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

David H. OI, Sanford D. PORTER & Steven M. VALLES

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.

David H. Oi (contact author), Sanford D. Porter & Steven M. Valles, United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, Florida, USA. E-mail: david.oi@ars.usda.gov

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, KRUSHELNYCKY & 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, TSCHINKEL 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 1960's and 1970's 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. (Labauchena) 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 *Orasema* (VARONE & BRIANO 2009, VARONE & al. 2010); the scarab beetle, *Martineziana dutertrei* (CHALUMEAU, 1983) (WOJCIK 1990); the strepsipteran *Caenocholax fenyesi* PIERCE, 1909 (KATHIRITHAMBY & JOHNSTON 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 *Kneallhazia 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 *Myrmecomyces annellisae* JOUVENAZ & KIMBROUGH, 1991 (JOUVENAZ & KIMBROUGH 1991) and *Myrmicinosporidium durum* HÖLLDOBLER, 1933 (PEREIRA 2004); *Wolbachia* and other bacteria specifically associated with fire ants (SHOEMAKER & al. 2000, BOUWMA & SHOEMAKER 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). CALCATERRA & 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 (CALCATERRA & 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-speciated 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 presumably first collected in *Solenopsis invicta* from Brazil in 1976 and later described in 1986 (JOUVENAZ & al. 1980, JOUVENAZ & ELLIS 1986). Fluctuating declines in field populations of *S. invicta*, 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

Tab. 1: Percent prevalence of microsporidian fire ant pathogens *Kneallhazia solenopsae* and *Vairimorpha invictae* for sites and among colonies in various South American surveys. [a] – Not available; [b] *Solenopsis interrupta* SANTSCHI, 1916 infected with *K. solenopsae;* [c] small area near Brazil / Argentine border north of Misiones Province, Argentina; [d] *Solenopsis gayi* (SPINOLA, 1851) collected in Chile not infected.

Survey	Species	% Prevalence, Sites (n infected / n sampled); Colonies (n infected / n sampled)		Reference
		K. solenopsae	*V. invictae*	
1988 Buenos Aires province	*S. richteri, S. quinquecuspis*	25 (46 / 185); 8 (145 / 1836)	5 (9 / 185); 1.1 (20/1836)	BRIANO (1993) BRIANO & al. (1995a)
1991 - 1999 northern Argentina	*S. invicta*	43 (66 / 154) 12 (303 / 2528)	13 (20 / 154) 2.3 (58 / 2528)	BRIANO & WILLIAMS (2002) BRIANO & al. (2012)
1993 Cuiabá, Brazil	*S. invicta*	–[a] (– / 18 sites) 21 (13 / 61)	– (– / 18 sites) 6.6 (4 / 61)	BRIANO & al. (2012)
2001 – 2005 Central & Northern provinces, Argentina	*S. invicta, S. richteri, S. interrupta*[b]	32 (59 / 184) 15 (262 / 1780)	22 (24 / 109) 13 (191 / 1435)	BRIANO & al. (2006)
2001 - 2005 Brazil[c], SE Paraguay, southcentral Bolivia, central Chile	*S. invicta, S. richteri, S. gayi*[d]	7.7 (6 / 78) 2.8 (8 / 284)	4.6 (7 / 153) 1.1 (7 / 629)	BRIANO & al. (2006)

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* are 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). Infection 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 & HASHIMOTO, 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, PATROCK & 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 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-

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 extirpated the native fire ants outweighed the risk that the *Pseudacteon* species would be a detriment to the native fire ants (PORTER 2000, PORTER & GILBERT 2004).

The host specificity testing also revealed *Pseudacteon* biotype host preferences. In the case of *Pseudacteon curvatus* BORGMEIER, 1925 obtained from *Solenopsis richteri* populations, attack frequencies were higher and developmental rates faster on *S. richteri* than on *S. invicta* (PORTER & BRIANO 2000). Conversely, *P. curvatus* originating from *S. invicta* populations exhibited greater host preference for *S. invicta* (VAZQUEZ & al. 2004). Post-release host specificity testing of *Pseudacteon tricuspis* BORGMEIER, 1925 and *P. curvatus* has confirmed the host range predicted by the pre-release studies (VAZQUEZ & PORTER 2005, MORRISON & PORTER 2006).

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, CALCATERRA & 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 (TSCHINKEL 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

Fig. 1: Phorid fly attempting to oviposit into a fire ant worker. Note the defensive C-shaped posture of the ant (photo: S.D. Porter, USDA-ARS).

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 & CALCATERRA 2013). SD = standard deviation.

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

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 the field and then holding colonies in buckets with sterilized soil. Buckets were enclosed within a plastic bag until adult flies were observed (PESQUERO & al. 1995). Subsequently, flies were reared through one complete generation, including mating and oviposition, in the laboratory on North American *Solenopsis invicta* and the *S. invicta* × *S. richteri* hybrid (PORTER & al. 1997a). Innovative improvements to rearing methods, such as exploiting 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 (CAMPIOLO & al. 1994, FOWLER & al. 1995, PESQUERO & al. 1996, MORRISON & al. 1997, ORR & al. 1997, FOLGARAIT & al. 2003, FOLGARAIT & 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 & CALCA-

TERRA 2013). This species is also abundant and rapidly expanding even in competition with *P. curvatus* (PORTER & CALCATERRA 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 biotypes 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*) *solenopsae*: 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-*
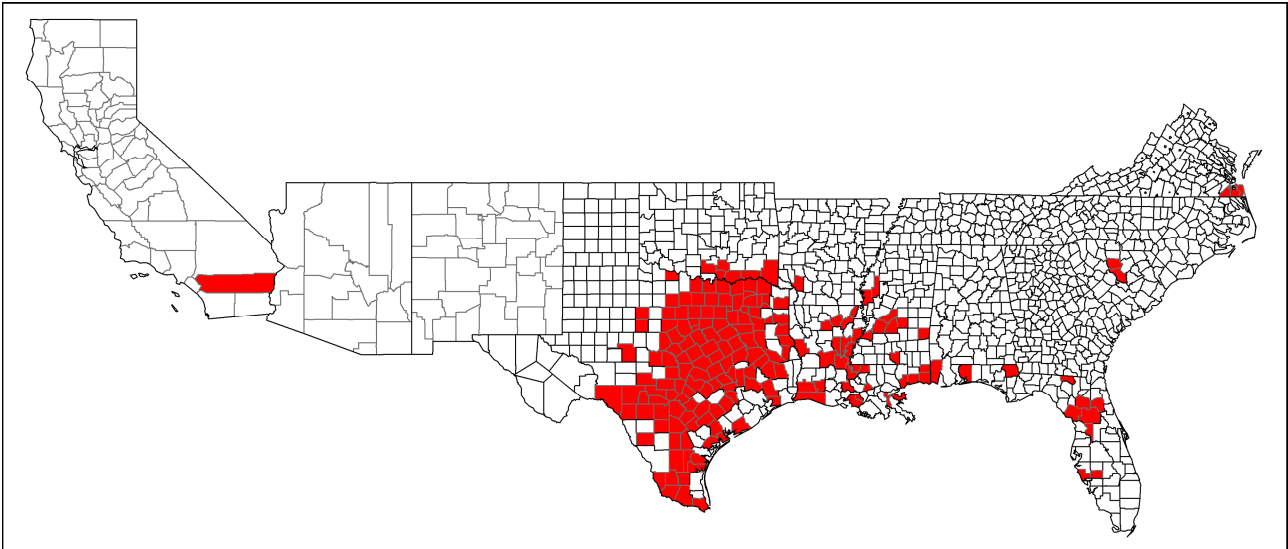
Fig. 2: Distribution of *Kneallhazia solenopsae* in the USA (in red, as of 2010) based on counties with confirmed detections of infection from past surveys and arbitrary sampling of study sites (map: D.H. Oi, USDA-ARS).

opsae causes a chronic, debilitative disease. Four spore types have been identified, but the functions of each as well as the life cycle are not definitively known (KNELL & 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 (BRIANO & 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 (BRIANO & 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 monogyne laboratory colonies were inoculated, while in polygyne colonies similar reductions occurred in about 11 months (WILLIAMS & al. 1999, OI & WILLIAMS 2002). In the field, *K. solenopsae* is more prevalent in polygyne *Solenopsis invicta* populations, although monogyne colonies are infected (OI & al. 2004, VALLES & BRIANO 2004, FUXA & al. 2005b, MILKS & al. 2008, VALLES & al. 2010b). The non-territorial behavior of polygyne colonies (GLANCEY & 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 (OI & WILLIAMS 2002). Hence, greater intercolony spread and persistent intracolony infections result in easier detection of *K. solenopsae* 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 spread and detection of *Kneallhazia solenopsae* are reduced (FUXA & al. 2005a, OI 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 states, primarily in polygyne sites (WILLIAMS & al. 1999, FUXA & al. 2005b; D.H. Oi, unpubl.). Subsequent surveys and arbitrary examinations of fire ants from study sites indicated *K. solenopsae* is widespread (Fig. 2) (STREETT & al. 2004, MITCHELL & al. 2006, ALLEN & al. 2010, ASCUNCE & al. 2010; D.H. Oi, unpubl.), and was in Texas at least since 1984 (SNOWDEN & VINSON 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, WOJCIK & al. 1987). With the exception of "virus-like particles" observed in an unidentified species of *Solenopsis* from Brazil (AVERY & 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 densovirus (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 Parvoviridae, 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 *Acyrthosiphon pisum* 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 titer), 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 (*Sf*21) 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 reinfestation of landscapes cleared of fire ants. Reinfestation was delayed by over a year where *Kneallhazia solenopsae* and *Pseudacteon tricuspis* 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 tricuspis* by *P. curvatus*) or potentially be an enhancement (*P. obtusus* and *P. curvatus*) (LEBRUN & al. 2009, PORTER & CALCATERRA 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, mircrosporidum, phorids) 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

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 irrigation. 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 millennia, 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, *Nylanderia 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.

Acknowledgments

References

ALLEN, G.E. & BUREN, W.F. 1974: Microsporidan and fungal diseases of *Solenopsis invicta* BUREN in Brazil. – Journal of the New York Entomological Society 82: 125-130.

ALLEN, G.E. & SILVEIRA-GUIDO, A. 1974: Occurrence of microsporidia in *Solenopsis richteri* and *Solenopsis* sp. in Uruguay and Argentina. – Florida Entomologist 57: 327-329.

ALLEN, H.R., VALLES, S.M. & MILLER, D.M. 2010: Characterization of *Solenopsis invicta* (Hymenoptera: Formicidae) populations in Virginia: Social form genotyping and pathogen / parasitoid detection. – Florida Entomologist 93: 80-88.

ALVES, S.B., STIMAC, J.L. & CAMARGO, M.T.V. 1988: Suscetibilidade de *Solenopsis invicta* BUREN e *Solenopsis saevissima* Fr. SMITH a isolados de *Beauveria bassiana* (BALS.) VUILL. – Anais da Sociedade Entomológica do Brasil 17: 379-387.

ANONYMOUS 2015: Imported fire ant: quarantine treatments for nursery stock, grass sod and other related articles. – United States Department of Agriculture, Animal and Plant Health Inspection Service, APHIS 81-25-001, Washington, DC, 18 pp.

ASCUNCE, M.S., VALLES, S.M., OI, D.H., SHOEMAKER, D., PLOWES, R., GILBERT, L., LEBRUN, E.G., SÁNCHEZ-ARROYO, H. & SANCHEZ-PEÑA, S. 2010: Molecular diversity of the microsporidium *Kneallhazia solenopsae* reveals an expanded host range among fire ants in North America. – Journal of Invertebrate Pathology 105: 279-288.

ASCUNCE, M.S., YANG, C.C., OAKEY, J., CALCATERRA, L., WU, W.J., SHIH, C.J., GOUDET, J., ROSS, K.G. & SHOEMAKER, D. 2011: Global invasion history of the fire ant *Solenopsis invicta*. – Science 331: 1066-1068.

AVERY, S.W., JOUVENAZ, D.P., BANKS, W.A. & ANTHONY, D.W. 1977: Virus-like particles in a fire ant, *Solenopsis* sp., (Hymenoptera: Formicidae) from Brazil. – Florida Entomologist 60: 17-20.

BEXTINE, B.R. & THORVILSON, H.G. 2002: Field applications of bait-formulated *Beauveria bassiana* alginate pellets for biological control of the red imported fire ant (Hymenoptera: Formicidae). – Environmental Entomology 31: 746-752.

BOUWMA, A.M. & SHOEMAKER, D. 2011: *Wolbachia* wSinvictaA infections in natural populations of the fire ant *Solenopsis invicta*: Testing for phenotypic effects. – Journal of Insect Science 11:11.

BRIANO, J.A. 1993: Effect of the pathogen *Thelohania* sp. (Microsporidia: Thelohaniidae) on field populations of the fire ant *Solenopsis richteri* (Hymenoptera: Formicidae) in Argentina. – M.Sc. thesis, University of Florida, Gainesville, FL, xv + 146 pp.

BRIANO, J.A. 2005: Long-term studies of the red imported fire ant, *Solenopsis invicta*, infected with the microsporidia *Vairimorpha invictae* and *Thelohania solenopsae* in Argentina. – Environmental Entomology 34: 124-132.

BRIANO, J.A., CALCATERRA, L.A., VANDER MEER, R.K., VALLES, S.M. & LIVORE, J. 2006: New survey for the fire ant microsporidia *Vairimorpha invictae* and *Thelohania solenopsae* in southern South America, with observations on their field persistence and prevalence of dual infections. – Environmental Entomology 35: 1358-1365.

BRIANO, J.A., CALCATERRA, L.A. & VARONE, L. 2012: Fire ants (*Solenopsis* spp.) and their natural enemies in southern South America. – Psyche: A Journal of Entomology: Article ID 198084.

BRIANO, J.A., CALCATERRA, L.A., WILLIAMS, D.F. & OI, D.H. 2002a: Attempts to artificially propagate the fire ant parasite

Solenopsis daguerrei (Hymenoptera: Formicidae) in Argentina. – Florida Entomologist 85: 518-520.

BRIANO, J.A., CALCATERRA, L.A., WOJCIK, D.P., WILLIAMS, D.F., BANKS, W.A. & PATTERSON, B.S. 1997: Abundance of the parasitic ant *Solenopsis daguerrei* (Hymenoptera: Formicidae) in South America, a potential candidate for the biological control of the red imported fire ants in the United States. – Environmental Entomology 26: 1143-1148.

BRIANO, J.A., JOUVENAZ, D., WOJCIK, D.P., PATTERSON, R. & CORDO, H. 1995a: Protozoan and fungal diseases in *Solenopsis richteri*, and *S. quinquecuspis* (Hymenoptera: Formicidae) in Buenos Aires Province, Argentina. – Florida Entomologist 78: 531-537.

BRIANO, J.A., PATTERSON, R.S., BECNEL, J.J. & CORDO, H.A. 1996: The black imported fire ant, *Solenopsis richteri*, infected with *Thelohania solenopsae*: intracolonial prevalence of infection and evidence for transovarial transmission. – Journal of Invertebrate Pathology 67: 178-179.

BRIANO, J.A., PATTERSON, R.S. & CORDO, H. 1995b: Long term studies of the black imported fire ant (Hymenoptera: Formicidae) infected with a microsporidium. – Environmental Entomology 24: 1328-1332.

BRIANO, J.A. & WILLIAMS, D.F. 2002: Natural occurrence and laboratory studies of the fire ant pathogen *Vairimorpha invictae* (Microsporida: Burenellidae) in Argentina. – Environmental Entomology 31: 887-894.

BRIANO, J.A., WILLIAMS, D.F., OI, D.H. & DAVIS, L.R., Jr. 2002b: Field host range of the fire ant pathogens *Thelohania solenopsae* (Microsporida: Thelohaniidae) and *Vairimorpha invictae* (Microsporida: Burenellidae) in South America. – Biological Control 24: 98-102.

BRIESE, D.T. 2005: Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedures. – Biological Control 35: 208-214.

BROOME, J.R. 1974: Microbial control of the imported fire ant, *Solenopsis richteri* FOREL (Hymenoptera: Formicidae). – Ph.D. thesis, Mississippi State University, Starkville, MS, vii + 66 pp.

BUREN, W.F. 1972: Revisionary studies on the taxonomy of the imported fire ants. – Journal of the Georgia Entomological Society 7: 1-26.

CALCATERRA, L.A., BRIANO, J.A. & WILLIAMS, D.F. 1999: Field studies of the parasitic ant *Solenopsis daguerrei* (Hymenoptera: Formicidae) on fire ants in Argentina. – Environmental Entomology 28: 88-95.

CALCATERRA, L.A., BRIANO, J.A., WILIAMS, D.F. & OI, D.H. 2001: Observations on the sexual castes of the fire ant parasite *Solenopsis daguerrei* (Hymenoptera: Formicidae). – Florida Entomologist 84: 446-448.

CALCATERRA, L.A., DELGADO, A. & TSUTSUI, N.D. 2008: Activity patterns and parasitism rates of fire ant-decapitating flies (Diptera: Phoridae: *Pseudacteon* spp.) in their native Argentina. – Annals of the Entomological Society of America 101: 539-550.

CALDERA, E.J., ROSS, K.G., DEHEER, C.J. & SHOEMAKER, D.D. 2008: Putative native source of the invasive fire ant *Solenopsis invicta* in the U.S.A. – Biological Invasions 10: 1457-1479.

CALLCOTT, A.M.A., PORTER, S.D., WEEKS, R.D., GRAHAM, L.C., JOHNSON, S.J. & GILBERT, L.E. 2011: Fire ant decapitating fly cooperative release programs (1994-2008): two *Pseudacteon* species, *P. tricuspis* and *P. curvatus*, rapidly expand across imported fire ant population in the southeastern United States. – Journal of Insect Science 11:19.

CAMPIOLO, S., PESQUERO, M.A. & FOWLER, H.G. 1994: Size-selective oviposition by phorid (Diptera: Phoridae) parasitoids on workers of the fire ant, *Solenopsis saevissima* (Hymenoptera: Formicidae). – Etologia 4: 85-86.

CHEN, J.S.C., SNOWDEN, K., MITCHELL, F., SOKOLOVA, J., FUXA, J. & VINSON, S.B. 2004: Sources of spores for the possible horizontal transmission of *Thelohania solenopsae* (Microspora: Thelohaniidae) in the red imported fire ants, *Solenopsis invicta*. – Journal of Invertebrate Pathology 85: 139-145.

CHEN, L. & FADAMIRO, H.Y. 2006: Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, *Pseudacteon tricuspis*. – Physiological Entomology 31: 46-56.

CHRISTIAN, P.D. & SCOTTI, P.D. 1998: The picorna-like viruses of insects. In: MILLER, L. & BALL, A. (Eds.): The insect viruses. – Plenum Press, New York, pp. 301-336.

COLLINS, H.L., CALLCOTT, A.M., LOCKLEY, T.C. & LADNER, A. 1992: Seasonal trends in effectiveness of hydramethylnon (AMDRO) and fenoxycarb (LOGIC) for control of red imported fire ants (Hymenoptera: Formicidae). – Journal of Economic Entomology 85: 2131-2137.

COLLINS, H.L., CALLCOTT, A.M., MCANALLY, L., LADNER, A., LOCKLEY, T. & WADE, S. 1999: Field and laboratory efficacy of *Beauveria bassiana* alone or in combination with imidacloprid against RIFA. – 1999 Accomplishment Report Gulfport Plant Protection Station, CPHST, PPQ, USDA, Gulfport, MS, 124 pp.

CONSOLI, F.L., WUELLNER, C.T., VINSON, S.B. & GILBERT, L.E. 2001: Immature development of *Pseudacteon tricuspis* (Diptera: Phoridae), an endoparasitoid of the red imported fire ant (Hymenoptera: Formicidae). – Annals of the Entomological Society of America 94: 97-109.

COOK, J.L., JOHNSTON, J.S., GOLD, R.E. & VINSON, S.B. 1997: Distribution of *Caenocholax fenyesi* (Strepsiptera: Myrmecolacidae) and the habitats most likely to contain its stylopized host, *Solenopsis invicta* (Hymenoptera: Formicidae). – Environmental Entomology 26: 1258-1262.

COOK, T.J. 2002: Studies of naturally occurring *Thelohania solenopsae* (Microsporida: Thelohaniidae) infection in red imported fire ants, *Solenopsis invicta* (Hymenoptera: Formicidae). – Environmental Entomology 31: 1091-1096.

COOK, T.J., LOWERY, M.B., FREY, T.N., ROWE, K.E. & LYNCH, L.R. 2003: Effect of *Thelohania solenopsae* (Microsporida: Thelohaniidae) on weight and reproductive status of polygynous red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), alates. – Journal of Invertebrate Pathology 82: 201-203.

DI PRISCO, G., PENNACCHIO, F., CAPRIO, E., BONCRISTIANI, H.F., EVANS, J.D. & CHEN, Y.P. 2011: *Varroa destructor* is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. – Journal of General Virology 92: 151-155.

DREES, B.M., VINSON, S.B., GOLD, R.E., MERCHANT, M.E., BROWN, E., ENGLER, K., KECK, M., NESTER, P., KOSTROUN, D., FLANDERS, K., GRAHAM, F., POLLET, D., HOOPER-BUI, L., BECKLEY, P., DAVIS, T., HORTON, P.M., GARDNER, W., LOFTIN, K., HOPKINS, J., VAIL, K., WRIGHT, R., SMITH, W., THOMPSON, D.C., KABASHIMA, J., LAYTON, B., KOEHLER, P., OI, D. & CALLCOTT, A.M. 2006: Managing imported fire ants in urban areas. – Texas Cooperative Extension, The Texas A&M University System, Technical Publication, B6043, College Station, TX, 22 pp.

FEENER, D.H., Jr. 1981: Competition between ant species: outcome controlled by parasitic flies. – Science 214: 815-817.

FEENER, D.H., Jr. & BROWN, B.V. 1992: Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* spp. (Diptera: Phoridae). – Annals of the Entomological Society of America 85: 80-84.

FERNÁNDEZ-MELÉNDEZ, S., GARCÍA-GONZÁLEZ, J.J., BARBER, D. & LOMBARDERO, M. 2007: Anaphylaxis caused by imported

red fire ant stings in Málaga, Spain. – Journal of Investigational Allergology and Clinical Immunology 17: 48-49.

FOLGARAIT, P.J., BRUZZONE, O.A. & GILBERT, L.E. 2003: Seasonal patterns of activity among species of black fire ant parasitoid flies (*Pseudacteon*: Phoridae) in Argentina explained by analysis of climatic variables. – Biological Control 28: 368-378.

FOLGARAIT, P.J., PATROCK, R.J.W. & GILBERT, L.E. 2007: Associations of fire ant phorids and microhabitats. – Environmental Entomology 36: 731-742.

FOWLER, H.G., PESQUERO, M.A., CAMPIOLO, S. & PORTER, S.D. 1995: Seasonal activity of species of *Pseudacteon* (Diptera: Phoridae) parasitoids of fire ants (*Solenopsis saevissima*) (Hymenoptera: Formicidae) in Brazil. – Científica, Saõ Paulo 23: 367-371.

FUXA, J.R., MILKS, M.L., SOKOLOVA, Y.Y. & RICHTER, A.R. 2005a: Interaction of an entomopathogen with an insect social form: an epizootic of *Thelohania solenopsae* (Microsporidia) in a population of the red imported fire ant, *Solenopsis invicta*. – Journal of Invertebrate Pathology 88: 79-82.

FUXA, J.R., SOKOLOVA, Y.Y., MILKS, M.L., RICHTER, A.R., WILLIAMS, D.F. & OI, D.H. 2005b: Prevalence, spread, and effects of the microsporidium *Thelohania solenopsae* released into populations with different social forms of the red imported fire ant (Hymenoptera: Formicidae). – Environmental Entomology 34: 1139-1149.

GILBERT, L.E., BARR, C., CALIXTO, A.A., COOK, J.L., DREES, B.M., LEBRUN, E.G., PATROCK, R.J.W., PLOWES, R., PORTER, S.D. & PUCKETT, R.T. 2008: Introducing phorid fly parasitoids of red imported fire ant workers from South America to Texas: Outcomes vary by region and by *Pseudacteon* species released. – Southwestern Entomologist 33: 15-29.

GLANCEY, B.M. & LOFGREN, C.S. 1988: Adoption of newly-mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* BUREN. – Florida Entomologist 71: 581-587.

GLANCEY, B.M., STRINGER, C.E., CRAIG, C.H. & BISHOP, P.M. 1975: An extraordinary case of polygyny in the red imported fire ant. – Annals of the Entomological Society of America 68: 922.

GRAHAM, L.C., PORTER, S.D., PEREIRA, R.M., DOROUGH, H.D. & KELLEY, A.T. 2003: Field releases of the decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) for control of imported fire ants (Hymenoptera: Formicidae) in Alabama, Florida, and Tennessee. – Florida Entomologist 86: 334-339.

HERATY, J.M. 1994: Biology and importance of two eucharitid parasites of *Wasmannia* and *Solenopsis*. In: WILLIAMS, D.F. (Ed.): Exotic ants: biology, impact, and control of introduced species. – Westview Press, Boulder, CO, pp. 104-120.

HERATY, J.M., WOJCIK, D.P. & JOUVENAZ, D.P. 1993: Species of *Orasema* parasitic on the *Solenopsis saevissima*-complex in South America (Hymenoptera: Eucharitidae, Formicidae). – Journal of Hymenoptera Research 2: 169-182.

HOFFMANN, B., DAVIS, P., GOTT, K., JENNINGS, C., JOE, S., KRUSHELNYCKY, P., MILLER, R., WEBB, G. & WIDMER, M. 2011: Improving ant eradications: details of more successes, a global synthesis and recommendations. – Aliens: The Invasive Species Bulletin 31: 16-23.

HOLWAY, D.A., LACH, L., SUAREZ, A.V., YSUTSUI, N.D. & CASE, T.J. 2002: The causes and consequences of ant invasions. – Annual Review of Ecology and Systematics 33: 181-233.

HUFFAKER, C.B., SIMMONDS, F.J. & LAING, J.E. 1976: The theoretical and empirical basis of biological control. In: HUFFAKER, C.B. & MESSENGER, P.S. (Eds.): Theory and practice of biological control. – Academic Press, New York, pp. 41-78.

ISHAK, H.D., PLOWES, R., SEN, R., KELLNER, K., ESTRADA, D.A., DOWD, S.E. & MUELLER, U.G. 2011: Bacterial diversity in *Solenopsis invicta* and *Solenopsis geminata* ant colonies characterized by 16S amplicon 454 pyrosequencing. – Microbial Ecology 61: 821-831.

JOUVENAZ, D.P. 1983: Natural enemies of fire ants. – Florida Entomologist 66: 111-121.

JOUVENAZ, D.P., ALLEN, G.E., BANKS, W.A. & WOJCIK, D.P. 1977: A survey for pathogens of fire ants, *Solenopsis* spp., in the Southeastern United States. – Florida Entomologist 60: 275-279.

JOUVENAZ, D.P., BANKS, W.A. & ATWOOD, J.D. 1980: Incidence of pathogens in fire ants, *Solenopsis* spp., in Brazil. – Florida Entomologist 63: 345-346.

JOUVENAZ, D.P. & ELLIS, E.A. 1986: *Vairimorpha invictae* n. sp. (Microspora: Microsporida), a parasite of the red imported fire ant, *Solenopsis invicta* BUREN (Hymenoptera: Formicidae). – Journal of Protozoology 33: 457-461.

JOUVENAZ, D.P. & KIMBROUGH, J.W. 1991: *Myrmecomyces annellisae* gen. nov., sp. nov. (Deuteromycotina: Hypomycetes), an endoparasitic fungus of fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae). – Mycological Research 95: 1395-1401.

JOUVENAZ, D.P., LOFGREN, C.S. & BANKS, W.A. 1981: Biological control of imported fire ants: A review of current knowledge. – Bulletin of the Entomological Society of America 27: 203-208.

KATHIRITHAMBY, J. & JOHNSTON, J.S. 1992: Stylopization of *Solenopsis invicta* (Hymenoptera: Formicidae) by *Caenocholax fenyesi* (Strepsiptera: Myrmecolacidae) in Texas. – Annals of the Entomological Society of America 85: 293-297.

KING, A.M.Q., ADAMS, M.J., CARSTENS, E.B. & LEFKOWITZ, E.J., (Eds.) 2012: Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. – Elsevier Academic Press, San Diego, CA, 1338 pp.

KLOTZ, J.H., JETTER, K.M., GREENBERG, L., HAMILTON, J., KABASHIMA, J. & WILLIAMS, D.F. 2003: An insect pest of agricultural, urban, and wildlife areas: the red imported fire ant. In: SUMNER, D.A. (Ed.): Exotic pests and diseases: Biology and economics for biosecurity. – Blackwell Publishing, Ames, IA, pp. 151-166.

KNELL, J.D., ALLEN, G.E. & HAZARD, E.I. 1977: Light and electron microscope study of *Thelohania solenopsae* n. sp. (Microsporida: Protozoa) in the red imported fire ant, *Solenopsis invicta*. – Journal of Invertebrate Pathology 29: 192-200.

KRUSHELNYCKY, P.D., HOLWAY, D.A. & LEBRUN, E.G. 2009 [2010]: Invasion processes and causes of success. In: LACH, L., PARR, C. & ABBOTT, K. (Eds.): Ant ecology. – Oxford University Press, Oxford, UK, pp. 245-260.

LACEY, L.A., FRUTOS, R., KAYA, H.K. & VAIL, P. 2001: Insect pathogens as biological control agents: Do they have a future? – Biological Control 21: 230-248.

LEBRUN, E.G., PLOWES, R.M. & GILBERT, L.E. 2009: Indirect competition facilitates widespread displacement of one naturalized parasitoid of imported fire ants by another. – Ecology 90: 1184-1194.

LOFGREN, C.S. 1986: History of imported fire ants in the United States. In: LOFGREN, C.S. & VANDER MEER, R.K. (Eds.): Fire ants and leaf cutting ants: Biology and management. – Westview Press, Boulder, CO, pp. 36-47.

LOWE, S., BROWNE, M., BOUDJELAS, S. & DE POORTER, M. 2000: 100 of the world's worst invasive species. – Aliens 12: s1-s12.

MANOUSIS, T. & MOORE, N.F. 1987: Cricket paralysis virus, a potential control agent for the olive fruit-fly, *Dacus oleae* GMEL. – Applied and Environmental Microbiology 53: 142-148.

McGlynn, T.P. 1999: The worldwide transfer of ants: geographical distribution and ecological invasions. – Journal of Biogeography 26: 535-548.

McNaught, M.K., Wylie, F.R., Harris, E.J., Alston, C.L., Burwell, C.J. & Jennings, C. 2014: Effect of broadcast baiting on abundance patterns of red imported fire ants (Hymenoptera: Formicidae) and key local ant genera at long-term monitoring sites in Brisbane, Australia. – Journal of Economic Entomology 107: 1307-1315.

Mehdiabadi, N.J. & Gilbert, L.E. 2002: Colony-level impacts of parasitoid flies on fire ants. – Proceedings of the Royal Society B-Biological Sciences 269: 1695-1699.

Mészáros, A., Oi, D.H., Valles, S.M., Beuzelin, J.M., Reay-Jones, F.P.F. & Johnson, S.J. 2014: Distribution of *Pseudacteon* spp. (Diptera: Phoridae), biological control agents of *Solenopsis* spp. (Hymenoptera: Formicidae), in Louisiana and associated prevalence of *Kneallhazia solenopsae* (Microsporidia: Thelohaniidae). – Biological Control 77: 93-100.

Milks, M.L., Fuxa, J.R. & Richter, A.R. 2008: Prevalence and impact of the microsporidium *Thelohania solenopsae* (Microsporidia) on wild populations of red imported fire ants, *Solenopsis invicta*, in Louisiana. – Journal of Invertebrate Pathology 97: 91-102.

Milner, R. & Pereira, R.M. 2007: Microbial control of urban pests – cockroaches, ants and termites. In: Lacey, L.A. & Kaya, K.H. (Eds.): Field manual of microbial control of insects. – Springer, Dordrecht, The Netherlands, pp. 695-711.

Mitchell, F.L., Snowden, K., Fuxa, J.R. & Vinson, S.B. 2006: Distribution of *Thelohania solenopsae* (Microsporida: Thelohaniidae) infecting red imported fire ant (Hymenoptera: Formicidae) in Texas. – Southwestern Entomologist 31: 297-306.

Morrison, L.W. 2012: Biological control of *Solenopsis* fire ants by *Pseudacteon* parasitoids: theory and practice. – Psyche: A Journal of Entomology, Article ID 424817.

Morrison, L.W., Dall'Aglio-Holvorcem, C.G. & Gilbert, L.E. 1997: Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). – Environmental Entomology 26: 716-724.

Morrison, L.W. & Porter, S.D. 2005a: Phenology and parasitism rates in introduced populations of *Pseudacteon tricuspis*, a parasitoid of *Solenopsis invicta*. – BioControl 50: 127-141.

Morrison, L.W. & Porter, S.D. 2005b: Testing for population-level impacts of introduced *Pseudacteon tricuspis* flies, phorid parasitoids of *Solenopsis invicta* fire ants. – Biological Control 33: 9-19.

Morrison, L.W. & Porter, S.D. 2006: Post-release host-specificity testing of *Pseudacteon tricuspis*, a phorid parasitoid of *Solenopsis invicta* fire ants. – BioControl 51: 195-205.

Moser, B.A., Becnel, J.J. & Williams, D.F. 2000: Morphological and molecular characterization of the *Thelohania solenopsae* complex (Microsporidia: Thelohaniidae). – Journal of Invertebrate Pathology 75: 174-177.

Mottern, J.L., Heinz, K.M. & Ode, P.J. 2004: Evaluating biological control of fire ants using phorid flies: effects on competitive interactions. – Biological Control 30: 566-583.

Nickle, W.R. & Jouvenaz, D.P. 1987: *Tetradonema solenopsis* n. sp. (Nematoda: Tetradonematidae) parasitic on the red imported fire ant *Solenopsis invicta* Buren from Brazil. – Journal of Nematology 19: 311-313.

O'Callaghan, M. & Brownbridge, M. 2009: Environmental impacts of microbial control agents used for invasive pests. In: Hajek, A.E., Glare, T.R. & O'Callaghan, M. (Ed.): Use of microbes for control and eradication of invasive arthropods. – Springer Science and Business Media B.V., Dordrecht, NL, pp. 305-327.

Oi, D.H. 2006: Effect of mono- and polygyne social forms on transmission and spread of a microsporidium in fire ant populations. – Journal of Invertebrate Pathology 92: 146-151.

Oi, D.H., Becnel, J.J. & Williams, D.F. 2001: Evidence of intracolony transmission of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in red imported fire ants (Hymenoptera: Formicidae) and the first report of spores from pupae. – Journal of Invertebrate Pathology 78: 128-134.

Oi, D.H., Briano, J.A., Valles, S.M. & Williams, D.F. 2005: Transmission of *Vairimorpha invictae* (Microsporidia: Burenellidae) infections between red imported fire ant (Hymenoptera: Formicidae) colonies. – Journal of Invertebrate Pathology 88: 108-115.

Oi, D.H. & Pereira, R.M. 1993: Ant behavior and microbial pathogens (Hymenoptera: Formicidae). – Florida Entomologist 76: 63-73.

Oi, D.H., Pereira, R.M., Stimac, J.L. & Wood, L.A. 1994: Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). – Journal of Economic Entomology 87: 623-630.

Oi, D.H., Porter, S.D., Valles, S.M., Briano, J.A. & Calcaterra, L.A. 2009: *Pseudacteon* decapitating flies (Diptera: Phoridae): Are they potential vectors of the fire ant pathogens *Kneallhazia* (= *Thelohania*) *solenopsae* (Microsporidia: Thelohaniidae) and *Vairimorpha invictae* (Microsporidia: Burenellidae)? – Biological Control 48: 310-315.

Oi, D.H. & Valles, S.M. 2009: Fire ant control with entomopathogens in the USA. In: Hajek, A.E., Glare, T.R. & O'Callaghan, M. (Eds.): Use of microbes for control and eradication of invasive arthropods. – Springer Science+Business Media B.V., pp. 237-257.

Oi, D.H. & Valles, S.M. 2012: Host specificity testing of the *Solenopsis* fire ant (Hymenoptera: Formicidae) pathogen, *Kneallhazia* (= *Thelohania*) *solenopsae* (Microsporidia: Thelohaniidae), in Florida. – Florida Entomologist 95: 509-512.

Oi, D.H., Valles, S.M. & Briano, J.A. 2010: Laboratory host specificity testing of the fire ant microsporidian pathogen *Vairimorpha invictae* (Microsporidia: Burenellidae). – Biological Control: theory and application in pest management 53: 331-336.

Oi, D.H., Valles, S.M. & Pereira, R.M. 2004: Prevalence of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in monogyne and polygyne red imported fire ants. – Environmental Entomology 33: 340-345.

Oi, D.H. & Williams, D.F. 2002: Impact of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) on polygyne colonies of red imported fire ants (Hymenoptera: Formicidae). – Journal of Economic Entomology 95: 558-562.

Oi, D.H. & Williams, D.F. 2003: *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in reproductives of red imported fire ants (Hymenoptera: Formicidae) and its implication for intercolony transmission. – Environmental Entomology 32: 1171-1176.

Oi, D.H., Williams, D.F., Pereira, R.M., Horton, P., Davis, T.S., Hyder, A.H., Bolton, H.T., Zeichner, B.C., Porter, S.D., Hoch, A.L., Boswell, M.L. & Williams, G. 2008: Combining biological and chemical controls for management of red imported fire ants (Hymenoptera: Formicidae). – American Entomologist 54: 44-53.

Orr, M.R., Seike, S.H., Benson, W.W. & Gilbert, L.E. 1995: Flies suppress fire ants. – Nature 373: 292-293.

ORR, M.R., SEIKE, S.H. & GILBERT, L.E. 1997: Foraging ecology and patterns of diversification in dipteran parasitoids of fire ants in South Brazil. – Ecological Entomology 22: 305-314.

OVERTON, K., RAO, A., VINSON, S.B. & GOLD, R.E. 2006: Mating flight initiation and nutritional status (protein and lipid) of *Solenopsis invicta* (Hymenoptera: Formicidae) alates infected with *Thelohania solenopsae* (Microsporidia: Thelohaniidae). – Annals of the Entomological Society of America 99: 524-529.

PASSERA, L. 1994: Characteristics of tramp species. In: WILLIAMS, D.F. (Ed.): Exotic ants: Biology, impact, and control of introduced species. – Westview Press, Boulder, CO, pp. 23-43.

PATROCK, R.J.W., PORTER, S.D., GILBERT, L.E. & FOLGARAIT, P.J. 2009: Distributional patterns of *Pseudacteon* associated with the *Solenopsis saevissima* complex in South America. – Journal of Insect Science 9:60.

PEREIRA, R.M. 2003: Areawide suppression of fire ant populations in pastures: Project update. – Journal of Agricultural and Urban Entomology 20: 123-130.

PEREIRA, R.M. 2004: Occurrence of *Myrmicinosporidium durum* in red imported fire ant, *Solenopsis invicta* and other new ant hosts in eastern United States. – Journal of Invertebrate Pathology 84: 38-44.

PEREIRA, R.M. & STIMAC, J.L. 1992: Transmission of *Beauveria bassiana* within nests of *Solenopsis invicta* (Hymenoptera: Formicidae) in the laboratory. – Environmental Entomology 21: 1427-1432.

PEREIRA, R.M., WILLIAMS, D.F., BECNEL, J.J. & OI, D.H. 2002: Yellow-head disease caused by a newly discovered *Mattesia* sp. in populations of the red imported fire ant, *Solenopsis invicta*. – Journal of Invertebrate Pathology 81: 45-48.

PESQUERO, M.A., CAMPIOLO, S., FOWLER, H.G. & PORTER, S.D. 1996: Diurnal patterns of ovipositional activity in two *Pseudacteon* fly parasitoids (Diptera: Phoridae) of *Solenopsis* fire ants (Hymenoptera: Formicidae). – Florida Entomologist 79: 455-457.

PESQUERO, M.A., PORTER, S.D., FOWLER, H.G. & CAMPIOLO, S. 1995: Rearing of *Pseudacteon* spp. (Dipt., Phoridae), parasitoids of fire ants (*Solenopsis* spp.) (Hym., Formicidae). – Journal of Applied Entomology 119: 677-678.

PLOWES, R.M., FOLGARAIT, P.J. & GILBERT, L.E. 2012: The introduction of the fire ant parasitoid *Pseudacteon nocens* in North America: challenges when establishing small populations. – BioControl 57: 503-514.

PLOWES, R.M., LEBRUN, E.G. & GILBERT, L.E. 2011: Introduction of the fire ant decapitating fly *Pseudacteon obtusus* in the United States: factors influencing establishment in Texas. – BioControl 56: 295-304.

PLUS, N. & SCOTTI, P.D. 1984: The biological properties of eight different isolates of cricket paralysis virus. – Annales de l'Institut Pasteur / Virologie 135: 257-268.

POINAR, G.O., JR., PORTER, S.D., TANG, S. & HYMAN, B.C. 2007: *Allomermis solenopsi* n. sp. (Nematoda: Mermithidae) parasitising the fire ant *Solenopsis invicta* BUREN (Hymenoptera: Formicidae) in Argentina. – Systematic Parasitology 68: 115-128.

PORTER, S.D. 1998: Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). – Florida Entomologist 81: 292-309.

PORTER, S.D. 2000: Host specificity and risk assessment of releasing the decapitating fly, *Pseudacteon curvatus*, as a classical biocontrol agent for imported fire ants. – Biological Control 19: 35-47.

PORTER, S.D. 2010: Distribution of the Formosa strain of the fire ant decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) three and a half years after releases in North Florida. – Florida Entomologist 93: 107-112.

PORTER, S.D. & BRIANO, J. 2000: Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*. – Florida Entomologist 83: 422-427.

PORTER, S.D. & CALCATERRA, L.A. 2013: Dispersal and competitive impacts of a third fire ant decapitating fly (*Pseudacteon obtusus*) established in north central Florida. – Biological Control 64: 66-74.

PORTER, S.D. & GILBERT, L.E. 2004: Assessing host specificity and field release potential of fire ant decapitating flies (Phoridae: *Pseudacteon*). In: VAN DRIESCHE, R.G., MURRAY, T. & REARDON, R. (Eds.): Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice. – FHTET-2004-03, USDA Forest Service, Morgantown, WV, pp. 152-176.

PORTER, S.D., GRAHAM, L.C., JOHNSON, S.J., THEAD, L.G. & BRIANO, J.A. 2011: The large decapitating fly *Pseudacteon litoralis* (Diptera: Phoridae) successfully established on fire ant populations in Alabama. – Florida Entomologist 94: 208-213.

PORTER, S.D. & HIGASHI, S. 2008: What should Japan be doing about *S. invicta*? In: HIGASHI, S., OGATA, K. & PORTER, S.D. (Eds.): Biology of *Solenopsis invicta*, especially behavioral ecology and molecular genetics. – Kaiyusha, Tokyo, pp. 163-175. (in Japanese)

PORTER, S.D., KUMAR, V., CALCATERRA, L.A., BRIANO, J.A. & SEAL, D.R. 2013a: Release and establishment of the little decapitating fly *Pseudacteon cultellatus* (Diptera: Phoridae) on imported fire ants (Hymenoptera: Formicidae) in Florida. – Florida Entomologist 96: 1567-1573.

PORTER, S.D., NOGUEIRA DE SÁ, L.A. & MORRISON, L.W. 2004: Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. – Biological Control 29: 179-188.

PORTER, S.D. & PESQUERO, M.A. 2001: Illustrated key to *Pseudacteon* decapitating flies (Diptera: Phoridae) that attack *Solenopsis saevissima* complex fire ants in South America. – Florida Entomologist 84: 691-699.

PORTER, S.D., VALLES, S.M., DAVIS, T.S., BRIANO, J.A., CALCATERRA, L.A., OI, D.H. & JENKINS, R.A. 2007: Host specificity of the microsporidian pathogen *Vairimorpha invictae* at five field sites with infected *Solenopsis invicta* fire ant colonies in northern Argentina. – Florida Entomologist 90: 447-452.

PORTER, S.D., VALLES, S.M. & OI, D.H. 2013b: Host specificity and colony impacts of the fire ant pathogen, Solenopsis invicta virus 3. – Journal of Invertebrate Pathology 114: 1-6.

PORTER, S.D., VALLES, S.M., WILD, A.L., DIECKMANN, R. & PLOWES, N.J.R. 2015: Solenopsis invicta virus 3: further host-specificity tests with native *Solenopsis* ants (Hymenoptera: Formicidae). – Florida Entomologist 98: 122-125.

PORTER, S.D., VANDER MEER, R.K., PESQUERO, M.A., CAMPIOLO, S. & FOWLER, H.G. 1995: *Solenopsis* (Hymenoptera: Formicidae) fire ant reactions to attacks of *Pseudacteon* flies (Diptera: Phoridae) in Southeastern Brazil. – Annals of the Entomological Society of America 88: 570-575.

PORTER, S.D., WILLIAMS, D.F. & PATTERSON, R.S. 1997a: Rearing the decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) in imported fire ants (Hymenoptera: Formicidae: *Solenopsis*) from the United States. – Journal of Economic Entomology 90: 135-138.

PORTER, S.D., WILLIAMS, D.F., PATTERSON, R.S. & FOWLER, H.G. 1997b: Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): an escape from natural enemies? – Environmental Entomology 26: 373-384.

PRESTON, C.A., FRITZ, G.N. & VANDER MEER, R.K. 2007: Prevalence of *Thelohania solenopsae* infected *Solenopsis invicta* newly mated queens within areas of differing social form distributions. – Journal of Invertebrate Pathology 94: 119-124.

SCOTTI, P.D., LONGWORTH, J.F., PLUS, N., CROIZIER, G. & REINGANUM, C. 1981: The biology and ecology of strains of an insect small RNA virus complex. Advances in Virus Research 26: 117-143.

SHAPIRO, A.M., BECNEL, J.J., OI, D.H. & WILLIAMS, D.F. 2003: Ultrastructural characterization and further transmission studies of *Thelohania solenopsae* from *Solenopsis invicta* pupae. – Journal of Invertebrate Pathology 83: 177-180.

SHIH, C.J., YANG, C.C. & WU, W.J. 2007: The battle of fire ant in Taiwan. – Proceedings, 19th Federation of Asian and Oceania Pest Managers Convention, Nov. 14-16, 2007, Chinese Pest Control Association, Taipei, Taiwan, pp. 16-30.

SHOEMAKER, D.D., AHRENS, M.E. & ROSS, K.G. 2006: Molecular phylogeny of fire ants of the *Solenopsis saevissima* species-group based on mtDNA sequences. – Molecular Phylogenetics and Evolution 38: 200-215.

SHOEMAKER, D.D., ROSS, K.G., KELLER, L., VARGO, E.L. & WERREN, J.H. 2000: *Wolbachia* infections in native and introduced populations of fire ants (*Solenopsis* spp.). – Insect Molecular Biology 9: 661-673.

SILVEIRA-GUIDO, A., CARBONELL, J. & CRISCI, C. 1973: Animals associated with the *Solenopsis* (fire ants) complex, with special reference to *Labauchena daguerrei*. – Proceedings Tall Timbers Conference on Ecological Animal Control by Habitat Management 4: 41-52.

SNOWDEN, K. & VINSON, S.B. 2006: Development of *Thelohania solenopsae* as an effective biological control agent for the red imported fire ant, *Solenopsis invicta* – <https://insects.tamu.edu/fireant/research/projects/pdf/06snowdenvinson.pdf>, retrieved on 25 November 2014.

SOKOLOVA, Y.Y. & FUXA, J.R. 2008: Biology and life-cycle of the microsporidium *Kneallhazia solenopsae* KNELL ALLAN HAZARD 1977 gen. n., comb. n., from the fire ant *Solenopsis invicta*. – Parasitology 135: 903-929.

SOKOLOVA, Y.Y., MCNALLY, L.R., FUXA, J.R. & VINSON, S.B. 2004: Spore morphotypes of *Thelohania solenopsae* (Microsporidia) described microscopically and confirmed by PCR of individual spores microdissected from smears by positron ablative laser microbeam microscopy. – Microbiology 150: 1261-1270.

SOLTER, L.F., BECNEL, J.J. & OI, D.H. 2012: Microsporidian entomopathogens. In: VEGA, F.E. & KAYA, H.K. (Eds.): Insect pathology, 2nd edition. – Elsevier, London, UK, pp. 221-263.

STIMAC, J.L. & ALVES, S.B. 1994: Ecology and biological control of fire ants. In: ROSEN, D., BENNETT, F.D. & CAPINERA, J.L. (Eds.): Pest management in the subtropics: biological control – a Florida perspective. – Intercept Ltd., Andover, UK, pp. 353-380.

STIMAC, J.L., ALVES, S.B. & CAMARGO, M.T.V. 1989: Controle de *Solenopsis* spp. (Hymenoptera: Formicidae) com *Beauveria bassiana* (BALS.) Vuill. em condições de laboratório e campo. – Anais da Sociedade Entomológica do Brasil 181: 95-103.

STIMAC, J.L. & PEREIRA, R.M. 2001: Methods and formulations for control of pests. – U.S. Patent No. 6,254,864.

STOREY, G.K., VANDER MEER, R.K., BOUCIAS, D.G. & MCCOY, C.W. 1991: Effect of fire ant (*Solenopsis invicta*) venom alkaloids on the in vitro germination and development of selected entomogenous fungi. – Journal of Invertebrate Pathology 58: 88-95.

STREETT, D.A., FREELAND, T.B., Jr. & PRANSCHKE, A.M. 2004: Distribution of *Thelohania solenopsae* in red imported fire ant populations in Mississippi. [poster presentation]. In: POLLET, D., JOHNSON, S., BECKLEY, P. & CLAYTON, S. (Eds.): Proceedings of the 2004 Annual Red Imported Fire Ant Conference, March 21-23, 2004. – Lousiana State University, AgCenter, Baton Rouge, LA, pp. 150-152.

SUAREZ, A.V., MCGLYNN, T.P. & TSUTSUI, N.D. 2009 [2010]: Biogeographic and taxonomic patterns of introduced ants. In: LACH, L., PARR, C. & ABBOTT, K. (Eds.): Ant ecology. – Oxford University Press, Oxford, UK, pp. 233-245.

THORVILSON, H., WHEELER, D., BEXTINE, B. & SAN FRANCISCO, M. 2002: Development of *Beauveria bassiana* formulations and genetically marked strains as a potential biopesticide for imported fire ant control. – Southwestern Entomologist 25, Suppl.: 19-29.

TSCHINKEL, W.R. 1988: Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. – Behavioral Ecology and Sociobiology 22: 103-115.

TSCHINKEL, W.R. 2006: The fire ants. 1st edition. – Harvard University Press, Cambridge, MA, 747 pp.

VALLES, S.M. 2012: Positive-strand RNA viruses infecting the red imported fire ant, *Solenopsis invicta*. – Psyche: A Journal of Entomology, Article ID 821591: 14 pp.

VALLES, S.M., ALLEN, C., VARONE, L. & BRIANO, J. 2010a: Complete genome sequence of an Argentinean isolate of Solenopsis invicta virus 3. – Virus Genes 40: 293-297.

VALLES, S.M., BELL, S. & FIRTH, A.E. 2014a: Solenopsis invicta virus 3: Mapping of structural proteins, ribosomal frameshifting, and similarities to *Acyrthosiphon pisum* virus and Kelp fly virus. – Public Library of Science One 9: e93497.

VALLES, S.M. & BRIANO, J.A. 2004: Presence of *Thelohania solenopsae* and *Vairimorpha invictae* in South American populations of *Solenopsis invicta*. – Florida Entomologist 87: 625-627.

VALLES, S.M. & HASHIMOTO, Y. 2009: Isolation and characterization of Solenopsis invicta virus 3, a new positive-strand RNA virus infecting the red imported fire ant, *Solenopsis invicta*. – Virology 388: 354-361.

VALLES, S.M. & OI, D.H. 2014: Successful transmission of Solenopsis invicta virus 3 to field colonies of *Solenopsis invicta* (Hymenoptera: Formicidae). – Florida Entomologist 97: 1244-1246.

VALLES, S.M., OI, D.H., PERERA, O.P. & WILLIAMS, D.F. 2002: Detection of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in *Solenopsis invicta* (Hymenoptera: Formicidae) by multiplex PCR. – Journal of Invertebrate Pathology 81: 196-201.

VALLES, S.M., OI, D.H. & PORTER, S.D. 2009: *Kneallhazia* (= *Thelohania*) *solenopsae* infection rate of *Pseudacteon curvatus* flies determined by multiplex PCR. – Florida Entomologist 92: 344-349.

VALLES, S.M., OI, D.H. & PORTER, S.D. 2010b: Seasonal variation and the co-occurrence of four pathogens and a group of parasites among monogyne and polygyne fire ant colonies. – Biological Control 54: 342-348.

VALLES, S.M., OI, D.H., YU, F., TAN, X.X. & BUSS, E.A. 2012: Metatranscriptomics and pyrosequencing facilitate discovery of potential viral natural enemies of the invasive Caribbean crazy ant, *Nylanderia pubens*. – Public Library of Science One 7: e31828.

VALLES, S.M. & PORTER, S.D. 2007: *Pseudacteon* decapitating flies: potential vectors of a fire ant virus? – Florida Entomologist 90: 282-283.

VALLES, S.M., PORTER, S.D., CHOI, M.Y. & OI, D.H. 2013a: Successful transmission of Solenopsis invicta virus 3 to *Solenopsis invicta* fire ant colonies in oil, sugar, and cricket bait formulations. – Journal of Invertebrate Pathology 113: 198-204.

VALLES, S.M., PORTER, S.D. & FIRTH, A.E. 2014b: Solenopsis invicta virus 3: Pathogenesis and stage specificity in red imported fire ants. – Virology 460: 66-71.

VALLES, S.M., SHOEMAKER, D., WURM, Y., STRONG, C.A., VARONE, L., BECNEL, J.J. & SHIRK, P.D. 2013b: Discovery and molecular characterization of an ambisense densovirus from South American populations of Solenopsis invicta. – Biological Control 67: 431-439.

VALLES, S.M., STRONG, C.A., DANG, P.M., HUNTER, W.B., PEREIRA, R.M., OI, D.H., SHAPIRO, A.M. & WILLIAMS, D.F. 2004: A picorna-like virus from the red imported fire ant, Solenopsis invicta: initial discovery, genome sequence, and characterization. – Virology 328: 151-157.

VALLES, S.M., STRONG, C.A. & HASHIMOTO, Y. 2007: A new positive-strand RNA virus with unique genome characteristics from the red imported fire ant, Solenopsis invicta. – Virology 365: 457-463.

VALLES, S.M., STRONG, C.A., HUNTER, W.B., DANG, P.M., PEREIRA, R.M., OI, D.H. & WILLIAMS, D.F. 2008: Expressed sequence tags from the red imported fire ant, Solenopsis invicta: Annotation and utilization for discovery of viruses. – Journal of Invertebrate Pathology 99: 74-81.

VAN SCHAGEN, J.J., DAVIS, P.R. & WIDMER, M.A. 1994: Ant Pests of Western Australia with particular reference to the Argentine ant (Linepithema humile). In: WILLIAMS, D.F. (Ed.): Exotic ants: Biology, impact, and control of introduced species. – Westview Press, Boulder, CO, pp. 174-180.

VANDER MEER, R.K. 1986: Chemical taxonomy as a tool for separating Solenopsis spp. In: LOFGREN, C.S. & VANDER MEER, R.K. (Eds.): Fire ants and leaf cutting ants: biology and management. – Westview Press, Boulder, CO, pp. 316-326.

VANDER MEER, R.K., PORTER, S.D., OI, D.H., VALLES, S.M. & PEREIRA, R.M. 2007: Area-wide suppression of invasive fire ant populations. In: VREYSEN, M.J.B., ROBINSON, A.S. & HENDRICHS, J. (Eds.): Area-wide control of insect pests: from research to field implementation. – Springer, Dordrecht, The Netherlands, pp. 487-496.

VARONE, L. & BRIANO, J. 2009: Bionomics of Orasema simplex (Hymenoptera: Eucharitidae), a parasitoid of Solenopsis fire ants (Hymenoptera: Formicidae) in Argentina. – Biological Control 48: 204-209.

VARONE, L., HERATY, J.M. & CALCATERRA, L.A. 2010: Distribution, abundance and persistence of species of Orasema (Hym: Eucharitidae) parasitic on fire ants in South America. – Biological Control 55: 72-78.

VAZQUEZ, R.J. & PORTER, S.D. 2005: Re-confirming host specificity of the fire ant decapitating fly Pseudacteon curvatus (Diptera: Phoridae) after field release in Florida. – Florida Entomologist 88: 107-110.

VAZQUEZ, R.J., PORTER, S.D. & BRIANO, J.A. 2004: Host specificity of a biotype of the fire ant decapitating fly Pseudacteon curvatus (Diptera: Phoridae) from Northern Argentina. – Environmental Entomology 33: 1436-1441.

VAZQUEZ, R.J., PORTER, S.D. & BRIANO, J.A. 2006: Field release and establishment of the decapitating fly Pseudacteon cur-

vatus on red imported fire ants in Florida. – BioControl 51: 207-216.

VOGT, J.T., PORTER, S.D., NORDLUND, D.A. & SMITH, R. 2003: A modified rearing system for production of Pseudacteon curvatus (Diptera: Phoridae), a parasitoid of imported fire ants. – Biological Control 28: 346-353.

WARD, D.F., GREEN, C., HARRIS, R.J., HARTLEY, S., LESTER, P.J., STANLEY, M.C., SUCKLING, D.M. & TOFT, R.J. 2010: Twenty years of Argentine ants in New Zealand: past research and future priorities for applied management. – New Zealand Entomologist 33: 68-78.

WILLIAMS, D.F. & BANKS, W.A. 1987: Pseudacteon obtusus (Diptera: Phoridae) attacking Solenopsis invicta (Hymenoptera: Formicidae) in Brazil. – Psyche: A Journal of Entomology 94: 9-13.

WILLIAMS, D.F., COLLINS, H.L. & OI, D.H. 2001: The red imported fire ant (Hymenoptera: Formicidae): an historical perspective of treatment programs and the development of chemical baits for control. – American Entomologist 47: 146-159.

WILLIAMS, D.F., OI, D.H. & KNUE, G.J. 1999: Infection of red imported fire ant (Hymenoptera: Formicidae) colonies with the entomopathogen Thelohania solenopsae (Microsporidia: Thelohaniidae). – Journal of Economic Entomology 92: 830-836.

WILLIAMS, D.F., OI, D.H., PORTER, S.D., PEREIRA, R.M. & BRIANO, J.A. 2003: Biological control of imported fire ants (Hymenoptera: Formicidae). – American Entomologist 49: 150-163.

WILLIAMS, R.N., PANAIA, J.R., GALLO, D. & WHITCOMB, W.H. 1973: Fire ants attacked by phorid flies. – Florida Entomologist 56: 259-262.

WILLIAMS, R.N. & WHITCOMB, W.H. 1974: Parasites of fire ants in South America. – Proceedings Tall Timbers Conference on Ecological Animal Control by Habitat Management 5: 49-59.

WOJCIK, D.P. 1990: Behavioral interactions of fire ants and their parasites, predators and inquilines. In: VANDER MEER, R.K., JAFFE, K. & CEDENO, A. (Eds.): Applied myrmecology: a world perspective. – Westview Press, Boulder, CO, pp. 329-344.

WOJCIK, D.P., JOUVENAZ, D.P., BANKS, W.A. & PEREIRA, A.C. 1987: Biological control agents of fire ants in Brazil. In: EDER, J. & REMBOLD, H. (Eds.): Chemistry and biology of social insects. – Verlag J. Peperny, München, pp. 627-628.

WUELLNER, C.T., DALL'AGLIO-HOLVORCEM, C.G., BENSON, W.W. & GILBERT, L.E. 2002: Phorid fly (Diptera: Phoridae) oviposition behavior and fire ant (Hymenoptera: Formicidae) reaction to attack differ according to phorid species. – Annals of the Entomological Society of America 95: 257-266.

YANG, C.C., YU, Y.C., VALLES, S., OI, D., CHEN, Y.C., SHOEMAKER, D., WU, W.J. & SHIH, C.J. 2010: Loss of microbial (pathogen) infections associated with recent invasions of the red imported fire ant Solenopsis invicta. – Biological Invasions 12: 3307-3318.

ZACARO, A.A. & PORTER, S.D. 2003: Female reproductive system of the decapitating fly Pseudacteon wasmanni SCHMITZ (Diptera: Phoridae). – Arthropod Structure & Development 31: 329-337.