The Effect of Iridomyrmecin on Native Ant Foraging Behavior

Miranda Thoenen

ABSTRACT

Linepthema humile are an invasive species that decimate native ant populations wherever they are found. One component of their trail pheromone, iridomyrmecin, is used by many other species as an ant repellant. Iridomyrmecin could be connected to the decline in native ant populations by excluding them from areas *L. humile* have colonized. I measured the effect iridomyrmecin has on native ant foraging behavior by comparing the number of ants that forage on baits treated with iridomyrmecin to a control. Iridomyrmecin significantly reduces the number of native ants that recruit to baits. These results suggest that Argentine ants could be using iridomyrmecin to passively suppress foraging behavior, lending them another advantage in their invasion.

KEYWORDS

Invasion ecology, Argentine ants, trail pheromones, defensive compounds, foraging suppression

INTRODUCTION

Invasive species are a pressing environmental concern in California today. A nonnative species can damage an ecosystem as the species outcompetes its native rivals (Holway 1999). Invasive ants are particularly adept invaders, and many displace native species without filling the same ecological niche (Rodriguez-Cabal et al. 2009). Of the invasive ants, Argentine ants (*Linepithema humile*) are among the most successful and damaging invasive species in the world (Moffett 2010). Originating in South America, Argentine ants stowed away on human ships and can now be found on every continent except Antarctica (Moffett 2010). As they invade, the ecosystem can become homogenized as plant species that were dependent on native ants are replaced by those that can survive without them (Moffett 2010). Invasions occur in swarms, overwhelming native ants with sheer numbers and a seemingly unstoppable expansion across territory (Erickson 1971, Moffett 2010). The full extent of their invasion potential is not yet known.

Argentine ants have several traits that aid their high rate of successful invasions and subsequent elimination of native ants. They are unicolonial, mixing freely between related nests and effectively eliminating intraspecific aggression and competition (Holway et al. 2002). Reproduction and expansion occurs via budding, with queens and workers leaving together to establish new nests that will be accepted as part of the original colony (Heller et al. 2008). Nests are often transient, under a rock or a few leaves, and workers and queens will move several times a day if necessary (Moffett 2010). With no natural enemies to suppress them, Argentine ants are free to expand into vast supercolonies, one of the biggest spanning most of California's coast (Moffett 2010). Their large numbers help them outcompete natives for resources like territory and food (Moffett 2010). Argentine ants patrol their territory constantly and if an ant that doesn't belong to the supercolony is found, it is quickly swarmed and killed, leaving a landscape devoid of ant diversity (Holway 1999, Human and Gordon 1996). When competing for food, Argentine ants find and recruit to baits in almost half the time it takes most native species (Holway 1999). Introducing Argentine ants to baits already exploited by native ants significantly reduced the number of workers of 6 out of 7 native species (Holway 1999). With an Argentine ant colony within 20m, foraging distance for a native Californian harvester ant (*Pogonomyrmex californicus*) was cut in half, reducing foraging behavior without direct contact (Erickson 1971). The mechanism behind this indirect suppression is unknown, but there is a possibility it is based on chemical interactions.

All ants use a variety of chemical compounds for communication, identification, and defense. They constantly secrete semiochemicals, chemical compounds that can indicate many things depending on surrounding context and which species is receiving the signal (Vander Meer et al. 1998). Trail marker pheromones are intraspecific semiochemicals that lead ants to food, nests, or battle. One potential trail pheromone identified for Argentine ants was Z-9-hexadecenal, which is found in the ventral gland and prompts trail-following behavior (Cavill et al. 1980). However, extracts from trails laid by living ants contained negligible amounts of Z-9-hexadecenal, and instead were comprised of iridomyrmecin and dolichodial, a mixture that also induced trail-following behavior (Choe et al. 2012). The mixture of iridomyrmecin and dolichodial has also been indicated as a vitality sign and its absence induces necrophoresis (removing dead nestmates to a waste pile), indicating that iridomyrmecin and dolichodial are produced and exuded constantly (Choe et al. 2009). Yet another study of all three chemicals found that only Z-9-hexadecenal induced trail following behavior (Neff 2015). If iridomyrmecin is not a trail marker pheromone, it is possible that its presence in trails has another purpose. In many species, iridomyrmecin has uses as an anti-ant defensive compound (Stökl et al. 2012, Smith et al 2014). Perhaps secreting iridomyrmecin repels ants not only from Argentine ants, but also their trails and is related to their high level of success as invaders.

In this study, I aim to answer the following questions: What effect does iridomyrmecin have on the foraging behavior of native ants? Does the presence of iridomyrmecin prevent ants from foraging in an area? Does adding iridomyrmecin prevent ants from returning to an area? How significant is this effect when compared to the presence of Argentine ants? I hypothesized that fewer ants will forage on a bait treated with iridomyrmecin than a bait treated with a control, and even fewer will forage on a bait with Argentine ants present. I measured foraging behavior as number of ants recruited to a bait. I also recorded alarm responses, such as gaster flagging or fighting.

METHODS

Study organisms

I conducted lab trials using velvety tree ants (*Liometopum occidentale*) collected from the Round Valley Regional Preserve. Velvety tree ants are native to the Western Coast of the United States, where they tend to be the most abundant ant species in oak and pine forests (Hoey-Chamberlain et al. 2013). They have a number of similarities to Argentine ants, including being dominant, establishing long foraging trails, raising Hemiptera, and seem to be unicolonial (Hoey-Chamberlain et al. 2013). Velvety tree ants have been documented as being particularly affected by the presence of Argentine ants (Holway 1999), perhaps because they occupy a similar niche. I conducted field experiments along well established foraging trails of velvety tree ants, and the baits were also visited by winter ants (*Prenolepis imparis*) and an unidentified *Formica* species. All Argentine ants I used came from colonies kept by the Tsutsui lab. I only brought workers into the field for trials.

Study site

I conducted field trials in Round Valley Regional Preserve. Round Valley is a part of the East Bay Park District located near Brentwood, California. The vegetation in Round Valley is a mixture of nonnative grassland, oak savannah, and shrub land. Native mammals include ground squirrels, rabbits, and kit foxes (East Bay Regional Park District 2017). Local ranchers graze livestock in the park as part of an effort to control the nonnative grasses. Human use of the park is primarily hiking, but some ride bikes or horses along the trails. Several creeks run through the park, but the soil is dry at higher elevations. I did my field trials in mid to late April on mostly sunny days.

Iridomyrmecin

I used synthetic iridomyrmecin supplied by Dr Kamal Chauhan of the USDA. An average Argentine ant worker contains around 7.6 micrograms of iridomyrmecin (Choe 2012). Using this number, Brian Whyte created an iridomyrmecin solution in 95% ethanol with the equivalent of 5 Argentine ants' worth of iridomyrmecin per 0.2mL, or 0.19mg/mL.

Lab trials

I conducted informal lab trials in mid-April to test the repellency of our iridomyrmecin solution. I placed a trimmed notecard in a petri dish and traced a line of the iridomyrmecin solution down one side and a line of ethanol down the other. I placed a droplet of sugar water beyond the lines on opposite sides of the petri dish. I placed one velvety tree ant worker in the petri dish and covered it so they could not escape. I recorded the behavior of the ant, paying close attention to signs of agitation or its response to touching the iridomyrmecin.

Pre-recruitment foraging trials

To test whether the presence of iridomyrmecin would prevent native ants from foraging in an area, I conducted paired foraging trials. I first located a foraging trail of velvety tree ants. I placed bait made of tuna fish and jelly on two identical notecards. I then used 0.2mL of 95% ethanol to draw a circle around the control bait. I did the same thing with the iridomyrmecin solution on the treatment bait. I placed both baits approximately 10-15cm away from the velvety tree ants' foraging trail and watched for about 10 minutes, counting the number of ants that crossed the circle to feed on the bait. I also kept a note if any of the ants exhibited agitated behavior such as cleaning themselves, gaster flagging, mandible spreading, or simply being repelled by the circle. I conducted 11 trials paired trials. I used R commander to run a t-test to see if there was a statistically significant difference between the average number of ants foraging on the control baits versus the treatment baits.

Post-recruitment foraging trials

To test whether the presence of iridomyrmecin would prevent ants from returning to a bait they had previously colonized, I conducted another set of paired foraging trials. Again, I located a foraging trail and placed two baits 10-15 cm away. I waited about 10 minutes for ants to find and recruit to the baits, then counted how many were actively feeding on the bait. I then used 0.2mL of ethanol or iridomyrmecin to draw a circle around the bait. I recorded the initial behavior of the ants and kept notes on how quickly they returned to feeding on the bait and any agitated behavior, making a final count at 10 minutes post exposure. I conducted 11 paired trials. I ran two separate t-tests on my data, comparing the before and after averages of the control and the before and after averages of the treatment.

Argentine ant trials

To test whether adding Argentine ants to a bait had a similar effect as iridomyrmecin, I set up a single bait notecard and left it 10-15cm away from another foraging trail for 10-15 minutes. After this time, I counted the number of native ants on the bait, then released 20 Argentine ant workers directly on top of it. I recorded signs of distress, including fighting and fleeing over the course of the next 10 minutes, after which I counted the number of native ants feeding on the bait. I conducted 10 Argentine ant trials. I ran a t-test comparing the before and after averages of the trials.

RESULTS

Lab trials

I found that velvety tree ants were generally repulsed by the iridomyrmecin solution. 12/20 ants were visibly repelled by the line of iridomyrmecin, and refused to cross it. All ants were distressed, patrolling the edges of the petri dish and looking for a way to escape. If they made contact with the iridomyrmecin solution, they would back away and clean themselves vigorously.

Pre-recruitment trials

I found that fewer native ants recruited to baits treated with iridomyrmecin than the control. There was a statistically significant difference between the number of ants that recruited to the control ($M=30.18$, $SD=19.67$) and the iridomyrmecin ($M=10.64$, $SD=10.30$) bait; $t(15.9)=2.92$, $p=0.0105$. There was a large variety in the number of ants that recruited to each type of bait, usually relating to the overall activity of the nearby foraging trail. Ants that found the control baits fed and recruited nestmates to the bait. In contrast, ants that found the iridomyrmecin bait tended to leave the bait after coming in contact with the circle, cleaning themselves compulsively or fleeing. This happened at least three times in every trial I conducted. Recruitment for both baits increased as the trial went on and more ants found the baits.

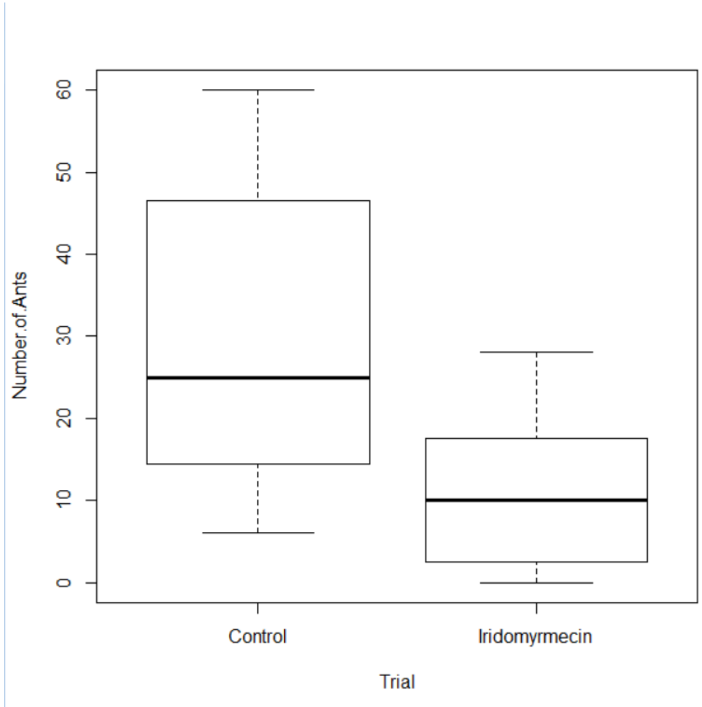

Figure 1: Number of ants recruiting to control (M=30.18) and iridomyrmecin (M=10.64) baits over the course of 10 minutes.

Post-recruitment trials

Fewer ants returned to post treatment iridomyrmecin baits than to control baits. My t test results indicated no statistical significance between initial (M=24.01, SD=15.92) and post-exposure (M=25.36, SD=17.89) counts of ants on the control bait t(19.74)=-0.063, p=0.95. There was, however, a significant difference between the initial number of ants (M=31.45, SD=17.60) and post exposure number of ants (M=17.0, SD=11.63) on the baits treated with iridomyrmecin t(17.33)=2.27, p=0.036. When either liquid was added to the bait, the ants would initially scatter. However, the ants that had been feeding on the control bait tended to return much quicker, in some cases, not even leaving. While they were agitated by the pipette, the damp notecard did not seem to be a deterrent. In contrast, the ants that had been feeding on the iridomyrmecin bait were slower in returning to the bait, and often didn't reach their original numbers by the end of 10 minutes. The ants also spent a great deal of time cleaning themselves.

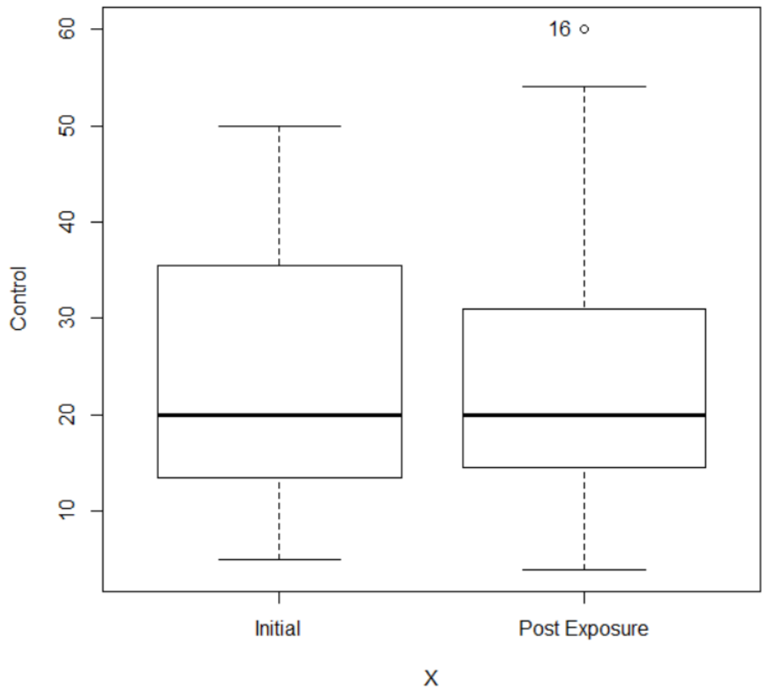

Figure 2: Initial number (M=24.91) and post exposure (M=25.36) number of ants on baits treated with 95% ethanol

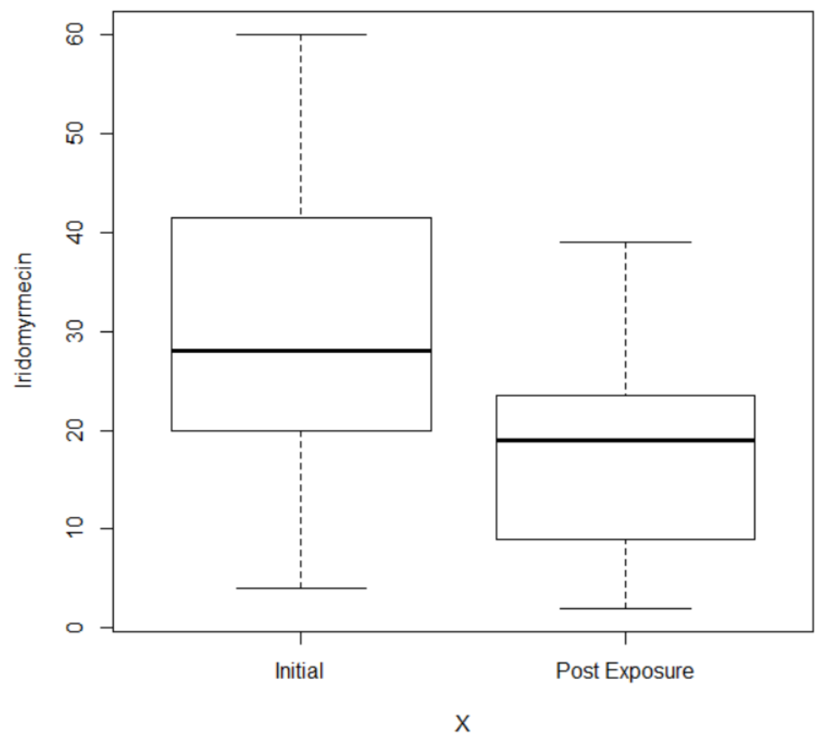

Figure 3: Initial number (M=31.45) and post exposure (M=17) number of ants on baits treated with iridomyrmecin.

Argentine ant trials

Adding 20 Argentine ants to baits colonized by velvety tree ants did not greatly reduce the number of native ants foraging on the bait 10 minutes later. There was not a statistically significant difference between the initial number of ants (M=32.6, SD=20.10) and the post-exposure number of ants (M=24.0, SD=13.98), $t(16.10)=1.11$, $p=0.28$. The immediate response of the velvety tree ants was to scatter, with most of the ants leaving the bait. Some continued foraging while other attacked the Argentine ants, grabbing them with their mandibles and attempting to smear them with the tip of their gaster, or clamping down on one of their legs and trying to pull them apart. There tended to be more velvety tree ants than the 20 Argentine ants I added. I saw several instances of Argentine ants hiding on top of the bait while the native ants foraged around the sides. After about 5 minutes or so, all the Argentine ants were dead, gone, or hiding. The velvety tree ants returned to foraging, although with more alertness, with some of them patrolling the edge of the notecard. I saw no instances of native ant mortality.

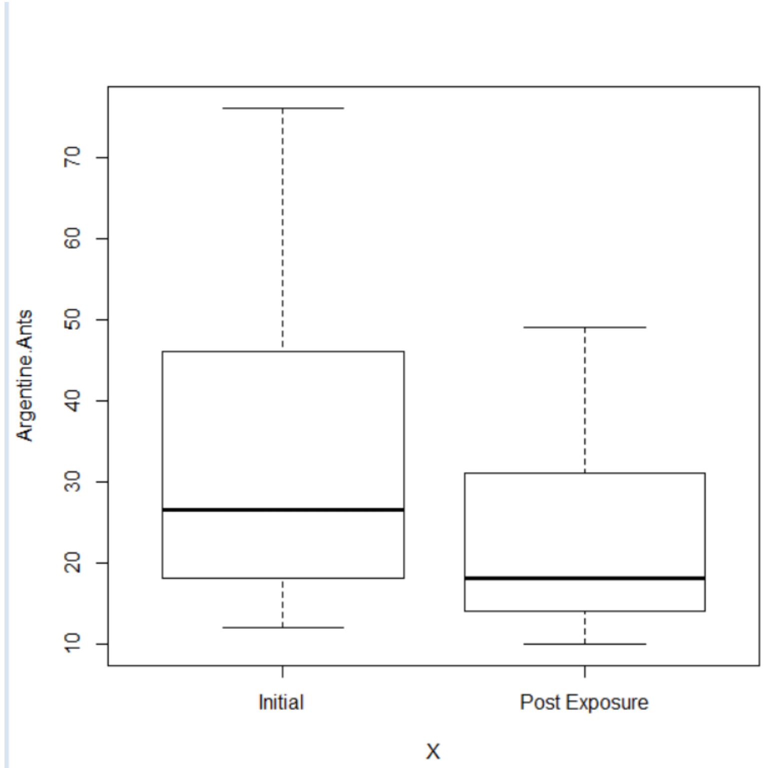

Figure 4: Initial number (M=32.6) and post exposure (M=24) number of native ants 10 minutes after Argentine ants were released onto the bait.

DISCUSSION

My study indicates that iridomyrmecin suppresses foraging behavior in a native ant species. Fewer ants foraged on baits that were surrounded by an iridomyrmecin solution than foraged on the control. Although workers foraging on the control and iridomyrmecin baits fled when liquid was added, fewer ants returned to the iridomyrmecin bait, which suggests that the iridomyrmecin may disrupt the foraging trails the velvety tree ants laid down. I also found that while the initial response to Argentine ants being introduced to a bait is to flee, the velvety tree ants were quick to overcome the limited number or Argentine ants, and several didn't move at all. Argentine ants are a highly successful invasive species that may use one of their trail pheromones to passively suppress the foraging of native ants; this could be an important factor in their success.

The many uses of iridomyrmecin

Iridomyrmecin is not an Argentine ant specific chemical. Several other species, mostly but not exclusively Hymenoptera, produce isomers of iridomyrmecin for a variety of uses.

Iridomyrmecin is found in the parasitoid wasp *Leptopilina heterotoma*, and they use it to repel ants (Stokl et al 2012). *L. heterotoma* also uses iridomyrmecin as a female sex pheromone and as a competition avoidance cue- that is, too much iridomyrmecin is taken as a signal that a potential nesting site is already taken and females will avoid it (Weiss et al 2013). This demonstrates at least one case in which insects use iridomyrmecin to discourage other insects from exploiting resources. I found that too much iridomyrmecin definitely repelled native ants from the baits initially. Iridomyrmecin is a highly volatile compound and evaporates quickly (Choe 2009). I found that as the notecard dried, the iridomyrmecin seemed to have less and less effect on new ants that found the bait. This could be because the lower concentration of iridomyrmecin was less repulsive to the native ants, or maybe their own recruitment trails were more attractive.

Iridomyrmecin and isoirdomyrmecin have also been found in the pygidial gland of several *Tapinoma* species of ants, including the widespread *Tapinoma sessile* (Tomalski et al 1986) who may use it as a defensive chemical. *Tapinoma* ants will produce a clear droplet at the tip of their gaster and attempt to smear it on an enemy. If they are successful, the enemy quickly retreats and frantically cleans itself, a pattern remarkably similar to ants that have been smeared by Argentine ants (Tomalski et al 1986, Martinez and Weis 2011, Holway 1999). I observed the same behavior in velvety tree ants that touched the circle of iridomyrmecin solution around my baits. Iridomyrmecin is stored in the pygidial gland along with dolichodial and released as a spray or droplet from the tip of the gaster (Neff 2015). Extracts from Argentine ants' pygidial glands have been shown to repel other ants and even have a knock down effect on some insects, causing a sort of partial paralysis (Holway 1999, Stokl et al 2012). Velvety tree ants that returned to the notecard immediately after I added iridomyrmecin to post recruitment baits were hesitant to cross the circle, and I observed several ants shake uncontrollably as they wandered around the edges of the notecard, perhaps because they were exposed to an unusually high dose of iridomyrmecin. When an Argentine ant sprays an enemy or lays down a trail, they don't release their entire store of iridomyrmecin, although Argentine ants in combat do release more iridomyrmecin than usual (Whyte, unpublished data). The concentration I used (about 5 ants worth per 0.2mL) would be hard to find in nature except in places Argentine ants are constantly patrolling, such as foraging trails or trails between nesting sites, or are actively fighting, such as the borders of supercolonies. The amount I used was enough to deter most ants for at least 5-7 minutes. A trail that is constantly being reinforced could deter ants indefinitely.

Foraging suppression as an invasion technique

The presence of Argentine ants is very effective at suppressing foraging in other species, even when they do not directly interact (Human and Gordon 1996). When an Argentine ant colony takes up residence, native species reduce their foraging distance from the nest. This could be partially due to the effects of using iridomyrmecin as a passive foraging suppressant. Interference and exploitation competition has been studied extensively as an invasion technique (Holway 1999, Human and Gordon 1996). Usually, an ant species will excel in either finding a resource first (exploitation) or invading and excluding the first species from the resource (interference). Argentine ants are adept at both. Previously it's been thought that Argentine ants find and recruit to baits quicker than native species because they have larger population numbers and are more active in their territory (Holway 1999). Their population numbers and activity level directly relates to the concentration of iridomyrmecin in an area. Perhaps they are so adept at exploitation interference because native ants avoid areas with a high concentration of iridomyrmecin, ergo avoiding areas colonized by Argentine ants. Because Argentine ants are unicolonial, as their population grows, their territory expands and the area left to the native ants shrinks until they can no longer support a colony.

Limitations

I studied the effects of iridomyrmecin on velvety tree ants. Other ant species may have a very different response, especially other ants that produce iridomyrmecin-like products. Additionally, all experiments took place in areas that Argentine ants had not yet colonized, making it unlikely that the native ants had come into contact with Argentine ants before. Previous exposure to the threat of Argentine ants could change the response of the native ants to their presence. All experiments took place over the course of two separate days, meaning the ants' activity level could have been influenced by the time of day and temperature. Bait placement could have been a factor in how many ants found and recruited to a bait. Bait placement was not random and could have unintentional bias. Future experiments could focus on different ant species, or different mechanics of using defensive chemicals as a passive defense against other ant species.

Broader implications

Argentine ants are a dangerous invasive species and I've demonstrated they have more of an advantage than we knew before. Argentine ants all over the world could be systematically suppressing the foraging behavior of native species and this already affecting the ecosystem (Moffit 2010). Argentine ants do not take on the ecological niches vacated by the removal of native ants and this can affect the ecosystem by disrupting ant-plant mutualisms or suppressing vertebrates that rely on larger ant species for food. I found that the presence of iridomyrmecin is enough to prevent velvety tree ants from accessing a valuable resource, and this may apply to other ant species as well. This does open up several possibilities to combat the invasion moving forward. Argentine ant foraging trails can be disrupted by spraying Z-9-hexadecenal over a field, thereby reducing the number of trails and ants foraging (Suckling et al. 2010). If Argentine ant trails can be disrupted by spraying one of their one pheromones, it's possible other ants might return to areas with fewer Argentine ants and therefore a lower concentration of iridomyrmecin. Additionally, an aggregation pheromone belonging to a native ant could be sprayed in an area to coax native ants back in. Although the Argentine ant invasion is widespread and damaging, future management could alleviate some of these effects.

ACKNOWLEDGEMENTS

Huge thanks to Brian Whyte for giving me the opportunity to spend my senior year watching ants do things and taking me anting and generally making this whole thesis possible. Thanks to Tina and Maggie and the whole 175 crew for thorough feedback and seemingly endless enthusiasm. I also need to thank JJ Kelly for reminding me it's okay to be a huge nerd about somethings. None of this would have been possible without Kamal Chauhan who provided the iridomyrmecin we used in the trials.

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