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DISTRIBUTION OF ^{14}C-LABELLED CARBOFURAN AND DIMETHOATE IN ROYAL JELLY, QUEEN LARVAE AND NURSE HONEYBEES

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SUMMARY

When sublethal levels of the ^{14}C-labelled systemic insecticides carbofuran and dimethoate were fed in sugar syrup to caged nurse bees (NB) in the laboratory, radioactivity was detected in both royal jelly (RJ) and queen larvae. Although ^{14}C-dimethoate was provided in syrup at lower concentrations than ^{14}C-carbofuran, higher levels of radioactivity were found in larval food and larvae with the former. In order to understand more fully the pathway of systemic insecticide into brood food, a distribution study of ^{14}C-insecticide within NB was conducted. When individual NB consumed sublethal levels ($< 1/30 \times LD_{50}$) of ^{14}C-insecticide, radioactivity was found to be largely confined to parts of the digestive system, including the honey sac (HS), with very little in various glands of the head and thorax. Results suggested that contamination of RJ occurred with the delivery of contents from the HS, rather than by secretion from the head glands responsible for the glandular component of larval food.

INTRODUCTION

Under natural conditions, queen honeybee larvae (QL), like those of worker and drone, are entirely dependent upon nurse bees (NB) for their food supply. Royal jelly (RJ), the special diet fed in abundance to QL, contains more lipids, much more sugar, and less water than the diet fed to young worker larvae (WL), and there is relatively little variation in the composition of RJ fed to younger or older QL (SHUEL and DIXON, 1959). Although bees aged 5-16 days are normally involved with the feeding of the immature worker stages of the colony (HAYDAK, 1963 ; SMITH, 1974), NB provisioning queen cells tend to be older, on average (SMITH, 1974), and have more developed hypopharyngeal glands (HPG) (HABOWSKY, 1962). Two types of servings, which together constitute RJ, are deposited by NB in queen cells : 1) a clear

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serving, and 2) a milky-white serving (VON RHEIN, 1933 ; SMITH, 1959 ; JUNG-HOFFMANN, 1960, 1966). The former serving is furnished by bees averaging 17 ± 2 days old, the latter by those 12 ± 2 days of age. The ratio of these offerings is approximately 1:1, with younger QL (< 72 h) being fed more of the white portion and older QL (> 72 h) receiving more of the clear component (JUNG-HOFFMANN, 1960, 1966). Furthermore, it has been established that these two servings have different origins in NB ; the clear serving is derived from the HPG secretion and from the honey sac (HS), while the milky-white portion is a mixture of the secretions of the HPG and mandibular glands (MG) (see HAYDAK, 1970).

Research on the direct effects of insecticide on honeybee larvae is scarce (NRCC, 1981). Larvae could receive unaltered insecticide with the sugar of their diet ; GILBERT and WILKINSON (1974) found the intact HS of workers to be void of mixed-function oxidase activity. Because the feeding of larvae by many different NB increases the probability of exposure of brood to insecticides (NRCC, 1981), queens, which receive 1 600 feedings (HABOWSKY, 1962 ; JUNG-HOFFMANN, 1966) and a total of 1.5 g food (JUNG-HOFFMANN, 1966), may have a higher likelihood than workers of receiving insecticide-contaminated food as larvae. The importance to the colony of rearing healthy QL is obvious ; queenless colonies undergoing supersedure would seem to be particularly vulnerable to insecticide-related problems with raising queens. In fact, two recent studies (NUNAMAKER, HARVEY and WILSON, 1984 ; STONER, WILSON and HARVEY, 1985) have indicated a decrease in queen-rearing success when honeybee colonies were exposed to insecticides.

Systemic insecticides have the capacity to be distributed throughout plants from the point of application, thereby conferring protection against phytophagous insects from within. Recently, investigations of a possible systemic action in the honeybee have been conducted. Carbaryl, a plant-systemic carbamate (C) insecticide (WORTHING, 1983), was absent in the brood-food glands of NB fed ^{14}C-carbaryl ; it was concluded that the insecticide does not accompany the glandular (secreted) portion of food supplied by NB to larvae, but probably rather contents from the HS (WITTMANN, 1981). However, STONER, WILSON and HARVEY (1985) concluded that acephate, a systemic organophosphorous (OP) insecticide (WORTHING, 1983), was indeed systemic in NB, the inference being that the insecticide was present in and secreted by the head glands responsible for the production of RJ. The purpose of the present study was to determine the distribution of carbofuran (C) and dimethoate (OP), two plant-systemic insecticides (WORTHING, 1983 ; MCEWEN and STEPHENSON, 1979), in NB, RJ, and QL, in order to better understand the nature of brood-food contamination in the honeybee.

MATERIALS AND METHODS

Supply of nurse bees and larvae

In mid-January, 1986, a queenright colony (hybrid of *Apis mellifera ligustica*) was transferred from the University of Guelph apiary to a flight room and maintained at a temperature and illumination regime which helped to stimulate brood rearing (DAVIS, SOLOMON and SHUEL, 1987). Pollen trapped in July, 1985, and stored at − 18 °C was supplied as a honey : pollen slurry (1:1, by vol). Frames of honey and a jar of water were also supplied continuously, so that food was always present in excess. To obtain newly-emerged workers, a frame of capped brood was removed from the colony, placed into a bee-tight cage and then into an incubator set at 34 ± 0.5 °C. This emergence cage was checked once daily ; all adults (< 24 h age) were distinctively marked on the thorax with coloured paints or discs, and then immediately introduced to the same colony. In this way, NB of a specific age could be obtained by collecting appropriately-marked workers from the surface of comb with larvae. Larvae were obtained from worker cells.

Distribution of systemic insecticide in nurse bees

The following tests were patterned after those of WITTMANN (1981), who fed carbaryl at 1/95 × LD_{50} per bee (LD_{50} = 1.34 µg/bee ; ATKINS, 1975). Eight-day-old NB were placed individually into glass vials (25 ml) and kept at 21 °C. After a 3-h starvation period, bees consumed a 25 µl aliquot of 2 M glucose solution, prepared immediately before use, containing [Ring-^{14}C] carbofuran (39.40 Ci/M, 95 %) or [O-methyl-^{14}C] dimethoate (17.0 Ci/M, 99 % ; purchased from Amersham Radiochemical Center, Arlington Heights, IL, U.S.A.) from a glass micropipette inserted through a hole in the vial cap. The insecticide levels consumed were sublethal ; the aliquot with carbofuran contained 1/29 × LD_{50} (LD_{50} = 0.160 µg/bee ; ATKINS, 1975), approx. 2 190 disintegrations per minute (dpm), while that for dimethoate was 1/30 × LD_{50} (LD_{50} = 0.146 µg/bee ; average of seven values reported in the literature), approx. 805 dpm. After feeding, bees were immediately transferred to an incubator (34 ± 0.5 °C), and removed for dissection at a specified time (0.25, 0.5, 1, 2, 4, 6, 12 or 24 h) later. Bees rarely survived longer than 24 h post-feeding. Any bees which regurgitated or defecated in the vials were rejected. Prior to dissection, bees were rapidly cooled at − 18 °C (3 min). After 0.5 µl blood (BL) was withdrawn at the scutum, the bee was decapitated and, using a dissecting microscope (9 × mag), pairs of HPG, post-cerebral glands (PCG) and MG were removed in Ringer's solution (WOODRING, 1985). The thoracic glands (TG) were extracted, and then the abdomen dissected in Ringer's to remove the HS (including proventriculus), ventriculus (V), Malpighian tubules (MT), small intestine (SI) and rectal sac (RS). Each dissected item was separately folded into Whatman No. 42 ashless filter paper (AFP) and placed into empty, labelled, glass scintillation vials. The Ringer's and the remainder of the bee (RB) were similarly collected. A typical dissection lasted 25 min ; three NB were dissected per time-interval per insecticide. Individual body parts were burned in a Packard Tri-Carb Sample Oxidizer. The resulting $^{14}CO_2$ was absorbed by 5 ml Carbosorb^R, and 6 ml Permafluor V^R added. Vials were analysed for 10 min each by a Packard 460C Scintillation Counter using the external-standard method. After feeding, the used micropipettes were also rinsed and counted, to determine any residual ^{14}C-activity in them. Background counts averaged 26.0 dpm and were automatically subtracted from all samples.

Rearing queens in cage experiments

Sixty worker bees, eight days old, were placed in Liebefeld cages and furnished with pollen : honey patties (4:1, by vol) and a vial each of honey and water and held in darkness at 34 ± 0.5 °C. Twenty h later the honey vial was replaced by a vial of 50 % (wt/vol) sucrose syrup without additions (control), or with either radiolabelled carbofuran or dimethoate at approx. 12 ppb (by vol). All food was supplied in excess. Twelve hours after the syrup was administered, two young larvae (1-2 days old) were grafted into beeswax queen cups melted onto a glass microscope slide and primed with a small amount of stored RJ. The cups were hung through a hole in the cage top, so that the queenless units would have an opportunity to rear queens.

In tests involving untreated syrup, NB were allowed to continually feed larvae and to cap the queen cells, and pupae and emerging adults were examined (head shape, compound eyes, mandibles, tongue

length, hind tibiae and basitarsi, sting lancets and ovaries) for queen-like characteristics. In experiments with insecticide-contaminated syrup, provisioned queen cells were harvested 72 h after the cups had been provided. Larvae were removed from their cells, blotted free of adhering food with preweighed pieces of AFP, weighed and then wrapped in AFP. Similarly, larval food was collected from cells with AFP and immediately weighed. Larvae and food were oxidized and counted for radioactivity as described above. Numbers of dead adults in the cages were determined at this time. Three cage experiments were conducted for each insecticide.

Observation of feeding activity and collection of food servings

Tests were conducted which, using a dissecting microscope, permitted the observation of food provisioning by NB and food consumption by larvae. This was accomplished by shaving off the bases of artificial beeswax queen cups, melting the cups to glass microscope slides, grafting larvae into the glass-bottomed cups (SMITH, 1959) and providing them to cages of NB as above. Because the individual servings of RJ deposited by NB can be distinguished and have different origins in NB (i.e., Clear - HPG & HS ; Milky - HPG & MG) (see HAYDAK, 1970), it was desirable to collect single servings for evidence suggesting the possible systemic movement of insecticide to the head glands responsible for the secreted component of larval food. Radioactivity in clear servings, with none in milky portions, would suggest the lack of (or very low) systemic action, to the brood-food glands, of systemic insecticide in NB. Accordingly, cages were provisioned with ^{14}C-carbofuran [specific activity (SA) 2.3 times higher than ^{14}C-dimethoate] in syrup at 13 ppb (by vol), pollen/honey patties and vials of water immediately after NB were collected, and then placed in the incubator. Approx. 24 and 48 h later, a cage was removed and placed on a lab bench in a darkened room of hive temperature, and a young larva (1-2 days old) grafted into an unprimed glass-bottomed cup was supplied to the cage. The cup was removed immediately after a serving was deposited, then the food was collected on preweighed AFP and weighed. A new larva, cup and slide were introduced for subsequent feedings. Similar servings from a cage were pooled and analysed for ^{14}C-activity. The number of inspections (entrance of the bee into the cup accompanied by antennation toward the larva ; SMITH, 1974) by various NB before a larva was eventually served, and the number of larvae cannibalised by the caged bees, were also recorded.

RESULTS

Distribution of systemic insecticide in nurse bees

Analysis revealed residual ^{14}C-activity of 208.48 ± 5.71 dpm (S.E.) (carbofuran, n = 3) and 49.16 ± 5.30 dpm (dimethoate, n = 3) in the micropipettes after NB were fed. Therefore, individual NB actually consumed, on average, 1 980 dpm (^{14}C-carbofuran) or 755 dpm (^{14}C-dimethoate), or approx. $1/32 \times LD_{50}$.

The distribution patterns of these two systemic insecticides in NB are shown in Fig. 1. Very low levels of radioactivity from each insecticide were detected in the HPG, MG, PCG, TG and BL. The highest levels recorded in glands were those in the HPG ; for NB fed ^{14}C-carbofuran and ^{14}C-dimethoate, only 24.54 ± 15.77 dpm (S.D.) (4 h) and 13.55 ± 11.10 dpm (2 h) were detected, respectively. Most radioactivity was located in various parts of the alimentary system, and by 24 h, had largely accumulated in the RS (Fig. 1). Similar results had been obtained earlier with carbaryl (WITTMANN, 1981) and diflubenzuron (WITTMANN, 1982). The quantity of radioactiv-

ity continually declined in the HS and RB of NB fed ^{14}C-carbofuran, but diminished more slowly in those fed ^{14}C-dimethoate. Radioactivity in the alimentary canal anterior to the HS may account, in part, for the levels detected in the RB. Total radioactivity recovered averaged 72.1 % (range : 64.2-79.2 %) for ^{14}C-carbofuran and 72.6 % (60.7-80.9 %) for ^{14}C-dimethoate, compared to approx. 31 % (approx. 8-60 %) for ^{14}C-carbaryl (WITTMANN, 1981).

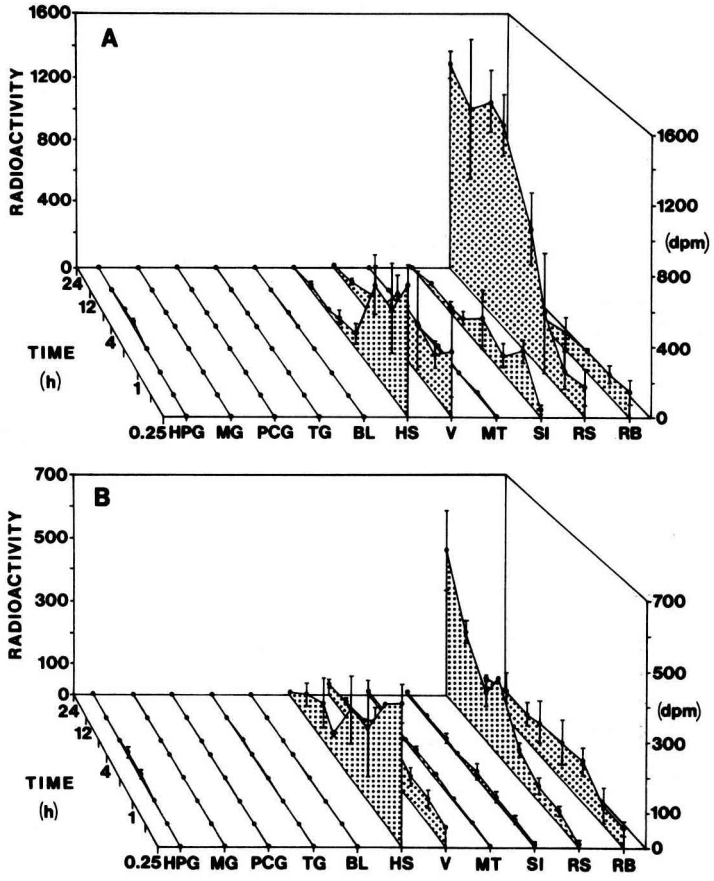

FIG. 1. — *Distribution of radioactivity in various parts of nurse honeybees over time after consuming radiolabelled systemic insecticide in 2 M glucose solution.*

A. Carbofuran ; B. Dimethoate

HPG = hypopharyngeal glands MG = mandibular glands
PCG = post-cerebral glands TG = thoracic glands
BL = blood HS = honey sac
V = ventriculus MT = Malpighian tubules
SI = small intestine RS = rectal sac
RB = Ringer's solution and remainder of body

Standard errors ≥ 10 dpm only, are shown as vertical bars at the data points.
Time is shown on a logarithmic scale.

Rearing queens in cage experiments

Although two queen cups were provided per cage, invariably only one larva, if any at all, was nourished by NB. The other larva was either abandoned or went missing. In the cages receiving untreated syrup, the occupied cup was elongated and sealed with wax from the neighbouring cup. PENG and JAY (1977) reared perfect queens and queenlike individuals in cages containing as few as 30 NB. Only perfect queens (not intercastes or worker-like individuals) were reared here by NB in cages supplied the control diet. This gave assurance that the experimental technique was biologically sound.

TABL. 1. — *Quantities of insecticide detected in queen larvae and larval food at 72 h, after ^{14}C-carbofuran or ^{14}C-dimethoate was administered in sucrose syrup to cages of nurse honeybees (60 bees/cage).*

Insecticide	Carbofuran			Dimethoate		
Syrup						
dpm/ml	5075			1865		
ng insecticide/ml (ppb)	12.8			11.4		
Cage no.	16	20	42	12	47	74
Larval food						
weight (mg)	110.71	244.15	220.09	258.91	126.78	282.63
radioactivity (dpm)	144.83	148.45	92.74	121.44	43.23	171.03
$\bar{X} \pm$ S.E. (dpm/mg)	0.7792 ± 0.2699			0.4717 ± 0.07625		
$\bar{X} \pm$ S.E. (pg insecticide [a]/mg)	1.972 ± 0.6829			2.871 ± 0.4641		
Larva						
weight (mg)	151.96	62.96	39.61	84.86	147.62	48.01
radioactivity (dpm)	88.73	38.98	13.84	45.33	49.71	25.60
$\bar{X} \pm$ S.E. (dpm/mg)	0.5175 ± 0.08452			0.4680 ± 0.06567		
$\bar{X} \pm$ S.E. (pg insecticide [a]/mg)	1.309 ± 0.2138			2.848 ± 0.3997		
No. adults dead [b]	1	0	1	0	0	0

a. The assumption is made that all radioactivity represents the parent insecticide molecule.
b. 84 h after ^{14}C-insecticide was provided.

The results of the tests involving ^{14}C-insecticide are shown in Table 1. In all six cages ^{14}C-activity was detected in both larvae and larval food. The latter appeared more watery and yellowish than RJ from queen cells under natural conditions. PENG and JAY (1977, 1979) found similar food deposited in queen cells by caged NB lacking their MG, and attributed this food to HPG secretion (and honey). As lipid components of RJ originate in the MG and defatted RJ is characteristically more yellow than the unaltered RJ, this conclusion seems reasonable. Queenlike pupae and adults developed from larvae which consumed this food (PENG and JAY, 1977, 1979). Relatively small larvae had a large surplus of food, similar to the case *in situ* (SMITH, 1954). Under the

assumption that measured radioactivity represented the unmetabolized parent insecticide, higher concentrations (pg/mg) of dimethoate than of carbofuran were found in larval food and larvae, despite higher levels of the latter in the syrup (Table 1). On a weight basis, concentrations of insecticide in syrup were 5.45×10^3 (carbofuran) and 3.33×10^3 (dimethoate) times higher than those in larval food. Larval condition appeared excellent. Adult mortality in the cages was very low, and no toxic effect was perceived.

Observations of feeding activity and collection of food servings

In preliminary experiments using the glass-bottomed cell technique, the provisioning of insecticide-contaminated food by NB and the consumption of such food by larvae, was observed. Results of the collection trials are shown in Table 2. Extremely low levels of radioactivity were detected in both clear and milky servings ; these were lower than expected from Table 1 (i.e., 0.7992 dpm/mg larval food). In cage 17, ten combined clear servings (13.59 mg) yielded the same level of radioactivity as a single milky feeding (0.55 mg).

TABL. 2. — *Results and observations of preliminary experiments involving collection of individual servings furnished by nurse honeybees to young female larvae residing in glass-bottomed queen cups. Syrup containing ^{14}C-carbofuran was provided to the cages (60 bees/cage).*

Syrup			
dpm/ml		5190	
ng carbofuran/ml (ppb)		13.1	
Cage no.	17		28
Time interval [a] (h)	24-31	45-49	20-25
No. pre-feeding inspections			
$\bar{X} \pm$ S.E.	4.3 ± 0.7	13.0 ± 3.1	3.0 ± 1.4
range	1-8	9-19	0-8
Servings			
CLEAR			
n	10	3	4
$\bar{X} \pm$ S.E. (mg)	1.36 ± 0.22	1.47 ± 0.59	2.21 ± 0.43
range (mg)	0.78-2.97	0.53-2.57	1.54-3.09
radioactivity (dpm) [b]	1.25 [c]	1.50	1.95
MILKY-WHITE			
n	1	0	1
$\bar{X}$ (mg)	0.55	0	0.45
radioactivity (dpm)	0.00 [c]	0	1.32
No. larvae eaten	3	5	2

a. Intervals in which larvae were hung into the cage and servings to them collected, after the start of the experiment.
b. All clear servings pooled into one sample vial.
c. Mean of three countings per vial.

JUNG-HOFFMANN (1966) found that female larvae in artificial queen cells of queenless colonies were provided food at the rate of 0.37 servings/h ; here, the average was 1.2 servings/h. More inspection visits occurred prior to feeding, and more larvae were cannibalised, when larvae were provided at 45-49 h than at 24-31 h (cage 17, Table 2). Inspections by various NB before a serving was given, and numbers of larvae cannibalised, were lowest at 20-25 h (cage 28). Cannibalism usually occurred with low numbers of previous inspection visits ; cage 17 [$\bar{X} = 0.57 \pm 0.65$ (S.E.), n = 8, range : 0-5], cage 28 (range : 2-20).

DISCUSSION AND CONCLUSIONS

Movement to the HPG has been shown for various ^{14}C-labelled materials injected or fed to NB (e.g., biopterin — REMBOLD and HANSER, 1960 ; leucine — HUANG and OTIS, 1986). Results of the tests with ^{14}C-carbofuran or ^{14}C-dimethoate suggest that these two plant-systemic insecticides are not secreted in appreciable quantity by the brood-food glands of NB into larval food, which is in agreement with the study of WITTMANN (1981), but not that of STONER, WILSON and HARVEY (1985). Only very low levels of radioactivity were detected in the dissected gland pairs. If this radioactivity corresponds to a true systemic movement, then that route into larval food would seem relatively unimportant. Furthermore, the presence of radioactivity in the HPG and MG does not necessarily mean that insecticide would be secreted by them. On the other hand, the HS contained relatively large quantities of radioactivity. It is probable that contamination of larval food in the cage experiments occurred from the HS, by delivery of insecticide with sugar (also see WITTMANN, 1981). Moreover, WITTMANN (1982) drew the same conclusion for ^{14}C-diflubenzuron, an insect-growth regulator (i.e., non-systemic ; WORTHING, 1983). There also exists some indirect supportive evidence : RJ itself contains 11-12 % sugar (SHUEL and DIXON, 1959), yet researchers have not identified sugar in the HPG and/or MG (GONTARSKI, 1958 ; HABOWSKY, 1962 ; JUNG-HOFFMANN, 1966). The latter researcher reported that only traces of fructose and other sugars could occasionally be found in the milky-white component of RJ.

Collection of individual servings failed to show conclusively the pathway of insecticide into larval food ; only minute levels of radioactivity were detected. This shortcoming is attributed to (a) the limitation on the amount of ^{14}C-insecticide that could be provided to the caged bees because of their high susceptibility (i.e., low LD_{50} value) to carbofuran, and (b) the low SA of the ^{14}C-insecticide material available. The use of radiolabelled material of high SA (eg. 750-1000 Ci/M) is recommended for future experiments involving insecticides highly toxic to worker adults. Also, adding more NB per cage (PENG

and JAY, 1979), conducting experiments at 24 h after the bees are caged and fed with radiolabelled insecticide (to minimize cannibalism and delays before feeding), and limiting the amounts of honey mixed with pollen and of water (to minimize dilution of the insecticide) could increase the rate of individual servings collected and the levels of radioactivity detected. Because worker bees consuming carbofuran and dimethoate may regurgitate their HS contents (BARKER, LEHNER and KUNZMANN, 1980 ; DAVIS, 1985 ; COX and WILSON, 1987), it is possible that insecticide-induced regurgitation may produce abnormalities in the ratio of clear : white servings, or in the proportions of the components (HPG secretion vs. HS content) involved in clear servings, provided by NB to larvae. This technique of collection of food servings may serve useful for such investigations.

JUNG-HOFFMANN (1966) reported that the ratio of the clear and milky components provided by NB was approx. 1 : 1 for QL. This ratio depended on age ; older NB provided less of the white serving, and young QL (< 72 h) received more of it (JUNG-HOFFMANN, 1960, 1966). Also, the proportion of the white component was particularly high on the second day of larval life (JUNG-HOFFMANN, 1966). The reason for the low rate of provisioning of the white component in the present cage tests is not known, because the larvae used were young. Perhaps the MG of the (initially) 8-day-old NB were not very active ; NB of 12 ± 2 days of age provided the milky serving (JUNG-HOFFMANN, 1966). SIMPSON (1960) found that in bees with large HPG, the MG often contained only a watery secretion. The HPG of the « winter » NB taken from the flight room colony at 8 days of age were enlarged (see also BROUWERS, 1983). That the serving initially provided to larvae is normally of the clear type, is another possibility. JUNG-HOFFMANN (1966) found that QL which were 1/4-1/2 day old received about 1.13 mg of the clear and 0.81 mg of the milky-white components per feeding. In the present study, utilizing larvae 1-2 days old, clear servings averaged 2.5-5 times larger than milky ones. These results suggest that higher quantities of a particular serving might be obtained by manipulating larval age.

From his distribution studies, WITTMANN (1981, 1982) concluded that the later instars (WL) would be the most likely to receive a direct delivery of insecticide from the HS. Ironically, older female larvae (e.g. 96 h) are also more susceptible to insecticides (carbofuran, dimethoate) in the diet than those < 72 h (DAVIS, SOLOMON and SHUEL, 1987). Besides differences in age, caste differences in exposure and susceptibility to insecticide in larval food may occur. Although QL < 72 h would appear more susceptible than larvae of similar age in the worker and drone castes, the latter would seem more vulnerable than QL when > 72 h, because the sugar content of RJ remains similar (11-12 %, wet weight) throughout the larval stage, whereas the low

level of sugar (3-4 %) in worker jelly (WJ) increases substantially (19-21 %) in the modified diet (MWJ) (SHUEL and DIXON, 1959). The change in nutrition with age of drone larvae is similar to that for WL (MATSUKA, WATABE and TAKEUCHI, 1973). Insecticide-contaminated pollen may be another source of brood-food contamination (see WITTMANN, 1982) leading to differential larval poisoning by caste ; pollen is also transferred to larval food from the HS. Very little pollen is present in RJ or diets fed to the other castes when < 72 h (SMITH, 1959 ; MATSUKA, WATABE and TAKEUCHI, 1973), but WL (SMITH, 1959 ; SHUEL and DIXON, 1959), and especially drone larvae (MATSUKA, WATABE and TAKEUCHI, 1973), receive large quantities of pollen in their diets after 72 h. Interestingly, WITTMANN (1981) found no difference in distribution of radioactivity in NB when the original glucose solution was replaced by a mixture containing sugar, honey and pollen.

Because queens apparently continue to be fed larval food as adults (see HAYDAK, 1970), they too may receive insecticide-contaminated (RJ). Adult queens heading colonies fed carbofuran experimentally (10 or 100 ppm) were among the last individuals to die (STONER, WILSON and RHODES, 1982), while queens of colonies supplied with dimethoate (10 ppm) died early (STONER, WILSON and HARVEY, 1983). Furthermore, in the latter case, colonies were unable to rear queens to replace those that died. STONER, WILSON and HARVEY (1985) suggested that differential systemic action to the head glands of bees feeding the queens may have been responsible for the above results. Apart from the possibility that queens have a higher susceptibility to dimethoate, another explanation may be that queens in the dimethoate experiments had received a higher concentration of insecticide in their food ; although cages of NB were provided with a 12 % higher concentration of carbofuran in syrup, the larval food elaborated by the bees contained a 46 % higher concentration of dimethoate. The reason for this disparity is unclear. It may be linked, however, to the relatively high levels of radioactivity which persisted in the HS, up to 12 h post-feeding, when individual NB were fed ^{14}C-dimethoate. Two factors (excluding regurgitation) appear responsible for dissipation of insecticide from the HS : (a) the rate of insecticide penetration through the foregut tissue, and (b) the rate of insecticide passage from HS to V. About the former case, some valuable information is known. CONNER, WILKINSON and MORSE (1978) found that when ^{14}C-insecticide was fed in both 0.5 M and 2 M sucrose solutions, penetration through the foregut occurred faster with the lower sugar concentration. Furthermore, uptake of insecticide from the lumen into foregut tissue was directly proportional to insecticide lipophilicity [dieldrin > parathion > carbaryl], but the rates of actual penetration were parathion > carbaryl > dieldrin, the explanation being that parathion had the optimal physicochemical properties required for penetration, while the slower uptake of carbaryl and slower release of dieldrin by foregut tissue limited their

rates of penetration (CONNER, WILKINSON and MORSE, 1978). Dimethoate is approx. 35 times more water soluble than carbofuran and 625 times more so than carbaryl (WORTHING, 1983 ; MCEWEN and STEPHENSON, 1979). Therefore, both carbofuran and dimethoate, but particularly dimethoate, might be expected to penetrate the HS tissue relatively slowly. At any rate, the evidence is abundantly clear that honeybee colonies are vulnerable, in a variety of ways, to dimethoate in food. Besides the problems associated with queen feeding and rearing (STONER, WILSON and HARVEY, 1983), dimethoate may arrive via the systemic route, unmetabolized, in nectar (LORD, MAY and STEVENSON, 1968 ; WALLER and BARKER, 1979), foragers can carry dimethoate in their HS to the hive at levels enough to kill several bees (JAYCOX, 1964 ; WALLER, BARKER and MARTIN, 1979), dimethoate residues can occur in honey (MIZUTA and JOHANSEN, 1972 ; WALLER and BARKER, 1979), and dimethoate levels not immediately lethal to adults can cause significant reductions in mature larval weights and problems at pupation (DAVIS, SOLOMON and SHUEL, 1987) and wing malformations at emergence (ATKINS and KELLUM, 1986).

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RÉSUMÉ

RÉPARTITION DU CARBOFURAN ET DU DIMÉTHOATE MARQUÉS AU ^{14}C DANS LA GELÉE ROYALE, LES LARVES DE REINE ET LES ABEILLES NOURRICES

Dans cette étude on a voulu déterminer la répartition d'insecticides systémiques (carbofuran et diméthoate) marqués au ^{14}C dans les nourrices, la gelée royale et les larves de reine, afin de mieux comprendre la nature de la contamination de la nourriture et du couvain chez l'abeille *Apis mellifica*. On a administré du sirop de saccharose contenant de l'insecticide marqué au ^{14}C à des doses sublétales (environ 12 ppb) à des groupes de 60 nourrices (âgées de 8 jours) maintenues au laboratoire dans des cagettes Liebefeld. On a détecté de la radioactivité aussi bien dans les larves que dans la nourriture larvaire (Tabl. 1). Bien que la concentration du sirop en diméthoate radiomarqué fût inférieure à celle en carbofuran radiomarqué, on a trouvé dans la nourriture larvaire des concentrations en diméthoate plus élevées.

Après la consommation par les abeilles d'une solution de glucose renfermant des doses sublétales (environ $1/32 \times DL_{50}$) d'insecticide marqué, on a effectué des dissections à intervalles réguliers

(0,25 - 24 h). Après oxydation, on a déterminé la radioactivité dans le corps à l'aide d'un compteur à scintillation liquide. On a trouvé très peu de radioactivité dans les glandes hypopharyngiennes, mandibulaires, post-cérébrales et thoraciques ; par contre des portions du tube digestif (y compris le jabot) présentaient une forte radioactivité, qui s'accumulait éventuellement dans l'ampoule rectale (Fig. 1). Les résultats laissent penser que la gelée royale est contaminée par le contenu du jabot plutôt que par les sécrétions des glandes céphaliques, qui sont à la source de la substance glandulaire de la nourriture larvaire.

Puisque la gelée royale est la combinaison de deux portions distinctes, qui ont chacune une origine différente chez la nourrice, il était souhaitable de récolter des portions individuelles de nourriture larvaire fournies par les nourrices aux jeunes larves royales. On a pu identifier et récolter des portions en greffant des larves dans des cellules royales ayant un fond en verre. Néanmoins, la radioactivité détectée dans les portions claires (qui renferment des sécrétions de la glande hypopharyngienne et du sucre du jabot) et les portions blanches (qui résultent des sécrétions combinées des glandes hypopharyngienne et mandibulaire) a été très faible (Tabl. 2) et leur récolte n'a pas fourni de plus amples résultats concernant le passage de l'insecticide vers la nourriture larvaire.

ZUSAMMENFASSUNG

VERTEILUNG VON ^{14}C-MARKIERTEM CARBOFURAN UND DIMETHOAT IN GELÉE ROYALE, KÖNIGINNENLARVEN UND AMMENBIENEN

Um die Vorgänge bei der Kontaminierung von Futter und Brut bei der Honigbiene *Apis mellifera* L. besser zu verstehen, wurde eine Untersuchung über die Verteilung von ^{14}C-markierten systemischen Insektiziden (Carbofuran und Dimethoat) in Ammenbienen, Gelée Royale und Königinnenlarven durchgeführt. Nach Verabreichung von subletalen Dosen (ca. 12 ppb) des ^{14}C-Insektizids in Saccharose-Sirup an Liebefeld-Kästchen mit je 60 (8 Tage alten) Ammenbienen wurde im Labor Radioaktivität sowohl in Larven als auch im Larvenfutter nachgewiesen (Tab. 1). Obwohl das ^{14}C-Dimethoat in geringerer Konzentration im Sirup vorlag als das ^{14}C-markierte Carbofuran konnte bei ersterem im Larvenfutter eine höhere Radioaktivität nachgewiesen werden.

Nach Anfütterung einzelner Ammenbienen mit subletalen Dosen (ca. $1/32 \times LD_{50}$) der ^{14}C-Insektizide in Glucoselösung wurden diese in verschiedenen Zeitintervallen (0.25 - 24 h) präpariert. Nach der Oxidierung wurden einzelne Körperteile mit Hilfe der Flüssigkeitsscintillationszählung auf Radioaktivität analysiert. In Hypopharynx-, Mandibel-, Kopf- und Brustspeicheldrüsen fand sich wenig Radioaktivität. In den verschiedenen Teilen des Verdauungssystems (einschließlich Honigmagen) wurde jedoch hohe Radioaktivität festgestellt, vermutlich akkumuliert sie im Rectum (Fig. 1). Aus den Ergebnissen konnte entnommen werden, daß die Kontaminierung des Gelée Royale durch die Beigabe des Inhalts aus dem Honigmagen entstand und nicht die Sekrete der Kopfdrüsen, als sekretorische Komponenten des Larvenfutters, verantwortlich sind.

Da das Gelée Royale eine Kombination aus zwei distinkten Portionen darstellt, die in den Ammenbienen verschiedenen Ursprungs sind, war es wünschenswert, einzelne Portionen des Larvenfutters, das von Ammenbienen an junge Königinnen verfüttert wird, zu sammeln. Dies geschah durch Umweiselung der Larven in Königinnenzellen mit Glasboden. Jedoch erwiesen sich die Anteile an Radioaktivität in der klaren Portion (Sekret der Hypopharynxdrüse und Zucker aus dem Honigmagen) und der weißen Portion (kombinierte Sekrete aus Hypopharynxdrüse und Mandibeldrüse) als sehr niedrig (Tab. 2). Die Analyse brachte also keine weiteren Erkenntnisse über den Weg von Insektiziden ins Larvenfutter.

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