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Impact of native vegetation cover near crops on the occurrence and molecular diversity of fire ants

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Abstract

Solenopsis invicta and *Solenopsis saevissima* are the most common fire ants in Brazil. Environmental disturbances favour the colony dispersal of both species, particularly those with an anthropogenic origin. However, the frequencies of the species can vary according to the amount of anthropogenic disturbances. Crops near native vegetation fragments is a common landscape in the Brazilian Atlantic Forest. Here, we analyse if there is influence of native vegetation cover on the occurrence of these fire ants and their molecular diversity in crops. Our hypothesis is that these species are antagonistic in terms of occurrence and molecular diversity when compared in the same habitat. We selected crops near fragments with around 50% of native vegetation cover, a percentage informative enough to detect biological responses from insects belonging to a same order. Nests were collected and the species were identified using external morphology, and mtDNA COI sequences. We reject our hypothesis, which suggest that the species colonize the vegetation patches around crops in a similar way. *Solenopsis invicta* is not limited by vegetation cover, and has a high haplotype diversity when compared to *S. saevissima*, particularly when the vegetation cover is greater than 50%. Additionally, the vegetation cover seems to not have influence in the total number of haplotypes, but the species have haplotypes that are exclusive to each landscape. Our findings suggest that *S. invicta* is expanding to native vegetation areas that it has not occupied before, as *S. saevissima* was dominant there.

Resumen

Solenopsis invicta y *Solenopsis saevissima* son las hormigas de fuego más comunes en Brasil. Las perturbaciones ambientales favorecen la dispersión de colonias de ambas especies, particularmente de aquellas de origen antrópico. Sin embargo, las frecuencias de las especies pueden variar según la cantidad de perturbaciones antropogénicas. Los cultivos cerca de fragmentos de vegetación nativa es un paisaje común en la Mata Atlántica brasileña. Aquí analizamos si existe influencia de la cobertura vegetal nativa en la ocurrencia de estas hormigas de fuego y su diversidad molecular en los cultivos. Nuestra hipótesis es que estas especies son antagónicas en términos de ocurrencia y diversidad molecular cuando se comparan en el mismo hábitat. Seleccionamos cultivos cercanos a fragmentos con alrededor del 50% de cobertura vegetal nativa, porcentaje lo suficientemente informativo como para detectar respuestas biológicas de insectos pertenecientes a un mismo orden. Se recolectaron los nidos y se identificaron las especies usando morfología externa y secuencias COI de mtDNA. Rechazamos nuestra hipótesis, lo que sugiere que las especies colonizan los parches de vegetación alrededor de los cultivos de manera similar. *Solenopsis invicta* no está limitada por la cobertura vegetal y tiene una alta diversidad de haplotipos en comparación con *S. saevissima*, particularmente cuando la cobertura vegetal es superior al 50%. Adicionalmente, la cobertura vegetal parece no tener influencia en el número total de haplotipos, pero las especies tienen haplotipos que son exclusivos de cada paisaje. Nuestros hallazgos sugieren que *S. invicta* se está expandiendo a áreas de vegetación nativa que no ocupaba antes, ya que *S. saevissima* era dominante allí.

KEYWORDS
agricultural landscapes, anthropogenic disturbances, Atlantic Forest, genetic diversity, human-modified landscape, olericulture

INTRODUCTION

Solenopsis (Hymenoptera: Formicidae) comprises 191 species and 22 subspecies of ants (Bolton, 2022). In the Neotropical Region, there are 116 species (Fernández et al., 2021), of which 24 are fire ants (Pitts et al., 2018). Most fire ants are native to South America (Trager, 1991; Pitts et al., 2018) and have a wide geographic distribution (Ascunce et al., 2011; Fernandes et al., 2016; Janicki et al., 2016). Identifying fire ants using morphology is difficult because the genus is highly diverse and there is a high degree of polymorphism in adult workers (Pitts et al., 2005; Pitts et al., 2018) and intraspecific variation in the larvae (Fox et al., 2012).

Solenopsis invicta Buren, 1972 and *S. saevissima* (Smith, 1855) are widely distributed fire ants in Brazil, especially the latter (Fernandes et al., 2016; Pitts et al., 2018; Supplementary material 1a in Figure S1). The first species is found mainly in the Pantanal region and around the headwaters of the Paraguay River, which are areas composed of savannas and seasonally flooded wetlands, but ranges from Porto Velho (Rondônia) to extreme southern Brazil (Pitts et al., 2018). *Solenopsis saevissima* is distributed along the entire Brazilian coast, including the Amazon basin region (Pitts et al., 2018).

The nests of *S. invicta* and *S. saevissima* occur in open, sunny places where workers, eggs, larvae and pupae are in direct contact with the soil (Almeida et al., 2007). Each nest consists of agglomerates of soil particles and has a labyrinth of galleries and chambers for food storage, larval rearing and garbage dumps (Fernandes et al., 2016). These ants have a generalist and opportunistic feeding habit. They look for live or dead invertebrates and small vertebrates (Asano & Cassill, 2012; Maciel et al., 2015), in addition to oily substances and secretions from extrafloral nectaries (Lanza et al., 1993; Le Bellec et al., 2006). Recruitment for foraging is massive and involves many workers (Bueno & Campos-Farinha, 1999). A colony can survive for many years, since the queen lives an average of 8 years and is commonly replaced after death (Tschinkel, 2006). *Solenopsis invicta* and *S. saevissima* have polymorphic castes, and the body length of workers ranges from 1.5 to 5 mm (Pitts et al., 2005; Tschinkel, 2006).

The geographic expansion of *S. invicta* and *S. saevissima* is often influenced by anthropogenic disturbances (Ascunce et al., 2011; Dejean et al., 2015). For example, the fragmentation of native vegetation to expand agricultural activities (Crist, 2009) creates open, sunny patches that these species like to establish (Almeida et al., 2007; King & Porter, 2007), especially in relation to the edge effect (Murcia, 1995). Furthermore, fragmenting native vegetation can cause a decrease in molecular diversity (Collevatti et al., 2020), as seen in vertebrates (Nöel et al., 2007; Björklund et al., 2010) and ant species, such as *Odontomachus rixosus* Smith, 1857 and *Pheidole annexa* Eguchi, 2001 (Bickel et al., 2006).

Agricultural areas are habitats with abiotic (e.g., humidity, temperature, and light [Almeida et al., 2007; Asano & Cassill, 2012; Lebrun et al., 2012; Chan & Guénard, 2019]) and biotic (e.g., food [Calcaterra et al., 2008; Chan & Guénard, 2019]) conditions favourable to *S. invicta* and *S. saevissima*. In addition, managing soil with machinery (e.g., clearing and ploughing [Holdefer et al., 2017; Atchinson et al., 2018; Chan & Guénard, 2019]) and using herbicides and insecticides (Fernandes et al., 2000) make ant colonies split and more readily disperse (Hays et al., 1982; Oliveira & Campos-Farinha, 2005). Thus, crops have characteristics that facilitate colonization by *S. invicta* and *S. saevissima*. Although these species colonize agricultural areas (Ramalho et al., 2022), *S. invicta* is dominant in places with more anthropogenic activity (e.g., without vegetation and with more buildings [Almeida et al., 2007; Gusmão et al., 2010; Ramalho et al., 2022]) and *S. saevissima* is more common in places with vegetation (Dejean et al., 2015; Ramalho et al., 2022). Therefore, does native vegetation cover from fragments around crops influence the number of nests and molecular diversity of *S. invicta* and *S. saevissima*? We hypothesized that the occurrence and molecular diversity of *S. invicta* and *S. saevissima* differ in crops under the influence of fragments with the same percentage of vegetation cover. Due to the geographic distribution of the species and the results of Ramalho et al. (2022), we expected to find more *S. invicta* nests and workers with less molecular diversity at collection sites where vegetation cover is less than 50%. The percentages of vegetation cover we used are based on studies with bees and wasps conducted by Fabian et al. (2013) and Montagnana et al. (2021). Landscapes with over 50% vegetation cover allow for greater richness and ecological interactions in these groups.

MATERIAL AND METHODS

Study area

The collections were made in the Southeast Region of Brazil between September 2015 and March 2017. Due to growing numerous vegetable crops, the region is known as the Paulista Green Belt (*Cinturão Verde Paulista*) (Pagani, 2012). The native vegetation in the region is Dense Ombrophilous Forest within the Atlantic Forest of Brazil (Suguituru et al., 2013). Six vegetable farms were chosen based on the calendar in the Brazilian Catalogue of Vegetables (*Catálogo Brasileiro de Olerícolas*) (Godim, 2010). The farms grow pumpkins, lettuce, beats, broccoli, chives, carrots, kale, cauliflower, spinach, turnips, okra, cabbage and parsley. All the crops (= collection sites) were in five to 10 rows that were 1 to 1.5 m wide and 100 m long, irrigated at least once a day, and had native fragments at a maximum distance of 30 m from the collection sites. Additionally, the collection sites were standardized in relation to use of insecticides, herbicides and synthetic fertilizers.

Vegetation cover

The vegetation cover in the native vegetation fragments near each collection site was measured using a 1 km radius buffer (Supplementary material 2 in Figure S2). This buffer size has been shown to be sufficient for insects for analyses in different types of landscapes (Helms, 2018; Corro et al., 2019; Ahuatzin et al., 2021; Montagnana et al., 2021). Additionally, this buffer size is the maximum area that *Solenopsis* can interact with the landscape through nuptial flight from the point of origin of the nest (Tschinkel, 2006; Lin et al., 2021). In the buffer, we characterized the native vegetation and agricultural areas, which was measured in m^2 (method adapted from Moreira et al., 2015, Nery et al., 2018, and Wazema et al., 2020). The landscape surrounding each crop was classified as greater than 50% native vegetation (percentage of native vegetation area > 50% [Supplementary material 3a in Figure S3]) and less than 50% native vegetation (percentage of agricultural area > 50% [Supplementary material 3b in Figure S3]). Aerial images were obtained using the Landsat 8 satellite (Bing aerial- Bing 2020 Microsoft Corporation Earthstar Geographics SIO, Microsoft Corporation). The scale adopted was 1:3000, using the software Qgis version 2.18.19 (Qgis Development Team, 2018). This scale provides greater detail to characterize the landscape and is used for landscape studies of invertebrates (Fabian et al., 2013; Montagnana et al., 2021).

Collecting the nests

To standardize the collections, the nests were collected by the same person. The search for nests lasted 4 h and was done during the day throughout and around the crop (500 to 750 m^2). All nests were collected. At each site, the nests (both ants and soil) were collected on sunny days when there had been at least 1 week without rain. The nests were recognized by the agglomerated soil particles above the surface. All the nest material above and below (to 5 cm deep) the soil surface was collected using a garden shovel. The nests were collected at a distance of 20 m apart. The collected material was placed in a plastic pot (5 L) previously coated with fluon (Polytetrafluoroethylene) on the upper part. In the laboratory, the ants from each nest were separated from the soil particles using the drip technique (Bueno, 2017). The specimens were stored in plastic tubes filled with 95% ethanol and stored in a freezer at −20°C. The fieldwork and collections were authorized by the Chico Mendes Institute for Biodiversity Conservation, Brazil (ICMBio/SISBIO permit n. 66500).

Species identification

Morphological identification was done using three major workers from each nest and a key for the *S. saevissima* species group (Pitts et al., 2018). The diagnostic characters used were the following: head shape and proportions, mesosoma shape, and shape and surface of the postpeciole (Trager, 1991; Pitts et al., 2018). High resolution images were taken with a multifocus image overlay system (AutoMontage software and Leica M205C stereomicroscope attached to a Leica DFC 295 camera) and used in the identification process. In addition, a comparison with specimens deposited at the Museum of Zoology of the University of São Paulo was also conducted.

We also identified the ants using molecular data of workers from all nests (Supplementary material 4 in Data S1). Total DNA of each worker ($n = 3$ per nest) was extracted separately using a protocol adapted from Martins et al. (2014). Subsequently, this was used to generate a fragment that varied from approximately 790 to 820 bp from the Cytochrome Oxidase I—COI gene and the CIJ and DDS primers described by Ahrens et al. (2005). The amplification program consisted of pre-denaturation at 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 48°C for 1 min, and 68°C for 2 min and a final extension at 72°C for 5 min. The confirmation of fragment amplification was performed in 1% agarose gel. Sequencing reactions were done with a reagent BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies—Applied Biosystems) and an ABI 3730 DNA Analyser (Life Technologies—Applied Biosystems). The sequences were edited with the software BIOEDIT (Hall, 1999) and MUSCLE 3.6 (Edgar, 2004) and compared to those in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and BOLDSYSTEM (http://www.boldsystems.org/) databases.

After these procedures, the tests were identified by DNA barcoding using the mtDNA cytochrome c oxidase subunit I (COI) (Hebert et al., 2003a, 2003b; Ratnasinghan & Hebert, 2007). The material obtained was compared with sequences deposited in databases, such

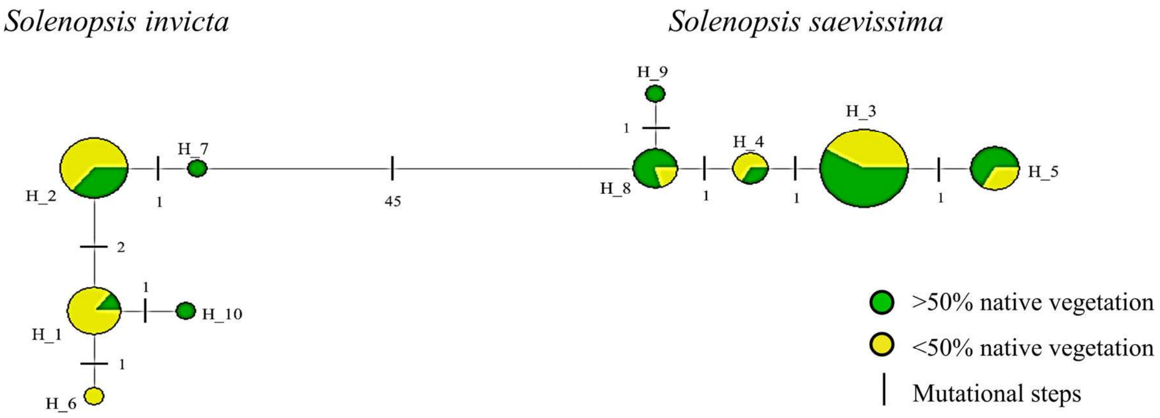

FIGURE 1 Haplotype network of *Solenopsis invicta* and *Solenopsis saevissima* according to native vegetation cover. The size of the circles in the network represents the number of times the haplotype (determined by the software DnaSP) was found.

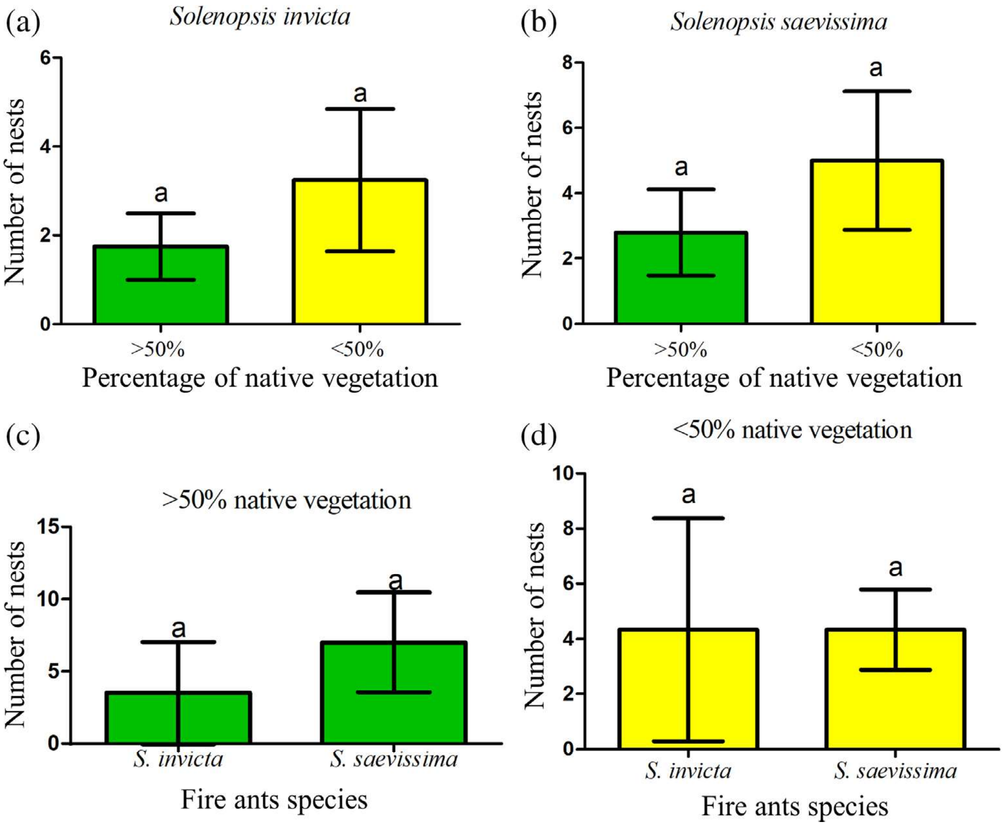

FIGURE 2 Number of nests of *Solenopsis invicta* (a) and *Solenopsis saevissima* (b) according to the native vegetation cover near crops. Number of nests of *Solenopsis invicta* and *Solenopsis saevissima* in sites with >50% (c) and <50% (d) native vegetation. Bar: standard deviation. Same letters: There is no significant difference (*t*-test; $p < 0.05$).

as GenBank and BOLDSYSTEM (Hebert et al., 2003a, 2003b; Ratnasinghan & Hebert, 2007). The comparison of the mtDNA of the collected individuals showed 98 to 99% similarity between haplotypes (Hebert et al., 2003a; Ronque et al., 2016). For *S. saevissima*, the haplotypes obtained were compared with the description by Ross et al. (2010) under accession numbers FJ467519.1, FJ467550.1, and FJ467540.1. For *S. invicta*, the haplotypes obtained were compared with the description by Shoemaker et al. (2006) and Caldera et al. (2008) under accession numbers AY950736.1, AY950742.1, AY950735.1, and EU352608.1. Low frequency haplotypes were sequenced again to exclude the possibility of a DNA polymerase error during the amplification process.

All sequences generated in the present study were deposited in GenBank. Vouchers were deposited in the Alto Tietê ant collection at the University of Mogi das Cruzes, São Paulo, Brazil (see Souza-Campana et al., 2020).

Statistical analysis

The number of nests between the sites near native vegetation fragments with different vegetation cover was compared using a t test. The same was done for the number of haplotypes. The haplotypes between species and native vegetation cover were compared using an ANOVA test (with Tukey posteriori) and a Venn diagram (Oliveiros, 2007). The significance level adopted was 5%. The tests were performed using the software BioEstat version 5.3 (Ayres et al., 2007). The haplotypic (h) and nucleotide (π) diversities were obtained using the software DnaSP. 4.9 (Rozas et al., 2003). The haplotype network was created using the software Network 4.5 (fluxus-enginnering.com) with the Median-Joining parameter (Bandelt et al., 1999). Linear models (GLM) with Poisson distribution were used to test differences in species occurrence among sites with different native vegetation cover. The same models were constructed using the haplotypes identified for each species. The analyses were performed using the software RStudio (version 1.3.959, package *Mass*) (Team R, 2021) with 5% significance.

TABLE 1 GLM results with a random intercept showing the comparison of the number of nests between the types of native vegetation cover and haplotypes

Model: Number of *S. invicta* nest ~percentage of native vegetation				
Predictors	Estimated	SE	*Z*	*p*
Intercept	653.574	115595.281	0.006	0.955
>50%	−6.536	1155.953	−0.006	0.955
<50%	−6.498	1155.953	−0.006	0.996
Model: Number of *S. invicta* nest ~Haplotypes				
Predictors	Estimated	SE	*Z*	*P*
Intercept	−23.30	49252.67	0.000	1
H1	−24.52	49252.67	0.000	1
H2	12.40	24626.34	0.001	1
H6	86.25	172384.36	0.001	1
H10	25.50	49252.67	0.001	1
Model: Number of *S. saevissima* nest ~ percentage of native vegetation				
Predictors	Estimated	SE	*Z*	*P*
Intercept	−50.9109	24.5025	−2.078	0.0377
>50%	0.5479	0.2552	2.147	0.0318
<50%	0.4902	0.2299	2.132	0.0330
Model: Number of *S. saevissima* nest ~ Haplotypes				
Predictors	Estimated	SE	*Z*	*P*
Intercept	1.0106	0.3148	3.21	0.00133
H8	0.5493	0.2463	2.23	0.02573

RESULTS

Species identification

Morphological and molecular analyses confirmed the occurrence of only *S. invicta* and *S. saevissima* in the sites. The morphological characters described by Pitts et al. (2018) were confirmed during the identification process. A molecular analysis showed that *S. invicta* and *S. saevissima* differ by 45 polymorphic sites (5.67%) between the H7 and H8 haplotypes. The haplotype network (Figure 1) indicates low variation for *S. invicta* with observed distances of one to two mutations, while for *S. saevissima* the variation was only one mutation.

Number of nests

In total, we found 55 nests, of which 19 were *Solenopsis invicta* and 36 were *S. saevissima*. The number of nests did not differ between species, when compared on sites with and without 50% native vegetation ($t = 1.237$; $p = 0.810$). Vegetation cover did not influence the number of *S. invicta* (Figure 2a) and *S. saevissima* (Figure 2b) nests. In addition, the species did not differ from each other in sites with native vegetation cover that was greater (Figure 2c) or less (Figure 2d) than 50%.

Molecular diversity

Solenopsis invicta has distinct haplotypic (h) and nucleotide (π) diversities when the vegetation cover is greater ($h = 0.714$; $\pi = 0.0019$) or less ($h = 0.604$; $\pi = 0.015$) than 50%. For *S. saevissima*, we observed similar values of haplotypic and nucleotide diversities between the types of vegetation cover (more than 50%: $h = 0.681$; $\pi = 0.0015$; less than 50%: $h = 0.615$; $\pi = 0.0011$). The results for *S. saevissima* were corroborated by the models, since they indicated that the species is similarly influenced by the two levels of native vegetation cover, especially haplotype H8 (Table 1).

Haplotype descriptions

For *S. invicta*, we identified the H1, H2, H7, H9, and H10 haplotypes and our data showed that the number of haplotypes does not differ between the native vegetation cover (Figure 3a–c). However, we recorded haplotypes exclusive to the two levels of vegetation cover (Figure 3d). For *S. saevissima*, we identified the H3, H4, H5, H8, and H9 haplotypes and our data showed que there was a difference in the frequency of some, depending on the vegetation cover (Figure 4a, b), but no difference in the total number of haplotypes (Figure 4c). For *S. saevissima*, we recorded haplotypes exclusive to one of the levels of vegetation cover (Figure 4d).

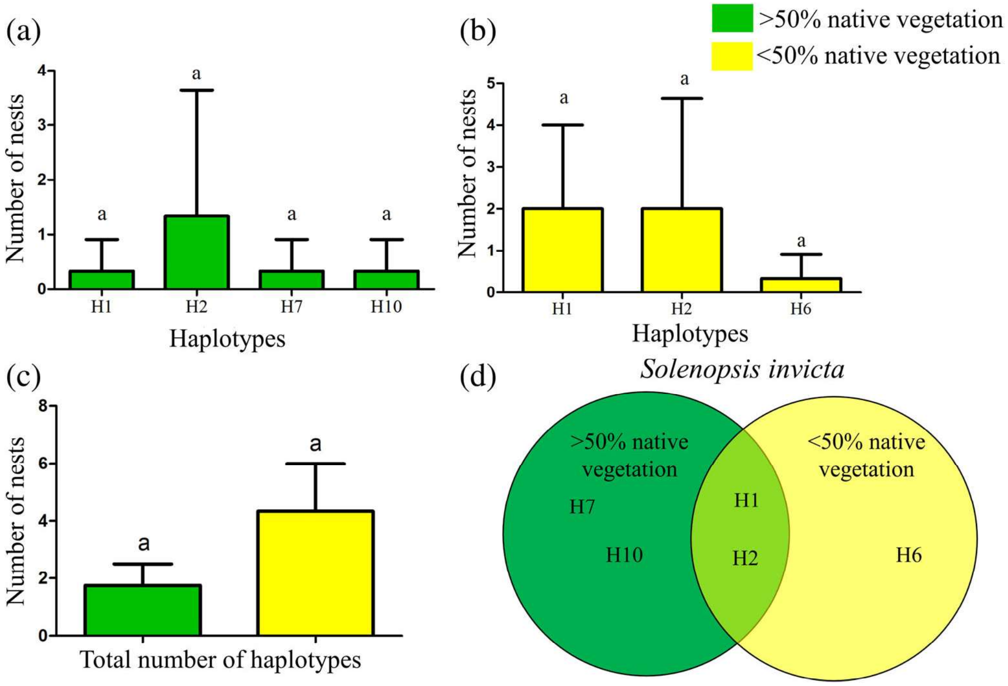

FIGURE 3 Number of nests of *Solenopsis invicta* according to haplotype types (a and b), total number of haplotypes (c) and Venn diagram, showing the sharing of haplotypes according to native vegetation cover (d). Same letters: There is no significant difference (ANOVA; $p < 0.05$; t-test; $p < 0.05$). The size of the circle in the Venn diagram is only illustrative.

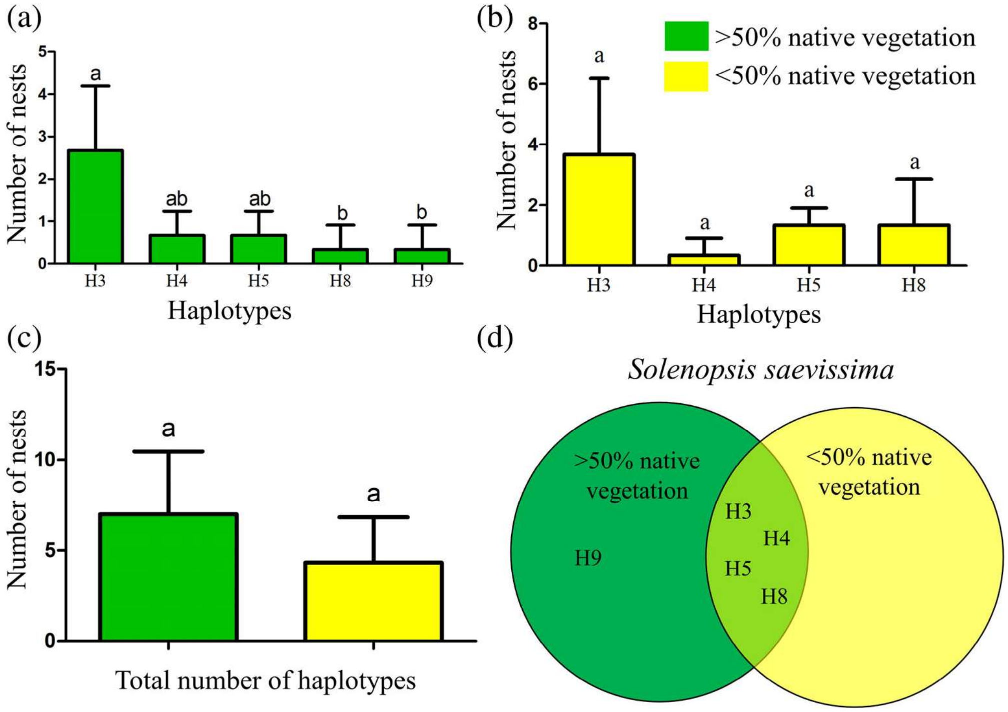

FIGURE 4 Number of nests of *Solenopsis saevissima* according to haplotype types (a and b), total number of haplotypes (c) and Venn diagram, showing the sharing of haplotypes according to native vegetation cover (d). Different letters: There is significant difference ($F = 3.0545$; $p = 0.0353$; t-test; $p < 0.05$). The size of the circle in the Venn diagram is only illustrative.

DISCUSSION

In this study, we compared the occurrence and molecular diversity of *S. invicta* and *S. saevissima* in sites near fragments with greater or less than 50% native vegetation cover. Even though the techniques used for identification are known, in this work we emphasize the importance of using morphology and molecular tools in combination when identifying *S. invicta* and *S. saevissima*. Our hypothesis was not corroborated, since the species have the same number of nests in the collection sites. Additionally, the molecular diversity is comparatively higher in *S. invicta* in sites with greater than 50% vegetation cover. In relation to *S. saevissima*, the molecular diversity is similar between the sites near native vegetation fragments, independent of vegetation cover.

These species have a high level of interspecific polymorphism (variable body size of workers) and lack intraspecific morphological characters that help in their differentiation (Pitts et al., 2005; Shoemaker et al., 2006; Fox et al., 2012; Pitts et al., 2018). Despite taxonomic lists (see Rossi & Fowler, 2004; Lapola & Fowler, 2008; Fernández et al., 2021) that have recorded *S. invicta* and *S. saevissima*, these species can be difficult to identify due to low taxonomic resolution (Kempf, 1961). Most of the time, *Solenopsis* workers are separated into morphospecies, as done in “ATLANTIC ANTS: a data set of ants in Atlantic Forests of South America” (Silva et al., 2022), which includes 64 nominal species (of the 965 recorded) and 701 morphospecies (of the 5285 recorded). The accuracy in identifying the species in our work was possible because we used an integrative taxonomic approach (Padial et al., 2010; Chialvo et al., 2018), including morphological characters and DNA barcoding, specifically the COI fragment, as an auxiliary tool (Hebert et al., 2003a, 2003b; Ratnasinghan & Hebert, 2007).

Our work also provides information about the genetic diversity of *S. invicta* and *S. saevissima*, which is poorly studied. Since the Atlantic Forest is very fragmented (Solórzano et al., 2021), and most fragments are less than 50 ha (Ribeiro et al., 2009) and interspersed with crops and other anthropogenic activities (Marques et al., 2022), knowledge about the occurrence and molecular diversity of *S. invicta* and *S. saevissima* is fundamental to control the invasive species and conserve the native species (Supplementary material 1b in Figure S1). In the study region, we consider *S. invicta* invasive and *S. saevissima* native based on the geographic origin of each species (see Pitts et al., 2005; Pitts et al., 2018).

Invasive species benefit from disturbances in natural ecosystems (Meyer et al., 2021), but our results show that *S. invicta* and *S. saevissima* occur in a similar manner in the collection sites, regardless of being near native vegetation fragments with greater or less than 50% vegetation cover. Opportunistic species (e.g., fire ants) benefit from fragmentation (Crist, 2009; Collevatti et al., 2020), which creates border areas (Ye et al., 2021). Forest borders resulting from anthropogenic activities especially exhibit sudden changes in temperature, humidity and light (Lourenco et al., 2019; García-Jain et al., 2021), which favours the colonization of *S. invicta* and *S. saevissima* (Almeida et al., 2007; Martins et al., 2014). For crops, there are also factors related to soil management that favour colonization by *S. invicta*. This species has increased its geographic distribution in the Southeast Region of Brazil due to the expansion of agricultural areas (Munhae et al., 2014), as suggested by our results in the metropolitan region of the State of São Paulo (Supplementary material 1b in Figure S1).

Our data also show that the fire ants analysed have similar haplotypic diversity (*S. invicta* [>50%: $h = 0.714$; <50%: $h = 0.604$] and *S. saevissima* [>50%: $h = 0.681$; <50%: $h = 0.615$]), except for *S. invicta* with greater vegetation cover. Haplotypic diversity values (h) greater than 0.5 are considered high (Grant & Bowen, 1998). Thus, what we found indicates high haplotypic diversity for *S. invicta* and *S. saevissima*. In the same region as our study, these species have similar high haplotypic diversity in areas with more vegetation (see Ramalho et al., 2022). However, *S. invicta* is associated with habitats with less vegetation and more construction, and its haplotypic diversity is usually lower in these areas (urban areas: $h = 0.352$; Ramalho et al., 2022). Thus, our results suggest that the vegetation cover cutoff (50%) we used was not enough to cause noticeable changes in the haplotypic diversity of *S. invicta*, as observed in urban areas by Ramalho et al. (2022). On the other hand, the haplotypic diversity of *S. invicta* in our work is within the range observed by Ahrens et al. (2005) ($h = 0.69$–0.95), but lower than that observed by Martins et al. (2014) ($h = 0.95$–0.96). A loss in diversity was also observed by Ross & Shoemaker (2005) during the invasion process in the United States, and these authors attributed the recent population expansion with a bottleneck introduction pattern. The haplotypic diversity values (h) found by us for *S. invicta* suggest that human activities are factors related to the dispersion of the species, as seen in the United States (Tschinkel, 2006).

The haplotypic diversity of *S. saevissima* was lower than that observed by Ross et al. (2010) ($h = 0.99$) and Martins et al. (2014) ($h = 0.95$–0.96). However, Ramalho et al. (2022) found values near those of Ross et al. (2010) and Martins et al. (2014), suggesting that the species is native to our study region. Additionally, the reconstruction of the ancestral state analysis by Ramalho et al. (2022) indicates a strong relationship with native vegetation, which corroborates our results. Our results are probably related to the smaller sampling of nests and geographic area (Muirhead et al., 2008), but they also suggest that populations of *S. saevissima* are derived from refugia or large isolated areas of endemism (e.g., forest fragments) (see Ahrens et al., 2005; Shoemaker et al., 2006; Martins et al., 2014). In this case, there is a reduction in the haplotypic diversity of populations (Nöel et al., 2007; Björklund et al., 2010) that is driven by human activities (Ahrens et al., 2005; Shoemaker et al., 2006).

In this work, we also found exclusive haplotypes of *S. invicta* and *S. saevissima*. For *S. invicta*, the H7 and H10 haplotypes were exclusive to sites that had greater than 50% native vegetation cover, while the H6 haplotype was exclusive to sites with less than 50% native vegetation cover. The H6 haplotype is derived from the H1 haplotype, which is more frequent in landscapes with less than 50% vegetation cover. This suggests a rapid expansion and recent establishment of the population in these landscapes (Dittrich-Schröder et al., 2018), which may be a specialization for anthropogenic habitats (Ascunce et al., 2011;

Lebrun et al., 2012; Ramalho et al., 2022). The study area is known as the Paulista Green Belt in São Paulo State (Rodrigues, 2014), where crops are grown in areas originating from fragmenting the Atlantic Forest. Thus, we suggest that these haplotypes may be associated with adaptations to the process of invasion and establishment of the species (see Ascunce et al., 2011). Disturbances, such as soil management, use of machinery and agrochemicals, facilitates the invasion (Crist, 2009; Wong et al., 2020; Herrero et al., 2021) of *S. invicta* (Fernandes et al., 2000). The H6 and H10 haplotypes show the emergence of new haplotypes that come from a probable recent population expansion (Bracco et al., 2007; Almada et al., 2008; Bachevskaya et al., 2014; Dittrich-Schröder et al., 2018). Thus, our results again suggest that *S. invicta* populations are expanding with a consistent pattern of a bottleneck introduction. The emergence of new haplotypes is similar to what happened in Taiwan and the United States, where evidence was found of recent introductions associated with bottleneck genes (Shoemaker et al. 2006; Nöel et al., 2007; Yang et al., 2008; Pelletier & Coltman, 2018).

For *S. saevissima*, the H9 haplotype was exclusive to sites with greater than 50% vegetation cover. This species is native to the region (Pitts et al., 2005; Pitts et al., 2018), and we suggest that this haplotype is related to the survival of this species in places with vegetation (Garvin et al., 2015; Novičić et al., 2015). We emphasize that *S. saevissima* is found near native forest fragments (Lunz et al., 2009; Dejean et al., 2015) and in naturally disturbed places (e.g., trails and clearings; Trager, 1991), which corroborates the reconstruction of the ancestral state analysis made by Ramalho et al. (2022). Furthermore, the lower occurrence of the H8 and H9 haplotypes and the increase of the H3 haplotype suggest the interaction of native vegetation and the occurrence of *S. saevissima*, as reported for other groups of animals (e.g., salamanders [Nöel et al., 2007] and birds [Björklund et al., 2010]). The replacement of native vegetation by agricultural areas may have isolated *S. saevissima* populations (Martins et al., 2014) and caused a bottleneck effect (Nöel et al., 2007; Pelletier & Coltman, 2018), which reinforces our suggestion. The H8 and H9 haplotypes also reinforce the suggestion that endemic populations of *S. saevissima* may be derived from refugia or large, isolated forest fragments.

Our studies found the occurrence of two species of fire ants and a total of 10 haplotypes, five for *S. invicta* and five for *S. saevissima*. The occurrence of these species is the same, regardless of the vegetation cover, and when native vegetation cover is more than 50%, molecular diversity is greater for both species, especially for *S. invicta*. This species is invasive in the region and understanding its process of expansion and genetic diversity could help when using techniques to control it that have less impact on the environment (e.g., gene silencing [see Meng et al., 2020; Zhang et al., 2022]). The use of environmentally friendly techniques could help conserve *S. saevissima* in its natural area of occurrence and regulate pest populations in agroecosystems.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. Material preparation and data collection were performed by Rodrigo F. Souza. The first draft of the manuscript was written by Victor H. Nagatani, Manuela O. Ramalho and Maria S.C. Morini, and all authors reviewed subsequent versions of the manuscript. Victor H. Nagatani managed and edited the manuscript. All authors read and approved the final manuscript and declare that they have no conflicts of interest.

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DATA AVAILABILITY STATEMENT

Data available in article supplementary material -The data that supports the findings of this study are available in the supplementary material of this article

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Geographic distribution of *S. invicta* and *S. saevissima* in Brazil (a) and São Paulo State (b). The arrow indicates the overlap of the species. Black circles indicate the study region. (a)—Adapted from Ross et al. (2010), Fox et al. (2012), Martins et al. (2014), Souza et al. (2014), and Pitts et al. (2018); (b)—Adapted from Fox et al. (2012), Munhae et al. (2014), Souza et al. (2014) Souza (2019).

Figure S2. Geographic location of *Solenopsis* nest collection sites according to the percentage of native vegetation in the vicinity of the crop.

Figure S3. Detailed characterization of the 1 km buffer showing, comparatively, the areas of native vegetation in the vicinity of the crop. (a) 1 km buffer of an area with >50% native vegetation; (b) 1 km buffer of an area with <50% native vegetation. Each central dot, in red, indicates the crop.

Data S1. Geographic characterization of nests, collection sites and similarity percentage of specimens from each nest of *Solenopsis* with haplotypes deposited in GenBank. NV, native vegetation; AA, agricultural areas.

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