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# Different Developments of Overwintered Larvae of *Monochamus alternatus* (Coleoptera, Cerambycidae) under a Constant Temperature

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**Abstract** The overwintered larvae of *Monochamus alternatus* were collected from dead pine trees in mid-March and placed individually into fresh bolts of *Pinus densiflora*. Thereafter the larvae were incubated for 196 days under constant conditions of 25°C and LD 16 : 8. The 4th (=final) instar larvae which had been in diapause emerged as adults 33–42 days after the beginning of incubation. The 4th instar larvae which had not been in diapause yet molted at least once, but did not emerge as adults during the incubation. The 1st to 3rd instar larvae developed into the diapausing 4th instar by the end of incubation. The overwintered 3rd and young 4th instar larvae skipped diapause during the final instar in our previous observations, whereas the chilled 3rd and young 4th instar larvae entered diapause in the present results. This discrepancy may have been caused by a constant temperature, fresh food, and/or adhesive cloth tape used in this experiment which prevented skipping diapause.

Key words: Cerambycidae; *Monochamus alternatus*; development; diapause; overwintered larvae.

## Introduction

The Japanese pine sawyer *Monochamus alternatus* HOPE is the primary vector of the pine wood nematode *Bursaphelenchus xylophilus* (STEINER et BUHRER) NICKLE in Japan (MAMIYA & IWASAKI, 1972; MORIMOTO & IWASAKI, 1972). This insect has 1 or 2-year life cycle depending on the oviposition time during the adult flight season from June to September (*e.g.*, OKUDA & SHIBATA, 1973; OCHI & KATAGIRI, 1974). It has 4 instars during larval stage in dead trees (MORIMOTO & IWASAKI, 1974) and overwinters as the 1st, 2nd, 3rd, or 4th instar larvae (TOGASHI, 1989 b). When larvae overwinter at the 1st or 2nd instars, they become adults in the following year (TOGASHI, 1989 a). Overwintered 3rd and 4th instar larvae develop into adults in the current year. *M. alternatus* has a diapause at the 4th larval instar, in which the larva becomes yellow (*e.g.*, KIMURA, 1974). Low temperature terminates the diapause (KIMURA, 1974).

TOGASHI (1989 a) developed a hypothesis on the regulation of the life cycle of this insect. Larvae overwintering as 1st or 2nd instars enter diapause at the 4th instar after overwintering. This results in a 2-year life cycle because they respond to the physical conditions of the 2nd winter. Larvae overwintering as 3rd or young 4th instars have not entered diapause yet. They skip diapause after overwintering due to the low temperatures of winter, resulting in a 1-year life cycle. When 4th

instar larvae are in diapause before winter, they terminate the diapause during the winter, resulting in a 1-year life cycle.

In this article an experiment was conducted to make clear the relationship between instars of overwintered larvae and developmental patterns following overwintering when larvae were held at a constant temperature.

## Materials and Methods

Dead trees of *Pinus thunbergii* PARL. and *P. densiflora* SIEB. et ZUCC., which had been killed in 1988, were felled and cut into logs of 1.5 m long on September 13 and 14, and November 10, 1988 in Shika, Ishikawa Prefecture, and transported to the Ishikawa Forest Experiment Station at Tsurugi, Ishikawa. The logs were placed in outdoor cages to keep at the natural climatic conditions.

The larvae were collected from the logs on March 11-15, 1989. Head capsule widths and larval weights were measured. Records were also kept of the larval color, the presence or absence of food within the intestine, and the location of larvae within the logs. Larval instars were determined by head capsule width (KOJIMA & KATAGIRI, 1964; OCHI, 1975): 0.585-1.170 mm, 1.260-1.935 mm, 1.980-3.150 mm, and 3.150-4.365 mm for 1st, 2nd, 3rd, and 4th instars, respectively.

The larvae were individually placed into *P. densiflora* bolts of 20 cm long with an average diameter of 7.0 cm (S.D.=1.3 cm) on the day they were collected. These bolts had been stored at 3°C from the day of felling (Aug. 4, 1988) through the day before larval inoculation. The bolts were placed on a tray with water at 10°C during a day before larval inoculation. The method of placing a larva within a bolt was as follows. A 6-27 $cm^2$ square of outer bark with inner bark attached was removed from each bolt. A larva was placed onto an artificial depression on the xylem surface made with chisel then covered with the bark which was fastened with adhesive cloth tape. The bolts were placed in small cages (20 cm × 20 cm × 30 cm) on March 15, 1989 and held at constant conditions of 25°C, 85-95% R.H., and LD 16 : 8. Daily observations were made to collect emerging adults.

The pine bolts were dissected 196 days (Sept. 27, 1989) after the start of incubation. Larval measurements mentioned above were made on all living larvae. Dead larvae were noted as well.

Live larvae recovered at the end of incubation were placed again into pupal chambers which they made within the xylem of the pine bolts. Five to six larvae were stored under one of five different temperature regimes; constant temperatures of 0, 5, 10, and 15°C, and a decreasing temperature from 15 to 0°C by letting ambient temperature lower 5°C every 15 days under a constant photoperiod of LD 12 : 12. After 60 days (Nov. 26, 1989), larvae were transferred to 25°C, 85-95% R.H., and LD 16 : 8, and their emergence was monitored for 80 days until February 15, 1990. Three larvae were not chilled and served as a control group.

## Results

The 1st to 4th instar larvae were collected from dead pine trees in mid-March, and were designated as L1, L2, L3, and L4 groups, respectively. Seventeen percent of them had already resumed feeding.

No adults appeared from the L1, L2, and L3 groups during the incubation of 196 days (Table 1). This was also true for the 3rd instar larvae which had constructed pupal chambers within xylem. Three females and three males emerged from the L4 group as adults 33–42 days (36 days on average) after the start of incubation. They did not construct pupal chambers in the xylem and did not feed on inner bark during their incubation, as no fecal material was found in the artificial depression. Their body was yellowish white or whitish yellow at the start of incubation. On the contrary, the milky white 4th instar larvae did not emerge as adults during the incubation of 196 days even if they were in pupal chambers within the xylem of the pine logs at sampling time. The larval head capsule width and mass of the L4's that emerged during incubation averaged 3.5 mm ($n=6$, S.D.$=0.3$ mm) and 356 mg ($n=6$, S.D.$=87$ mg) at the beginning of incubation. Mean values for the individuals of the L4 group which remained in larval stage during the incubation averaged 3.4 mm ($n=8$, S.D.$=0.1$ mm) and 354 mg ($n=8$, S.D.$=77$ mg). There was no significant difference in head capsule width and mass between emerged and non-emerged individuals ($t$-test, $0.2<P$).

The dissection of bolts after incubation indicated that 4 larvae of L3 group died with a little or no feeding, and that a larva of L4 group died under bark after considerable feeding on inner bark. The remaining larvae were alive.

Individuals of the L1, L2, and L3 groups developed into the 4th instar and their body became yellow without any food in the intestine, indicating that they were in diapause (Table 1). All of them constructed pupal chambers in xylem. This was true for two 3rd instar larvae which had completed their pupal chambers in the xylem by the time of sampling. Individuals in the L4 group which did not emerge also made pupal chambers in xylem. Their body became yellow except for a whitish yellow larva. Interestingly, their head capsule width and mass increased during the incubation of 196 days; from 3.4 to 4.0 mm for average head capsule width and from 354 to 838 mg for average mass (Table 2). These increases were confirmed for each individual. This fact indicated that the larvae judged as the final (=4th) instar from head capsule width molted at least once during the incubation.

Table 2 shows the average head capsule width and mass of each larval group before and after the incubation of 196 days. There was no significant difference in head capsule width and mass among 3 larval groups of L2, L3, and L4 at the end of incubation (one-way ANOVA, $0.1<P$).

Individuals within pupal chambers were alive as larvae after the incubation of 80 days following chilling of 60 days: such chilling at 5 kinds of temperature

Table 1. Development of overwintered *Monochamus alternatus* larvae of different instars during a 196 day incubation under constant conditions of 25°C and LD 16 : 8.

| Beginning of incubation (March 15, 1989) | | | | | End of incubation (September 27, 1989) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Larval instar | Body color[a] | Location[b] | No. of used[c] | mass (mg) (mean±SD[d]) | No. of adults[e] | No. of dead[f] | No. of alive[c][f] | Larval instar[g] | Body color[a][g] | Location[b] | mass[g][h] (mg) (mean±SD[d]) |
| First | MW | A | 1 (0) | 9 | 0 | 0 | 1 (0) | Fourth | Y | E | 933 |
| Second | MW | A | 11 (1) | 27±10 | 0 | 0 | 11 (0) | Fourth | Y | D, E | 782±277 (10) |
| Third | MW | A | 11 (3) | 169±105 | 0 | 3 | 8 (0) | Fourth | Y | D, E | 744±137 (6) |
| | MW | B | 1 (1) | 63 | 0 | 0 | 1 (0) | Fourth | Y | E | 1,150 (1) |
| | MW | D | 2 (0) | 158±76 | 0 | 0 | 2 (0) | Fourth | Y | D, E | 550±307 (2) |
| | YW | D | 1 (0) | 180 | 0 | 1 | 0 (0) | — | — | — | — |
| Fourth | MW | A | 2 (1) | 306±57 | 0 | 0 | 2 (0) | Fifth? | WY, Y | E | 820±10 (2) |
| | MW | C | 3 (1) | 315±115 | 0 | 1 | 2 (0) | Fifth? | Y | E | 724±117 (2) |
| | MW | D | 2 (0) | 305±55 | 0 | 0 | 2 (0) | Fifth? | Y | D | 850±392 (2) |
| | MW | E | 1 (0) | 452 | 0 | 0 | 1 (0) | Fifth? | Y | D | 1,164 (1) |
| | YW | A | 1 (0) | 297 | 1 | 0 | 0 (0) | — | — | — | — |
| | YW | D | 1 (0) | 408 | 1 | 0 | 0 (0) | — | — | — | — |
| | YW | E | 1 (0) | 424 | 0 | 0 | 1 (0) | Fifth? | Y | E | 756 (1) |
| | WY | D | 3 (0) | 400±68 | 3 | 0 | 0 (0) | — | — | — | — |
| | WY | E | 1 (0) | 228 | 1 | 0 | 0 (0) | — | — | — | — |

[a] MW=milky white, YW=yellowish white, WY=whitish yellow, Y=yellow. [b] A=under bark, B=in straight tunnel within xylem, C=in curved tunnel within xylem, D, E=in pupal chambers with short and long plugs, respectively, within xylem. For further explanation, see TOGASHI (1989b). [c] The numeral in parentheses indicates the number of larvae with food in intestine. [d] SD=standard deviation. [e] The adults emerged during the incubation. [f] They were larvae. [g] Live larvae. [h] The numeral in parentheses indicates the number of larvae examined.

Table 2. *Monochamus alternatus* larval head capsule widths and masses before and after a 196 day incubation following overwintering.

| Beginning of incubation (March 15, 1989) | | | End of incubation (September 27, 1989) | |
|---|---|---|---|---|
| Larval instar | Head capsule width[a] (mm) (mean ± SD[b]) | Mass[a] (mg) (mean ± SD[b]) | Head capsule width[a] (mm) (mean ± SD[b]) | Mass[a] (mg) (mean ± SD[b]) |
| First | 1.1 (1) | 9 (1) | 3.9 (1) | 933 (1) |
| Second | 1.6±0.2 (11) | 27±10 (11) | 3.9±0.4 (11) | 782±277 (10) |
| Third | 2.6±0.3 (11) | 148±105 (11) | 3.7±0.3 (11) | 746±231 (9) |
| Fourth | 3.4±0.1 (8) | 354±77 (8) | 4.0±0.2 (8) | 838±210 (8) |

The data contain no individuals emerging as adults during the incubation. [a] The numeral in parentheses is the number of larvae examined. [b] SD = standard deviation.

regimes could not terminate the diapause of the 4th and possibly 5th instar larvae. The same was found for larvae which were not chilled.

## Discussion

The individuals overwintering as 1st, 2nd, and 3rd instar larvae did not develop into adults, but developed to the diapausing final instar larvae, when they were kept at a constant temperature of 25°C after the hibernation.

Some 4th instar larvae emerged as adults about one month after the start of incubation. Others remained in larval stage during a 196 day incubation. Fourth instar larvae that exhibited different developmental patterns under a constant temperature, were also different in body color. Individuals which emerged as adults had been yellowish white or whitish yellow, while the non-emerging ones had been milky white. There was no difference in head capsule width, mass, or location within pine logs between the groups.

Larvae are yellow when they are in diapause during the 4th instar (*e.g.*, KIMURA, 1974). The results of this experiment showed that diapausing 4th instar larvae terminated diapause by mid-March. Larvae which had not undergone diapause, entered diapause when kept at 25°C after hibernation.

A previous study (TOGASHI, 1989 a) suggested that the overwintered 3rd and 4th instar larvae developed into adults in June or July of the current year when held under natural conditions. Overwintered 1st and 2nd instar larvae entered diapause during the 4th instar, resulting in the adult emergence in the following year (TOGASHI, 1989 a). This differed from the results of the current experiment. This discrepancy may have been due to the difference in the ambient temperature and food quality between the natural and experimental conditions, although an effect of adhesive cloth tape was also conceivable. Under natural conditions, the temperature changed daily. Furthermore, the inner bark (food for the 3rd and 4th instar larvae) was no longer fresh when the larvae resumed feeding in spring. Food quality is considered to be important because non-diapausing, overwintered

larvae feed on the inner bark after overwintering. Thus, to understand the regulation of the *M. alternatus* life cycle, research on the effect of temperature and food quality on the development of non-diapausing, overwintered 3rd and 4th instar larvae is needed.

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