Attraction of *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae) to Linoleic Acid in Oil

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Abstract: Red imported Fire Ants (*Solenopsis* invicta) are well known pests that can lead to ecological destruction. An important industry regarding Red Imported Fire Ants (RIFA) is how to get rid of them—this includes baits. For RIFA ant baits it is important that fire ants need to have maximum attractivity to them, so the poison can eradicate the fire ant problem. Previous research has been conducted to see what is attractive to *S. invicta* and it appears that certain fatty acids are attractive to them. This study aims to examine different levels of attraction of *S. invicta* to a variety of oils with different fatty acid levels. It was expected that oils with higher levels of linoleic acid would be the most attractive to RIFA, as that seems to be one of the more nutritional oils for them. What was observed was that there was a higher attraction to macadamia nut oil, but only with a 90% confidence interval from the least attractive oil—pistachio nut oil. What may have happened in this experiment is that there was a higher attraction rate of oils with more intense aromas instead of attraction to the fatty acids themselves.

Keywords: Solenopsis invicta, linoleic acid, pine nut oil, bait

The Red Imported Fire Ant, *Solenopsis invicta* (hereafter RIFA), is an invasive ant species that threatens biodiversity and agriculture. The RIFA were introduced to the United States in the 1930's, and in 1957 a federal-control program has been launched and is still in place today (Williams 1983). The goal of this program is to discover and apply procedures for the management of RIFA populations that would limit their ecological and economical damages. The most effective method developed uses baits laced with slow acting insecticides.

From these programs, baits using soybean oil as the main attractant were developed. This attractant is still used today in most ant baits used by the pest management industry. However, there is always the possibility of improving these baits to attract RIFA more effectively to the insecticides being used

The main component of vegetable oils are triglycerides (98%), themselves composed of three fatty-acids connected to a glycerol backbone. The attractiveness of RIFA to fatty acids has been previously studied, but the exact relationship between them is still fairly unknown (Hughes et al. 1994). The main result of these early studies shows a greater attraction to linoleic acid, with the assumption that this fatty acid is nutritionally more important than others and is therefore more phagostimulatory. However, other fatty acids have been associated with non-feeding behaviors such as necrophoric behaviors. The goal of our experiment is to compare RIFA attraction to oils of different fatty compositions and determine if the sole linoleic acid content of an oil is the best marker of its attractiveness to RIFA.

RIFA mounds were observed in abundance at a pecan orchard, and it was questioned why there were so many there. There has been a high attraction to pecan oil observed in the past—it may be because of its high linoleic oil concentration (Ryan et al. 2006). Because of this observation, pecan oil and primarily taxonomically relatives were tested for comparison to soybean oil.

Cooking oils were used in this experiment to ensure that there were no additives. Cosmetic oils were used for some oils where cooking oils could not be found. To account for this difference, both cosmetic and cooking versions of pecan oil were used to calculate if there was a difference between the attractivity of the different types of oil based on intended use. The expected outcome is that pecan oil will be associated with the highest number of ants recruited and will not be directly linked to its sole linoleic acid content, but to a more complex blend of compounds.

Cooking oils were used in this experiment to ensure that there were no additives. Cosmetic oils were used for some oils where cooking oils could not be found. It is assumed that there were also no unstated additives in these oils. To account for this difference, both cosmetic and cooking versions of pecan oil were used to calculate if there was a difference between the attractivity of the different types of oil based on intended use.

Methods and Materials

Set up. A total of eleven different oils were tested: six were cooking oils (treatments A-F): pecan nut (Kinloch Plantation, Winnsboro, LA), hickory nut (Forager's Harvest, Weyerhaeuser, WI), walnut (Spectrum Organics, Petaluma, CA), almond (BetterBody Foods, Lindon, UT), macadamia nut (Roland Foods, New York, NY), and soybean oil (Hill Country Fare, TX). The other five were natural cosmetic oils (treatments G-K): pecan nut (Mayan's Secret, Canoga Park, CA), Brazil nut (Hyderabad, India), cashew nut (Deve Herbes, Una, India), pistachio nut (Botanical Beauty, Miami, FL), and Siberian pine nut (Flora Aromatics, Orlando, FL). Two milliliter Eppendorf tubes (Eppendorf, Oldenburg, Germany) were stuffed with cotton (AF&F Fiber & Finishing, Albermarle, NC) to the 0.75mL line, and three hundred milliliters of oil were added to a prepared Eppendorf tube. Each oil had 10 repetitions in each transect. There were a total of six transects, resulting in a total of 660 tubes.

Collection. The experiment was conducted at 4PM because the foraging activity of the RIFA was at its highest point in the evening. The ground surface temperature was 24℃, a temperature that allows foraging activity by RIFA. The beginning of each transect was marked with a flag and a tube was opened and placed on the ground one meter apart from each other. The end of the transect was marked with another flag. There was at least 20 meters of space between each transect. After an hour from the time the first tube was set down, tubes from each transect were collected. The tubes were kept separated for each transect for ant identification and counting. In the time between collection and identification, the tubes were kept at -20°C.

Identification and counting. After collection, tubes in each transect were separated by oil type. The number of ants in each tube in the transect were counted and the species of ant was recorded.

Analysis. These results were analyzed using one-way ANOVA tests, followed by Tukey-Kramer post-hoc tests if necessary. Three different variables were tested: the combined number of *S. invicta* counted in each transect, the number of tubes in each transect to attract *S. invicta*, and the ratio of how many ants per tube on average there were in each transect. Further analysis could be run with a Principal component analysis (PCA) to visualize the relationships between the different components of each oil.

Results

The total number of ants recruited was compared between the different oils. This was further broken down to compare the different species seen in each oil (Figure 1). The top three most effective oils at attracting ants were pine, macadamia, and hickory. The least attractive oils to S. invicta appear to be cashew, cosmetic pecan, pistachio, and walnut. The majority of the ants (90.04%) that were collected were the targeted species, S. invicta. However, because there were other species of ants, the data was divided and only the results with RIFA were accounted for in the statistical analysis.

The difference between the number of S. invicta between each transect had a p-value of 0.062. This means that the hypothesis can be accepted with 90% confidence. When analyzing this with a Tukey post hoc, it can be seen that, within the 90% confidence, the only statistically different treatments are the pine nut oil and pistachio nut oil (Figure 2).

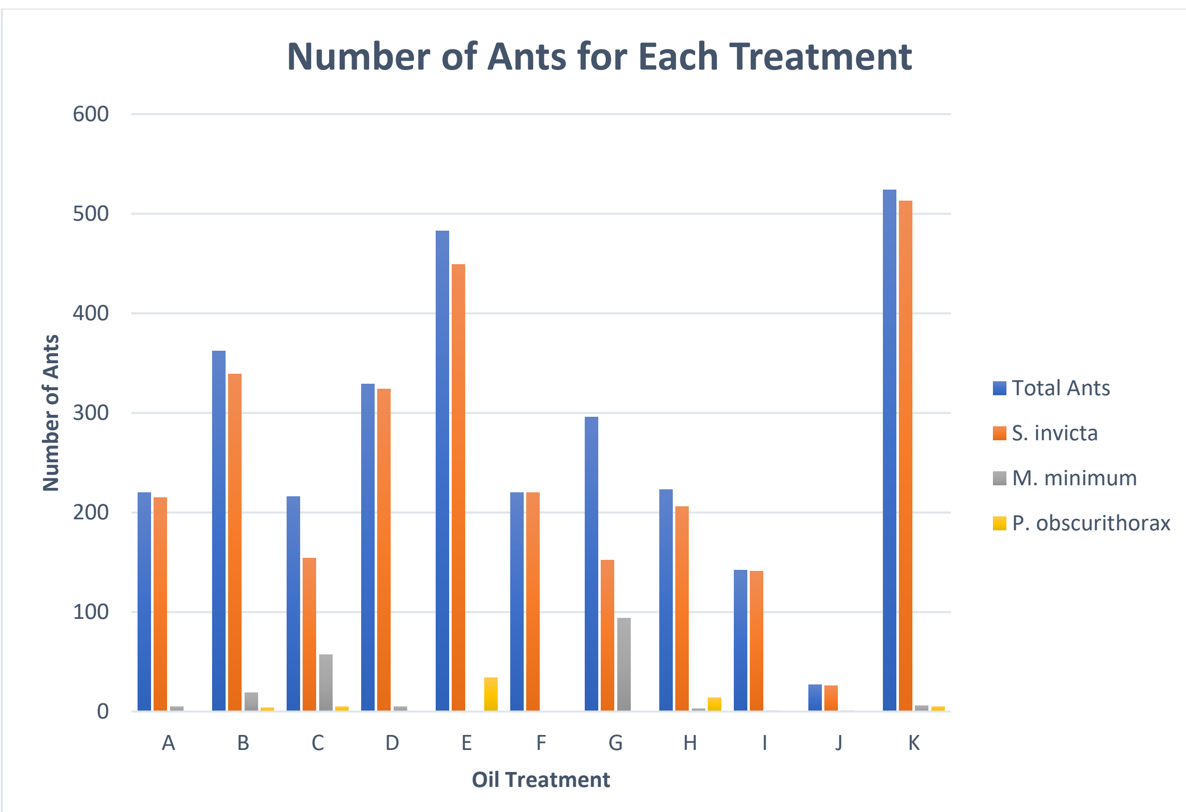

Figure 1. Ants collected from all transects. The total ants are then separated by species

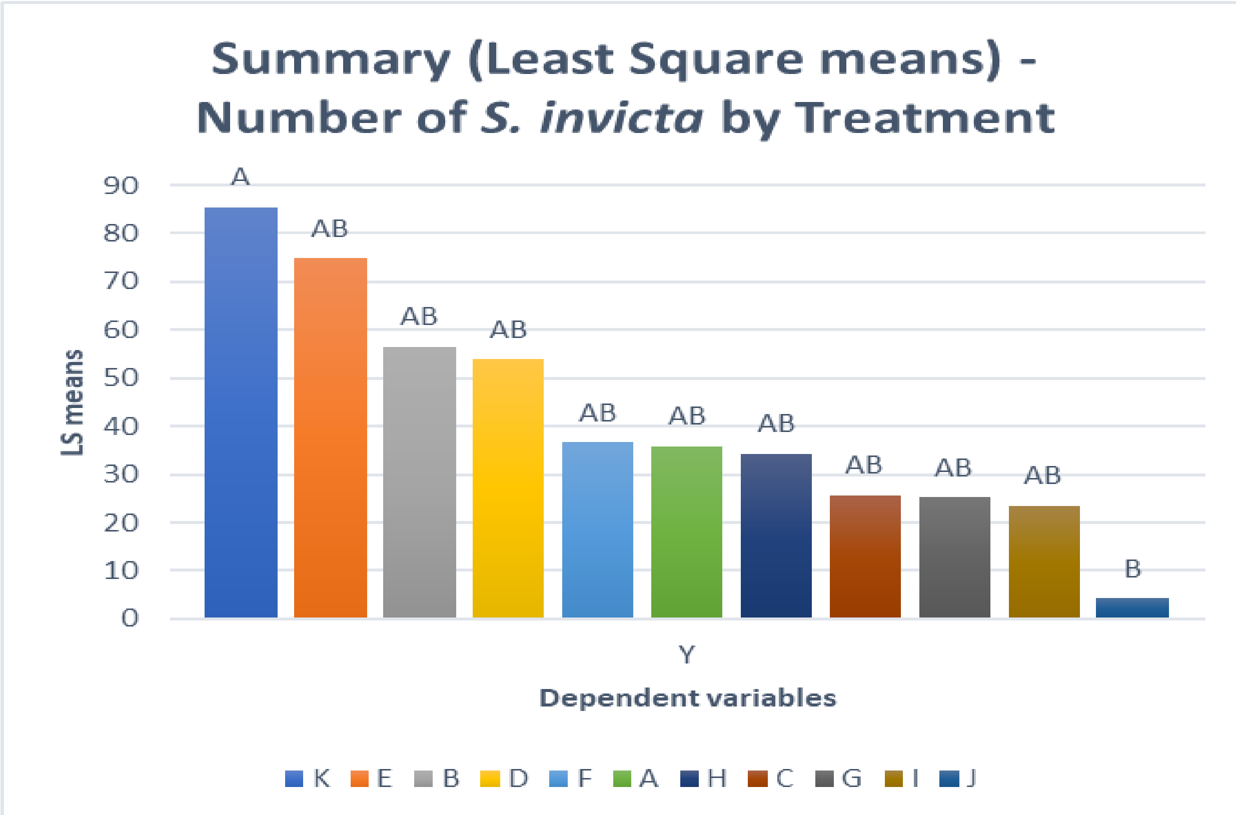

Figure 2. Tukey post hoc test analyzing the number of S. invicta by oil treatments.

Analysis of the difference between the number of tubes that attracted *S. invicta* was the least statistically significant data. This is because of the limited range of possible data. The p-value for this test was 0.304. This means that this could only be accepted with less than 70% confidence. When conducting a Tukey pot hoc with 60% confidence, it is seen that the only treatments with the potential to have significant differences are both macadamia nut oil and pine nut oil, when compared with pistachio nut oil (Figure 3).

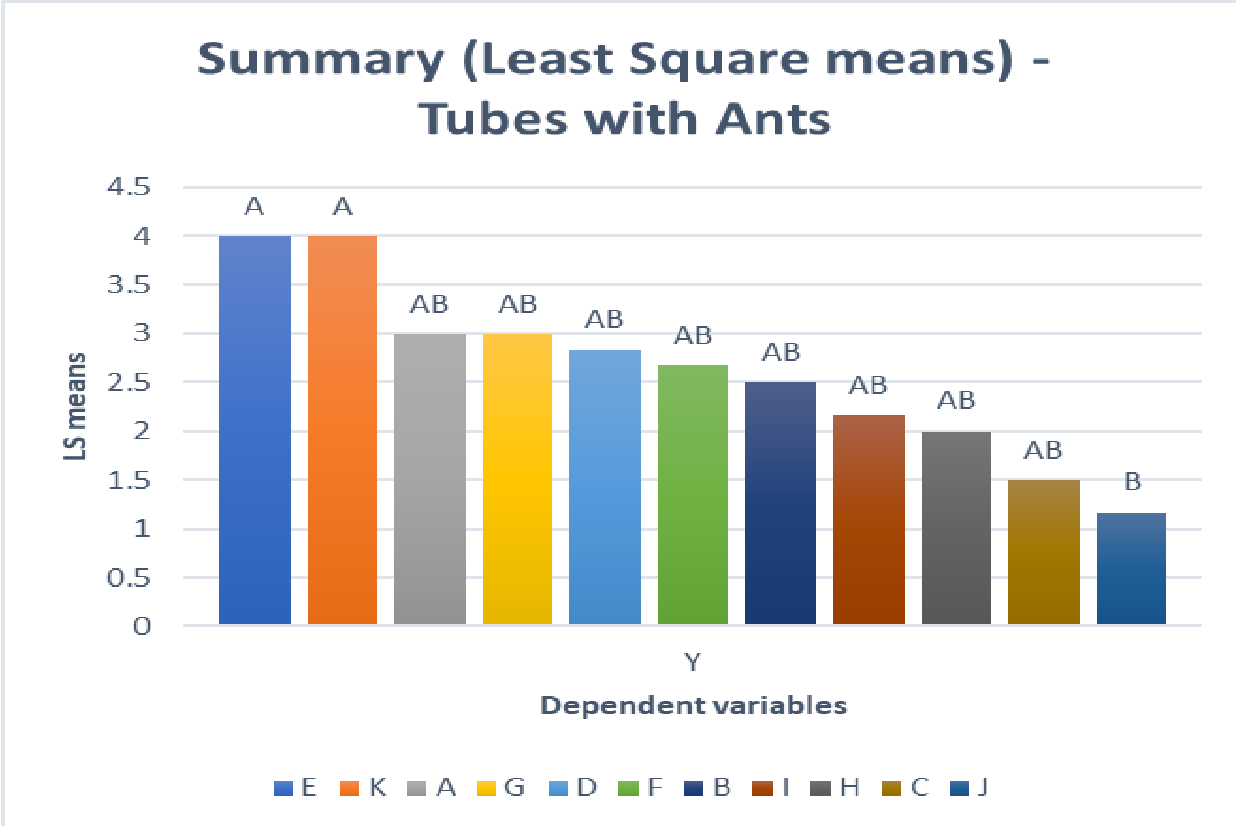

Figure 3. Tukey post hoc test of the number of tubes containing any ants by oil treatment.

The third statistical analysis was the ratios of the number of *S. invicta* in each transect to the number of tubes with specimens. This is used to project the average specimens per tube in each transect. The p-value for this analysis was 0.095, which means that there is a statistical significance within a 90% confidence interval. Within this confidence interval, the difference between hickory nut oil and pistachio nut oil was significant (Figure 4). Additionally, when a two-sided Dunnett analysis was ran the hickory nut oil ratio was found to be statistically different from soybean oil where the p-value is 0.069.

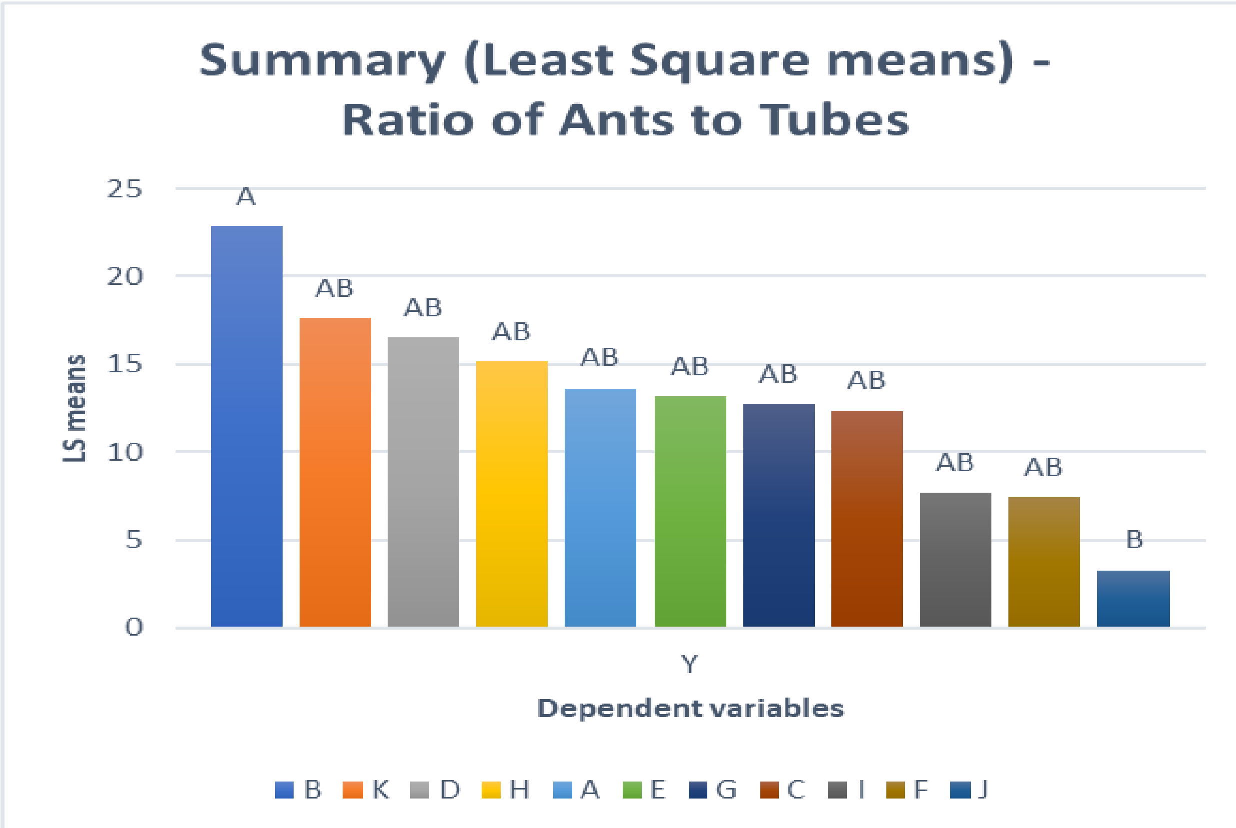

Figure 4. Tukey post hoc test of the projected average ants per tubes by oil treatment.

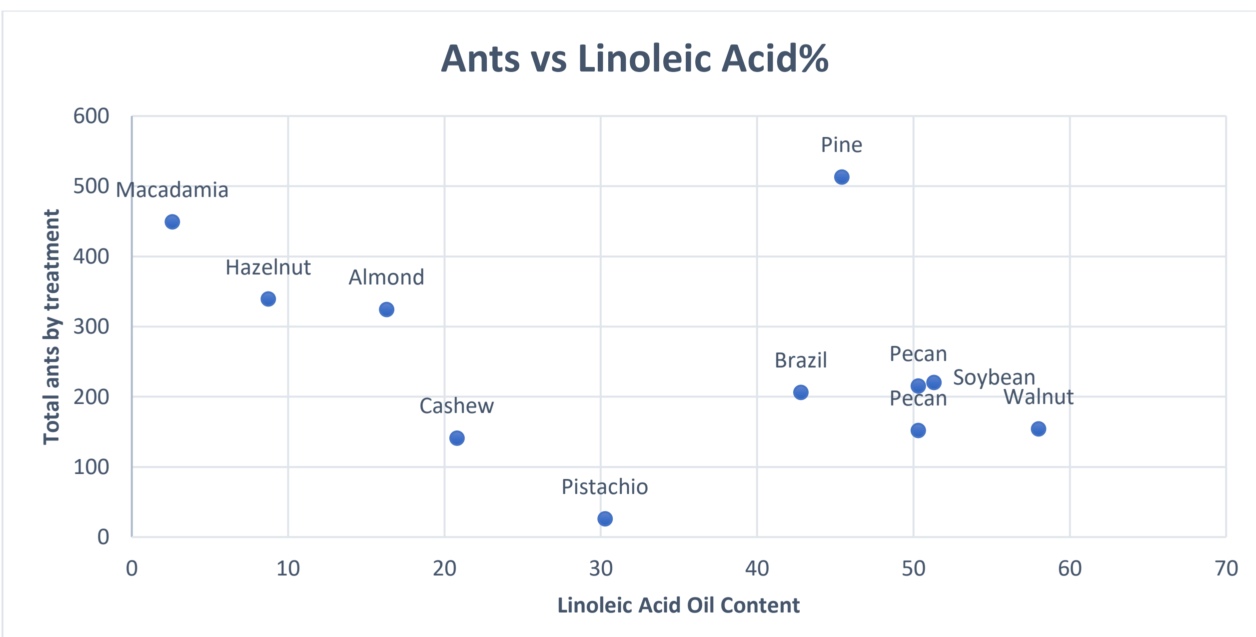

Figure 5. Relationship between ants and linoleic oil content.

Discussion

These oils were chosen for their varying linolenic, linoleic, and oleic acid percentages. When the number of S. invicta was plotted against linoleic acid content there was not a trendline to quantify the results (Figure 5) (Ryan et al. 2006) (Sun et al. 2022) (Sathe et al. 2008, Nogales-Bueno et al. 2021) (Aquino-Bolaños et al. 2017) . This paired with the quantifiable results leads to conclusion that the hypothesis that pecan oil would attract more *S. invicta* individuals than oils with a lower linoleic acid percentage was incorrect.

With the information present, the conclusion that can be made is that there is a significant difference between Siberian pine nut oil and pistachio nut oil. As stated before, there was no correlation of fatty acid contents when analyzing the data as a whole. However, fatty acid content can be compared between pine nut and pistachio nut oil. The two differences in fatty acid in contents are oleic acid (18:1) and linoleic acid (18:2). For pine nut oil, it has an oleic acid content of 38.55% and linoleic acid content of 45.41%. Pistachio nut oil has an oleic acid content of 58.39% and linoleic acid content of 30.27%.

It has been found that both oleic acid and linoleic acid are seen in necrophoric behavior in *S. invicta (Xu et al. 2018)*. The other side of this is the expectancy of linoleic acid to be connected more to necrophoric behavior and linolenic acid to be more connected with attractive food behaviors. In leaf-cutter ants it has been found that they exhibit aggression to high linolenic acid (18:3) and high attraction to linoleic acid (18:2) (Khadempour et al. 2021) . For these reasons, the inverse relationships of the oleic and linoleic acid contents in pine and pistachio nut oil seems to be better understood.

This sense of understanding is not supported by the remainder of the data. There are also high oleic acid contents in hazelnut, almond, and macadamia nut oil—all of which had higher recruitment than pistachio nut oil. This is likely due to the combination of foraging pathways and olfactory attraction to oils with a ‘stronger’ scent (Gordon 1988) (Drees 2011).

There are different pathways used by ants when they are patrolling in the absence of food and with the presence of food (Gordon 1988). What most likely occurred in this experiment was that on a standard patrol (in the absence of food) an ant came within a range of be able to recognize food was nearby (which could be a larger range for oils with more intense scents). Once discovering the food, a pheromone trail was left if the ant determined the oil was a good food source. Theoretically, there should be a higher occurrence of finding oils with a larger scent range. This is something that was not tested in this experiment, however future research could explore this to test scent attractivity to ants versus nutritious value of the oil once attracted. With this information, it may be possible to more accurately determine if oils with higher linoleic acid are more attractive to *S. invicta*. With the combination of these findings, an optimally effective bait could be developed for RIFA.

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