



Assessing Presence of Argentine Ants Using the Bait Method

Survey Protocol

U.S. DEPARTMENT OF THE INTERIOR
U.S. GEOLOGICAL SURVEY
WESTERN ECOLOGICAL RESEARCH CENTER

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Introduction

Argentine ants (*Linepithema humile*) are small uniformly brown ants (figure 1). Colonies have multiple queens, thousands of workers, and lack intraspecific aggression and displace native ant species in invaded areas (Holway 1999). Argentine ants are behaviorally dominant in invaded communities in North America (Andersen 1997). Argentine ants are omnivorous. Colonies enter the reproductive phase during the summer months increasing protein intake (Rust et al. 2000). Winter and Spring food intake consists mainly of carbohydrate rich plant and insect exudates (Rust et al. 2000). Foraging activity is limited to moderate temperatures. Argentine ants are not tolerant of cold or hot temperatures and therefore are not active during the hottest part of the day and overall less active during the winter.



Figure 1 Argentine ant (from Antweb.org)

Figure 2 Argentine ants on tuna bait

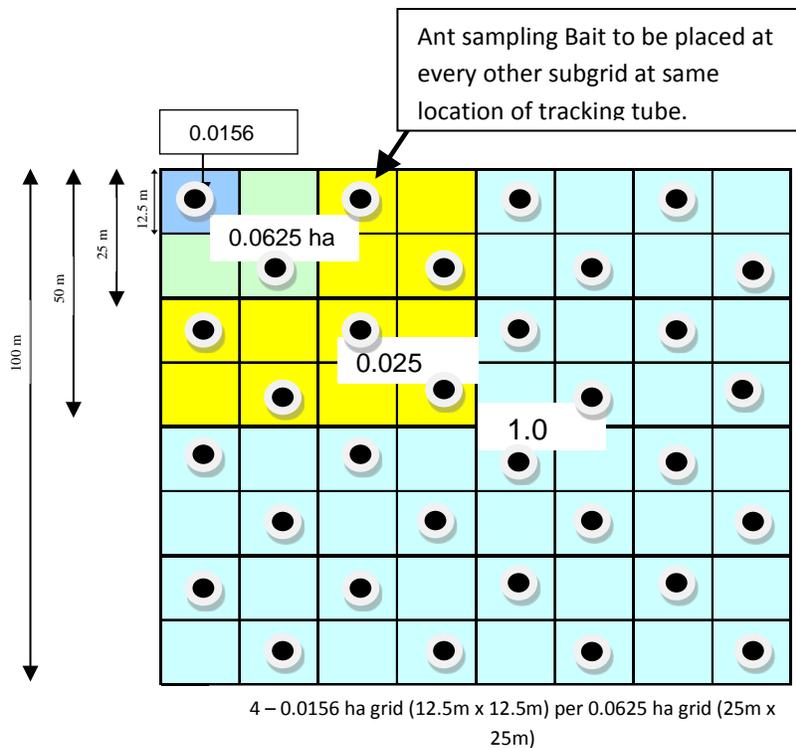
Various field techniques can be used to study ant communities (see Bestelmeyer et al 2000 for specifics on particular techniques). Selecting a method will depend on the purpose of the study and time and effort available. In this protocol, the baited card method is employed to assess presence of Argentine ants. Baiting consists of a food substance and a bait platform left out for a period of time, after which observations or collections are made. The method is biased towards recruit-foraging, behaviorally dominant species (Andersen 1997), and therefore well suited for assessing presence Argentine ants (Figure 2). Baiting is also a quick and cost effective method. Compared to some of the other techniques available, it requires less time and effort and does not require much specialized equipment or training.

This protocol is geared towards recruiting Argentine ants to bait locations. Preferences for timing of sampling are recommended for optimizing collection of Argentine ants. Results from this type of bait study will not provide an accurate measure of what native ants are present, as many ants will not be attracted to the bait, may not be active during the baiting period, or will be competitively excluded once dominance over the bait is established. Results from use of this baiting protocol at 43 locations in Pacific Pocket Mouse habitat on Marine Corp Base Camp Pendleton yielded 8 other species besides Argentine ants. Comparatively, nearly 60 species were found when using the pitfall method (buried container for ant collection with opening flush to the ground) for other studies done across southern California (Mitrovich et al. 2010, Matsuda et al. 2011).

Methods

Placement of sites will depend on the goals of the study. For example, our Pacific Pocket Mouse study, ant baits were set over 1ha (100m x 100m) grids. The mouse grids consisted of 64 12.5m x 12.5m sub-grid locations set up in an 8 by 8 square. An index cards with bait were placed on every other 12.5m x 12.5m grid (fig. 3) for 32 baits total. This setup allows for interpreting results at various spatial scales. Argentine ants foraging distance to bait in Vega and Rust 2003 was 61m and foraging trails from nests have been reported at much larger distances. As an alternate set up, cards could be reduced to 16 baits placed at the center of 25m x 25m grids within the larger 1 ha grid. For the rare plant monitoring project (SDMMP 2014), the bait station should be placed at the most suitable site (as described below) within or near the sampling area (i.e., 10-m radius circle).

Field Surveys should be conducted when temperatures are at least 15°C (59°F) and conclude before reaching 27°C (~80°F). Morning sampling is suggested, as decreased surface activity has been observed in the afternoon. For optimal sampling, conduct surveys during the warmer months of the year. Tuna in oil is suggested for baiting. Cookies, such as pecan sandies, also work well since they have a high sugar and butter content as well as nuts for protein. Ideally, the bait card would be placed in a shaded spot or near an area of moisture. If neither condition is present, select the most suitable space within the survey area, which at minimum will be cleared open ground. If possible, wait 45 minutes from the start time before checking the bait card. However, if the plant monitoring takes less time, then check the card when the monitoring is completed and be sure to record the time. Between 60 and 90 minutes is considered sufficient time to recruit ants to the bait (Bestelmeyer et al. 2000). Therefore, it is suggested that baits not be left unchecked after 90 minutes. The more time the bait is left out, the risk of bait being removed by other animals increases (i.e. crows may take bait off card).



4 – 0.0625 ha grid (25m x 25m) per 0.25 ha grid (50m x 50m)

Figure 3 Ant bait Grid set up for Pacific Pocket Mouse Study

Setting out Baits:

1-Record the start time and temperature. The start time is the time at which you set the first index card with bait down. For the rare plant monitoring project (SDMMP 2015), enter the start time and temperature on the rare plant monitoring data form in the section for Argentine Ants (Section V).

2-Label the front of the index card with the location. Location names should have GPS points associated with at least each sampling location. Ex Sample Grid #25, Card 1A would be labeled 25-1A and GPS coordinates would be taken for the center of Plot 25. For the rare plant monitoring project, record on the index card the Occurrence ID and Sample Point (if more than one point sampled/occurrence). On the rare plant monitoring form in the section for Argentine Ants (Section V) enter the coordinates where the bait station is placed.

3-Place the index card flush with the ground. Raised edges on the index card can delay bait discovery. If the substrate is not level, create a clearing for the card that allows the card to be flat on the surface.

4-Drop a small chunk of cookie on the card. Continue steps 2-4 until you have set out bait at all locations. Record the bait end time and temperature after setting the last index card.

Checking on Baits:

1-Record the time the bait was checked and the temperature.

When approaching each bait, try not to disturb any ants present. For the rare plant monitoring project (SDMMP 2015) the check time will be the time that you finish the plant sampling. Ideally, this will be

around 45 minutes. Enter the time finish and temperature on the rare plant monitoring form in the Argentine ants section (Section V). Record whether an ant sample was collected.

2- Take a picture of the index card if there is any ant activity. This will be to gauge the abundance of ants present at each grid. Make sure the picture includes the grid number that is written on each index card. You can skip this step if no ants were present at the bait. For the rare plant monitoring project, if a photo is taken enter a photo number on the rare plant monitoring form section for Argentine ants (Section V).

Don't forget to check the underside of the index card to see if ants are present. If ants are present take a picture of the underside and then of the label on the top side of the index card. While more aggressive ants would be found on top of the index card, subordinate or cryptic species might be found on the underside.

3- Collect a few ants (5-10 individuals is sufficient) of each species present on the index card. Place ants into the 1.5mL microcentrifuge tube filled with 95% ethanol. Tweezers either directly or using water tension can be used to pick up ants (pipette will also work with water tension). An aspirator can also be used to collect ants.

Some ants are polymorphic, meaning there are various sizes and/or types of workers. It is most important to collect the different types when there are major and minor castes (fig 4) of workers present, as identification is sometimes based on the major workers.

4-Once ants are collected; make sure tube is labeled properly. Label the tube with location, date, and collector using an alcohol proof pen (such as VWR markers)

Examples for labels:

Camp Pendleton Grid 294-3C 15Jul2013 USGS

5- Shake or blow any remaining ants off card and discard remaining bait and index card in trash bag.

6- After the last card has been checked, record check end time and temperature,

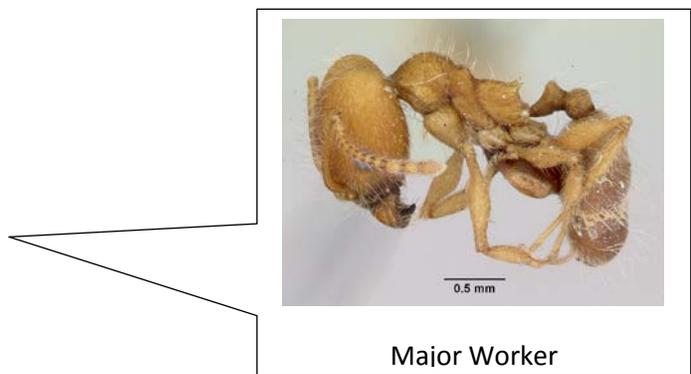




Figure 4 Example of *Pheidole* sp. with major and minor workers, (inserts from antweb.org)

Processing Samples

Collected specimens should be identified under a microscope. Identification of ants can be determined to the genus level using Ward 2005. Field photos will be used to determine ant abundance using the abundance categories: no ants (N/A or “-“ depending on data entry format), 1= 1-50, 2= 50-100, and 3= 100+ (see appendix for examples).

Equipment List

- Pecan Sandie cookies
- Gloves
- Index Cards
- 1.5ml microcentrifuge tubes
- Storage box with divider for 1.5ml vials
- 95% ethanol
- Tweezers or pipette, aspirator
- Alcohol proof pen, such as VWR markers
- Writing utensil (for writing start/end times and temps, labeling cards)
- Camera
- GPS Unit
- Thermometer or location based real time weather data (weather app)
- Trash bags

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Appendix

Argentine Ants Category 1 (ct 27)



Argentine Ants Category 2 (ct 74)



Argentine Ants Category 3 (ct >250)



Comparing species similar to Argentine Ants

Dorymyrmex



Forelius



Argentine Ant

