochromes: sensory reception, transduction, and clock functions subserving circadian systems. *Curr. Opin. Neurobiol.* 10:456–86.

Kou R. and Chen, S. J. (2000). Allatotropic and nervous control of corpora allata in the adult male loreyi leafworm, *Mythimna loreyi* (Lepidoptera: Noctuidae). Physiol. Entomol. 25: 273-280.

Lee K-Y. and Denlinger D. L. (1997). A role for ecdysteroids in the induction and maintenance of the pharate first instar diapause of the gypsy moth, *Lymantria dispar*. *J.Insect Physiol.* 43:289–96

Mansingh, A. (1971). Physiological classification of dormancies in insects, *Canadian Entomologist* 103, 983–1009 pp.

Meola R. W. and Adkisson P. L. (1977). Release of prothoracicotropic hormone And potential of developmental ability during diapauses in the bollworm, *Halitosis zeal*. *J.Insect Physiol.* 23:683–88

Noguchi H. and Hayakawa, Y. (2001). Dopamine is a key factor for the induction of egg diapause of the silkworm, *Bombyx mori*. *Eur. J. Biochem.* 268:774–80

Richard D. S. and Saunders D. S. (1987). Prothoracic gland function in diapause and non-diapause *Sarcophaga argyrostoma* and *Calliphora vicina*. *J. Insect Physiol.* 33:385–92

Rinehart J. P. and Denlinger D. L. (2000). Heat shock protein 90 is down regulated during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*, but remains responsive to thermal stress. *Insect Mol. Biol.* 9:641–645

Rinehart J. P., Cikra-Ireland R. A., Flannagan R. D. and Denlinger D. L. (2001). Expression of ecdysone receptor is unaffected by pupal diapause in the flesh fly, *Sarcophaga crassipalpis*, while its dimerization partner, USP, is downregulated. *J. Insect Physiol.* 47:915–21

Rinehart J. P., Denlinger D. L. (2000). Developmental upregulation of inducible hsp70 transcripts, but not the cognate form, during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*. *Insect Biochem. Mol. Biol.* 30:515–21

Sato Y, Oguchi M, Menjo N, Imai K, and Saito H. (1993). Precursor polyprotein for multiple neuropeptides secreted from the subesophageal ganglion of the silkworm, *Bombyx mori*: characterization of the cDNA encoding diapause hormone precursor and identification of additional peptides. *Proc. Natl. Acad. Sci.USA* 90:3251–55

Schotland P. and Sehgal A. (2001). Molecular control of *Drosophila* circadian rhythms. 28: 15–30 pp.

Singtripop, T., Wanchacheewa, S., Tsuzuki, S. and Sakurai, J. (1999). Larval growth and diapause in a tropical moth *Omphisa fuscidentalis* Hampson. *Zool. Sci.* 16, 725-733.

Smith, S. L. (1985). Regulation of ecdysteroid titre: synthesis. *In* GA Kerkut, LI Gilbert, eds. Comprehensive insect physiology, biochemistry and pharmacology. Vol 8. Oxford: Pergamon Press. 295-341, pp.

Suzuki, K., Minagawa, T., Kumagai, T., Naya, S., Endo, Y., Osanai, M. and Kuwano E. (1990). Control mechanism of diapause of the pharate first–instar larvae of the silkmoth *Antheraea yamamai*. *J. Insect Physiol.* 36:855–60

Takeda M. and Skopik S. D. (1997). Photoperiodic time measurement and related physiological mechanisms in insects and mites. *Annu. Rev. Entomol.* 42:323–49.

Tammariello, S. P. and Denlinger, D. L. (1998). G0/G1 cell cycle arrest in the brain of *Sarcophaga crassipalpis* during pupal diapause and the expression pattern of the cell cycle regulator, proliferating cell nuclear antigen. *Insect Biochem. Mol. Biol.*28:83–89

Tanaka H, Sudo C, An, Y., Yamashita T, and Sato K. (1998). A specific peptide produced in adult diapause of the leaf beetle, *Gastrophysa atrocyanea* Motschulsky (Coleoptera: Chrysomelidae). *Appl. Entomol. Zool.* 33:535–43

Ushatinskaya, R. S. (1987). Summer diapause (aestivation) in insects, Nauka, Moscow 140-173.

Xu W-H, Sato Y, Ikeda M, and Yamashita O. (1995). Stage-dependent and temperature controlled expression of the gene encoding the precursor protein of diapause hormone and pheromone biosynthesis activating neuropeptide in the silkworm, *Bombyx mori*. *J. Biol. Chem.* 270:3804–3808

Yamashita O. (1996). Diapause hormone of the silkworm, *Bombyx mori*: structure, gene expression and function. *J. Insect Physiol.* 42:669–79

Yhoungaree, J. and Puwastien, P. (1997). Edible insects in Thailand: An unconventional protein source. *Eco. Food Nut.*, 8 pp

Yin C. M., Chippendak, M. (1994). Juvenile hormone III bisepoxide: new member of Insect Juvenile hormone family. Zool. Stud. 33: 237-245.

Yocum G.D., Joplin K. H. and Denlinger D. L. (1998). Upregulation of a 23 kDa small heat shock protein transcript during pupal diapause in the flesh fly, *Sarcophagacrassipalpis*. *Insect Biochem. Mol. Biol.*28:677–82

Zaslavski V. A. (1988). *Insect Development, Photoperiodic and Temperature Control*. Berlin: Springer-Verlag.