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Effects of Temperature and Photoperiod on Reproduction of the Rice Black Bug, *Scotinophara coarctata* (Fabricious)

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Abstract

The rice black bug, *Scotinophara coarctata,* was recorded as an occasional pest but has now become a potential major pest of rice in Thailand. This study investigates the influence of temperature and photoperiod on reproduction of *S. coarctata.* Newly-hatched first instar nymphs were reared in a constant conditions at 25, 30 and 35°C in 10:14, 11:13, 12:12 and 13:11 LD. The findings suggest that temperature is the dominating factor on nymphal development of *S. coarctata.* The study finds that more than 50 percent of fertile mating pairsare found both at 25 and 30°C. Thus, here isno reproductive diapause in *S. coarctata* under constant conditions. However, the number of surviving adults significantly decreased when kept at 30°C and no nymphs survived at 35°C, so the selected rearing temperatures for investigating the effect of changing photoperiod on reproduction of *S. coarctata* is 25°C. To determine how reproduction of *S. Coarctata* is affected by photoperiod, first instar nymphs were reared under both constant (10:14 and 13:11 LD) and induced-changing photoperiod conditions. In the latter case, first instar nymphs were pre-maintained at 25°C in a short day (10:14 LD) and long day (13:11 LD) conditions. Until the third instar, nymphs were transferred into the opposite photoperiod conditions from short to long day and long to short day conditions. The results show that the fecundity of *S. coarctata* reared at 25°C under changing photoperiod conditions are not significantly affected when photoperiod was changed. The critical information derived from this study indicates that temperature is the dominating factor on reproduction and there is no reproductive diapause occurring under either constant or induced-changing photoperiod conditions.

Introduction

Rice and its derived products is the main staple food for over half of the world's population and is the most rapidly growing food resource in Asia in terms of the area under cultivation (Diouf, 2003; Barrion et al., 2008). The rice sector in Asia is facing the enormous challenge in managing insect pests because of the extensive use of pesticides leading to the destruction of the natural enemies of rice pests (Heong & Samson, 2012).

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The total rice production losses in tropical Asia to pests are estimated in a range of 120 to 200 million tons of grain yield (Willocquet et al., 2004), but a significant demand for rice productions has increased to attain food security (ESCAP, 2009; Redfern et al., 2012). Understanding of biology of the pest species is the first and most important step in long-term successful pest management (Witmer, 2007). The rice black bug (*Scotinophara coarctata* (Fabricious)) is the sap-sucking insect belonging to Order Hemiptera and Family Pentatomidae. It attacks rice plants from early vegetative to maturity resulting in stunned growth significantly after one or more days of feeding (Morrill et al., 1995). It has been reported to be predominantly invasive insect pest to rice plants in many parts of Asia, particularly in the Philippines, Indonesia, and Malaysia (Rombach et al., 1986; Pathak & Khan, 1994; Cuaterno, 2007; Sepe & Demayo, 2014). Earlier, *S. coarctata* was regarded as a minor rice pest and economic insignificance that was found only in the southern part of Thailand, but it has become a potential major pest that can be found throughout various regions from South to North Thailand. Several studies in Thailand have been carried out on field survey and insecticide application to eradicate *S. coarctata,* rather than rely on sustainable approaches. Besides, no resistant cultivars to this pest species are available at this time (Suwat, 2001). The outbreak trends of *S. coarctata* are unpredictable and lacks biological information about this specie. There is thus an urgent need for understanding their biology. It is known that climatic conditions not only affect the status of pests, but also affect their population dynamics (Nylin, 2001; Gutierrez & Ponti, 2014). Cho et al. (2008) reports that *Scotinophora lurida* (Burmeister) distributed in temperate zone entered reproductive diapause induced by short photoperiod and low temperature. The critical photoperiods are between 9 and 10 hour at 25°C and between 12 and 16 hour at 20°C. Whilst, at low temperature (15°C) all adults entered into reproductive diapause even under long-day length photoperiod. Therefore, we assume that reproduction of *S. coarctata* might be affected by changes in temperature and photoperiod. Against this background, the main aims of this project is to check whether *S. coarctata* hasreproductive diapause and investigate the ability to reproduce eggs by comparing between the groups reared at constant and induced-changing photoperiod conditions. The important data will lead to understanding the plasticity of *S. coarctata* in reproductive capacity and their population dynamics.

Materials and methods

1. Insect cultures

Field sampling was conducted in different rice infested areas in the central plain of Thailand. The eggs, nymphs and adults of *S. coarctat*a were collected from the fields and maintained in the greenhouse at the Rice Department of Thailand on 5-6 week old seedlings of *Oryza sativa* L. cv. *Prachinburi 2.* All experiments were carried out in October 2014 to July 2015 with insects from the second generation of the mass-reared populations.

2. Experimental procedures

Prior to starting the experiments, appropriate rearing temperature was investigated in order to produce correct amount of *S. coarctata* adults for the experiment. Newly-hatched first instar nymphs of *S. coarctata* were reared under short day (10:14 and 11:13 LD), equinox-day (12:12 LD) and long day (13:11 LD) photoperiod at 25, 30 and 35°C in a Perspex rearing box containing a rice seedling. Daily observations were made to record the time taken to moult to adult. An appropriate rearing temperature was chosen from counting the highest amount of the surviving adult rice black bugs.

To investigate effects of altering photoperiod on reproduction, newly-hatched first instar nymphs of *S. coarctata* were maintained at the selected temperature. Briefly, first instar nymphs were pre-maintained at selected temperature in a short day (10:14 LD) and long day (13:11 LD) conditions. Until the third instar, the surviving nymphs were transferred into the opposite photoperiod conditions from short to long day (from 10L to 13L) and long to short day conditions (from 13L to 10L) and reared until the late fifth instar, after which males and females were reared separately on rice seedlings to obtain unmated adults. A total of 30 male and female pairs weretaken from this stock and then allowed to mate and oviposit under the same conditions. The occurrence of reproductive diapause can take place if females do not produce eggs above 50% of all mating pairs (Danks, 1987). Thus, eggs laid by each female were recorded and fertile mating pairs were compared.

3. Data analysis

The growth status includes alive and death in different rearing conditions is compared using Pearson's Chi-square test in SPSS 17.0 software. Differences at a probability level of $P < 0.05$ are considered significant.

A split-plot method is used to determine the main effects of treatment on the development of *S. coarctata* using temperature and photoperiod as fixed factors. In the split plot design, photoperiod is a split plot factor within the temperature treatment. One way analysis of variance (ANOVA) is used to determine the photoperiod effects on the mean viable eggs per female of *S. coarctata*. Where significant differences occurred, the data were further analysed by Tukey's honest significance difference post-hoc test and the Games-Howell test to separate statistically heterogenous and non-heterogenous groups, respectively.

Results and discussion

1. Effects of temperatures and photoperiods on reproductive ability of *S. coarctata* under constant conditions

In comparison of the growth status of *S. coarctata* reared in the incubators of two temperatures (25 and 30°C) and four photoperiods (10:14, 11:13, 12:12 and 13:11 LD), the data indicates that the environmental growth conditions significantly affectes growth status (including alive and death) under 25°C (χ^2=31.251, d.f.=9 P<0.001) and 30°C (χ^2=18.375, d.f.=9 P=0.031), whereas no nymphs survived at 35°C (Table 1).

Table 1 The number of death in various nymphal stages and surviving adults of *Scotinophara coarctata* under constant rearing conditions

Parameter	Temperature / Photoperiod											
	25 °C				30°C				35°C			
	10:14	11:13	12:12	13:11	10:14	11:13	12:12	13:11	10:14	11:13	12: 12	13: 11
Dying nymphs												
N1	20	35	17	26	14	11	12	15				
N2	32	32	44	39	44	28	31	47	136	68	150	119
N3	22	16	31	27	12	16	10	9	414	249	400	381
N4	41	20	33	21	11	8	10	10				
N5	53	35	34	48	28	17	18	11				
Surviving adults	132	162	141	139	53	68	67	81				
Male: Female	57:75	78:84	70:71	71:68	25:28	33:35	27:40	35:46				
Total number	300	300	300	300	162	148	148	173	550	317	550	500

The mean development times from nymph to adult under different constant photoperiod conditions at 25 and 30°C are shown in Fig. 1. There is no difference in nymphal period between the photoperiods (F=2.257, P=0.081), nor in the interaction between the photoperiod and temperature (F=0.720, P=0.541), but there is a significant effect of temperature on the nymphal development time (F=41.156, P<0.001).

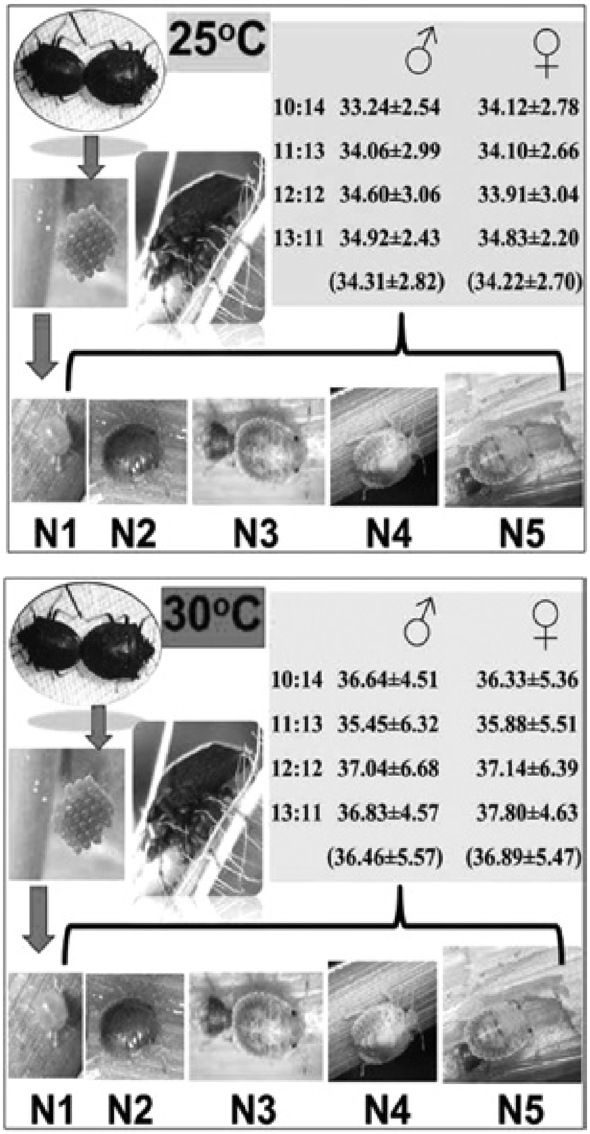

Fig. 1 Effects of temperatures and photoperiods on nymphal development time of *Scotinophara coarctata* under constant rearing conditions

Even though *S. coarctata* could complete their life cycle at 25 and 30°C, the number of surviving adults of *S. coarctata* significantly decreased at 30°C. Therefore, the selected rearing temperature for investigating the effect of changing photoperiod on reproduction of *S. coarctata* is at 25°C. However, the surviving adult females and males of *S. coarctata* kept at both at 25 and

30°C were transferred as pairs into separate rearing boxes with a rice seedling and maintained in the same rearing conditions to examine the effects of photoperiod and temperature regimes under constant rearing conditions. The results shows that *S. coarctata* produced eggs in all mating groups at 25°C and there were more than 50% of fertile mating pairs at 30°C under different photoperiod conditions. The important result from this experiment reveals that there is no reproductive diapause occurring under constant rearing conditions as the percentages of fertile females is over half of all mating pairs (Fig. 2).

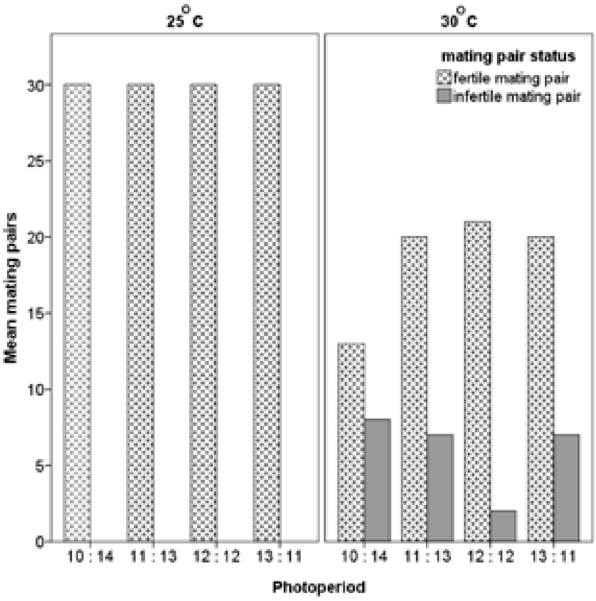

Fig. 2 Effects of temperatures and photoperiods on mating status of *Scotinophara coarctata* under constant rearing conditions.

2. Effects of changing photoperiods on fecundity of *S. coarctata*

As the gender of *S. coarctata* could not be determined at the first instar stage, the male and female sample sizes were not equal. Thus, the initial mating pairs used in this study at 25°C were 35, 40, 36, and 35 pairs at 10L, 13L, 10L to 13L, and 13L to 10L conditions, respectively. The total number of fertile mating pairs, and mean egg laid per female are summarized in Table 2. The result shows that 100 percent of fertile mating pairs are found in all photoperiod combination conditions at 25°C. The highest number of viable eggs per female is obtained under a constant photoperiod condition. However, the response to a change of photoperiod results indicates that mean egg viability percentages of *S. coarctata* reared at 25°C are not significantly affected when photoperiod is changed ($F_{3,142}$=0.345, P=0.793). This experiment results confirms that thereis no reproductive diapause occurring under either constant or induced-changing photoperiod conditions.

Table 2 Fecundity of *Scotinophara coarctata* at 25°C under constant and induced-changing photoperiod conditions

Rearing conditions	Fertile mating pairs	Mean viable eggs (eggs/ female)
Constant condition 10:14 LD	35	79.1±5.8
Constant condition 13:11 LD	40	70.9±4.9
Changing condition (from 10L to 13L)	36	65.2±4.2
Changing condition (from 13L to 10L)	35	55.2±3.4

Across the world, pests, especially herbivorous insects cause devastating damage to the world's agricultural products (van der Goes van Naters & Carlson, 2006; Oliveira et al., 2014). Similarly, one of the common problems encountered by Thai farmers are pest and disease outbreaks because they do not know when is the right time to check and control until the pest levels are above the economic threshold due to the unmanaged pest problems. Thus, data on biology and ecology of pests are useful to predict and control the outbreak of particular pest species. Very little is known about the biology and life history of the occasional pest such as the rice black bug. Many abiotic factors such as temperature and photoperiod have an influence on the population dynamics (Van Lenteren et al., 2005; Pan et al., 2014) in terms of diapause (Saunders, 2002; Belozerov, 2008) and reproduction of insect. Some studies show that *S. lurida* adults exhibit reproductive diapause induced by short photoperiod and low temperature (Cho et al., 2007; 2008). This study assumed that the rice black bugs in Thailand, *S.coarctata,* might be affected when they experienced changing environmental conditions. This study focused on the effects of temperature and photoperiod on reproduction of *S. coarctata* which are the potential pest and becoming a serious threat to rice production in Thailand.

After maintaining *S. coarctata* at 25 and 30°C under four constant photoperiods including 10:14, 11:13, 12:12 and 13:11 LD, the results show that the duration of *S. coarctata* development varied in response to temperature and photoperiod conditions. The critical results derived from this study show that photoperiod is not significantly affected on development time and survival in nymphal stages, but temperature does have an effect. Moreover, all nymphs of *S. coarctata* are find to die at 35°C and the survival of *S. coarctata* adults significantly decreasing when reared at 30°C. The results reveal that *S. coarctata* might be susceptible to high temperature. There are a range of possible effects of extreme temperatures that can explain the decreae in survival of *S. coarctata* at above 30°C rearing condition. Firstly, high temperatures denature proteins (Salvucci et al., 2000) and these transport proteins might play a crucial role in metabolism and energy acquisition (Wood & Trayhurn, 2003; Kikuta et al., 2010). Many important protein enzymes found in insects function as catalysts in cells and regulate metabolism (Thompson & Lee, 1994) and high temperatures may modify the structure of transporting by disrupting weak interactions such as vander Waals, ionic and hydrogen bonds that stabilize conformation (Neven, 2000). Although some proteins can return to a functional conformation after denaturation, little is currently known regarding such mechanisms in insects (Kanamori et al., 2010). However, the average hot season temperatures in Thailand where the outbreak of *S. coarctata* occur range from 25 to 35°C (Mazur, 2011). In general, distribution of organisms is limited within a range of temperature at the limit of their survival. In nature, *S. coarctata* have to experience fluctuated temperature conditions and they also have evolved enormous adaptation including behavioral and physiological adaptations in response to these unfavorable conditions (Abram et al., 2017). At present, there is still a lack of information on the behavior of *S. coarctata* in natural habitats, this data will be useful as a supportive information.

In general, most seasonal biology research has intensively focused on effects of photoperiod and temperature on insects in temperate zone communities because these factors are distinctively different among seasons. Therefore, organisms will have ability to detect seasonal changes in the timing of photoperiodic stimuli and respond to them in adaptive way such as entering into diapause which enables them to survive in the unfavorable environmental conditions (Beck, 1980). As mentioned previously, the reproductive diapause took place at the adult stage of *S. lurida.* However, from our findings the results reveal that *S. coarctata* are able to produce viable eggs in all conditions including constant and induced-changing photoperiod conditions because the number of fertile mating pairs are above 50 percent. So, the changing of photoperiod in nature as seasonal changesis not the key factor that affects reproduction of *S. coarctata*. The findings do not answer what environmental factors play a key role in keeping *S. coarctata* in a low or high population density and how the changes in population status influence an outbreak in Thailand.

Listinger (2007) supports the idea that the role of alternate plant hosts might fulfill functions for the rice black bugs to improve their fitness e.g. supplying sustenance for development, providing a depository for eggs, and shelter from the natural enemies. There are several alternative host plants for *S. coarctata* such as corn (*Zea mays* L.); barnyard grass (*Echinochloa crus-galli* L.P. Beauv), purple nutsedge (*Cyperus rotundus* L.); umbrella sedge (*Cyperus iria* L.), jungle rice (*Echinochloa colona* L. Link.), pickerel weed (*Monochoria vaginalis* (Burm.f.) Presl.), red sprangletop (*Leptochoa chinensis* Nees) and water primrose (*Jussiaea linifolia* Vahl) in non-rice growing season (Royboon & Wantana, 2017). A relationship between biotic and abiotic factors on life history traits of *S. coarctata* has far eluded demonstration. However, this suggestion could be a starting point for future research in order to combine both the effects of biotic and abiotic components on the biology of this pest species. Overall this study determined that temperature is the most important abiotic factor for development and reproduction compared with photoperiod. The results lead to a better understanding of the biology of the rice black bugs which would be useful for forecasting their population dynamics and aid in determining a pest management system.

Conclusion

This study is the first investigations involved the effects of temperature photoperiod on the populations of *S. coarctata.* The critical information derived from the biological studies indicate that temperature was the dominating factor on development of *S. coarctata*. Another important results revealed that there was no reproductive diapause occurring under constant rearing conditions as the percentages of fertile females was over half of all mating pairs. Such insights on biology and ecology of *S. coarctata* relating to different environmental conditions may lead to understand population structure and dynamic and prove crucial in explaining rapid spread of *S. Coarctata* populations in Thailand. It also help define forecasting and managing protocols.

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