When applied in the field, such a solution stength may be effective in driving the worms out of their burrows and making them more susceptible to other control measures. A concentration of 150 p. p. m. is less than the concentration produced by the technique of application (10-30 lb/acre)[6]. However, 2, 4-D acid is safer to use even at a greater application rate because it is effective with water hyacinth in rice paddies and it does not affect rice[7].

It is noteworthy that the aquatic oligochaete *B. sowerbyi* is more resistant to herbicides than the terrestrial oligochaete *Eisenia foetida*. This latter species had a survival of 90% at 1 p. p. m., 80% at 10 p. p. m., and nil at 100 p. p. m. and 1,000 p. p. m. monuron solutions after an exposure of 26 hours[5]. Other experiments[8] have shown that the resistance of *Allolobophora*, *Pheretima*, and *Alma* to the same herbicides belongs to the class of the *Branchiura* resistance. This means that the resistance of *Eisenia foetida* may be exceptionally lower than many other oligochaetes.

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Seasonal Changes of the Waxy Covering and its Components of a Scale Insect, *Ceroplastes pseudoceriferus* Green. Yoshio Tamaki and Shozo Kawai (Agricultural Chemicals Inspection Station, Kodaira, Tokyo, and Tokyo Agricultural Experiment Station, Tachikawa, Tokyo) Received September 9, 1966. *Botyu-Kagaku*, 31, 148. 1966.

21. ツノロウムシ *Ceroplastes pseudoceriferus* Green の虫体被覆物およびその構成成分の季節的変化

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ツノロウムシの真の虫体重は8月から12月にかけて，ほぼ直線的に増加するが，虫体被覆物の生成速度は8～10月に大，10～12月に小であり，虫体被覆物の生成が生育の初期に活発であることを推定させた．虫体被覆物の全虫体に対する割合は，9月に最高値80%を示し，このときの被覆物中の水性物質（interior honeydew）とロウ質物の比率は77対23であったが，1月にはこれが64%に減少し，interior honeydew とロウ質物の比率は58対42となった．

1～2令幼虫のロウ質物はその後の時期のロウ質物と質的に異なるものであることがうかがわれたが，interior honeydew 中のアミノ酸と糖類の構成は調査した6カ月間でほとんど変わらなかった．

被覆物中の interior honeydew は肛門から排泄される dropped honeydew とくらべてアミノ酸，糖類の構成が非常に異なり，これら2種の物質はたがいに異なった生物的意義を有するものと考えられた．

Many kinds of scale insects have become serious pest of various plants in Japan. However, they are rather difficult to control by insecticides because of the waxy covering which protect the insect body against insecticidal spray. The waxy covering of *Ceroplastes pseudoceriferus* Green which constitutes about 73% of intact adult female on fresh weight basis, is composed of 28% waxy substance and 72% aqueous substance designated as a honeydew (Tamaki 1963).

The present study was undertaken with a view to elucidate the quantitative and qualitative change of the waxy covering during the growth of *C. pseudoceriferus*, and to obtain some informations on biological significance of the aqueous substance in the waxy covering.

Materials and Methods

All the individuals of *C. pseudoceriferus* were collected from twigs of a tea plant at Fujimi-cho, Tachikawa-shi, where eggs had hatched at the end of June. Appropriate number of individuals ranging from 200 to 1,200 were sampled every month from July to January. They were treated with chloroform as already described (Tamaki 1964), and aqueous substance and crude wax were separated from real insect body. On the other hand, eggs oviposited under the female adults were collected from the tea plant at Tachikawa at the beginning of June, and were inoculated on fruits of a pumpkin variety, Hyuga (*Cucurbita moschata* Duch.). The growth and development of the scale insects reared on the pumpkin fruits were closely coincided with those on the tea twigs; details on the results of the rearing experiment will be reported elswhere. All the individuals reared on the pumpkin were sampled in November and treated as those collected from the tea plant.

Honeydew excreted from anus of the insects was recieved on glass plates which had been placed under tea twigs or pumpkin fruits. Droplets of the honeydew deposited on the glass plate were wiped off by absorbent cotton soaked with water. The honeydew was extracted with hot water from the absorbent cotton and concentrated *in vacuo*. The honeydew deposited on the glass plate, referred to as dropped honeydew, and the aqueous substance contained in the waxy covering, referred to as interior honeydew, were respectively analyzed.

Crude wax was examined by the following thin layer chromatography. Glass plates (20 × 20cm) were coated with Wakogel B-10 (Wakō Pure Chemicals Ind.) and activated at 120~130°C for 40 minutes. Each 10 μl of 5% chloroform solution of the crude wax was spotted on the plate. The plates were developed with a mixture of *n*-hexane:diethyl ether:glacial acetic acid (70:30 :1). After development the plates were sprayed with 40% sulfuric acid and charred for one hour in an oven at 130°C.

Amino acids and carbohydrates were analyzed by paper chromatography according to Tamaki (1964 a, b). A two dimensional ascending technique was applied for amino acid analysis with butanol:glacial acetic acid:water (4 : 1 : 2) and phenol:water (5:1), and one dimensional ascending technique was employed for carbohydrate analysis with triple solvent irrigation of butanol: glacial acetic acid:water (4:1:2) or ethylacetate: glacial acetic acid:water (3:1:1). Amino acids were detected by spraying ninhydrin reagent, sugars by *p*-amino hippuric acid-phthalic acid reagent, and sugar alcohol by bromocresol purple reagent. In all cases 5 or 10 μl of 10% aqueous solutions of honeydew dry matter was spotted on paper sheets.

Results

Seasonal changes of weight of intact insect and its main components

Figure 1 shows seasonal changes in weight of intact insect, real insect body, waxy covering,

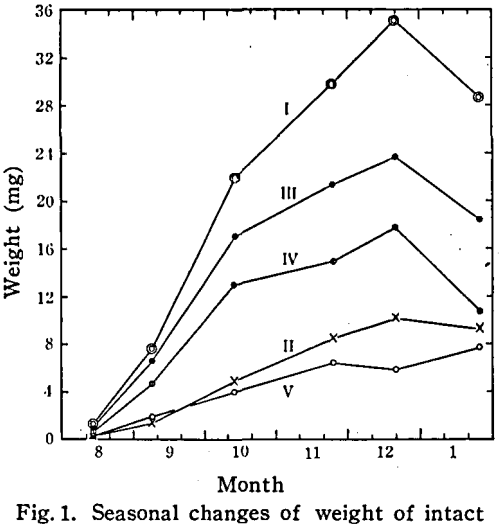
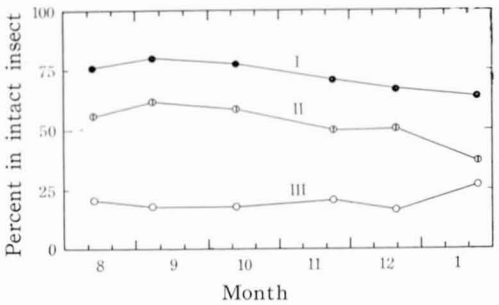


Fig. 1. Seasonal changes of weight of intact insect (I), real insect body (II), waxy covering (III), and its components, interior honeydew (IV), and crude wax (V).

and its components. The average weight of intact insect was 1.3 mg at the middle of August when the insects were second and third instar larvae, and increased to a maximum 35.1 mg at the middle of December, that is 27 times of the weight in August. The weight then decreased to 28.6 mg at the end of January. Accompanying with the seasonal change of the intact insect's

weight, the waxy covering and the insect body increased in weight until December and then decreased in January. The interior honeydew, a main component of the waxy covering, was also parallel to the waxy covering and intact insect in changing pattern of the weight. The sharp decrease of the weight of the waxy covering in December to January is due to the decrease of interior honeydew resulting from evaporation of water. While the weight of crude wax, another maining component of the waxy covering, did not decrease in January. Production rate of waxy covering was larger than growth rate of real insect body from August to October, while the both appeared to be equal from October to December.

Figure 2 shows a seasonal changes of compositions of the waxy covering and its maining components expressed as per cent of intact insect. Per cent of the waxy covering increased from August to the beginning of September, showing a maximum value of 80%, and decreased until the end of January, showing a value of 64%.



Eig. 2. Seasonal changes of percentage of waxy covering (I), and its components, interior honeydew (II), and crude wax (III), in intact insect.

The remainders of these values of the waxy covering show the per cent of real insect body. The interior honeydew was parallel to the waxy covering in its changing pattern, while per cent of crude wax gradually increased from August until January. At the beginning of September the interior honeydew and crude wax amounted to 77% and 23% of the waxy covering, respectively. But these values changed to 58% and 42%, respectively, at the end of January.

Seasonal difference of crude wax

Representative thin layer chromatograms of crude wax are shown in Figure 3. As seen in the chromatogram No. 7, crude wax obtained from the insects which had been collected in July when the insects were of first and second

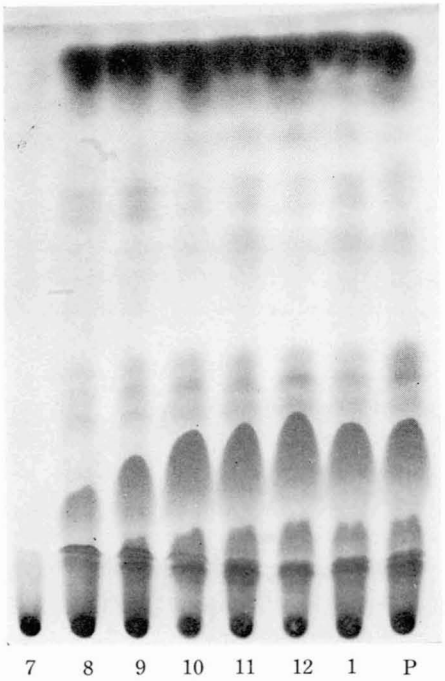


Fig. 3. Thin-layer chromatograms of crude wax in the waxy covering of *C. pseudoceriferus*. Numbers indicated below each chromatogram show the month when the samples are collected. Chromatogram P is of the insect reared on pumpkin fruit.

instar larvae, was quite different from the other chromatograms. From August to October some quantitative differences could be found on the chromatograms, but thereafter the chromatograms were almost same until January. Of particular interest is the fact that the chromatogram P, the crude wax of individuals reared on pumpkin fruits, is almost same as the other chromatograms of crude waxes of the insects collected from tea plant.

Seasonal difference of amino acids and carbohydrates in interior honeydew

Little or no qualitative difference was found in amino acid composition of the interior honey-

dew from August to January. Twelve kinds of amino acid, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, theanine, valine, arginine, glutamine, leucine and/or isoleucine, and cystine were detected in all the interior honeydew during six months.

However, the amount of each amino acid was so small, that differences of the amount of amino acids were not inferred from the chromatograms.

Pattern of carbohydrates in the interior honeydew was quite same throughout six months. Glucose, ribitol, mannitol and an unknown sugar alcohol were detected in all the insects collected from August to January.

Difference between interior and dropped honeydew

Eight amino acids, aspartic acid, glutamic acid, glycine, alanine, theanine, valine, glutamine, and leucine and/or isoleucine were detected in the dropped honeydew of the insects grown on tea plants. Of these amino acids, alanine was of the smallest amount. However, in the case of the interior honeydew alanine was of the largest amount.

Carhohydrates in the interior and dropped honeydew on two kinds of hosts are shown in Table 1. Six sugars, fructose, glucose, sucrose, raffinose, stachyose, and an unknown oligosaccharide, were detected in the dropped honeydew excreted by the insects grown on tea, but only glucose in the interior honeydew. In the case of the insects reared on pumpkin fruit the sugar composition also differs between the interior and the dropped honeydews. The most remarkable difference between the two honeydews was found in sugar alcohol. Ribitol, mannitol, and an unknown sugar alcohol were usually found in the interior honeydew regardless of the kind of hosts. But ribitol was not detected in the dropped honeydew of the insects from both tea and pumpkin fruit. Confirmation on the absence of mannitol and an unknown sugar alcohol in the dropped honeydew was impossibble bcause of interferrence by some substances of similar Rf values on the chromatogram.

Table 1. Difference of carbohydrate composition between interior and dropped honeydews of *C. pseudoceriferus* grown on tea and pumpkin.

Carbohydrate	Rs	Tea		Pumpkin	
		interior	dropped	interior	dropped
Unknown	1.51*			+	+
Fructose	1.20*		+	+	+
Glucose	1.00*	+	+	+	±
Sucrose	0.82*		+	±	+
Maltose	0.65*			±	+
Raffinose	0.48*		+	±	+
Unknown	0.35*				+
Stachyose	0.26*		+		+
Unknown	0.15*		+		±
Ribitol	1.00**	+		+	
Mannitol	0.96**	+		+	
Unknown	0.77**	+		+	

* Standard: Glucose
Solvent : Butanol:acetic acid:water (4:1:2), with triple irrigation

** Standard: Ribitol
Solvent : Ethyl acetate:acetic acid:water (3:1:1), with triple irrigation

Discussion

Ceroplastes pseudoceriferus Green hatched at the end of June on tea plant continues to grow up until December, but thereafter no growth seemed to occur. This growth pattern is almost same as the observation of Kajita (1965) on *C. pseudoceriferus* infesting *Podocarpus Nagi* Zool. *et* Moritzi at Fukuoka, Kyushu.

Newly hatched larvae of *C. pseudoceriferus* begin to secrete a white waxy substance from the body surface just after settling on host (Tamaki 1963). The rate of the wax secretion in larval period, expressed as weight of crude wax, is larger than that of the growth of real insect body. However, the reverse is true in and after October, when the most individuals are of adult stage. Rate of the interior honeydew secretion is also larger in August to October than in October to December. Thus, the rate of production of the waxy covering of the scale insect is larger in August to October than in October to December, while the growth of the real body continues until December at rather constant rate. These facts suggest that waxy covering is actively produced in the period from larval stage to the beginning of adult stage rather than from middle to late adult stages. In view of biological significance of the waxy covering,

it seems that the waxy covering is actively produced in the first half of growth period of the insect and it serves as a protective agent.

The amino acid and carbohydrate compositions of the interior honeydew did not change throughout the growth period, and were different from that of the dropped honeydew. The waxy covering of the first and second instar larva of the scale insect seems to contain only waxy substances ; and water soluble substances, the interior honeydew, are produced after the third instar, and seem to be secreted from the body surface and not from anus (Kawai and Tamaki, unpublished observations). The dropped honeydew is considered to be of the same category to honeydews excreted by aphids and some scale insects which have been believed to be a mixture composed of excessive nutrients from plant sap and some metabolic waste products of the insects (Gray and Fraenkel 1953, Ewart and Metcalf 1956, Bacon and Dickinson 1957, Mittler 1958, Maltais and Auclair 1962). The interior honeydew, however, is considered to be quite different in its biological meaning from the honeydews produced by aphids and some scale insects. Hackman and Trikojus (1952) separated aqueous substances from waxy coverings of *Ceroplastes ceriferus*, *C. rubens*, and *C. destructor*, Gilby and Alexander (1957) from *C. destructor*, and Tamaki (1963) from *C. pseudoceriferus*, and they designated as honeydew. The designation as only "honeydew", however, may be unsuitable to apply against these aqueous substances contained in the waxy covering of scale insects belonging to genus *Ceroplastes*.

The interior honeydew of *C. pseudoceriferus* amounts to 58~78% of the waxy covering from the third instar to adult, and is considered to be an important principle of the covering. A dry matter comprises only 5~9% of the interior honeydew, and water is its main component.

The fact that ribitol is present in the interior honeydew and not in the dropped honeydew without regard to the kind of host plants, shows the possibility of biosynthesis of the sugar alcohol in the insect. Furthermore, it suggests that the polyhydroxy alcohol plays an important role in the physiology of the scale insect. Mouse bone-marrow cells are protected against freezing and thawing damage when these are frozen as suspensions in glycerol, erythritol, ribitol, mannitol or sorbitol (Bender *et al* 1960). Ethylene glycol, glycerol and mannitol showed protection against freezing in gardenia leaf cells (Sakai 1960). Qualitative relation between freezing-tolerance of insects and the presence of glycerol has been well established (Salt 1961). It could be imagined that ribitol and other sugar alcohols in the interior honeydew of *C. pseudoceriferus* play an important role in freezing-tolerance of the waxy covering. Decreasing water content in the interior honeydew throughout winter season results in increasing concentration of sugar alcohols, and the waxy covering would serve as freezing-tolerance of the scale insect.

Summary

The amount of the waxy covering and its two main components, waxy substance and interior honeydew, of *Ceroplastes pseudoceriferus* Green increase with increase of the weight of real insect body ; but the rate of production of the waxy covering is larger in the beginning of the growth period than in the middle and late adult stages.

The waxy substances secreted by the insect in July seemed to be qualitatively different from these of the other seasons. Little or no difference was found in amino acid and carbohydrate compositions of the interior honeydew from August to January.

The interior honeydew, a main component of the waxy covering, and the dropped honeydew excreted from anus were different each other in amino acid and carbohydrate compositions. Particularly ribitol was unable to detect in the dropped honeydew, while it was detected in the interior honeydew.

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Utilization of Sterols in Clothes Moths, *Tinea pellionella* and *Tineola bisselliella*[1] Shoziro Ishii and Sachio Kawahara* (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto) Received October 1, 1966. *Botyu-Kagaku*, 31, 153. 1966.

22. イガおよびコイガのステロール要求

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イガ *Tinea pellionella* とコイガ *Tineola bisselliella* はいずれも羊毛害虫として知られている．イガは羊毛を含む動物質食物しか寄主とし得ないが，コイガは動物質，植物質両方を寄主とすることができる．各種の飼育試験の結果，イガとコイガとではステロールの要求に相違があることがわかった．すなわちイガは食物中のステロールがコレステロールでないと成育しないが，コイガはコレステロールの他に植物ステロールである β-シトステロール，スティグマステロールをも利用する．このステロール要求の差が寄主の範囲を規定している．イガ類のステロール要求を利用して，コレステロールを含まぬ米ぬかで飼育したコナマダラメイガ *Ephestia cautella* 幼虫のステロールを，ステロール源とした飼料でイガを飼育すると成育することから，コナマダラメイガ幼虫は植物ステロールからコレステロールへ変えることを証明した．一方ガスクロマトグラフにより米ぬかステロールと，コナマダラメイガステロールを定量し，化学的にもこの変換を裏付けた．

The case-bearing clothes moth, *Tinea pellionella* and the weebbing clothes moth, *Tineola bisselliella* are known to be serious pest insects of woolen products. In experimental conditions, the webbing clothes moth is able to rear by feeding plant marerials such as rice bran, while case-bearing clothes moth is not by feeding them.

It is of interest to clarify why the webbing clothes moth can develop by feeding either plant or animal origin product, and the case-bearing clothes moth can not develop by feeding plant products.

a) Feeding tests on rice bran and fish meal.

Rice bran and fish meal were used for food of both clothes moths. These two food materials were mixed at various proportions. Five eggs collected from the rearing of both moths were transferred in small vials (16 mm × 60 mm) containing 1 g of rice bran and/or fish meal. Each experiment was replicated two or three times. The feeding experiments were carried out at 25°C. Number of adults emerged and the developmental period from egg to adult were recorded. The results are given in Table 1.

The results clearly indicated that the webbing clothes moths can develop by feeding either fish meal or rice bran even though the latter food was not so suitable, while the case-bearing clothes moth can develop by feeding only the fish meal. If rice bran was mixed with fish meal at a ratio of 1:1, larvae of the case-bearing clothes moth could not develop and died.

b) Improvement of amino acid composition in rice bran.

In order to improve amino acid composition of

1) This paper has been read at the Annual Meeting of Japanese Society of Applied Entom. and Zool. held at Tokyo, April, 1965

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