Journal of Stored Products Research 63 (2015) 47–50

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Interactive effects of temperature and relative humidity on oviposition and development of *Callosobruchus chinensis* (L.) on azuki bean

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ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form
12 May 2015
Accepted 12 May 2015
Available online 24 June 2015

Keywords:
Seed beetle
Oviposition
Developmental time
Adult emergence
Longevity

ABSTRACT

The effects of three temperatures, 20 °C, 25 °C and 30 °C, and four relative humidity (RH) levels, 30–35%, 50–55%, 70–75%, and 90–95%, on oviposition and development of *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) were investigated. *C. chinensis* were introduced to 30 g azuki bean seeds (cultivar Hongeon) one day post emergence. Five pairs of male and female beetles were used for each combination of temperature and RH, and replicated ten times. The introduced adults were allowed to lay eggs for 72 h. Temperature significantly influenced oviposition with increased number of eggs at 30 °C. Relative humidity had no effect on egg laying. Development time was affected by both temperature and RH. Development time was shortest at 30 °C and 70–75% RH. Adult emergence rate was affected by both temperature and RH; adult emergence rate increased with the increase in temperature and RH (up to 70–75%) levels. The results suggest that rather than the interactive effect of temperature and RH, individual effects of temperature and RH on *C. chinensis* are profound and *C. chinensis* is likely to cause greater damage to azuki bean seeds stored at 30 °C and 70–75% RH.

1. Introduction

Grain legumes are considered an important source of protein in Asia (Stanton et al., 1966). Among leguminous seeds, azuki beans are popularly used in desserts, snacks and confectionery items in East Asian countries and its use is increasing (Yousif et al., 2003). They are, however, susceptible to several insect species belonging to the family Bruchidae in both field and storage (Lale and Vidal, 2003; Gbaye et al., 2011). Azuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), infests legumes in the field and damages stored leguminous seeds. They render the seeds unappealing with their presence and emergence holes (Bellows, 1982; Southgate, 1984; Gbaye et al., 2011). Seed damage severely affects nutritional quality and germination potential of leguminous seeds (de Sá et al., 2014; Bae et al., 2014a, 2014b). Damage severity caused by any pest species depends on the rate of population increase of the species, which is influenced by temperature, humidity, and nutritional quality of the host species (Howe, 1965). Studies have reported that egg-laying behavior and development of *C. Chinensis* are influenced by environmental factors, and chemical and physical characteristics of host species (Messina, 2004; Mainali et al., 2015).

Temperature and humidity changes are known to affect oviposition and development of bruchids in storage (Lale, 1998; Lale and Vidal, 2003). Several studies on different species of bruchids addressing the temperature and humidity effects have been conducted (Howe and Currie, 1964; Mookerjee and Chawla, 1964; Giga and Smith, 1983). Most of the studies available are related to ovipositional and developmental performances of *C. chinensis* at different temperatures or at different humidity levels apart from a study carried out on *Callosobruchus maculatus* (F.) (Lale and Vidal, 2003). Study on the interactive influence of temperature and humidity on life processes of *C. chinensis* is sparse. We, thus, conducted this study to investigate the interactive effect of the temperature and humidity conditions on oviposition, development, survival, and adult longevity of *C. chinensis* on azuki bean (*Vigna angularis* [Willd.] Ohwi & Ohashi).

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http://dx.doi.org/10.1016/j.jspr.2015.05.008
0022-474X/

2. Materials and methods

2.1. Insect rearing

Callosobruchus chinensis were collected from azuki bean seeds (cultivar Hongeon) harvested from the crop grown in the fields of National Institute of Crop Science, Rural Development Administration, Miryang, Korea in 2011. Newly emerged adults of *C. chinensis* were sexed (Halstead, 1963). The adults were exposed to azuki bean seeds placed in a square shaped transparent petri plate (24 L × 2.5H cm) at 28 °C and 16L: 8D h photoperiod in the laboratory. After adult emergence, in the presence of directional light on the top, the adults together with the seeds were transferred to a Petri dish covered by an inverted funnel in order to facilitate their upward movement directed to one particular outlet for easy collection. The beetles are positively phototactic and negatively geotactic.

2.2. Influence of temperature and relative humidity on oviposition, development, emergence and adult longevity

Five pairs of male and female *C. chinensis* collected from the laboratory colony were released onto 30 g of azuki bean seeds (cultivar Hongeon) which were dried to a consistent moisture level, and then placed inside humidity chambers maintained at one of four different relative humidity (RH) levels. The RH levels within the chambers were maintained by using saturated salt solutions as described in Winston and Bates (1960). Salts used were $MgCl_2$, Mg $(NO_3)_2.6H_2O$, NaCl, and K_2SO_4 for 30–35, 50–55, 70–75, and 90–95% RH, respectively. Humidity chambers were then placed inside incubators set at 20, 25, or 30 °C. All chambers were held at a photoperiod of 16:8 (L: D) hour. Oviposition was allowed for 72 h. A 72-h period for egg laying ensures that sufficient amount of eggs are laid. After 72 h, released *C. chinensis* were removed and number of eggs laid on the azuki bean seeds were counted Developmental times from egg to adult were recorded and rates of adult emergence were calculated based on the number of total eggs and total number of adults emerged. The procedure was replicated 10 times for each temperature and RH condition. The emerged adults from each treatment combination were collected and held in a Petri dish, and they were observed for longevity providing respective temperature and RH combination used during their development.

2.3. Statistical analysis

Data on the number of eggs laid, developmental times, rates of adult emergence, and adult longevity were analyzed by a two-way analysis of variance (ANOVA), and the Tukey test was used as a post hoc analysis for the interaction and effect of temperature and RH (PROC GLM; SAS Institute, 2000). Mean values of developmental time and adult longevity of each individual within a replication of each treatment were calculated and the data on developmental time and adult longevity were then analyzed by a two-way ANOVA and Tukey test as mentioned above.

3. Results

3.1. Influence of temperature and relative humidity on oviposition, development, emergence and adult longevity

The maximum number of eggs laid per female was 17.7 (±3.2SD) at 30 °C and 70–75% RH. There was significant effect of temperature ($F = 739.7$, $df = 2, 108$; $P < 0.0001$). Number of laid eggs increased with increased temperature. However, there was no significant effect of RH ($F = 1.1$, $df = 3, 108$; $P = 0.354$) and interactions between temperature and RH ($F = 1.7$, $df = 6, 108$; $P = 0.134$; Fig. 1).

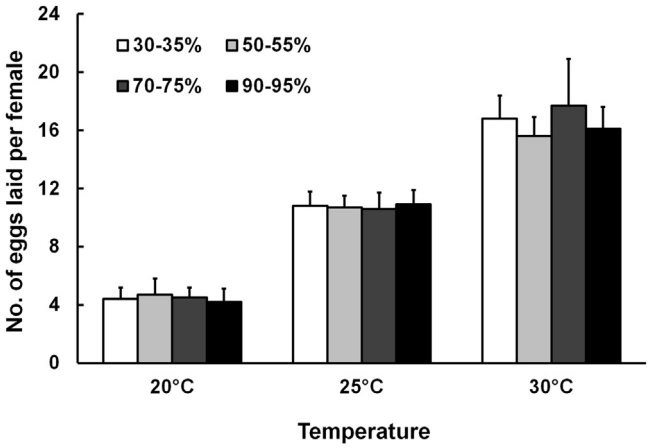

Fig. 1. Influence of temperature (20, 25, and 30 °C) and RH (30–35%, 50–55%, 70–75%, and 90–95%) on oviposition of *Callosobruchus chinensis* on azuki bean seeds in a period of 72 h. Ten replications were made. See text for the statistics.

The developmental time was shortest at 30 °C and 70–75% RH. There was significant effect of temperature ($F = 869.4$, $df = 2, 108$; $P < 0.0001$) and RH ($F = 8.3$, $df = 3, 108$; $P < 0.0001$). Development time decreased as temperature and RH increased. However, RH levels higher than 70–75% had negative effect on developmental time as formation of mold was found on a few of the seeds, and for each temperature condition developmental time increased at RH 90–95%. There was no significant effect of interactions between temperature and RH on developmental time ($F = 0.8$, $df = 6, 108$; $P = 0.594$; Fig. 2).

The highest adult emergence rate was 90.6% ± (3.1SD) at 30 °C and 70–75% RH. There was a significant effect of temperature ($F = 51.3$, $df = 2, 108$; $P < 0.0001$), RH ($F = 92.18$, $df = 3, 108$; $P < 0.0001$), and interactions between temperature and RH ($F = 9.2$, $df = 6, 108$; $P < 0.0001$). Emergence rate increased as temperature and RH increased. However, RH level higher than 70–75% had negative effect on adult emergence rate of *C. chinensis* probably because of mold formation on a few of the seeds (Fig. 3).

The adult longevity was directly proportional to increase in RH and inversely proportional to increase in temperature. Adults lived

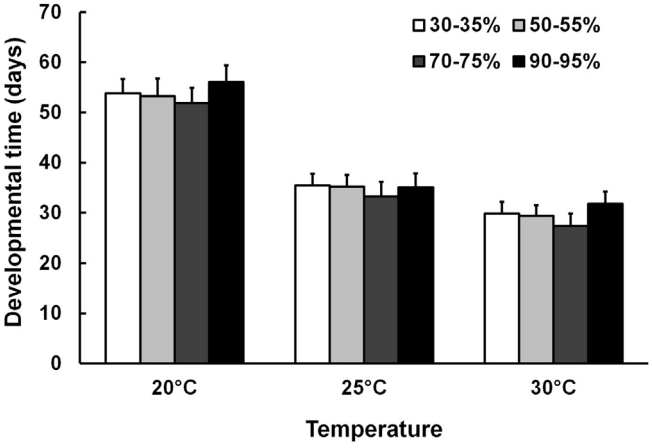

Fig. 2. Influence of temperature (20, 25, and 30 °C) and RH (30–35%, 50–55%, 70–75%, and 90–95%) on developmental time (days ± SD) of *Callosobruchus chinensis* on azuki bean seeds. Ten replications were made. See text for the statistics.