Disruptions of *Solenopsis invicta* Pheromone Trails

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Abstract. *Solenopsis invicta,* commonly known as the red imported fire ant, is an invasive species to the United States whose history began in the early 20th century. Given time and increasing human interaction, the species was able proliferate in much of the Southwest. This artificial migration, coupled with the species' anatomy, defense mechanisms and aggressiveness. has caused increasingly important issues with colonies forming near households and affecting crop yields. The highly potent venom can cause painful skin rashes and can even induce death in certain members of population. However, these ants rarely sting as individuals, but rather as a group- as a mechanism of defense in response to, for example, habitat disruption. To achieve such collaborative action, these insects release pheromones. And one in specific, Z, Z, Z-Allofarnesene, aids in the formation of ant trails. Therefore, given the medical impact of the species, one can see the importance of developing alternative methods of disrupting this specific pheromone transmission. This study utilizes common household items (lemon juice, vinegar, black pepper, cinnamon, and paprika) to test the efficacy of pheromone disruption. The results yielded the conclusion that products containing highly volatile functional groups in a certain odorous physical state have a significantly higher impact on pheromone trails.

Keywords: Invasive species, Pheromone, disruption, *Solenopsis invicta*

Solenopsis invicta, more commonly known as the red imported fire ant, was introduced to the United States in the 1930s from South America and has successfully established itself in 14 states. The ant initially arrived in Mobile, Alabama, but was able to infest other states, such as Texas and California (U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, 2006). The imported fire ant has a limited dispersal ability, but can move great distances when transported in soil, plants or even vehicles. These types of human actions have allowed the accidental movement of *Solenopsis invicta* into new environments, where they consequently destroy crops and harm wildlife (Ascunce et al., 2011).

The red imported fire ant, or *Solenopsis invicta,* can be identified by its various physical features. These features include a three-segmented body that is red to brown in color with the gaster (posterior segment) being black. The worker ants vary in size from 2.4 to 6.0 mm. Their mandibles consist of four distinct teeth. There are also a pair of 10-segmented antennae, which end in a two-segmented club. Another characteristic to help distinguish from the domestic species is that the domestic species workers will have square shaped heads that are very large relative to the rest of their bodies. *S. invicta* workers have proportional body segments (Collins and Scheffrahn, 2018).

Solenopsis invicta contains one of the most potent venoms in all of Hymenoptera, as nanogram doses can cause sensitization in 25% of those exposed to their venom (Hoffman, Dove and Jacobson, 1988). On average, a single red imported fire ant sting will contain 10 to 100 ng of protein, so even a single sting can provoke production of immunoglobulin E and induce anaphylaxis in allergic individuals. Unfortunately, it is far more likely for the victim to experience multiple injections of venom, as red imported fire ants tend to attack in large masses and each ant can sting the victim multiple times. Due to *S. invicta*'s aggressive behavior, there are more severe cases that have been documented and involve multiple-organ shutdowns, with symptoms including renal failure, liver injury, coagulopathy, and rhabdomyolysis**.** These symptoms can be traced back to the formic acid and piperidines, toxic alkaloids, found in the venom, as they can have hemolytic and cytotoxic effects (Cochran et al., 2013). These symptoms only occur in rare circumstances, as anaphylaxis is more common in allergic individuals, while non-allergic individuals only experience localized pain and the formation of sterile pustules. However, the initial insect attack is not the only concern, as a reported 6.8% of individuals that sought medical attention following a sting from *S. invicta* experienced a secondary infection (Lofgren and Meer, 1986). Overall, *Solenopsis invicta* is an extremely dangerous arthropod, and as it continues to become more widespread, a single sting can mean life or death.

Solenopsis invicta produce scented chemicals, known as pheromones, in order to communicate with each other. The red imported fire ants can use pheromones for a number of tasks, such as marking the location of food sources, creating the tunnels in their colonies, and letting other fire ants know when danger is nearby. The red imported fire ant can detect these pheromones, along with other external stimuli, using the sensory receptors on the tips of their antennae (Renthal et al., 2003). The left and right antennae tell the fire ants what direction to head based on the strength of the pheromone, as they can detect the concentration of the present pheromones. In this experiment, we will be focusing specifically on Z, Z, Z-Allofarnesene, the trail pheromone of *Solenopsis invicta* (Williams et al., 1981). Overall, the use of pheromone communication by *Solenopsis invicta* has contributed greatly to the success of their species.

The notorious role of fire ants in our world has been common knowledge since the invasive insects first established themselves in the region. Between their enormous economic impact on the agriculture industry and the disruption of native ecosystems, the impact of these Hymenopterans cannot be underestimated. However, the primary significance of fire ants in everyday life (and thus, in this study) resides in their medical implications in regards to humans (Suckling et al., 2012). Individuals become concerned when fire ants infest a home or yard, as the painful, potentially dangerous stinging will almost assuredly follow. The aggressions of these invaders seemingly demand toxic counter-measures. Yet, such treatments often have dire effects on non-target organisms and sensitive ecosystems. One alternative approach has grown to garner both civilian and professional attention: Household products as fire ant deterrents (Suckling et al., 2010).

As previously illustrated, fire ants form their characteristic trails through the deployment of signature pheromones. Creating disorientation among said trails through the introduction of pheromone-clouding chemicals would suppress mobilization of foraging efforts. Through the amateur trials of defensive homeowners, some standard kitchen supplies have received attention as potential pest control agents. This study serves to test the effectiveness and safety of various household substances as disruptors of fire ant trails, in the efforts of preventing medical harm to humans.

Materials and Methods

For this experiment, our group went to Spence Park on campus at the University of Texas A&M in College Station, Texas, from 5:45pm to 7:00pm. There, we looked for ant hills. There were three ant hills identified at the park. Pieces of dried pineapple (International Foodsource LLG, Dover, NJ) were set around each ant hill to bring out the ants and cause them to start forming pheromone trails. We continually monitored each ant hill for pheromone trail formation. Once noticeable trails began to form, we started testing our methods of disruption. First, paprika (McCormick & Company, Sparks, MD) was dusted on top of one of the ant trails by a group member. We recorded the effects and moved on to another ant hill. Black pepper (McCormick & Company, Sparks, MD) was then sprinkled on the different ant hill, and results were recorded. We waited for the other ant hills to form trails. Once a trail was finally formed, vinegar (HJ Heinz Company, Chicago, IL) was sprayed on the ants using a Bajer Spray Bottle (EACH, (H-E-B), College Station, TX). Results were recorded. We moved back to the first hill, and sprinkled cinnamon (McCormick & Company, Sparks, MD) on a different section of a new ant pheromone trail. Results were recorded and that ant hill was no longer used. For our last method, we went to a new ant hill and sprayed Italian Lemon Juice (Concord Foods, Brockton, MA) on an ant trail using a new spray bottle (EACH, (H-E-B), College Station, TX). During the process, pictures were taken, and observations of the effects of each method (regarding their ability to cloud ant pheromones) were recorded.

Results

With the application of paprika, the ant trail became disrupted. However, the ants began to restart another trail over the paprika, and also made trails to avoid the substance. This was done on the first ant pile, and it took nearly 3 minutes for the ants to reconstruct their trails once the paprika had been applied. The lemon juice was sprayed roughly 6 inches out from another ant pile, and it took the ants approximately 30 seconds to recreate their trails. On the original pile, cinnamon caused the ants to become disoriented, and it took about 3 minutes for the first ant to return to the nest. However, even after regaining some navigation, the ants still remained individually scattered. Conversely, among the final ant pile, the black pepper did not cause any disruption to the ant trails. This was the same for the vinegar, as the ants were apparently not affected, and therefore their trails were not disrupted.

Table 1: How the different substances affected the pheromone trails

Substance Tested:	Observations:
Paprika	Disrupted the pheromone trails of the fire ants for approximately 3 minutes.
Lemon Juice	Disrupted the pheromone trails for 30 seconds before a new pheromone trail was formed.
Cinnamon	Disrupted the fire ant pheromone trails for over 3 minutes. No new pheromone trail was formed by the affected fire ants.
Black Pepper	Had no effect on pheromone trails. The ants ignored this substance and continued using their pheromone trail.
Vinegar	Had no effect on pheromone trails. The ants ignored this substance and continued using their pheromone trail.

Discussion

According to the collected data, cinnamon proved to be the most effective product of those tested in disrupting the pheromone trails of *Solenopsis invicta*, while vinegar and black pepper proved to be the least effective. Vinegar and black pepper were determined to be the least effective because the pheromone trails were not disrupted, and the applications had no actual effect on the fire ants present. Lemon juice was slightly effective, causing a 30 second period of confusion amongst the red imported fire ants. Of note, this would not prove an effective method for long term pheromone disruption. Paprika was the second most effective product, as it caused disorientation amongst the fire ants for approximately 3 minutes before they were able to rebuild another pheromone trail avoiding the paprika. It should be noted that *Solenopsis invicta* refused to rebuild their pheromone trails through the paprika, as it demonstrates that even once the fire ants get over their confusion, the paprika will still repel them. Cinnamon was deemed the most effective, as it resulted in the longest period of disorientation and did not result in a new pheromone trail being formed around the product, as the fire ants refused to approach the cinnamon.

Two liquid household products, lemon juice and vinegar, were tested to disrupt the pheromone trails of *Solenopsis invicta*. Lemon juice was selected as a possible obstructer due to its acidic properties, owing to the presence of d-limonene. D-limonene is considered a toxic substance to fire ants, hence its presence in many commercially available products used to destroy fire ant hills. Vinegar was selected purely on account of its strong smell, which could in turn overwhelm the fire ant's antennal sensilla. As a result, massive confusion would theoretically ensue, and a disruption amongst the communication system would result in many fire ants being lost. However, both of these liquid products proved to be ineffective, as lemon juice only distracted the fire ants for approximately 30 seconds, while vinegar was completely ignored. A possible explanation for the ineffectiveness of these products would be there physical state. Liquids can easily evaporate when present in small amounts or exposed to direct sunlight, both of which were conditions present during the experiment. Furthermore, if the liquids were not highly concentrated, then a small amount of the product would not result in a response. In addition to those products, cinnamon, paprika and black pepper were the solid materials tested. The cinnamon was observed to be the most effective at deterring ants and disrupting their pheromone trails. This solid contains many different volatile compounds, such as esters, aldehydes, and carboxylic acids. However, its most effective compound is cinnamaldehyde, which gives cinnamon its strong distinctive odor and flavor. As a result of this compound, the ants became disoriented and were unable to follow the pheromone trails because the odor was too strong to overcome. The scent also did not evaporate or easily disperse from the affected area, due to the fine, ground-up nature of the cinnamon. The ants became lost and even had trouble going around the cinnamon. Paprika was also able to disrupt pheromone trails, but the ants were able to rebuild these trails- unlike with cinnamon. Capsicum oleoresin is found in paprika and is a bio-repellent that comes from the family of peppers known as Capiscum (Wiersma et al., 1997). It is the ingredient that causes the spiciness of peppers. The spiciness and 'heat' associated with peppers and paprika, and the consequential strong odor, were expected to be effective at disrupting the ants. Although the spice had an effect, the results did not meet the expectation. The concentration of capsicum oleoresin in the paprika powder may not have been high enough to confuse the ants. In regards to these two fine powders, perhaps their structure acted in the irritation/obstruction of the spiracles and/or tracheae of the insects. Finally, the black pepper did not have an effect on the ants. Although it contains piperine, which is the alkaloid responsible for its pervasive odor, there was no disruption of pheromone trails. The consistency of the black pepper was thicker and coarser than the powdered forms of cinnamon and paprika, and this physical characteristic may have been the reason for the black pepper's inability to cause disruption among the ants. Overall, the solid products tested were observed to have a greater effect on disrupting the pheromone trails created by the ants than the liquid products did.

Future research is recommended to determine whether the physical state and consistency of the household product had a significant effect on the ability of the product to disrupt the pheromone trails. In this experiment, the solid powders were observed to be much more effective in disrupting the pheromone trails than the liquids and the black pepper. Further research could then focus on determining if this was due to the consistency of the substance, its physical state, or both. Furthermore, such research should be conducted to determine a more definitive answer as to the effectiveness of black pepper on pheromone trail disruption. There is a lack of information available on the science behind the use of black pepper as an insect repellant, despite many claims of its effectiveness. Therefore, future studies could explore the properties of the substance, to determine why ants may avoid the pepper in some cases, and not in others. Another main focus would be to determine if the black pepper is scaring or disrupting the ants, rather than their pheromone trails. Lastly, future research should be conducted to determine more possible household products that could be used to disrupt *Solenopsis invicta* pheromone trails or act as natural repellents for this invasive species.

Pesticides can best be described as poisons, as they are designed to kill the target individual upon ingestion. Humans that ingest, inhale, or absorb high enough doses of these pesticides can experience adverse effects, including nausea, headaches, muscle aches, dyspnea, and even death. In fact, as many as 81 of the 276 approved substances of pesticides in Europe were considered toxic (Damalas and Eleftherohorinos, 2011). Moreover, these pesticides can be very costly depending on the area of application, which can prove to be quite large in farms and other centers of agriculture. As a result, pesticides should be replaced by natural household deterrents as often as allowed, to prevent pesticide poisoning and unnecessary costs. Such an opportunity has been exhibited by this study. With consequential control of fire ants, risk of incidental stinging would significantly lower. Overall, natural deterrents, namely cinnamon and paprika, act as a safer, cheaper, and effective alternative to pesticides, and can be utilized to lower the risk of injection with *Solenopsis invicta* venom through pheromone trail disruption.

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