

ASPECTS OF NECROPHORIC BEHAVIOR IN THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*

by

DENNIS F. HOWARD and WALTER R. TSCHINKEL ¹⁾

(Dept. of Biological Science, Florida State University, Tallahassee, Fla., USA)

(With 5 Figures)

(Acc. 17-I-1975)

INTRODUCTION

Among the social Hymenoptera, the most conspicuous exhibition of nest cleaning activity is the stereotypic removal of dead colony members from ant nests. The term "necrophoric behavior" has been applied to distinguish the disposal of corpses from other sanitation tasks (WILSON *et al.*, 1958) and WHEELER (annotation in translating REAUMUR, 1926) indicates that in many species of ants, workers discard uneaten food and other wastes with corpses at common refuse piles. The bulk of the literature on formicid necrophoric behavior is descriptive and ancient naturalists particularly were inclined toward anecdotal discourses on "ant funerals" (WHEELER, in REAUMUR, 1926).

McCOOK's (1879, 1882) account of corpse-disposal behavior in the harvester ant, *Pogonomyrmex barbatus* F. Smith and several species of honey ant was among the earliest of more objective studies. His observations revealed that dead workers are transported out of the nest by their sisters and deposited in a heap near the entrance. More recently, MAETERLINCK (1930) cited the occurrence of necrophoric behavior in the ant, *Solenopsis fugax* Latreille and similar observations have been recorded for the fungus gardening ant, *Atta texana* Buckley (MOSER, 1963; WEBER, 1972), and the primitive Australian ant, *Myrmecia vindex* Clark (HASKINS, 1970). Even the nomadic species of *Eciton*, the army ants, remove their dead from temporary bivouac sites (RETTENMEYER, 1963).

Experimental investigations of sanitation behavior have been limited to a few studies of necrophoric behavior in ants. Pioneering work was under-

1) We wish to thank Duane MEETER of the Statistical Consulting Center at Florida State University for his competent statistical advice and aid, William F. HERRNKIND for help with the analysis of orientation data and Charles R. STASEK for critical reading of the manuscript.

taken by WILSON *et al.* (1958), who studied the nature of stimuli releasing corpse-transport in the harvester ant, *Pogonomyrmex badius* Latreille. Filter paper squares treated with acetone extracts of *P. badius* corpses were carried by workers to the refuse pile in the same manner as corpses. Although the active component could not be identified, the fatty acids accumulating as a result of decomposition were implicated and bits of paper coated with synthetic oleic acid typically elicited a necrophoric response.

BLUM (1970) confirmed WILSON *et al.*'s conclusion that oleic acid and other unsaturated fatty acids release corpse-removal behavior in *Solenopsis invicta*. Even the primitive ant, *Myrmecia vindex*, responds to objects coated with oleic acid by carting them to the refuse pile (HASKINS, 1970).

Evidence that cues other than chemicals serve to release necrophoric behavior has not been reported. When *P. badius* colonies were offered corpses that had been leached in acetone for as much as three weeks and thoroughly dried, the corpses were either dragged into the nest or dismembered (WILSON *et al.*, 1958). Freshly frozen workers of *S. invicta* are also treated unlike corpses by their nest mates (BLUM, 1970). Furthermore, WILSON *et al.* (1958) have furnished convincing evidence for the effectiveness of a chemical in provoking necrophoric behavior. After healthy *P. badius* workers received a light application of oleic acid and were returned to their nest, workers encountering the treated ants quickly seized them and transported them to the refuse pile. Apparently, any signals the treated ants may normally offer are overcome by this substance.

While experimental studies have yielded insight into the chemical mediation of necrophoric behavior in ants, other aspects of the problem have been ignored or require clarification. That the releaser is purely chemical has not been rigorously tested in a species other than *P. badius*. The appearance of the signal after death and factors that lead to refuse pile formation are other interesting questions. This paper represents an effort to provide answers to these and other questions relating to the disposal of corpses from the nest of the red imported fire ant, *Solenopsis invicta* Buren.

MATERIALS AND METHODS

Colony maintenance.

Eight colonies of *Solenopsis invicta* Buren were collected in February, 1973, from a vacant pasture in Leon County, Florida. Each colony included a reproductive queen, copious supply of workers, male and/or female alates, and brood in various stages of development. Each colony was separated into two populations: a) an "experimental colony", consisting of the queen, 5000 randomly selected workers, and 50 virgin sexuals and b), a "stock population" comprising the balance of individuals. Stock populations were situated in soil-filled fiberglass bins, and the experimental colonies were transferred to eight circular arenas, each constructed by securing a 6" high strip

of clear stiff acetate around the perimeter of a plywood circle 2' in diameter. The floor of the arena was covered with brown paper taped at the perimeter and a 100 mm plastic petri dish in the center of each arena served as a nest. Twelve equally-spaced entrance grooves were cut on the rim of the bottom plate of each petri dish and a sheet of clear red acetate was affixed to the top plate to darken the nest. A 3/8" hole was drilled through the top plate to facilitate placement of objects into the nest. A core of wooden toothpicks encased in plastic tubing functioned as a water wick by passing through a hole in the nest and arena floors to a cup of water below. The acetate walls of the arenas were dusted with talc and potential avenues of escape were sealed with wax. Four of these arena-nests were used for releaser experiments (Colonies A, B, C, D) and four for orientation experiments (Colonies I, II, III, IV).

All populations were given an artificial diet (BHATKAR & WHITCOMB, 1970) as well as peanut butter and minced mealworm larvae. In order to preserve the radial symmetry of the arenas, food was placed atop the center of each nest on small trays of acetate or aluminum foil. The colonies were not fed while experiments were in progress and the food trays were removed at least 20 minutes prior to the initiation of any experiment. Temperatures recorded in the laboratory ranged from 18 to 22°C. and the light regime varied according to the experiment.

Bioassay of necrophoric releasers.

WILSON *et al.* (1958) found that the effectiveness of materials as releasers of necrophoric behavior in *P. badius* could be estimated by placing baits in groups of five a short distance from the nest and monitoring the proportion of baits carried to the refuse pile within five minutes. We used this procedure with minor modifications. Two groups (treatment and control) of five baits each were placed on two, 1/2" X 1-1/2" paper cards in a line midway between the nest and the refuse pile. If a worker removed a bait within five minutes and carried it to the refuse pile or arena wall within eight minutes, the bait was defined as having released necrophoric behavior. Any bait not treated in this manner was considered a negative response. Four experimental colonies (henceforth referred to as Colony A, B, C, and D) were subjected to two replicates of this assay, both replicates constituting a trial. In a single trial, a maximum score of 40 is possible for all colonies and the results are reported as a proportion, X/40, where X is the total positive responses. Unless otherwise specified, statistical significance was determined by X² analysis.

The positions of treatment and control baits were reversed between replicates and the sequence of colony testing randomized.

RESULTS

RELIABILITY OF THE BIOASSAY

The criteria of the bioassay allow necrophoric behavior to be distinguished from unrelated, but superficially similar worker activities. When worker-sized bits of wood, paper, or metal shavings were placed between the nest and a discrete refuse pile, the objects were ignored or hauled over short distances, but never toward the refuse pile. Food was transported directly into the nest. The controls, 24-hour dead corpses, placed in the same position were invariably carried away from the nest in the general direction of the refuse pile. Although many corpses were not taken directly to the pile but to the nearby arena wall, they were eventually deposited at the refuse pile. The time limits adopted in the bioassay were sufficient for workers con-

sistently to remove most, but not necessarily all, of the control corpses (24 hours-decomposed). When placing baits in the arena, care was taken to avoid alarming the colony since severe disturbances brought large numbers of frenzied workers into the foraging area and corpses that were normally inspected and removed were now ignored or even attacked.

Because no effort was made to maintain each colony at its original size and experiments were conducted at differing times, fluctuations in the number of workers available may have altered a colony's capacity for necrophoric tasks. However, a comparison of the total positive responses within any experiment reveals that no significant differences occurred among colony scores (Goodness of fit, X^2). Moreover, the similarity in mean scores for control corpses in each experiment precludes the possibility that the necrophoric response changed significantly during the course of the investigation ($p > 0.05$, by single factor analysis of variance). After corpses were placed on the assay site, workers appeared to discover them by chance contact. The discovering worker probed the corpse with antennae and forelegs for less than one minute, moving slowly, giving equal attention to all parts of the inert body. Thereafter, the corpse was usually lifted by grasping the petiole or pronotum of the dead ant with the mandibles and leaning back while hoisting the ant with a slow, upward movement of the head. The corpse was adjusted by manipulation with the forelegs and mandibles until it was turned nearly vertically, with its legs facing away from the carrier. Events leading to the deposition of corpses are described later.

APPEARANCE OF THE SIGNAL AFTER DEATH

BLUM (1970) reported that *S. invicta* workers treat recently killed workers differently than those that have been dead a few hours. In a preliminary experiment, we tested the appearance of the necrophoric releaser by simultaneously bioassaying three types of baits on each colony: a) five workers killed by freezing in a vial in a dry ice — acetone bath, thereafter left in an open container at room temperature for 24 hours, b) five workers identically prepared but refrozen and thawed immediately before presentation to the colony and c) five workers killed by flash freezing and presented directly to the colony. To obtain a rough estimate of the frequency with which corpses were visited, the number of workers inspecting each type of corpse was noted at one minute intervals beginning at 30 seconds. A total of 30 corpses were presented per colony in two replicates. No colonies were tested consecutively and the configuration of the cards was randomized with respect to replicate and colony.

The experiment revealed distinct differences between the necrophoric

releaser effect of freshly-killed and aged corpses. Both groups of corpses that had decomposed for 24 hours were carried toward the refuse pile soon after their introduction and no significant differences in worker responses to these groups existed either for mean time until necrophoric release (24 hr-dead, 2.0 min. \pm 1.6 S.D.; 24-hour dead-refrozen, 2.1 min. \pm 1.5 S.D.; $p > 0.05$, analysis of variance) or number of visits prior to removal (X^2 analysis). Of the 80 one day-decomposed corpses presented, only five were not removed within five minutes, while 39 of 40 (95%) freshly-killed workers still remained on cards (Mean response time: 31.3 min., S.D. = 14.9). This was significantly greater than either group of one day-decomposed corpses ($p < 0.01$ by Mann-Whitney rank test within each colony across tests).

As indicated by BLUM (1970), workers responded to fresh corpses with vigorous and extended inspection, and as a result, they tended to congregate around these corpses in greater proportions than others. This is reflected in the higher mean number of visitors to fresh *vs.* refrozen corpses in the first few minutes of the experiment: X^2 analysis confirms that these differences are significant ($0.01 < p < 0.025$) for the first three minutes. Thereafter, the number of refrozen ants remaining is insufficient to permit statistical comparison between groups. For fresh corpses, there is an approximately 50% reduction in worker visits followed by stabilization to about one visit per sample after 20 minutes. Variability increased with time due to the gradual depletion in sample size. Curiously, none of the corpses were taken toward the nest.

No postural changes in the freshly killed workers could be correlated with the delay in releasing necrophoric behavior. When removed from the freezing bath, the gaster of each ant was already curled under its thorax and the legs folded in a position typical of older corpses. The similarity in treatment of refrozen and non-refrozen 24 hours-dead corpses discounts the possibility that a temporary chill from the bath seriously affected the necrophoric response.

If a chemical cue is solely responsible for the release of necrophoric behavior, it must be generated or unmasked very quickly. Indeed, more than half of the freshly-killed workers had been transported toward the refuse pile within 30 minutes. While a postural necrophoric signal now seemed unlikely, the onset of rigor *per se* as a potential cue remained to be tested. Rigor appeared gradually in freeze-killed workers but could be achieved immediately by killing ants with a 15 second exposure to 100°C. This experiment would also discern enzymatic from non-enzymatic processes of signal generation. In two separate experiments, workers were killed by either

method, held at room temperature for predetermined intervals of 0-80 min. (in 5 min. increments) and subjected to the bioassay. Controls consisted of 24 hours-dead workers re-treated with the applicable temperature.

The results of these experiments (Fig. 1) reveal that the necrophoric signal appears gradually over about an hour and is independent of the degree of stiffness in freshly killed corpses. No significant differences existed in scores for heat and freeze-killed workers at any stage of decomposition. Both types of corpses elicited a small proportion of positive responses (3/40) within five minutes of their demise and analysis by Kolmogorov-Smirnov

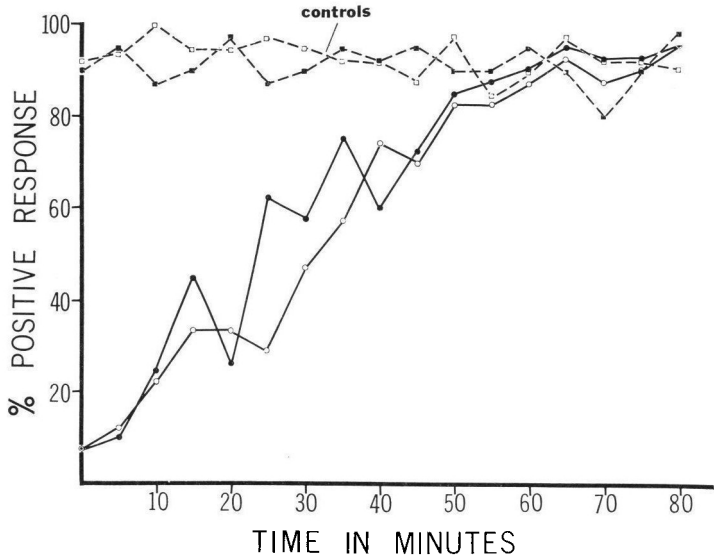


Figure 1. Percent positive necrophoric responses to heat and freeze-killed workers as a function of time since death. Solid circles joined by solid lines: heat-killed workers. Solid squares joined by broken lines: 24 hours-dead, reheated workers. Open circles joined by solid lines: freeze-killed workers. Open squares joined by broken lines: 24 hours-dead, refrozen workers. $n = 40$ for all cases.

goodness of fit shows that responses increased from 0 to 50 minutes ($p < 0.001$) and were evenly distributed after 50 minutes ($p > 0.05$). When heat and freeze-killed workers had been dead for 55 minutes or more, they were treated like controls ($p > 0.05$, by X^2 analysis). Scores were more variable for ants that had died 10 to 50 minutes before assaying (S.E. = 20-50%) and approximated the variance for controls at other times (S.E. = 18-30%).

Results with the bioassay are in agreement with those of the preliminary experiment. The T_{50} (time at which corpses elicited a 50% response to

freeze-killed workers) was about 30 min. and agrees with the mean 31.3 minutes for the first experiment. If the intensive investigations of corpses by workers altered the development of the necrophoric signal, disparity would be expected between the results of the bioassay, which limited such interactions to five minute periods, and the preliminary experiment, where worker-corpse contact was permitted for prolonged sessions. Since no obvious divergences occurred, intrinsic changes in fresh corpses must be primarily responsible for the appearance of the death signal.

At least some workers are able to evoke necrophoric behavior minutes after death, but is mortality a necessary prerequisite for this capacity? If not, workers might treat motionless, but living workers like fresh corpses. A sample of workers was thus collected from Colonies A-D, anesthetized at 15°C. with carbon dioxide, and presented in their home arenas for bioassay in two trials. Although this procedure kept the ants quiescent for only two to four minutes, coordinated locomotory functions did not return until after the bioassay. Anesthetized ants drew a wide range of responses (ignoring, inspection, tugging) from workers, but not necrophoric behavior. Controls (24 hours-dead workers) were hauled to the refuse pile rapidly. The results suggest that immobility in itself plays an insignificant role in releasing necrophoric behavior. However, bait movement during assay and the low expected frequency of positive responses prevents stronger interpretation.

REMOVAL OF THE SIGNAL BY EXTRACTION

In the final application of the bioassay, worker responses to extracted corpses were tested for evidence of a non-chemical necrophoric signal. A large number of corpses was subjected to serial Soxhlet extractions with *solvents* (methanol, tetrahydrofuran, ether, each for two 12-hr periods) to remove all extractable chemical cues. The cumulative removal of signal was determined by assaying corpse (dried 15 min.) and extract samples (3 ant-equiv. applied to each 2 × 4 mm twisted filter paper bit) after each 12 hour extraction. Corpse controls were 24-hr dead workers, extract controls were an equal volume of clean solvent on filter paper bits. The results are summarized in Table 1.

The first and second extractions in methanol did not diminish the proportion of positive responses to the corpses even though worker treatment of extracts showed that some signal was removed. Following extraction with tetrahydrofuran (THF), the corpses elicited significantly fewer necrophoric responses, but scores for the resultant THF extracts indicated that a relatively small quantity of chemical cue had leached out in this solvent.

TABLE I

Fraction of positive responses to serially extracted corpses and resultant extracts

Solvent	Extracted corpses		Extract on filter paper	
	Treatment	Control	Treatment	Control
1st Methanol	37/40	35/40	32/40	0/40
			38/40 *	6/40 *
			39/40 **	0/40 **
			p < 0.001	
2nd Methanol	37/40	36/40	12/40	0/40
			p < 0.005	
1st Tetrahydrofuran	35/40	39/40	4/40	1/40
			p < 0.005	
2nd Tetrahydrofuran	22/40	35/40	2/40	0/40
1st Ether	17/40	38/40	0/40	0/40
2nd Ether	15/40	37/40	0/40	0/40
			p < 0.005	
1st KOH, water rinse	2/40	39/40	—	—
2nd KOH, water rinse	0/40	36/40	—	—

* Extract tested on completely extracted corpses, dried 15 min.

** Extract tested on completely extracted corpses, dried 30 min.

Further extraction with ether did not significantly reduce the releaser effectiveness of the corpses and no necrophoric stimulant appeared in the extract. After the last ether extraction, the corpses were cleared in potassium hydroxide for two 24 hour periods, and this caused them to lose all capacity to activate necrophoric behavior. Workers contacting these bodies rarely stopped to inspect them, and just one corpse was lifted from the card only to be dropped seconds later. The KOH extract was not tested.

It seemed possible that thoroughly extracted corpses did not release necrophoric behavior because of some repellent arising from the extraction or hydrolysis. Such repellency might also interfere with removal of cleared corpses to which known releasers had been added. Colonies were therefore offered corpses that had been subjected to the entire extraction and clearing series and treated with three ant-equivalents of the first methanol extract. A control was provided by adding an equal volume of clean methanol to extracted workers. Both groups were dried for 15 minutes before testing. In the first trial, the extract-readded corpses consistently elicited positive responses in the bioassay, demonstrating no adverse effect from a repellent. Unexpectedly, a small fraction of the control corpses were also carried toward the refuse pile. An additional trial with a 30 minute drying period did not affect the scores for the extract-readded baits but eliminated necro-

phoric responses to controls. Thus, residual solvent probably resulted in the positive responses to the controls. Completely extracted and dried corpses are void of necrophoric signals.

DIVISION OF NECROPHORIC LABOR BY SIZE

In a mature colony of *Solenopsis invicta*, the worker population is moderately polymorphic, with worker size ranging monophasically from minors to majors. Although distinct morphological castes do not occur, workers of a particular size class might indulge in necrophoric activity at a greater than random frequency. Using scape length as a convenient index of worker size (WILSON, 1953; WALSH, 1972), the size distribution of 150 randomly sampled, freeze-killed workers from colony A was compared against that of 150 workers performing necrophoric labor. One day after death, corpses were placed in the nest of their home colony. Workers were captured as they carried the dead (these were not collected) to the arena wall, killed and their scapes measured.

The resulting distributions for the two samples were nearly superimposable. The mean scape length of the random sample [$0.68 \text{ mm} \pm 0.09$ (S.D.)] and that of the corpse-carrying workers, [$0.69 \text{ mm} \pm 0.09$] were not significantly different ($p > 0.2$, log transformation of the data and t-test).

WORKER ORIENTATION DURING NECROPHORIC BEHAVIOR IN THE FIELD

Just as the presence of a releaser is critical to the initiation of necrophoric behavior, the direction of movement chosen by corpse-bearing workers decides the ultimate location of the dead. We thus investigated where, relative to the nest, fire ant workers deposit corpses both in laboratory and field colonies. In addition, the effects of a variety of environmental stimuli on the

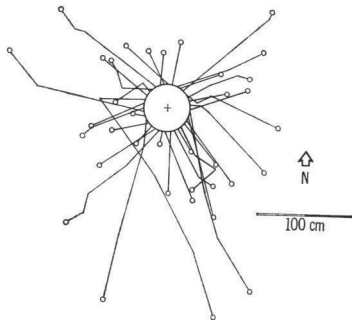


Figure 2. Scale diagram of paths taken by necrophoric workers in relation to the mound. The large circle represents the perimeter of a single mound and the small circles indicate corpse deposition sites (see text).

direction and site of corpse deposition were examined. Through careful examination of several fire ant mounds and their vicinity (4m radius) scattered corpses and fragments of insect cuticle were located but no discrete piles were found. One colony on a level lawn was chosen to verify that refuse piles were not being overlooked. Over a six day period, the colony was provided with a total of 36 corpses, placed at 10° intervals (compass bearings) on the perimeter of the mound. At three minute intervals, color-coded pins were used to mark the trail of corpse-carrying workers, shown in Figure 2 as lines radiating from the mound. Since the duration of corpse-transport exceeded three minutes in all but two cases, the tendency of workers to travel straight, more or less radial paths is clearly demonstrated. The dead were carried from 7 to 315 cm and only 5 of the 36 corpses were deposited within 31 cm of the mound border (the approximate radius of an experimental arena), but there was no common deposition site.

Events leading to the deposition of corpses varied considerably. Workers sometimes dropped their burden upon interruption by other ants, or when the dead occasionally became entangled in vegetation. Typically, however, no obvious extrinsic stimuli were observed. In such instances, the dead were lowered to the ground and, without any attempt to bury their former burden, workers returned to the mound.

The ability of corpse-bearing workers to travel long distances in a straight line suggested sun-compass orientation. Therefore, the same colony was tested by the classical technique of SANTSCHI (1911). As soon as the heading of a necrophoric ant was ascertained, its view of the sun was blocked by a sheet of cardboard and a mirrored solar image was provided from a radically different horizontal bearing but the original vertical angle. If the ant navigated by maintaining a constant bearing relative to the sun, it would be expected to adjust its heading to the same azimuth for the "new" sun. After testing 15 workers by this procedure, a sun-compass was clearly implicated. Table 2 shows that the ants responded to apparent changes in solar position ranging from 70° to 155° with a maximum directional error of only $\pm 45^\circ$. Rayleigh's test revealed that the errors were not uniformly distributed over all possible bearings whereas the original and adjusted headings were. The V-test indicated that the errors were concentrated around a predicted value of 0° and further analysis by STEPHENS' test (1962) proved that the mean directional error did not deviate significantly from 0° ($p > 0.05$).

In order to characterise corpse disposal in a larger number of colonies, active mounds on terrain ranging from nearly level lawns to sparsely weeded, 15° inclines (measured with an angular level, Warren-Knight Co.) were selected for study. Over a period of two weeks, 250 workers were collected

TABLE 2

Deviations between headings taken by necrophoric workers and those predicted by sun-compass orientation; data in degrees

Original heading	Difference between sun and "new" sun azimuths	Expected adjusted heading	Actual adjusted heading	Error	
215°	— 110	105	110	+ 5	
285	— 115	170	150	— 20	
275	— 135	140	95	— 45	
130	+ 115	245	215	— 30	
120	+ 140	260	270	+ 10	
125	+ 95	220	240	+ 20	
200	+ 110	310	290	— 20	
125	— 135	350	10	+ 20	
10	+ 155	165	190	+ 25	
145	+ 120	265	250	— 15	
165	+ 130	295	305	+ 10	
0	+ 150	150	180	+ 30	
135	+ 120	255	235	— 20	
125	+ 100	225	235	+ 10	
335	+ 70	45	90	+ 45	
Mean vector length:	0.31	—	0.35	0.36	0.92
Mean angle:	146.3°	—	226.5°	217.7°	1.8°
Rayleigh test:	n.s.	—	n.s.	n.s.	p < 0.001
V-test:	—	—	—	—	p < 0.0001

from each colony, killed by freezing, and lightly sprayed with red, fluorescent paint. At dawn of experimental days, the marked corpses were returned to the galleries of the nest through a small hole cut at the apex of the mound. The hole was recapped and, owing to early morning low temperatures, alarm responses by the disturbed colonies were avoided. Shortly after sunset of the same day, the mound and its vicinity were carefully inspected for the marked ants with the aid of a portable ultra-violet lamp. The location of corpses was recorded and the region was further examined in daylight.

Only a small fraction of the marked corpses were recovered, although later excavation of the mounds confirmed that all had been moved. As in the preliminary study, corpses were dispersed in each of the foraging areas, but refuse piles were also discovered near 15 of the 20 mounds surveyed (Fig. 3). Unlike those formed in the laboratory, refuse piles in the field were inconspicuous and often situated near the border of the nest. An at-

tempt to correlate corpse and refuse pile locations with potential directional information (slope, positions of landmarks and the sun, magnetic north) showed only the downhill bearing from the mound to have any relationship. In colonies established on inclines ranging from 5 to 15° no corpses were deposited uphill of the mound. For mounds on slopes of less than 5° this relationship partially held for refuse piles but not for individual corpses. However, unavoidable inaccuracy arose in assigning downhill vectors and gradients to mounds on slight inclines and may have exaggerated this effect. A total of eighty-four fire ant colonies established on slopes of various magnitude were surveyed for refuse piles. Single refuse piles were discovered at 19 nest sites, multiple piles at 32, and for 33 colonies, no refuse piles could be found. Again, the location of refuse piles correlated with the downhill vector (Fig. 3), but not with the other orientation cues. Moreover, as slope increased, the proportion of mounds with refuse piles also increased: Of colonies surveyed on grades of less than 5°, only 46% had at least one refuse pile. For slopes of 6 to 10°, 69% had refuse piles and for slopes of 11 to 20°, 86% showed refuse pile formation.

Many of the refuse piles were located just beyond tunnel openings, from which workers periodically emerged carrying bits of debris and an occasional corpse. Upon reaching the refuse pile, they either dropped their cargo immediately or began a series of slowly executed, tight turns across the pile.

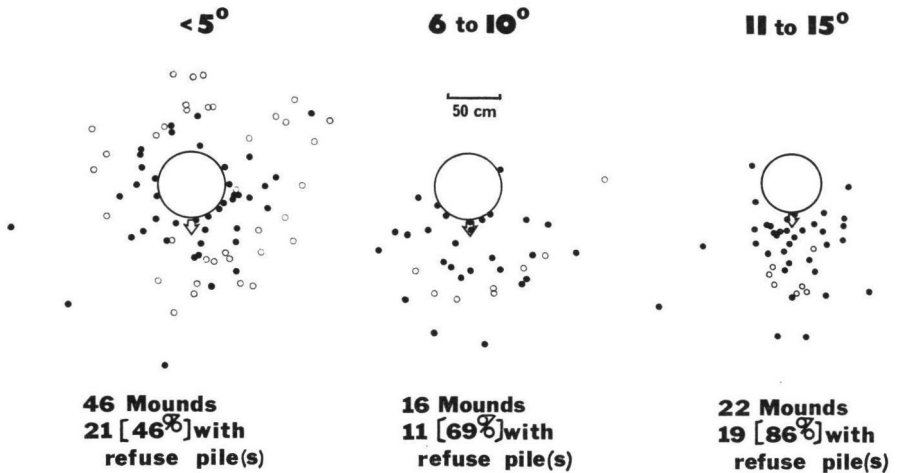


Figure 3. Refuse pile and corpse locations in relation to the mound for various slopes. Large circles: mound perimeter; arrow: downhill direction; small, open circles: individual marked corpses; small, closed circles: refuse piles. Number of mounds comprising each composite figure is indicated, as are the number and proportion near which refuse piles were found. Data from surveys and marked-corpse experiments are combined.

This locomotory pattern was often interrupted as the workers stopped to probe through the rubbish until the burden was finally deposited. Although some workers stayed on at the pile, picking up and dropping assorted refuse, none made obvious attempts to bury its contents.

The refuse piles were heavily infested by scavenging mites that apparently fed on decomposing corpses and rubbish but could not be found on living ants. Also located in the piles were numerous patches of tacky, dark brown, fecal material.

Only one instance of cannibalism was observed at refuse piles in the field (a dead female alate). Many of the unmarked corpses in the refuse pile had been dismembered, but this may have resulted from erosion or other factors unrelated to cannibalism. Observations of laboratory colonies confirmed that partial cannibalism of deceased sexuals frequently occurred, but consumption of dead workers was limited to the gasters of a few major workers.

Workers from two other ant species were seen gathering material from fire ant refuse piles. In sunny, open areas, colonies of *Conomyrma pyramica flavopectus* Smith were often intermingled with fire ant mounds. These workers were treated aggressively by *S. invicta*, but they occasionally took corpses from the refuse pile to their own nest. WILSON *et al.*, (1958) reported that *C. pyramica* collects corpses from *Pogonomyrmex badius* colonies as a source of food. A regular visitor to fire ant refuse piles on borders of wooded zones was the primitive fungus gardening ant, *Cyphomyrmex rimosus minutus* Mayr., which is not treated aggressively by *S. invicta*. *Cyphomyrmex* workers incorporate discarded fire ant corpses and fragments of insect cuticle into the substrate of the fungus garden. *Cyphomyrmex* and *Conomyrma* may partly account for the low rate of recovery of marked corpses, although recovery was also low where neither was present.

LABORATORY STUDIES OF WORKER ORIENTATION DURING NECROPHORIC BEHAVIOR

Field surveys were useful for correlating patterns of corpse-disposal with orientation cues, but causal relationships could only be established through carefully controlled experiments. Corpse-bearing workers leaving the nests in arenas I through IV were supplied with various directional cues in the laboratory to determine which cues favored refuse piles over dispersal. The colonies had been established for several months and in three of the four (colonies I, II, and IV) a single pile of rubbish and corpses had been formed at the periphery of the arena. Colony III maintained two refuse piles approximately six inches apart on the arena border. Five experiments tested four different potential directional cues: 1) no horizontal

directional cues (*i.e.* a radially symmetrical field); 2) half of the arena wall was lined with black posterboard, half with white; 3) a 1½" vertical black stripe was provided on a white arena wall; 4) an incandescent light was provided from a 45° angle rather than directly over the nest; 5) the inclination of the arena floors was changed from level to sloped at 5°, 10° and 15°. In each experiment, dead workers were taken from the refuse pile, poured into the center of the nest and the headings of 45 necrophoric workers observed. We also noted whether each worker dropped its corpse at the first point of contact with the arena wall or turned to carry it clockwise or counterclockwise. Corpses were only rarely deposited other than at the base of the arena wall and were never returned to the nest.

Analysis of the necrophoric headings (Rayleigh's test) indicated that these were randomly distributed ($p > 0.05$) for all but the last set of experiments (slope) indicating that the refuse pile, landmarks and position of light do not affect the headings of necrophoric workers. Furthermore, there was no significant difference in choice of clockwise or counterclockwise directions, except for the experiment in which light was presented from an angle. The ants were significantly positively phototactic ($p < 0.001$ Yate's corrected, goodness of fit). In the first three experiments, the paths to the arena wall followed by necrophoric workers were often very looping and indirect, but when light was presented from an angle these changed to straight radial courses.

In the final experiments, colonies were tested with the floor of the arena tilted at various angles (5, 10, 15°). The refuse pile was situated directly uphill of the nest for each slope and downhill on one 10° incline. Because the shield encompassing a tilted arena was no longer perpendicular to the foraging floor as viewed from within, it would appear higher at the downhill side and lower at the uphill side. A modified shield, cut diagonally from the top to a point 16 cm lower on the opposite side was utilized to simulate the visual field surrounding an arena tilted at 15°. This shield was placed around a level arena with the refuse pile at the simulated downhill side. Under these conditions, all colonies responded with uniformly distributed headings ($p > 0.05$), indicating that an unmodified shield could be used without artifact.

Unlike other cues, slopes clearly affected the headings taken by corpse-bearing workers, even on an incline as small as 5°. Necrophoric workers displayed a decisive downhill preference, independent of whether the refuse pile was uphill or downhill (Fig. 4). Many workers that began their journeys uphill gradually changed to downslope and workers moving downhill often traveled a pendular course, oscillating about the true downhill direction. Ray-

leigh's test for uniformity was rejected at $p < 0.01$. Strengthened by the concentration of headings around the downhill direction, the V-test was rejected at $p < 0.0001$ for all distributions except colony I at 5° , where $p < 0.005$. Furthermore, no mean heading deviated significantly from directly downhill of the center of the nest (Stephens' test, $p > 0.05$). As shown in

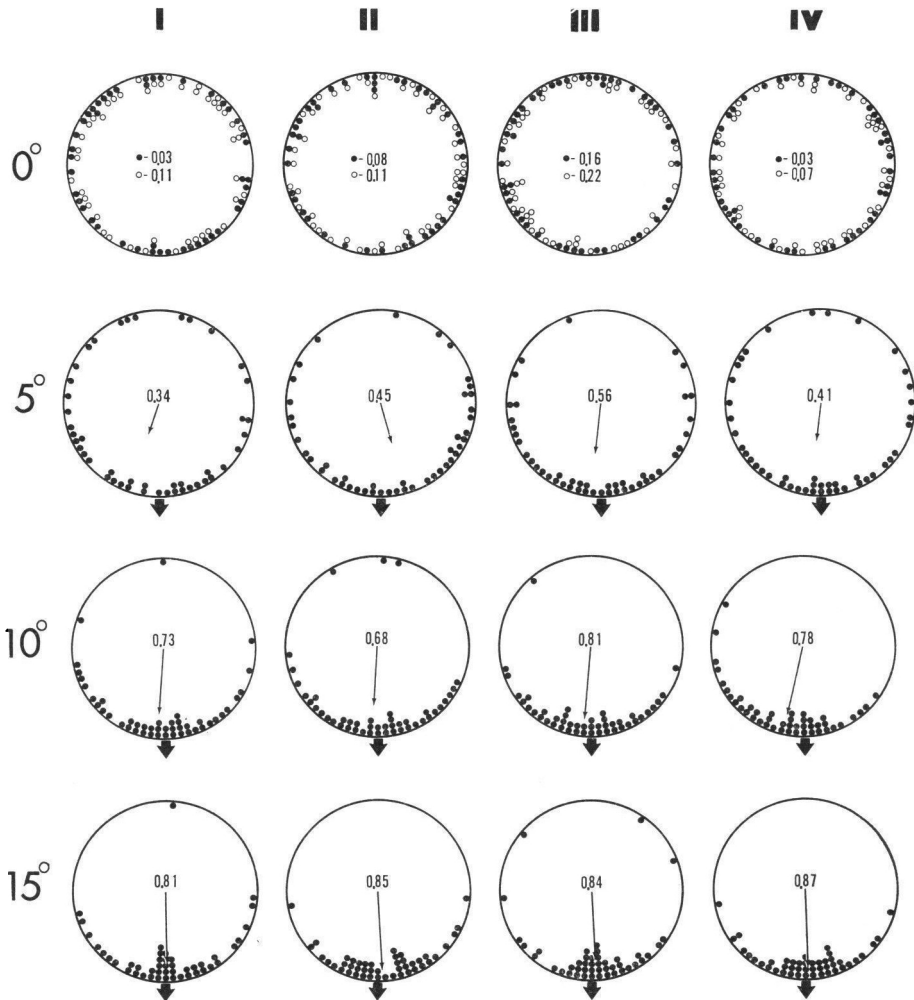


Figure 4. Circular distribution of necrophoric headings on various inclines for colonies I through IV. Slope is indicated to the left of each row. Downhill direction (bold arrow) is opposite the refuse pile. Thin arrow in circle indicates the mean direction and vector length (also given numerically at the center of the circle). For 0° slope, two trials of 45 worker headings were carried out and are denoted as open and closed circles.

Figure 5, the concentration (mean vector length) of the distributions increased linearly with slope, until a plateau was reached between 10 and 15°. Conversely, the variance in mean vector length among colonies decreased with increasing slope. These relationships confirmed that necrophoric workers respond to slopes by positive geotaxis. Light is not necessary for this behavior, since even in the dark, corpses added to colonies tilted 10° (with the refuse pile uphill) were deposited at the base of the incline. While down-slope orientation was demonstrated regularly by necrophoric workers, foraging ants moved in various directions across the arena floor, without apparent geotaxis.

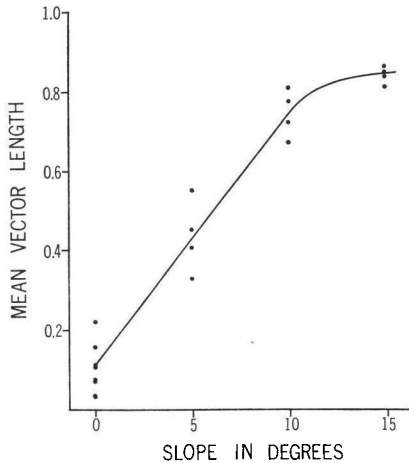


Figure 5. Mean vector length as a function of the magnitude of slope. Each point represents the mean vector length (r) for a single distribution of 45 necrophoric headings. Data from slope experiments (Fig. 4) where the refuse pile was uphill of the nest.

For $r = 1$, all 45 ants must intercept the same point ($\pm 2.5^\circ$) on the arena wall, and for $r = 0$, the headings must be maximally dispersed around the arena wall.

THE REFUSE PILE AS A TERMINUS FOR NECROPHORIC BEHAVIOR

It was evident that the refuse pile held no long-range attraction for necrophoric workers, but both in the laboratory and field, debris and corpse-laden workers that contacted refuse piles stereotypically discarded their burden. To determine the nature of the signal(s) responsible for the termination of necrophoric behavior, refuse piles were altered to present limited cues to corpse-bearing workers. All refuse and corpses were removed from arena II, and a new layer of masking tape was fixed to the entire perimeter of the arena floor. For the first of three related experiments, four items were prepared: a) a "unit" refuse pile, of 250 worker corpses and a small amount of debris, both originating from the stock population's refuse pile, spread

evenly over a 2.5 cm filter paper square. b) Another "unit" refuse pile placed in a "contact-allowed cage", constructed by fastening a sheet of slightly convex, medium-mesh screen over the pile and wedging the pile upward against the screen with a piece of filter paper, thus allowing workers to contact the refuse pile through the screen. c) A unit refuse pile in a "contact-prevented cage", built as above but without elevating the refuse pile so that workers could pass closely over the pile but could not contact it. d) A control screen cage with no refuse pile. The items were taped at equal intervals along the border of the arena and talcum-coated guides were added to direct necrophoric workers toward the test items.

Approximately 50 corpses were added to the interior of the nest. As a

TABLE 3

Fraction of positive responses by necrophoric workers to variously treated refuse piles

Test no.	Item presented	Type screen cage	Response	p *
1	Refuse pile	None	77/80	< 0.001
	Refuse pile	Contact-allowed	61/80	
	Refuse pile	Contact-prevented	9/80	n.s.
	Screen control	—	7/80	
2	Refuse pile	None	80/80	< 0.001
	Ether extract	Contact-allowed	37/80	< 0.001
	Ether extract	Contact-prevented	11/80	
	Ether control	Contact-allowed	5/80	n.s.
	Ether control Screen control	Contact-prevented —	8/80 9/80	
3	Refuse pile	None	79/80	< 0.001
	Extract readded	Contact-allowed	58/80	< 0.001
	Extract readded	Contact-prevented	6/80	
	Extracted refuse pile	Contact-allowed	15/80	n.s.
	Extracted refuse pile	Contact-prevented	10/80	
	Screen control	—	12/80	

* X² analysis (Yates' corrected where applicable).

necrophoric worker passed over a test item, a positive response was scored if the corpse was deposited or a negative response if the ant proceeded on with its cargo. Corpses deposited at any of the test areas excluding the unscreened standard pile were quickly removed. Additional corpses were periodically supplied the nest until a total of 40 binomial responses had been recorded for each experimental item. Thereafter, the configuration of the test items was altered and a replicate conducted.

As shown in Table 3 (test no. 1) almost all necrophoric workers encountering the unscreened refuse pile added corpses to it. Corpse deposition required worker contact with the refuse pile, since the "contact-prevented" pile elicited only a small fraction of the positive responses as that of the "contact-allowed" pile. Moreover, there were no differences in worker behavior on the "contact-prevented" and control areas. Both were traversed without radical changes in direction. The few corpses deposited on these areas result from the general scatter around the arena border. The significantly lower proportion of positive responses at the "contact-allowed" cage versus the unscreened pile may be explained by partial interference of the screen with the worker's ability to contact the pile. Worker behavior at both piles was characterized by sharp turns and probing movements with the antennae and forelegs.

This procedure was later used with six test items: An unscreened refuse pile and screen control were retested to provide a standard for comparison. Substituting for the "contact-allowed" and "prevented" refuse piles were the ether extracts prepared by 48 hour soxhlet extraction of two unit refuse piles evaporated on small bits of filter paper and screened in the two types of cages. The remaining items were solvent (ether) controls on filter paper bits placed in "contact-allowed" and "prevented" cages.

Worker responses revealed that a chemical signal was at least partially effective in terminating necrophoric behavior. Refuse pile extracts that could be touched evoked a significantly greater proportion of positive responses than any of the three controls (Table 3, test no. 2), indicating that the signal is nearly non-volatile or that the worker threshold to it is very high and its diffusion rate low. Furthermore, necrophoric workers crossing over "contact allowed" extract frequently displayed haphazard turns while this was not seen at the "contact-prevented" extract.

For the final test, worker responses to extracted and extract-readded refuse piles were examined. Four unit refuse piles were cleared for three days in 10% potassium hydroxide and rinsed in distilled water. After drying, the piles were soxhlet extracted in ether for 48 hours and dried for two hours. Two of the extracted piles were transferred directly to the two types

of cages. The remaining piles were treated with one pile-equivalent each of ether extract from other unit piles and were dried for an additional two hours before placement in the cages. Cross-test standards were included. Of the refuse piles presented, only the unscreened standard and the extract-readded, "contact-allowed" piles received a significantly greater proportion of positive responses than the screen control (Table 3, test no. 3). Thus contact chemoreception plays an important role in terminating necrophoric behavior. If visual cues were important, more positive responses would have been expected at contact-prevented refuse piles than at the screen controls. A purely tactile cue is also improbable, since extracted refuse piles received similar scores in both contact-prevented and contact-allowed situations.

To determine if the "terminating signal" originated from corpses, or from other material in the refuse pile, colony II was offered a refuse-free pile of 250 48-hour dead worker corpses on a square of filter paper. Foraging workers began immediately to dismantle the heap of corpses, carting the dead clockwise and counterclockwise along the base of the arena wall, and depositing most of corpses on the control refuse pile. A secondary pile was formed at another area of the arena border. Closer inspection revealed a zone of 40-50 streaks of fecal material on the tape beneath this pile. Several days had passed since the tape had been replaced and fecal smudges were scattered across it, but were particularly dense underneath the newly-formed pile. After peeling the tape containing the fecal zone and a piece of tape of equal size (control zone) from the arena perimeters, a border of fresh tape was installed. The fecal and control zones were then refastened on opposite sides of the arena and corpses were added to the interior of the nest. Of 80 necrophoric workers encountering the control zone, 11 dropped corpses on it, while 49 deposited corpses on the fecal zone (significantly different, $P < 0.0001$, by Yates' corrected X^2), and this area elicited the same locomotory patterns seen at the refuse piles. When necrophoric workers were offered the zones under screened "contact prevented" cages, the fecal area score dropped significantly ($p < 0.001$) to 8/80 and the control zone remained essentially as before (7/80). Examination of other laboratory colonies confirmed that feces were concentrated at refuse piles compared to other regions of the arenas. Because the material adhered firmly to corpses and refuse in the piles, it could not be collected in sufficient quantity to prepare an uncontaminated extract.

DISCUSSION

The refuse pile appears to be a ubiquitous feature of ant nests and speaks for the adaptive advantage of necrophoric and sanitation behavior. Such

behavior allows continued occupation of a nest and favors colony health by reducing contact with potential pathogens. Past studies have concentrated on the release of necrophoric behavior and have done little to account for the formation of the refuse pile. From the present study we can give a general account of the entire process of necrophoric behavior and refuse pile formation: contact chemical cues arising within a short time after death cause workers to pick up the dead ants and transport them from the nest on a more or less random radius unless a slope is present. The increased probability of moving downslope increases the chance of coming upon fecal deposits and other corpses which, because of contact chemical cues, in turn increases the probability of dropping the transported corpse and initiating a refuse pile. By carrying the corpses downhill, workers consume less energy and eliminate the problem of having the dead wash back to the mound during heavy rains.

Necrophoric behavior in *Solenopsis invicta* is released exclusively via the chemical mode. The possibility that auditory cues emanate from the dead can be dismissed outright. Visual signals do not significantly affect the necrophoric response, for workers regularly transport filter paper bits treated with corpse extracts to the refuse pile but ignore thoroughly extracted corpses. This also makes a purely tactile signal unlikely. In contrast, the extraction experiments confirm the conclusions of WILSON *et al.* (1958) and BLUM (1970) that recognition of the dead is achieved through chemoreception, for the signal capacity of corpses could be abolished by extraction and hydrolysis and most of it can be returned by addition of the resultant extract. Further, the low volatility of fatty acids (shown by BLUM (1970) to release necrophoric behavior) and the lack of worker orientation prior to contact with corpses suggest that the dead are identified through contact (or near contact) chemoreception. It would appear, then, that corpses are removed only from areas of the nest where the colony is active and might contact them.

Other authors have documented examples of contact chemoreception among ants. SCHMIDT (1938) demonstrated that *Lasius niger*, *Manica rubida*, and *Myrmica rubra* sense various carbohydrates by contact with the antennae and mouthparts. BRIAN (1970) found that the queen pheromone of *M. rubra* has an extremely small active space and that workers probably detect it by contact. Finally, WALSH & TSCHINKEL (in press) showed that *Solenopsis invicta* workers recognize brood by a contact pheromone.

The range of substances releasing necrophoric behavior may be broader than previously reported. BLUM (1970) determined that releasers are limited to the fatty acid fraction of *S. invicta* corpses, but in the present study,

methanol stimulated workers to transport a small proportion of thoroughly extracted corpses to the refuse pile. Similarly, if filter paper bits coated with incompletely dry shellac or oil-free paints are placed in a nest, they are quickly taken to the refuse pile. If the same material is dried it is tolerated by workers, implicating the solvents as releasers (HOWARD, unpublished). Perhaps naturally occurring volatile substances also provoke necrophoric behavior, despite the absence of fatty acids.

The latent period between death and release of necrophoric behavior is remarkably brief for a purely chemical signal, increasing from zero to a plateau in an hour. WILSON *et al.* (1958) postulated that decomposition of the body lipid increases the titer of fatty acids to above threshold. BLUM (1970) cited the presence of lipolytic bacteria in older fire ant corpses, but reserved judgement on the possibility that this flora accounts for the initial appearance of the signal. It is unlikely that bacteria or enzymes account for the early signal, for its appearance would probably have been retarded in heat-killed as compared to freeze-killed workers, but this is not the case. It is conceivable that the releaser is masked by a competing odor which gradually fades after death. For instance, HASKINS (1970) demonstrated that *Myrmecia vindex* workers respond to oleic acid-treated cocoons by promptly dumping them at the refuse pile, but larvae soaked with the same substance are retrieved to the nest. HASKINS presumed that the necrophoric signal is overridden by larval odor, but unfortunately, offered no proof that a larval odor exists. However, WALSH & TSCHINKEL (1974) furnished convincing authentication of a brood pheromone in *S. invicta* and noted that brood retrieval is not significantly diminished even six hours after death. There is considerable evidence that fire ant workers are marked by a chemical odor (WILSON, 1962) and perhaps this scent partially nullifies pre-existing, near threshold concentrations of fatty acids. Whatever the underlying mechanism, it confers the advantage of rapid removal of corpses from the nest.

In the field on a horizontal plane, corpse-bearing ants do not move toward or away from the sun, but use it as a reference during navigation from the nest. *S. invicta* workers also orient by photomenotaxis when returning from foraging expeditions (WILSON, 1962; MARAK & WOLKEN, 1965). Necrophoric workers in arenas initially move away from the nest at various headings relative to the light source, but turn toward the light after reaching the arena wall. This secondary phototaxis probably contributes little to refuse pile formation, since few insuperable obstacles exist for workers in the field.

That necrophoric activity is not carried out by workers of restricted size classes fits the established pattern of weak caste polyethism in *S. invicta*. Mound repair, defense, foraging (WILSON, 1971) and brood retrieval

(WALSH, 1972) are also divided equally among workers of various sizes. Age polyethism may explain why some workers in laboratory colonies remove corpses from the nest proper, but do not venture onto the foraging area with them.

It is difficult to conceive of an obvious advantage for the formation of concise refuse piles over random disposal of wastes outside the nest, particularly in *S. invicta* where refuse piles may or may not occur, depending on the terrain. WILSON (1971) indicated that *S. invicta* workers kept in artificial nests exploit corpses as a source of food, thus it is possible that the refuse pile serves as a convenient storage area. Subsequent cannibalism of the corpses has been suggested (WILSON, 1971), but we found only limited evidence for cannibalism and many other species which form refuse piles do not cannibalize the dead.

SUMMARY

Removal of dead ants from the nest (necrophoric behavior) is released solely by contact chemical cues in the fire ant, *Solenopsis invicta* Buren. Exhaustively extracted corpses do not release necrophoric behavior, but the extracts do when applied to filter paper bits. The necrophoric releaser is absent at death but appears rapidly and reaches a plateau within about an hour. The rate of signal appearance is identical in heat and freeze killed workers, implying a non-enzymatic origin. There is no specialized caste or size of worker which carries out necrophoric labor. In the field, in the absence of slope, corpse-bearing workers head outward from the nest on random radii and drop their corpses at unpredictable distances, making refuse piles rare. There is a positive relationship between slope and the presence of refuse piles, and these are located downhill from the mound. When the headings of necrophoric workers were measured in circular arenas in the lab, the only potential orientational cue (tested: landmarks, light, 5°, 10°, 15° slope) which resulted in non-random distribution of headings was slope. The concentration of the headings was a direct function of the slope and seemed to plateau at about 15°. Corpse-bearing ants show stereotyped behavior upon encountering refuse piles and adding their burden to it. Chemical stimuli probably issuing from the feces in the refuse pile bring about the end of necrophoric behavior and maintain the refuse pile. These chemical cues, as also those initiating necrophoric behavior, must be contacted to be effective.

REFERENCES

- BHATKAR, A. & WHITCOMB, W. H. (1970). Artificial diet for rearing various species of ants. — Florida Entomol. 53, p. 229-232.
- BLUM, M. S. (1970). The chemical basis of insect sociality. — In: Chemicals controlling insect behavior. Ed. by Morton BEROZA, Academic Press, New York.
- BRIAN, M. V. (1970). Communication between queens and larvae in the ant, *Myrmica*. — Anim. Behavior 18, p. 467-472.
- HASKINS, C. P. (1970). Researches in the biology and social behavior of primitive ants. — In: Development and evolution of behavior. Ed. by L. R. ARONSON, E. TOBACH, D. S. LEHRMAN & J. S. ROSENBLATT, W. H. Freeman and Company, San Francisco.
- MAETERLINCK, M. (1930). The life of the ant. — (English tr. by B. Miall), G. Allen and Unwin, Ltd. London.