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Rearing of the Brown Citrus Aphid,
Toxoptera citricidus (KIRKALDY),
(Homoptera: Aphididae), on a Synthetic
Diet with Reference to pH and
Sucrose Concentration of Diet
and Rearing Density^{1,2}

Shinkichi KOMAZAKI

Okitsu Branch, Fruit Tree Research Station,
MAFF, Shimizu, Shizuoka 424-02, Japan

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The artificial rearing method of an aphid on a chemically defined diet is applicable for studying nutritional requirement and feeding behaviour (PARRY and FORD, 1969; MITTLER, 1976; SRIVASTAVA and AUCLAIR, 1974), morph determination (SUTHERLAND and MITTLER, 1971; LECKSTEIN and LLEWELLYN, 1974; WHITE, 1972) and virus transmission (KUNKEL, 1977). Several species of aphids were reared successfully on a synthetic diet for more than several generations or almost permanently (AKEY and BECK, 1972; CHAWLA et al., 1974; DADD and KRIEGER, 1967; DADD and MITTLER, 1966; KRIEGER, 1971). In this study, *Toxoptera citricidus* which attacks citrus (FURUHASHI and NISHINO, 1968) and transmits the citrus tristeza virus (SASAKI, 1974), was reared on a synthetic diet based on that of MITTLER et al. (1970).

The apterous viviparae were collected from a citrus grove at the Okitsu Branch, Fruit Tree Research Station in Shimizu. Stock cultures of aphids were maintained on new shoots of potted *Citrus aurantium* in a growth cabinet at 20°C with natural long daylength. Before experimental use, the aphids were reared more than three generations on young shoots of the plants under an uncrowded

condition. Newly molted (within one day old) apterous adults, denoted 'parents,' were taken from cultures and individually transferred into a glass cup (3 cm in diameter and 3 cm high) after being weighed by a torsion balance. The newly deposited nymphs were individually caged in other cups except those used for testing the rearing density. Fifty aphids were used in each experiment. The diet was enclosed in Sealon film® sachets which covered the glass cup. The aphids in the cups were kept in a growth chamber at 20°C under illumination of 16 hr per day; the diet was renewed every two days. They were examined daily and aphid mortality, molting, nymph becoming adult, adult survival and fecundity were recorded, and newly matured aphids were weighed.

The weight of parents showed very little fluctuation ranging from 0.9 to 1.0 mg. Longevity was significantly correlated with the fecundity in parents ($r=0.79$, $n=855$) and this was also true of later generations ($r=0.49$, $n=175$). The nymphal period correlated with the fecundity ($r=-0.45$, $n=175$) and adult weight ($r=-0.29$, $n=94$). The longevity and nymphal period are shown in the following results.

The effects of sucrose concentration on aphid growth are shown in Table 1. The longevity of parents with no food and those fed only distilled water was 0.54 day and 0.90 day respectively and the aphids did not produce progeny. A diet of 25% sucrose (MD25) was most suitable for parents but not as good as the plant on which they survived for 18.61 days. The aphids grew well in the first two generations but did not mature in the fourth generation on MD25. On MD20 the aphids completed six generations, but in the second generation the growth was not as good as on MD25. On MD15 the second generation did not reach the adult stage. TAHORI and HAZEN (1970) reported that

¹ *Appl. Ent. Zool.* **19** (1): 114–117 (1984)

² Contribution from the Fruit Tree Research Station, B-98.

for *Toxoptera aurantii* (BOYER) a 55% sucrose diet was the most suitable but the aphid could be reared only two generations on it. These kin species differed in the most suitable sucrose concentration of diet.

In the next experiment, the most suitable diet pH was examined; pH was adjusted with KOH. The results are illustrated in Fig. 1. Parents' longevity

which also indicates reproductivity, was the longest on a diet of pH7. In the first generation, the longevity was the shortest on a diet of pH6 and development was rather fast on pH7. In the second generation, longevity was shorter on pH6 than on pH8 but the nymphal period did not differ among these three diet pHs. In the third generation, the longevity was the same on each pH but

Table 1. The growth of *T. citricidus* on different sucrose concentrations of diet

		Sucrose concentration in MD-diet		
		15%	20%	25%
Parent	Adult longevity	4.02 ^a	4.32 ^a	7.65 ^b
1st generation	Longevity	7.65 ^a	6.73 ^a	9.38 ^a
	Nymphal period	15.14 ^a	16.32 ^a	16.13 ^a
2nd generation	Longevity	3.02 ^a	5.96 ^a	10.96 ^b
	Nymphal period	—	22.15 ^a	19.31 ^b
3rd generation	Longevity	—	9.53 ^a	6.81 ^a
	Nymphal period	—	14.20	— ^c
4th generation	Longevity	—	10.95 ^a	4.00 ^a
	Nymphal period	—	19.62	—

^{a, b} Numbers followed by the same letter in each line are not significantly different by LSD test ($p < 0.05$). ^c Data omitted because only one aphid matured.

Fig. 1. The growth of *T. citricidus* on different pH of diet. pH 6: open circles; pH 7: closed circles; pH 8: triangles. Longevity: solid line; nymphal period: dotted line. The symbols with the same letter in each generation are not significantly different in longevity (capital letters) or nymphal period (small letters) by the LSD test ($p < 0.05$).

Fig. 2. The growth of *T. citricidus* reared at various densities. Longevity: open circles or triangles; nymphal period: closed circles or triangles; the control reared on plants: triangles. The symbols with the same letter are not significantly different in longevity (capital letters) or nymphal period (small letters) by the LSD test ($p < 0.05$).

nymphal period was the shortest on pH6. No marked improvement in aphid growth was observed in the range of tested pH, but growth of the aphid on the diet with pH6 became better as the generations advanced.

For the experiment on rearing density, a diet with 20% sucrose and pH7 was used. The first generation was reared under various densities ranging from one to ten aphids per cup. The results compared with data from the control which were reared on plants are illustrated in Fig. 2. Longevity was prolonged when more than two aphids were placed together in one cup. The developmental period was the shortest in cups containing four aphids. The percent reaching adult stage, which is not shown in the figure, was also the highest in this density. Even in this case, however, the aphid was less fecund compared with aphids reared on plants. No alate was produced in any of the experiments.

In conclusion, the diet with 20% or 25% sucrose and pH7, and four to six aphids per cup was found suitable for rearing this aphid. But the growth and reproduction of the aphid on a synthetic diet does not compare with those raised on a plant. The composition of amino acids in the diet and addition of secondary plant substances (SCHOON-

HOVEN and DERKSEN-KOPPERS, 1976) or growth factors (CHAWLA et al., 1974) to the diet remain to be tested.

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Host Selection Behavior of *Anicetus*

beneficus ISHII et YASUMATSU

(Hymenoptera: Encyrtidae)

II. Bioassay of Oviposition Stimulants

in *Ceroplastes rubens* MASKELL

(Hemiptera: Coccidae)¹

Shozo TAKAHASHI and Junji TAKABAYASHI

*Pesticide Research Institute, Faculty of Agriculture,
Kyoto University, Kyoto 606, Japan*

(Received July 25, 1983)

The encyrtid parasitoid, *Anicetus beneficus* ISHII et YASUMATSU preferentially oviposits on the natural host, *Ceroplastes rubens* MASKELL in the field. The ovipositional behavior of *A. beneficus* toward this natural host has been observed and the sequential behavior reported (NODA et al., 1982). The behavior can be induced not only on a living natural host but also on a scale wax extract of a hemispherical shape (3 mm in diameter and 2 mm high). *A. beneficus* started random walking on the extract with antennal flagellation (drumming) and drills its ovipositor after choosing an oviposition site. Crude extracts of *C. rubens* scale wax were absorbed on filter paper in a spot 3 mm in diameter. *A. beneficus* showed no ovipositional behavior toward this spot. For a bioassay of oviposition stimulants in the crude extracts, a paraffin model was made in a hemispherical shape (3 mm in diameter and 2 mm high) and impregnated with a chloroform solution of the crude extracts (0.1 mg=0.1 insect equivalent). The model was kept at room temperature overnight and placed in a test tube (6 mm in diam-

eter and 50 mm long). In a test female *A. beneficus* was confined with the model in the test tube and bioassayed. The bioassay was repeated 12 times for a sample. The random walking with antennal flagellation and drilling of the ovipositor shown by the female was taken as a criterion in the bioassay (Fig. 1. a, b, c). Bioassay results are shown in Table 1.

Water soluble components have been suggested as ovipositional stimulants of scale insects (QUDNAU and HÜBSCH, 1964; WESELOH and BARTLETT, 1971). In the present study, a water soluble fraction of *C. rubens* scale wax was inactive in bioassay, but chloroform extracts proved to be active. The chemical structures of scale wax composition in chloroform extracts are complex and numerous terpenoids have been identified in the scale extracts of the genus *Ceroplastes* (MIYAMOTO et al., 1979; NAYA et al., 1980, 1981; PAWLAK et al., 1983). In *Ceroplastes rubens* MASKELL, a fair portion of the terpenoids in the wax may consist of diterpenoids, which have been called rubabietic acid, rubenic acid and rubenol (KONO and MARUYAMA, 1936, 1937). However, the proposed structures need revision in light of modern methods of structure analysis. Recently, cerorubenic acid-I, II, III and cerorubenol-I, II have been characterized to be the first representatives of two new skeletal classes of sesterterpenoids (TEMPESTA and NAYA, in preparation). It is very interesting to study the correlation of the wax composition of the genus *Ceroplastes* and the oviposition stimulation toward the encyrtid parasitoid using the bioassay described above.

¹ Appl. Ent. Zool. **19** (1): 117–119 (1984)