



Mitigating the allergic effects of fire ant envenomation with biologically based population reduction

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Purpose of review

To describe the current efforts to use biological control agents to reduce fire ant population levels, thus ultimately reducing the number of human sting and allergic reaction incidents.

Recent findings

Climate change and worldwide fire ant expansion will increase the frequency of human encounters and allergenic events, putting additional pressure on the public health sector. Six species of fire ant decapitating flies are now established in the United States. The microsporidium *Kneallhazia solenopsae* is well established and new fire ant hosts have been identified. The fire ant virus *Solenopsis invicta* virus 3 shows good potential for use as an environmentally friendly biopesticide because of its virulence and host specificity.

Summary

During separate founding events in the United States, Australia, mainland China, and Taiwan, fire ants native to South America escaped their native pathogens and parasites. Consequently, fire ant populations in these introduced regions pose a serious public health threat to the human populations by envenomation and subsequent allergic reactions. Specific, self-sustaining biological control agents have been discovered, studied, and released into fire ant populations in the United States in an effort to re-establish an ecological/competitive balance, resulting in reduced fire ant densities and human exposure.

Keywords

biocontrol, biological control, fire ant, microsporidia, parasites, *Pseudacteon*, sting, venom, virus

INTRODUCTION

The invasive fire ant, *Solenopsis invicta*, is present in the south and southeastern United States at population densities 5–10 times greater than those found in their native South America [1], most likely because they left their numerous natural enemies behind [2^{***}]. Currently, fire ants infest 130 000 000 hectares in 14 states and Puerto Rico [3], with population densities reaching 140 colonies per hectare (200 000 workers per colony). The economic impact of this ant species exceeds \$5 billion annually and covers multiple economic sectors. In addition, human health risks from allergic responses to envenomation by these ants are a major factor driving efforts to develop effective control measures.

Most hymenopteran venoms are protein based; however, fire ant venom is almost entirely composed of piperidine alkaloids that have a variety of physiological activities, some of which result in the sterile pustule associated with fire ant stings [4]

(Fig. 1). An accumulation of medical reports of allergic reactions to fire ant stings in the 1960s and 1970s stimulated the discovery of venom proteins that comprise 0.1% of the venom [4]. Unlike bees and wasps, 30–60% of the human population in infested areas is stung at least once each year by the ubiquitous fire ant. Thus, with 1–5% of the population susceptible to the development of hypersensitivity, millions of people are at risk [5,6].

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KEY POINTS

- Climate change and worldwide fire ant expansion will dramatically increase the frequency of human encounters and allergenic events.
- Fire ant decapitating flies have been successfully established in the United States, including two species with wide geographic distribution.
- Microsporidian pathogens debilitate fire ant colonies and can be an important component of sustained suppression of fire ant populations.
- Development of viruses as biopesticides offers a species-specific, nontoxic alternative to traditional insecticides for controlling fire ants.

In the last decade, imported fire ant populations have been discovered in Australia, Taiwan and mainland China [7], and additional habitat suitable to sustain fire ant populations worldwide is large [8]. As a result, the range of this invasive species is likely to expand and the impact of fire ants on public health will likely increase correspondingly. In China, the fire ant has rapidly expanded into four provinces since the initial discovery in 2004 and 33% of people in the infested area have been stung and 10% of those experienced allergic reactions, including anaphylactic shock [9]. Increases in allergic incidents in the

United States have been correlated with climate change, including those from fire ants [10]. Global warming associated with climate change is projected to expand the range of the fire ant 133 km northward by the year 2100 [11] in the United States and likely also in mainland China. It is clear that climate change and accidental world-wide expansion of fire ants will place a significant burden on healthcare providers and their facilities.

BIOLOGICALLY BASED FIRE ANT CONTROL

Fire ant control with classical biological agents is sustainable, benefits the environment by reducing pesticide use in urban and agricultural areas, and helps restore the natural biodiversity of ground dwelling vertebrates and arthropods that existed before the fire ant invasion [12]. In addition, reduced fire ant populations would decrease the incidence of human exposure to fire ant envenomation and resulting allergies. A wide array of natural enemies have been found in South America including viral and fungal/microsporidian pathogens, nematodes, a parasitic wasp, a parasitic ant, and several species of decapitating flies [2]. We focus on the current research with decapitating flies, viruses, and microsporidian pathogens in U.S. fire ant populations; however, as current infestations in Australia, Taiwan, and mainland China are derived



FIGURE 1. Typical pustules from fire ant stings. Fire ant stings on hand (left), leg (center), and closeup view by millimeter scale (right). The sterile pustules result from piperidine venom alkaloids. Reproduced by courtesy of USDA-ARS (for photographs).

from the USA [7], our success is expected to translate well to other fire ant infested countries.

Fire ant decapitating flies

Phorid decapitating flies in the genus *Pseudacteon* were selected for release in the United States because they are very host specific [13–15], they have no negative effects on plants or nontarget animals [15], and fire ants have evolved a suite of defenses against decapitating flies, which demonstrate that these flies have impacted South America fire ant populations [16]. Decapitating flies have the potential to reduce fire ant populations by direct parasitism, disruption of daytime foraging efforts, and possibly as vectors of fire ant diseases [17].

Female *Pseudacteon* flies, each with several hundred eggs [16], appear to locate their hosts by detecting the social pheromones ants use to communicate [18,19]. Female flies hover several millimeters above fire ant workers (Fig. 2) before diving in and rapidly injecting an egg into the ant thorax with a short hypodermic-shaped ovipositor. Shortly after hatching, maggots move into the heads of their hosts where they develop for several weeks (Fig. 2) [20]. Just prior to pupation, parasitized ants leave their nest looking for a place to die, apparently zombies under the control of their parasite [21]. Once out of the nest, the maggot releases a chemical which results in the decapitation of its living and often still twitching host [16]. The maggot then eats everything in the head and pupates within the empty

head capsule, using it as a pupal case. After several more weeks, adult flies emerge ready to attack more fire ants (Fig. 2).

Six species of phorid decapitating flies have been released and established in the United States as fire ant biocontrol agents. The first species was *Pseudacteon tricuspidatus* Borgmeier from São Paulo Brazil (1997). This species attacks the medium-sized and large-sized fire ants and was released at multiple sites across the Southeast [21–23]. It currently occupies about two-thirds of the fire ant quarantine area. *Pseudacteon curvatus* Borgmeier is the second and most successful species of decapitating fly established in the United States (2000 and 2003). This species attacks small fire ant workers. A biotype from Las Flores, Argentina, has been established on black and hybrid fire ants in Alabama, Mississippi, and Tennessee. A second biotype from Formosa, Argentina, has been established on red fire ants in Florida, South Carolina, North Carolina, Alabama, Louisiana, Arkansas, Oklahoma, and Texas [21–23]. *P. curvatus* often accounts for more than 90% of decapitating flies collected at sample sites [24] and currently occupies almost all of the fire ant range in the United States. A third species of decapitating fly, *Pseudacteon litoralis* Borgmeier was established in south-central Alabama in 2005 [25]. This fly attacks the largest sizes of fire ant workers. It has expanded several miles out of its release site, but populations remain small and it has only been collected intermittently over the last several years. A fourth species, *Pseudacteon obtusus* Borgmeier was recently



FIGURE 2. Female decapitating fly about to attack host fire ant (left), decapitated fire ant worker (center), and fly emerging from head capsule of host (right). Reproduced by courtesy of USDA-ARS (for photographs).

established in Florida in 2008 [24] and several sites in Texas in 2006 [26]. This species attacks the medium-sized and large-sized fire ants. In the Gainesville, Florida area, *P. obtusus* is competing well with *P. curvatus*, but it is displacing *P. tricuspidis*. A fifth species, *Pseudacteon nocens* Borgmeier was successfully released in Texas beginning in 2006 [27]. This large species is active mostly in the early morning and evening hours like *P. litoralis* above. Finally, the very small decapitating fly *Pseudacteon cultellatus* Borgmeier has recently been established at sites (2010) in North Central and South Florida (unpublished observation).

Multiple species of decapitating flies were released because different species attack different sizes of fire ants, during different times of the day, and in different habitats [16]. Competition among species occurs [24], but it was expected that a community of parasitic flies which attacks and parasitizes all sizes of fire ant workers would have more impact than one or two species which only parasitize a portion of fire ant workers. Two of the six species of decapitating flies released are relatively abundant and widespread; nevertheless, current impacts on fire ants have not been strong enough to reliably measure because of the highly variable nature of fire ant populations and rapid expansion of the fly populations [28].

MICROSPORIDIAN PATHOGENS

Two microsporidian pathogens, *Kneallhazia solenopsae* and *Vairimorpha invictae*, were described from South American fire ants in the 1970s and 1980s, respectively. These microsporidians are among the few fire ant natural enemies with documented colony level declines [29,29]. *K. solenopsae*, which was previously known as *Thelohania solenopsae* [30], was initially found in Florida in 1996 [31]. Although it was not purposely introduced from South America, *K. solenopsae* was distributed from Florida to several other states and it has spread naturally throughout the USA [29]. Typical of microsporidiosis in other insects, *K. solenopsae* causes a slow debilitation and sometimes death (Fig. 3). Obvious effects of *K. solenopsae* include a significant reduction in fecundity and premature death of the queen, which can lead to the slow demise of a colony. Under field conditions however, reductions in fire ant populations were attributed more to the smaller size of the colonies rather than a reduction in the number of colonies [32]. Establishment of both *K. solenopsae* and the decapitating flies has been associated with slower re-infestations by fire ants [33].

Part of the persistence of infected field colonies was the preponderance of *K. solenopsae* infections in

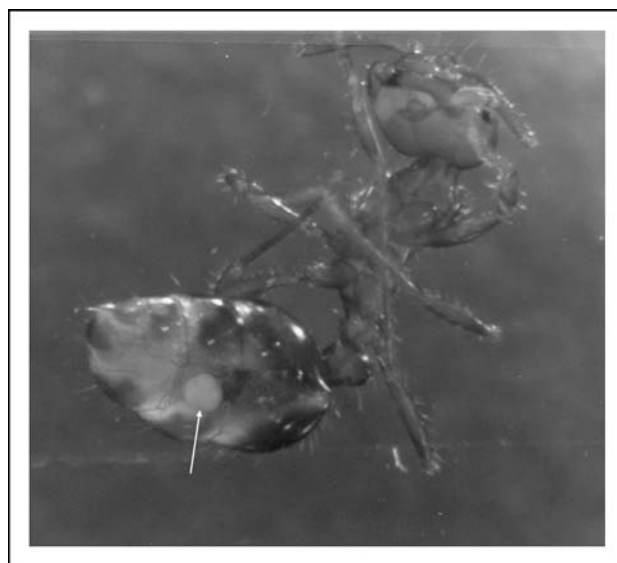


FIGURE 3. A pathogen-infected fire ant. Red imported fire ant infected with *Kneallhazia solenopsae*. Cyst (arrow) contains spores of the microsporidium. Reproduced by courtesy of USDA-ARS (for photographs).

colonies of the polygyne social form. These colonies have many queens, which can be asynchronously infected, and because polygyne workers are not territorial, infected and noninfected workers move between colonies, which maintains the infection throughout the polygyne population. In contrast, colonies that have one queen per colony (monogyne social form) do not interact with other colonies, thus inhibiting the spread of the infection [29].

V. invictae has not been detected in the USA [34], which restricts studies on this microsporidium to quarantine facilities. Laboratory inoculations of fire ant colonies with either live, infected immature fire ants, or with infected dead workers resulted in infections and poor colony growth. Monitoring of infected fire ant populations in Argentina suggests this pathogen to be more virulent, with wider and more abrupt fluctuations in prevalence, than *K. solenopsae*. Moreover, areas infected with both *K. solenopsae* and *V. invictae* exhibited substantial and sometimes lasting declines in fire ant populations [35].

Efforts are continuing to obtain approval to introduce *V. invictae* into the USA. Host specificity studies were promising, in which *V. invictae* infections were limited to the *Solenopsis saevissima* species group, of which the black and the red imported fire ants are the only susceptible species in North America [36–38]. However, the host specificity of *K. solenopsae* has seen some unexpected host range expansion. Similar to *V. invictae*, the host range of

K. solenopsae was thought to be restricted to the *S. saevissima* species group, but it has been detected in the tropical fire ant, *Solenopsis geminata*, and the hybrid of the tropical and southern fire ant, *S. geminata* × *S. xyloni*. Analysis of the genetic diversity of *K. solenopsae* from fire ants in North and South America suggested the existence of variants of this pathogen [39]. Interestingly, sampling of nonfire ant species in the vicinity of early U.S. detections of *K. solenopsae* 12 years later have not revealed any infections in addition to red imported fire ants.

Ecological niche modeling of the distribution of red imported fire ants in the southeastern USA and matching distribution projections of *K. solenopsae* and *V. invictae* in the USA indicated that *K. solenopsae* would spread throughout the fire ant invaded regions. *V. invictae*'s projected distribution encompassed a limited portion of the host distribution. It was concluded that *K. solenopsae* was potentially a more effective biocontrol than *V. invictae* [40]. However, predictions may not have accounted for fire ant social form relative to disease prevalence, virulence, and other biotic factors. Nevertheless, ecological niche modeling should be a useful tool in projecting the establishment and spread of the fire ant microsporidians when being considered as biological controls in other invaded countries.

FIRE ANT VIRUSES

Viruses can be effective control agents against insects and provide an alternative to traditional insecticides in sensitive areas. They can be used as biopesticides or released as classical biological control agents. In an effort to identify virus infections of *S. invicta*, a metagenomics approach was employed and resulted in discovery of the first viral infections of any ant species [41]. The currently described fire ant viruses all possess positive sense, single-stranded RNA genomes and include *Solenopsis invicta* virus 1 (SINV-1), SINV-2, and SINV-3 [42[¶]]. All three viruses are found in South American and U.S. populations of fire ant, but the initial discovery was made in U.S. populations. Although all three viruses are likely members of the *Picornavirales*, only SINV-1 has been formally placed taxonomically; it has been assigned to the *Dicistroviridae* [43]. SINV-1 and SINV-2 appear to cause chronic, asymptomatic disease under normal conditions. However, when SINV-1-infected or SINV-2-infected ant colonies experience certain stressors, the viruses often lead to phenotypic changes and colony mortality – a common trait among many known insect-infecting positive sense single-stranded RNA viruses [44]. Conversely, SINV-3 appears to be consistently associated with significant fire ant colony mortality [45], reminiscent of

colony collapse disorder in honey bees – which is also associated with a positive sense, single-stranded RNA virus [46]. Thus, our research efforts are now focused on the efficacy and development of SINV-3 as a control agent against *S. invicta*.

SINV-3 possesses an A/U-rich genome (70.9% A/U; 29.1% G/C), 10386 nucleotides in length encoding two large open-reading frames (ORFs) (Genbank accession FJ528584). The 5' proximal ORF possesses domains with strong identity to an RNA-dependent RNA polymerase, protease, and helicase. For comparison, the genome of an Argentinean isolate obtained from the Santa Fe region of Argentina was sequenced in entirety [47]. Argentina is thought to be the region from which the U.S. *S. invicta* population originated [48]. The U.S. and Argentinean isolates exhibited identical start and stop codon positions for ORFs 1 and 2, and the genome lengths were identical. Further comparisons revealed no significant directional selection despite significant separation of the ant host populations geographically (Argentina/United States) and temporally (approximately 70 years). Thus, SINV-3 may have been a relatively recent introduction into the North American *S. invicta* population.

Unlike SINV-1 and SINV-2, SINV-3 exhibits a broad tissue tropism [42[¶]] and is detected in *S. invicta* queens, workers, and larvae. This systemic characteristic appears to coincide with the association between SINV-3 infection and significant mortality among *S. invicta* laboratory colonies [45]. Signs of infection include large midden piles of ants, brood mortality, and colony collapse. SINV-3 can be successfully delivered in various bait formulations to fire ant colonies [49]. Water-based and oil-based bait formulations effectively transmitted SINV-3 infections to *S. invicta* colonies with corresponding detrimental effects to colony health and productivity. Fire ant colonies exposed to a single 24-h pulse treatment of SINV-3 became infected with the virus regardless of the bait formulation. However, water-based, bait-treated colonies become infected more rapidly and exhibited significant declines in their brood ratings compared with untreated controls and oil bait-treated colonies. SINV-3-treated colonies usually exhibit decreased quantities of workers, brood, and total colony weight. In addition, worker mortality is higher, queen weight is lower, and the number of eggs laid by queens is lower in SINV-3-treated fire ant colonies.

In addition to virulence, SINV-3 also exhibits host specificity [50]. Nineteen species of ants in fourteen genera and four subfamilies were exposed to SINV-3 under laboratory conditions. Despite extreme exposure, active, replicating SINV-3 infections were only detected in *S. invicta* and hybrid

(*S. invicta* × *Solenopsis richteri*) fire ant colonies. The lack of infections in *S. geminata* fire ants from the United States indicate that SINV-3 is restricted to the *saevissima* complex of fire ants.

SINV-3 negatively impacts fire ant colonies, is amenable to bait formulation, exhibits environmental durability [51], and is host specific; all attributes conducive to use as a biopesticide or biological control agent of fire ants. Additional studies required for SINV-3 development include understanding the replication strategy of the virus, production of pure virus in quantity, and field trials.

CONCLUSION

Although a number of highly effective insecticides are available for fire ant control, they must be used on a regular basis to maintain an area (e.g., school playground or park) free of fire ants. Discovery and utilization of effective natural enemies, including pathogens and parasitoids, will provide a sustainable, permanent reduction of fire ant populations. Furthermore, natural enemies will help to establish an ecological equilibrium without harming the native community. Establishment of natural enemies as biological control agents perhaps represents the most feasible strategy to suppress populations of well entrenched populations of invasive ants to tolerable levels.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 454).

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