



Determining Presence of Argentine Ants Using the Rapid Assessment Protocol

Survey Protocol



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

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Table of Contents

Introduction	1
Methods	2
Conducting Surveys.....	3
Processing Samples.....	5
Example Datasheet	6
Equipment List	7
References	7
Appendix	9

Introduction

Argentine ants (*Linepithema humile*) are small brown ants that have a head and thorax lighter brown than the rest of the body (Figure 1). Colonies consist of multiple queens and have a unicolonial social structure, where multiple nests behave as a single large colony. In introduced areas they have reduced intraspecific aggression, functioning as a super colony capable of displacing native ant species (Hölldobler and Wilson 1990, Holway 1999). Argentine ants are behaviorally dominant in invaded communities in North America (Andersen 1997). They are highly omnivorous and opportunistic foragers (Suarez et al. 1998, Rust et al. 2000). 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 plants and insect exudates (Rust et al. 2000). Argentine ant activity is limited by low winter temperatures and high summer temperatures (Abril et al. 2007). The species is not very active during the hottest part of the day and is less active overall during the winter.

Argentine ants not only have a negative impact on the native ant community, they also displace other invertebrates (Human and Gordon 1997), often changing the structure of the invertebrate community. Argentine ants can indirectly affect other, larger species, including both plants and animals. Animals that depend on ants as a major part of their diets, as with horned lizards (Suarez and Case 2002) can be impacted when Argentine ants become the dominant species in the invertebrate community. Argentine ants have been shown to disrupt seed dispersal in certain plant species (Gómez et al. 2003). They can be predators of eggs and hatchlings in bird nests (Sockman 1997, Choe et al. 2010).



Figure 1 *Linepithema humile* (Argentine ant)
(Photographer: April Nobile, from www.Antweb.org)



Figure 2 Argentine ants on card baited with cookie crumbs

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 the time and effort available. Here, the rapid assessment protocol employs the baited card method to determine the 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 the presence of Argentine ants (Figure 2). The rapid assessment protocol provides information on the

current condition of habitat and extent of invasion. Compared to some of the other techniques available, the baited card method does not require much specialized equipment or training, and requires little time and effort, making it cost effective and well suited for the rapid assessment of an area.

This protocol is geared towards recruiting Argentine ants to the bait locations. The recommendations included here are intended to optimize the detection and collection of Argentine ants only. Results from this type of survey will not provide an accurate measure of what native ants are present, as some 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.

Methods

Surveying for and documenting Argentine ants is a multi-step process: 1) a preliminary survey and 2) follow-up surveys where the species is detected. Preliminary surveys should be conducted to determine the need for and locations of secondary surveys. The perimeter of the property should be scouted for locations most likely to harbor Argentine ants. This can be an area where there is an urban interface, building structures, paved roads, or any areas with a water source or high moisture levels. Along the property boundary, preliminary survey locations should be spaced no more than 200 meters apart, as we expect favorable conditions in an area invaded with Argentine ants to allow spread along the perimeter and a similar distance inland from the edge. Preliminary survey points should also be placed at locations within the property that have favorable conditions, such as a pond or creek running through the middle of the property.

If Argentine ants are discovered during preliminary surveys, a series of secondary surveys should be done to determine how far into the property Argentine ants occupy from the initial discovery point. For each preliminary survey location, set up a linear series of secondary survey locations extending from the preliminary location towards the interior of the property (Figure 3). The secondary survey transects within a property may need to be divided into several subunits that can be completed within the temperature and time limits of the protocol. The field personnel will need to return to each survey location within 90 minutes of setting the baited card to check the results.

The distance between the secondary survey locations is based on the foraging behaviors of the species. The foraging distance to bait observed by Vega and Rust (2003) for Argentine ants was 61 meters, with foraging trails from nests reported at much larger distances. Therefore we have decided secondary survey locations will continue from the preliminary point every 50 meters, extending into the center of the property. Mitrovich et al. (2010) found there was a high likelihood (~75%) of Argentine ant invasion within the first 200 meters in from the urban edge, decreasing to 10% after this distance. In Suarez et al. (1998) and Bolger et al. (2000), Argentine ants were rarely detected beyond 200 meters. Given this information, points along the transect can be discontinued if no Argentine ants have been found over the previous 200 meters of survey locations, whether that detection was the preliminary observation or a subsequent secondary survey location. However, if your transect crosses a water source or road running through the property, this point should be treated like a preliminary point (if it was not already) and surveys should extend out in both directions from this point.

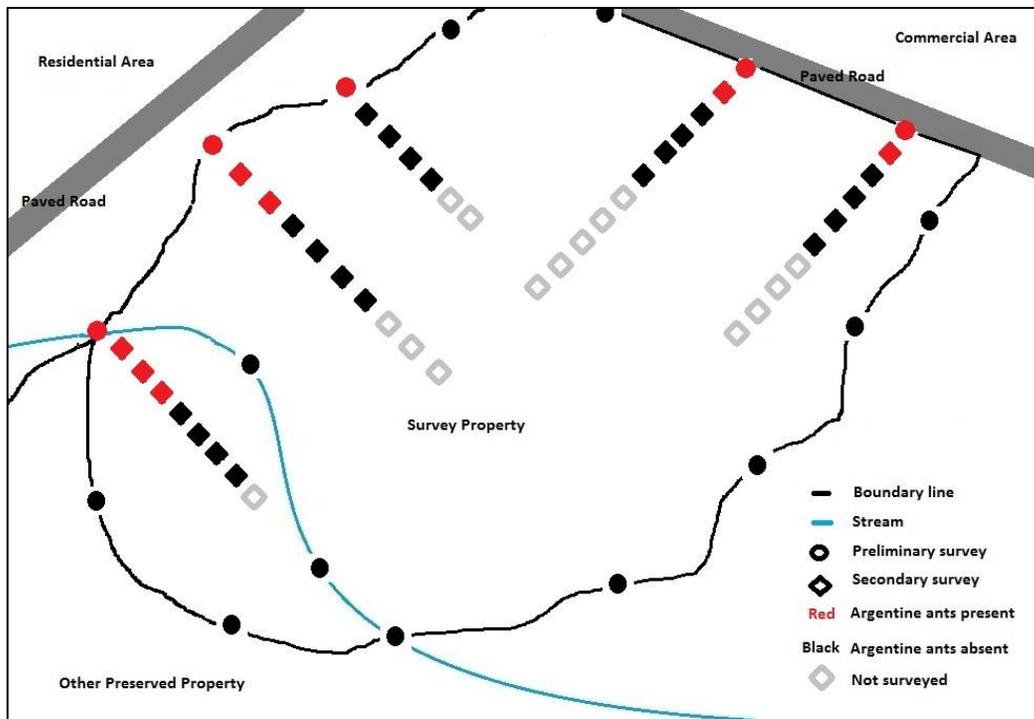


Figure 3. Example map of survey points

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. If temperatures are warm enough at night or early morning, samples can be conducted as needed to allow for sufficient time to complete the survey. For optimal sampling, conduct surveys during the warmer months of the year.

Conducting Surveys

Start a new datasheet for each day for each property; record the name of the property, the observer, and the date at the top of the form. Record the start time and start temperature when you get to your first survey point. Before placing the baited cards at each survey site, the area should be searched to see if there is any ant activity within a 25 meter radius. If ants are discovered, they should be documented and/or collected. The observation of three independent centers of ant activity within the 25 meter radius of the survey point means that no return trip is required to collect the baited cards. If Argentine ants are detected during the visual inspection of the survey point, only one instance is sufficient. If a single instance of Argentine ants has been discovered or three independent centers of activity without Argentine ants have been observed before setting bait cards, you can move on to the next survey point.

If you do not find ants at the survey site, you will set out three baited cards within a 25 meter radius of the point. Ideally, the baited 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 (clear an area if necessary). The card needs to be accessible to the ants and easy for the surveyor to observe and collect. Trails, nest entrances, and under shrubs are good places to put index cards.

Label the front of each index card with the property name, transect name, and survey point. Assign each of the three cards for that site a letter (ex. Crestridge G-50m card A). In this example, the property name is Crestridge, the preliminary survey point along the perimeter is "G", this is the secondary survey point 50 meter in from the edge, and this is "card A". 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. Cookies, such as pecan sandies, are used as bait since they have a high sugar and butter content as well as containing nuts for protein. Cookies are easy to carry and set out as compared to other possible baits. Crumble a small chunk of cookie on the labeled card.

Wait a minimum of 45 minutes from the time you set the first baited card. 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 completely removed by the ants or being removed by other animals increases (i.e. crows may take bait off the card).

When approaching each baited card, try not to disturb any ants present. Make sure the 1st picture at each location includes the information that is written on each of the three index card. This will designate which set of pictures belongs with each survey point. It is important that you attempt to get a clear picture of the specimens on the card, as some ants are skittish and will abandon the card when disturbed. Your picture may serve as the only potential means of visual identification. Photos should be taken at an angle versus top down, to attempt to capture a side view of ants. The distinct identifying features of many ant species are easier to see in profile. It is recommended that a few pictures be taken from further away from the card, since these should be more likely to be clear and focused than pictures taken with the macro setting on the camera. These photos will have better detail and can be enlarged when reviewing them for ant identification. If possible, take photos using the macro setting as these will also help with identification. You can skip this step if no ants were present at the bait. Record "None" for each empty card.

Collect a few ants (2-5 individuals is sufficient) of each species present on the index card. Place ants into a 1.5mL microcentrifuge tube filled with 95% ethanol. Tweezers, either directly or using water tension, can be used to pick up ants (a pipette will also work with water tension). An aspirator can also be used to collect ants. Don't forget to check the underside of the index card to see if ants are present. While more aggressive ants would be found on top of the index card, subordinate or cryptic species might be found on the underside.

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 (Figure 4) of workers present, as identification is sometimes based on the major workers.

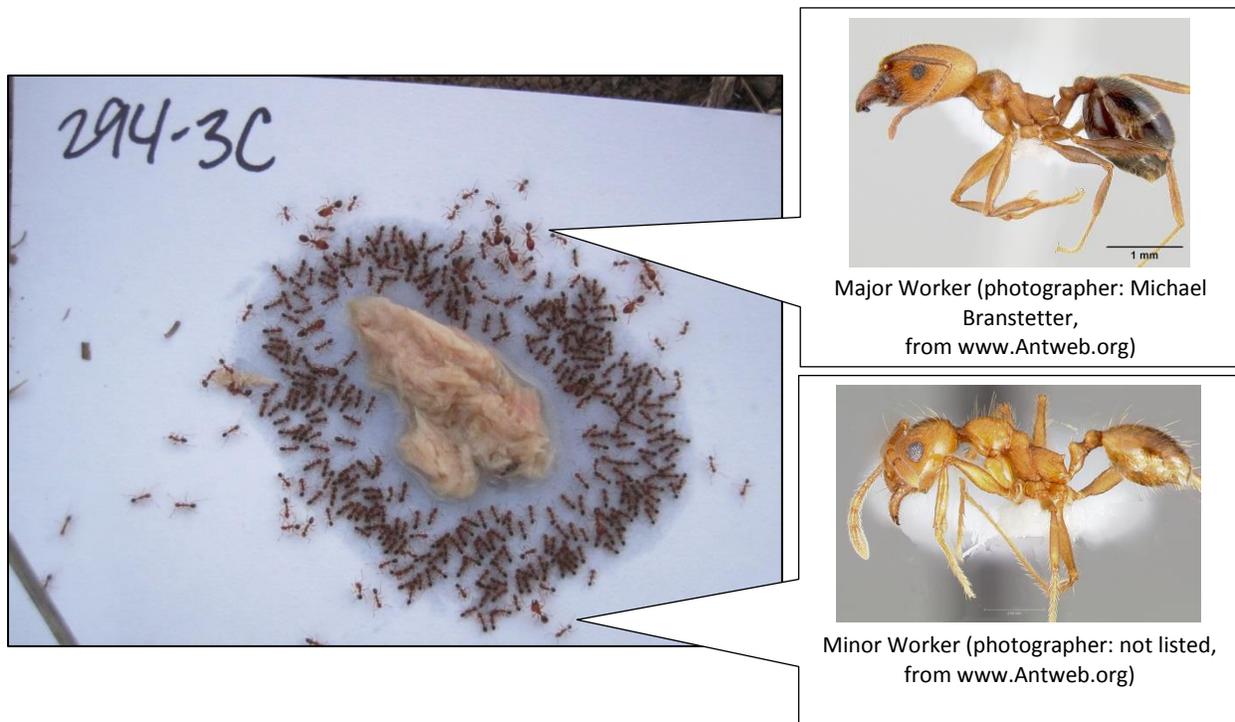


Figure 3. Example of *Pheidole* spp. with major and minor workers

Once ants are collected; make sure each tube is labeled properly with all of the site information using an alcohol proof pen (such as VWR markers). Record if specimens were collected on the data sheet. Shake or blow any remaining ants off the card. Discard the remaining bait and index card in a trash bag. After the last card has been checked, record the end time and end temperature.

Processing Samples

Species identifications should be verified after returning to the office. Collected specimens should be identified under a microscope. If the baited card was photographed, the image can be enlarged at a desktop computer to confirm the reported species. Identification of ants in San Diego can be determined to the genus level using Ward 2005. Representative photos of ant species in California can be found on www.antweb.org to aid in identification. For any records where the original species identification was incorrect, document the changes on the original paper datasheet and any subsequent data files.

Equipment List

Pecan Sandie cookies

Index Cards

1.5ml microcentrifuge tubes

Storage box with divider for 1.5ml microcentrifuge tubes

95% ethanol

Tweezers, pipette, or aspirator

Alcohol proof pen, such as VWR markers

Writing utensil (for writing start/end times and temps, labeling cards)

Datasheet

Camera

GPS Unit

Thermometer or location based real time weather data (weather app)

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Appendix

Comparing species similar to Argentine Ants

Dorymyrmex sp.



Photographer: Jen Fogarty, from Antweb.org

Forelius sp.



Photographer: Ryan Perry, from Antweb.org

Linepithema humile



Photographer: April Nobile, from www.Antweb.org