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FILTRATION OF MICROPARTICLES FROM LIQUIDS INGESTED BY THE RED IMPORTED FIRE ANT *SOLENOPSIS INVICTA* BUREN (*)

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SUMMARY

The imported fire ant, *Solenopsis invicta*, has a highly efficient mechanism for filtering particles from food material. Latex microspheres as small as 0.88 μm were filtered and concentrated in the infrabuccal pocket. The compacted mass was then ejected as a pellet. The maximum size latex spheres swallowed by larvæ were 45.8 μm, which approximately corresponds to the diameter of the larval esophagus. The efficient filtration of particles offers little hope for the success of controlled release insecticide formulations that require ingestion of solid particles.

ZUSAMMENFASSUNG

Die Filtrierung von anwesenden Mikropartikelchen in den eingenommenen Flüssigkeiten von der roten importierten Feuerameise Solenopsis invicta **Buren**

Die importierte Fenerameise hat einen besonders wirksamen Mechanismus Fremdpartikelchen die in der Nahrung anwesend sind zu filtrieren. Latex Partikelchen von 0.88 μm Durchmesser wurden im infrabukalen Beutel filtriert und eingeengt. Die kompakte Masse wurde nachher als Kügelchen ausgestossen. Die von den Larven verschluckten Latex Kügelchen hatten einen Durchmesser, der eine Länge von 45.8 μm erreichen Konnte und dem Durchmesser der Speiseröhre der Larven entsprach. Die wirksame Filtrierung der Partikelchen bietet wenig Hoffnung auf Erfolg das Durchlassen der Kontrollierten Insektenvertilgungsmittel zu gestatten, wenn sie in harter Form verabreicht werden müssen.

Control of the red imported fire ant, *Solenopsis invicta* Buren, depends on the delayed action of a toxicant, which allows foraging workers to distribute the toxicant to the rest of the colony and queen via trophallaxis before they are killed. Although many fast-acting insecticides are effective against

* Mention of a commercial or proprietary product in this paper does not constitute an endorsement or recommendation of this product by the U.S.D.A.

foraging workers, none is currently available with delayed toxicity over a wide range of concentrations. Matrix-bound and encapsulated toxicants represent potential methods for delaying the activity of fast-acting insecticides (PAUL and HARRIS, 1976). Both could be formulated as dispersions in a food attractant and after ingestion by foraging ants, would be distributed throughout the colony by trophallaxis. Ideally, lethal concentrations of the toxicant would buildup in the recipients of the bait via diffusion through or decomposition of the matrix or wall of the microcapsules.

These methods depend on trophallaxis and ingestion of the dispersed solid by all or most workers and larvae. Fourth-instar *S. invicta* larvae are known to ingest solids (PETRALIA and VINSON, 1978), but earlier instars and adults are regarded as liquid feeders. Most ants have a diversified diet that includes debris and other particles, which necessitates a mechanism for removing these solids from food material. The infrabuccal pocket was first implicated as a receptacle for solids by WHEELER (1910). Once the pocket becomes full, its contents are ejected as a compact pellet (JANET, 1895). EISNER and HAPP (1962) showed conclusively that in the ant *Campanotus americanus*, the infrabuccal pocket and buccal tube act as a filtration device preventing solid particles > 150 μm from entering the crop and possibly blocking the passage of fluids through the proventriculus. More recently, QUINLAN and CHERRETT (1978) reported that the leaf cutting ant, *Acromyrmex octospinosus*, filters particles as small as 10 μm in diameter. In addition, the queens were able to transmit the symbiotic fungus associated with this species to newly founded colonies via the infrabuccal pocket contents. In the subfamily Pseudomyrmecinae, larvae are fed food pellets formed in the infrabuccal pockets of workers (WHEELER and BAILEY, 1920). TICE (1967), in studies of *Solenopsis saevissima richteri (=S. invicta)* males, found fine hairs on the ridged buccal tube, which she believed served a straining function.

We report here the results of our investigations of the ability of *S. invicta* workers and larvae to ingest microparticles and observations on the structures responsible for filtration, and the impact of our findings on the possible use of microparticles as vehicles for delaying the toxicity of bait toxicants.

MATERIALS AND METHODS

Adults and 4th-instar larvæ used in our studies were obtained from well established laboratory colonies originating locally from either newly-mated field-collected queens or from the excavation of mature field colonies. All colonies were maintained under ambient laboratory temperature and humidity with irregular illumination. Minor workers were selected at random for the experiments and then starved for a week or longer. Fluorescent latex microspheres (Polyscience, Inc. Paul VALLEY Industrial Park, Warrington Park, PA 18976) were obtained in 3 sizes, 4.5 ± 0.162 μm, 1.83 ± 0.58 μm and 0.88 ± 0.02 μm, as concentrated aqueous suspensions (2.5 % solids). Microsphere suspensions acceptable to

Table I. — Microfiltration by red imported fire ant workers. Analysis of ants fed honey-water containing fluorescent microspheres. Observations made within 1 hr of feeding.

Tabelle I. — Mikrofiltration der roten importierten Feuerameisenarbeiter. Analyse der Ameisen die mit Honigwasser genährt wurden, das fluoreszierende Mikrokügelchen enthält. Die Beobachtungen wurden binnen einer Stunde nach Fütterung gemacht.

		Avg. no particles/microscopic field *a/*	
Microsphere size (microns)	No. ants examined	Crop *b/*	Infrabuccal pocket *b/*
0.88	5	3.16	77.28 *c/*
1.8	8	0.02	44
4.6	5	0.007	26.0

a/ One microscopic field equals 1.33 mm^2.

b/ Five microscopic fields per ant examined.

c/ Buccal pellet ejected in 3 cases. One pellet count = 1760 particles/1.33 mm^2.

the ants as a food were prepared by dilution of the concentrated suspension with distilled water and raw honey (1:4: 5) (WILLIAMS et al., 1980). Evidence of ingestion was monitored by the addition of red food coloring to the final suspension.

Particle suspensions were offered to the workers by placement of a drop of the formulation in a shallow pan followed by introduction of about 30 workers. Workers were allowed to feed until satiated. The duration of feeding was recorded, then the ants were removed and placed in plastic cups. Ingestion was confirmed by dissection and microscopic examination of the crop, infrabuccal pocket, and infrabuccal pellets. Individual 4th-instar larvæ were removed from the colony and placed ventral-side-up on moist tissue paper in a petri dish. Latex spheres (5.70 ± 0.205 µm, 25.7 ± 10.0 µm, 45.5 ± 8.9 µm and 90.7 ± 17.7µm) from Polyscience, Inc. were suspended in water, mixed with dried egg yolk, and the mixture redried. This food formulation was placed on the anteroventral feeding region of the larvæ with a minuten pin mounted on a matchstick. After 2.5h (for 5.70µm spheres) or 13h (for other spheres), larvæ were dissected and the midgut contents examined for microspheres with compound and stereo microscopes.

For morphological and histological examinations, the infrabuccal pocket and the buccal tube were sectioned, prefixed in osmium tetroxide vapors, fixed in hot 2.5 % glutaraldehyde 1 % acrolein, and postfixed in osmium tetroxide. The fixed specimens were *en bloc* stained with 0.5 % aqueous uranyl acetate, dehydrated with 2,2-dimethoxy-propane, and embedded in a Spurr-Poly Bed® mixture. The 0.5 µm sections were stained with toluidine blue.

RESULTS

The data in table I clearly show that very few particles of any size reached the crop of minor workers and that the particles were being effectively concentrated in the infrabuccal pocket. The increase in numbers

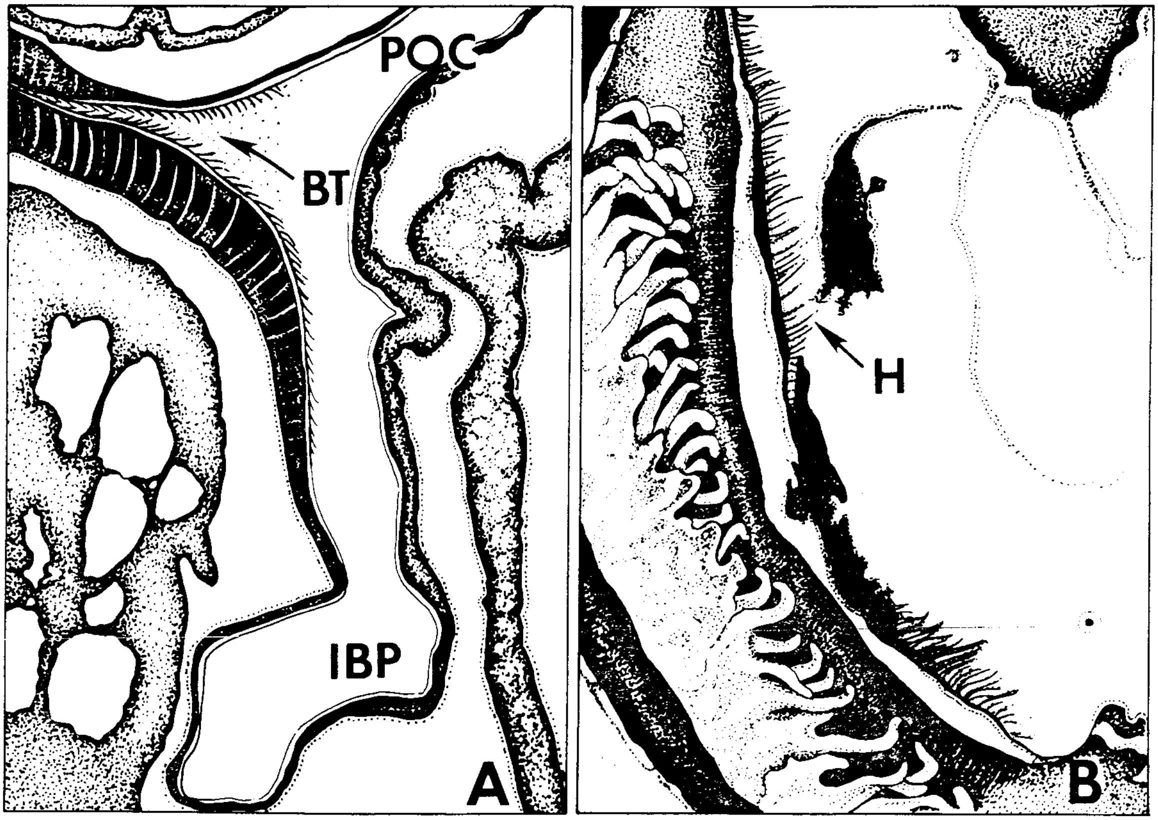

Fig. 1. — Section through head of worker ants of *S. invicta*. A. Showing the infrabuccal pocket (IBP), the lined buccal tube (BT), and the preoral cavity (POC). B. Section showing long hairs (H) lining the anterior margin of the buccal tube (X625).

Abb. 1. — Kopfschnitt von Ameisenarbeiter *Solenopsis invicta*. A. Sichtbar sind infrabukaler Raum (IBP), die bukale Röhre (BT), und der Mundersvorraum (POC). B. Langes Haar (H) am vordern Rand der bukalen Röhre (X625).

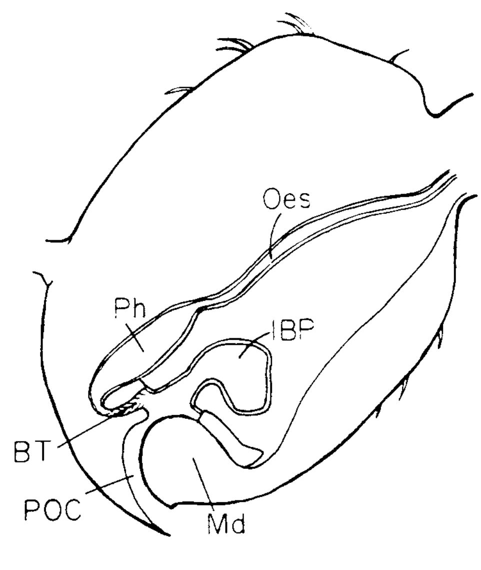

Fig. 2. — Cross section of an ant head showing the mandible (MD), preoral cavity (POC), buccal tube (BT), pharynx (Ph) and œsophagus (Œs).

Abb. 2. — Kreuzschnitt durch einen Ameisenkopf. Sichtbar sind Kiefer (MD), Mundersvorraum (POC), Bukale Röhre (BT), Rachen (Ph), und Speiseröhre (Oes).

of particles in the infrabuccal pocket as particle size decreased is consistent with the closer packing possible with smaller particles. Although particles of 0.88 μm started to show some breakthrough into the crop, we were unable to determine the exact lower limits of the filtration mechanism because smaller particles cannot be reliably detected by light microscopy. Ants examined 1 hr after feeding had significantly lower numbers of particles in their infrabuccal pocket then those examined immediately after feeding because of ejection of the pocket contents. In the single case in which red food color was observed in the midgut, only 3 microspheres (1.8 μm) were found in the entire sample. The microparticles in the infrabuccal pellets were so concentrated (1435-1760 particles/field) that counting was difficult.

Microscopic examination of prepared sections of the preoral cavity, the infrabuccal pocket, and the buccal tube showed the presence of very stiff, hair-like projections on both the upper and lower walls of the buccal tube (fig. 1 A). In addition, long hairs were found to line the anterior margin of the dorsal lip of the tube (fig. 1 B). The spatial relationship of these ingestion elements in the head are shown in figure 2.

The maximum size of the latex spheres swallowed by larvae were in the 45.8 μm range. Dissection revealed that these spheres were approximately the same diameter as the esophagus where it enters the midgut via the cardiac valve. Sphere in the 90.7 μm range were not ingested but remained on the anteroventral body region of the larvae after the egg yolk had been ingested.

DISCUSSION

Our experiments showed that adult red imported fire ants have a highly efficient mechanism for filtering small particles from ingested fluids. Although our observations did not elucidate the mechanism in detail, it does involve ridges and hairs at the entrance to and in the buccal tube. These structures provide a system of passive filtration in which semi-liquid food is taken into the infrabuccal pocket, and by compression, the liquid is forced through the filtering hairs into the buccal tube. The resulting buccal pellet is then ejected or fed to larvae. In the course of trophallaxis, the distributed liquid is subjected to many filtrations.

Although the occurrence of this mechanism is not surprising (adult Hymenoptera are generally regarded as being liquid feeders), the extremely small particle size ($<$ 1μm) capable of being filtered was not expected. In consideration of development of controlled-release formulations of fast-acting toxicants involving any kind of solid dispersion, these data offer little hope for success. Present technology is capable of producting 1-5 μm microencapsulated toxicants, but these would be readily filtered. Matrix formulations, when ground to fine particle size, offer such a large surface area that rapid diffusion into the carrier food source simply mimics the activity of the unadulterated toxicant. The data on particle ingestion by 4th-instar larvae suggests that this stage could be a target for entrapped toxicants. However, this approach would not be effective for killing an entire colony unless the toxicant was constantly available to the larvae and thus prevented replacement of adult workers as they died of natural causes.

EISNER and HAPP (1962) reported that individuals of *C. pennsylvanius* were capable of infrabuccal pocket filtration of particles as small as 150 μm and that these filtration capabilities were magnified during trophallaxis by repeated regurgitation and refiltration of crop contents. They further stated that this mechanism was probably more important in the subfamilies Formicinae and Dolichoderinae than other subfamilies because their proventriculus is especially adapted to dam the posterior outlet of the crop and is thus more susceptible to particulate blockage. Consequently, a replete caste has evolved in these two subfamilies. In contrast, the proventricular structure of the myrmicines can allow ingestion of particles and may reflect feeding habits involving solids (EISNER, 1957). However, in opposition to this scheme are the results of QUINLAN and CHERRETT (1978) on the myrmicine leaf cutting ant, *Acromyrmex octospinosus*, which is capable of filtering particles of 10 μm diameter from liquid food. The highly efficient filtration mechanism was suggested to be necessary because *A. octospinosus* does not practice trophallaxis and therefore requires a very efficient single-pass filter (QUINLAN and CHERRETT, 1978). Our data on *S. invicta*, another myrmicine, reveal that it has a filtration system at least 10 times more effective than that of *A. octospinosus*, and that *S. invicta* actively exchanges food by crop regurgitation. These data suggest that either particle filtration is unrelated to the structure of the proventriculus or that the proventriculus structure varies a great deal within subfamilies. The report that *S. invicta* has a replete caste (GLANCEY, *et al.*, 1973) lends some support to the latter

Table II. — A comparison of particle size filtered and average worker size for three species of ants.

Tabelle II. — Grössevergleich der filtrierten Partikelchen und Durchschittsgrösse von drei Ameisenspezies.

Species	Smallest particle size filtered (μm)	Average worker size (mm)
Campanotus pennsylvanius	150 *a/*	9.5 *c/*
Acromyrmex octospinosus	10 *b/*	6.0 *d/*
Solenopsis invicta	> 0.88	4.4 *c/*

a/ EISNER and HAPP, 1962.
b/ QUINLAN and CHERRETT, 1978.
c/ SMITH, 1965.
d/ WEBER, 1972.

possibility. Until a thorough survey of ant proventriculi and filtration capabilities is made, a definitive answer to their relationship is not possible.

The three species investigated are obviously capable of filtration at considerably different levels of efficiency. This may not reflect a difference in filtration mechanism but instead simply the relative size of the ants investigated. QUINLAN and CHERRETT (1978) have already shown, in their studies of particle ingestion in minima, media, and maxima workers of *A. octospinosus,* that filtration capabilities within a species vary with worker size. The size of particles filtered by the infrabuccal pocket apparatus does decrease with the average worker size of the species so far investigated (table II).

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