大量飼育のためのナミテントウの低コスト,低労力飼料の開発

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Development of Low-Cost and Labor-Saving Artificial Diet for Mass Production of an Aphidophagous Coccinellid, *Harmonia axyridis* (Pallas)

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Introduction

Harmonia axyridis is an Asiatic polyphagous ladybug with large variability in its color pattern; it is an important species for biological control of aphids and has the following characteristics: (1) high voracity in both larval and adult stages; (2) wide food range, preying on many species of aphids, and also on other Homopteran such as psyllidae and scale insects; (3) high reproductive capacity — a single female can lay up to 3800 eggs (Broun *et al.* 1996); (4) multivoltine with more than 10 generations a year under laboratory conditions.

These favorable pest-control characteristics have resulted in introduction of the species into Europe and North America, and its distribution is widening (Dreistadt *et al.* 1995).

Mass production is indispensable for practical use in pest control. In many cases of commercial production of other predators, indigenous or substitute living prey is used, so rearing usually involves mass production of prey or substitute insects (Finney 1948, Morrison and Ridgeway 1976). In this situation, development of a low-cost artificial diet would be an advantage. Artificial diets for aphidophagous coccinellids have been studied and improved for a long time (Smirnoff 1958, Smith 1965, Attallah and Newsom 1966, Kariluoto *et al.* 1976, Singh *et al.* 1985). Some diets succeeded partially but none has been adopted for practical mass production.

Lyophilized and pulverized drone honeybee brood (drone powder, DP) is well known as an excellent substitute diet for rearing various predacious insects, including *H. axyridis* (Matsuka *et al.* 1972, Okada *et al.* 1972, Matsuka and Niijima 1985). Nutritional studies have led to some improvement of DP (Matsuka and Okada 1975, Matsuka and Takahashi 1977, Niijima *et al.* 1977). However, modified DP has some difficulties from both the cost and labor viewpoints. This paper re-examines the improvement of DP for *H. axyridis* and discusses the possibilities for actual use.

Materials and Methods

Insects and rearing test

Harmonia axyridis (Pallas) in these experiments were the offspring (second and third generations) of adults collected from the field. They were reared on DP, and newly-hatched larvae were used for rearing tests.

Generally, individual rearing tests in small Petri dishes (6-cm diameter) in a dark incubator at 24±2℃ were used to prevent cannibalism. Test diets and a water-saturated sponge were supplied and renewed everyday. The mortality, developmental period, and adult body weight within 24 hours after emergence were measured to evaluate the test diets. DP was used as a control.

Sugar preference test

Sugar preference was examined to determine a suitable sugar for the diet. Young fourth-instar larvae were starved for 12 hours before the experiment to activate their searching behavior. Each sugar was dissolved in distilled water (0.1 or 1 mol/l) and used to saturate a piece of cellulose sponge (1×1cm). Trehalose, melezitose and raffinose were dissolved until the saturation point because they could not be dissolved to 1 mol/l. Two sponges (one saturated with the sugar solution and the other with distilled water) were placed 2-cm apart in the center of a Petri dish (9-cm diameter). Five larvae were released into the Petri dish and their response to the sponges was checked at 15-minute intervals for 1 hour. Mouth contact with a sugar-saturated sponge was scored as a positive response. The contact to the water-saturated sponge was always very low (<5%), so it was neglected. Twelve replicates were conducted for each sugar at 24±2℃ in daylight.

Diet materials and preservatives

The materials which showed good results in this experiment were shown in Table 1. For the insect

Table 1 Diet materials and cost

Material	Commercial name	Company or source	Price/100 g (¥)
Honeybee powder (DP)	---	made in lab	1600[a]
Aphid powder	---	made in lab	?
Fruitfly powder	---	made in lab	?
Mealworm powder	---	made in lab	720[a]
Silkworm powder	---	made in lab	?
Food for mynah bird	Q-chan	Nippon Pet Food Co. Ltd.	60
Food for carp	Swimy	Nippon Pet Food Co. Ltd.	70
Food for goldfish	Asakaze	Nippon Formula Feed Manuf. Co. Ltd.	240
Fish meal	Fish meal	Nippon Formula Feed Manuf. Co. Ltd.	48
Yeast	AY-65	Asahi Beer Co. Ltd.	200
Chicken liver powder	---	made in lab	290[a]

a) Calculated from raw material purchased from grower/market powders, aphids (*Lachnus tropicalis*), young pupae of silkworm (*Bombyx mori*), old fruitfly larvae and pupae (*Dacus dorsalis*) and mealworm (*Tribolium* sp.) were lyophilized after homogenizing in the same way as making DP. The mealworms were obtained from a pet shop. Sucrose was added to all the commercial materials, such as animal feed, and was ground into a fine powder. The AY-65™ is brewers' yeast autolyzed at 65%. It originates from the brewing industry and is sold commercially.

To save labor feeding the diets, several preservatives used commonly in insect diets were examined. Powdery diet with a preservative and a water-saturated sponge were put on glass slides placed in a lidded Petri dish and kept in an incubator at 25℃. Ten replicates were used for each preservative. Water was supplied every day and the quality change in diets and degree of mold infection were monitored.

Gelatinization and diet coating

To save labor supplying water, powdery diet was mixed with water, and gelation was tried using gelatinizers. Preliminary tests suggested a moisture content of 80% as best from both the physical and nutritional viewpoints. Suitable gelatinized diets were made using two gelatinizers: agar (0.5%), and sodium alginate (1%). Agar was dissolved into half of the water and heated and then this solution was mixed well with the other half of the water suspending the powdery diet. The agar-gelatin diet was supplied as a small cube. The alginate-gelatin diet was made by dripping it into a solution of calcium chloride (1%) giving spherical beads, 2 to 3 mm in diameter. These gelatinized diets were also evaluated by rearing tests. Attempts to maintain the moisture content of these gelatinized diet were tried using shellac (GSN™ Gifu Shellac Co.), and paraffin. Shellac was dissolved in ethanol and sprayed on. Paraffin was melted by heat and dripped on.

Results

Role of sugar in diets

The feeding responses to several sugar solutions are shown in Fig. 1. There is a considerable difference in the response to the two concentrations(0.1mol and 1.0mol/l). The larvae responded strongly to the higher concentration and the response percentage was more than double in the case that larvae showed remarkable response. Fructose, glucose, and sucrose generally elicited a good response. Trehalose, which is major sugar in insect blood, and melezitose which is contained in the honeydew of aphids, showed high responses. Some sugars and derivatives, such as xylose, mannitol, mannose, and lactose did not produce any response.

When sucrose was added to AY-65 at several ratios, the larval development varied (Table 2). Just AY-65 itself could not sustain development and all larvae died in a few days at the first instar. Although AY-65 fortified with 50% sucrose produced a high emergence ratio, the development period

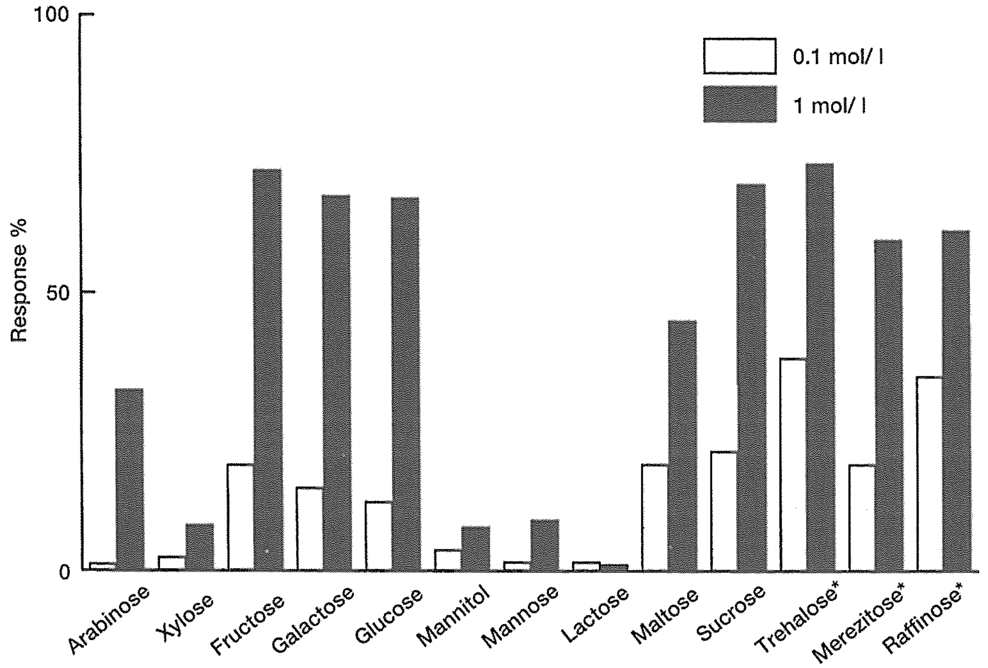

Fig. 1 Response of 4th instar larvae of *H. axyridis* to sugar solution (* Saturated solutions were used instead of 1mol/l/)

Table 2 Phagostimulative and additional effects of sucrose added to AY65™

Sucrose content %	n	% of insects reaching			Developmental period (Days±SD)	Adult weight (mg±SD)	
		4-L[a]	pupa	adult		female	male
0[b]	20	0					
10.0	20	0					
12.5	20	30	15	15	30.7±0.9	19.3	14
16.6	20	20	20	20	28.8±1.3	17.2±2.1	20.5
20.0	20	30	20	20	30.3±5.4	14.8	21.5±0.1
25.0	20	45	40	20	24.8±1.8	---	22.2±2.2
33.3	20	60	40	35	25.3±1.3	23.6±2.6	20.9±7.5
50.0	20	95	95	85	26.5±1.8	19.3±2.0	20.8±2.3

a) 4th instar larva, b) AY65™ only

and adult body weight were similar to AY-65 fortified with 33.3% or 25% sucrose addition. Even the best result with fortified AY-65 was inferior to that of DP only, and fortified AY-65 is inadequate for use as a single diet.

Low-cost materials for diet and additives

Finding low-cost materials is very important. Over 30 materials including insect powders, commercial animal feeds, and even cooking supplies were tested. The results for several

Table 3 Larval development of *H. axyridis* on several foods

Diet or material[a)]	n	% of insects reaching			Developmental period (Days±SD)	Adult weight (mg±SD)
		4-L[b)]	pupa	adult		
Honeybee powder	20	90	90	90	18.9±0.5	27.5±0.8
Aphid powder	20	80	80	80	20.1±0.3	32.5±3.0
Fruitfly powder	40	20	10	5	25	6
Mealworm powder	20	20	5	5	25	18.9
Silkworm powder	20	90	70	55	20.3±0.4	16.2±0.6
Food for mynah bird	20	50	20	10	27.7±1.3	16.5±0.8
Food for carp	20	50	50	30	27.1±0.8	16.5±0.5
Food for goldfish	20	40	30	10	29.2±0.2	16.8±0.7
Fish meal	20	20	10	5	24	18.5
Dry yeast	20	60	40	35	25.3±1.3	22.9±4.4
Chicken liver	20	70	70	60	22.9±1.6	23.1±5.2

a) Sucrose was added at a ratio of 30% except to insect powder. See also Table 1.
b) 4th instar larva

materials that sustained larval development are shown in Table 3. Both aphid powder and honeybee powder (DP) showed good and comparable development. Silkworm, fruit fly and mealworm powder are materials that can be obtained in large quantities, but the results of rearing tests were poor, although the development period was shorter than with other commercial materials. Several animal feeds also sustained larval development at low emergence, long developmental period, and small body size. AY-65 and chicken liver gave relatively good results compared to other commercial materials. Several yeast such as Ebios™ (dried *Saccharomyces cerevisiae*), and Torula yeast™ (*Torula* sp.) were tested, but gave similar or inferior results to AY-65 (unpublished data).

Although the single materials are all inferior to DP, they could be effective additives for lowing the cost of the DP diet. AY-65 and sucrose mixture (2:1) was added to DP at different ratios and the fortifying effects were investigated (Table 4). There is almost no significant difference in

Table 4 Effects of additives in honeybee powder (DP) on larval development of *H. axyridis*

Additive	Concentration %	n	% of insects reaching			Developmental period (Days±SD)	Adult weight (mg±SD)	
			4-L[a)]	pupa	adult		female	male
None[b)]	0	20	100	100	100	16.8±0.7	38.3±4.2	31.9±5.4
AY65™[c)]	30	20	90	90	90	16.3±1.0	38.2±3.8	32.0±2.2
	40	20	95	95	95	16.8±0.9	36.5±3.1*	30.5±3.1
	50	20	100	100	100	16.1±0.3	32.5±4.2**	28.8±3.6*
	60	20	100	95	95	18.9±1.6**	30.9±3.3**	28.1±2.3*
	70	20	90	90	90	19.2±2.3**	28.6±3.0**	26.6±3.3**
	80	20	95	95	95	19.6±1.1**	28.6±1.2**	26.8±3.7**
Chicken liver[c)]	40	20	90	90	90	18.9±2.8**	31.0±2.8**	29.1±3.3**

a) 4th instar larva, b) DP only c) Additives contained 30% sucrose
* and ** indicate significant difference to control at $p<0.05$ and $p<0.01$

development period and body weight between the control and diets fortified with up to 40% AY-65 and sucrose. Chicken liver was also a good additive .

Preservatives and labor saving

Several preservatives to prevent molding were examined. As shown in Fig 2, dehydroacetic acid, methyl-*p*-hydroxybenzoic acid (MpHB), and sorbic acid were effective for over 10 days at 1% (Table 5). Their effectiveness varied with concentration and fell down at levels below 0.1%. MpHB (1%) remained effective for about 3 weeks, which covers most of the larval period. These three

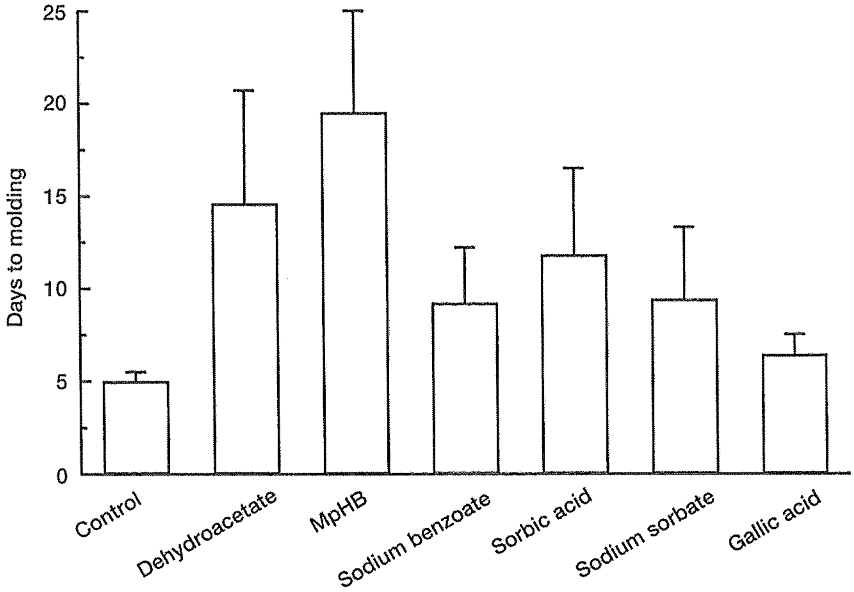

Fig. 2 The effects of 1% preservatives against DP

Table 5 Effects of preservatives in DP

Preservative	Concen-tration (%)	Days (±SD) to molding Appearance	Cover
Control	---	4.0±0.8	5.0±0.5
Methyl-*p*-hydroxybenzoic acid	1	14.0±6.1	19.5±5.5
	0.1	5.9±1.2	6.8±1.0
	0.01	4.5±0.7	5.4±0.5
Sorbic acid	1	9.6±2.7	11.8±4.7
	0.1	4.9±1.4	7.4±2.7
	0.01	5.7±1.4	7.0±1.2
Dehydroacetic acid	1	8.8±0.8	14.6±6.1
	0.1	6.3±1.3	6.0±1.8
	0.01	4.4±1.4	7.3±2.1

Table 6 Effect of several preservatives on larval development of *H. axyridis*[a)]

Preservative[b)] (1%)	n	% of insects reaching			Developmental period (Days±SD)	Adult weight (mg±SD)	
		4-L[c)]	pupa	adult		female	male
None (control)	20	80	80	80	17.5±0.5	36.5±3.9	34.8±2.1
Methyl-*p*-hydroxybenzoic acid	20	80	75	70	19.2±0.7	36.0±2.5	30.9±2.8
Sorbic acid	20	95	80	80	18.3±0.8	38.1±3.4	32.5±1.8*
Dehydroacetic acid	20	85	70	70	21.0±1.1**	29.5±4.3**	26.6±3.6**

a) Diets were renewed each day.
b) Preservatives were added to DP at a ratio of 1%.
c) 4th instar larva
* and ** indicate significant difference to control at $p<0.01$ and $p<0.05$.

Table 7 Effect of frequency of feeding on larval development of *H. axyridis*

Frequency of feeding[a)]	n	% of insects reaching			Developmental period (Days±SD)	Adult weight (mg±SD)	
		4-L[b)]	pupa	adult		female	male
Everyday	20	90	90	90	20.6±1.4	33.0±2.7	29.6±2.7
Every 2 days	20	95	85	85	20.4±1.2	30.0±2.5	27.9±2.3
Every 3days	20	95	95	90	21.1±1.0	33.3±1.7	29.7±1.8
Every 5 days	20	95	85	85	22.2±1.3	31.1±2.6	28.4±2.6
Once	20	85	85	80	22.4±0.8	30.1±2.2*	26.5±2.0*
Never	20	85	85	80	22.8±2.1	29.6±3.4*	26.8±2.3*

a) Lower-cost diet (DP:AY-65:Sucrose=6:3:1) with preservative (MpHB)
b) 4th instar larva
* indicates significant difference to control (every day) at $p<0.05$.

Table 8 Larval growth of *H. axyridis* fed on powdery and gelatinized diet

Gelatinizer	Physical condition	n	% of insects reaching			Developmental period (Days±SD)	Adult weight (mg±SD)	
			4-L[a)]	pupa	adult		female	male
Control	powdery	20	95	95	95	19.1±1.0	32.1±3.1	29.4±2.2
Agar	gel	20	85	85	85	20.5±1.3	32.7±2.1	29.0±1.3
Sodium alginate	gel	20	70	70	65	24.1±2.6**	23.0±4.2**	23.0±3.0**
Sodium alginate	powdery	20	70	65	65	24.9±3.2**	23.9±3.7**	20.8±3.4**

a) 4th instar larva
** indicates significant difference to control at $p<0.01$

preservatives had no remarkable effect on larval development even at 1% addition, although dehydroacetic acid caused some reduction (Table 6). These preservatives were also effective in diets containing AY-65, and even in moist gelatinized diet for over 20 days.

Rearing tests were conducted at different food-supply intervals using the low-cost diet with MpHB (Table 7). Supplying food only once or twice during the larval period sustained larval development and had only a small impact on development period and body weight. Supplying water

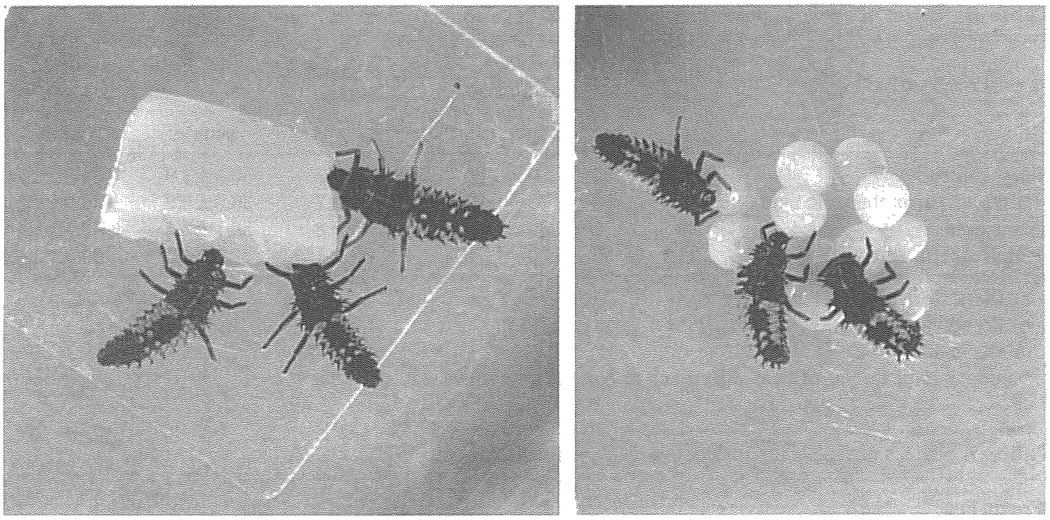

Fig. 3 Fourth instar larvae eating gelatinized diet by agar (left) and sodium alginate (right)

is another labor-intensive factor and gelatinization of the low-cost diet was attempted (Table 8). The two gelatinizers gave suitable physical properties to the diets and the larvae ate well (Fig. 3). The agar-gel diet gave a similar result to powdery diet (control), but the alginate-gel diet caused some deterioration. This is not due to physical factors because powdery diet containing the same amount of alginate caused a similar deterioration.

Discussion

Sugar is an important constituent of the diet of these insects both for nutrition and as a phagostimulant. Figure 1 shows that this species is not fastidious about the type of sugar, and sucrose is sufficient for meeting the nutrition requirements. DP might be an excellent diet because it contains plenty of sugars (Niijima 1995). AY-65 fortified with sugar sustained larval development although none of larvae grew up without the addition of sugar. The quantity of sugars also seems an important factor as shown in Fig. 2. These results suggest either that AY-65 has the minimum nutrients for larval development but insufficient sugar as the feeding stimulant, or that fortification with sucrose masks an inadequacy in the diet.

Addition of sugar improved the quality of diets that are otherwise unsuitable (Table 3). Several commercial animal feeds also sustained larval development when fortified with sucrose at 30%. All these commercial feeds contained fish meal; Fish meal™ with sucrose also sustained development (Table 3). Fish meal could be an effective diet for larval development of this species. Cheaper additives reduce the cost of the diet.

Several insect powders that could be obtained cheaply in large quantities were tried; DP was confirmed to be the second-best for development after aphid powder, which is the species' natural food (Table 3). Addition of sucrose might improve the dietary quality of other insect powders. Singh *et al.* (1985) succeeded in rearing *Leis conformis* using a diet consisting of lyophilized potato tuber worm, yeast hydrolyzate, sucrose and vitamins. Iwasa has also succeeded in rearing *H. axyridis* on a large scale using live diamondback moth larvae (personal communication). However, production of these prey insects is very labor intensive compared to honeybee brood, which can be produced easily by beekeepers. Broun *et al.* (1996) established a technique for rearing large numbers of *H. axyridis* using eggs of *Ephestia kuehniella*. They pointed out that the problem with this technique is the very high cost of the eggs (FFr10,000/kg). Hongo and Obayashi (1997) also succeeded in rearing *H. axyridis* using diapause eggs of brine shrimp, *Artemia salina*, which are relatively cheap. Niijima and Matsuka (1990) calculated the cost of DP as US2.25 cents per chrysopid. Since this would be close to the cost per coccinellid, compared with lepidopteran eggs, DP does not seem expensive.

Addition of additives including AY-65 at a ratio of 40% to DP produced the same development as 100% DP diet, which cuts the cost of the diet. The results suggest that a suitable low-cost diet could be formulated as DP : AY-65 : sucrose at a ratio of 6 : 3 : 1. Chicken liver or fish meal could be substituted for AY-65.

The insects in the experiments represented in Table 4 might be a good strain, because the control results (DP) showed high emergence, short development period and heavy weight compared with controls in other series. Selection of such superior strain would be improve the quality of the controling agent.

DP is stable for some months when it is kept dry (Sakai *et al.* 1978), but when kept in high-moisture conditions, it absorbs water and becomes moldy in a few days, so this diet must be changed every other day. MpHB was the best preservative and had no effect on the larval development. Kariluoto (1978) has reported the efficiency of sorbic acid and MpHB in an artificial diet for *Adalia bipunctata*; 0.2% sorbic acid prevented molding of diet for 3 weeks at 25℃ and was superior to MpHB. In DP, 1% sorbic acid was effective for about 10 days but was inferior to MpHB. This difference may result from the different diet components and form. The *A. bipunctata* diet was gelatinoid and heat-processed. Addition of preservative reduces the need for frequent food renewal; the low-cost diet with 1% MpHB sustained the entire development of this species, eliminating the need to change the food.

Gelatinized diet with agar did not affect larval development of this species. Spherical diet gelatinized with sodium alginate showed some adverse impact due not to the physical factor, but to the sodium alginate itself. Spherical diet is easy to handle for supplying or coating. However, the gelatinized diet dried out in a few days, and our intention to reduce labor in supplying water was not attained. Some coating processes were tried to prevent evaporation of moisture. Shellac has been used to coat human foods, such as chocolate. It is easy to make a very thin film but it does not match this hydrophilic diet. Although shellac-coating was tried after covering the diet with stearic acid, the larvae could not bite through it. Paraffin has been used for aqueous diets for chrysopids (Vanderzant 1969), but it is difficult to form a thin coat and the results were unstable.

Another problem in rearing predacious insects is cannibalism. It can be avoided by individual rearing using cell-unit chambers in the same way as for chrysopids (Morisson and Ridgway, 1976). However, a water-supply system is needed, so a water-containing gelatinized diet would be very advantageous. One reason for the successful mass production of other insect predators using lepidopteran eggs and living larvae, is that eggs and larvae have a high moisture content. We successfully gelatinized the low-cost diet, but failed to maintain the moisture content. Future studies on coating techniques are needed. A simplified and automated rearing method would reduce labor costs, making future mass production of these ladybirds with a strong possibility.

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Summary

Development of low-cost and labor-saving artificial diet for mass production of an aphidophagous coccinellid, *Harmonia axyridis* (Pallas). Keiko Niijima, Wataru Abe and Mitsuo Matsuka (Fac. Agric., Tamagawa Univ., Machida-shi, Tokyo 194) Bull. Fac. Agric., Tamagawa Univ. No. 37: 63-74

Several materials such as insect powders, commercial animal feeds, and yeast, which could be obtained cheaply in large quantities, were tried as artificial diets for mass production of *Harmonia axyridis*. Drone honeybee brood powder (DP) was most effective. Sugar acts as a phagostimulant, and sucrose improves nutritive condition for this species. Autolyzed yeast, chicken liver and fish meal could be useful additives to reduce the cost of DP-diet.

Methyl-*p*-hydroxybenzoic acid, sorbic acid and dehydroacetic acid were effective preservatives and the diet could be preserved with 1% methyl-*p*-hydroxybenzoic acid for the entire larval development period, eliminating the need to change the diet.

Gelatinized diet was tried to save labor supplying water. Agar and sodium alginate were useful gelatinizers, but the diet still dried out within a few days and needs further improvement.

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要　　約

大量飼育のためのナミテントウの低コスト，低労力飼料の開発

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アブラムシ類の天敵としてその利用の必要性が高まっているナミテントウ，*Harmonia axyridis* (Pallas) の大量飼育のための人工飼料の改良を試みた．本種の人工飼料として，ミツバチ雄蜂児粉末(DP)と同等以上に有効で，安価な素材は見つけられなかった．自己分解イースト（AY-65[TM]），トリレバーあるいは魚粉はショ糖を30%加えることにより成虫を出すことができたが，羽化率の低下，生育期間の延長，羽化成虫の小型化などが認められ，単独での飼料化は難しいことが分かった．市販のペットフードでもショ糖を添加することによりわずかに成虫を出すことができるものが見つかり，ショ糖が本種の摂食刺激的役割を果たしていることが分かった．本種幼虫の種々の糖への摂食反応を調べたところ多くの糖に反応し，糖に対する選好性は低いことが分かった．AY-65はDPの増量剤として有効であり，40%加えても本種の生育に遜色はなく，飼料の低コスト化につながった．以上のことからDP：AY-65：ショ糖が6：3：1の低コスト飼料が考案された．

防腐剤の検討をしたところパラヒドロキシ安息香酸メチル（Methyl-*p*-hydroxybenzoic acid；MpHB)，ソルビン酸，デヒドロ酢酸がDPに対して強い防腐効果をもち，本種の生育にも影響が少かった．特にMpHBを1%加えると幼虫期に1回も餌換えをせずに成虫まで飼育できることが分かり，労力の削減につながった．もう一つ残された問題である水分の供給法として，この低コスト飼料に水をまぜ，ゲル化した飼料を試作した．ゲル化にはアルギン酸と寒天が有効であったが，アルギン酸は本種の生育に悪影響が認められた．これらの飼料は数日で乾燥してしまい給水労力の削減にはつながらず，さらにパラフィンの様な疎水性の被膜の検討が必要であることが分かった．