The Red Imported Fire Ant (Hymenoptera: Formicidae) in the West Indies: Distribution of Natural Enemies and a Possible Test Bed for Release of Self-Sustaining Biocontrol Agents

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The red imported fire ant (Hymenoptera: Formicidae) in the West Indies: distribution of natural enemies and a possible test bed for release of self-sustaining biocontrol agents

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Abstract

Sample collections of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) were taken from 20 islands of the West Indies and evaluated for the presence of key pathogens and parasites of this invasive pest ant. We hypothesized that bottleneck events during the introduction of this ant species in the West Indies would have resulted in populations devoid, or nearly so, of natural enemies. Monogyne and polygyne social forms were found throughout the islands surveyed with monogyny being more prevalent (65%) compared with polygyny (35%). Among 254 samples, only 25 (~10%) tested positive for the presence of pathogens or parasites. The microsporidian *Kneallhazia solenopsae* was the most prevalent pathogen detected; it was found in 20 colonies. A second microsporidian species, *Vairimorpha invictae*, was shown to be present in a polygyne sample collected from St. Croix—the first detection of this pathogen outside South America. Similarly, *Solenopsis invicta densovirus* (SiDNV) was detected in one polygyne sample from Anguilla. SiDNV is not found in *S. invicta* U.S. populations, so this detection also represents the first geographic discovery outside of South America. Two species of *Pseudacteon* decapitating flies were found to have dispersed into the Bahamas. Utilization of the islands of the West Indies for release, establishment, and impact assessment of *S. invicta* natural enemies is discussed.

Key Words: *Kneallhazia solenopsae*; *Vairimorpha invictae*; SINV; SiDNV; *Pseudacteon*; pathogen; parasite

Resumen

Colecciones de muestras de *Solenopsis invicta* Buren (Hymenoptera: Formicidae) fueron tomadas de 20 islas Antillanas y evaluadas para determinar la presencia de patógenos y parásitos claves de esta hormiga plaga invasora. Presumimos que los eventos de cuello de botella durante la introducción de esta especie de hormiga en el Caribe se deben a la población carente, o casi, de enemigos naturales. Las formas sociales monoginias y poliginias fueron encontradas en todas las islas examinadas siendo las monoginias las más frecuentes (65%) en comparación con las poliginias (35%). Entre las 254 muestras, solamente 25 fueron positivas para la presencia de patógenos o parásitos. El microsporidio *Kneallhazia solenopsae* fue el patógeno más prevalente detectado; fue encontrado en 20 colonias. Una segunda especie de microsporidio, *Vairimorpha invictae*, fue encontrado en una muestra poliginia de St. Croix—la primera detección de este patógeno fuera de Sudamérica. Del mis mo modo, *Solenopsis invicta densovirus* (SiDNV) fue detectado en una muestra poliginia de Anguila. SiDNV no se encuentra en las poblaciones de *S. invicta* en EE.UU., por lo que esta detección también representa el primer descubrimiento geográfico fuera de América del sur. Se encontró que dos especies de moscas decapitadoras *Pseudacteon* se han dispersado hasta las Bahamas. Se discute la utilización de las Islas del Caribe para a liberación, el establecimiento y la evaluación del impacto de enemigos naturales de *S. invicta.*

Palabras Clave: *Kneallhazia solenopsae*; *Vairimorpha invictae*; SINV; SiDNV; *Pseudacteon*; patógenos; parásitos

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) has proven to be a formidable invasive pest species. Indigenous to South America, *S. invicta* has established invasive populations in the United States (Callcott & Collins 1996), mainland China, Taiwan, Australia, Mexico, and across the Caribbean (Davis et al. 2001; Wetterer & Snelling 2006; Wetterer & Davis 2010; Wetterer et al. 2014). In the United States alone, cost estimates for controlling and repairing damage caused by *S. invicta* exceed $6 billion annually (Pereira 2003). A number of insecticidal baits are available to control *S. invicta*, which are highly effective against this ant pest (Williams et al. 2001). Unfortunately, these baits are expensive to use in large areas, and because the ant is so prolific and ubiquitous, they often must be used several times a year to maintain acceptable levels of control (Collins et al. 1992; Drees et al. 2009), otherwise fire ant populations quickly re-infest untreated areas. Also, some fire ant baits are not suitable for nature preserves and other natural areas because they kill a wide range of native ants (Zakharov & Thompson 1998).

In South America, fire ant population densities are generally 10 to 20% of invasive populations found in North America (Porter et al. 1992, 1997). Although several hypotheses have been posited to explain the intercontinental differences (Wilder et al. 2012), the most widely accepted is that *S. invicta* has escaped its natural enemies during found-

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ing events (Porter et al. 1997). Classical or self-sustaining biological control agents from South America are important components of a biological control program because they offer the possibility for permanent, regional suppression of fire ants (Williams et al. 2003). At least 44 fire ant natural enemies have been identified in South America: *Pseudacteon* fly parasitoids (23 species), microsporidia (*Vairimorpha invictae* and *Kneallhazia solenopsae),*fungus (*Myrmecomyces annellisae*), nematodes (*Allomermis solenopsi*, *Tetradonema solenopsis*, and *Hexamerma* spp.), eucharitid wasps (5 species)*,* scarab beetle (*Martineziana dutertrei*), strepsipteran (*Caenocholax fenyesi*), parasitic ant (*Solenopsis daguerrei*), densovirus (*Solenopsis invicta densovirus*, SiDNV), and RNA viruses (3 viruses) (Wojcik et al. 1987; Williams et al. 2003; Briano et al. 2012). By way of comparison, only 6 natural enemies have been found in the United States: the microsporidian *K. solenopsae,* the *Martineziana* scarab beetle, the *Caenocholax* strepsipteran, and 3 RNA viruses as above (Wojcik et al. 1987; Williams et al. 2003; Briano et al. 2012). Successful releases of self-sustaining parasites and pathogens will not eradicate fire ants but could help tilt the ecological balance in favor of native arthropods (Porter 1998). If this happens, fire ant populations in introduced ranges could be reduced to levels similar to those in South America where fire ants are not considered an important problem (Porter et al. 1997).

The *S. invicta* infestation in the West Indies offers excellent opportunities to examine and quantify the effectiveness of specific natural enemies, alone or in combination, on *S. invicta* populations. The islands are relatively small, making biological control efforts (introductions and evaluations) manageable. Because the *S. invicta* populations established on each of these islands represent separate and unique infestations, the first task is to screen the populations of *S. invicta* for known pathogens. Thus, our objective was to conduct a survey for the presence of pathogens among *S. invicta* populations of many of the islands of the West Indies in which *S. invicta* is established.

Materials and Methods

Solenopsis invicta workers were sampled by hand-collecting ants from nest mounds and attracting ants to tuna lures during surveys of fire ant distribution in the Caribbean (Wetterer & Snelling 2006; Wetterer & Davis 2010; Wetterer et al. 2014). The ants were placed in 99% ethanol. Detailed collection notes for all samples evaluated are provided in the Supplementary Material for this article in Florida Entomologist 98(4) (Dec 2015) online (http://purl.fcla.edu/fcla/entomologist/browse). Red imported fire ant workers (*S. invicta*) were discriminated from native tropical fire ant workers (*Solenopsis geminata* [F.]) by the isometry of large workers compared with small workers and by the presence of a medial clypeal tooth in minor workers (Wojcik et al. 1976).

Pathogen/parasite infections were determined by molecular methods because they offer the most sensitive approach for detection. *Solenopsis invicta* ant samples were first processed to extract RNA by the Trizol (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA) method and DNA by a method described previously (Valles et al. 2002). A pooled sample of 10 worker ants was used for each nucleic acid preparation. In some cases, fewer ants were available. In these instances, the ants were divided in half for each preparation (DNA and RNA). DNA was used as template to conduct polymerase chain reaction (PCR) to determine the social form of the ants by genotyping the *Gp-9* alleles (Valles & Porter 2003), and to detect the presence of *K. solenopsae* (Valles et al. 2002), *V. invictae* (Valles et al. 2004), SiDNV (Valles et al. 2013b), and *Pseudacteon* decapitating parasitic flies (Oi et al. 2009). RNA from each sample was evaluated by reverse transcriptase PCR (RT-PCR) for the presence of *Solenopsis invicta* viruses SINV-1, SINV-2, and SINV-3 (Valles et al. 2009). Retrospective screening for these viruses in ant samples stored in ethanol is possible for years after collection (Valles 2007; S. M. V., unpubl. data).

Samples testing positive by generation of an amplicon of anticipated size were repeated to ensure that the result was not a false positive. In addition, gel-purified amplicons were ligated into the pCR4-TOPO vector, transformed into TOP10 competent cells (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and sequenced at the Interdisciplinary Center for Biotechnology Research (University of Florida, Gainesville, Florida, USA) by the Sanger method to verify that the amplicon was from the suspected pathogen or parasite. Sequences were analyzed by Blast analysis to establish their identity (Altschul et al. 1997).

Results and Discussion

In total, 254 *S. invicta* samples were collected from across the West Indies; the ants were genotyped at the *Gp-9* locus to assign social form and evaluated by molecular methods for the presence of key pathogens and parasites. Monogyny, or single-queen colonies, was the most prevalent social form, representing 65% (182/254) of the samples. This percentage was less than in most areas in the United States (80–85%, Porter 1992; Porter et al. 1992, 1997) and South America (~90%, Porter et al. 1997) but more than in Texas (~50%, Porter et al. 1991). Because monogyne and polygyne colonies are genetically distinct, it is somewhat surprising that 80% of the islands surveyed had both polygyne and monogyne fire ants present (16/20; Fig. 1). Only the monogyne social form was detected on Aruba, Barbuda, Grand Cayman, and Jamaica (Fig. 1). Conversely, only polygyne colonies were detected on Great Stirrup Cay. However, of sites with only one social form, only Barbuda had enough samples to expect the possibility of finding both forms. Also consider that the ant samples collected at lures were pooled for analysis and likely represented a mixture of colonies. A single polygyne ant included in this sampling method would mask any workers from monogyne colonies included in the samples. Thus, monogyny may be under-represented with this sampling method.

Regardless, the high frequency of both social forms on these islands suggests two possibilities. First, most islands experienced multiple independent invasions of each social form. This possibility seems a bit unlikely, but monogyne queens may be able to disperse long distances (20–30 km) on wind currents after mating 50 to 100 m in the air (Vinson & Greenberg 1986), and the polygyne social form may have spread via island-to-island transport of infested nursery stock or other commercial products in contact with soil; alate polygnye queens are rather feeble and do not easily found independent claustral colonies (Preston et al. 2007). The second, intriguing possibility for the high frequency of both social forms is that most dispersal occurs from inter-island transport of polygyne colonies, and heterozygous polygyne queens are capable of producing homozygous monogyne populations. Genetic analyses among colonies on these islands could discern their relatedness and provide insight into the introduction and spread of *S. invicta* on these islands (Ascunce et al. 2010). The answer to this question also would help establish whether long-distance transport of monogyne queens after mating flights has been an important mechanism in the invasion and dispersal of *S. invicta* across the West Indies. A better understanding of the mechanisms of inter-island invasions could be useful in restricting the dispersal of this pest among islands or island groups in the Pacific.

Our tests detected the presence of 2 microsporidian pathogens and 2 fire ant viruses. The microsporidian *K. solenopsae* was detected and verified from 20 *S. invicta* samples collected from 10 of the 20 is-

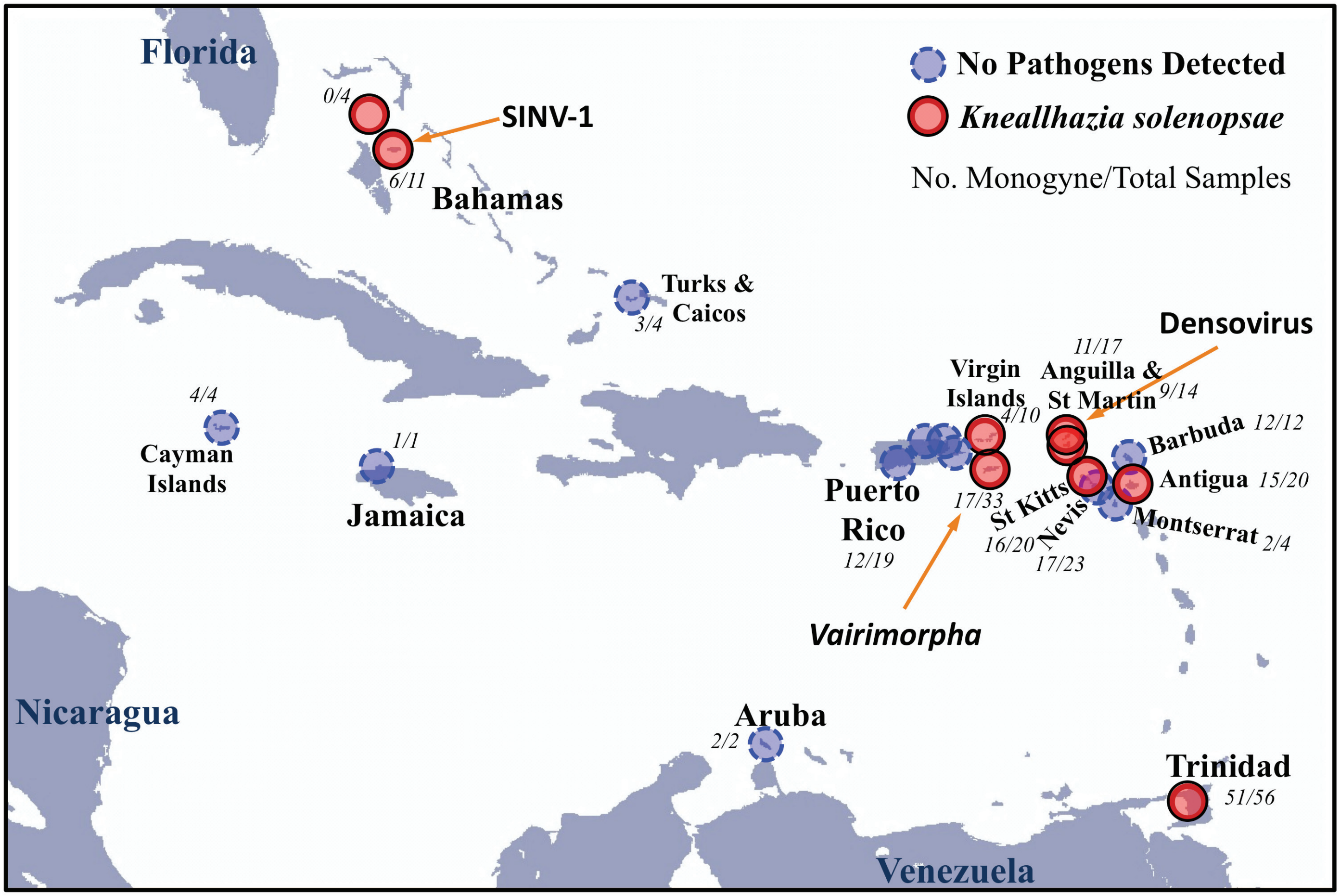

Fig. 1. Distribution of 2 fire ant microsporidian pathogens (*Kneallhazia solenopsae*, *Vairimorpha invictae*) and 2 fire ant viruses (SINV-1, SiDNV) among collections of the red imported fire ant, *Solenopsis invicta*, from islands in the West Indies. The fire ant RNA viruses SINV-2 and SINV-3 were not detected in any of the collections. The number of collections from monogyne colonies is shown over the total number of collections for each island or island group (Tortola [1/5], St. John [0/1], and St. Thomas [3/4]).

lands, including Anguilla, Antigua, Great Stirrup Cay, New Providence, St. Kitts, St. Croix, St. Martin, St. Thomas, Tortola, and Trinidad. The majority of these infections (15/20) were detected in polygyne colonies, which is consistent with pathogen surveys conducted in the United Sates (Oi et al. 2004; Valles et al. 2010; Allen et al. 2011).

Two monogyne samples from New Providence tested positive for SINV-1. SINV-1 exhibits widespread distribution in U.S. and South American fire ant populations and is sometimes very prevalent in localized populations (often >80%) (Valles et al. 2009; Valles 2012). This virus often accompanies founding populations (Yang et al. 2010).

Another microsporidian species, *V. invictae*, was shown to be present in a polygyne sample collected from St. Croix. *Vairimorpha invictae* is limited to *S. invicta* populations in its native range (Oi et al. 2012), so this is the first detection of this pathogen outside South America. Curiously, we also found the virus SiDNV in 1 polygyne sample from Anguilla. SiDNV is also not found in *S. invicta* U.S. populations (Valles et al. 2013b), so detection of this virus on Anguilla represents a new geographic discovery. The presence of *V. invictae* in St. Croix and SiDNV in Anguilla suggests some fire ants in the West Indies may originate directly from South America rather than from introduced U.S. populations. Another possibility that needs to be investigated is that these pathogens jumped from the tropical fire ant (*S. geminata*) populations, which are common in the West Indies, or that the 2 samples in question may have included a few *S. geminata* workers by accident. The problem with this hypothesis is that *V. invictae* has not been detected in *S. geminata* (Oi et al. 2010) and we do not know whether *S. geminata* is capable of serving as a host for SiDNV.

The absence of SINV-2 and SINV-3 in West Indies *S. invicta* samples is of considerable interest because it suggests that these 2 pathogens, and some of the pathogens above, could be released in the West Indies as self-sustaining biological control agents. These viruses have been reported to be absent in founding populations in China, California, and Australia (Yang et al. 2010). We are particularly interested in the possibility of releasing SINV-3 because laboratory studies have shown it to be highly pathogenic, virulent, and host specific to fire ants in the *saevissima* complex from South America (Porter et al. 2013, 2015).

DNA evidence of *Pseudacteon* decapitating flies was found in 1 ant sample from Great Stirrup Cay. This result was confirmed by collection of an adult female *Pseudacteon curvatus* Borgmeier fly from this location. In addition, 2 *P. curvatus* females and 1 male *Pseudacteon tricuspis* Borgmeier fly were collected from New Providence Island about 100 km to the southeast. The discovery of these flies was surprising because the collection sites were separated by 100 to 200 km of open sea from mainland Florida where *P. curvatus and P. tricuspis* had been released 8 and 11 yr earlier, respectively. Bulk transport of parasitized fire ants in nursery plants from Florida or transport by large wind storms are possible explanations for their presence on these 2 islands. The absence of DNA evidence of flies from other samples was expected because these samples were all collected in years and at locations not likely to have been exposed to *Pseudacteon* parasitoids.

As hypothesized, the introduction(s) of *S. invicta* onto the islands of the West Indies occurred largely without accompanying natural enemies. No natural enemies were detected on half of the islands examined, and on the remaining islands, *S. invicta* populations supported only 1 or 2 natural enemies. These data provide further support for the hypothesis that escape from natural enemies contributes to the successful invasive capacity of this ant (Porter et al. 1992). Lack of natural enemies within these discrete populations and the relatively small, isolated nature of the islands indicate that they may serve as suitable test locations for the intentional introduction and subsequent evaluation of natural enemies against *S. invicta*. The isolated habitat offers the ability to quantify the impact of different natural enemies alone, or in combination, on *S. invicta* populations in a relatively short time frame. Additionally, introductions can be more closely monitored providing a better understanding of the epidemiology in the case of pathogens.

The successful utilization of islands for this purpose has been reported for a number of invertebrate invasive pests. One of the best documented examples is the rhinoceros beetle (*Oryctes rhinoceros* [L.]; Coleoptera: Dynastidae) in the South Pacific (Young 1986). A baculovirus was discovered in the beetle, but there was doubt about the impact of this virus on the beetle population (Marschall 1970). However, carefully planned and executed introductions into beetle populations onto Tongatapu and certain islands of Fiji resulted in an epizootic and corresponding decline in the beetle populations (Young 1974; Bedford 1976). The researchers introduced the virus onto one side of the island and were able to follow its spread and estimate the rate of spread of the epizootic across to the other side. Island biological control efforts have been reported also for Schizophora flies on Easter Island (Ripa et al. 1995) and *Schistosoma* snails in the Caribbean (Pointier & McCullough 1989).

Laboratory tests have shown that SINV-3 causes significant mortality in *S. invicta* colonies (Porter et al. 2013; Valles et al. 2014). SINV-3–treated colonies suffer collapse and elimination within 30 to 60 d of infection depending on the virulence of the strain (Valles et al. 2013a). Successful field releases of SINV-3 have been accomplished in Florida (Valles & Oi 2014) and introductions established in California (S. M. V. and D. H. Oi, unpublished results). However, the impact of the virus on the *S. invicta* field population has not been determined. Our goal is to release SINV-3 alone or in combination with other *S. invicta* natural enemies (SiDNV, *V. invictae*, SINV-1, SINV-2, *Pseudacteon* species) into the ant populations on islands of the West Indies and monitor the impact and spread of the agents. The data reported in this study demonstrate the suitability of these islands to study *S. invicta* biological control under controlled conditions.

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