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An observation of a potentially novel defensive behavior against pesticides: *Messor oertzeni* build a defensive wall against ant traps in the field (preliminary note)

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Abstract

In the course of conducting honey bee experiments on the Greek Island of Lesbos we took the opportunity to observe the reactions of ants, *Messor oertzeni* Forel (Hymenoptera Formicidae Myrmicinae), to a baited ant trap placed in its main foraging path (active ingredient: sodium cacodylate). Each trap had three entrances and we tested five nests. For 14 days we observed the nests and photographs were taken daily to document our observations. Following a baseline condition in which none of the three entrances were open, one entrance was open. Several days later the entrance we opened was turned 90 degrees away from the main foraging trail and a second entrance was opened and placed in the same orientation as the first entrance (i.e., in the main foraging path). Our observations revealed that for four of the five ant colonies, the ants built a barrier around the opened entrance preventing other ants from entering the trap. The materials they used to bar the entrance was composed of twigs, pebbles and soil. We believe that the apparent ability of ants to avoid the effects of an insecticide by baring the entrance to a bait trap is a novel finding and should be replicated under more controlled conditions.

Key words: ants, insecticide, baited traps, avoidance.

Introduction

Ants and other insects use a variety of mechanisms to avoid danger including mimicry, threats, attack, and learning (for a review see: Hutchins *et al*., 2003). For example, desert ants learn to associate visual cues with falling into a pit trap and on subsequent trips use the visual cues to avoid falling into the trap (Wystrach *et al*., 2020) and trap-jaw ants can modulate their mandibles in response to surprising events (Aonuma, 2020).

Studies on the aversive conditioning of ants from a comparative psychological perspective are now rare. However, the early studies support the more recent experiments on the ability of ants to escape and avoid aversive events (Abramson, 1994). This early research repeatedly showed that ants escape and avoid a variety of stimuli including vibration, radiation and aversive odors such as peppermint. The California harvester ant for instance can learn to remain stationary to terminate and subsequently avoid an intense vibration in a passive avoidance task (Abramson, 1981) and carpenter ants can escape the odor of peppermint oil by moving into a safe space (Hoagland, 1931). In an experiment investigating time allocation in carpenter ants to different rates of peppermint odor, the behavior of ants varied systematically with the local rates of peppermint delivery (DeCarlo and Abramson, 1989). Carpenter ants also avoid X-ray exposure in a passive avoidance situation by escaping into a protected area (Hug, 1960; Martinsen and Kimeldorf, 1972).

The ability of ants to escape and avoid aversive events suggests that they can also avoid the dangers associated with insecticides. We have found no studies investigating whether ants can avoid such dangers. In the course of conducting honey bee studies on the Greek Island of Lesbos we took the opportunity to observe the behavior of ants, *Messor oertzeni* Forel (Hymenoptera Formicidae Myrmicinae), when poison bait is placed near the colony in the main foraging path.

Materials and methods

We used a three-entrance circular ant trap (Gel Pro Dobol, manufactured by DAPNI Biocides Ltd, Greece). The trap is approximately 7 cm in diameter and consists of three entrances with a single reservoir of poison (active ingredient: sodium cacodylate) connecting all three wells. Each entrance is 1.5 cm wide and 1 cm high. To enter one of the three wells, an ant must climb over an 8 mm ledge (i.e., the entrance to the wells is not flush with the ground). We placed the trap in the main foraging path of an ant colony.

Five ant colonies were observed with four of the five colonies approximately 18.29 meters apart (approximately 20 yards). The fifth colony was located approximately 36.58 meters from the others (approximately 40 yards). We observed each colony for two weeks and took photographs of the colonies at approximately 7:00 AM and again at 7:00 PM daily.

All of our observations followed the same basic plan. Each colony was observed for 14 days. For the first three days the colonies were left undisturbed. The purpose here was to determine if the ants naturally made barriers in the location where we expected to place the trap (the main foraging path). Following the three day undisturbed period (days 1-3), we place an unopened trap in the location of the main foraging path for an additional three days (days 4-6). The rationale for this manipulation was to determine of the ants would “unconditionally” attempt to cover the trap. After this control period, we opened one of the tabs with the open tab facing the entrance of the nest. The purpose here was to ensure the ants would visit the trap. This phase lasted for 4 days (days 7-10). For the next four days (11-14), we turned the trap 90 degrees and opened a new tab; the original tab was still opened but no longer faced the entrance. The rationale here was to determine if the ants would block the new entrance providing at least some replication of our initial observations.

Results and discussion

As expected during the first control period none of the five colonies built any barriers on the path we expected to place the trap. During the second control period, when we placed the trap in the main foraging path, the ants explored the trap but the only building we observed was the addition of some dirt to the surrounding area (figure 1). We believe that this additional dirt only added to the colonies circular mound structure by excavation and removing waste form the nest. There was no indication that the ants were disturbed by the presence of the unopened trap.

Once the traps were opened it took 1-2 days to see any significant building around the entrance. One ant trap had a pile of plant material placed along the side of the ant trap that bordered the main trail (figure 2). There were variable responses to the traps among our five colonies. Some colonies placed dirt in the entrance; while another colony used dead ants to partially block the well. Sites 2 and 3 plugged the wells with dirt. We also noticed that the colony entrances at sites 2 and 3 both moved about a foot away from their original placement. In one colony (site 4) no significant blocking of the entrance was observed. The colonies from three of the sites clearly exhibited building activity blocking the entrance. Figure 3 shows one such example.

Supporting our observations that the ants built a barrier around the bait entrance are the results of turning the trap 90° (days 11-14). Figures 4 through 8 shows a sequence of photographs that when the trap is turned and the bait entrance opened, the ants began to plug the new opening with sticks, dirt, and rocks. A video showing the behavior of plugging an entrance is available at https://youtu.be/WNJUpdPpQyI.

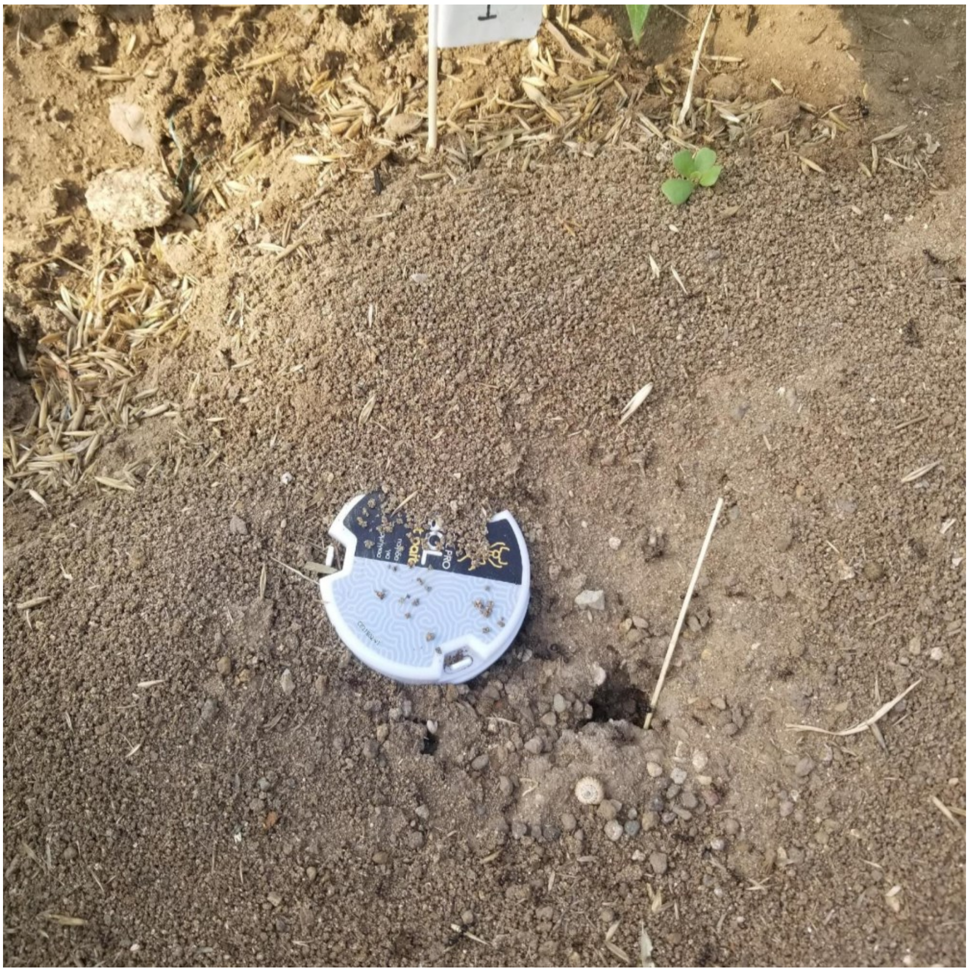


Figure 1. The last day of the control period of the experiment.

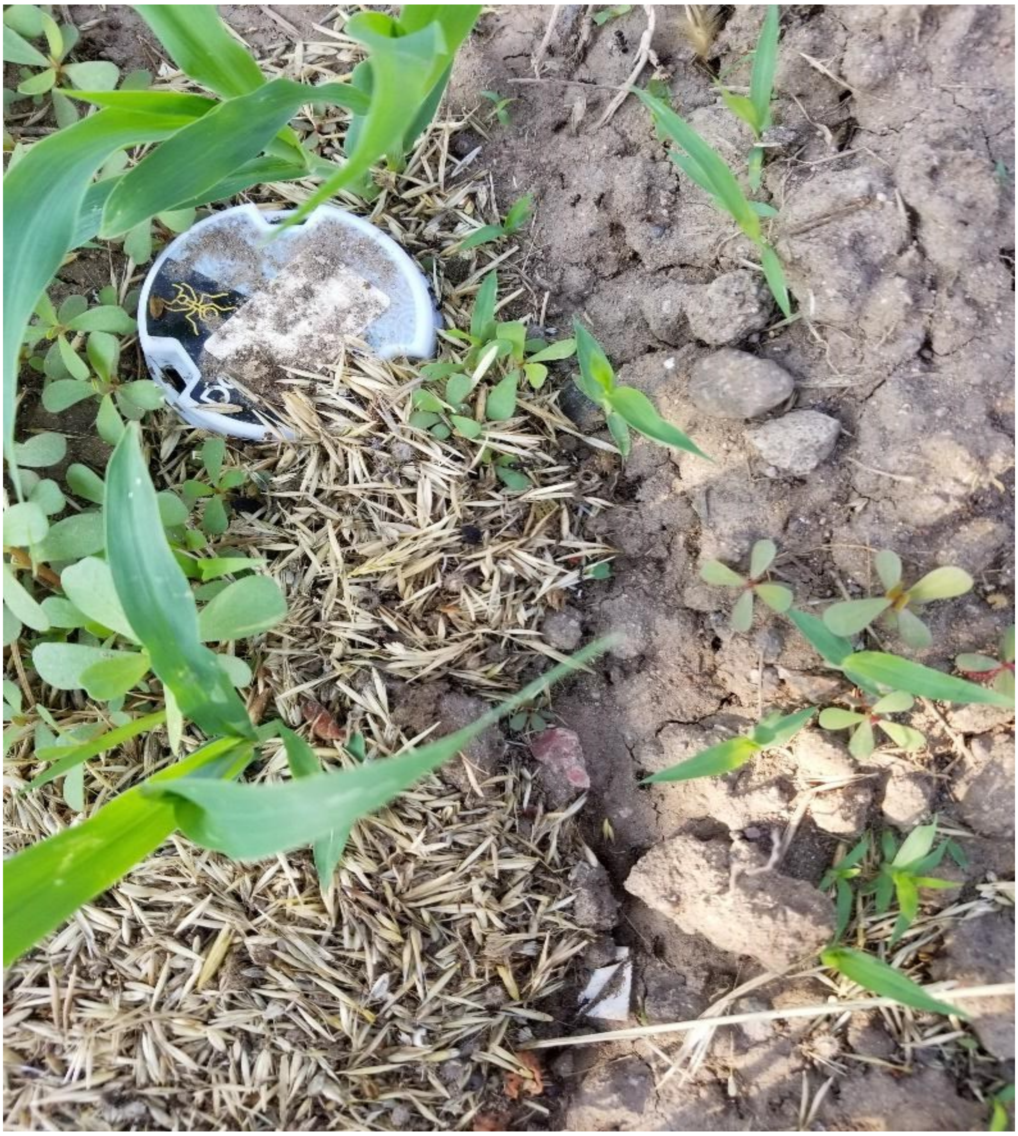
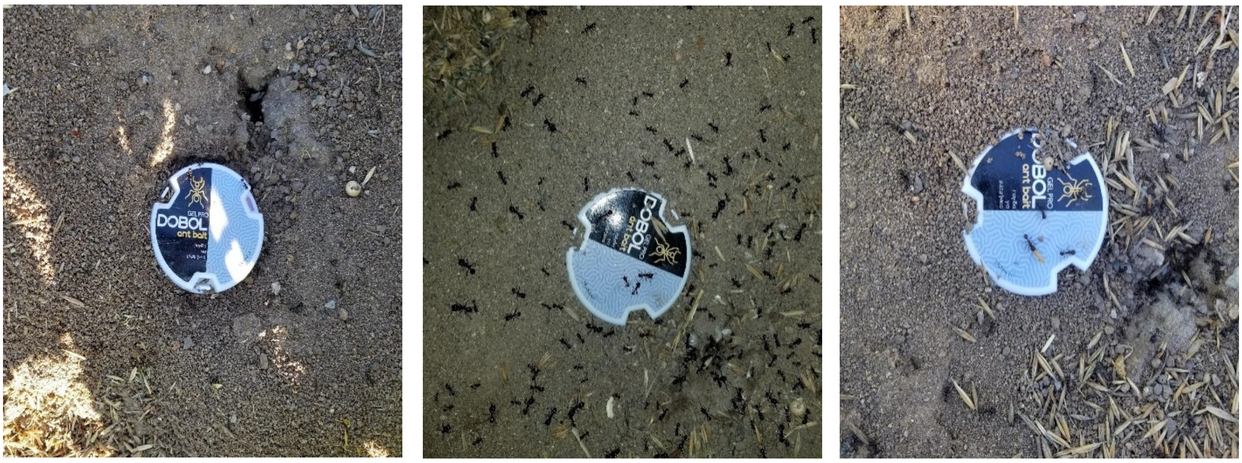


Figure 2. The defensive building around the exploratory ant trap.



A B C

Figure 3. Sequence of blocking the insecticide entrance over the course of three consecutive days: **A)** 6/28, morning first tab was opened; **B)** 6/29, evening of the second day; **C)** 6/30, morning of the third day.

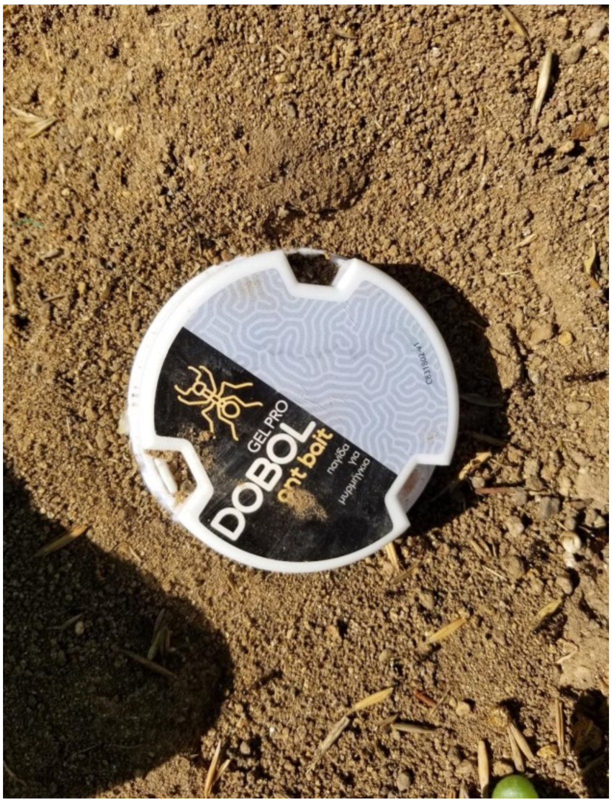


Figure 4. Photograph of site 1 on day 10, the first day the trap was turned 90°. Note that the opening to the right was previously plugged. The entrance to the bottom left will be opened.

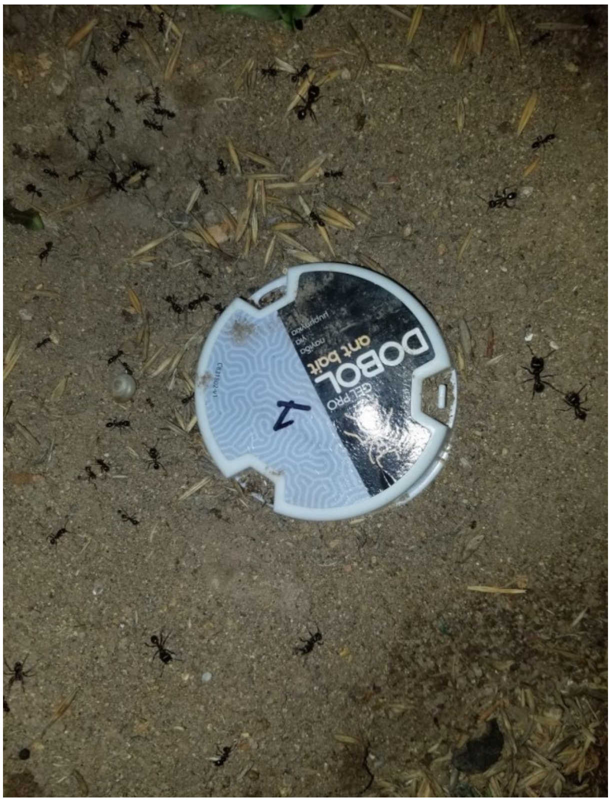


Figure 5. Photograph of site 1 from the 7:00 PM observation period on Day 10. This is the first photograph taken 12 hours after the new entrance was opened. Note the sticks being piled up in front of the entrance.

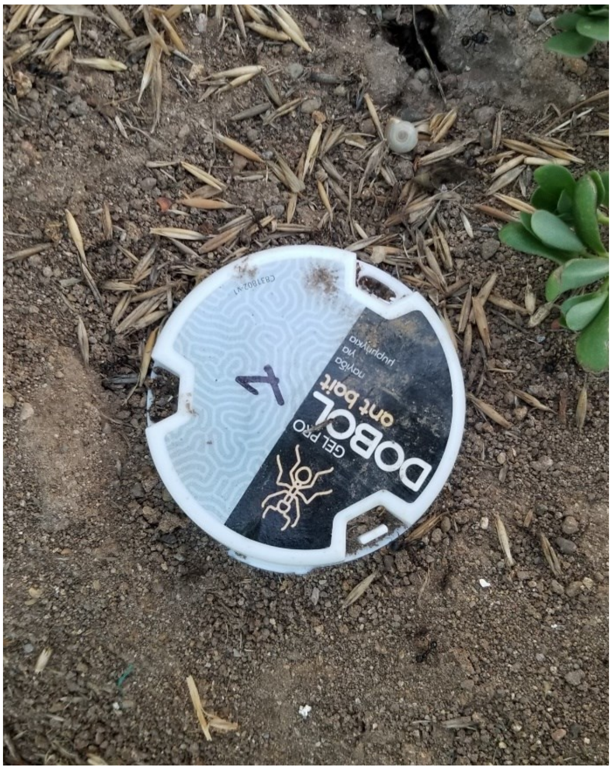


Figure 6. Photograph of site 1 from the 7:00 PM observation period on Day 11.

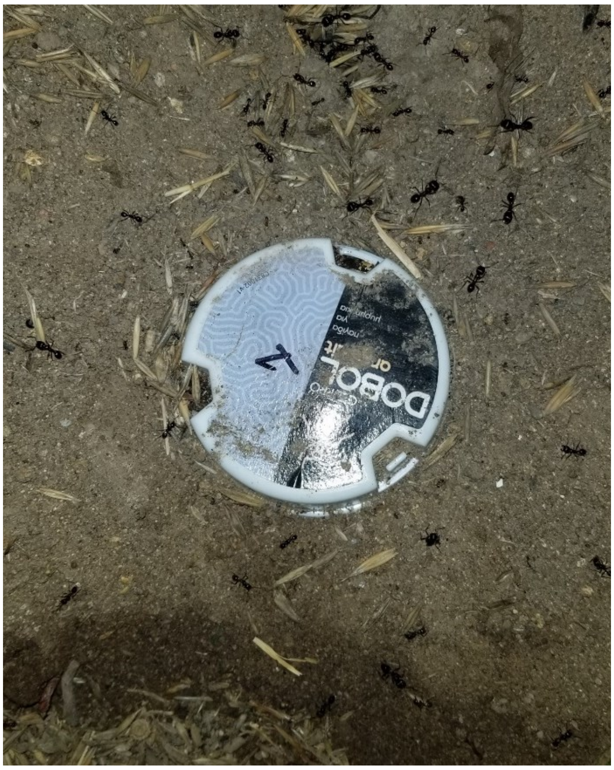


Figure 7. Photograph of site 1 from the 7:00 PM observation period on Day 12.

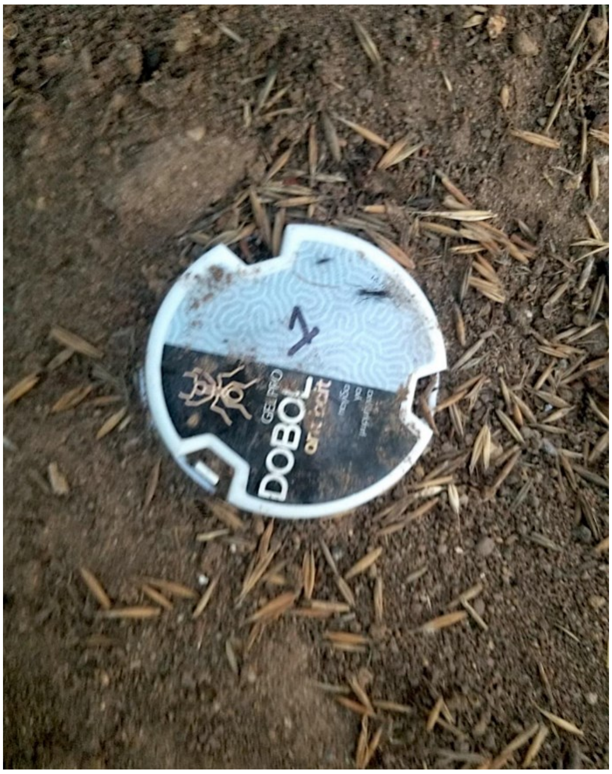


Figure 8. Photograph of site 1 from the 7:00 PM observation period on Day 13.

We would like to note that it was not feasible to provide any quantitative analysis of the photographs. The debris covering the entrances to the trap could not be weighed without disturbing the ants nor was it possible to control for the possibility that other organisms would visit the observation sites. Moreover, the photographs were taken under field conditions making it difficult to provide an accurate quantitative analysis because of shifting light conditions. The best we can offer are the photographs themselves and the video clip.

Conclusion

The results in this paper consists of observations that need to be replicated. We believe that the observation of ants forming a barrier around an insecticide trap to prevent other members of the colony from being exposed is a novel and potentially important observation. It suggests the tantalizing possibility that at least some ant species work together to prevent nest mates from dying of insecticide exposure. We view this behavior as analogous to the balling behavior that is observed with honey bees fighting parasitic wasps (Kamdemir *et al*., 2012).

An extensive literature search failed to uncover similar observations. However, Wheeler (1910, pgs. 179-180) noted, "A peculiar reaction is exhibited by nearly all ants in the presence of some substance that they cannot remove, such as a strong-smelling liquid. They throw pellets of earth or any other debris on the substance, sometimes in sufficient amount to bury it completely. The origin of this reaction which is often manifested in artificial nests, is very obscure." Wheeler goes on to suggest that such behavior "…has all the characteristics of a pure reflex, although, curiously enough, its manifestation under certain conditions has been regarded as a demonstration of reasoning power." Unfortunately, Wheeler provides no citations related to these observations.

What we observed is what Wheeler observed. During our study, ants avoided the trap by either building walls, plugging an entrance, or relocating the entrance to its colony. Blocking the entrance to the poison bait was observed in four of our five sites, and in three of the five sites, the colony eventually moved away from the area of the poison bait. In one colony no significant building activity was observed.

The behavioral mechanism to account for the activities we observed is unknown at this time. The best we could do was to make a series of observations. Frankly, we were surprised that ants would exhibit such behavior. It is known that cockroaches, *Blattella germanica* (L.), can learn to avoid an area treated with an insecticide (Ebeling *et al*., 1966). However, the key difference between Ebeling *et al*. (1966) and our study is that we observed ants actively building a barrier to prevent nest mates from consuming a toxic substance rather than simply not entering an area previously treated with an insecticide.

One suggestion is that ants "unconditionally" fill in holes that they encounter. We discount this for three reasons. First, when the trap was placed unopened during the baseline period, there was little activity associated with blocking an entrance to the poison. It was only when the poison was exposed did the ants cover an entrance. Second, it is known from a study by Sokolowski *et al*., (2010) that blow flies (*Phormia terraenovae* Robineau-Desvoidy) seldom enter a hole without receiving a reward to do so. Third, it took time (about one to two days) before the ants began to block off an entrance leading to the poison bait. If the response we observed were an unconditioned reflex, the behavior of blocking the poison would have begun immediately. Whether an interpretation of the behavior we observed can be couched in terms of traditional principles of learning is an interesting challenge and remains to be determined.

Under normal conditions, an ant trap designed for home use would not be placed in the field. When used in the home, the targeted ants would not have access to the type of sticks, pebbles, and soil present in field conditions. Thus, we do not know if ants exposed to the poison in the home would exhibit the behavior we observed in the field. Perhaps if ants living in a home environment were provided with sticks, pebbles and soil, the behavior we observed in the field may be a common avoidance mechanism of ants in response to poison bait traps.

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