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Oogenesis in summer females of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), in southern Zhejiang, China*

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Abstract: The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, has two generations in southern Zhejiang, China. To determine oogenesis in first-generation females (summer females) and its relations to temperature, females were collected from a rice field in early and mid-July and reared on young rice plants at 28, 31 and 34 °C in the laboratory. Percentage of females having oocytes, number of oocytes of different stages (stage-I, from early previtellogenesis to middle vitellogenesis; stage-II, late vitellogenesis; and mature-oocyte stage), and length of ovarioles were determined every 10 d of feeding. At each temperature, oogenesis took place in over 40% of females after 20~40 d of feeding, but only 0.0~3.3 stage-I, 0.0~0.8 stage-II and 0.0~1.1 mature oocytes were observed at each observation date. Temperature had significant effect on number of stage-I oocytes but not on number of stage-II and mature oocytes in early July females; temperature had no significant effect on number of oocytes of either stage in mid-July females. Conclusively, in southern Zhejiang, summer *L. oryzophilus* females have great potential to become reproductive on rice, but their oogenesis activity is very low, with the overall procedures little affected by temperature.

Key words: *Lissorhoptrus oryzophilus*, Oogenesis, Ovarian development, Reproduction
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INTRODUCTION

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is an important insect pest of rice in the United States (Way and Wallace, 1993; Stout *et al*., 2001) and northeastern Asia (Lee and Uhm, 1993; Chen *et al*., 2005; Saito *et al*., 2005). Adult weevils feed on the foliage of rice plants leaving longitudinal slit-like scars, larvae prune root causing extensive damage to root systems, stunting plant growth and reducing grain yields (Way, 1990). This weevil can have two generations annually in China (Shih and Cheng, 1993; Zhai *et al*., 1997) and Japan (Sato, 1986), and two or more generations in the United States (Muda *et al*., 1981; Shang *et al*., 2004) if young rice is continually available.

In China, there have been several reports on the reproduction of summer females (first-generation females), all of which targeting the population in southern Zhejiang (Zhai *et al*., 1997; 1998; 1999; Jiang *et al*., 2004a). In this area, most summer females emigrate from rice fields for summer and subsequently winter hibernation, and only 5%~8% of the females that remain in the fields before rice harvest reproduce on transplanted late-season rice (Zhai *et al*., 1998). Under laboratory conditions, however, 53.6% of summer females collected in this area produced mature eggs in the ovary after three weeks of feeding on young rice (Zhai *et al*., 1998). Jiang *et al*.(2004a)

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also observed a rapid increase in ovipositing individuals after feeding of summer females on plants extended to three weeks. These reproductive females deposit 7~20 eggs under favorable laboratory conditions (Jiang *et al*., 2004a). Summer reproduction of this weevil is greatly affected by food quality and temperature. Their reproductive development was more rapid feeding on the grasses *Echinochloa crusgalli* (L.) Beauv., *Leptochloa chinensis* (L.) Nees. and rice seedlings than on ineffective tillers of aging rice (Zhai *et al*., 1998), more rapid feeding on young rice seedlings than on old ones (Jiang *et al*., 2004a). When given young rice seedlings, more productive individuals could be produced at 35 °C than at 25 and 30 °C (Zhai *et al*., 1998).

Overall the ecology of reproduction in summer *L. oryzophilus* females is well known but much is less clear about its precise physiological bases. In the present study, oogenesis and ovarian development were quantified in summer females at different temperatures, aiming at enriching our understanding on their reproductive physiology.

MATERIALS AND METHODS

Insects

Summer *L. oryzophilus* females were collected in a rice filed (0.08 ha) located in Yueqing (28.1° N, 120.9° E), southern Zhejiang. In this area, only parthenogenetic females occur. Rice is normally grown in two seasons, transplanted in early May and around end of July respectively. The weevil has two generations annually, attacking the early- and late-season rice respectively; the first generation can attain high densities and causes economic loss, while density of the second generation is very low (Zhai *et al*., 1997).

Rice plants (cv. Zhongsi 2, the National Rice Research Institute, China) were transplanted in early May 2005 and spaced 18~20 cm apart, with three plants per hill. Normal water management and fertilization were resorted to, no insecticides were applied to the field throughout the growth season. In this field, the authors observed that the majority of summer females emerged during early July. As reproductive behavior of summer females is related to collection time (Jiang *et al*., 2004a), they were collected in early July and mid-July separately. Collection was conducted by gently placing a ferrous plate (35 cm×50 cm×3.5 cm) near to the base of the plants and patting the plants at 15~20 cm height.

Temperature treatments and adult rearing

Three temperatures were used, 28, 31 and (34±1) °C, which is typical of summer temperatures in Yueqing. The collected females were placed in 2.5 cm diameter, 20 cm long glass tubes containing tap water to 6~7 cm depth, three 10~14 d old rice plants (cv. Shanyou 63), and 10 females per tube. Then, they were maintained in climatically controlled chambers at each of the three temperatures with a photoperiod of 16 h:8 h (light:dark). Plants in tubes were replaced every 3~4 d with new plants.

Quantitative determination of oogenesis and ovarian development

Oocyte number and ovariole length were determined after feeding for 10, 20 and 30 d in the females collected in early July, and after feeding for 10, 20, 30 and 40 d in the females collected in mid-July. According to Kurihara and Matsuzaki (1989), we divided the oocytes in summer weevils into three groups based on their age and morphology. The first group was in early previtellogenic to middle vitellogenic stages, 100~300 μm in length. The second group was in late vitellogenic stage, >300 μm in length; oocyte volume had not reached the maximum, and chorion formation had not completed. The third group was mature eggs, ca. 600 μm in length with maximum volume. For descriptive convenience below, the three groups of oocytes were named as stage-I, stage-II and mature oocytes, respectively.

At each observation date, females were sampled (n=20~30 in most samplings) randomly from tubes at each temperature. Abdomen of sampled females was dissected on a glass slide with a small drop of saline (9.0 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$ and 4.0 g sucrose per 1 L), ovaries were exposed by grasping the tip of the abdomen with fine forceps and gently pulling the reproductive organs in a posterior direction until free from other tissues. Then, morphology of each oocyte in ovarioles and egg calyxes was observed under a dissecting microscope, and number of oocytes falling in each stage was counted. Thereafter, the ovariole end was grasped and pulled gently across a thin layer of saline until the ovarioles were straightened, then ovariole length was measured from the base to terminal using an ocular micrometer inserted in the microscope.

Data analyses

For females subjected to each temperature and feeding-duration combination, percentage of individuals having oocytes (oogenesis percentage) and mean value of oocyte number and ovariole length were calculated. Chi-square tests were conducted to analyze effects of temperature on oogenesis percentage at the level P=0.05. A two-way analysis of variance (ANOVA) was conducted to analyze effects of temperature and feeding duration on oocyte number and ovariole length. Before subjected to ANOVA, values were transformed using square root (sqrt): sqrt(n+1) and sqrt(n) for oocytes number and ovariole length, respectively. Tukey test was used to compare means from three or more treatments, and t test to compare means from two treatments, at the level P=0.05 (SPSS, 1999).

RESULTS AND DISCUSSION

For females collected in early July, oocytes occurred in a similar percentage (45.8%~57.7%) of females after 20 d of feeding at different temperatures, and occurred in 72.4% and 88.2% after 30 d at 28 and 31 °C, respectively. No significant (P>0.05) effect of temperature on these percentages was observed (Table 1). However, for females collected in mid-July, percent oogenesis increased as temperature increased, with a significant (P<0.05) effect of temperature observed in females feeding for 20 and 30 d (Table 1).

The above results suggest that relations between oogenesis in summer *L. oryzophilus* females and temperature may vary with time, their oogenesis appears to be more temperature-sensitive during later times. Such reproductive behavior changes along with time were also detected in a previous study (Jiang *et al*., 2004a), where females collected later (in mid-July) oviposited earlier than those collected earlier (in late June and early July) when given young rice in the laboratory. The reasons responsible for these differences are not clear to date.

It is still unclear as to the actual proportion of summer females that can reproduce on late-season rice in southern Zhejiang. This proportion was below 10% according to the estimation of Zhai *et al*.(1998). Under laboratory conditions, however, it appeared to be much higher with the maximum estimated to be about 70% (Jiang *et al*., 2004a), and this estimation was supported by the present study where oogenesis took place in about 70% of summer females after 20~40 d of feeding at 28 °C (Table 1).

For early-July females, temperature affected significantly (P<0.05) only the number of stage-I oocytes, but not significantly (P>0.05) the number of stage-II or mature oocytes (Table 4); at higher temperatures more stage-I oocytes were observed in the females feeding for 20 and 30 d (Table 2). For mid-July females, temperature had no significant (P>0.05) effect on the number of oocytes of either stage (Table 4), but more stage-I oocytes were also observed at higher temperatures in the females feeding for 20 and 30 d (Table 3). This indicates that more stage-I oocytes can be produced at higher temperatures in the regime of 28~34 °C, although the overall

Table 1 Percentage of summer *L. oryzophilus* females having oocytes after a period of feeding on rice at different temperatures

Adult source	Feeding days	Females having oocytes (%)			Chi-square tests		
		28 °C	31 °C	34 °C	χ^2	*df*	*P*
Collected from a rice field in early July 2005	10	0.0 (20)	0.0 (20)	0.0 (20)	–	–	–
	20	45.8 (24)	57.7 (26)	50.0 (26)	0.730	2	0.694
	30	72.4 (29)	88.2 (17)	–	1.577	1	0.209
Collected from a rice field in mid-July 2005	10	0.0 (20)	0.0 (20)	0.0 (20)	–	–	–
	20	43.3 (30)	53.3 (30)	87.1 (31)	13.610	2	0.001*
	30	60.0 (30)	93.3 (30)	96.7 (30)	17.760	2	0.000*
	40	67.7 (31)	87.5 (8)	100.0 (5)	3.204	2	0.201

Values in parentheses were the number of females observed. * indicates significant effects of temperature on oogenesis percentage (P<0.05)

Table 2 Number of oocytes and length of ovarioles in summer *L. oryzophilus* females after a period of feeding on rice at different temperatures: females collected in early July 2005

	Feeding days	28 °C	31 °C	34 °C
No. of stage-I oocytes	10	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
	20	1.3±0.4 a	1.7±0.4 a	2.7±0.8 a
	30	0.3±0.2 bB	2.5±0.8 aA	–
No. of stage-II oocytes	10	0.0±0.0	0.0±0.0	0.0±0.0 b
	20	0.4±0.2	0.4±0.2	0.8±0.3 a
	30	0.1±0.1	0.2±0.1	–
No. of mature oocytes	10	0.0±0.0	0.0±0.0 b	0.0±0.0
	20	0.4±0.3	0.1±0.1 ab	0.3±0.2
	30	0.1±0.1	0.7±0.4 a	–
Ovariole length (μm)	10	169.5±4.8 b	183.0±6.2 b	179.3±4.6 b
	20	248.4±12.2 a	248.5±9.2 a	254.6±10.1 a
	30	197.9±11.0 bB	240.8±14.4 aA	–

In a row, means followed by the same uppercase letter were not significantly different ($P>0.05$); in a column, means followed by the same lowercase letter were not significantly different ($P>0.05$). Rows or columns without letters indicate no significant difference among means ($P>0.05$)

Table 3 Number of oocytes and length of ovarioles in summer *L. oryzophilus* females after a period of feeding on rice at different temperatures: females collected in mid-July 2005

	Feeding days	28 °C	31 °C	34 °C
No. of stage-I oocytes	10	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
	20	1.4±0.4 aB	2.3±0.5 aAB	3.3±0.4 aA
	30	0.6±0.2 abB	1.8±0.5 aAB	2.8±0.6 aA
	40	1.7±0.4 a	1.1±0.8 ab	0.0±0.0 b
No. of stage-II oocytes	10	0.0±0.0	0.0±0.0 b	0.0±0.0 b
	20	0.3±0.2	0.5±0.2 a	0.6±0.2 a
	30	0.0±0.0	0.2±0.1 ab	0.1±0.0 ab
	40	0.1±0.0	0.0±0.0 ab	0.0±0.0 ab
No. of mature oocytes	10	0.0±0.0	0.0±0.0 b	0.0±0.0
	20	0.3±0.2	1.1±0.4 a	0.7±0.3
	30	0.0±0.0	0.2±0.1 b	0.2±0.2
	40	0.2±0.1	0.0±0.0 b	0.0±0.0
Ovariole length (μm)	10	167.5±2.9 c	155.2±5.2 b	157.0±3.4 c
	20	231.1±13.5 abB	258.1±16.3 aAB	287.2±12.8 aA
	30	198.1±9.9 bcB	254.3±8.1 aA	246.3±6.6 bA
	40	238.2±11.0 a	267.3±21.8 a	263.3±9.0 ab

In a row, means followed by the same uppercase letter were not significantly different ($P>0.05$); in a column, means followed by the same lowercase letter were not significantly different ($P>0.05$). Rows or columns without letters indicate no significant difference among means ($P>0.05$)

oogenesis in summer females appeared to be little affected by temperature.

For both early and mid-July females, feeding durations had significant ($P<0.05$) effect on the number of oocytes of each stage (Table 4). But in each feeding-duration treatment, only 0.0~3.3 stage-I, 0.0~0.8 stage-II and 0.0~1.1 mature oocytes were observed at each temperature (Tables 2 and 3). This indicates that very few oocytes are produced in summer females although their oogenesis can take place in over 40% of individuals. Such a weak oogenesis in summer females should have contributed a lot to the low density of their offspring on late-season rice in southern Zhejiang.

Both temperature and feeding duration had significant ($P<0.05$) effect on ovariole length of both

Table 4 ANOVA on effects of temperature and feeding duration on oocyte number and ovariole length in summer *L. oryzophilus* females described in Tables 2 and 3

Dependant variables	Source	Females collected in early July			Females collected in mid-July		
		df	*F*	*P*	*df*	*F*	*P*
No. of stage-I oocytes	T	2	3.457	0.034*	2	1.338	0.264
	FD	2	16.023	0.000*	3	19.722	0.000*
	T×FD	3	2.494	0.062	6	3.213	0.005*
No. of stage-II oocytes	T	2	1.063	0.348	2	0.515	0.598
	FD	2	7.534	0.001*	3	8.683	0.000*
	T×FD	3	0.720	0.541	6	0.496	0.811
No. of mature oocytes	T	2	0.461	0.631	2	0.908	0.404
	FD	2	4.260	0.016*	3	8.459	0.000*
	T×FD	3	2.085	0.104	6	1.248	0.282
Ovariole length	T	2	3.226	0.042*	2	6.888	0.001*
	FD	2	41.966	0.000*	3	44.866	0.000*
	T×FD	3	1.692	0.171	6	2.878	0.010*

T: Temperature; FD: Feeding duration. * indicates significant effects of temperature at the level P=0.05

early and mid-July females (Table 4). Ovarioles of early July females were significantly (P<0.05) longer at 31 °C than at 28 °C after feeding for 30 d (Table 2); ovarioles of mid-July females were significantly (P<0.05) longer at 34 °C than at 28 °C after feeding for 20 d, and longer at 31 °C and 34 °C than at 28 °C after feeding for 30 d (Table 3). Ovarioles elongated significantly at each temperature as feeding extended from 10 to 20 d (Tables 2 and 3).

Summer reproductive capacity of *L. oryzophilus* may have geographic differences. At Taoyoung (25.1° N, 121.2° E) of Taiwan, about two summer females and eight offspring larvae were ever observed at the peak on per hill of late-season rice (Shih and Cheng, 1993). However, in southern Zhejiang, there were only 0.1~0.4 summer females and 0.6~1.0 offspring larvae on per hill of late-season rice (Zhai *et al.*, 1997; 1999). In Louisiana, densities of summer females and their offspring appear to be much higher than those reported in Asian areas (Shang *et al.*, 2004). We suggest that reproduction in summer females be compared among geographic populations with emphasis both on their potential of developing into reproductive individuals and on their subsequent oogenetic activity (number of oocytes produced in ovaries).

Our earlier studies found that overwintered *L. oryzophilus* females could lay an average of over 45 eggs (Jiang and Cheng, 2003a; 2003b). Summer females could lay 60 eggs after they previously experienced 15 °C for 50 d (Jiang *et al.*, 2004b). Compared with these females, the fecundity of summer females is apparently much lower, and they appear to be in a “transitional” state between diapause and reproduction. Significance of the formation of such females to their life history is unknown.

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