# Assessment of Artificial Rearing Method for the Pine Sawyer Beetle, *Monochamus alternatus* (Coleoptera: Cerambycidae)

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#### Introduction

Japanese pine forests are threatened by the Japanese pine sawyer (*Monochamus alternatus* HOPE,) the vector of pine wood nematode (*Bursaphelenchus xylophilus*) which causes extensive pine mortality (Kiyohara and Tokushige, 1971; Mamiya and Enda, 1972; Morimoto and Iwasaki, 1972). Due to the nature and biology of this insect, (i. e. it spends most of its life cycle in the wood) there is a need to rear this insect in artificial diets to study its behaviour, physiology, bio-chemistry and the use of control measures.

The present study was carried out to investigate the most suitable artificial diet for rearing nematode free beetles and to determine nutritional requirements for developing a mass rearing technique by which pine sawyer beetle could be reared from larvae to adults without need for periodic care.

Entomologists need laboratory reared insects of uniformly high quality for applied and basic research. There are relatively few studies on methods of rearing Monochamus species in particular. This could be due to insect's feeding behaviour (Singh and Moore, 1985). Studies on Cerambycidae were in most cases either on chemical diet or plant tissues alone (Emori, 1986; Akutsu et al. 1980; Murakoshi et al. 1981; and Gardiner, 1970). Yamane (1974 and Yamane (1973) studied on larval mortality only. On the other hand Enda and Kitajima (1990). Kosaka and Ogura (1990) conducted studies on *Monochamus alternatus*, although survival rate were good they did not compare artificially reared with the wild beetles. Ito, (1982) compared his reared insect's body length, body width and pre-wing length with the wild ones. It could also be said that lack of suitable rearing techniques has hampered research on the biology and control of many species of wood boring insects injurious to forest and shade trees. The advantage of artificial diet over natural food stuffs is that rearing is generally easier and life histories and behaviour can be precisely studied with less effort.

#### **Materials and Methods**

Eleven different artificial diets were investigated (Table 1) They were grouped into two categories: Chemical and natural diets.

#### Chemical diets.

Chemical diets were prepared after Singh (1983); Galford (1969) and Gardiner (1970) with some modifications. The composition of each diet is shown in Table 2 and 3.

# Diet 1.

Mould inhibitor and vitamin mixtures were first prepared separately. Ingredients in (a) were mixed for 15 minutes in a flask using a magnetic stirrer and the contents were stored in the refrigerator. Ingredients in (b) were prepared similarly. Items in group 1 were well

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Table	1	Artificial	diets tested	d

Diet	Description	Diet	Description
1	Chemical diet 1	7	Xylem diet
2	Chemical diet 2	8	Ground tissue diet
3	Chemical diet 3	9	Silkworm diet
4	Bark diet	10	10 cm * 10 cm log diet
5	Leaves diet	11	50 cm log diet
6	Sapwood diet	12**	Natural diet

<sup>12\*\* 4</sup>th instar larvae collected from the forest received the same treatment as those reared on artificial diet.

Table 2. Composition of Chemical diet 1

Ingredients	Quantity	Ingredients	Quantity
Group 1	(g)	(a) Mould inhibitor	(g)
Agar powder	15.0	Methy-parahydroxybenzoate	0.7941
Caseine (from milk)	21.0	Sorbic acid	1.0588
Cellulose powder	60.0	95% alcohol	9 m <i>l</i>
Wesson's salt	6.0		10.8529
Wheat germ	18.0		
Group 2		(b) Vitamin mixture	(mg)
Cholesterol	0.3	Niacin	500
Linolic acid	1.5	Riboflavin	250
Dichloromethane (evaporate)	30 m <i>l</i>	Folic acid	125
Group 3		Biotin	10
Distilled water	390 m <i>l</i>	Calcium pentothanate	500
Potassium hydroxide 4M	3.0m $l$	Thiamine hydrochoride	125
Group 4		Vitamin B <sub>12</sub>	1
Vitamin mixture	12.0	Distilled water	500 m <i>l</i>
Sucrose	18.0		2011
Dextrose	3.0		
Distilled water	$43.02 \; \mathrm{m}l$		
Streptomycin sulphate (BP)	0.09		
Benzlpenicillin (sodium)	0.09		
Group 5			
Mould inhibitor	9 m <i>l</i>		
Plant material (cambium)	150.0		
	750.0		

mixed with items in group 2. Dichloromethane was used to dissolve cholesterol and linolic acid so that they could mix well with other items in group 1. Group 1 and 2 were then mixed with group 3. The mixture was blended thoroughly and autoclaved at 125°C for 20 minutes. Group 4 and 5 were then mixed together and added to the warm autoclaved mix. Finally cambium tissue was chopped into small pieces, frozen and blended by mixer and then added by hand. The diet was then poured into a copper container to cool. Twenty five rearing petri dishes were also autoclaved for 20 minutes at 125°C. Each petri dish was filled with 25 g of the artificial diet ready for inoculation.

#### Diet 2.

Agar and sugar were boiled in distilled water by steam. Sorbic acid and methyl

Table 3. Composition of Chemical diets 2 and 3

Chemical diet 2		Chemical diet 3		
Ingredients	Quantity	Ingredients	Quantity	
	(g)		(g)	
Agar	26.88	Distilled water	132.0	
Sucrose	13.44	Vitamin free catsein	21.0	
Levulose	6.72	Potassium hydroxide 4M	3 m <i>l</i>	
Dextrosehydrous	6.72	Cellulose powder	3.0	
Vitamin mix	10.08	Wesson's salt mixture	6.0	
Dry yeast	33.60	Sucrose	21.0	
Soy bean protein	13.44	Wheat germ	18.0	
Wesson's salt mix	16.80	Choline chloride	0.6	
Cholesterol	0.67	Ascobic acid	2.4	
Wheat germ	16.80	Nutrient agar 4%w/v	384.0	
Vitamin B <sub>12</sub>	0.067	Vitamin mixture	6.0	
Sorbic acid	1.68			
Methyl-parahydroxybenzoate	0.84	Microbial agent:		
Cellulose powder	266.80	Formalin (37%formaldehyde)	0.3	
Wheat germ oil	3.36	Parahydroxybenzoate	0.9	
Water	672 m <i>l</i>	Chlorotetracycline	0.18	
Plant tissue (cambium)	218.38	Plant tissue (cambium)	150.0	
	$1\overline{362.40}$		748.38	

parahydroxybenzoate were dissolved in 95% alcohol and added to an agar/sugar solution, and further boiled until the alcohol evaporated. The solution was then hand blended with the remaining ingredients. Petri dishes and diet were autoclaved as above, and 25 g of diet supplied per dish.

#### Diet 3.

Ingredients were hand blended except for nutrient agar which was first dissolved in cold water and liquified over steam while adding cambium tissue. Petri dishes and diet were autoclaved as above, and 25 g of diet supplied per dish.

#### Natural diets.

Bark diet, leaf diet, sapwood diet, xylem diet, ground tissue diet and silkworm diet (Silk mate 3M) were prepared. 700 g of silkworm diet, 7 g dried yeast, and 525 ml distilled water were added to each of the plant tissue mentioned above. Table 4 shows the composition of

Table 4. Composition of the natural diets

	Ingredients						
Diet/name	Silkorm diet (g)	Dried yeast (g)	Distilled water (ml)	Plant tissue (g)			
4 Bark	700.0	7.0	525.0	350.0			
5 Needles	700.0	7.0	525.0	350.0			
6 Sapwood	700.0	7.0	525.5	350.0			
7 Xylem	700.0	7.0	525.5	350.0			
8 Ground tissue	700.0	7.0	525.0	350.0			
9 Silkworm diet	700.0	7.0	525.0				

natural diets.

#### **Diets 4-8.**

Frozen plant tissue from red pine tree (Pinus densiflora) were chopped into small pieces, blended by mixer and then by hand. Each treatment comprised of 25 petri dishes, each filled with 60 g of the diet, compressed to half the weight of the dish and autoclaved for 20 minutes at 125°C prior to inoculation. Plant tissue was not added into silkworm diet (diet 9).

#### Diet 10: (Log diet).

Twenty year old red pine trees in Tanashi forest were felled and crosscut into 10 cm long logs with a diameter of about 10 cm. To prevent evaporation both ends of the log were treated with molten wax and then placed into a covered glass jar prior to inoculation.

# Diet 11: (50 cm log).

One of the 50 cm long logs used during oviposition was left as a treatment to investigate the effect of non disturbed insects on growth and development. Only 25 visible oviposition scars were left on the log. However, the exact number of eggs laid was not known.

## Inoculation and rearing

The laboratory reared stock originated from two sources: Larvae of. *M. alternatus* collected from the University experimental station at Tanashi, and larvae of *M. alternatus* obtained from the Entomology section of the Forestry and Forest Products Research Institute in Tsukuba, Japan.

A small hole was made into the diet or the log, and second instar larvae transferred into the diet/log with their head pointing downwards, one larva per petri dish/log. Petri dishes were sealed with parafilm (Trade name) to prevent the entry of micro organisms. Petri

Table 5. Effects of artificial diets and temperature on growth and development of *M. alternatus* larva

Diet No.	Survival rate (%) 2nd-4thinstar		Head capsule	e width (mm)	Larval weight (g)	
	R	С	R ±SD	C ±SD	R ±SD	C X±SD
1	28	36	$3.29 \pm 0.20$	3.38±0.93	0.445±0.21	0.585±0.37
2	28	48	$3.35 \pm 0.30$	$3.98 \pm 0.27$	$0.883 \pm 0.19$	$0.881 \pm 0.13$
3	28	40	$3.40 \pm 0.25$	$3.63 \pm 0.45$	$0.438 \pm 0.15$	$0.340 \pm 0.24$
4	76	100	$4.22 \pm 0.30$	$4.17 \pm 0.34$	$1.275 \pm 0.29$	0.340 - 0.24
5	72	96	$3.51 \pm 0.22$	$3.67 \pm 0.28$	$0.794 \!\pm\! 0.21$	$0.977 \pm 0.26$
6	16	40	$3.34 \pm 0.35$	$3.06 \pm 0.42$	$0.386 \pm 0.13$	$0.357 \pm 0.18$
7	32	36	$3.47 \pm 0.17$	$3.04 \pm 0.48$	$0.467 \pm 0.13$	0.584
8	28	60	$3.44 \pm 0.10$	$3.29 \pm 0.33$	$0.732 \pm 0.17$	$0.638 \pm 0.31$
9	28	8	$3.27\!\pm\!0.25$	$2.40 \pm 0.20$	$0.547 \pm 0.16$	$0.331 \pm 0.22$
10	100	92	$3.47\!\pm\!0.29$	$3.72 \pm 0.27$	$0.810 \pm 0.19$	$0.987 \pm 0.28$
11	44	40	$3.65 \pm 0.16$	$3.62 \pm 0.16$	$0.711 \pm 0.21$	$0.652 \pm 0.21$
12**	100		$3.92 \pm 0.30$		$0.708 \pm 0.14$	_

R: Roon temp., C: Controlled temp.

dishes/logs were labelled and divided into two groups; controlled rearing conditions ( $25\pm1^{\circ}$ C, RH 50-60%, LD 16:18) and room temperature conditions. Each treatment contained 25 insects.

Insects were observed every week after inoculation for 3 months and daily after hibernation through to emergence.

After 95 days larvae were transferred into clean petri dishes containing moist blotting paper, weighed, larval head capsule width measured, and then placed into a rearing chamber at 10°C in total darkness for 127 days to hibernate. Temperature was then raised to 25°C under these conditions. Number of days from 4th instar larva to pupa and pupal weight were recorded. Adults were raised under the same conditions as pupae. At two days after emergence, adult weight, wing length and sex were recorded.

Twenty five 4th instar larvae and 25 wild adults from infested trees were collected from the field and the larvae were reared through adults. Growth parameters of those taken in the field were compared to those raised on the artificial diet.

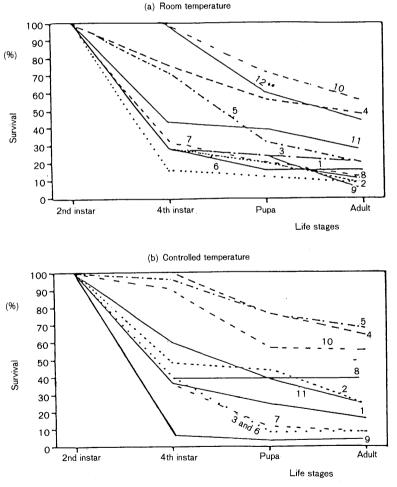


Fig. 1. Survival curves of *M. alternatus* reared on artificial diets and temperature regimes (Number represents diets; for adult survival rate refer Table 7).

Table 6.	Effects of artificial	diets and temperature on growth and development of M. alternatus	
	pupa		

Diet No.	Survival rate (%) 4th-pupa		Pupal weight (g)		Pupation (%)	
	R	С	R ±SD	C X±SD	R	С
1	16	24	0.382±0.38	$0.444 \pm 0.13$	57	67
2	20	44	$0.337 \pm 0.04$	$0.548 \pm 0.16$	71	92
3	24	12	$0.357 \pm 0.06$	$0.442 \pm 0.11$	86	30
4	56	76	$0.707 \pm 0.23$	$0.997\!\pm\!0.19$	74	76
5	32	76	$0.530 \pm 0.21$	$0.726\!\pm\!0.17$	44	79
6	12	8	$0.248\!\pm\!0.03$	$0.430 \pm 0.03$	75	20
7	20	12	$0.259\!\pm\!0.14$	$0.486 \pm 0.08$	62	37
8	20	40	$0.562 \pm 0.20$	$0.474 \pm 0.27$	71	67
9	24	4	$0.370 \pm 0.13$	0.496	86	50
10	72	68	$0.694 \pm 0.17$	$0.777 \pm 0.14$	72	74
11	40	40	$0.568 \pm 0.20$	$0.500 \pm 0.14$	90	100
12**	60		$0.700 \pm 0.12$	_	60	_

R: Room temp., C: Controlled temp.

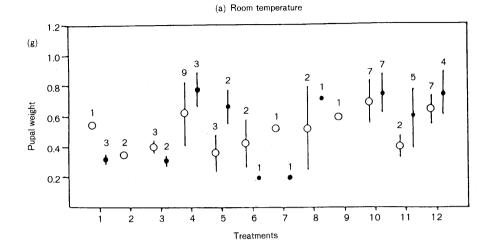
#### Results

Larval growth and development of the Japanese pine sawyer beetles (*M. alternatus*) on the diets and two temperature regimes were evaluated using Duncan multiple comparison test. Fourth instar larvae were determined by head capsule width following the method of Kojima & Katagiri (1964). Larvae were considered to be in a diapause when they were yellowish-white or yellow in colour and had no food in their gut. The rating system used the following data:—4th instar head capsule width and weight, percentage pupation, percentage pupal yield, pupal weight, percentage adult emergence, percentage normal adult, adult weight, adult wing length, days to pupation, days to eclosion, days from second 2nd instar larvae to adult and from pupa to adult. However, the most important criteria of the value of larval diet are the yield and quality of adult produced. The latter was used as the basis for final evaluation of the diets in this study. The relationship between different growth and development parameters was also noted.

Most larvae pupated within the first 20 days following hibernation and emergence was completed after 302 days. Artificial diet had a significant influence on survival percentage of larvae, pupae and adults while temperature had no significant effect on survivals. Table 5 shows that diet 10R(diet 10 room temp.) and diets 4C, 5C, and 10C (diet 4, diet 5, and diet 10 controlled temp. respectively) are relatively efficient for supporting larval growth and development. Mortality was higher among larvae at room temperature than in controlled temperature treatments. The highest larval mortality (92%) was observed in diet 9C and (84%) in diet 6R.

The yield of adults is usually determined by the yield of pupae and by the number of healthy adults emerging from puparia. Least pupal mortality was observed in diets 4C, 5 C, and 10 of both room & controlled temperature treatments (Table 6). Survival curves showed that mortality was highest during the second larval instar and relatively low during the pupal stage (Fig. 1).

There was a significant difference in larval head capsule width between diets, and the



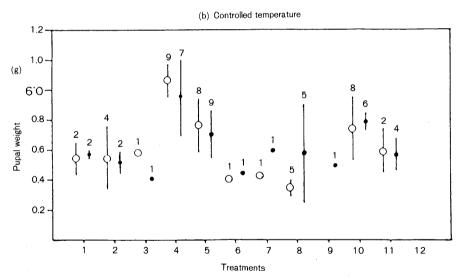
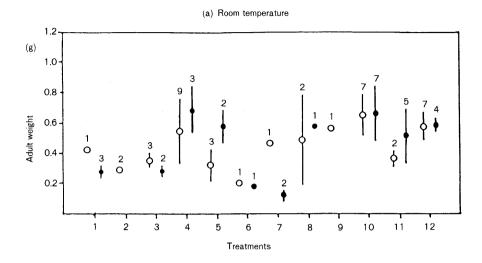


Fig. 2. Female (○) and male (●) pupal weight of *M. alternatus* reared on artificial diets and two temperature regimes (No. indicates sample size).

interaction of diet and temperature. Temperature was found not to have any influence on head capsule size development. Fourth instar larvae which fed on the bark diet had the largest head capsule width  $(4.22\pm0.30~\text{mm})$  and  $4.17\pm0.34~\text{mm}$  (Table 5).

Both artificial diet and temperature had a significant influence on the weights of larvae, pupae and adults. An interaction effect of the two factors was observed on larval and pupal weight. Table 5 shows that average weight of the 4th larval instar in diet 4 of both room  $(1.275\pm0.29~\mathrm{g})$  and controlled  $(1.259\pm0.28~\mathrm{g})$  temperature treatments was higher than in other diets. Controlled temperature treatments had a higher overall mean pupal weight value than room temperature treatments (Table 6).

Figure 2 shows that mean pupal weight of females and males reared under controlled



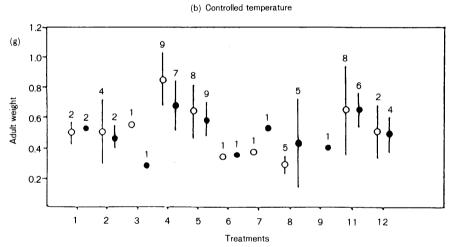


Fig. 3. Effect of artificial diet and temperature on adult female (○) and male (●) weight of *M. alternatus* larvae reared on artificial diet (No. indicate sample size).

temperature were higher than those reared under room temperature. Pupae from larvae reared in diets 4, 10C and 5C were slightly larger than those collected from the forest (Table 6).

Adult weight was measured two days after emergence. Table 7 shows that the highest adult weights were recorded in diets 4C ( $0.78\pm0.20g$ ). Average weight of beetles collected from the forest under natural condition  $12^{**}$  ( $12^{**}$  Larvae collected from the forest) was slightly lower (0.407+0.08g) but showed less variation. Controlled temperature treatments seem to be efficient in rearing M. alternatus larvae to adult. Differences in weight of adults resulting from different diets and temperatures were more pronounced among females than males (Fig. 3).

Artificial diet and the interaction between the diet and temperature had a significant

Table 7.	Effects of artificial diets and temperature on growth and development of the adult $M$ .
	alternatus beetle

	Survial rate (%)		Adult w	eight (g)	Adult wing length (mm)	
Diet No.	R	С	$_{ar{ ext{X}}\pm ext{SD}}^{ ext{R}}$	$\overset{C}{ar{\mathtt{X}}\pm\mathtt{SD}}$	R X±SD	C X±SD
1	16	16	$0.322 \pm 0.07$	0.513±0.06	13.15±0.82	14.30±0.47
2	8	24	$0.296 \pm 0.02$	$0.500 \pm 0.20$	$13.25 \pm 1.15$	$15.11 \pm 2.24$
3	20	8	$0.357 \pm 0.06$	$0.422 \pm 0.13$	$13.76 \pm 1.63$	$13.80 \pm 1.60$
4	48	64	$0.557 \!\pm\! 0.21$	$0.780 \pm 0.20$	$16.06 \pm 2.00$	$18.06 \pm 2.07$
5	20	68	$0.422 \pm 0.18$	$0.613 \pm 0.15$	$14.84 \pm 2.17$	$14.80 \pm 3.60$
6	8	8	$0.198 \pm 0.01$	$0.356 \!\pm\! 0.002$	$11.35 \pm 1.15$	$14.50 \pm 0.50$
7	12	8	$0.246 \pm 0.16$	$0.452 \!\pm\! 0.08$	$12.43 \pm 3.40$	$15.80 \pm 0.20$
8	12	44	$0.515 \pm 0.25$	$0.350 \pm 0.16$	$15.32 \pm 2.60$	$11.33 \pm 2.87$
9	4	4	0.576	0.471	16.80	12.30
10	56	56	$0.651 \pm 0.17$	$0.645 \pm 0.18$	$16.81 \pm 1.74$	$16.47 \pm 4.74$
11	28	24	$0.74 \pm 0.17$	$0.491 \pm 0.16$	$15.51 \pm 2.03$	$15.51 \pm 0.97$
12**	44	_	$0.618 \pm 0.13$		$16.57 \pm 1.23$	_
12N	25insects		$0.407\pm0.08$	_	17.04±1.44	<del></del> .

NB. 12N = Beetles colleted from the forest (wild beetles).

R: Room temp., C: Controlled temp.

effect on adult wing development. Adults collected from the forest two days after emergence had shorter wing lengths compared to those reared from diet 4C (17.05 $\pm$ 1.44 mm, Table 7). Females had longer wings than males. Adults from larvae collected from the forest and adults collected from the forest also showed a similar result (Fig. 4).

Artificial diet and temperature had no significant effect on larval developmental period. Comparison between duration of larval stages, pupae and adults reared on artificial diet is presented in Table 8.

In general, Larval head capsule width, pupal weight, adult body weight and wing length values of larvae reared under controlled temperature treatments were higher than those obtained from room temperature treatments. Sex ratio ranged from 0.44–0.64.

Experimental results showed that larval body weight was positively correlated to the head capsule width in both room (r=0.64; Y=0.93x+2.90; n=125) and controlled (r=0.47; Y=0.96x+2.83; n=148) temperature treatments. Also a significant correlation was observed between body weight and wing length in the room temperature treatment (r=0.87; Y=10.53x+10.02; n=51) but in controlled temperature treatments the r value was lower (r=0.30; Y=9.40x+9.88; n=81). In larvae collected from the forest a positive correlation was also recorded. Positive correlation was also noted between larval body weight and head capsule width, and between adult weight and wing length both in adults reared from larvae collected from the forest and in the adults collected from the forest two days after emergence.

#### Discussion

When all 13 criteria used to evaluate the diets were considered separately, it was very difficult to decide objectively which was the best diet. Unless one is looking specifically for particular differences, a rating system by which each criterion is given equal weight will measure the overall value of diet. If, for example, one is looking for high fecundity in beetles more emphasis may be given to beetle weight than survival rate. However, Singh

Table 8.	Effects of artificial	diets and	temperature on	developmental	period of. M.	alternatus
	larvae					

Temp. Trt.	Diet No.	Individual No.	Larva-Pupa (days) X±SD	Individual No.	Pupa-Adult (days) X±SD	Individual No.	Larva-Adult (days) X±SD
	1	4	19.5±1.12	4	12.2±3.50	4	253±4.60
	2	5	$22.8 \pm 7.36$	2	$15.0 \pm 4.00$	2	$257 \pm 5.00$
	3	6	$22.7 \pm 6.72$	5	$16.0 \pm 6.34$	5	$261 \pm 9.91$
	4	20	$21.3 \pm 3.60$	12	$15.1 \pm 5.15$	12	$257 \pm 6.75$
	5	8	$21.0 \pm 4.58$	5	$10.6\!\pm\!2.24$	5	$253 \pm 1.41$
(R)	6	3	$38.7 \pm 4.10$	3	$13.0 \pm 0.00$	2	$271 \pm 2.00$
(K)	7	5	$35.4 \pm 5.61$	3	$14.3 \pm 20.5$	3	$272 \pm 5.18$
	8	5	$16.6 \pm 4.22$	3	$18.0 \pm 6.68$	3	$257 \pm 10.4$
	9	6	$23.8 \pm 2.61$	1	14.0	1	260
	10	18	$16.1 \pm 4.66$	14	$14.8 \pm 2.93$	14	$252 \pm 3.68$
	11	10	$25.3 \pm 6.88$	7	$12.7 \pm 3.06$	7	$258 \pm 7.50$
	12**	15	$23.3 \pm 10.0$	11	$13.2 \pm 12.0$	11	$256 \pm 7.92$
	1	4	35.3±7.00	4	16.0±3.50	4	273±6.40
	2	11	$38.2 \pm 4.30$	6	$14.2 \pm 3.20$	6	$273 \pm 3.80$
	3	3	$29.3 \pm 2.50$	2	$16.0 \pm 2.00$	1	266
	4	19	$19.7 \pm 5.20$	16	$17.0 \pm 4.80$	16	$258 \pm 7.4$
	5	19	$18.7 \pm 3.60$	17	$15.1 \pm 4.10$	17	$256 \pm 3.90$
(C)	6	2	$46.0\pm1.00$	2	$11.0 \pm 3.00$	2	$279 \pm 2.00$
	7	3	$14.7 \pm 4.10$	2	$13.5 \pm 5.50$	2	$247 \pm 3.50$
	8	10	$17.0 \pm 2.60$	9	$14.0 \pm 3.40$	9	$252 \pm 2.60$
	9	1	15.0	1	19.0	1	256
	10	17	$20.2\!\pm\!4.70$	14	$13.9 \pm 3.90$	14	$256 \pm 3.30$
	11	10	$19.0 \pm 5.90$	6	$14.3 \pm 3.70$	6	$253 \pm 4.30$

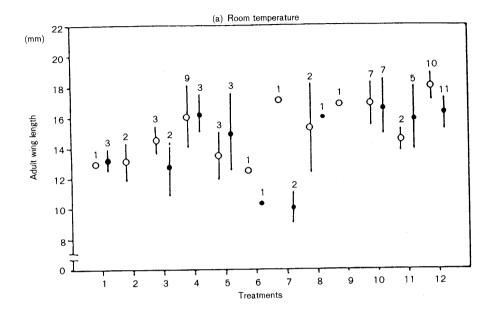
12\*\*: 4th instar larvae collected from the forest received the same treatment as those reared on artificial diet.

R: Room temp., C: Controlled temp.

(1983) reported that one of the qualities of an ideal diet for a mass rearing programme should produce an average yield of adults of at least 75% from initial viable eggs, and the size and rate of development of insects be similar to those in nature.

Rearing temperature had a significant influence on growth and development of *M. alternatus* larvae. Studies on Cerambycidae have shown that insects with life cycles of up to 2 years in nature were reared in artificial media in less than one year (Galford, 1979; Gardiner, 1970). In the Honshu area *M. alternatus* larvae which survive winter low temperatures sometimes take two years to complete the generation (Igarashi, 1976; Kimura, 1974; Takizawa *et al.*, 1979).

Pan and Long (1961) reported that plant tissue diets were slightly more efficient in rearing sugar cane borer than chemical artificial diets. The same was reported by Kieckhefer and Deer (1967). This argument was refuted by Ito (1960) who reported that Yoshida and his coworkers were able to rear silkmoth larvae from hatching through to the middle of the 5th instar, but never to maturity on a diet containing over 50% of mulberry powder starch, sucrose and soy bean. Wongsiri and Radolf (1962) found that mortality of sugar cane borer was higher among larvae reared on host plant tissue than in chemical diets. Addition of host plant tissue to the diet did not further increase the development rate of



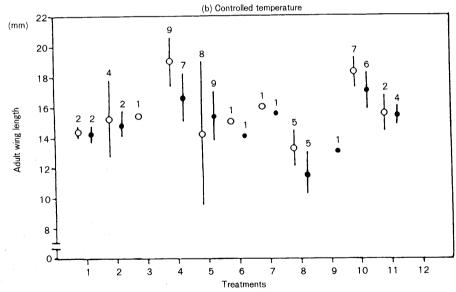


Fig. 4. Effects of artificial diets and temperature on wing develoment of adult M. alternatus female  $(\bigcirc)$  and male  $(\bigcirc)$  (no. indicate sample size).

cerambycidae larvae (Harley and Wilson, 1968). Despite the contradictory results of others shown above, results in the present study showed that inclusion of host plant tissue had a significant influence on larval growth and development of. *M. alternatus*; diet 10 (Log) was the superior diet followed by diets 4 (bark) and 5 (needles) respectively.

Highest adult survival rates are obtained in diet 5C (68%) and diet 10 (56%) in both

5C

10R

10C

12\*\*

12N

collected in the forest and adults collected from the forest 2 days after emergence						
Diet	Adult		Male		Female	
	body weight $\bar{X}\pm SD$ (g)	wing length X±SD (mm)	body weight $\bar{X}\pm SD$ (g)	wing length X±SD (mm)	adult weight $\bar{X}\pm SD$ (g)	wing length X±SD (mm)
4C	$0.78 \pm 0.2$	18.1±2.1	$0.69 \pm 0.2$	16.9±1.6	0.85±0.2	19.0±1.6

(7)

 $15.1 \pm 1.6$ 

(9)

 $16.6 \pm 1.9$ 

(7)

 $17.0 \pm 1.2$ 

(8)

 $16.4 \pm 1.0$ 

(4)

 $16.1 \pm 0.1$ 

(15)

(9)

 $0.64 \pm 0.2$ 

(8)

 $0.65 \pm 0.1$ 

(7)

 $0.64 \pm 0.2$ 

(6)

 $0.59 \pm 0.1$ 

(7)

 $0.41 \pm 0.0$ 

(10)

(9)

 $14.2 \pm 4.0$ 

(8)

 $16.8 \pm 1.3$ 

(7)

 $18.2 \pm 1.0$ 

(6)

 $16.6 \pm 1.3$ 

(7)

 $18.0 \pm 1.2$ 

(10)

(7)

 $0.59 \pm 0.1$ 

(9)

 $0.65 \pm 0.2$ 

(7)

 $0.65 \pm 0.1$ 

(8)

 $0.67 \pm 0.1$ 

(4)

 $0.39 \pm 0.1$ 

(15)

Table 9. Comparison between adults of *M. alternatus* reared on artificial diets from larvae collected in the forest and adults collected from the forest 2 days after emergence

(16)#

 $0.61 \pm 0.1$ 

(17)

 $0.65 \pm 0.2$ 

(14)

 $0.64 \pm 0.2$ 

(14)

 $0.62 \pm 0.1$ 

(11)

 $0.41 \pm 0.1$ 

(16)

 $14.8 \pm 3.6$ 

(17)

 $16.8 \pm 1.7$ 

(14)

 $16.4 \pm 4.7$ 

(14)

 $16.6 \pm 1.2$ 

(11)

 $17.0 \pm 1.4$ 

(25)

room and controlled temperature treatments. This could have been caused by early acceptance of the diet and provision of rearing conditions similar to larval natural habitat respectively. Enda and Kitajima (1990) and Kosaka and Ogura (1990) reared adults and larvae of pine sawyer beetles on plant tissue diets reported a high mortality during early developmental stages. This is in agreement with the present study.

Insects provided with bark tissue had the widest head capsule width and were heavier than in any other treatments. Head capsule width had a very strong relationship with larval weight and larvae with larger head capsule widths were found to be more active and produced heavier females. Since mean female pupal weights were heavier than males, larval head capsule width can be indirectly related to fecundity in females. The rate of development on each medium to the 4th instar, pupal and adult stages was somewhat shorter than that observed in natural conditions. The difference does not appear to be a question of temperature alone as pointed out before, it is thus possible that the difference in developmental period may be due to nutritional factors.

Diet  $10~(10\times10~{\rm cm}~{\rm log})$  was the superior diet followed by the bark diet treatment. Based on the objectives of the study and the need to produce individuals with similar traits that can compete with wild beetles in the field, data from wild beetles and adults raised from larvae collected from the forest were compared to adults from diets 4,5 and 10 (Table 9). It was recommended that diet 4 (bark tissue) be used as a standard rearing methods to rear pine sawyer beetle M. alternatus due to ease of preparation cost (materials and labour) space saving, and environmental factors since mass rearing will need many  $10~{\rm cm}~{\rm logs}$ , this is equivalent to felling  $20~{\rm year}$  old red pine trees (deforestation).

#### Summary

Studies were carried out to determine the most suitable diet for rearing beetle and to develop a mass rearing technique by which pine sawyer beetle (*Monochamus alternatus*) could be reared from larvae to adults without need for periodic care.

Eleven diets and two temperature regimes were tested. Diets were grouped into:

<sup>(25) (25) (25)</sup> 

Chemically defined diets; plant tissue diets; natural diets and silk worm diet. Larvae were reared individually in petri dishes. Their growth rate and body sizes were compared with the pine sawyer beetles collected from the forest. Suitability of the diet was evaluated using a 13 point rating system. Controlled temperature treatments were superior to room temperature treatments. Log diet was the best diet tested but bark diet was selected as the standard diet to be used for rearing *Monochamus alternatus* larvae due to technical, environmental and economic reasons.

Key words: Monochamus alternatus, Artificial diet, Growth, Development

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# マツノマダラカミキリの人工飼育法の検討

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# 要 旨

マツノマダラカミキリ虫を飼育するのに最適な人工飼料を決定し、定期的な世話をしなくてもマツノマダラカミキリ幼虫が成虫になるような大量飼育法を開発するために試験を行った。11種類の飼料と2段階の温度条件下で調べた。飼料は化学合成飼料、植物組織からなる飼料、自然条件下の餌からなる飼料、およびカイコ用飼料に分けられる。幼虫はシャーレ内で個体飼育し、羽化後に成長率と体の大きさについて野外のものと比較した。

飼料の好適さは 13 point rating system により評価した。温度調節された場合の方が室温の場合より優れていた。丸太からなる飼料が最適であったが、技術的、環境的および経済的理由から、マツノマダラカミキリ幼虫の飼育には Bark diet が標準的な飼料であると判定された。

キーワード: マツノマダカミキリ, 人工飼料, 成長, 発育