Research Summary for Young Scientist of the Year Application

Necrophoric Behavior in the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), and Its Implication on the Horizontal Transfer of Slow-Acting Insecticides

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Main Research Issues

(1) Necrophoric behavior of Argentine ants
(2) Horizontal transfer of insecticides within the colony through necrophoric behaviors of Argentine ants
(3) Developing effective virtual baiting strategy against Argentine ants

Necrophoric behavior of Argentine ants

Among ants, one of the most conspicuous nest cleaning activities is the stereotypic removal of dead nestmates from the nest (Fig. 1). The term 'necrophoric behavior (or necrophoresis)' has been applied to distinguish the disposal of corpses from other sanitation tasks in the colony. Dead workers are transported out of the nest by their nestmates and deposited in piles near the nest entrance. These piles are referred to as 'graveyards', 'refuse piles', or 'bone piles'. We will adopt the anthropomorphic term 'graveyard' throughout the paper when referring to them.

Most studies so far suggest that chemical cues on the ant corpse release necrophoric behavior. Some experimental studies suggest that the mediation of necrophoric behavior is purely chemical. Oleic acid and other unsaturated fatty acids initiated corpse-removal behavior in *Solenopsis invicta* Buren, *Myrmecia vindex* Smith, and *Pogonomyrmex badius* Latreille. Wilson et al. (1958) hypothesized that the releaser compounds such as fatty acids accumulate in the ant's corpse as a result of decomposition after death. According to Howard and Tschinkel (1975), the necrophoric signal appears gradually over an hour after ants are killed. This led them to postulate that the releaser chemical is masked by a competing odor which gradually fades after death, however, the exact mechanism remains as a mystery.

Field and laboratory observation showed that Argentine ants frequently move about their dead nestmates and deposit them on the graveyard or refuse piles (Fig. 1). We located the ant graveyard in the field by providing several Argentine ant corpses coated with a red fluorescent pigment (Radiant Color Co., Richmond, CA), and detected with a portable UV light (365 nm) at night. Most of the graveyards were located about 2-3 m away form a nest entrance. The observed graveyard (i.e., refuse pile) contained at least 3,000 conspecific ant corpses (counted head capsules), other ant species including *Pyramica* spp. (Myrmicinae), *Hypoponera* sp. (Ponerinae), and other insect parts (e.g., honeybee head capsules, beetles).

Laboratory study using experimental nest and arena (Fig. 2) indicated that Argentine ants respond differently toward freshly killed nestmates and aged corpses. Argentine ants killed by freezing after anesthetizing with CO_2 take about six times longer to be removed from ant nest tube (556.0 ± 105.1 sec, mean ± SEM, n = 9) than do the 24-h-old dead corpses (89.3 ± 17.0 sec, mean ± SEM, n = 9). However, all of the fresh-killed ants were carried out within 30 min after introduction into the nest. This period of time was not considered to be long enough to allow considerable amount of enzymatic decomposition occurs. Thus, we hypothesized that live ant workers already have enough titers of necrophoric chemicals in their body, but those cue may be rapidly exposed after death or some other competing odors rapidly disappear.

If the necrophoric chemical cues already exist in the ant's body, we might be able to specifically locate the source of the cues. If they come from the fat bodies, some type of secretary glands, or digestive system, ant's gaster will be the source. If they mainly come from the muscle, thoracic segment of the ants (i.e., trunk) will be the source. If the necrophoric cues are originated from mandibular or esophageal glands, the source would be ant head. To examine these, fresh-killed Argentine ants were cut into three parts – head, trunk, and gaster. Small wood pellet (fecal pellets of Western Drywood Termites, *Incisitermes minor*; washed with acetone in Soxhlet extractor for at least 24h) was treated with head, trunk, and gaster extract (apporoximately, 0.03-ant equivalent). These wood pellets treated with each extract were place on the bottom of Argentine ant colony simultaneously, and the number of remaining pellets on their original small filter paper (4.25cm diameter) was recorded (Fig. 3). The result of study indicated that plenty of necrophoric cues exist in fresh-killed ants, especially in the gaster (Fig. 4).

Preliminary TLC (thin layer chromatography) studies indicated that oleic acid is not major component of freshly-killed Argentine ants, even though the fresh corpse would elicit nestmate's necrophoric responses (Fig. 5). This is particularly interesting because other former studies regarding necrophoresis have suggested that oleic acid as one of the most important necrophoric cues in other ants. Some of the major compounds in the Argentine ant gaster do not exist in large amount in the other areas of body (Fig. 5, arrow). Chemical isolation (flash liquid chromatography) and characterization (NMR, GC-MS) of these compounds should be followed. Bioassay with isolated compounds will be conducted to test if the necrophoresis is elicited by the particular compounds.

Fig. 1. Argentine ants are carrying dead ants or other insect to graveyards. All the ants here were photographed in the graveyards found in the field. Ants typically deposit the corpses when they encounter an existing graveyard.

Fig. 2. Experimental arena for necrophoresis bioassay. In the center of large plastic Petri dish, Tygon vinyl tubing with small experimental ant nest was connected. Ants in the red chamber will maintain their brood and queens in the tubing compartment. Moisture was provided by wetting a dental wick plugged in the other end of nest tubing. Small piles of dead nestmates are found along the Petri dish edge.

Fig. 3. Pellet bioassay setup. 30 treated pellets were place in the center of small filter paper (4.25-cm diameter) within 8mm circle. All the filter papers were placed on the bottom of nest box simultaneously. Ants found the pellets removed or ignored them.

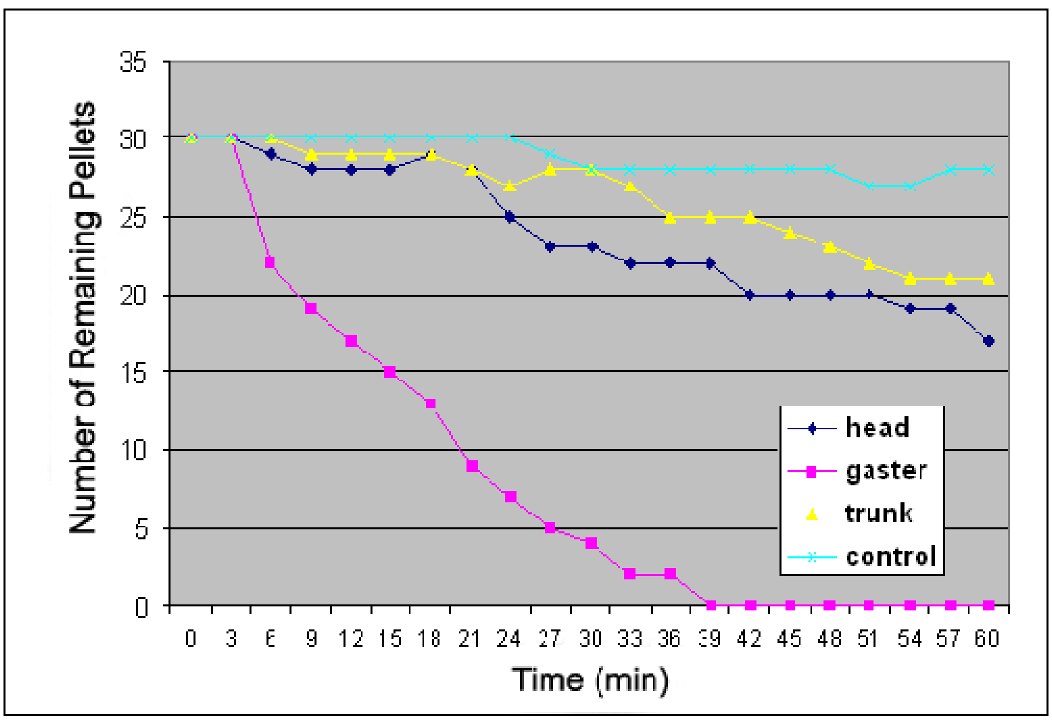

Fig. 4. Number of treated pellets remaining on the filter paper center. 30 pellets were treated with each extract and placed on the bottom of Argentine ant colony. Each pellet was treated with acetone extract of crushed head, gaster, and trunk (approximately, 0.03-ant equivalent). Control pellets were treated with solvent only. All the removed pellets were placed on or around exiting refuse piles (graveyards).

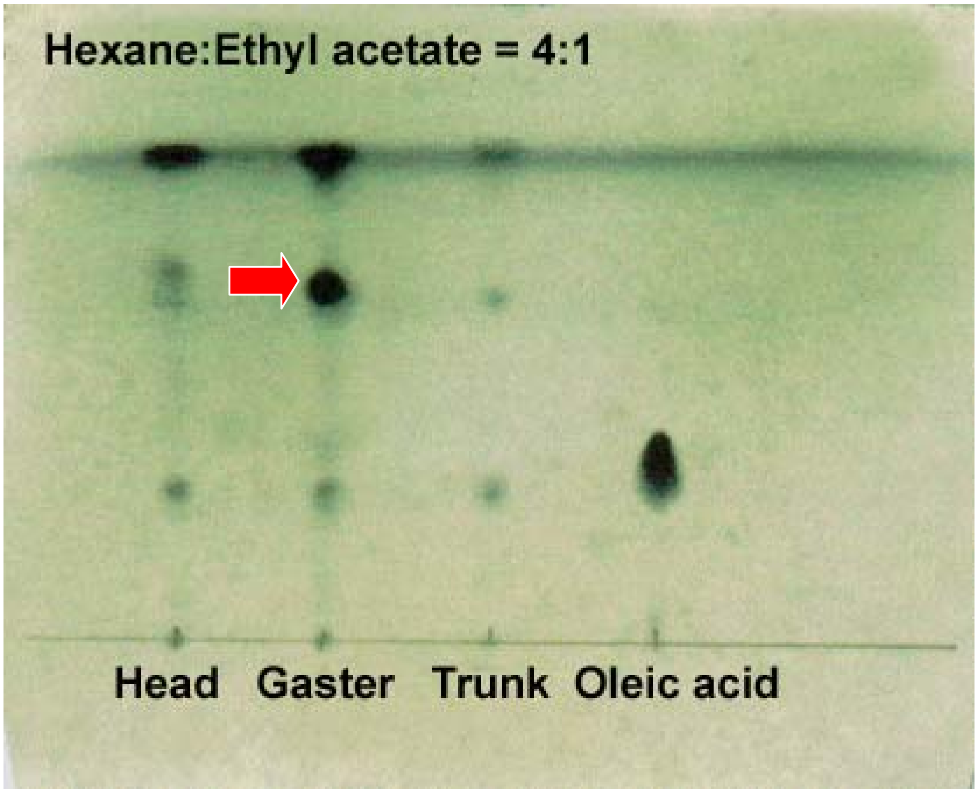

Fig. 5. TLC on silica gel of the extracts of Argentine ant body parts. Extracts were initially collected in acetone, and solvent was evaporated to dryness. The sample was again dissolved in hexane / methanol mixture, and only hexane soluble part was analyzed using TLC. Solvent system used was hexane / ethyl acetate (4:1, vol:vol).

Horizontal transfer of insecticides within the colony through necrophoric behaviors of Argentine ants

Five insecticides used by urban pest management professionals (PMPs) for ant control and three experimental insecticides were tested to determine if these insecticides are horizontally transferred among individuals in colonies of Argentine ants, *Linepithema humile* (Mayr). Ants were exposed to insecticide-treated sand for 1 min and then placed in a colony of untreated ants. When ants were exposed to 20 and 40 ppm of fipronil, it was readily transferred to other individuals in the colony, resulting in the greatest mortality (Fig. 6). Ants exposed to 20 and 40 ppm fipronil deposit provided significant transfer and kill for 3 and 7 d, respectively. Experiments in large foraging arenas (Fig. 7) demonstrated that necrophoresis was an important behavior facilitating the horizontal transfer of fipronil. When ants contacted contaminated corpses in the process of removing them to refuse piles, they subsequently died. Fipronil-contaminated dead ants that were placed in the vicinity of the nest resulted in significantly higher mortality than did corpses placed in a distant foraging arena (Fig. 8). Most of the dead ants accumulated in the vicinity of nest rather than in the foraging arena (Fig. 9), workers retrieving dead ants to refuse piles from the foraging arena.

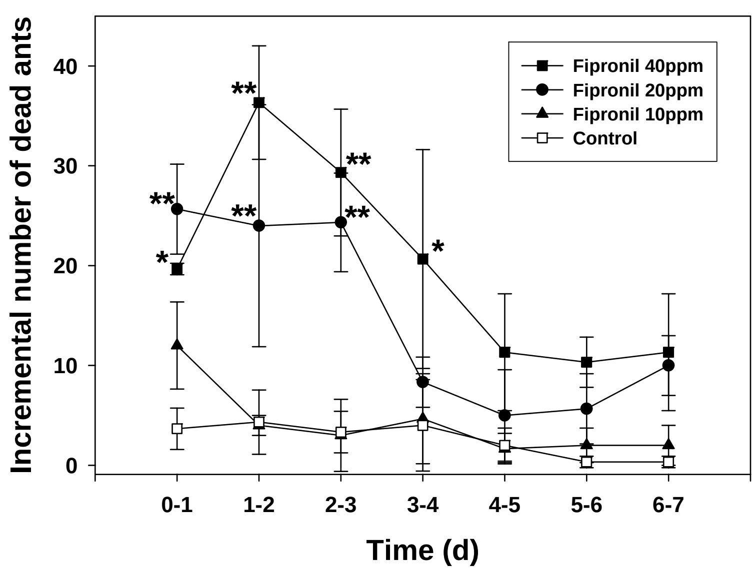

Fig. 6. Incremental number of dead ants between two consecutive days in three fipronil treatments and control. Values represent means ± SD. An asterisk (*) represents significant differences between the treatment group and corresponding controls (Dunnett's test; **, $P \leq 0.0001$; *, $P < 0.01$).

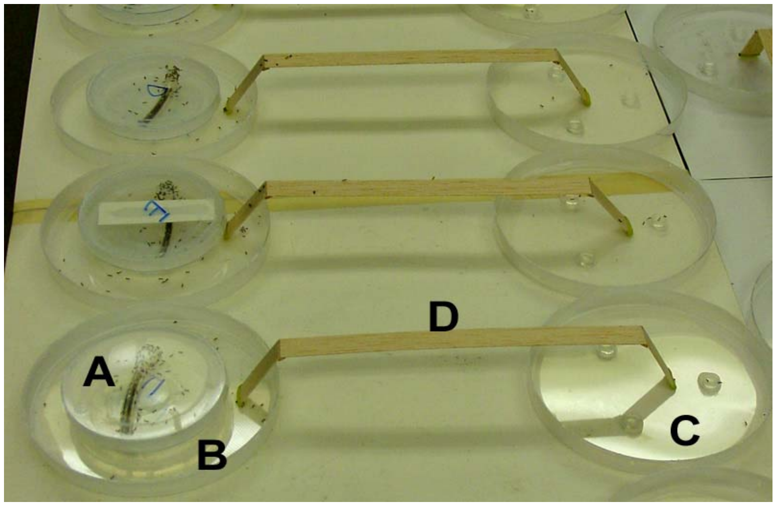

Fig. 7. 'Three-chamber experimental arena' with three different compartments: (A) nest (one-chamber nest model), (B) area around nest, and (C) foraging arena. To allow ants to forage, chamber B and C were connected with a wooden bridge (D).

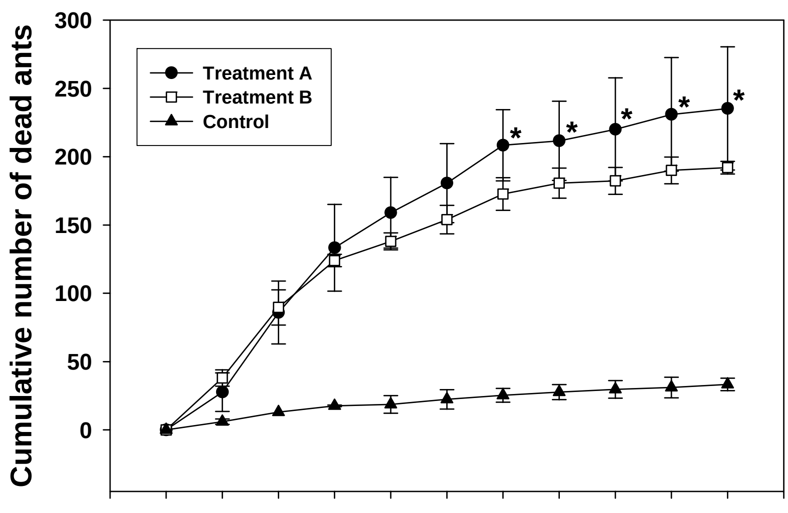

Fig. 8. Cumulative number of dead ants in the three-chamber experimental arena after the addition of 10 freeze-killed ants which were previously exposed to fipronil on sand (40 ppm). Values represent means ± SD. An asterisk (*) indicates that treatments A and B differed from one another on that day (Fisher's LSD; $P < 0.05$). In treatment A, dead ant were initially placed in the nest; in treatment B, dead ants were initially placed in the foraging arena.

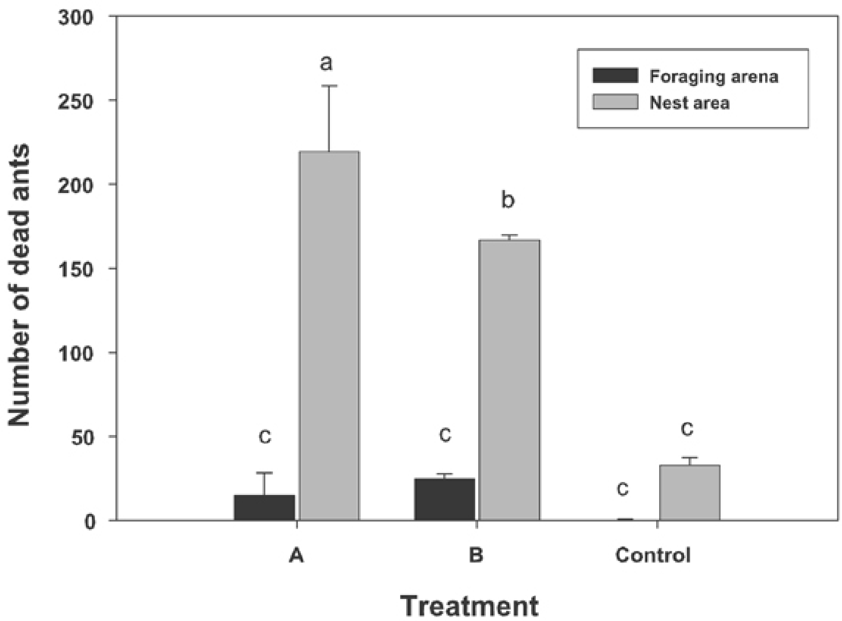

Fig. 9. Mean ± SD number of dead ants recovered in foraging arena and nest area at the end of trial. Means with the different letter are significantly different (Fisher's LSD; $P < 0.05$). The insecticide-treated dead ants were initially placed in the nest chamber (treatment A) or in the foraging arena (treatment B). At day 7, ant corpses in the nest area (nest chamber + area around nest) and foraging arena were counted and compared.

Developing effective virtual baiting strategy against Argentine ants

As recent studies conducted in my laboratory indicate, it is obvious that the necrophoresis (i.e., carrying dead nestmate) is one of the most important mechanisms by which horizontal transfer of some effective insecticide occurs. Thus, understanding how the necrophoresis is happening in Argentine ant colony will be greatly beneficial for developing effective control strategies against them. By exploiting ants' necrophoric behaviors for control, we have a new control approach that we refer to as "virtual baiting" (Fig. 10). This strategy would be most preferred for sensitive sites where broadcast insecticide applications might be of concern. Virtual baiting exploits the foraging / recruitment behavior and necrophoresis of worker ants and avoids the need of formulating special toxic baits. Our recent study indicated that dead ants accumulated in the bait station made sucrose solution less attractive to the foragers. My next research themes for this coming summer will include developing stable and effective virtual bait system for urban and agricultural applications.

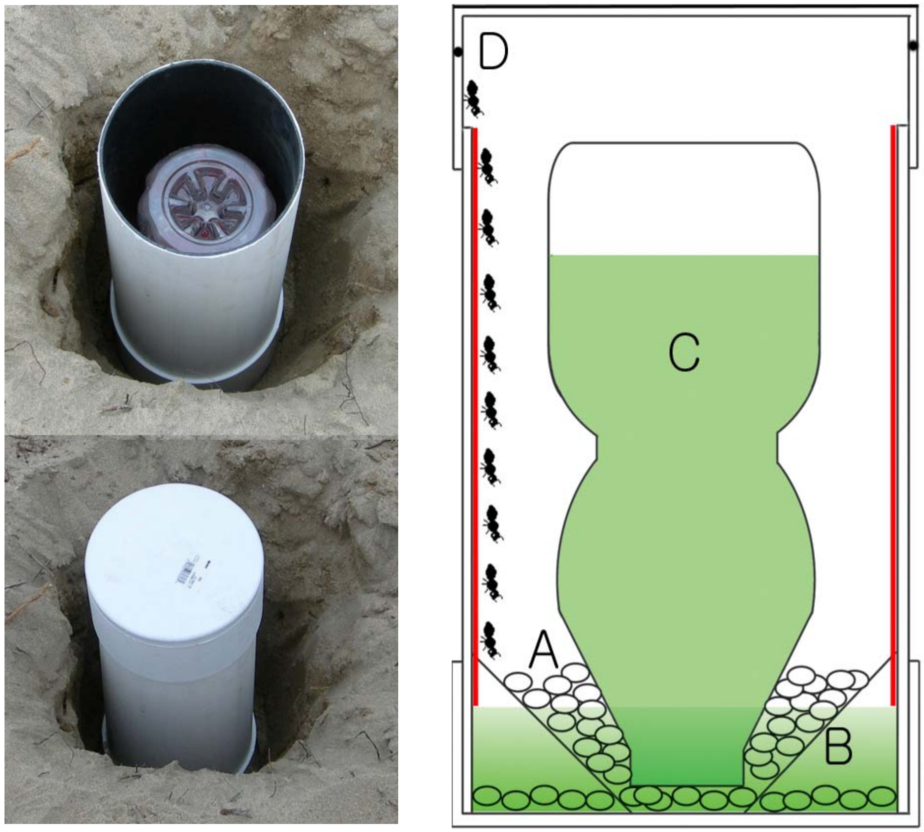

Fig. 10. "Virtual Bait Stations." The inner surface of PVC pipe was treated with fipronil 2SC at a rate of 8.3 µg/cm2. A layer of smooth rocks (A) and an inverted cone (B) were placed in the bottom. A 2-liter plastic bottle of 25% sugar water (C) was inverted into the rocks. The top of the pipe was sealed and ants had access to the station through holes (D) in the top of the PVC. Thus, ants foraged across the fipronil treatments (in red) to access the sugar water.

References Cited

Howard, D. F., and W. R. Tschinkel. 1975. Aspects of necrophoric behavior in the red imported fire ant, *Solenopsis invicta*. Behavior 56: 157-180.

Wilson, E. O., N. I. Durlach, and L. M. Roth. 1958. Chemical Releasers of Necrophoric Behavior in Ants. Psyche 65: 108-114.