Effects of temperature and winter diapause on survival and development in bivoltine and trivoltine ecotypes of the rice stem maggot, *Chlorops oryzae* Matsumura (Diptera: Chloropidae), reared on a winter host

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(Received 28 April 1997; Accepted 20 August 1997)

Abstract

In *Chlorops oryzae*, winter diapause in the first larval stage is induced by short days in the egg stage and maintained by short days in the larval stage. On wheat plants (winter host), most diapause and non-diapause larvae failed to pupate and died within 50 days at 23°C regardless of photoperiod. When diapause larvae were reared on wheat plants at 10, 15 or 20°C under LD12 : 12, the survival rates increased with decreasing temperatures. Diapause development at 10°C was completed about 70–90 and 40–50 days in the Akita (bivoltine, 39°N) and Aichi (trivoltine, 35°N) strains, respectively. The survival rates on wheat seedlings at 23°C in diapausing larvae of the Akita strain increased with increasing periods of previous exposure to 10°C from 41 to 80 days. Aichi larvae showed higher survival rates regardless of the period at 10°C. It appears that low temperature promotes survival of the larvae on wheat plants, and larvae which complete diapause development can grow to the pupal stage on winter hosts.

Key words: *Chlorops oryzae*, host plants, diapause development, photoperiod, low temperature

INTRODUCTION

The rice stem maggot, *Chlorops oryzae* Matsumura (Diptera: Chloropidae), is an important pest of paddy rice plants. This species is distributed throughout Japan, and northern bivoltine and southern trivoltine ecotypes have been recognized (Iwata, 1963; Hirao, 1970). In the bivoltine area (Hirao, 1970), the maggots overwinter in the first stadium inside the stem of Gramineae. In June, they feed on young panicles of their hosts and pupate inside the leaf sheath. The adults of the overwintering generation oviposit on rice plants at the tillering stage from late June to early July. Newly hatched larvae burrow into the stem of rice plants and move to the growing points where they feed on developing leaves. First-generation larvae remain in the first larval stage while feeding on developing leaves. They mature after feeding on young panicles and pupate after head formation of their hosts. Adults of the first generation emerge in early September and oviposit on

Gramineae. Second-generation larvae overwinter inside the stem of winter hosts. In the trivoltine area (Iwata, 1963), the first stadium larvae hibernate as in the bivoltine area, but the adults emerge and oviposit on rice plants from mid-May to early June. First-generation larvae grow fast and pupate, feeding on only developing leaves of rice plants at the early tillering stage. Adults of the first generation again oviposit on rice plants at the panicle formation stage in mid-July. Second-generation larvae mature after feeding on young panicles. Adults of the second generation oviposit on Gramineae from late September to early October. Third-generation larvae overwinter inside the stem of winter hosts.

Oryza sativa L. and *Leersia japonica* Makino have been recorded as summer hosts of *C. oryzae* by Okamoto (1970), who also reported 17 species of Gramineae as winter hosts in Japan. Wheat and barley are economically important among the winter hosts. The winter hosts germinate before the ovipositing period of

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the last brood of *C. oryzae* in autumn, and persist until the emerging period of the overwintering generation the following spring. The effects of different winter hosts on post-diapause development under natural conditions have been studied (Iwata et al., 1957; Tamura et al., 1959; Hirao, 1970). Kishino (1960) reported that some larvae of the third generation in the trivoltine strain developed to the pupal stage on wheat plants (winter host) in a green house under natural daylength. However, hibernation of this insect had been ascribed to a cold quiescence in the first stadium (cf. Takeda and Nagata, 1992).

Recently, photoperiodic control of development, winter and summer diapauses of *C. oryzae* has been elucidated (Takeda and Nagata, 1992). When the egg stage is exposed to long days (as in summer under natural conditions), photoperiod during the larval stage influences larval development and also the incidence of summer diapause, which occurs in the mature larval stage (Takeda, 1997). Larvae of the bivoltine strain develop without delay under short and intermediate days, but their development is retarded under long days. On the other hand, larval development in the trivoltine strain is fast under both short and long days, but delayed under intermediate days. Thus, the larvae of the first generation in the bivoltine strain enter summer diapause under long days (as in summer under natural conditions), but those in the trivoltine strain develop without delay. This difference in the photoperiodic response between the two geographic ecotypes is the principal factor that determines the number of generations each year. Furthermore, the effects of photoperiod and age of rice plants on larval development have been elucidated (Takeda and Nagata, 1997).

The effects of photoperiod and temperature on the induction and maintenance of winter diapause have been elucidated by rearing on young rice (summer host) seedlings (Takeda, 1996). Short days during the egg stage induce a winter diapause in the first stadium. Once winter diapause has been induced, the diapause is maintained by short days, but can be terminated by long days. The critical photoperiods at 20°C for the induction and maintenance of winter diapause in the bivoltine (Akita) and trivoltine

(Aichi) strains are about 14 and 13 h, respectively. The critical photoperiods in both strains are shortened with increasing temperature. However, little is known about the influence of winter hosts on survival and diapause in this species.

In some species of insects from the temperate regions, a period of low temperatures (as in winter under natural conditions) promotes diapause development (Tauber et al., 1986; Danks, 1987). Low temperatures in winter may also favor survival by reducing the rate of metabolism of the insects.

In this study, therefore, we analyzed the thermal requirements and winter diapause in relation to survival and development in the two geographic ecotypes of *C. oryzae* reared on winter hosts.

MATERIALS AND METHODS

Rearing procedure. Two strains of *C. oryzae* used in the experiments were maintained in our laboratory as follows. The Akita (bivoltine) strain was established in 1989 from a field site at Sen-nan (39°22'N, 70 m altitude), Akita Prefecture. The Aichi (trivoltine) strain was originally collected at Inazawa (35°13'N, 2 m altitude), Aichi Prefecture, in 1989. Eggs of their progeny were exposed to long day (LD15:9) conditions to avert winter diapause and larvae were reared under short day (LD12:12) conditions to avert summer diapause (Takeda, 1996, 1997). The rice cultivar "Kenbaiwai" was used for maintaining cultures.

About 100 adults were released into a rearing cage with rice seedlings for oviposition (Takeda and Nagata, 1992). Adults and eggs were kept under controlled environmental conditions in incubators illuminated with two 15 W fluorescent lamps. The wheat (*Triticum aestivum* L.) variety "Kitakami" was used for rearing the larvae. Three to 5 days before the introduction of newly hatched larvae, 20 germinated wheat seeds were sown in a plastic cup (6.0 cm diameter, 8.0 cm high) filled with a sufficient amount of soil and 0.4 g of a compound fertilizer containing N, P and K. Newly hatched larvae were inserted individually into the leaf sheath of wheat plants of the first or second leaf stage (Takeda and Nagata, 1992). The wheat plants were kept un-

der controlled environmental conditions in bioclimatic chambers with four 250 W incandescent lamps.

Survival and development of non-diapause and diapause larvae at 23°C. Eggs of both strains were exposed to a photoperiod of LD15:9 at 20°C to avert winter diapause. Newly hatched larvae from these eggs were inserted individually inside the leaf sheath of wheat plants and kept under LD12:12, 14:10 or 15:9 at 23°C. The infested wheat plants in two or four cups were dissected to collect larvae and pupae at 10 day intervals. Living larvae were classified into the three stadia by the morphology of the posterior spiracles and third instars were further divided into the feeding and mature stages (Takeda, 1993; Takeda and Nagata, 1992).

To compare with the above treatments, eggs were kept under LD12:12 at 20°C to induce winter diapause, and larvae were reared on wheat plants under LD15:9 at 23°C for diapause termination. The developmental stages were determined as described above.

Effects of temperature on survival and diapause development. Eggs of both strains were kept under LD12:12 at 20°C to induce winter diapause. Newly-hatched larvae were inserted individually inside the leaf sheath of wheat seedlings and kept one night under LD12:12 at 20°C to allow them to burrow into the stem. Subsequently, the infested wheat plants were kept under LD12:12 at 10, 15 or 20°C. The wheat plants from two cups were dissected at 10 day intervals to collect larvae and then the first instars were transferred to rice seedlings. These first instars were maintained under LD12:12 at 20°C, the persisting winter diapause condition (Takeda, 1996). At day 7 after the transfer, the rice plants were dissected to collect larvae and their developmental stages were determined.

To analyze the effect of low temperature (10°C) on the survival of non-diapause larvae reared on wheat plants, eggs of both strains were exposed to LD15:9 at 20°C. The stages of these larvae were checked as described above.

Effect of diapause development on survival and development on wheat plants. Eggs of both strains were kept under LD12:12 at 20°C to induce winter diapause. Newly-hatched larvae

were held on wheat seedlings under LD12:12 at 20°C for one night and transferred to LD12:12 at 10°C. The infested wheat plants were dissected at day 41, 61 or 79–80 after hatching and then the first instars were again inserted into the leaf sheath of wheat seedlings of the first or second leaf stage. These larvae were transferred to LD15:9 at 23°C and their developmental stages were determined 35 days after the transfer.

Survival and development of overwintering larvae collected from the bivoltine area. Winter host plants (*Agrostis clavata* Trin. var. *nukabo Ohwi*) were collected from Sen-nan in late October, 1993. They were transplanted at the farm of the Tohoku National Agricultural Experiment Station (39°29'N, 30 m altitude). These winter hosts were sampled at 14 to 20 day intervals from October 26 to January 8, and on March 19. The host plants were dissected, and the overwintering larvae were transferred to wheat or rice seedlings under LD15:9 at 23°C. Their developmental stages were checked after 28 and 35 days of the transfer to rice and wheat plants, respectively. Pupae from the samples of March 19 were placed in a Petri dish with moist filter paper. After emergence, head width was measured under a binocular microscope with an ocular micrometer.

Growth of overwintering larvae collected from the bivoltine area. Winter hosts (*Agrostis clavata* Trin. var. *nukabo Ohwi*) were collected from Sen-nan on April 8 in 1994. They were dissected to collect overwintering larvae. First instars were moved to wheat or rice seedlings and kept under LD12:12, 14:10 or 15:9 at 23°C. Pupation was recorded daily.

RESULTS

Survival and development of non-diapause and diapause larvae at 23°C

In this study, the larvae from eggs exposed to LD12:12 are called diapause larvae, and those exposed to LD15:9 as non-diapause larvae. Figure 1 shows the changes in the survival rates and developmental stages of the Akita (bivoltine) strain during the period when the larvae were reared on wheat plants under various photoperiods at 23°C. At day 10 after hatching, the survival rates of the non-diapause larvae

and diapause larvae ranged from 32.5 to 47.5% and there was no significant difference in the survival rates among different treatments (Fisher's exact probability test, $p > 0.05$). At day 20 after hatching, however, the survival rates of the non-diapause larvae were significantly higher when they were kept under LD15:9 than those under LD12:12 or 14:10. This difference might have been caused by the retardation of larval development in the bivoltine strain under long day (Takeda and Nagata, 1997). Thereafter, the survival rates of the non-diapause larvae decreased rapidly, and no larvae survived 50 days after hatching under any of the three photoperiods. Although there was no significant difference in the survival rates between diapause and non-diapause larvae reared under long day (LD15:9), 10% of the diapause larvae survived and one pupated at day 50 after hatching under this diapause-terminating condition.

In the Aichi (trivoltine) strain, there was no significant difference in the survival rates at day 10 after hatching among the different treatments (Fig. 2). Some non-diapause insects pupated,

but the survival rates at day 50 after hatching were 10% or less. The survival rate at day 50 after hatching was higher in the diapause larvae than in the non-diapause larvae under LD15:9. The trivoltine strain tended to show higher survival rates than the bivoltine strain under every photoperiod.

Effects of temperature on survival and diapause development

Figure 3A shows the effect of temperature on the survival of diapause and non-diapause larvae in the Akita strain reared on wheat plants under LD12:12. At day 10 after hatching, the survival rate ranged from 62.5 to 74.4%, and there was no significant difference in the survival rates among the different treatments. When reared at 10°C, more than 60% of the diapause larvae survived on day 80 and 47.5% on day 90. At this temperature, there was no significant difference in survival rate between the diapause and non-diapause larvae until day 80, but more diapause larvae survived than non-diapause larvae on day 90 (Fisher's exact probability test,

Fig. 1. Survival rates and developmental stages in the Akita (bivoltine) strain of *C. oryzae* reared on wheat plants at 23°C under various photoperiods as given in the figure for eggs (E) and for larvae (L). Developmental stages given in the right side of the column as 1, 2, 3 and P indicate first, second, third instars and pupae, respectively. Same letters above bars indicate no significant difference when the survival rates among different photoperiodic treatments were compared for the same period of incubation (Fisher's exact probability test, $p > 0.05$). Diapause and non-diapause given in the figure indicate the larvae from eggs exposed to short day (LD12:12) and long day (LD15:9), respectively.

Fig. 2. Survival rates and developmental stages in the Aichi (trivoltine) strain of *C. oryzae* reared on wheat plants under various photoperiods at 23°C. M: mature third instar. For other keys, see Fig. 1.

$p < 0.05$). At 15°C, the survival rates of diapause larvae decreased from 82.5 to 30.8% by day 90. Furthermore, the survival rates at 20°C of the diapause larvae decreased rapidly and only 2.6% of them survived at day 80 after hatching. Thus, the survival rate in the diapause larvae of the bivoltine strain tended to decrease with increasing temperature.

Figure 3B shows the changes in the percentages of first instars in the bivoltine (Akita) strain reared on wheat plants. Most diapause larvae were still in the first larval stage at day 80 or 100 after hatching. By contrast, more than 50% of the non-diapause larvae reached the second or later stages between days 80 and 90 after hatching.

After the larval development of the first instars was checked in this experiment, they were transferred to rice seedlings under LD12:12 at 20°C, conditions which maintain winter diapause (Takeda, 1996). Most non-diapause larvae developed to the second or later stages at day 7 after the transfer (Fig. 3C). On the other hand, more than 90% of the diapause larvae remained in the first larval stage after 10 to 50 days at 10, 15 or 20°C. About 50% of the diapause larvae started development after 60–80 and 80–90 days of incubation at 10 and 15°C, re-

spectively. A few larvae reared at 20°C on wheat plants survived at day 60 after hatching (Fig. 3A), and thus, the resumption of larval development could not be determined.

More than 70% of Aichi (trivoltine) larvae reared on wheat plants under LD12:12 survived 10 days after hatching (Fig. 4A), and there was no significant difference among the three temperature treatments. When larvae were reared at 10°C, the survival rates of the diapause and non-diapause larvae ranged from 72.5 to 92.5% and from 39.5 to 80%, respectively. The difference between the diapause and non-diapause larvae was not significant except for the survival rates on days 30, 80 and 90. Diapause larvae died earlier at 20 and 15°C than at 10°C.

Figure 4B shows the changes in the percentages of first instars reared on wheat plants. The percentages of first instars in the non-diapause larvae reared at 10°C decreased rapidly between days 30 and 40. On the other hand, more than 50% of the diapause larvae remained in the first larval stage on days 60–70 at 15 and 20°C. At 10°C, about 50% of diapause larvae molted to the second stage at day 90 after hatching.

The first-stadium larvae found in the above experiment were transferred to rice seedlings un-

Fig. 3. Effects of temperature, photoperiod and host plant on survival and development in the Akita (bivoltine) strain of *C. oryzae*. A: The survival rates of larvae reared on wheat seedlings (winter plants) at three different temperatures (see below) under LD12:12. The asterisks show significant differences in survival rate from diapause larvae reared at 10°C (Fisher's exact probability test, $p < 0.05$). B: Percentages of first instars reared on wheat plants at three different temperatures under LD12:12. C: Percentages of first instars at day 7 after the transfer to rice seedlings under LD12:12 at 20°C, after being kept at different temperatures for the period given in the abscissa. Eggs were exposed to LD12:12 (diapause, solid line), or LD15:9 (non-diapause, broken line) at 20°C. Temperature in the larval stage on winter plants was 10 (circle), 15 (triangle) or 20°C (square).

Fig. 4. Effects of temperature, photoperiod and host plants on survival and development in the Aichi (trivoltine) strain of *C. oryzae*. A: The survival rate of larvae reared on wheat plants at three different temperatures under LD12:12. B: Percentages of first instars reared on wheat plants at three different temperatures under LD12:12. C: Percentages of first instars at day 7 of rearing on rice seedlings under LD12:12 at 20°C after exposure to different temperatures for the period given in the abscissa. For keys, see Fig. 3.

Fig. 5. Effect of diapause development on survival and larval development of *C. oryzae* reared on wheat plants. Diapause larvae were exposed to LD12:12 at 10°C for 41, 61 and 79–80 days, and then transferred to LD15:9 at 23°C to terminate diapause. The survival rates and developmental stages were determined by dissecting the wheat plants 35 days after the transfer. Upper: Akita (bivoltine) strain. Lower: Aichi (trivoltine) strain. 3: third instars; M: mature third instars; P: pupae. Same letters above bars indicate no significant difference in the survival rate at the 5% level (Fisher's exact probability test).

der LD12:12 at 20°C. Most non-diapause larvae developed to the second or third stadium at day 7 after the transfer, regardless of being reared on wheat plants during preceding periods (Fig. 4C). When diapause larvae were transferred after being reared on wheat plants for 20 days at 10, 15 or 20°C, more than 90% of them remained in the first larval stage. Thereafter, the percentage of first instars decreased rapidly with increasing length of the previous rearing on wheat plants. Thus, diapause was terminated in about 50% of Aichi larvae by 40 to 50 days of rearing on wheat plants at all of the three different temperatures.

Effect of diapause development on survival and development on wheat plants

Diapause first instars of both strains were reared on wheat plants under LD12:12 at 10°C for 41, 61 or 79–80 days, and then transferred to wheat seedlings under LD15:9 at 23°C to terminate diapause. Figure 5 shows the survival

rates and developmental stages at day 35 after the transfer. As the period of rearing at 10°C increased, the survival rate and percentage pupation in the Akita (bivoltine) strain increased. When Akita larvae were held at 10°C for 79–80 days, the survival rate was 66%, and 65% of the survivors pupated. On the other hand, the survival rates and percentage of pupation in the Aichi (trivoltine) strain were not influenced by the duration of 10°C exposure. More than 79% of Aichi larvae survived and more than 85% of the survivors pupated.

Survival and development of overwintering larvae collected from the bivoltine area

Overwintering larvae were collected in the bivoltine area and reared for 28 and 35 days on rice and wheat seedlings, respectively, under LD15:9 at 23°C. The survival rates on wheat plants ranged from 17.5 to 53.3% in the samples collected on January 8 or earlier (Fig. 6). By contrast, the survival rate of March-collected larvae was 83.8%, being significantly higher than in the earlier samples. When reared on rice seedlings, more than 83% of larvae collected on any date survived and the percentage of pupation ranged from 30% (November sample) to 74% (March sample).

There was no significant difference in head width between adults reared on wheat and on rice plants (males on wheat 0.81 ± 0.03 mm, on rice 0.80 ± 0.03 mm; females on wheat 0.89 ± 0.05 mm, on rice 0.91 ± 0.02 mm, *t*-test, $p > 0.05$).

Growth of overwintering larvae collected from the bivoltine area

Winter host plants were dissected to collect larvae on April 8 in the bivoltine area, when all larvae were in the first larval stage. They were transferred to rice or wheat seedlings at 23°C. Table 1 shows the effects of photoperiod and host plants on survival and development of these larvae. On wheat plants, the survival rates of individuals attaining the pupal stage ranged from 25.0 to 38.3%, and there was no significant difference in the values among the photoperiods. However, the mean duration of the larval stage was significantly longer under LD14:10 than under LD12:12.

Fig. 6. Survival rates and developmental stages of overwintering first-stage larvae of *C. oryzae* collected in the bivoltine area on the dates (abscissa), when reared on wheat or rice plants under LD15:9 at 23°C. The developmental stages were determined 35 and 28 days after rearing on wheat and rice plants, respectively. Sample sizes at the start were from 38 to 80 for wheat plants and from 24 to 29 for rice plants. Same letters above bars indicate no significant difference in the survival rate at the 5% level (Fisher's exact probability test). For key, see Fig. 5.

Table 1. Pupation and larval development of larvae collected in the bivoltine area of *C. oryzae* when reared on wheat or rice plants under three photoperiods at 23°C

Host plant	Photoperiod L:D	No. of insects tested	No. of insects that bored	No. of pupae ¹ (% pupation) ²	Mean duration of the larval stage (day ± S.D.) ³
Wheat	12:12	60	48	15 (25.0 c)	32.5 ± 8.9 b
	14:10	60	46	15 (25.0 c)	43.2 ± 13.5 c
	15:9	60	44	23 (38.3 c)	34.0 ± 12.8 bc
Rice	12:12	47	40	39 (83.0 a)	21.3 ± 7.6 a
	14:10	48	39	36 (75.0 ab)	22.5 ± 9.2 a
	15:9	48	31	29 (60.4 b)	19.6 ± 4.8 a

¹Twenty-one percent of pupae were parasitized by *Coelinidae oryzicola* Watanabe.

²Percentages followed by the same letter are not significantly different at the 5% level (Fisher's exact probability test).

³Means followed by the same letters are not significantly different at the 5% level (*t*-test).

On rice plants, the survival rates ranged from 60.4 to 83.0%, and there was a significant difference in these values between LD12:12 and LD15:9. The mean durations of the larval stage ranged from 19.6 to 22.5 days, and there was no

significant difference among the photoperiods. The larval stage was significantly longer when the larvae were reared on wheat plants than on rice plants.

DISCUSSION

In *C. oryzae*, short days in the egg stage induce winter diapause in the first stadium, but long days avert it. Once diapause has been induced, short days maintain but long days terminate the diapause (Takeda, 1996). For non-diapause larvae of both the bivoltine and trivoltine strains reared on rice seedlings under short days, a temperature of 23°C appears to be nearly optimum for development (Takeda, 1997). In the present study, the non-diapause larvae of the bivoltine strain (Akita, 39°N) reared on wheat plants under three photoperiods at 23°C failed to pupate and most died before day 50 after hatching (Fig. 1). Although a few insects of the trivoltine strain (Aichi, 35°N) reached the pupal stage under these conditions, the survival rate at day 50 after hatching was very low (Fig. 2). These data suggest that wheat plants are not suitable for non-diapause larvae.

The survival rate of diapause larvae of both strains on wheat plants under short day (LD12:12) was higher at 10°C than at 20°C (Figs. 3 and 4). Non-diapause larvae of both strains also showed considerably high survival rates at 10°C. Thus, low temperature exerts a direct effect on survival by reducing the metabolic requirements of *C. oryzae*.

In many species of temperate insects, a period of low temperatures allows diapause development to be completed, so that development resumes when temperature rises in spring (Tauber et al., 1986; Danks, 1987). The survival rates in the diapause larvae of both strains reared on wheat plants decreased earlier at 20 and 15°C than at 10°C (Figs. 3 and 4). It is difficult to determine whether the mortality occurred in diapausing larvae or diapause-completed larvae. However, the resumption of development after transfer to growth-permitting conditions can be used as an index of diapause completion (Danks, 1987). Non-diapause first instars of both strains kept at 10°C resumed development as soon as they were transferred to rice seedlings at 20°C. This indicates that the development of non-diapause first instars was inhibited by the low temperature. On the other hand, diapausing larvae were characterized by delayed development on transfer to rice seed-

lings at 20°C. In the Akita strain, however, 50% of diapause larvae resumed development immediately after transfer to rice seedlings at 20°C after 60 to 80 days at 10°C (Fig. 3C). Although the duration of diapause on wheat plants at 20°C could not be determined in this study, diapause larvae of the Akita strain reared on rice seedlings reached the second stage after 80–90 (including 7 days to resume development) days under LD12:12 at 20°C (Takeda, 1996). It appears that low temperature is not indispensable for the completion of diapause in the Akita strain, although the rate of diapause development was lower at 15°C than at 10°C. Further support is furnished by the results obtained for the Aichi (trivoltine) strain: diapause was terminated in 40 to 50 days when larvae were reared on wheat plants at the three different temperatures (Fig. 4C). Thus, diapause development in the Aichi strain is completed regardless of temperature.

In the present study, to understand the effect of diapause development on survival and pupation in *C. oryzae* reared on wheat plants, diapause larvae were kept at a low temperature (10°C) for various periods, then transferred to wheat seedlings and reared for 35 days under LD15:9 at 23°C. The survival rates and percentage of pupation in the Akita (bivoltine) strain increased with increases in the period spent at the low temperature, and about 40% of Akita (bivoltine) larvae reached the pupal stage after 80 days of rearing at 10°C (Fig. 5). This suggests that the survival rate of the bivoltine strain on wheat plants increased with the progress of diapause development. In contrast, 40 days of low temperature was sufficient for the completion of larval development on wheat plants in the Aichi (trivoltine) strain. This difference in the low temperature requirement for pupation on wheat plants between the two strains corresponds to the difference in the duration of diapause development between them. Thus, after the completion of diapause, larvae could grow to the pupal stage even on wheat plants. Coinciding with these results, the larvae collected from the bivoltine area in March could pupate on wheat plants (Fig. 6). For *C. oryzae*, winter diapause should end at or shortly after the winter solstice (Takeda, 1996). However, the

survival rate and percentage of pupation at 23°C from January-collected bivoltine larvae were significantly lower than those from March-collected larvae (Fig. 6). This indicates that diapause development was not as progressed in January as in March.

When reared on rice plants, the average duration to pupation in April-collected larvae from the bivoltine area was not affected by photoperiod (Table 1). This suggests that post-diapause development on the summer host plants was not influenced by photoperiod, as reported previously (Takeda and Nagata, 1992). Although the survival rate was much lower and the duration of the larval stage was longer when April-collected larvae were reared on wheat plants than on rice plants, some larvae pupated on the winter host at 23°C.

The results of the present study showed that the survival on wheat plants was higher in the trivoltine strain than in the bivoltine one. The cause of this difference between the two geographic ecotypes is not clear, but it may be related to the different thermal conditions in their habitats. Adults of the last brood in the bivoltine and trivoltine ecotypes lay eggs in early September and late September, respectively. The optimum temperature for germination of winter hosts ranges from 15 to 20°C (Arai, 1961), and the average temperature drops to 20°C in early September in Akita (39°N) and late September in Aichi (35°N), and further to 15°C in Akita 20 days later and in Aichi 30 days later. Thus, overwintering larvae encounter low temperatures earlier in the bivoltine area than in the trivoltine area. Furthermore, the bivoltine strain completes development after feeding on young panicles of winter hosts, but most larvae of the trivoltine strain reach the third larval stage before feeding on young panicles (Iwata, 1963). The rearing procedures in the present study are more suitable for the trivoltine strain than for the bivoltine strain.

Variation in resistance of rice plant cultivars to the rice stem maggot is well-known, and most larvae die in the early larval stage on highly resistant rice varieties (Yushima and Tomisawa, 1957; Takeda and Suzuki, 1986). Kishino (1960) also reported that the survival of the trivoltine strain varied with wheat and barley (winter

hosts) varieties. It is assumed that survival and development of *C. oryzae* vary with species and varieties of winter hosts. Although only one variety of wheat plant was used as a winter host in this study, the results clearly show that low temperatures in fall and winter promote survival of *C. oryzae* on winter hosts, but are not indispensable for the completion of diapause development. Furthermore, the survival rate to the pupal stage on wheat plants was higher in larvae after completion of diapause development (or overwintering) than in non-diapause larvae.

ACKNOWLEDGEMENTS

We gratefully thank Dr. Sinzo Masaki for his critical review and improvement of the manuscript. We also thank Dr. Kaoru Maeto, Forestry and Forest Products Research Institute, for identification of the parasitoid specimens. Thanks are due to Dr. Seiji Ito, Tohoku National Agricultural Experiment Station, for providing the wheat seeds. We are indebted to Mrs. Junko Kusanagi for assistance with rearing insects.

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