Spread of the red imported fire ant, *Solenopsis invicta*, in Virginia and effects of sub-lethal exposure to agrochemicals on its behavior

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SCIENTIFIC ABSTRACT

Solenopsis invicta is an invasive ant that has caused detrimental impacts to ecosystems and economies in the Southeastern United States, recently including Virginia. In this study, we explored the invasion ecology of *S. invicta* through two main objectives. First, we established a comprehensive distribution map of *S. invicta* in Virginia through multiple survey techniques. We then compared our findings with published models quantifying the potential spread of *S. invicta* and created our own species distribution model. In 2020-2021, *S. invicta* occurrences were found in 7 counties beyond the current Quarantine and our data show that *S. invicta* has spread further than predicted. Our own species distribution model suggests that the distribution area for *S. invicta* is likely to increase under the projected climate change. This study provides insights into the range expansion of *S. invicta* at the border of its suitable habitat and allow for improvements to models of its spread under these conditions. Additionally, it provides useful information to inform county extension agents to know where they are to expect new infestations of *S. invicta*. Second, we investigated the impacts of pesticide residue on the behavior of *S. invicta* through neonicotinoid exposure. We found detectable levels of neonicotinoids in the soil of the ant mounds as well as in the ants themselves. In addition, we investigated the effects of dietary exposure to imidacloprid on foraging behavior in a laboratory setting. We found that unexposed colonies were able to locate the food source more quickly during the second trial while exposed ants were unable to improve their performance. We also found that more exposed ant workers were unable to successfully navigate the maze as compared to unexposed workers. Our results suggest impaired learning of maze tasks and impaired navigational skills in neonicotinoid-exposed ants.

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GENERAL AUDIENCE ABSTRACT

The red imported fire ant (RIFA) is an invasive ant species found throughout the Southeastern United States that has negatively impacted ecosystems and economies. In the past few decades, RIFA has invaded the Coastal Plain of Virginia, resulting in legislation that restricts the movements of soil, plant products, and some equipment in and out of several southeastern counties and independent cities. To develop better management practices, there is a critical need to understand the spread, establishment, and impacts of RIFA in greater detail. We aimed to do this by surveying the current distribution of RIFA in Virginia and investigate the impact of insecticide use on their spread and behavior. In 2020-2021, we found RIFA occurrences in 7 counties beyond the current Quarantine, which is further than previously predicted. We then built a model using climatic variables that predicts the distribution of RIFA and found their habitable range is likely to increase under the projected climate change. Additionally, we investigated the impacts of common agricultural pesticides on the behavior of RIFA. We found these chemicals present in both the soil of the ant mounds and in the ants themselves. We also found that dietary exposure to imidacloprid, a neonicotinoid pesticide, altered the foraging behavior of RIFA. This study provides useful information to advise county extension agents to know where they are to expect new infestations of RIFA. Our results also suggest that human activity alters the invasion ecology of recent arrivals such as the red imported fire ant.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1 Introduction

Biological invasions threaten environmental, economic and social wellbeing across the globe (Pejchar & Mooney, 2009). The spread of invasive species is largely caused by human activity, such as trade and transportation (Di Marco & Santini, 2015). As the impacts of climate change and globalization increase, so too will the threat of invasive species (Hellmann et al., 2008; Seebens et al., 2017). Invasive ants are particularly noteworthy because of their ability to rapidly spread into an area causing damages to native species, agriculture, and human health (Lessard et al., 2009; Lowe et al., 2000). The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae; Figure 1.1), is a notoriously invasive species and is listed as one of the world's 100 worst invasives by the Global Invasive Species Database (Lowe et al., 2000).

Native to South America, the red imported fire ant (RIFA) was accidentally introduced to North America in the 1930s (Vinson, 1997) and has since spread across the southeastern United States and to other regions of the world (Sung et al., 2018; Sutherst & Maywald, 2005). RIFA causes major damages to agriculture, human health, and ecosystem processes (Adams et al., 1983; Gutrich et al., 2007; Wojcik et al., 2001). In the United States, RIFA and the closely related black imported fire ant (*Solenopsis richteri*) cause an estimated economic impact of $6 billion annually (Gutrich et al., 2007). Climate change has and will continue to affect distribution of pest species with likely negative economic and environmental consequences (Ziska & McConnell, 2016). Additionally, globalization and increased global trade is assisting in the spread of invasive pest species (Hulme, 2009).

Although RIFA are widely established across the southeastern US, it has only recently become established in Virginia. Systematic records of where RIFA are in Virginia have been lacking, and it is unclear where the invasion front is. To establish the boundaries of the range expansion it is crucial to investigate their spread in Virginia using systematic surveys. Therefore, the goals of this project were to 1) investigate RIFA distribution in Virginia and 2) investigate impacts of land use on its spread.

2 Invasion and Distribution

Native to South America, the red imported fire ant (RIFA) was accidentally introduced in the United States through the port of Mobile, Alabama in the 1930s (Buren et al., 1974). From there colonies spread rapidly throughout the southeastern United States and to many other parts of the world (Wetterer, 2013). Many recent introductions in China and Australia have likely originated in the US rather than from their native South American range (Ascunce et al., 2011). Climate factors, such as temperature and precipitation, are often the main constraint to the natural spread (Sutherst & Maywald, 2005) while human activity is considered one of the driving forces behind its spread (King & Tschinkel, 2006). RIFA colonies spread through mating flights and human transportation at a rate of one to three miles per year (Callcott & Collins, 1996). Recently the spread of RIFA has slowed as it reaches its northern limit dictated by temperature and moisture (Callcott & Collins, 1996). However, due to the impacts of global warming and human development the spread is likely to continue (Korzukhin et al., 2001; Morrison et al., 2005; Sutherst & Maywald, 2005).

The Federal Imported Fire Ant Quarantine (7 CFR 301.81) was established in 1958 to prevent the artificial spread of fire ants (APHIS, 2019; Figure 1.2). Currently, 14 states (Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia) and the Commonwealth of Puerto Rico are included, fully or partially, in this regulation. The focus of this effort is to limit human assisted spread of fire ants rather than eliminate the species from existing areas. It requires items capable of carrying ants such as soil, plant products, and equipment to be inspected when moving from within the Quarantine region to outside of it. (APHIS, 2019). The Federal Quarantine was established in Virginia in 2009, and was recently updated in 2019 (Miller & Allen, 2019; Figure 1.3).

3 Impact

Due to its aggressive nature and painful sting, RIFA is considered a threat to humans, infrastructure, and ecosystem processes. They are omnivorous feeders and aggressive foragers that will feed on almost anything, including insects and invertebrates (Tschinkel, 2006). RIFA prefers open sunny environments and can often be found in agricultural settings. Infestations of RIFA in farmland across the southeastern US has caused damage to many crops and livestock (Adams et al., 1983). RIFA also has been reported to suppress pest species as well as natural enemies (Eubanks, 2001) and cause damage to farm equipment. Infestations cause stress and ailment to farmers who work in infested fields (Chan & Guénard, 2019). Despite these negative impacts on agricultural systems, RIFA may be an important beneficial predator in agricultural systems and an effective biocontrol agent for many common arthropod pests (Harvey & Eubanks, 2004; Vogt et al., 2001; Wang et al., 2016). In urban areas it is often found along sidewalks, parks, and playfields where it is considered a pest due to its painful and potentially dangerous sting. In addition to agricultural and urban environments, RIFA also pose a threat to natural ecosystems by reducing biodiversity (Allen et al., 2016; Gotelli & Arnett, 2000; Wang et al., 2019; Wojcik et al., 2001). RIFA displaces native ant species through competition for resources (Gotelli & Arnett, 2000; Wang et al., 2019). RIFA invasions also directly impact the diversity of invertebrates, birds, and mammals (Wojcik et al., 2001). Its presence may also alter abiotic factors such as soil structure and fertility (Lafleur et al., 2005).

4 The invasive success of RIFA

The invasive success, or ability to invade a new environment, of a species is linked to many different factors, including human activities, ecological characteristics, and phenotypic characteristics (Ascunce et al., 2011; Estoup & Guillemaud, 2010; Holway & Suarez, 1999). Invasive species more readily adapt to changes in the environment than native species (Sorte, 2014; Wolkovich & Cleland, 2014). It is well known that invasive species change the structure of communities they invade (Blois et al., 2013), however these relationships are complex and there is a need to study them in greater detail, especially in the light of climate change (Diez et al., 2012; Wolkovich & Cleland, 2014).

Behavior is an essential component of the invasive success of a species (Holway et al., 2002; Holway & Suarez, 1999; Wolf & Weissing, 2012). The most successful invasive ants have many shared traits, including: multiple queen colonies or polygyny (Hölldobler & Wilson, 1977), large interconnected super colonies (Chen & Nonacs, 2000), high rates of aggression towards other species (Kirschenbaum & Grace, 2007), and monopolization of resources (Holway & Suarez, 1999). Invasive ants in their introduced range may have higher rates of exploration, aggression, food exploitation, and expansion then in their native ranges (Blight et al., 2016). Invasive ants often form symbiotic relationships with honeydew producing Hemiptera, which contributes to colony growth and high density populations (Helms & Vinson, 2008). Individual route learning is key to the foraging success of mass-recruiting ants (Pasquier & Grüter, 2016) such as fire ants.

The dispersal of invasive ants is often caused by human commerce, where fragments of colonies can be carried long distances with cargo (Mack et al., 2000). Along with their aggressive behavior, human activity is a major force behind the spread of RIFA (King & Tschinkel, 2008). Humans not only aid in the physical movement of RIFA through global trade, but this species is also more likely to invade recently disturbed habitats (King & Tschinkel, 2008). It prefers to build colony nests in warm, open environments such as pastures, parks, and along roadways. The potential range of RIFA is limited by temperature and moisture (Morrison et al., 2005; Tschinkel, 2006). As the impacts of climate change and globalization increase, the habitable area of RIFA is likely to increase as well (Morrison et al., 2005; Sung et al., 2018; Sutherst & Maywald, 2005).

5 RIFA and neonicotinoid exposure

Because fire ants prefer recently disturbed habitats such as agricultural fields (King & Tschinkel, 2008), they are likely to be exposed to agrochemicals that are not specifically targeted towards them. Neonicotinoids are a common group of insecticides often used in farm and urban landscapes (Jeschke et al., 2011). There is evidence that neonicotinoids have left persistent residue in the soil and waterways in and around agricultural areas (Bonmatin et al., 2015). The negative impacts of non-target exposure to neonicotinoids in invertebrates is well-documented (Bonmatin et al., 2015; Johnson et al., 2010; Pisa et al., 2014). Exposure to sublethal concentrations of neonicotinoids has been demonstrated to alter the behavior of many arthropod species (Desneux et al., 2007; Pisa et al., 2014; Tappert et al., 2017; Thorne & Breisch, 2001). Imidacloprid is a common neonicotinoid with many different uses and applications. It is used to control termites, soil insects, fleas on pets, and sucking insects (Gervais et al., 2010). It is often applied as a seed coating in agricultural systems (Gervais et al., 2010) and has been found in soil at varying concentrations (Jones et al., 2014).

Many non-target organisms are affected by neonicotinoids through long-term sublethal exposure (Halm et al., 2006). There are many likely exposure routes to neonicotinoids for non-target species, including accumulation in the soil and water (Bonmatin et al., 2015; Goulson, 2013; Pisa et al., 2014). They are predominantly used in artificial seed coatings (Jeschke et al., 2011), where only 2-20% of the active ingredient is taken up by the plant (Sánchez-Bayo, 2014). The remaining active ingredient can leach into the soil and surrounding environment (Radolinski et al., 2018). Additionally, non-target species may be exposed through their diet and accumulation through the food chain (Desneux et al., 2007). The excretion product (honeydew) of sap feeding insects targeted by neonicotinoids contain neonicotinoid residues (Calvo-Agudo et al., 2019). Many ant species including RIFA feed on this honeydew product (Styrsky & Eubanks, 2010; Wilder et al., 2013). As a soil dwelling insect found in agricultural systems throughout the southeastern US, fire ants are likely to have a variety of exposure routes to neonicotinoids, such as their environment (Bonmatin et al., 2015; Pisa et al., 2014; Thompson et al., 2020) and diet (Calvo-Agudo et al., 2019; Wilder et al., 2013). The many potential exposure routes make it difficult to calculate exposure levels of neonicotinoids within ants. Field realistic exposure has not been quantified in RIFA.

Pollinators, specifically honeybees, have been the primary focus of behavioral research of the sub-lethal effects of neonicotinoids (Johnson et al., 2010; Mason et al., 2013; Prisco et al., 2013). Neonicotinoids target receptors in insects' nervous system (Brown et al., 2006) and therefore can impair cognitive function of insects and alter behavior such as foraging (Mason et al., 2013; Muth & Leonard, 2019), learning and memory (Stanley et al., 2015; Williamson & Wright, 2013) and social behavior (Boff et al., 2018). Even at low concentrations, they have sublethal effects that adversely impact the behavior, fertility, and immune functions of insects, including ants (Galvanho et al., 2013; Pisa et al., 2014; Schläppi et al., 2021; Wang et al., 2015a).

While there are likely many consequences due to the unintentional effects of neonicotinoids on ants, data are largely lacking (Schläppi et al., 2021).

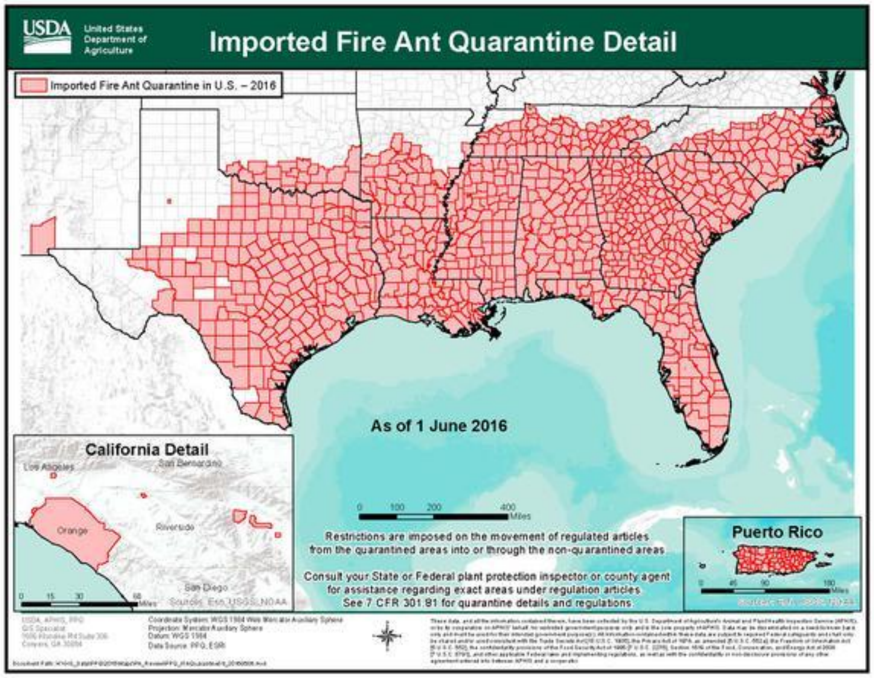
A few studies have looked at the impact of sublethal exposure of neonicotinoids on ants (Barbieri et al., 2013; Galvanho et al., 2013; Wang et al., 2015b). These studies show neonicotinoid exposure may have a range of behavioral effects on ants, however, the results of these studies are often not consistent. For example, Barbieri *et al.* (2013) found changes in aggressive behavior and not foraging. However, a similar study using different species found that aggressive behavior was altered in one species but not the other (Thiel & Köhler, 2016). Contrary to Barbieri *et al.* (2013), they observed a change in foraging behavior (Thiel & Köhler, 2016). Another study found that imidacloprid seed coating altered ant community composition in the field and had lethal and sublethal effects on *Tetramorium caespitum* in a laboratory environment (Penn & Dale, 2017). A recent study investigated the effects of long-term exposure in *Lasius niger*, finding reduced colony sizes thus suggesting a trade-off between detoxification and fertility (Schläppi et al., 2020). Because of the long-life span and sedentary nature of many ant colonies, long-term exposure is extremely likely.

Neonicotinoids are a common ingredient in baits used to control RIFA populations (Chen & Oi, 2020). While this intentional exposure can be an effective tool when used correctly, RIFA is likely subjected to unintentional exposure to neonicotinoids (Schläppi et al., 2021) possibly causing sublethal exposure. Wang *et al.* (2015) found that sublethal exposure of neonicotinoids impacted RIFA behavior including digging and foraging (Wang et al., 2015b). Sublethal exposure to new queens may affect colony development (Wang et al., 2015a). A recent study found that long-term exposure to neonicotinoids reduced colony size in *Lasius niger*, but was only noticeable during the second year (Schläppi et al., 2020). In the long run, even low concentrations of neonicotinoids may have subtle but lasting effects (Schläppi et al., 2020). However, the effects of long term exposure on soil insects is still poorly understood (Schläppi et al., 2020). These studies suggest that sublethal exposure of neonicotinoids may have complex and varying effects on ant behavior.

Behaviors such as foraging and competition are important for the invasive success of a species (Holway et al., 2002; Wolf & Weissing, 2012). Exploiting a food source is an important factor in the invasive success of fire ants, and changes in behavior may alter their ecological impacts. Field realistic exposure of neonicotinoids has not been quantified in fire ants, and consequently, the behavioral effects of exposure have not been studied at field realistic amounts. The foraging efficiency of fire ants relies on their ability to exploit a food source quickly and therefore out compete other species (Wilder et al., 2013). Changes in these behaviors could alter the interactions between native and invasive species, and therefore change the success of invasive species. Different species may have different levels of exposure to pesticides and exposure may also have different effects on different individuals. Understanding how RIFA invasion is affected by anthropogenic disturbances, such as neonicotinoid exposure, can lead to better management practices. Additionally, it will lead to more knowledge on the environmental impacts of these insecticides.

6 Figures

Figure 1.1: Image of red imported fire ant. Photo by David Almquist, University of Florida. Source: https://entnemdept.ufl.edu/creatures/urban/ants/red_imported_fire_ant.htm



Restrictions are imposed on the movement of regulated articles from the quarantined areas into or through the non-quarantined areas

Consult your State or Federal plant protection inspector or county agent for assistance regarding exact areas under regulation articles See 7 CFR 301.81 for quarantine details and regulations

Figure 1.2: Map of Imported Fire Ant Quarantine from 2016. Source: USDA-APHIS

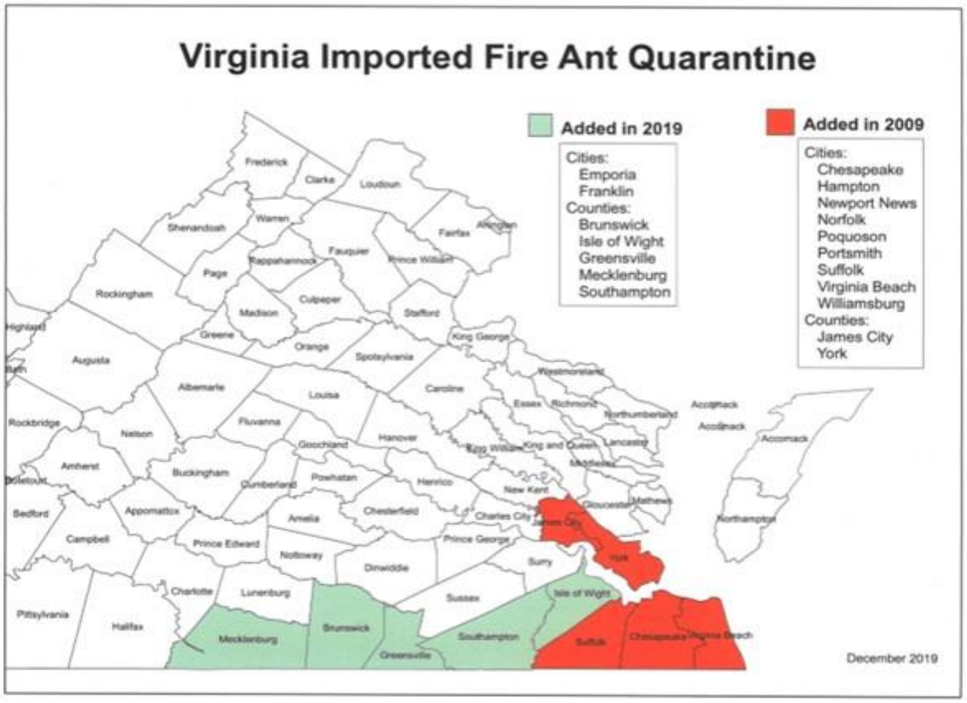


Figure 1.3: Federal Fire Ant Quarantine Map in Virginia. Red counties and cities were added in 2009. Green counties and cities were added in 2019.

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CHAPTER 2: UNEXPECTED FAST RANGE EXPANSION OF THE RED IMPORTED FIRE ANTS IN VIRGINIA AND PREDICTION OF FUTURE SPREAD

1 Abstract

The red imported fire ant, *Solenopsis invicta*, is a notorious invasive species with a broad array of detrimental impacts to ecosystems, economies, and human health. Once established, it rapidly spreads through the area and is virtually impossible to eradicate. Early detection is a key factor in managing invasive species and limiting their damaging impacts. The full extent of spread of the red imported fire ant (RIFA) in Virginia, situated along the northernmost edge of its introduced range in North America, is currently unknown. In this study, we examined the current distribution of RIFA in Virginia using multiple data sources. During the summers of 2020 and 2021, we conducted a series of visual surveys along public roadways. In addition, we used multi-year infestation reports from the Virginia Department of Agriculture and Consumer Services collected between 2016 and 2021. Lastly, we surveyed local naturalists, county extension agents, landscape and nursery business owners, and land managers. We compared our findings to previously published models quantifying the potential spread of RIFA, investigated deviations from these models, and constructed a species distribution model to predict the potential range expansion of RIFA. In 2020-2021, RIFA was found in 7 Virginia counties beyond the current Quarantine zone and our data show that RIFA has already spread further than predicted as recently as 15 years ago. Our species distribution model suggests that the range of RIFA is likely to increase under the currently projected climate change scenarios. This study provides insights into the range expansion of the red imported fire ant at the border of its suitable habitat and allows for corrections to models of its future spread. In addition, it provides information to advise sound management practices and eradication efforts.

2 Introduction

As the impacts of climate change and globalization continue to increase, so will the threat of invasive species (Seebens et al., 2017). The spread of invasive species is often facilitated by humans, who move them, intentionally or accidentally, through trade and transportation (Di Marco & Santini, 2015). The effects associated with climate change can also cause shifts in species' ranges and increase the risk of invasive species (Bertelsmeier et al., 2015).

Many ants are particularly problematic invaders as they rapidly spread into an area and cause damages to native species, agriculture, and human health (Lessard et al., 2009; Lowe et al., 2000). The red imported fire ant, *Solenopsis invicta* (Buren), is a notorious invasive species in many parts of the world including the southern United States (Wetterer, 2013). The Global Invasive Species Database lists the red imported fire ant (RIFA) as one of the world's top 100 worst invasive species (Lowe et al., 2000). Native to central South America, the red imported fire ant was introduced in the United States through the port of Mobile, Alabama in the 1930s (Buren et al., 1974). From there colonies expanded rapidly throughout the southern United States (Sung et al., 2018). In 2000, the US Department of Agriculture estimated that RIFA was expanding approximately 120 miles (193 km) per year (Kemp et al., 2000). The drier conditions of the American West, and the colder temperatures in the north, have slowed its spread (Vinson, 1997). Due to RIFA's high adaptive and reproductive capacity (Ross & Shoemaker, 2008), global climate change, and current urbanization trends, its range is likely to continue to expand (Morrison et al., 2005).

In the United States, the red imported fire ant causes an estimated economic impact of $6 billion annually (Drees & Lard, 2006). It is an aggressive species, known for its painful sting which can have negative health effects on wildlife, livestock, and humans (Langkilde, 2009; Vinson, 2013; Wang et al., 2019). RIFA impacts infrastructure and businesses by infesting public areas such as schools, businesses, and recreational areas (Gutrich et al., 2007). Additionally, RIFA is pest in agricultural systems, impacting farm equipment and reducing crop yields (Adams et al., 1983; Vinson, 2013). In its introduced range, the red imported fire ant favors disturbed sites such as pastures, lawns, gardens, urban parks, and roadsides (Porter & Tschinkel, 1993; Rosson, 2004). Mounds vary in size and shape depending on the size and age of the colony and soil type, and are usually found in open and sunny habitats such as fields and pasture (Rosson, 2004; Figure 2.1).

While RIFA can spread through a variety of avenues, human activities such as trade and transportation are major forces behind its spread (King & Tschinkel, 2008). In addition, this species readily spread into recently disturbed environments such as agricultural fields and new development (Tschinkel, 2006). Human activities also contribute to its long-distance dispersal, when ants are transported along with agricultural and landscaping materials. Temperature (Morrill, 1977; Porter, 1988), precipitation (Thorvilson et al., 1992; Wojcik, 1983), vegetation type (Rosson, 2004), and human activity (Bertelsmeier et al., 2015; King & Tschinkel, 2008) influence the areas suitable for RIFA establishment and continued persistence. The main constraints limiting RIFA spread are temperature and precipitation (Morrison et al., 2005; Chen et al., 2020). Urbanization may facilitate the spread of the red imported fire ant, especially in colder climates where ants can overwinter in electrical equipment and insulation (Thorvilson et al., 1992).

Managing invasive species is extremely difficult, especially once they become established (Hoffmann et al., 2011). Early detection and subsequent limit on spread are important mitigation tools for preventing the establishment and spread of such taxa (Lodge et al., 2006). The Federal Imported Fire Ant Quarantine was established in Virginia in 2009 and included the independent cites of Chesapeake, Hampton, Newport News, Norfolk, Poquoson, Portsmouth, Suffolk, Virginia

Beach, and Williamsburg and the counties of James City and York. In the 2019, the independent cities Emporia and Franklin and the counties Brunswick, Isle of Wight, Greensville, Mecklenburg, and Southampton were added (Miller & Allen, 2019). This legislation restricts the movement of soil, plant products, equipment used for moving soil, and other items that are capable of harboring ants and requires the inspection of such items before transportation (APHIS, 2019). Mapping and modeling species distributions is another important tool for tracking and managing the spread of invasive species as well as for predicting the impacts of climate change on their distributions (Miller, 2010). Such models have become increasingly important in monitoring species distribution especially in regards to environmental change (Miller, 2010). Previous models have shown that the range expansion of the red imported fire ant is likely to continue to increase (Chen et al., 2020; Korzukhin et al., 2001; Morrison et al., 2005; Sung et al., 2018; Sutherst & Maywald, 2005).

In this study, we investigated the current spread of *S. invicta* in Virginia (along the northernmost edge of its introduced range in North America) and qualitatively compared it to an earlier predictive model (Morrison et al., 2005). In addition, we constructed a species distribution model and explore the effects of climate on RIFA range expansion in North America. Although some opportunistic data on occurrence is being collected by the Virginia Department of Agriculture and Consumer Services (VDACS), we complement these data with our own extensive, systematic presence / absence surveys. Our mapping efforts allow us to study the effects of climate conditions on these recent trends of expansion. Mapping and modeling species distribution is important for the management of invasive species and gives insight to how global warming may effect the distribution of species (Miller, 2010).

3 Methods

3.1 Current range of the red imported fire ant in Virginia

3.1.1 Road-side Surveys

During the summers of 2020 and 2021, we conducted a series of visual surveys along public roadways in southern Virginia. The survey methods were adapted from the United States Department of Agriculture "Imported Fire Ant Program Manual"(APHIS, 2019).

We selected 23 roadways that extended 25 miles more or less perpendicularly to the current Imported Fire Ant Quarantine zone (Figure 2.2). Roadways were selected using ArcGIS Online (Esri, 2020) and were situated approximately 10 miles apart from each other. During each survey, we stopped every 5 miles along each roadway (5 stops per roadway; total of 123 stops) and looked for evidence of RIFA. Due to the orientation of some roadways several stops overlapped and were sampled only once. Surveys were conducted during daylight hours when air temperatures were between 65 and 90°F. Land cover in the surveyed region primarily consists of cropland, forests, and disturbed areas. In 2021, we limited the roadway surveys to within 10 miles of known RIFA presence.

Before surveying each site, we examined its suitability using Google Maps. A location was deemed unsuitable if there was construction, if the location had no safe turnoff, or if it was located on private property. If a site was unsuitable, we choose a new site 1 km away. Exact stopping location was determined at the time of the survey and was adjusted for parking and safety of the surveyor (MM). Roadways were grouped by convenience and randomly selected in advance via a random number generator. At each stop, we walked one-quarter mile along each side of the road and visually surveyed for mounds, small piles of exposed soil, or other soil disturbances (APHIS, 2019). RIFA mounds are easily distinguished from other ant mounds because they do not have visible entrances. When we suspected a mound to be RIFA, we disturbed the mound using a stick or shovel. When disturbed fire ants rushed out of the mound and climbed vertically, we deemed them to be fire ants. In addition, we identified RIFA based on visual characteristics. RIFA are red to brown in color, between 2.4 to 6 mm in body size, have a two segmented pedicel, and have a sting present (Collins & Scheffrahn, 2001).

3.1.2 Reports from Virginia Department of Agriculture and Consumer Services

In addition to road surveys, we used multi-year infestation reports from the Virginia Department of Agriculture and Consumer Services (VDACS) from 2016-2020. VDACS provided coordinates with ¼ mile accuracy to obscure sensitive information. The focus of these reports is in areas outside of the Imported Fire Ant Quarantine zone.

3.1.3 Community Science Reports and Other Data Sources

We used community science, and other efforts to gather RIFA occurrence data from 2020 to 2021. Community science efforts included participating individuals from the Virginia Master Naturalist program who provided locality data, and research grade records harvested from iNaturalist. iNaturalist is a community science platform for mapping and sharing observations of biodiversity world-wide. In addition, we surveyed county Cooperative Extension offices and while no specific locations were recorded through this survey, we were able to gain a better understanding of RIFA's spread in the study area. Presnce absence data were also provided by the Virginia Museum of Natural History (KI).

3.2 Comparison to previous model

To compare the current range of *S. invicta* in Virginia with a previously published model (Morrison et al. 2005) we overlaid our occurrence data with the model's maps and visually compared the two distributions because the original data and model are no longer available. We used geographic landmarks to align the two maps as accurately as possible and utilized the same 0.5° x 0.5° cell grid used in the Morrison *et al.* (2005) model. We then qualitatively compared the differences between the model's predictions and our data.

3.3 Prediction of habitable range with current and future climate predictions

3.3.1 Species Distribution Model

The methods and code for creating the species distribution model (SDM) follows Zurell and Engler (2019) with a few revisions to adapt to our data using R version 4.0.5 (R. Core Team, 2021). The SDM was constructed to model the potential distribution of RIFA under historical and future climate conditions (see section 3.3.3). Each of the environmental variables was entered as a linear and quadratic term. For model fitting, we used a binomial generalized linear model (GLM) available through the *mecofun* package (Zurell et al., 2018), and we trained the model on all US data available through the Global Biodiversity Information Facility (see section 3.3.2). The two-sided significance level was set to $P < 0.05$. After training the model, we evaluated its accuracy by projecting the distribution of RIFA under historical climate conditions. Once the species distribution model was fitted and assessed, we projected future spread over geographic space.

3.3.2 RIFA Presence and Absence Data

We collected presence data from the Global Biodiversity Information Facility (GBIF), a free open access source of global biodiversity data (GBIF.org, 2021; Appendix A). We collected all data points recorded in the United States and removed any records with incorrect coordinates or those with reported uncertainty in the coordinates (final data: n=7574). We then created 7000 pseudo absence points uniformly across the United States.

3.3.3 Environmental Data

The spread and establishment of RIFA is influenced by many factors including climate. According to previous findings, temperature and precipitation are the main climatic factors limiting the spread of RIFA (Morrison et al., 2005; Chen et al., 2020). We used the bioclimate variable data set from WorldClim, which offers a free high spatial resolution dataset of global weather and climate for both past data and future predictions (Fick & Hijmans, 2017). The dataset includes 19 bioclimate variables. Among these, we picked 5 variables associated with *S. invicta* spread (Sung et al., 2018): annual mean temperature (Bio1), maximum temperature of the warmest month (Bio5), minimum temperature of the coldest month (Bio6), annual precipitation (Bio12), and precipitation of the driest month (Bio14). We collected historical (current) data at a spatial resolution of 30 seconds or approximately 1 km^2. We collected future climate data at a resolution of 2.5 minutes (highest resolution available) for 2050 and 2070.

4 Results

4.1 Current range of Red Imported Fire Ant in Virginia

We recorded a total of 153 confirmed RIFA occurrences in the period 2020-2021 (Figure 2.3A). The number of observations reported from VDACS increased every year since 2016: 5 reports in 2016, 17 reports in 2017, 65 reports in 2018, 71 reports in 2019, and 108 reports in 2020. From the 2020 roadway surveys, we found RIFA present at 9 of 123 sites; 8 of these sites occurred at the site closest to the starting point (within 5 miles of the Imported Fire Ant Quarantine zone). From the 2021 roadway surveys, we found RIFA present at 6 of the 50 sites, 5 of these sites occurred at the site closest to the starting point. Prior to 2020, no RIFA observations had been recorded in Virginia through iNaturalist. Between 2020 and 2021, there were 25 observations reported through iNaturalists: 24 of the observations were confirmed and 1 could not be substantiated. Additional 13 observations were collected through the other sources. We found a total of 537 absence points collected from the roadway surveys, VDACS, and data sourced from the Virginia Museum of Natural History (KI; Figure 2.3A).

In total, 111 RIFA occurrences were reported outside of the quarantine boundaries in 2020 and 2021. Most of these observations were in Halifax, Charlotte, Lunenburg, and Sussex counties, with a few observations in Pittsylvania Co., Dinwiddie, and Chesterfield counties. Prior to 2020, VDACS reported a few isolated RIFA observations in the Richmond area (Figure 2.3B), however these sites appear to be isolated and were not confirmed in 2020 through our prospective survyes.

4.2 Comparison to previous models

Both westward and northward expansion are greater than expected 15 years ago (Morrison et al., 2005). By 2020, the range has expanded 5 cells further than predicted, including the potential range predicted by 2080-2089. Along the North Carolina border, expansion has increased by 3 cells, or approximately 128 km past the range expected by Morrison *et al.* (2005). Northward expansion is 1 cell or approximately 55 km past the expected range from the Morrison *et al.* 2005 model (Figure 2.4).

4.3 Species Distribution Model for the red imported fire ants

We found four of the five bioclimatic variables we selected *a priori* for the model building to be significant predictors of RIFA presence: annual mean temperature, minimum temperature, average precipitation, and minimum precipitation; maximum temperature was not a significant predictor of RIFA presence (Table 2.1). Because of both significant linear and quadratic terms present, the partial response curves (Figure 2.5) provided a better impression than the coefficient table of how these variables are connected to the RIFA occurrence. If the annual mean temperature is below 5°C RIFA cannot persist in an area (Figure 2.5A). Based on our predictions mean annual temperatures above 30°C (Figure 2.5A) and minimum temperature below -20°C (Figure 2.5C) restrict RIFA range.

Based on these model parameters, we made predictions regarding the spread of *S. invicta* under current and future conditions (Figure 2.6). The prediction of current spread is reflective of the training data on a larger scale, except west of the Mississippi where our model predicts suitable conditions, but there is a gap in empirical occurrence data (Figure 2.6A). Figure 2.6B shows the level of risk of RIFA infestation in Virginia under current climatic conditions on a continuous scale from 0 to 1. High risk areas are mostly located along the border with North Carolina and along Virginia's Coastal Plain and the Delmarva Peninsula. Lower suitability areas are situated through much of central Virginia. Most of the western part of the state is predicted to be unsuitable for RIFA establishment under current climatic conditions (Figure 2.6). Future predictions show the habitable area of RIFA in the southeastern United States is likely to increase by 2050 and 2070, especially through the lower Midwest. Westward expansion through Texas and parts of the southwest is less likely (Figure 2.6 C &D).

5 Discussion

In this study we outline the current spread of *S. invicta* in Virginia, one of the current range expansion fronts in the United States. Our results show that both westward and northward RIFA expansion in the state is greater than expected 15 years ago (Morrison et al., 2005). If we trained the species distribution model on US data not including the recently collected Virginia data, the model seemingly predicts the current range we observed. This indicates that the earlier model by Morrison *et al.* (2005) either underestimated the speed of change in global climate, or that earlier models did not capture the climatic factors affecting RIFA spread well enough. In either case, our data suggests that updated models are needed to more accurately predict where RIFA may be expected in the future.

From 2016-2020, RIFA observations have expanded west into Virginia’s Halifax Co. and north into the City of Richmond. In 2020-2021, expansions continued westward into Halifax Co. and towards Pittsylvania Co. Northward expansions continued into Charlotte, Lunenburg, Dinwiddie, Sussex, Surry, and Chesterfield counties. While most RIFA observations were located just outside the current quarantine boundaries, isolated observations have been made as far as the City of Richmond. In 2020-2021, RIFA occurrences were found in 7 counties beyond Quarantine. In 2020, using model measurements from Morrison *et al.* (2005), expansion has increased by 5 cells, including areas beyond the 2040-2049 predicted potential range. Chances of *S. invicta* occurrence are likely to vary from year to year based on changes in temperature, moisture conditions, and land management practices. For example, an unusually cold winter or unusually dry summer could lower ant survival. Land management practices such as treating RIFA mounds with insecticides or repeated mowing may cause ants to relocate or disappear from an area.

VDACS documentation of RIFA occurrences has increased steadily since 2016. VDACS efforts focus on limiting spread beyond the quarantine boundaries and preventing new infestations across Virginia. When possible, active mounds outside of the quarantine zone are treated with insecticidal bait. Although time consuming, roadside surveys, such as the ones used in this study, are a common method for detecting introduced ant populations (Gippet et al., 2016; Tong et al., 2018). Although early detection is key for reducing the spread and eradicating newly established colonies (Lodge et al., 2006), proactive surveillance such as delimiting and roadside surveys is rare in invasive ant monitoring and most early detection relies on public reporting (Cooling & Hoffmann, 2015). Since the establishment of the Virginia Imported Fire Ant Quarantine in 2009, the red imported fire ant has increasingly become a concern for the public.

While species distribution models rely on a number of underlying assumptions, they are useful tools for managing the spread of invasive species. We used a newly created species distribution model to estimate the potential distribution of RIFA under current and future climate projections. Our model shows that: 1) RIFA is not occupying the currently suitable habitat in its entirety, and 2) the suitable habitat for RIFA is to increase under future climate change especially if current conditions (climate change and globalization) do not change. Based on these results, RIFA is likely to continue to expand its range northward along the Atlantic coast as well further north into the Midwest. One potential reason why there are no records of RIFA from the Delmarva Peninsula could be the barriers set by the Chesapeake Bay. Based on the results from this model and previously published models, measures should continue to be put in place to reduce the chance of RIFA establishment, especially in Virginia. Continued monitoring is needed for early detection of newly invaded areas, which is in turn critical to managing the spread of invasive species. Validating species distribution models is important for model accuracy and can help with early detection and managing spread.

Management of invasive species is difficult and complete eradications are extremely rare (Hoffmann et al., 2011). In order to develop better management practices, there is a crucial need to better understand the current spread and possible future distribution of the red imported fire ant in greater detail (Hoffmann et al., 2011). Model accuracy can help with early detection and with limiting the spread of this, and other, invasive species. Models predicting RIFA expansion could be continually validated as the expansion continues. Moreover, there is a need for better understanding of the environmental factors underlying RIFA’s distribution and spread. This study provides an insight into the range expansion of *S. invicta* at the border of its suitable North American habitat and elucidates some of the environmental factors associated with its current and future spread.

6 Figures and Tables

Figure 2.1: Red imported fire ant mounds in Chesapeake Virginia. Photos by Morgan Malone

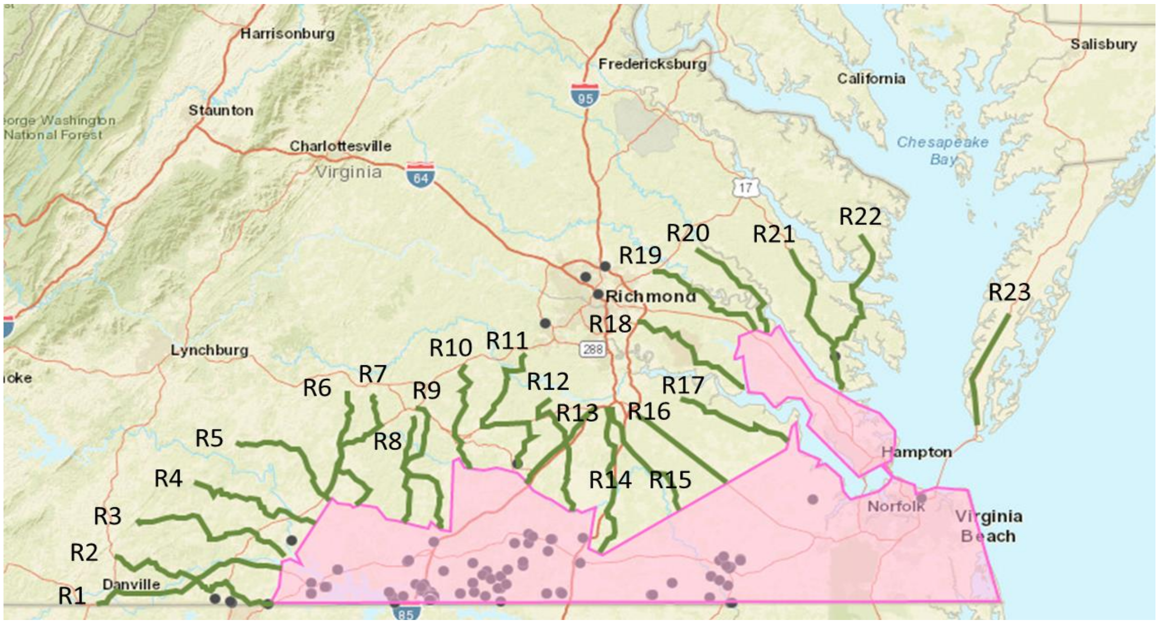

Figure 2.2: Roadside survey ArcGIS Online map (Esri, 2020) with World Street basemap (Esri, 2009). The pink region represents Virginia’s Imported Fire Ant Quarantine zone. Each green line represents a road or conglomerate of roads surveyed. Each black point represents fire ant occurrence from VDACS data 2016-Feb. 2020.

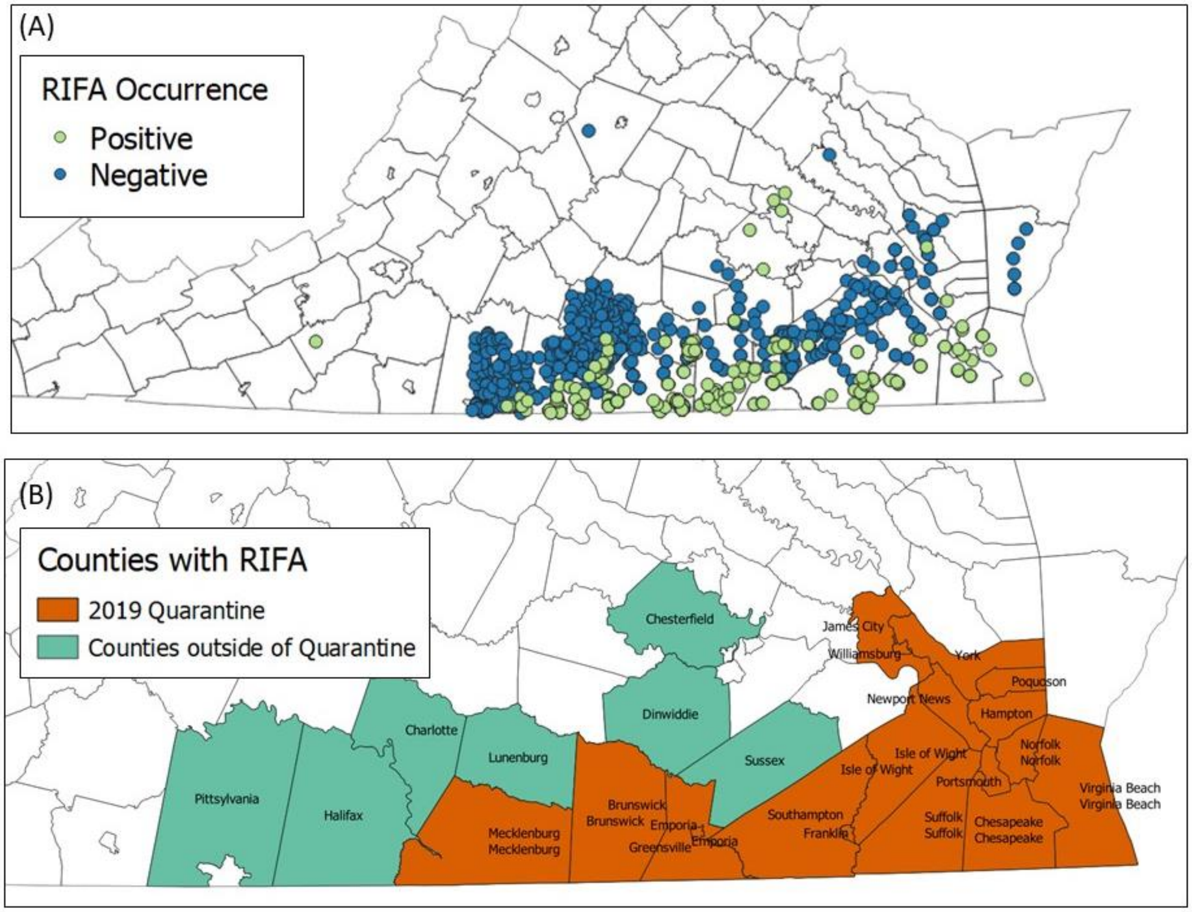

Figure 2.3: Red imported fire ant occurrences in Virginia. A) RIFA presence in Virginia based on data from all survey methods from 2016 to 2021. Light green points represent positive occurrences, dark blue points represent negative occurrences. B) VA counties with RIFA occurrences in 2020 and 2021. Areas in orange represent counties part of the Imported Fire Ant Quarantine, areas in green represents counties outside the Quarantine zone. Maps created using QGIS (QGIS Development Team, 2019).

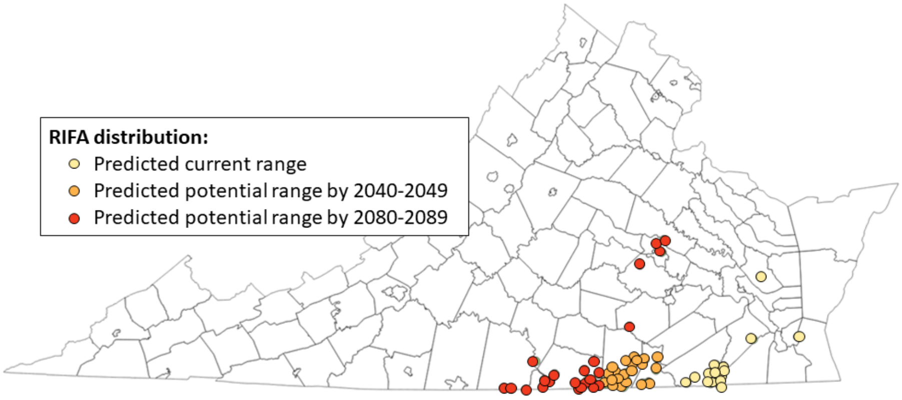

Figure 2.4: Overlay of Virginia current range with model predictions from Morrison et al. 2005. Individual points compared with model results. Each cell represents a 0.5° x 0.5° gride (area varies with latitude).

Table 2.1: Coefficient table for the species distribution model. The square terms are given in bold.

Climate Variable	Point Estimate (upper to lower)	Pr(>|z|)	
	0.00 (0.00 to 0.00)	< 0.001	*
Annual mean temperature	17.56 (9.73 to 31.72)	< 0.001	*
Annual mean temperature^2	**0.93 (0.91 to 0.94)**	**< 0.001**	*****
Maximum temperature	1.08 (0.53 to 2.22)	0.824	
Maximum temperature^2	**1.00 (0.99 to 1.01)**	**0.662**	
Minimum temperature	1.28 (1.18 to 1.39)	< 0.001	*
Minimum temperature^2	**1.01 (1.00 to 1.01)**	**0.032**	*****
Average precipitation	1.00 (1.00 to 1.00)	0.428	
Average precipitation^2	**1.00 (1.00 to 1.00)**	**< 0.001**	*****
Minimum precipitation	1.15 (1.12 to 1.18)	< 0.001	*
Minimum precipitation^2	**1.00 (1.00 to 1.00)**	**< 0.001**	*****

*significance P < 0.05

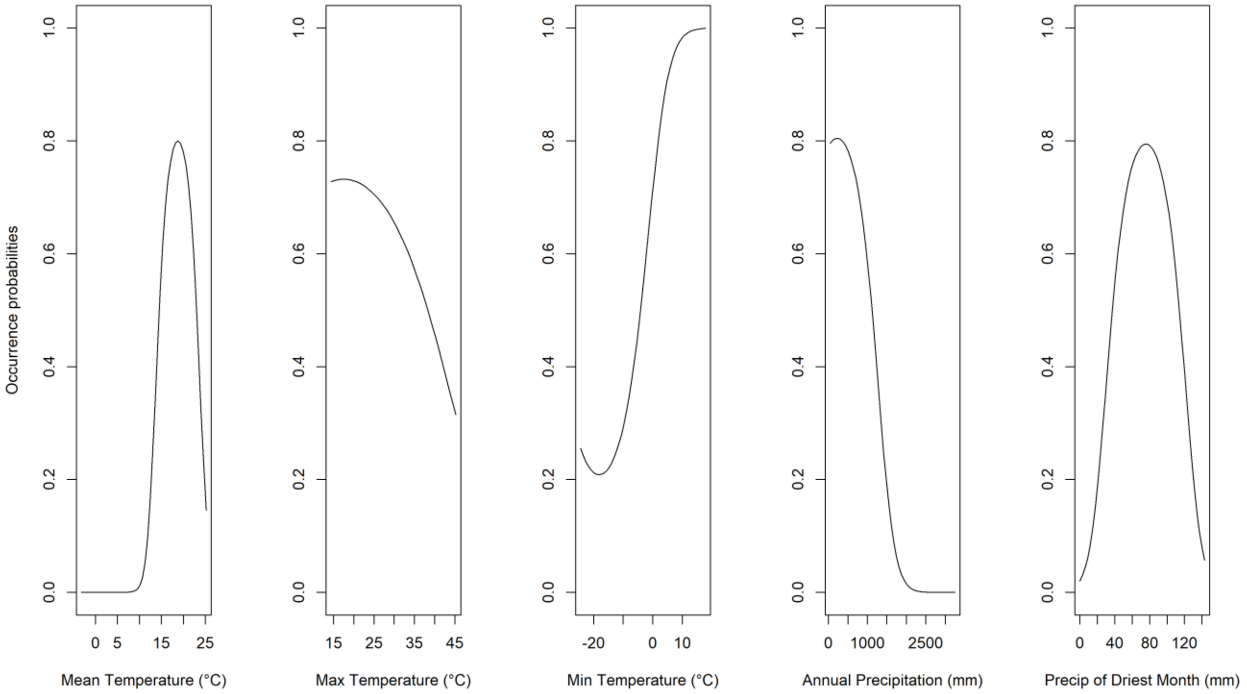

Figure 2.5*:* Partial response curves showing RIFA occurrence probability for each environmental variable.

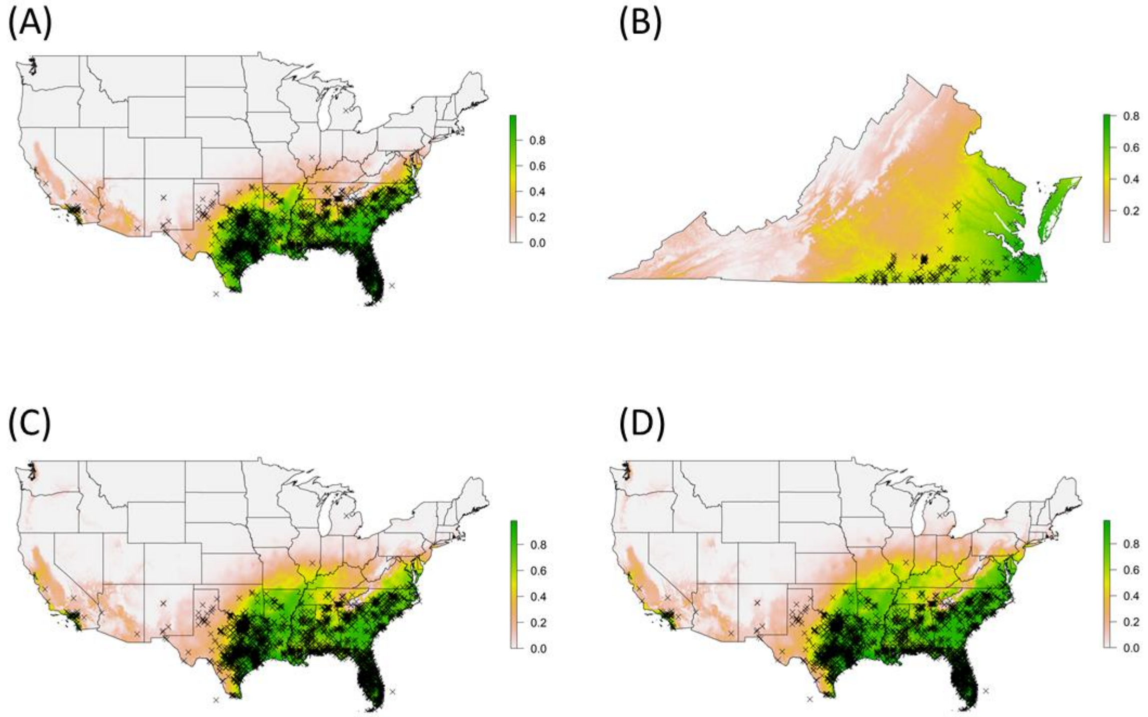

Figure 2.6: Level of risk of *S. invicta* spread in the United States on a continuous scale from 0 to 1 (dark green represents high risk areas, yellow represents mid risk areas, red represents low risk areas, and gray represents no risk areas). Black crosses represent RIFA occurrence data from GBIF and this study. A) Current possible range based on current climate data, B) Current possible distribution in Virginia. Note that the Virginia occurrence data displayed here was not used for prediction, C) Future possible range for 2050 based on future climate data from World Clim, and D) Future possible range for 2070 based on future climate data from World Clim.

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CHAPTER 3: CHRONIC SUB-LETHAL EXPOSURE OF RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*, WORKERS TO THE NEONICOTINOID IMIDACLOPRID LOWERS THEIR ABILITY TO NAVIGATE AND EXPLOIT FOOD SOURCES IN A LABORATORY SETTING

1 Abstract

Neonicotinoids are insecticides commonly used to minimize production costs and crop losses in agricultural settings. However, neonicotinoids have been shown to have harmful impacts on non-target organisms including changes in cognitive function, behavior, and fitness of pollinators. Compared to their effects on pollinators, the unintentional effects of neonicotinoids on ants remain poorly understood. In this study, we examined levels of exposure to common nicotinoids in the invasive *Solenopsis invicta*, the red imported fire ant, and the effects of neonicotinoid dietary exposure on its foraging behavior. We measured the exposure of red imported fire ants to two commonly used neonicotinoids, clothianidin and imidacloprid, in an agricultural setting in southeastern Virginia. We found detectable levels of both compounds in the soil of the ant mounds as well as in the ants themselves. In addition, we tested unexposed and imidacloprid-exposed colonies, after two and three weeks of treatment, regarding the time it took foragers to discover a food source and their ability to navigate a raised maze in a laboratory setting. Imidacloprid-exposed and control colonies did not differ in their time to food discovery in the initial trial. However, control colonies were able to locate the food source more quickly during the second trial while exposed ants were unable to improve their performance. We also found that more imidacloprid-exposed ant workers were unable to successfully navigate (i.e., fell off) the maze as compared to untreated workers. Our results suggest impaired learning of maze tasks and impaired navigational skills in neonicotinoid-exposed ants.

2 Introduction

Neonicotinoids are a common group of insecticides often used in agricultural and urban landscapes (Jeschke et al., 2011). Neonicotinoids target receptors in insects' nervous system (Brown et al., 2006) and therefore can impair cognitive function and alter behaviors such as foraging (Gill et al., 2012; Henry et al., 2012), learning and memory (Stanley et al., 2015; Williamson & Wright, 2013) and social behavior (Boff et al., 2018). There is evidence that neonicotinoids have left persistent residue in the soil and waterways in and around agricultural areas (Bonmatin et al., 2015). While these insecticides are often relied on to control pest species, especially in food production, they also cause harmful effects to non-target species (Pisa et al., 2014) and have been identified as a driver of insect declines (Hallmann et al., 2014; Sánchez-Bayo & Wyckhuys, 2019). Exposure to neonicotinoids, even at low concentrations, has been demonstrated to alter the behavior of many arthropod taxa (Desneux et al., 2007; Pisa et al., 2014; Tappert et al., 2017; Thorne & Breisch, 2001).

Ants play an important ecological and economic role in terrestrial environments (Holldobler & Wilson, 1990) and invasive ants are considered some of the world's worst invasive species (Lowe et al., 2000). The red imported fire ant, *Solenopsis invicta*, (Hymenoptera: Formicidae) is invasive across much of the southeastern United States, recently including Virginia (Miller & Allen, 2019). While neonicotinoid baits are often used to control invasive ants (Rust et al., 2004), there are also likely unintentional exposure routes to neonicotinoids for ants including persistency in the soil and water (Bonmatin et al., 2015; Pisa et al., 2014). In addition, ants may be exposed to neonicotinoids through their diet. Many ant species consume the excretion product (honeydew) of sap feeding insects (Wilder et al., 2012) and neonicotinoid residues have been found in their honeydew excretion (Calvo-Agudo et al., 2019). Ants may also feed on crop seeds treated with neonicotinoids (Morrison et al., 1999; Thompson et al., 2020). The many potential exposure routes make it difficult to trace neonicotinoid exposure in ants. While field exposure to neonicotinoids in bees has been quantified multiple times (Alkassab & Kirchner, 2017; Bonmatin et al., 2015; Tsvetkov et al., 2017), field realistic exposures to neonicotinoids in ants has not been extensively quantified (Schläppi et al., 2021).

Pollinators, specifically honey bees, have been the primary focus of behavioral research on the sublethal effects of neonicotinoids (Henry et al., 2012; Rundlöf et al., 2015; Whitehorn et al., 2012). Neonicotinoids have been found to lower the foraging success of honey bees due to impaired homing success (Henry et al., 2012), reduce bumble bee colony growth and reproduction (Whitehorn et al., 2012), and decrease wild bee nesting activity (Rundlöf et al., 2015). Only a few studies have examined the impact of sublethal exposure of neonicotinoids on ants, however, these studies have demonstrated a wide range of behavioral effects (Barbieri et al., 2013; Galvanho et al., 2012; Thiel & Köhler, 2016; Wang et al., 2015). Barbieri *et al.* (2013) found that sublethal neonicotinoid exposure had different impacts on the foraging and interspecific interactions of a native ant, *Monomorium antarcticum*, and an invasive ant, *Linepithema humile*. A study methodologically similar to Barbieri *et al.* (2013) found that sublethal exposure to imidacloprid reduced foraging activity in *Lasius niger* greater than in *Lasius flavus* (Thiel & Köhler, 2016). Wang *et al.* (2015) found that sublethal exposure of imidacloprid suppressed feeding, digging, and foraging behavior in *Solenopsis invicta*. These studies suggest that sublethal exposure to neonicotinoids may have complex and varying effects on ant behavior.

In this study, we quantified the amount of neonicotinoid residue in soil samples and in *Solenopsis invicta* workers to determine field-realistic levels of exposure. We also examined the effect of neonicotinoid exposure on *S. invicta* behavior via laboratory foraging experiments.

Foraging behavior is of particular interest because foraging efficiency of *S. invicta* relies on their ability to exploit a food source quickly and therefore outcompete other species for the resource (Wilder et al., 2012). Specifically, we tested whether chronic exposure to neonicotinoids has the potential to alter behaviors relevant to the invasion success of this species. *S. invicta* is considered one of the world's worst invasive species due to its detrimental impacts to ecosystems, economies, and human health (Lowe et al., 2000) and such data have the potential to provide an insight into the individual and colony responses of *S. invicta* to environmental residues.

3 Methods

3.1 Determining field-realistic exposure to neonicotinoids in agricultural fields

To determine the field-realistic exposure to common neonicotinoids we collected ant and soil samples from field sites at the Tidewater Agricultural Research & Extension Center (TAREC), in Suffolk, Virginia during the summer of 2021. The TAREC conducts research on common row crops grown in southeastern Virginia including corn, cotton, peanuts, and soybeans. Additionally, neonicotinoids are used as a pest management tool both as a seed dressing and broadcast applications. We collected control samples from South Quay Sandhills Natural Area Preserve, in Suffolk, Virginia where neonicotinoids are not used.

At South Quay, control *Solenopsis invicta* samples were collected on June 28, 2020. At TAREC, samples were collected on May 19 and June 01, 2021. We collected soil samples from fields planted with seed treatments of clothianidin and imidacloprid (Figure 3.1). Approximately 10 samples were collected randomly throughout each field from the top 6 inches of the soil profile using a hand trowel. We removed plant debris from the samples, then combined the samples together. We also collected samples from two mounds both located along a drainage ditch within 5 m of the sampled agricultural fields that used clothianidin and imidacloprid as a seed treatment (Figure 3.1). At each of the two mounds, we collected *S. invicta* workers using a shop-vacuum and soil using a hand trowel. Samples were placed into a paper bag and stored at 20°C. Collection equipment was washed with soap and water between sampling events.

To measure neonicotinoid residue, the ant and soil samples were transported to the Xia Lab at the Virginia Tech School of Plant and Environmental Sciences. During transportation, the samples were stored in an ice filled cooler and freeze-dried upon arrival. The samples were tested for the presences of two neonicotinoids, clothianidin and imidacloprid, using Liquid chromatography-mass spectrometry/ mass spectrometry method (LC-MS/MS). Concentrations are reported in ng of neonicotinoid per g of sample (ppb).

3.2 Foraging effects of sublethal exposure to Imidacloprid

3.2.1 Study Species

We collected *Solenopsis invicta* colonies (n=10) with permission from the Virginia Department of Conservation and Recreation at South Quay Sandhills Natural Area Preserve, in Suffolk, VA, where insecticides are not used. Colonies were extracted using the methods presented in Banks et al. (1981) and Chen (2007). Each colony consisted of at least one queen, approximately 150 workers, and brood. Colonies were housed in plastic boxes (13" x 7-1/2" x 4-1/4") at the Tidewater Agricultural Research and Extension Center, under standard laboratory conditions (23-25°C, 12-hour light/dark cycle). Each colony box had a plaster of Paris floor, a nest area made from an upside-down petri dish covered with paper towel, and a water source. Colonies were fed a 2 M sugar water solution via a soaked cotton ball and mealworms cut in half. The food source was replaced 3 times a week.

3.2.2 Insecticide, Dosage, and Randomization

To test the impacts of sublethal contamination of neonicotinoids on foraging behavior, we used chronic dietary exposure to imidacloprid (IMD; > 99.9% purity, Sigma-Aldrich, St. Louis, MO). Due to the widespread use across many different crops IMD is a likely cause of sublethal exposure to neonicotinoids in many insect species including ants (Jeschke et al., 2011). Colonies were randomly assigned to one of two treatments: no exposure to IMD (n=5) or chronic dietary exposure to IMD (n=5). After a one-week acclimation period, unexposed colonies were fed a 2 M sugar water solution and exposed colonies were fed a 2 M sugar water solution containing 1.25 μg/ml IMD for 2 weeks and 3 weeks. Methods were adapted from Thiel and Köhler (2016).

Solutions were refreshed every 2 days. The IMD concentration used in this study corresponds to field application rates for crop protection (Jeschke et al., 2011). Due to delays caused by the COVID-19 pandemic, our own concentration measurements were not yet available to inform treatment decisions. All randomization was conduction via a random number generator.

3.2.3 Foraging Bioassay

To assess the impacts of sublethal exposure to IMD on *S. invicta* foraging activity we examined the recruitment and exploitation time of the unexposed and exposed colonies after 2 weeks (trial 1) and 3 weeks (trial 2) of chronic exposure. We measured foraging activity through food discovery time and the number of ants foraging over a 2-hour period (feeding, exploring, and walking speed).

After the initial 2-week exposure time, all food sources were removed from the nest box for 24 hours. After the starvation period, each nest box was given access to a foraging maze (Czaczkes, 2018). The maze was constructed from 3D printed structures designed by Dr. Tomer J. Czaczkes and followed a similar layout as that of Thiel and Köhler (2016) (Figure 3.2). The base of each maze was filled with soapy water to prevent ants from escaping. At the start of each experiment each maze was washed, and the paper overlays were replaced. A 2 M sugar water solution was randomly placed at one of two terminal ends (A or B in Figure 3.2). All experiments took place during the 12-hour light period (7 AM to 7 PM) and colonies were tested in a random sequence over 2-3 days. After all foraging experiments were conducted, exposed/ unexposed solutions were replaced, and the experiment was repeated one week later (trial 2).

3.2.4 Measurements

At the start of each foraging period, we counted the number of workers outside of the nest (baseline active workers). We then connected each colony box to the foraging maze with a bridge and ants were allowed to forage for 2 hours. In situ, we recorded the time in minutes to locate the food source and the number of ants at the food source, the number of ants on the maze, and walking speed every 10 minutes. At each recording point we also took a picture of the maze as a backup recording method. Walking speed was measured by how long it takes for an ant to walk across a 20 cm segment of the maze towards the food source. Walking speeds were recorded for 3-4 ants, when possible, during each 10-minute period. At the end of the 2-hour foraging period we counted the total number of ants that fell into the surrounding water (drowned ants). The number of drowned ants was used as a proxy for lost navigational skills and compromised ability to navigate hazardous environments.

3.3 Statistical Analyses

As we were interested in the presence/ absence of neonicotinoid exposure in ants in natural environments, we present the raw data from the soil and ant samples. All data from the behavioral experiments were analyzed using R version 4.0.5 (R Core Team, 2021). Significance was set to $P < 0.05$ for all tests and when possible, point estimates and two-sided 95% confidence intervals are also presented. For the time to finding the food source variable, we present the median and the lower and upper quartiles.

Regarding the behavioral experiment component of our study, we focused on the following: 1) if the treatment had an effect on food discovery time at the colony level, and 2) how many ants were unable to navigate the maze and fell into the surround water; a proxy of potentially lost navigational skills. We used a time-to-event (or survival) analysis to model how long it took ants to discover food with the packages *survival* (Therneau, 2021) and *frailtypack* (Rondeau et al., 2012) in R. Because colonies were tested twice, we use a survival model that allows for shared frailty within colonies to account for the non-independence of the two runs per colony. Similarly, we used a generalized mixed model (Poisson with log-link) to model the number of ants that had drowned, with random intercepts for the colonies using the *glmer* function in the *lme4* package (Bates et al., 2015) in R. In both cases, our predictor of interest was ants exposed to IMD.

Model specifications were made prior to running the analyses. Because there might be learning effects or effects of chronic exposure for the second trial, we modeled the exposure (IMD vs. control) as an interaction with trial (Exposure × Trial interaction). As both the time to finding food and the number of ants falling off the maze may be a function of how many ants were on the maze at the start of the trial, we adjusted our models by adding the baseline number of active ants as a fixed effect. These primary analyses were conducted by RS while blinded to the treatment levels. MM blinded all treatments by randomly labeling exposure factor levels as A or B. After the primary analysis, RS was unblinded while conducting the exploratory analysis.

In addition to the primary analysis, we also examined how the number of ants that exploit the food source changed over time, and how many ants were active on the Y-maze over time. We calculated pointwise geometric means and 95% confidence intervals for the number of ants on the maze, the number of ants at the food source, and ant speed for each time step these variables were measured. We then qualitatively described the observed patterns.

4 Results

4.1 Neonicotinoid concentrations in field soil, red imported fire ant, and their mounds

All samples from South Quay NAP tested negative for clothianidin and imidacloprid. At TAREC, clothianidin was detected in the soil from the corn field (0.731 ppb), cotton field (0.534 ppb), mound 1 (detectable <=but lowest std), and mound 2 (0.409 ppb) for the first set of samples collected on May 19, 2021. Imidacloprid was detected in the soil from the corn field (5.971 ppb) and cotton field (1.113 ppb), but not in the soil from mounds 1 and 2. Clothianidin was detected in the soil from the corn field (0.488 ppb) and cotton field (0.639 ppb), but not from mounds 1 and 2 for the second set of samples collected on June 01, 2021. Imidacloprid was detected in the soil from the corn field (4.398 ppb) and cotton field (3.463 ppb), but not in the soil from mounds 1 and 2 (Table 3.1). Neither of the first set of ant samples from mounds 1 and 2 had detectable levels of clothianidin or imidacloprid. Both clothianidin (4.502 ppb) and imidacloprid (detectable but lowest std) were detected in the second set of ant samples from mound 2. Four weeks after planting there was no ant activity at mound 1, therefore samples could not be collected (Table 3.1).

4.2 Foraging effects of sublethal exposure to Imidacloprid

4.2.1 Untreated but not treated colonies shortened time to food discovery in second trial

All colonies discovered the food source during the allotted 2 hr. foraging period. In trial one, the median time to food discovery was the same for both the control and treated colonies (median [upper quartile; lower quartile] time to food discovery - controls: 5.0 [5.0; 7.0] minutes; treatment: 5.0 [4.0; 6.0] minutes). During the second trial, it took the controls less than half that time to discover the food source, whereas the treated colonies took longer to locate the food (median time to food discovery - controls: 2.0 [2.0; 4.0] minutes; treatment: 6.0 [3.0; 6.0] minutes). Overall, this resulted in a significant interaction between treatment and trial. As a result, while there was no difference between the control and exposed colonies in the chance to discover food at any given time during trial 1, untreated colonies had a higher chance of finding the food at any given time during trial 2 (hazard ratios; trial 1 hazard ratio (treatment / control): 1.71 (0.47 to 6.25), p = 0.42; trial 2 hazard ratio (treatment / control): 0.11 (0.02 to 0.79), p = 0.0282; see Figure 3.3).

4.2.2 More ants of IMD exposed colonies fell off the maze during exploration

Overall, at the end of the foraging period ants from colonies exposed to IMD had more than two and a half times the number of ants falling off the maze as compared to ants from untreated colonies (control mean: 5.68 (3.16 to 10.20); treatment mean: 15.15 (8.78 to 26.15); mean ratio (treated / control): 2.67 (1.20 to 5.94); p = 0.0162). However, control colonies had approximately double the numbers of ants falling off the maze during the second trial as compared to the first trial (mean trial 1: 3.87 (1.99 to 7.56); mean trial 2: 8.32 (4.57 to 15.14); mean ratio (trial 2 / trial 1): 2.15 (1.14 to 4.06); p = 0.0108). This was not the case with the colonies exposed to IMD (mean trial 1: 14.32 (8.13 to 25.22); mean trial 2: 16.04 (9.12 to 28.20); mean ratio (trial 2 / trial 1): 1.12 (0.76 to 1.65); p = 0.876). This interaction meant that while exposed colonies lost significantly more ants to falling off the maze in trial 1 (mean ratio (treated / control): 3.70 (1.18 to 11.60); p = 0.0175), this difference was not significant in trial 2 (mean ratio (treated / control): 1.93 (0.65 to 5.68); p = 0.401; Figure 3.4).

4.2.3 Minimal differences in number of ants on maze and number of ants at food source, but decreased ant walking speed in IMD exposed ants

Ants from treated and untreated colonies did not differ from one another regarding the number of ants on the maze, the number of ants at the food source, or walking speed. Naturally, there were no ants on the maze or at the food source at time 0. This number then increased in both groups as ants began to explore the maze. Generally, exposed colonies were slightly more active after 30 minutes, although point-wise confidence intervals are rather wide (Figure 3.5). Similarly, the number of ants at the food source increased after discovery, but then fluctuated in both groups (Figure 3.5). Ant walking speed was faster in the control ants during trial 1, as evidenced by the higher geometric means of the control group as compared to the exposed colonies. However, the point-wise confidence intervals are also wide, and during the second trial mean walking speed was faster at 8 of the 11 timepoints in control ants (Figure 3.5).

5 Discussion

We found detectable exposure of ants to neonicotinoid pesticides in an intense agricultural setting. In a laboratory setting, we demonstrated that chronic consumption of imidacloprid has a stark effect on the foraging efficiency of *S. invicta*. These results suggest that insecticide residue may alter the foraging behavior of recent arrivals such as the red imported fire ant with potential implications for their invasion ecology.

5.1 Neonicotinoid concentrations in soil from fire ant territories and in ants

We found residue of clothianidin and imidacloprid in soil from agricultural fields that use these neonicotinoids as seed and in-furrow treatments. Furthermore, we found residue of clothianidin in the soil taken directly from the fire ant mounds during the first set of sampling, but not two weeks later during the second sampling event. Imidacloprid was not detected in the soil from the ant mounds in either of the two sampling events. (Table 3.1). Our findings are consistent with the published literature. Neonicotinoids have been documented to be persistent at variable concentrations in soils (Thompson et al., 2020). For example, a study investigating imidacloprid concentrations in farm soil in France found 59% of farms sampled contained over 1 ppb, and soil concentrations were higher after two years of treatment than after one year (Bonmatin et al., 2005). In soils planted with seed treatments of clothianidin in Ontario, concentrations ranged from 0.53 to 38.98 ppb immediately after planting (Schaafsma et al., 2015). Neonicotinoids generally have long half-lives in the soil which is affected by soil type, moisture, temperature, pH, and ultraviolet radiation (Thompson et al., 2020). For example, the reported half-life of clothianidin in the soil is 148–6931 DT50 and is 100–1250 DT50 for imidacloprid (Thompson et al., 2020).

In addition, we detected both clothianidin and imidacloprid in our ant samples. To our knowledge, this is the first study to demonstrate that environmental contamination in agricultural settings ultimately percolates through to higher trophic levels. One potential pathway for ant exposure to neonicotinoids may be via trophic transfer (plant to pest to enemy). For example, neonicotinoids are often used to target phloem-feeding insects such as aphids (Jeschke et al., 2011). Insects that survive this treatment excrete honeydew that contains residue of neonicotinoids (Calvo-Agudo et al., 2019), which then may be consumed by fire ants. Alternatively, fire ants may feed directly on coated seeds (Morrison et al., 1999). Our study, however, did not focus on the factors leading to the high concentration of clothianidin and detectable levels of imidacloprid we observed in *S. invicta*. Exposure may vary due to environmental conditions such as rain and alternative food sources (Thompson et al., 2020). In our specific case, there was only one rain event occurring a few days prior to the second sampling. We also could not confirm if ants were only foraging in the closest fields or at other locations. Fire ant territories can range from 12 to 197 m^2 in size (Adams, 2003). Despite all these limitations, our data confirm that fire ants are likely to be exposed to neonicotinoids in southeastern Virginia agricultural fields.

While *S. invicta* mounds were located in close proximity to the agricultural fields, we were unable to find fire ant mounds within the fields. The soil properties between the area where the mounds are located and the fields varies due to the way the soil is manipulated. The soil directly within the fields is subject to tillage, whereas the soil around the fields is subject to compaction from the movement of equipment. Soil properties affect the half-life and movement of neonicotinoids (Thompson et al., 2020) and the changes in soil properties between the fields and mounds likely have an effect on the way neonicotinoids move through the soil. Neonicotinoids are water-soluble and can runoff into waterways after rainfall events (Thompson et al., 2020).

5.2 Foraging effects of sublethal exposure to Imidacloprid

All colonies were able to discover the food source and recruit nestmates within a fraction of the upper time limit of our trials. However, our results suggest that ants exposed to imidacloprid were impaired in two crucial ways during foraging. First, unexposed ants discovered the food source faster during the second trial as compared to the first, whereas exposed ants did not improve their time to food discovery (Figure 3.3). Second, ants lost their way more often when exposed to neonicotinoids and fell off the maze more frequently (Figure 3.4). In a real-world scenario, these two impairments might affect overall foraging performance. As the exploitation of food resources is an important factor in the invasive success of *S. invicta* (Holway et al., 2002), any changes in foraging efficiency may alter the ecological impacts of this species.

We suggest three non-mutually exclusive scenarios regarding the patterns of time to food discovery we observed. First, chronically exposed colonies may have impaired learning and memory. Most ants use a combination of pheromone trails and route learning to exploit a food source (Czaczkes et al., 2013; Ozaki et al., 2005). As neonicotinoids act on specific neuronal pathways (Williamson & Wright, 2013), they are likely to have different effects on different species. Previous studies have documented that sublethal exposure to neonicotinoids can alter ant behavior depending on species, dosage, and exposure route (Barbieri et al., 2013; Galvanho et al., 2012; Penn & Dale, 2017; Thiel & Köhler, 2016). Neonicotinoid exposure in bees has been previously shown to impact learning and memory (Stanley et al., 2015; Williamson & Wright, 2013). Second, ants of exposed and unexposed colonies may differ in their baseline activity levels (due, for example, to the additional week of exposure in the second trial). Imidacloprid exposure has been demonstrated to impair locomotion but not foraging capacity in *Tetramorium caespitum* (Penn & Dale, 2017). Our data suggest that ants from the control treatment may be faster, although this pattern manifested itself more strongly during the first trial (see Figure 3.5). Third, our treatment may not have been completely sublethal and could have led to differential mortality between the treatment groups. In turn, colony memory might have been lost between the first and the second trial because exposed foragers died at a higher rate. Although we did not quantitatively assess the differential mortality between the two treatment groups, we suggest that this is an unlikely scenario as treatment groups did not appear to differ in mortality rates between the two trials.

Imidacloprid-exposed ants seemingly had a harder time navigating the maze without falling off, which may be a result of impaired cognitive abilities or impaired motor skills. In studies using similar experimental designs, sublethal exposure to imidacloprid did not significantly affect the rate at which ants fell off a maze (Barbieri et al., 2013; Thiel & Köhler, 2016). Our data on walking speed suggests a potential impairment of individuals from exposed colonies. During the initial trial, individuals from untreated colonies moved faster as compared to individuals from exposed colonies at all measured time points, and in the second trial they were faster in 8 out of 11 measured time points. Our results are similar to those of Wang *et al.* (2015) who reported slower walking speed in *S. invicta* workers exposed to imidacloprid.

5.3 Conclusions

Our study demonstrates that neonicotinoid exposure negatively affects the foraging ability of *S. invicta*. To fully understand the unintentional effects of neonicotinoids on an ecosystem, it is important to look at all species, including invasive ones. In addition, we found that ants are exposed to neonicotinoid pesticides in agricultural settings. Although the degree of exposure and possible exposure routes are unclear, our study shows that imidacloprid at sublethal concentrations impacts the foraging activity of fire ants. Even small changes in activity may be biologically relevant.

These results suggest that human activity may alter the invasion ecology of recent arrivals such as the red imported fire ant.

6 Figures and Tables

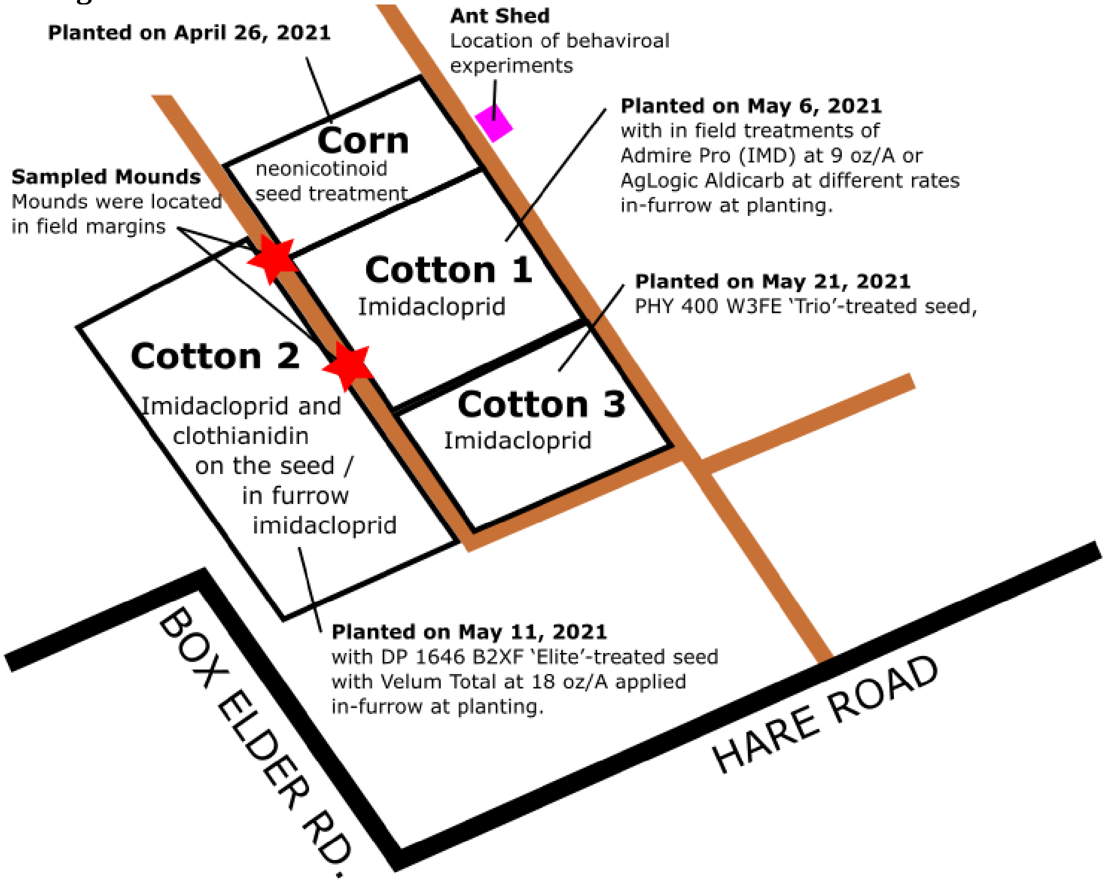

Figure 3.1: Map of sampling locations at Tidewater AREC, Suffolk, VA. Star symbol represents the location of the fire ant mounds. Corn planted on April 26, 2021, with neonicotinoid (application rate NA) seed treatment. Cotton 1 planted on May 6, 2021, with in field treatments of Admire Pro (imidacloprid) @ 9 oz/A or AgLogic Aldicarb at different rates in-furrow at planting. Cotton 2 planted on May 11, 2021, with DP 1646 B2XF 'Elite'-treated seed (contains imidacloprid and clothianidin on the seed), with Velum Total (contains imidacloprid) at 18 oz/A applied in-furrow at planting. Cotton 3 planted on May 21, 2021, with PHY 400 W3FE 'Trio'-treated seed (includes imidacloprid on the seed).

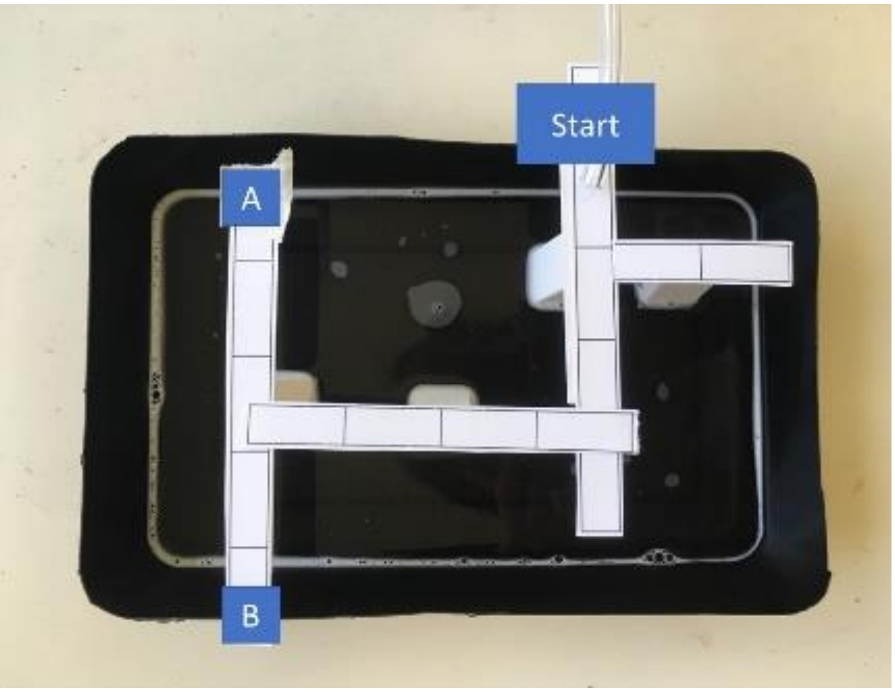

Figure 3.2: Foraging maze. A bridge connected to the nest box was placed at the start of each foraging trial. A food source was randomly placed at A or B. The base was filled with soapy water to simulate habitat complexity and prevent escaping.

Table 3.1: Concentration (ppb, ng/g) of clothianidin (CLO) and imidacloprid (IMD) from freeze-dried soil and ant samples from May 19 (first sample collection) and June 01 (second sample collection).

	CLO (ppb)		**IMD (ppb)**	
	May 19	*June 1*	*May 19*	*June 1*
Ants				
Mound 1	ND	-	ND	-
Mound 2	ND	4.502	ND	detectable[1]
Soil				
Mound 1	detectable[1]	ND	ND	ND
Mound 2	0.409	ND	ND	ND
Corn	0.731	0.488	5.971	4.398
Cotton	0.534	0.639	1.113	3.463

[1] detectable at lowest std

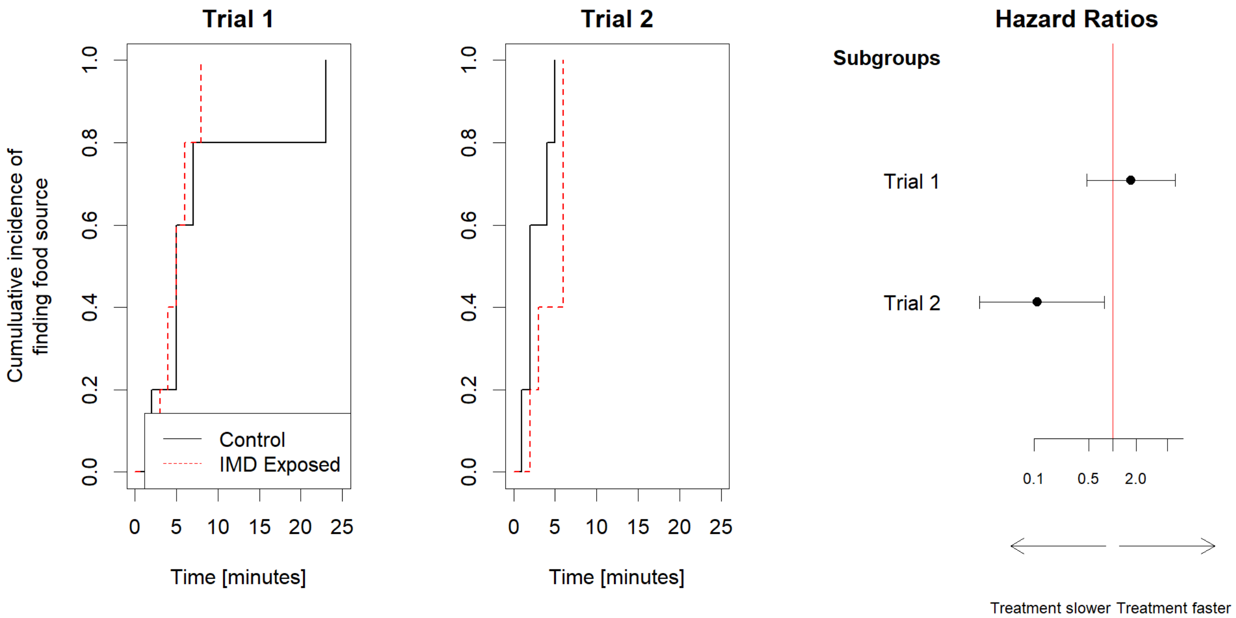

Figure 3.3: Time to food discovery for treated (red) and control (black) colonies and hazard ratios (treatment / control) for trials 1 and 2.

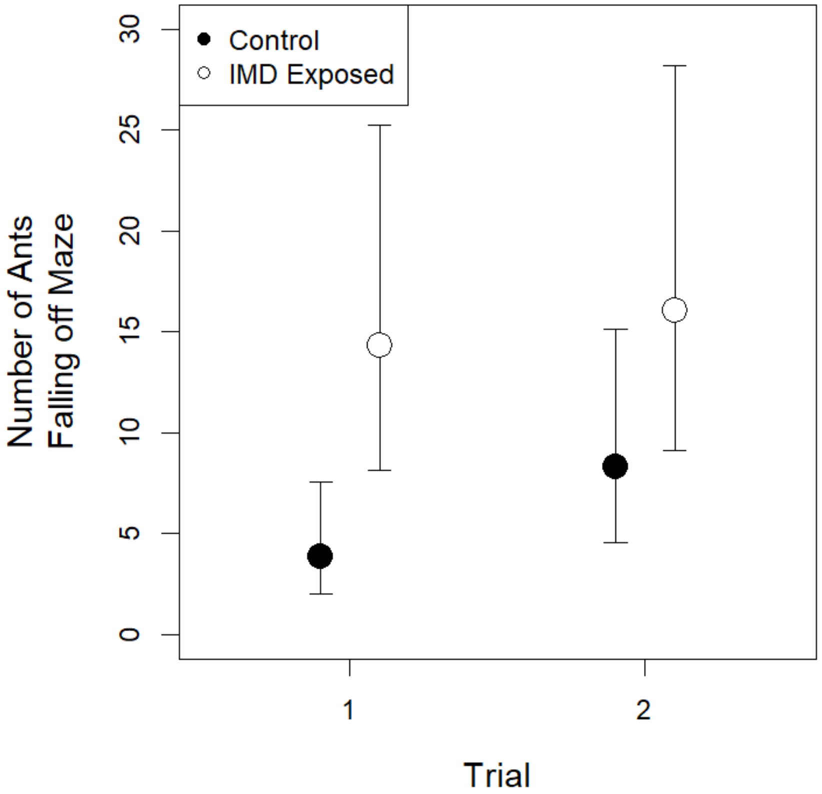

Figure 3.4: Number of ants that fell of the maze into surrounding water (drowned) for controls (black circles) and treatment (open circles) for trial 1 and 2.

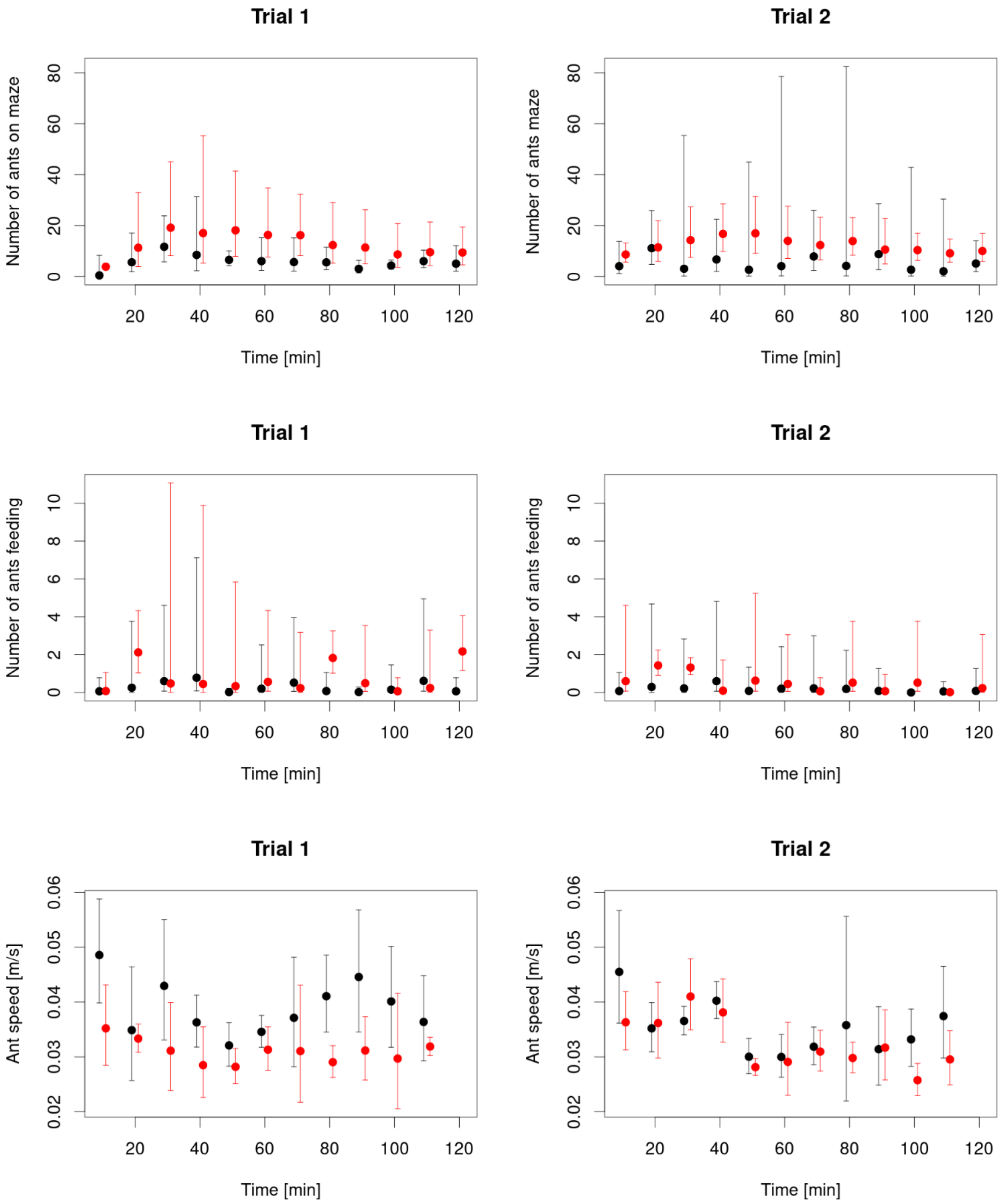

Figure 3.5: Number of ants on the maze and at the food source, and ant walking speed over time. Shown are geometric means (point-wise 95% confidence interval). Black is control, red is treatment.

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CHAPTER 4: FINAL DISCUSSION

The red imported fire ant, *Solenopsis invicta* Buren, is a notoriously invasive species throughout the southern United States (Lowe et al., 2000). Our study explored the current and potential range of fire ants in Virginia and the United States as well as the factors affecting their spread. Understanding how fire ant invasion is affected by anthropogenic disturbances, such as neonicotinoid exposure, can lead to better management practices. Additionally, it will lead to more knowledge on the environmental impacts of these insecticides on ants (Schläppi et al., 2021).

Results from road and public fire ant surveys confirmed that the red imported fire ants is established outside of the current Imported Fire Ant Quarantine in Virginia. RIFA surveys primarily relied on roadside surveys, which are time consuming yet critical for early detection of introduced ant populations (Lodge et al., 2006). Additionally, we created a species distribution model to estimate the possible distribution of the red imported fire ant under current and future climate projections. While species distribution models rely on underlying assumptions, they are useful tools for managing the spread of invasive species (Miller, 2010). Our model shows that the suitable habitat for fire ants is likely to increase under future climate projections especially if current conditions such as global temperature warming due to anthropogenic climate change and increases in urbanization continue.

In our study on the impact of neonicotinoid exposure on fire ant behavior, we found that sublethal exposure to imidacloprid can alter the foraging activity of red imported fire ants. While all colonies were able to find the food source and recruit nestmates, imidacloprid-exposed colonies took longer to find the food source during the second trial and were more likely to drown during exploration. Exploiting a food source is an important factor in the invasive success of fire ants, and changes in behavior may alter their ecological impacts. Neonicotinoids have been shown to negatively affect the navigation of honeybees (Fischer et al., 2014). In our study, once ants discovered the food source, all colonies returned to the food source and recruited nestmates regardless of exposure. Previous studies have document that sublethal exposure to neonicotinoids can alter ant behavior at different concentrations (Barbieri et al., 2013; Galvanho et al., 2013).

To our knowledge, this study is the first to provide a field-realistic measure of neonicotinoid exposure to RIFA in southeastern Virginia cropping systems. This study shows that imidacloprid and clothianidin persists in the soil, even one month after planting, which may lead to long term exposure. In this study, we only measured soil exposure and did not consider other possible unintentional exposure routes such as foliage, water, and diet. Future research is needed to explore exposure routes in greater detail.

Due to limited time (COVID-19 pandemic lockdowns), we had to conduct foraging experiments before our results from the neonicotinoid analysis were ready. Rather we used a concentration rates reported in previous studies (Barbieri et al., 2013; Thiel & Köhler, 2016). The distribution of red imported fire ants is linked with anthropogenic activities such as agriculture (King & Tschinkel, 2008). These areas often use neonicotinoids as a pest management tool and fire ants may come in contact with them through various pathways (Schläppi et al., 2021). This study only examined short-term exposure of worker ants to neonicotinoids. However, colonies may experience long-term exposure due to sedentary colonies and queens with long life spans (Schläppi et al., 2021). A recent study found that long-term exposure to neonicotinoids reduced colony size in *Lasius niger*, but was only noticeable during the second year (Schläppi et al., 2020). In the long run, even low concentrations of neonicotinoids may have subtle effects with lasting effects (Schläppi et al., 2020). However, the effects of long term exposure on soil insects is still poorly understood (Schläppi et al., 2020).

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APPENDIX A

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