A review of the biological control of fire ants (Hymenoptera: Formicidae)

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Abstract

The suppression of established invasive ants will likely require biological control by natural enemies. This approach is self-sustaining and can impact undetected or inaccessible populations that are a source of the continual presence and expansion of the invaders. There is an ongoing effort to introduce and distribute biological control agents against fire ants, Solenopsis invicta Buren, 1972 and Solenopsis richteri Forel, 1909, in the USA. Eight natural enemies, consisting of two pathogens, a microsporidium, Kneallhazia solenopsae, and a virus, Solenopsis invicta virus-3, plus six species of parasitoid flies in the genus Pseudacteon are now established (i.e., reproducing in the field). These biological control agents are host specific and negatively impact fire ants to varying degrees. Fire ant populations in the presence of biological control agents are often reduced (based on number of ants per nest), but the number of nests may not decline. It remains to be seen if the continued release and establishment of a suite of biological control agents can result in the complete control of fire ants. The currently established biocontrol agents are acting as expected by weakening fire ant colonies, and contributing to the potential cumulative attrition of colony vigor. Knowledge gained from establishing and disseminating biological control agents of fire ants in the USA will allow for faster progress in the utilization of biological control for fire ants in other regions of the world as well as providing guidance for the importation and establishment of natural enemies for other invasive ants.

Key words: Solenopsis invicta, red imported fire ant, Solenopsis richteri, black imported fire ant, classical biological control, biocontrol, natural enemies, parasites, pathogens, Pseudacteon, Kneallhazia solenopsae, Thelohania, SINV, Solenopsis invicta virus, Dicistroviridae, single-stranded RNA virus, invasive species, integrated pest management, control.

Introduction

Several ant species are highly invasive in various regions of the world (McGlynn 1999, Lowe & al. 2000, Suarez & al. 2009). Biotic attributes including highly abundant populations, efficient dispersal, omnivorous feeding behavior, and unicoloniality, in addition to the preference for disturbed habitats, have allowed these species to establish, spread, and become dominant in many ecosystems (Passera 1994, Holway & al. 2002, Krushelnicky & al. 2009). Invasive ant incursions elicit a range of responses that include eradication programs, aggressive containment, and management. Eradication is often desired once an incursion is detected, and this approach has been successful when infestations are very small and localized (often < 1 ha) (Hoffmann & al. 2011). In contrast, infestations that are entrenched across various habitats and geographically expansive, such as the Argentine ant, Linepithema humile (Mayr, 1868) in Western Australia and New Zealand (van Schagen & al. 1994, Ward & al. 2010) and the red imported fire ant, Solenopsis invicta Buren, 1972, in the southern USA (Williams & al. 2001) have not been eliminated despite extensive eradication attempts. Eradication programs in the late 1990s and 2000s for S. invicta in California (USA), Queensland (Australia), and Taiwan (Klotz & al. 2003, Shih & al. 2007, McNaught & al. 2014) also have not achieved eradication to date, and eradication is no longer an objective in the USA.

If eradication is not feasible, regulatory programs of aggressive containment can be instituted. Quarantines, generally initiated during eradication, are maintained, and the elimination of new infestations both inside and outside the quarantined areas are attempted with contact insecticides and / or insecticidal ant baits to hinder, or ideally, stop the further spread of infestations (Porter & Higashi 2008). The major difference between eradication and aggressive containment is that the goal of aggressive containment is only to suppress populations rather than to find and kill the last colony. While aggressive containment requires far fewer treatments than eradication, it is still a relentless program that needs substantial funding for many years. Realistically, containment eventually will fail as populations colonize inaccessible habitats or simply escape detection.

Management of invasive ants encompasses several control methods that attempt to slow the spread and lessen their impacts on humans and the environment. In urban settings, contact insecticides and baits applied to infested
properties temporarily suppress populations to tolerable levels (DREES & al. 2006). Commodity treatments use insecticides to disinfest host / harborage material and quarantines restrict movement of readily infested articles such as nursery stock (ANONYMOUS 2015). A more sustainable management approach is classical biological control, where natural enemies are introduced and established to reduce pest populations. Ideally, natural enemies will be self-sustaining and spread naturally into untreated or inaccessible populations and significantly reduce the ant population's reproductive rate and dissemination. Lastly, integrated pest management (IPM), utilizes a combination of control methods (e.g., insecticides and biological control) to facilitate near-and long-term suppression of invasive ants.

The biological control of invasive ants is underutilized perhaps because of the emphasis on eradication and chemical control. In addition, the potential for negative, unintended consequences, for example the biocontrol agent becoming a pest, from introducing exotic organisms for biological control should not to be overlooked. Thorough evaluation of host specificity before introductions and post-release monitoring is imperative to minimize and determine non-target impacts (O'CALLAGHAN & BROWNBRIDGE 2009). In some cases, non-targets may serve as hosts and thus the potential benefit of establishing an effective biocontrol agent must be evaluated against the risk of harming non-targets (PORTER & GILBERT 2004). The biological control of invasive ants should be a high priority management option. Here we review the utilization of biological control with natural enemies to suppress the fire ant population in the USA.

Status of potential biological control agents for fire ants in the USA

Solenopsis richteri FOREL, 1909 and Solenopsis invicta were accidentally introduced separately into the USA from South America in 1918 and the 1930s, respectively (LOFGREN 1986, TSCHEINKEL 2006). At the time of these introductions both were considered to be two forms of the same species, and it was not until 1972 that the two forms were formally designated separate species (BUREN 1972). The more prevalent fire ant species in the USA is S. invicta, and together with S. richteri and their hybrid (VANDER MEER 1986), invasive fire ants occupy an estimated 148 million hectares in 2014 (A.-M. Callcott, pers. comm. [based on USA hectares under the Federal Imported Fire Ant Quarantine]).

Surveys and studies on parasites and pathogens of Solenopsis invicta and S. richteri for potential use as biological control agents initially were conducted in the 1960s and 1970s among South American and southern USA populations (ALLEN & BUREN 1974, ALLEN & SILVEIRA-GUIDO 1974, BROOME 1974, JOUVENAZ & al. 1977, JOUVENAZ & al. 1981). These included extensive studies on the parasitic ant S. (Labiачhena) daguerrei (SANTSCHI, 1930) (SILVEIRA-GUIDO & al. 1973), observations of scuttle flies in the genus Pseudacteon attacking ants in the S. saevissima species complex (WILLIAMS & al. 1973), and the discovery of a microsporidium infection in S. invicta (ALLEN & BUREN 1974, KNELL & al. 1977). To date, at least 40 parasites, parasitoids, and pathogens of S. invicta or S. richteri have been reported. This number includes 23 species of fly parasitoids in the genus Pseudacteon; the parasitic ant S. daguerrei; five species of eucharitid wasps in the genus Ornithopus (VARONE & al. 2009, VARONE & al. 2010); the scarab beetle, Myrmarzania dutertrei (CHALMEAU, 1983) (WOCIK 1990); the strepsipteran Caeno-chola ntenysei PIECE, 1909 (KATHIRITHAMBY & JOHNSON 1992, COOK & al. 1997); nematodes Tetradonema solenopsis NICKLE & JOUVENAZ, 1987 and Allomermis solenopsi POINAR, PORTER, TANG & HYMAN, 2007 (NICKLE & JOUVENAZ 1987, POINAR & al. 2007); the microsporidia Knealhazia solenopsae (KNELL, ALLEN & HAZARD, 1977) and Vairimorpha invictae JOUVENAZ & ELLIS, 1986 (KNELL & al. 1977, JOUVENAZ & ELLIS 1986); the neogregarine protozoan Mattesia sp. (PEREIRA & al. 2002); fungi Myrmeconyces amnelliae JOUVENAZ & KIMBROUGH, 1991 (JOUVENAZ & KIMBROUGH 1991) and Myrmicinosporidium durum HOLLDOBLER, 1933 (PEREIRA 2004); Wolbachia and other bacteria specifically associated with fire ants (SHOE-MAKER & al. 2000, BOUWMA & SHOE-MAKER 2011, ISHAK & al. 2011); RNA viruses Solenopsis invicta virus 1, 2, and 3; and the DNA virus Solenopsis invicta densovirus (SiDNV) (VALLES 2012, VALLES & al. 2013b). While their potential as biological control agents is often mentioned, only a limited number of these natural enemies have had their host specificity and/or their life histories investigated. Several reviews provide the chronology of surveys for, and descriptions of natural enemies of fire ants (WILLIAMS & WHITCOMB 1974, JOUVENAZ 1983, WILLIAMS & al. 2003, OI & VALLES 2009, BRIANO & al. 2012, VALLES 2012). In this review, we summarize the evaluations and current status of natural enemies that were considered, are being assessed, or were released for the biological control of fire ants in the USA.

Solenopsis daguerrei, Orasema spp., Allomeris solenopsi, and Mattesia sp. have had various aspects of their life histories and effects on imported fire ants evaluated for their biological control potential. While none of these species are being actively pursued as biological control agents, they are associated with reduced vigor or even death of individual fire ants.

Parasitic ant Solenopsis daguerrei: In the 1960s Silveira-Guido and colleagues described how queens of the parasitic ant Solenopsis daguerrei, attach, or yoke, to the neck of a S. richteri queen and lay eggs simultaneously with the yoked host queen. Both the parasite and host eggs are reared by the fire ant colony and thus the parasite is considered to be a drain on colony resources. Heavily infested fire ant colonies were reported to have fewer major workers and alate reproductives (SILVEIRA-GUIDO & al. 1973). CALCATERA & al. (1999) reported significantly fewer host queens in infested field colonies of S. richteri, as well as, decreased worker brood in the fall and spring. These results implied a detrimental impact of S. daguerrei on fire ant colonies, however, findings were qualified by high variation and the need for more sampling locations of longer duration. Prevalence of S. daguerrei in South American surveys was reported to be low (1.4% of 12,180 colonies) but not unexpected given the behavior of workerless parasitic ants and the sampling methods used (BRIANO & al. 1997). Attempts to propagate Solenopsis daguerrei in the laboratory and field have not been successful, and have precluded further efforts to utilize it as a biological control agent. Transferring S. daguerrei queens to non-parasitized
colonies resulted in execution of the parasite. In addition, the sustained survival of adult reproductives did not occur after the introduction and eclosion of S. daguerrei pupae in non-parasitized colonies (CALTERRA & al. 2001, BRIANO & al. 2002a). It has been speculated that S. daguerrei may be a complex of cryptic species associated with specific host fire ant species. This may contribute to the reported unsuccessful intercolony transfer of this parasite. While low mtDNA sequence divergence among S. daguerrei haplotypes does not support the hypothesis that social parasites co-specified with its hosts, the possibility of the existence of multiple cryptic species cannot be discounted (SHOEMAKER & al. 2006). Certainly a better understanding of the systematics of S. daguerrei could revive consideration of its use as a biological control agent.

Eucharitid wasps in the genus Orasema: Several species of eucharitid wasps in the genus Orasema parasitize Solenopsis fire ants. The life cycle for Orasema xanthopus (CAMERON, 1909), typical of other Orasema species, begins with adult wasps ovipositing in a variety of plants, including crops, apparently causing minor damage on some plant tissues. Planidia (mobile, 1st instar larvae) eclose and attach to foraging worker ants which transport them back to their colony. Within the colony, planidia move to the ant larvae where they develop endoparasitically, then after host pupation, they feed on the pupae as an ectoparasite. Mature Orasema larvae detach from the now deformed host and pupate with the host brood. Orasema adults emerge in host nests and adults exit the nest for mating and oviposition (WILLIAMS & WHITCOMB 1974, HERATY & al. 1993, HERATY 1994). Orasema simplex HERATY, 1993 was the most common species recovered from fire ant nest excavations in surveys across southern South America. The transfer of planidia to laboratory S. invicta ant colonies has been accomplished, but only 12 adults were reared from 385 transfers, a 3.1% rate (VARONE & BRIANO 2009, BRIANO & al. 2012). Intracolony prevalence of parasitism varied widely with reports of an average of 18.3 to 22 Orasema individuals (larvae, pupae, and adults) per parasitized colony, with a maximum of 598 individual per colony (HERATY & al. 1993, VARONE & BRIANO 2009). The impact of Orasema on imported fire ant colonies is not known (HERATY 1994), but appears to be low given the small number of recovered parasites relative to the tens of thousands of fire ants within a colony. The biological control potential of O. simplex, the most studied Orasema species parasitizing S. invicta, is hindered by the broad range of plants used for oviposition (VARONE & BRIANO 2009) and potentially by difficulties in laboratory propagation and minimal effects on fire ant populations.

Nematode Allomeris solenopsi and protozoan Mattesia sp.: Infections in Solenopsis invicta by the nematode Allomeris solenopsi and the neogregarine (protozoan) pathogen Mattesia sp. were discovered in the 2000s (PEREIRA & al. 2002, POINAR & al. 2007). Allomeris solenopsi has an intriguing water dependent life history which is not fully resolved given the paucity of water within an S. invicta nest (POINAR & al. 2007). BRIANO & al. (2012) reported reduced survivorship, aggression, and stinging behavior in infected ants. In addition, field parasitism averaged 52 workers per colony which encompassed a wide variation in the number of infected adults (range: 1 - 500 per colony). Laboratory transmission was accomplished, but at a low rate which will likely hinder evaluation of A. solenopsi for biological control. Unsuccessful laboratory transmission of the Mattesia sp. also has deterred the further evaluation of this protozoan as a biological control despite some promising attributes. The Mattesia sp. is widely distributed in the state of Florida, USA, and there is evidence that infected ants die sooner than healthy ones when observed in the laboratory (PEREIRA & al. 2002). However, with both the nematode and the neogregarine, the intracolony prevalence appears to be limited and the impacts on fire ant colonies are unknown.

Entomopathogenic fungus Beauveria bassiana: Unlike the fire ant natural enemies discussed previously, the fungus Beauveria bassiana (BALSAMO-CRIVELLI) VUILLEMIN, 1912 is a generalist entomopathogen, infecting numerous species across several insect orders. Beauveria bassiana has been isolated from S. invicta in Brazil, with one virulent isolate, #447, evaluated as a potential biological control agent (ALVES & al. 1988, STIMAC & al. 1989, STIMAC & ALVES 1994). Spores of the isolate have been mass produced on rice media and directly applied to individual fire ant nests using various spore formulations. OI & al. (1994) collected infected workers from treated field nests and piles of infected cadavers were observed outside of these nests. However, cadaver piles are indicative of the presence of survivors that removed infected ants from the nests to create the piles. The majority (48 - 100%) of inoculated colonies either relocated or remained active (OI & al. 1994). Bait formulations containing the 447 isolate spores, as well as with alginate encapsulated mycelia using an isolate from the Mexican leaf-cutting ant, Atta mexicana (SMITH, 1858), have also been tested with variable reductions in laboratory and field colonies (COLLINS & al. 1999, STIMAC & PEREIRA 2001, BEXTINE & THORVILSON 2002, THORVILSON & al. 2002, MILNER & PEREIRA 2007). Despite recovery of dead and infected fire ants after B. bassiana applications, other factors such as the hygienic behavior of ants, microbial antagonism, and the fungistatic effect of fire ant venom may limit the intracolony transmission of the pathogen (STOREY & al. 1991, PEREIRA & STIMAC 1992, OI & PEREIRA 1993). Thus, the pathogen may be more amenable as a biopesticide requiring extensive spore contact with the colony. In addition, the intercolony spread between treated and untreated nests has not been documented and have discouraged the further pursuit of B. bassiana as a biological control agent.

Microsporidium Vairimorpha invictae: Currently under consideration for the biological control of fire ants is the microsporidian pathogen Vairimorpha invictae. This pathogen was presumed first collected in Solenopsis invicta from Brazil in 1976 and later described in 1986 (OUVENAZ & al. 1980, JOUVENAZ & ELLIS 1986). Fluctuating declines in field populations of S. invictae, which included 100% reductions for over 31 months, were associated with natural infections of V. invictae in Argentina (BRIANO 2005). Evidence of V. invictae pathogenicity include laboratory studies documenting dead workers having higher infection prevalence than live workers in diseased colonies, and faster mortality of starved infected workers than starved, uninfected workers (BRIANO & WILLIAMS 2002). In addition, small laboratory colonies infected with V. invictae had reductions in colony growth of greater than 80% (OI & al. 2005). The host range of V. invictae is limited to...
fire ants in the *S. saevissima* species group, which includes *S. invicta* and *S. richteri*. Host range was based on field surveys in South America and laboratory host specificity tests on North American *Solenopsis* fire ants (BRIANO & al. 2002b, PORTER & al. 2007, OI & al. 2010).

The natural mechanism of intracolony and intercolony transmission of *Vairimorpha invictae* is unknown. Detection of infected eggs and queens has been rare, thus the importance of transovarial transmission for *V. invictae* is uncertain (BRIANO & WILLIAMS 2002). However, *V. invictae* can be transmitted by adding live, infected brood or dead, *V. invictae*-infected adults into *S. invicta* colonies (OI & al. 2005). Infestation by isolated spores has not been accomplished, but pupae became infected when reared from larvae by infected adult workers (JOUVENAZ & ELLIS 1986, OI & al. 2010).

Potential limitations to the utilization of *Vairimorpha invictae* as a biological control agent are its somewhat low field prevalence among colonies (Tab. 1), although epizootics at some sites have been reported. Epizootics of over 50% have been followed by abrupt declines in infection prevalence, and at some sites *V. invictae* was not detected for several years (BRIANO & WILLIAMS 2002, BRIANO 2005, BRIANO & al. 2006, BRIANO & al. 2012). The fluctuating presence of *V. invictae* has made it challenging to find and maintain consistent sources of inoculum. Similarly, consistent laboratory propagation, which is necessary for field releases, has yet to be achieved. Furthermore, constant changes in regulatory requirements to export biological control agents have significantly diminished access to *V. invictae*. Nevertheless, efforts are continuing to gain approval to employ this pathogen of fire ants as a classical biological control agent in the USA.

**Pathogens and parasites used for the biological control of fire ants in the USA**

To date eight natural enemies have been released or distributed as self-sustaining biological control agents of fire ants in the USA. Two pathogens found in South America, the microsporidium *Kneallhazia solenopsae* and the RNA virus *Solenopsis invicta* virus 3 VALLES & HASEMOTO, 2009, were detected in *Solenopsis invicta* populations in the USA and have since been introduced into uninfected populations in geographically dispersed sites. The classical biological control approach of importing, releasing, and establishing a natural enemy from the native range of *S. invicta* and *S. richteri* has been accomplished with parasitoid phorid flies in the genus *Pseudacteon*. A total of six *Pseudacteon* species from South America have been imported and established in USA populations of fire ants. Below we discuss the background and current status of the biological control of fire ants with all of the above agents.

**Parasitoid phorid flies in the genus Pseudacteon:** At least 24 species of *Pseudacteon* flies attack fire ants in South America (PORTER & PESQUERO 2001, PATRICK & al. 2009). Females hover several millimeters above fire ant workers before diving in and rapidly injecting an egg into the ant thorax with a short hypodermic needle-like ovipositor. After eclosion, the maggot makes its way to the head of the fire ant worker, where it develops and decapitates its host prior to pupariation (PORTER 1998, CONSOLI & al. 2001). The maggot then pushes the mandibles aside and pupariates inside the empty head capsule. After several weeks, adult flies emerge ready to mate and attack more fire ants. Only a single fly emerges per parasitized ant, with egg to adult development time ranging from four to 12 weeks depending on temperature and species. Adult flies live only a few days and a female can have 100 - 200 eggs (PORTER & al. 1997a, PORTER 1998, ZACARO & PORTER 2003, CHEN & FADAMIRO 2006).

Host specificity testing of *Pseudacteon* species considered for introduction into the USA was reviewed in detail by PORTER & GILBERT (2004). Six categories of host range studies were conducted: 1) literature searches, which provide field host data as well as geographic distributions; 2) field observations on behavior and ecology to supplement literature data; 3) field testing in the native range to confirm literature documentation of general host specifi-

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**Tab. 1: Percent prevalence of microsporidian fire ant pathogens** *Kneallhazia solenopsae* and *Vairimorpha invictae* for sites and among colonies in various South American surveys.

<table>
<thead>
<tr>
<th>Survey</th>
<th>Species</th>
<th>% Prevalence, Sites (n infected / n sampled); Colonies (n infected / n sampled)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1988</td>
<td></td>
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<tr>
<td>Buenos Aires province</td>
<td><em>S. richteri</em>, <em>S. quinquoccuspis</em></td>
<td>25 (46 / 185); 8 (145 / 1836)</td>
<td>BRIANO (1993)</td>
</tr>
<tr>
<td>1993</td>
<td><em>S. invicta</em></td>
<td>2 (– / 18 sites)</td>
<td>BRIANO &amp; al. (2012)</td>
</tr>
<tr>
<td>Cuiabá, Brazil</td>
<td><em>S. invicta</em>, <em>S. richteri</em>, <em>S. interrupta</em></td>
<td>6.6 (4 / 61)</td>
<td>BRIANO &amp; al. (2006)</td>
</tr>
<tr>
<td>2001 – 2005</td>
<td><em>S. invicta</em>, <em>S. richteri</em>, <em>S. interrupta</em></td>
<td>7.7 (6 / 78); 2.8 (8 / 284)</td>
<td>BRIANO &amp; al. (2006)</td>
</tr>
<tr>
<td>Central &amp; Northern provinces, Argentina</td>
<td><em>S. invicta</em>, <em>S. richteri</em>, <em>S. interrupta</em></td>
<td>32 (59 / 184); 15 (262 / 1780)</td>
<td>BRIANO &amp; al. (2006)</td>
</tr>
<tr>
<td>2001 - 2005</td>
<td><em>S. invicta</em>, <em>S. richteri</em>, <em>S. guyi</em></td>
<td>4.6 (7 / 153); 1.1 (7 / 629)</td>
<td>BRIANO &amp; al. (2006)</td>
</tr>
<tr>
<td>Brazil*, SE Paraguay, southcentral Bolivia, central Chile</td>
<td><em>S. invicta</em>, <em>S. richteri</em>, <em>S. guyi</em></td>
<td>6.6 (4 / 61)</td>
<td>BRIANO &amp; al. (2006)</td>
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city to fire ants (tests with congeners are particularly useful if they occur in the USA); 4) laboratory testing in quarantine; 5) testing for attraction to an assortment of foods to determine if flies could be a nuisance or potential disease vector; and 6) post-release field testing to confirm pre-release host specificity determinations.

Laboratory tests in quarantine focused on the tropical fire ant, Solenopsis geminata (FABRICIUS, 1804) and the southern fire ant, S. xyloni MCCOOK, 1880, which are native to the USA and similar in size to S. invicta and S. richteri. In theory (BRIESE 2005), flies that do not attack fire ant congeners are unlikely to attack other ant genera. If flies developed in either of the native fire ants, then additional non-Solenopsis ants were tested. No-choice tests were utilized initially to determine if attacks would occur without alternative hosts. Paired choice tests between Solenopsis species were conducted to assess whether the presence of the preferred host facilitated or suppressed attacks on a secondary host. If ant species were observed to be attacked, they were maintained for four to five weeks and checked for evidence of parasitism (PORTER & GILBERT 2004).

Pseudacteon species released in the USA had high host specificity to Solenopsis invicta, S. richteri, and/or their hybrid. Some species could develop in S. geminata and S. xyloni in the laboratory. However, the high preference for the target hosts, and the relative benefit of releasing flies against the invasive fire ants species which have ex-istence for Pseudacteon flies against the invasive fire ants species which have ex-
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sistence for Pseudacteon flies against the invasive fire ants species which have ex-

The impact of the parasitic phorid flies on fire ants at the colony level is associated with the behavioral reactions of the ants to the presence of flies. The presence of a single fly usually stops or greatly inhibits the foraging efforts of hundreds of workers (FEENER & BROWN 1992, ORR & al. 1995, PORTER & al. 1995). Reduced foraging appears to facilitate competition from ants that might otherwise be excluded from food sources in fire ant territories (FEENER 1981, ORR & al. 1995). In contrast, maximum direct parasitism of fire ants was reported to be < 1% for phorid species released in Florida and < 3% for assemblages of native phorid species in Argentina (MORRISON & PORTER 2005a, CALCATERA & al. 2008, VALLES & al. 2010b). More recent data suggest that up to 5% of colony workers can be parasitized (S.D. Porter, unpubl.). Given that a colony can easily contain over a hundred thousand fire ant workers (TSCHINNEL 1988), the effect of direct parasitism on colony populations is presumably limited.

MORRISON & PORTER (2005b) reported that the effects of a single species of fly, Pseudacteon tricuspis, did not rise above background variability in the field (10 - 30%). Assessments of colony level impacts by single species of phorid flies in the laboratory have resulted in contrasting conclusions. Inhibition of fire ant foraging in the presence of flies together with a competing ant species resulted in less protein consumption by colonies, smaller numbers of intermediate-sized workers, and significantly decreased worker populations (MEHDIABADI & GILBERT 2002). In contrast, MOTTERN & al. (2004) reported that significant inhibition of foraging did not affect colony growth; however, an unexplained 50% mortality of their test colonies during four weeks of observations almost certainly masked any effective assessment of fly effects on colony growth. Furthermore, whatever impact Pseudacteon flies have on fire ant populations it is clearly sufficient to have caused the evolution and maintenance of a number of phorid-specific defense behaviors such as seeking refuge, curtailing movement, and assuming specialized defensive postures (Fig. 1) (WILLIAMS & BANKS 1987, FEENER & BROWN 1992, PORTER & al. 1995, WUELLNER & al. 2002). These behaviors could have only evolved if Pseudacteon flies have population-level impacts on the survival of fire ant colonies or the production of the reproductive caste (PORTER & al. 1995, PORTER 1998). Alternatively, MORRISON (2012) contended that the regulation of fire ant populations by Pseudacteon parasitoids is unlikely because compensating behaviors of fire ants allows them to acquire sufficient nutrition despite reduced foraging when flies are present. Compensating behaviors include foraging when flies are not active (e.g., at night), utilizing underground foraging tunnels that limit their accessibility to flies, and guarding food resources from competitors even when flies arrive (PORTER & al. 1995). In fact, the proliferation of the introduced Pseudacteon flies in the USA may simply be a product of the availability of abundant host populations of fire ants (MORRISON 2012).
Tab. 2: Traits of *Pseudacteon* species established in the USA for fire ant biological control (adapted from PORTER & CALCATERA 2013). SD = standard deviation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Average female thorax width (mm ± SD, n = 10)</th>
<th>Host size preference</th>
<th>Year of first successful release</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. tricuspis</em></td>
<td>0.51 ± 0.03</td>
<td>medium-large</td>
<td>1997</td>
<td>PORTER &amp; al. (2004)</td>
</tr>
<tr>
<td><em>P. curvatus</em></td>
<td>0.33 ± 0.03</td>
<td>small</td>
<td>2000 Las Flores; 2003 Formosa biotypes</td>
<td>GRAHAM &amp; al. (2003)</td>
</tr>
<tr>
<td><em>P. litoralis</em></td>
<td>0.57 ± 0.02</td>
<td>large</td>
<td>2005</td>
<td>PORTER &amp; al. (2011)</td>
</tr>
<tr>
<td><em>P. obtusus</em></td>
<td>0.53 ± 0.02</td>
<td>medium-large</td>
<td>2006</td>
<td>PLOWES &amp; al. (2011)</td>
</tr>
<tr>
<td><em>P. nocens</em></td>
<td>0.49 ± 0.05</td>
<td>medium-large</td>
<td>2006 - 2010</td>
<td>PLOWES &amp; al. (2012)</td>
</tr>
<tr>
<td><em>P. cultellatus</em></td>
<td>0.33 ± 0.02</td>
<td>small</td>
<td>2010</td>
<td>PORTER &amp; al. (2013a)</td>
</tr>
</tbody>
</table>

Despite the inconclusive determination of *Pseudacteon* impact on fire ants at the colony level, the flies have been established in the USA as classical biological control agents. In addition to being highly host specific, of critical importance was the development of efficient rearing methods to provide flies for studies and releases. Methods initially entailed exposing fire ant colonies in trays to flies in anticipation of mating and oviposition, in the laboratory on North American *Solenopsis invicta* and the *S. invicta X S. richteri* hybrid (PORTER & al. 1997a). Innovative rearing methods, producing in controlled environments, are hereby exploitable brood tending behavior to overcome the defensive reactions to avoid *Pseudacteon* attacks, have resulted in successful mass rearing operations that can produce thousands of flies per day (PORTER 2000, VOGT & al. 2003). Modifications in rearing and release methods continued to be adopted in response to the nuances in behavior and traits of the various *Pseudacteon* species (GILBERT & al. 2008, CALLCOTT & al. 2011, PLOWES & al. 2011, PORTER & al. 2013a).

In South America, different species of decapitating flies partition niche space by host, host size, season, time of day, and host location (CÂMPOLO & al. 1994, FOWLER & al. 1995, PESQUERO & al. 1996, MORRISON & al. 1997, ORR & al. 1997, FOLGARAT & al. 2003, FOLGARAT & al. 2007). Similarly, the six species established in the USA (Tab. 2) parasitize different sized workers, and attack at different times of the day and host location (i.e., nests vs. foraging trail).

*Pseudacteon tricuspis* and *P. curvatus* are now widely distributed across the southeastern USA (CALLCOTT & al. 2011). Densities of *P. curvatus* are about 10-fold those of *P. tricuspis*, probably because of the greater abundance of small workers in fire ant colonies. *Pseudacteon tricuspis* parasitizes the less prevalent medium sized workers. A third species, *P. litoralis* BORGMEIER, 1925, which attacks large fire ants, was previously uncommon around its release site in Alabama (PORTER & al. 2011), but as of 2013 was found to be abundant and has expanded outward dozens of kilometers (L.C. Graham, pers. comm.). A fourth species that targets medium-large workers on mounds and at foraging trails, *Pseudacteon obtusus* BORGMEIER, 1925, has been established at multiple sites in Texas and Florida (GILBERT & al. 2008, PLOWES & al. 2011, PORTER & CALCATERA 2013). This species is also abundant and rapidly expanding even in competition with *P. curvatus* (PORTER & CALCATERA 2013). A fifth species, *P. nocens* BORGMEIER, 1925, has been established in Texas where it attacks large fire ant workers especially during twilight (PLOWES & al. 2012), unlike the other released species, besides *P. litoralis*, which are diurnal. The sixth established species, *P. cultellatus* BORGMEIER, 1925, is a very small decapitating fly which parasitizes the smallest of fire ant workers. It has been recovered at two sites in Florida from 2010 through the last survey in 2013, where populations remain low (PORTER & al. 2013a). As previously indicated, one purpose of introducing several species of *Pseudacteon* was to exploit their varied activity patterns and host preferences to increase the parasitism pressure on the fire ants. While co-occurrence of several fly species in the USA is documented, there is evidence of competitive displacement among some of the species. As mentioned above, *P. curvatus*, which was released after *P. tricuspis*, has become the most abundant species, while *P. tricuspis* is becoming rare in some locations (LEBRUN & al. 2009, PORTER & al. 2013a). Likely mechanisms of the displacement are that *P. curvatus* parasitizes the more prevalent minor fire ant workers, thus it is more abundant. Moreover, the abundant *P. curvatus* will arrive at disturbed nests first and illicit the refuge seeking response by the fire ants which makes the less prevalent major workers even less available for attacks by *P. tricuspis* (LeBRUN & al. 2009, PORTER 2010). Nevertheless, the introduction of several species and bio-types of biocontrol agents and subsequent displacement of the less efficient or poorly adapted agent(s) with a better agent(s) is a common tenant of successful classical biological control (HUFFAKER & al. 1976).

**Microsporidium pathogen Kneallhazia** (formerly *Thelohania*): The fire ant pathogen *Kneallhazia solenopsae* was discovered in "cyst-like" bodies in alcohol-preserved specimens of *Solenopsis invicta* collected in Brazil and described as a new species, *Thelohania solenopsae* KNELL, ALLEN & HAZARD, 1977 (ALLEN & BUREN 1974, KNELL & al. 1977). It has since been placed in a new genus *Kneallhazia* (SOKOLOVA & FUXA 2008). Over 20 years later (1996), *K. solenopsae* was observed in *S. invicta* samples collected in Florida, USA (WILLIAMS & al. 2003). *Kneallhazia solenopsae* and other microsporidia were considered to be protozoa, but have been reclassified as a phylum within the kingdom Fungi (SOLTER & al. 2012). Similar to other entomopathogenic microsporidia, *K. solen-
K. solenopsae causes a chronic, debilitating disease. Four spore types have been identified, but the functions of each as well as the life cycle are not definitively known (Kneall & al. 1977, Shapiro & al. 2003, Sokolova & al. 2004, Sokolova & Fuxa 2008). All castes and developmental stages of the fire ant can harbor K. solenopsae infections. Infections in queens as well as in eggs provide evidence of transovarial transmission (Briono & al. 1996, Valles & al. 2002, Sokolova & Fuxa 2008). Furthermore, infected colonies have been reared from naturally infected S. invicta queens collected after mating flights (Oi & Williams 2003).

Intercolony transmission of Kneallhazia solenopsae has been accomplished in the laboratory and field by transferring live, infected larvae and/or pupae into the nests of uninfected colonies (Williams & al. 1999, Oi & al. 2001, Oi & al. 2008). However, the mechanism of infection between colonies and between individual workers or queens is not known. Inoculations with suspensions of isolated spore types per os have been unsuccessful (Shapiro & al. 2003) and transmission by the ingestion of spore containing fecal fluid and meconium has been proposed but not confirmed (Chen & al. 2004). One potential pathway of intercolony transmission was demonstrated in the laboratory through colony raiding, i.e., the abduction of brood from small infected colonies by large, uninfected colonies (Oi & Williams 2003).

Based on extensive field surveys in South America, the host range of Kneallhazia solenopsae was limited to six species of Solenopsis fire ants, all in the S. saevissima species group, which includes S. invicta and S. richteri (Oi & Valles 2009). Species-specificity tests in the USA with laboratory colony inoculations and field sampling within K. solenopsae infected areas in Florida did not find infections in 15 non-S. invicta ant species (Oi & Valles 2012). However, infections have since been detected in S. geminata and the S. geminata × S. xyloni hybrid, both fire ants in the S. geminata species group. Examination of the genetic diversity of the K. solenopsae infecting these species in North and South America supported the existence of two major clades, with most of the K. solenopsae isolates from S. geminata in one clade (Ascunce & al. 2010). Interestingly, Moser & al. (2000) reported that K. solenopsae in S. invicta from Florida and Brazil, and S. richteri from Argentina were all genetically distinct, but closely related and indistinguishable morphologically. Further studies are needed to confirm if the different K. solenopsae host isolates differ biologically (e.g., pathogenicity among host species).

Colony declines and population reductions have been documented in Kneallhazia solenopsae infected Solenopsis invicta and S. richteri. Infections in inoculated laboratory colonies of S. invicta resulted in lower queen weights, diminished oviposition rates, and faster queen death. Significant reductions in brood and overall colony decline also were observed in infected colonies (Williams & al. 1999, Oi & Williams 2002). Additionally, colony founding by infected queens is impaired, possibly because of reduced lipid reserves (Cook & al. 2003, Oi & Williams 2003, Overton & al. 2006, Preston & al. 2007). Field population reductions associated with K. solenopsae were reported for S. invicta in the USA (Cook 2002, Oi & Williams 2002, Fuxa & al. 2005b) and S. richteri in Argentina (Briono & al. 1995b). For both hosts, population reductions fluctuated over time, with maximum reductions of 63% in the USA and 83% in Argentina. Reductions were often attributed to smaller colony sizes as opposed to declines in the number of fire ant nests (Oi & Valles 2009).

Social form (i.e., monogyny or polygyny) appears to play an important role in moderating the effect of Kneallhazia solenopsae on fire ant populations. Brood reductions of > 90% were recorded approximately six months after monogyny laboratory colonies were inoculated, while in polygynous colonies similar reductions occurred in about 11 months (Williams & al. 1999, Oi & Williams 2002). In the field, K. solenopsae is more prevalent in polygynous Solenopsis invicta populations, although monogynous colonies are infected (Oi & al. 2004, Valles & Briono 2004, Fuxa & al. 2005b, Milks & al. 2008, Valles & al. 2010b). The non-territorial behavior of polygynous colonies (Glancy & al. 1975) permits movement of infected ants between colonies thus allowing for the intercolony spread of the pathogen. Conversely, the interchange of uninfected
ants, including the adoption of queens (GLANCEY & LOFGREN 1988), may contribute to the prolonged decline in infected polygyne colonies. In addition, asynchronous infections among the many queens per colony allows for the production of uninfected progeny (OY & WILLIAMS 2002). Hence, greater intercolony spread and persistent intracolony infections result in easier detection of *Solenopsis* in polygyne populations.

In contrast, infections in the territorial monogyne colonies can be isolated and demise of the single infected queen results in a rapid colony decline. Thus, opportunities for companies can be isolated and demise of the single infected queen (FUXA & al. 2005a, OY 2006). However, epizootics in monogyne populations have occurred, but the pathogen was not sustained (FUXA & al. 2005b). In limited sampling from Argentina, the prevalence of *K. solenopsae* in monogyne *Solenopsis invicta* was 55%, and it was speculated that a genetic basis or an intermediate host were responsible for the high prevalence (VALLES & BRIANO 2004).

*Kneallhazia solenopsae* has been distributed as a biological control agent by harvesting live, infected brood collected in Florida and introducing it into ten states in the USA from 1998 - 2004. Establishment and spread was documented in five sites, primarily in polygyne sites (WILLIAMS & al. 1999, FUXA & al. 2005b; D.H. OY, unpubl.). Subsequent surveys and arbitrary examinations of fire ants from study sites indicated *K. solenopsae* is widespread (Fig. 2) (STREET & al. 2004, MITCHELL & al. 2006, ALLEN & al. 2010, ASCUNCE & al. 2010; D.H. OI, unpubl.), and was in Texas at least since 1984 (SNOWDEN & VISON 2006), well before its initial USA detection in 1996. The slow demise of laboratory colonies and the fluctuating reductions in infected fire ant populations suggest that field declines caused by *K. solenopsae* could be obscured by the immigration of colonies or the founding of new colonies.

**RNA virus, *Solenopsis invicta virus-3***: Viruses can be important biological control agents against pest insect populations (LACEY & al. 2001). However, extensive searches for viruses infecting *Solenopsis invicta* in the introduced (USA) and native (South America) ranges using traditional microbiological methods had failed to identify ant-specific viruses (JOUVENAZ & al. 1977, JOUVENAZ & al. 1981, JOUVENAZ 1983, WOICK & al. 1987). With the exception of "virus-like particles" observed in an unidentified species of *Solenopsis* from Brazil (AYER & al. 1977), no viruses had been described by these methods. Conversely, the metatranscriptomics approach (VALLES & al. 2008) has resulted in the discovery of four unique fire ant viruses: *Solenopsis invicta* virus 1 (SINV-1) (VALLES & al. 2004), *Solenopsis invicta* virus 2 (SINV-2) (VALLES & al. 2007), *Solenopsis invicta* virus 3 (SINV-3) (VALLES & HASHIMOTO 2009), and *Solenopsis invicta* densivirus (SiDNV) (VALLES & al. 2013b). These discoveries represent the first described viruses known to infect insects in the family Formicidae. SINV-1, -2, and -3 are positive-sense, single-stranded RNA viruses, each with a unique genomic architecture. SINV-1 has been placed taxonomically into the order Picornavirales, family Dicistroviridae, genus *Aparavirus*. SINV-2 and SINV-3 are currently unclassified (KING & al. 2012). Unofficially, SiDNV (a DNA virus) belongs in the family Paroviridae, subfamily Densovirinae (VALLES & al. 2013b).

SINV-1, -2, and -3 are found currently in the USA and Argentinean fire ant populations while SiDNV is limited to Argentinean populations. SINV-1 and -2 appear to cause chronic infections under normal conditions with no clearly defined etiology described to date. However, when SINV-1- or SINV-2-infected ant colonies experience certain stressors, the viruses often lead to colony member mortality (VALLES & al. 2007). Conversely, SINV-3 appears to be consistently associated with significant fire ant colony mortality (VALLES & HASHIMOTO 2009) reminiscent of colony collapse disorder in honey bees – which is also associated with a positive sense, single-stranded RNA virus (DI PRISCO & al. 2011). Thus, research efforts have focused on development of SINV-3 as a classical biological control agent and as a biopesticide. Current efforts are also underway to characterize, determine host specificity, import, and release SiDNV into the USA as a classical biological control agent (S.M. VALLES & S.D. PORTER, unpubl.).

Molecular characterization of USA and Argentinean SINV-3 revealed a positive-sense, single-stranded RNA comprised of 10,386 nucleotides, and polyadenylation at the 3′ terminus with minor intercontinental differences noted (VALLES & HASHIMOTO 2009, VALLES & al. 2010a). The genome revealed two large open reading frames (ORFs) in the sense orientation with an untranslated region (UTR) at each end and between the two ORFs. The 3′-proximal ORF2 of the genome was shown to be expressed via ribosomal frameshifting and the sequences encoding the structural proteins map to both ORF2 and the 3′ end of ORF1 (VALLES & al. 2014a). This genome organization and structural protein expression strategy resemble that of *Acrystaphthon pism* virus (APV), an aphid virus. SINV-3 was also shown to produce subgenomic RNA to express its structural proteins suggesting a distant relationship to members of the family Caliciviridae (VALLES & al. 2014a).

SINV-3 was found to infect all life stages of *Solenopsis invicta*, including the eggs. Quantitative PCR of different stages showed that immature ants contained significantly lower quantities of SINV-3 compared with adults (workers, alates and queens) (VALLES & HASHIMOTO 2009). Time-course experiments further revealed infection to be specific to adult workers, while SINV-3 did not appear to replicate in larvae (VALLES & al. 2014b).

SINV-3 has been confirmed as the etiological agent causing significant mortality in fire ant laboratory colonies (VALLES & al. 2014b). Fire ant colonies infected with SINV-3 exhibit a characteristic etiology, including the cessation of feeding / retrieval of solid food, brood and worker mortality (with a corresponding increase in SINV-3 titre), decreased egg production / ovary wasting and finally colony collapse. We hypothesize that this etiology is all based on altered worker behavior which prevents them from acquiring and / or distributing food to the larvae and queen, resulting in malnourishment of colony members (VALLES & al. 2014b).

In order to establish the host range of SINV-3, 23 species of ants in 14 genera and four subfamilies were exposed to this virus (PORTER & al. 2013b, PORTER & al. 2015). Despite extreme exposure to the virus, active, replicating infections occurred only in colonies of *Solenopsis invicta* and the *S. invicta × S. richteri* hybrid. These results strongly support a conclusion that SINV-3 can be safely used as either a biopesticide or a self-sustaining bio-
logical control agent in regions of the world where this virus does not occur. However, as a precaution for other regional introductions, further host specificity testing may be required with local native ants.

Bait formulations were prepared by homogenizing live and frozen infected Solenopsis invicta (all developmental stages and castes) and mixing them with either 10% sugar solution, cricket paste, or soybean oil adsorbed to defatted corn grit. All formulations effectively transmitted SINV-3 infections to S. invicta colonies (VALLES & al. 2013a). Fire ant colonies exposed to a single 24 hour pulse treatment of SINV-3 became infected with the virus regardless of the bait formulation. However, sugar and cricket bait-treated colonies became infected more rapidly than oil-treated colonies. Sugar and cricket bait-treated colonies exhibited significant declines in their brood ratings compared with the untreated control and oil-bait-treated colonies. Significant declines in the quantity of live workers, live brood, total colony weight, proportion of larvae and queen weight were reported in SINV-3-treated colonies. Thus, SINV-3 is amenable to bait formulation – the preferred method of toxin dissemination for ant control. In addition, transmission of SINV-3 to field colonies of S. invicta in Florida has been reported, using infected ant homogenate in 5% sucrose solution (VALLES & OI 2014). While methods for large area inoculations need to be developed, local inoculative releases have been successfully accomplished with the sucrose solution formulation in California (S.M. Valles & D.H. Oi, unpubl.). Current studies are underway to evaluate the impact of the virus among field colonies.

Development and use of RNA viruses as insect control agents has been proposed (SCOTTI & al. 1981, CHRISTIAN & SCOTTI 1998) and demonstrated under controlled conditions for a limited number of insect pests (PLUS & SCOTTI 1984, MANOUSIS & MOORE 1987). A major limitation of the use of RNA viruses in insect control is large-scale production of virus. Virus production can be accomplished if a host cell line is available. However, insect host cell lines supporting viral production are available for only a handful of viruses. Indeed, the absence of a fire ant cell line has hampered development of SINV-3 as a biopesticide or to improve the production of virus particles for inoculative releases. Attempts to utilize a baculovirus-driven expression system to produce SINV-3 infectious particles in quantity have been conducted (H.R. Allen & S.M. Valles, unpubl.). A full-length copy of the SINV-3 genome was assembled successfully and a heterologous bacmid-SINV-3 vector was produced. The insect cell line Spodoptera frugiperda 21 (Sf21) supported production of baculovirus expressing full-length SINV-3 transcripts. However, while successful transcription of the SINV-3 genome occurred, evidence that translation was occurring could not be produced. Thus, production of SINV-3 remains the major obstacle for use of this virus as a biopesticide.

Fire ant biological control and IPM

The classical biological control of fire ants offers a self-sustaining, long-term, non-pesticide alternative capable of negatively impacting fire ants across their entire introduced range. Fire ant populations in the presence of biological control can be smaller based on estimated number of ants per nest, even though entire colonies within nests are not exterminated (OI & WILLIAMS 2002, FUXA & al. 2005b). Moreover, with the current biocontrol agents it must be emphasized that a realistic goal is to weaken, instead of eliminate, the majority of fire ant colonies (FUXA & al. 2005b). It remains to be seen if the continued release and establishment of a suite of biological control agents can result in the sustained suppression of fire ants such that their populations are not perceived to be an important pest (i.e., complete control).

One measure of weakened fire ant populations is reducing the rate of recolonization within the context of integrated pest management. When an area is cleared of fire ants using insecticides, it is normally re-infested within months by the immigration of colonies from the surrounding area as well as from colonies founded after mating flights (COLLINS & al. 1992). Establishment of biological control agents in untreated, or unmanaged, landscapes has been used to slow the reinfection of landscapes cleared of fire ants. Reinfestation was delayed by over a year where Kneallhazia solenopsae and Pseudacteon tricuspid were released and established (OI & al. 2008). This concept was extended to large scale “area-wide” demonstration sites (120 ha in five states) and resulted in greater fire ant suppression where biocontrols and fire ant baits were combined relative to bait applications alone (PEREIRA 2003, VANDER MEER & al. 2007).

The introduction and establishment of different types and species of fire ant specific parasites and pathogens provides opportunities for interactions among these biocontrol agents. The effects of these interactions can be unpredictable, perhaps resulting in displacement (e.g., Pseudacteon tricuspid by P. curvatus) or potentially be an enhancement (P. obtusus and P. curvatus) (LEBRUN & al. 2009, PORTER & CALCATERA 2013). Four species of the Pseudacteon flies can acquire Kneallhazia solenopsae by parasitizing infected Solenopsis invicta. Infection by K. solenopsae is apparently not detrimental to Pseudacteon development and infected flies have been field collected widely in Louisiana and Florida (OI & al. 2009, VALLES & al. 2009, MÉSZÁROS & al. 2014). It has been speculated that the flies may vector K. solenopsae, but transmission has not occurred in preliminary studies (D.H. Oi, S.D. Porter & S.M. Valles, unpubl.). Conversely, SINV-1 was not acquired nor appeared to be mechanically vectored by Pseudacteon flies (VALLES & PORTER 2007). All three types of fire ant biocontrols (virus, microsporidium, phorid) can co-occur within the same field colony and their presence does not seem to preclude or predispose infections or parasitism (VALLES & al. 2010b).

Fire ants dominate broad expanses of natural, agricultural, and urban habitats which makes it difficult, if not impossible, to implement a single strategy for effective control. The classical biological control approach of establishing natural enemies and relying on self-dissemination and proliferation is likely the most sustainable method of suppressing widely distributed invasive species like fire ants (PORTER & al. 1997b). Depending on the level of tolerance to the presence of fire ants, an IPM approach may be required, where biological controls are integrated with insecticide and/or alternative control measures. With the spread of fire ants to other countries throughout the world, there are further opportunities to utilize classical biological control. Indeed, Kneallhazia solenopsae and SINV-3

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were not detected in surveys of *Solenopsis invicta* in Australia, New Zealand, China, and Taiwan (Yang & al. 2010), and a project to release the two pathogens and *Pseudacteon* flies in Taiwan is in progress. Similarly, *Pseudacteon* flies, *K. solenopsae*, and SINV-3 have been successfully released in 2014 in the Coachella Valley of California. Prior to the releases, there were no flies, and a paucity of pathogens in *S. invicta* populations that were sustained by irradiation. It was encouraging to recover flies and the pathogens, which apparently have survived the extreme summer temperatures and established in this urbanized, desert habitat (D.H. Oi, S.D. Porter & S.M. Valles, unpubl.).

Classical biological control implemented in the USA for fire ants is the product of decades of past and ongoing research. New knowledge and technologies have and will continue to improve the implementation of biological control for invasive ants. The source population of USA fire ants has been redefined (Caldera & al. 2008) and the worldwide invasion history of fire ants determined (Ascunce & al. 2011). These findings have guided current explorations for additional biological control agents to the source populations of the introduced USA fire ants. Invasion history for more recent fire ant incursions, for example Taiwan, also supports the utilization of the fire ant natural enemies established in the USA as a source of biocontrol agents.

This review attempts to capture pertinent aspects of the research conducted to implement classical biological control of *Solenopsis invicta* in the USA. Fire ant biological control is an ongoing process, with documented successes in establishing natural enemies and demonstrating population reductions of fire ants. Fire ants have invaded two additional continents in the new millennium, and with fire ant incursions reported in Europe (Fernández-Meléndez & al. 2007), further geographic range expansion of this pest seems inevitable. Invaded locations without the considerable resources needed for eradication, could conceivably afford and benefit from the classical biological control approach. Likewise, eradication programs deemed unsuccessful or unsustainable, could transition to classical biological control. The fire ant biocontrol in the USA has served as an impetus to initiate the search for biological control agents for other invasive ant species. Metagenomic surveys for pathogenic biocontrols have identified viruses infecting the tawny crazy ant, *Nylamberia fulva* (Mayr, 1862), another invasive ant currently plaguing the southern USA (Valles & al. 2012). Coupling new technologies with the logistical knowledge gained from establishing biological control agents of fire ants will allow for faster progress in the utilization of biological control for other invasive ants.

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