SHORT COMMUNICATIONS

Feeding Stimulants for the Larvae of the Yellow Butterfly, *Eurema hecabe mandarina* DE L'ORZA (Lepidoptera: Pieridae)¹

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(Received November 5, 1981)

Previously, we reported that some of the biting stimulants for the larvae of the yellow butterfly, *Eurema hecabe mandarina* DE L'ORZA, were isolated from their favored host plant, *Lespedeza cuneata* G. DON (a species of bush-clover; "Medohagi" in Japanese), and presumed that a swallowing factor is necessary in addition to the biting factors for continuation of the feeding response (NUMATA et al., 1978, 1979). This paper deals with the

examination of the swallowing factor.

As reported previously, the E₅ fraction from the leaves of *L. cuneata* (Fig. 1) stimulated the biting action of the larvae, but did not induce the feeding response unless it was combined with one of the residues, R₃ or R₄, which alone did not exert any apparent stimulative effect on either the biting or feeding actions (NUMATA et al., 1979). These results suggest that R₃ and R₄ contain a certain constituent which induces swallowing. R₄ was further extracted with boiling-water, and then treated with several reagents by the method (TIMELL, 1961) (Fig. 1).

Feeding responses of the larvae of *E. hecabe mandarina* to residues obtained were evaluated on the basis of the total number of frass pellets excreted by 5 final-instar larvae, which were starved for 4 hr 1 day after the last larval molt, then placed individually on the test diets for 20 hr at 25°. These diets were prepared by incorporating the powdered test samples into a basal diet containing the extract E₅ (NUMATA et al., 1979). The results of 3–20-fold replications are shown in Table 1.

As reported previously, the mean frass count for 4 replications was 156.0 ± 1.5 for fresh leaves of *L. cuneata*, and 125.9 ± 3.8 for the agar diet containing dried leaf powder. Positive feeding

Fig. 1. Procedure of fractionation of the swallowing factor from *L. cuneata* leaves.

¹ Appl. Ent. Zool. **17** (4) : 582–584 (1982)

Table 1. Feeding responses of the larvae of *E. hecabe mandarina* to residual fractions from *L. cuneata* leaves^a

Fraction	No. of replicates	No. of frass pellets per 5 individuals ^c
Basal diet (1% agar)	4	14.0±0.8 ^w
R ₃	20	66.7±2.7 ^x
R ₄	3	53.0±5.3 ^x
R ₅	9	53.2±4.2 ^x
R ₆	4	37.5±1.2 ^y
R ₇	8	50.9±3.8 ^x
R ₈ ^b	3	55.0±3.5 ^x

^a The test diets were prepared by incorporating each residue (0.26 g) into the extract E₅ (0.12 g) in 1 ml of 1% agar.

^b The residue extracted by boiling water from *C. camphora* leaves.

^c Figures followed by different letters are significantly different at $P < 0.01$.

Table 2. Feeding responses of the larvae of *E. hecabe mandarina* to some commercial cellulose powders^a

Kind of cellulose powder	Mesh	Opening (μm)	No. of replicates	No. of frass pellets per 5 individuals ^e
Basal diet (1% agar)			4	14.0±0.8 ^w
Toyo A	100—200	47—149	4	38.5±2.8 ^x
Toyo C	≥300	≤46	4	20.3±1.1 ^y
Whatman CF-11	≥200 : <200 (1 : 2) ^b	≤74 : >74 (1 : 2) ^{b, c}	4	52.0±4.1 ^z
Whatman CC-11	≥200	≤74	3	20.7±1.8 ^y
Merck mikrokristallin	≥200 : <200 (3 : 1) ^b	≤74 : >74 (3 : 1) ^{b, d}	5	25.2±2.7 ^y

^a The test diets were prepared by incorporating each cellulose powder (0.26 g) into the extract E₅ (0.12 g) in 0.8 ml of 1% agar.

^b Indicates the weight ratio of different mesh or opening of powders.

^c The sample contains about 67% of powder above 74 μm long.

^d The sample contains about 25% of powder above 74 μm long.

^e Figures followed by different letters are significantly different at $P < 0.01$.

responses were obtained with all residues from extraction of R₃, although a slight depression in response was found in some of them when compared with R₃ (Table 1). It was proved that residual biting stimulants were removed from R₃ by hot MeOH extraction, since they were detected by gas-liquid chromatography in E₄. The slight depression in effectiveness of some residues seems to be partially attributable to this reason. Likewise, the leaves of the camphor tree, *Cinnamomum camphora* SIEB., a non-host plant of the butterfly, were fractionated in the same way as *L. cuneata*. Fraction R₈ of the boiling-water extraction of this plant was found to have an ef-

fect on feeding response comparable to R₅ from *L. cuneata*. The positive feeding responses of the larvae to cellulose (R₇) from *L. cuneata* and the residue R₈ from *C. camphora* led to the assumption that cellulose, one of the common constituents of every plant, is effective in stimulating the continuous swallowing action of this larva, as shown by the result obtained in *Bombyx mori* (HAMAMURA et al., 1962). In order to confirm this assumption additional feeding tests were carried out with some of the commercial cellulose powders (Table 2).

The results were not, however, always in accord with the expectation that all the cellulose

powders would induce a similar level of feeding. The two commercial cellulose powders Toyo A and Whatman CF-11 were more effective as a swallowing stimulant than the others. Although there was no difference in x-ray diffraction spectra between these cellulose powders, some difference in their size was observed. Two of more effective cellulose powders contain about 70 to 100 per cent of powder above 74 μm long, while the other less effective powders contain about 75 to 100 per cent below 74 μm long. Thus, cellulose powder above 74 μm long may act as a main continuous swallowing stimulant for the larvae of *E. hecabe mandarina*.

It is evident that the effective residue R_4 contains a smaller amount of cellulose than R_7 . Nevertheless, R_4 induces feeding activity equivalent to the effect of R_7 . From this fact some of constituents such as lignin and hemicellulose, which were removed on the way of extraction

to R_7 , may induce feeding activity equivalently to cellulose or promote the effect of cellulose.

ACKNOWLEDGMENTS

The authors are grateful to Prof. M. INOUE and Dr. T. ISHIDA of this college for the x-ray diffraction spectral measurements.

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Preliminary Characterization of the Aggregation Pheromone in the Sow Bug, *Porcellionides pruinosus* (BRANDT) (Isopoda: Oniscoidea)¹

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(Received January 29, 1982)

Gregariousness has been commonly found in some terrestrial isopod crustaceans. The feces of these isopods were shown to contain the active principle responsible for the initiation and maintenance of aggregation. The active principle was produced by the intestines and excreted with the feces. Therefore, the active principle

was suggested to be an aggregation pheromone (TAKEDA, 1980 a, b). The chemical nature of this aggregation pheromone, however, has remained to be elucidated.

In the present paper, the chemical nature of the aggregation pheromone in *Porcellionides pruinosus* is reported.

The activity of the aggregation pheromone was assayed by the use of 16 sets of Petri dishes. The inner bottom and sides of each Petri dish were covered with moistened filter papers. The area of the filter paper on the side was divided into 8 equal parts. Ten to 50 μl of a sample solution was spotted on the center of one of the 8 parts (S-spot) and an identical amount of the solvent was spotted on the center of each of the other 7 parts (C-spots). Then one worm was put into every Petri dish, and the position of the worm was recorded after 30 min. It was significant that in more than 6 sets of Petri dishes the worm was found beside the S-spot ($p < 0.01$). The subjects were adult worms collected in the fields and kept in the laboratory.

Five g of feces was extracted with 200 ml of diethyl ether or acetone for 7 hr using a Soxhlet

¹ Appl. Ent. Zool. **17** (4) : 584-586 (1982)

powders would induce a similar level of feeding. The two commercial cellulose powders Toyo A and Whatman CF-11 were more effective as a swallowing stimulant than the others. Although there was no difference in x-ray diffraction spectra between these cellulose powders, some difference in their size was observed. Two of more effective cellulose powders contain about 70 to 100 per cent of powder above 74 μm long, while the other less effective powders contain about 75 to 100 per cent below 74 μm long. Thus, cellulose powder above 74 μm long may act as a main continuous swallowing stimulant for the larvae of *E. hecabe mandarina*.

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