

Effects of Ant Presence on *Nicrophorus investigator*'s Reproductive Success

Student: Omar Ortuno

Mentor: Rosemary Smith

Independent Research and Course/REU

2018

Abstract

Burying beetles require carrion in order to successfully reproduce and they compete with vertebrate scavengers, other burying beetles, ants, flies, fungi, and microbes for this ephemeral resource. Burying beetles have mechanisms to combat competition from fly eggs, fungi, and microbes, but interactions between ants and beetles have not been extensively studied, and are not yet well understood. Interactions between the ant species *Tapinoma sessile* and the burying beetle *Nicrophorus investigator* may yield a diffuse mutualistic relationship involving the predation of undesired dipteran eggs from the carrion. This study found fly presence reduced successful carcass sequestration by *Nicrophorus investigator*, with fly presence at the carcass leading to the beetle's complete failure to bury. Ants (*T. sessile*, *Formica fusca*, *Formica rufa*, *Myrmicinae* subfamily) were found to have a conditional interaction with flies, for their presences in tandem increased beetle burial success, while pure ant presence was still negative for successful carcass burial.

Introduction

Carrion is an ephemeral, nutrient-rich resource, appearing only after the death of an organism, and one that attracts competition between vertebrates, invertebrates, fungi, and microbes (Hoback et al. 2004). Among these invertebrates, are the carrion beetles, including burying beetles, which play an important role in the break down and recycling of organic material back into the ecosystem (Kalinová et al. 2009).

Nicrophorus is a genus of carrion beetles that contains about 75 species and is found mainly in the northern hemisphere, most likely due to increased competition found in the southern locales from ants, flies, and vertebrates, as well as increased rates of carrion decomposition due to warmer climates (Scott 1998). *Nicrophorus* species are known for their unique use of teamwork for sequestration of carrion among beetles (Milne and Milne 1976), as well as their extended biparental care of young (Scott 1998).

The reproduction process of *Nicrophorus* begins by first locating a suitable, fresh carcass that hasn't been overrun by competitors, and that is small enough to bury. Carcasses used for reproduction are found via chemosensors found on the antennae of the beetles, which detect odors of decay (Kalinová et al. 2009). A single carcass may be found by multiple beetles, but a single pair eventually drives off the other beetles (Wilson et al. 1984), and this pair then begins to bury the carcass once a proper location is found. Once underground, the beetles copulate, work the carrion into a compact ball, and remove the fur or feathers (Milne and Milne 1976) before covering it in oral and anal secretions with antimicrobial properties (Hoback et al. 2004). A conical depression is then made at the top of the compacted carcass, into which the pair of beetles regurgitate partially digested tissue, which will be used to feed the larvae (Milne and Milne 1976). The eggs are then laid in the surrounding soil, and hatch approximately 56 hours later (Solis and Smith 2017). Complete consumption of the carcass occurs 15-20 days after hatching, and the larvae enter

underground chambers for overwintering (Otronen 1988, Smith et al. 2000), and only then do the parent beetles leave the chamber, except for the male, which may leave earlier.

If vertebrate scavengers do not find and take the carrion, the main competition is between flies, ants, and other beetles (Trumbo 1990a). Intraspecific competition between *Nicrophorus* species have been shown to be settled by size differences, with the larger individuals winning significantly more often (Otronen 1988). Trumbo (1990a) found that within 24 hours after setting the carrion, ants were active on over 95% of them, and flies had oviposited on the majority of the carcasses. This can prove to be a great problem for the burying beetles, as they will abandon carcasses that are heavily exploited by competitors, with the majority of carcasses that aren't successfully buried by the beetles being completely consumed by fly larvae (Trumbo 1994).

In order to combat dipteran competitors, burying beetles will either destroy the fly eggs and/or larvae themselves, or mutualistic phoretic mites that live on the beetles will eat them (Scott 1998). Although some studies have questioned the effectiveness of these phoretic mites, as they sometimes target *Nicrophorus* eggs, too.

Depending on the abundance, the presence of ants could lead to abandonment of the carrion by the beetle. Scott et al. (1987) hypothesized that the finding of a large, concentrated resource, such as a dead vertebrate, could elicit a massive recruitment response from an ant colony, which would likely drive off any prospecting burying beetles. One possible counter to the competition from ants could be the nocturnal activity associated with some *Nicrophorus* species, such as *N. defodiens*. In this case, early detection of a carcass could lead to successful sequestration by the beetle. Yet, burying beetle and ant interactions have not been extensively examined (Scott et al. 1987, Scott 1998), and an attempt to further our understanding of ant-beetle interactions was made by studying the effects of ant presence on burial success for various *Nicrophorus* species, but no significant effect was found, and burial failure was instead attributed to intraspecific and intrageneric conflict (Solis and Smith 2017); however, an observation was made regarding a specific species of ant, *Tapinoma sessile*, which was seen carrying fly eggs away from the carrion.

If this observation was accurate, and representative of an as-of-yet unknown interaction between *Tapinoma sessile* and *Nicrophorus* beetles at a potential burial site, then it raises the question of what the effects of *Tapinoma sessile* are on *Nicrophorus* reproductive success. Ant-dipteran interactions have been observed previously, with Fuller (1934) claiming that ants add complexity to the carrion-feeding communities by consuming both carrion and dipteran larvae (as cited in Trumbo 1990), but a potential interaction involving *Nicrophorus*, in which the burying beetles benefit, has not yet been observed. The focus of this study is to test the hypothesis that presence of *T. sessile* increases the reproductive success of *N. investigator*, a common *Nicrophorus* beetle found in Gothic, Colorado.

Methods

Study Organisms

Our study focused on *Nicrophorus investigator* due to its relative abundance compared to the other native *Nicrophorus* species, *N. guttula* and *N. defodiens*, found in Gothic, Colorado. *N. investigator* appears to be univoltine (Smith et al. 2000), and the adults are diurnal with a preference for warm, open meadows. If a male finds the carrion first, it will release pheromones in an attempt to attract a partner. Other males may be attracted to the pheromones, which would cause intraspecific conflict that usually results in the victory of the larger beetle. The parents attend to the brood for 12 to 18 days (Smith et al. 2000), and the larvae enter the underground overwintering chambers in July and August, where they stay for as long as 11 months as prepupa (Smith 2002). The following summer they pupate and eclose as reproductive adults, which reproduce throughout July and August. .

The ant species *Tapinoma sessile*, is known for its occasional extreme polygyny, colony reproduction by budding, reduced internest aggression, polydomy, and a generalist diet, which may consist of ephemeral foods such as insect cadavers and refuse (Buczkowski and Bennett 2006), with an increase in protein intake during the summer, possibly due to increased brood production (Barbani 2002). Their nests can be found under stones, and are usually shallow. *T. sessile* is known to move its nests in response to adverse conditions; however, the abandoned nests may be re-colonized if conditions improve (Buczkowski and Bennett 2008). They also exhibit high foraging site fidelity, which is a common attribute of many ants.

Study Sites

This study was conducted at sites around the Rocky Mountain Biological Laboratory, in Gothic, Colorado. More specifically, they were located along the Judd Falls trail (Figure 1).

T. sessile's* effect on Reproductive success of *N. investigator

To determine the effects of *Tapinoma sessile*, 31 nests were located along the Judd Falls trail, but only 29 were used. These 29 nests were split into 3 different sets, and used at different times according to the table below.

Set number	Sites	Date of utilization
1	1, 2, 4, 5, 6, 7, 8, 9, 10	June 30 – July 24
2	11, 12, 13, 14, 15, 16, 17, 18, 19, 20	July 8 – August 1
3	3, 21, 22, 23, 25, 26, 27, 28, 30, 31	July 16 - August 10

At each nest site, 2 cans were placed about 1 meter from each other and from the nest. The distance of 1 meter was determined based on Solis's findings, which

determined distance from the nest to be arbitrary with regards to the ant abundance (Solis and Smith 2017).

The steel cans used were 15 cm diameter, and 17 cm deep. The cans were buried deep enough to leave approximately a 5 cm rim exposed. Metal mesh screens were fixed on top of the cans using wire and a rubber band. Small holes were cut into the top of the screens to allow ants and flies to enter and exit the can. The bottom of the cans had 5 holes punctured into them to prevent water from stagnating. One previously frozen dead mouse, collected from the cabins at RMBL, was placed into each can, each one having been weighed and within a 17-25 gram range, which is in accordance with Smith's findings for *N. investigator's* preferred rodent size (Smith and Merrick n.d.). One of the cans at each site was designated as the ant-exclusion can, which was achieved by applying tangle foot on both the outside and inside of the exposed rim.

Fly egg abundance, fly larvae abundance, and ant abundance were assessed every day at 9:00 am. Burial success was assessed for a minimum of 4 days, after which failed burials were removed from the set. Brood size was assessed 19 days after the introduction of beetle pairs into the can.

Ant nests were baited with vials containing tuna for two days to assess ant presence, after which the cans were buried as described previously. Each can was then baited with tuna to allow the ants to become accustomed to finding food in the cans. The bait remained in the cans for two days, during which ant presence was assessed. These were referred to as "prep days."

Prep Day 1	Set up the cans and place tuna in each one
Prep Day 2	Check cans for ants present

The experiment for each set began on the day after "Prep Day 2" and used the following schedule every week for the first three weeks:

Day 1	Placement of mouse carcasses in each can
Day 2	Check for flies and ants (counting and identifying)
Day 3	Introduction of a beetle pair (male and female) into each can
Day 4 - 6	Check for burial, ant presence, and fly presence
Day 7	Check for burial, ant presence, and fly presence. Remove cans with failed burials.
Day 21	Collect, count, and weigh larvae

The beetles needed for the experiment were collected from 10 beetle traps, baited with chicken drumsticks, set up around the Gothic town site, specifically past

Ore House, along the tree line, and 3 more traps found around the Gothic Research Center (GRC). The beetles captured were kept in plastic containers in the lab, with food and water as needed, until used for the experiment. Any beetles collected from the cans after collecting the broods were returned to the lab. Larvae obtained from the broods were reburied for a separate experiment after being counted and weighed.

Results

The total number of ants present within the ant exclusion cans and the ant cans was analyzed using a two-way ANOVA/ two-sample t-test. The results showed a statistical difference between the mean number of ants that were present in the two different treatments, with the greater number of ants appearing in the ant treatment cans (Figure 2, $F_{1,55} = 14.0064$, $p\text{-value} = 0.0004$).

Fly presence, as determined by presence of adult flies, fly eggs, or fly larvae, and mouse burial success were analyzed using a contingency table and a chi-squared test. There is a statistically significant difference in burial success when fly presence was accounted for, with the most successful burials being those without fly presence (Figure 3, $X^2 = 9.604$, $p\text{-value} = 0.0019$).

Ant exclusion cans contained ants in some cases, and the ant treatment cans didn't contain any ants in other cases. Due to this, the cans were reclassified based on what was observed within them. Four new treatments were created: no ants, no flies (nanf); no ants, flies present (naf); ants present, no flies (anf); and ants present, flies present (af), table 1. These new treatments and burial success were analyzed using a contingency table and a chi-squared test. The least successful treatment, in terms of burial success, was no ants, flies present, with 100% failure ($n=5$); the most successful was no ants, no flies, with an 80% success rate ($n=10$). The second and third most successful treatments were the ants present, no flies treatment (64%, $n=22$) and the ants present, flies present treatment (35%, $n=20$) respectively. (Figure 4. $X^2 = 14.254$, $P\text{-value} = 0.0026$). A chi-squared analysis of the "naf" and "af" treatments on burial success found no statistical difference (Figure 5. $X^2 = 3.750$, $P\text{-value} = .0528$).

Overall, of the 58 cans buried, 29 contained buried carcasses. Of those 29, 16 managed to yield a brood. Sets 1 and 3 both contained 9 successful burials, with set 2 having the most at 11. Sets 2 and 3 both contained 5 broods, with set 1 containing the most at 6.

Discussion

Our findings showed that the ant exclusion and ant treatments were capable of creating a statistically different mean number of ants; however, the ant exclusion can design wasn't perfect as it allowed the entry of ants, albeit significantly less. Flies were not found to be consistent either, as not all cans contained fly presence. These two details were the reasoning behind the recategorizing of the cans into 4 new treatments based upon what was observed within the cans at the end of the experiment.

Flies were determined to be the most important determining factor when assessing burial success in the cans. This may be due to the great number of eggs that they can oviposit in the approximate two days that it takes *Nicrophorus* to reach the carcass (Smith and Heese 1995), which then hatch into maggots that can consume a great part of the resource before the beetle gets a chance to bury it (Trumbo 1994).

Also, although the experiment was designed to target *T. sessile* presence, other ant species were found within the cans, specifically *Formica fusca*, *Formica rufa*, and ants belonging to the subfamily Myrmicinae. We failed to find any statistically significant results involving any one specific type of ant; so, all ants were grouped together for our analysis. Ants were found to decrease burial success for the beetles; however, their relationship is more interesting, as ants were determined to have conditional interactions. Ants, regardless of species, when present at a carcass that lacks fly presence, decrease burial success for the beetles, most likely due to usage of the resource, or direct conflict with the present beetles, but when present in tandem with flies, they increased burial success.

Ants reducing the number of fly eggs present by eating them or destroying them might explain this result; however, this behavior was not observed directly.

Follow-up studies may have more success in keeping ants out of the ant exclusion can by reworking the design, or simply adding additional tangle foot. Also, because four different ant species entered the cans, it's possible to recreate this experiment and instead look for equal numbers of different ant species' nests, which could possibly increase the number of ants found other than *T. sessile*, and yield more interesting results. Lastly, this experiment tried to focus on reproductive success, but it failed to yield sufficient broods, with only 16 out of 58 cans having broods, so future experiments with more successful yields may be able to analyze them and see if ant presence has an effect on brood size.

Acknowledgements

I would like to thank Dr. Rosemary Smith for her input and contributions toward every aspect of this experiment; Arabelle Osicky and Barrett Gray for manual labor; National Science Foundation for the REU award that funded this experiment, and the staff at the Rocky Mountain Biological Laboratory for allowing me to carry out my research.

References

- Barbani, L. E. 2002. Foraging Activity and Food Preferences of the Odorous House Ant (*Tapinoma sessile* Say) (Hymenoptera: Formicidae):87.
- Buczkowski, G., and G. Bennett. 2008. Seasonal polydomy in a polygynous supercolony of the odorous house ant, *Tapinoma sessile*. *Ecological Entomology*.
- Buczkowski, G., and G. W. Bennett. 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker. *Insectes Sociaux* 53:282–290.
- Hoback, W. W., A. A. Bishop, J. Kroemer, J. Scalzitti, and J. J. Shaffer. 2004. Differences Among Antimicrobial Properties of Carrion Beetle Secretions Reflect Phylogeny and Ecology. *Journal of Chemical Ecology* 30:719–729.
- Kalinová, B., H. Podskalská, J. Růžička, and M. Hoskovec. 2009. Irresistible bouquet of death—how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. *Naturwissenschaften* 96:889–899.
- Milne, L. J., and M. Milne. 1976. The Social Behavior of Burying Beetles. *Scientific American* 235:84–89.
- Otronen, M. 1988. The effect of body size on the outcome of fights in burying beetles (Nicrophorus). *Annales Zoologici Fennici* 25:191–201.
- Scott, M. P. 1998. The Ecology and Behavior of Burying Beetles. *Annual Review of Entomology* 43:595–618.

- Scott, M. P., J. F. Traniello, and I. A. Fetherston. 1987. Competition for prey between ants and burying beetles (*Nicrophorus* spp): differences between northern and southern temperate sites. *Psyche: A Journal of Entomology* 94:325–332.
- Smith, R. J. 2002. Effect of larval body size on overwinter survival and emerging adult size in the burying beetle, *Nicrophorus investigator*. *Canadian Journal of Zoology* 80:1588–1593.
- Smith, R. J., and B. Heese. 1995. Carcass Selection in a High Altitude Population of the Burying Beetle, *Nicrophorus investigator* (Silphidae). *The Southwestern Naturalist* 40:50–55.
- Smith, R. J., A. Hines, S. Richmond, M. Merrick, A. Drew, and R. Fargo. 2000. Altitudinal Variation in Body Size and Population Density of *Nicrophorus investigator* (Coleoptera: Silphidae). *Environmental Entomology* 29:290–298.
- Smith, R. J., and M. J. Merrick. (n.d.). Resource availability and population dynamics of *Nicrophorus investigator*, an obligate carrion breeder. *Ecological Entomology* 26:173–180.
- Solis, A., and R. J. Smith. 2017. Native ant (*Formica rufa*, *Formica fusca*), *Nicrophorus* spp. interactions at small mammal carcass resources in the southern Rocky Mountains, Gunnison National Forest, Colorado.
- Trumbo, S. T. 1990. Reproductive Success, Phenology and Biogeography of Burying Beetles (Silphidae, *Nicrophorus*). *American Midland Naturalist* 124:1.
- Trumbo, S. T. 1994. Interspecific Competition, Brood Parasitism, and the Evolution of Biparental Cooperation in Burying Beetles. *Oikos* 69:241.

Wilson, D. S., J. Fudge, and K. B. Station. 1984. Burying beetles: intraspecific interactions and reproductive success in the field. *Ecological Entomology*:195–203.

Tables and Figures

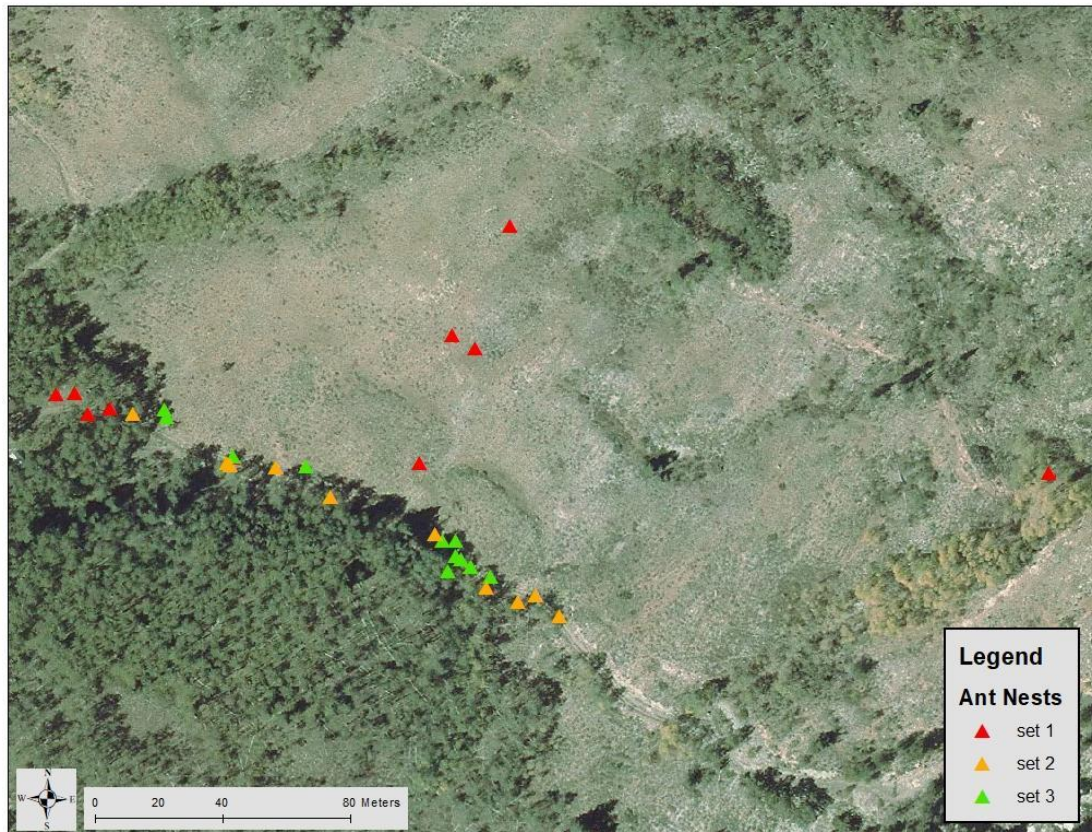


Figure 1. Map of sites along the Judd Falls trail, Gothic, Co.

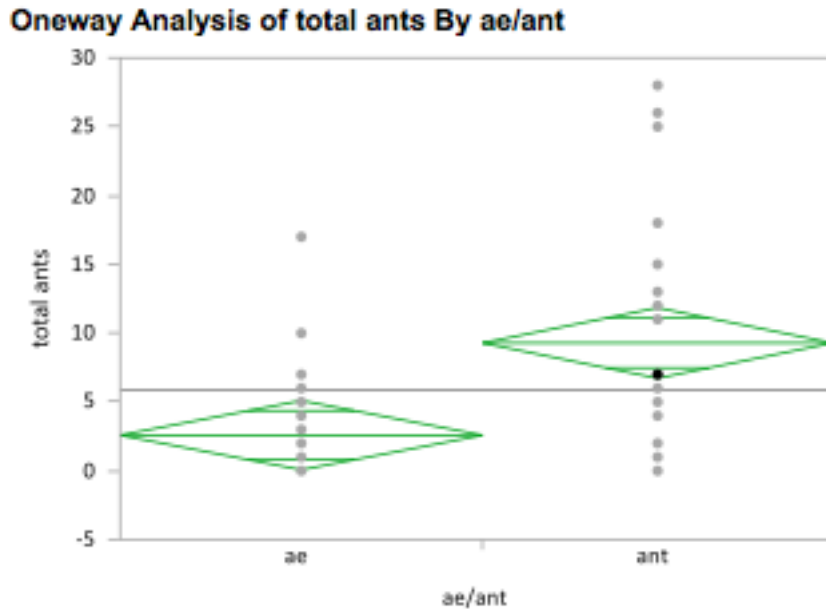


Figure 2. Two-way ANOVA analysis of ant-exclusion treatment (ae) and the ant treatment (ant). $F_{1,55} = 14.0064$, $p\text{-value} = 0.0004$

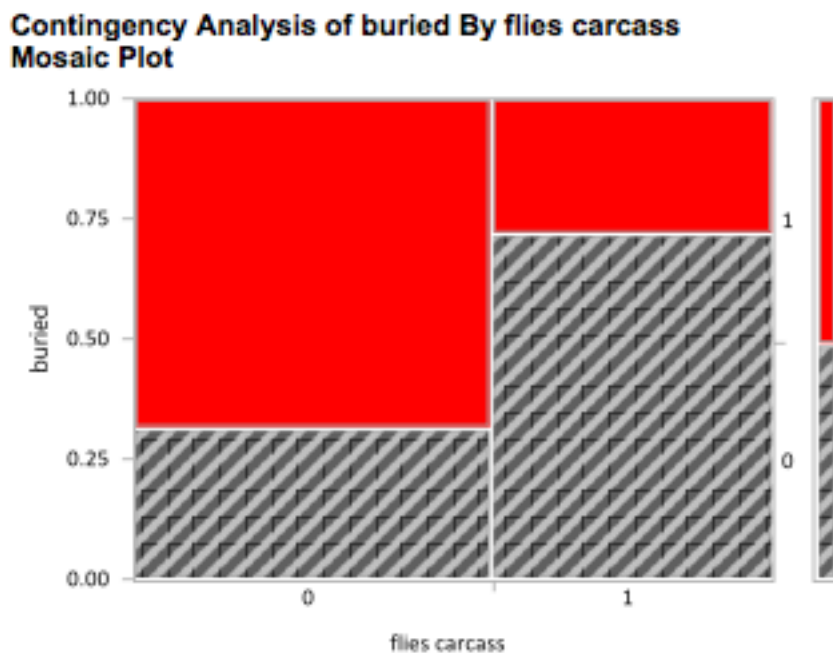


Figure 3. Contingency table of fly presence and burial success. Y-axis: 0 represents failure to bury, 1 represents successful burial. X-axis: 0 represents lack of fly presence, 1 represents fly presence. $\chi^2 = 9.604$, $p\text{-value} = 0.0019$

Contingency Analysis of buried By trt Mosaic Plot

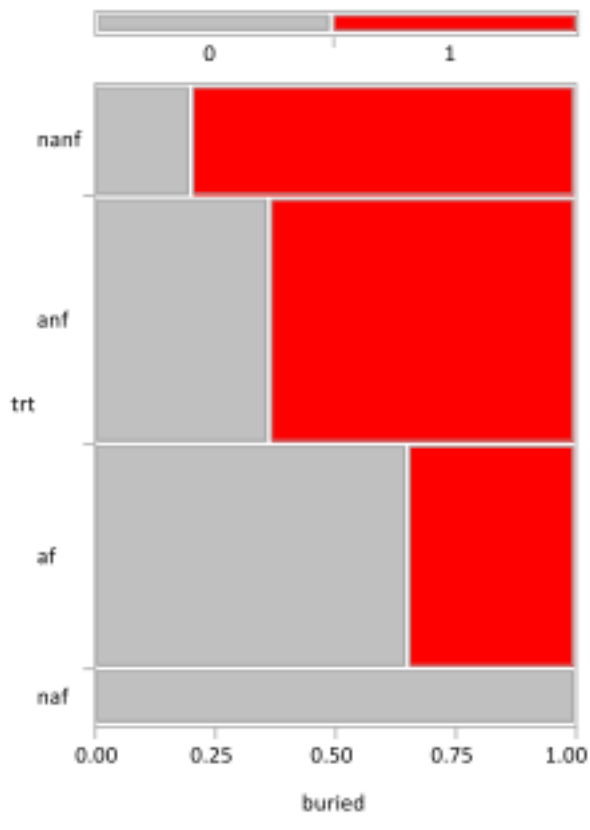


Figure 4. Contingency table for effectiveness of treatment and beetle burial success. 0 represents failure to bury, 1 represents successful burial. Treatments: nanf= no ants, no flies; anf= ants present, no flies; af= ants present, flies present; naf= no ants, flies present. $X^2 = 14.254$, P-value= 0.0026

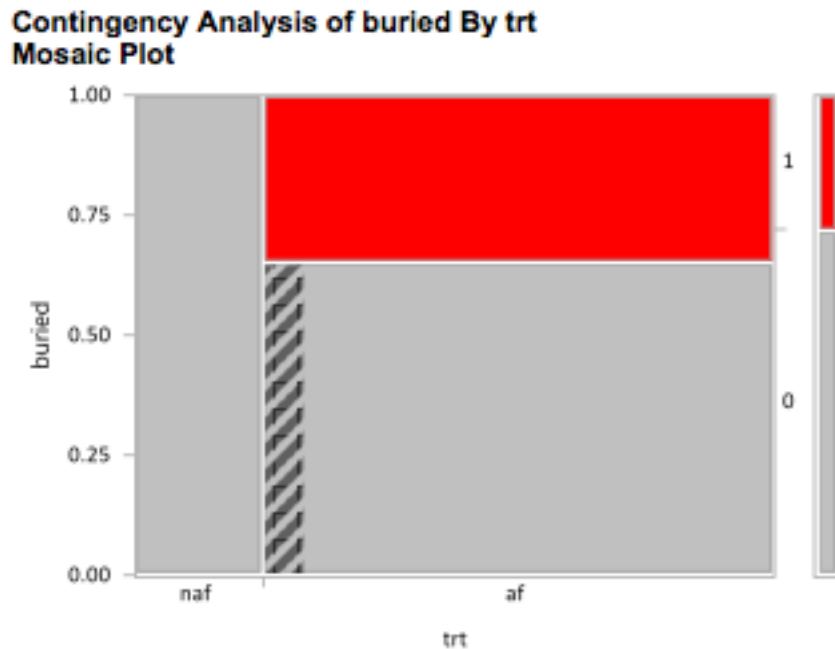


Figure 5. Contingency table for effectiveness of af and naf treatments on burial success, af= ants present, flies present; naf= no ants, flies present. $X^2= 3.750$, P-value= .0528

Treat ment	Not Buried	Buried	Total
naf	5	0	5
af	13	7	20
anf	8	14	22
nanf	2	8	10
Total	28	29	57

Table 1. Table showing new treatments based upon observed ant/fly presence within each can.