Ant allergens and hypersensitivity reactions in response to ant stings

Rutcharin Potiwat1 and Raweerat Sitcharungsri2

Summary

Hypersensitivity reactions caused by ant stings are increasingly recognized as an important cause of death by anaphylaxis. Only some species of ants (e.g. Solenopsis spp., Myrmecia spp., and Pachycondyla spp.) cause allergic reactions. Ant species are identified by evaluating the morphologic structures of worker ants or by molecular techniques. Ant venom contains substances, including acids and alkaloids, that cause toxic reactions, and those from Solenopsis invicta or the imported fire ant have been widely studied. Piperidine alkaloids and low protein contents can cause local reactions (sterile pustules) and systemic reactions (anaphylaxis). Imported fire ant venoms are cross-reactive; for example, the Sol i 1 allergen from S. invicta has cross-reactivity with yellow jacket phospholipase. The Sol i 3 allergen is a member of the antigen 5 family that has amino acid sequence identity with vespid antigen 5. The clinical presentations of ant hypersensitivity are categorized into immediate and delayed reactions: immediate reactions, such as small local reactions, large local reactions, and systemic reactions, occur within 1–4 hours after the ant stings, whereas delayed reactions, such as serum sickness and vasculitis, usually occur more than 4 hours after the stings. Tools for the diagnosis of ant hypersensitivity are skin testing, serum specific IgE, and sting challenge tests. Management of ant hypersensitivity can be divided into immediate (epinephrine, corticosteroids), symptomatic (antihistamines, bronchodilators), supportive (fluid resuscitation, oxygen therapy), and preventive (re-sting avoidance and immunotherapy) treatments. (Asian Pac J Allergy Immunol 2015;33:267-75)
notabilis) were originally described from Thai territories and are located in agricultural fields and forests.5

Invasive ants are exotic species that establish colonies outside their native areas and can cause a decline or a change in diversity, community, and populations of native invertebrates, vertebrates, and plants by their invasion and displacement.6 However, invasive ant species, such as Solenopsis geminata and Tetraponera rufonigra, can also play an important role in the impact on clinical hypersensitivity reaction.7, 8 The important invasive ant species are widely distributed worldwide (Table 1).9-11 Of these invasive ant species, Anoplolepis gracilipes (yellow crazy ant), Pheidole megacephala (big-headed ant), and Solenopsis geminata (tropical fire ant) are all present in Thailand.6 The yellow crazy ant is thought to decrease the diversity and population of native fauna and flora in ecosystems due to its replacement of natural native species, but information on its distribution is rarely reported.

**The medical importance of ant-induced hypersensitivity**

Thailand has a number of ants of medical importance. The red imported fire ant (Solenopsis invicta, Buren) is an important invasive ant species that is also present in many other parts of the world, and is considered to cause hypersensitivity in both children and adults. Other fire ants in the genus Solenopsis, including S. invicta, S. richteri, S. geminata, S. saevissima, S. xyloni, and S. aurea can cause allergic reactions.10, 12 Importantly, there is cross-reactivity among venoms of the Solenopsis species, such as S. xyloni and S. aurea, and other hymenoptera.13, 14 The tropical fire ant (S. geminata) is a natural native species with a wide distribution throughout Thailand whose stings can cause allergic reactions. Another group of ants that cause hypersensitivity belong to the genus Myrmecia, especially M. pilosula, which are found in Australia.15-17 However, the ant species that are most commonly responsible for anaphylaxis in Thai patients are Solenopsis geminata, Tetraponera rufonigra, and Odontoponera denticulata (Figure 1). Patients with stings from these ants were referred to tertiary care hospitals in Bangkok for immunotherapy, and ants were sent for species identification at the Faculty of Tropical Medicine, Mahidol University (unpublished data). All three species are aggressive ants with painful bites or stings. Both T. rufonigra and O. denticulata have a stinger at the end of the gaster (Figure 2). T. rufonigra-induced hypersensitivity has been reported in humans after stings and can cause severe anaphylaxis.18 The first case of T. rufonigra-induced anaphylaxis was reported from Thailand. The patient was a 17-month-old girl who presented with two episodes of urticaria, angioedema, dyspnea, and loss of consciousness.7

**Species identification of the most common causative species of ant anaphylaxis in Thailand**

The most common causative species of ant anaphylaxis in Thai patients are Solenopsis geminata, Tetraponera rufonigra, and Odontoponera denticulata. S. geminata belongs to the family Formicidae, subfamily Myrmicinae, genus Solenopsis, species geminata. The morphology of Solenopsis can be confused with a smaller species of Monomorium, although Solenopsis is distinguished by the presence of a two-segmented petiole.19, 20 Identification of fire ant species is very difficult and involves evaluating the morphology of worker ants rather than just one specimen. S. geminata workers have a polymorphic size of 3–8 mm body length. The body has a reddish brown color and is mostly smooth and shiny, without sculpture. The head of the S. geminata worker is divided into two notches, while in S. invicta workers these notches are absent (Figure 2). The differentiation of tropical fire ant morphology is determined using worker ants but can also be characterized using molecular techniques based on mitochondrial DNA encoding cytochrome oxidase subunit I (COI) or the internally-transcribed spacer (ITS) gene.21 The differentiation of Solenopsis species by morphology alone is difficult and can often be confused with other species; therefore, molecular identification is now widely used. PCR amplification of the COI gene followed by cutting nucleotide sequences with the HinI restriction enzyme can be used to distinguish S. invicta, S. geminata, and S. xyloni from Florida.22 However, even though molecular amplification is now widely used, it is expensive.

T. rufonigra belongs to the family Formicidae, subfamily Ponerinae, genus Tetraponera, species rufonigra. It is black and shiny except on their alitrunk that has an orange line, which is the specific characteristic of T. rufonigra. Differentiation of
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**Table 1. The geographical distribution of major invasive ant species.**

<table>
<thead>
<tr>
<th>Ant species</th>
<th>Geographical Range</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoplolepis gracilipes*</td>
<td>Africa, Tropical Asia?</td>
<td>91</td>
</tr>
<tr>
<td><strong>(Yellow crazy ant)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinoponera gigantea</td>
<td>South America, Argentina</td>
<td>92</td>
</tr>
<tr>
<td>Hypoponera sp.</td>
<td>Brazil, Malaysia</td>
<td>93, 94</td>
</tr>
<tr>
<td>Linepithema humile (Argentine ants)</td>
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<td>95, 96</td>
</tr>
<tr>
<td>Myrmecia pilosula</td>
<td>Far East, Australia</td>
<td>47, 66, 71</td>
</tr>
<tr>
<td>Odontomachus hauri</td>
<td>Caracas, Venezuela</td>
<td>71, 97</td>
</tr>
<tr>
<td>Pachycondyla chinensis (Chinese</td>
<td>Japan, Asia</td>
<td>98, 99</td>
</tr>
<tr>
<td>needle ant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pachycondyla semnaarensis (Samsun</td>
<td>West Africa</td>
<td>100, 101</td>
</tr>
<tr>
<td>ants)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheidole megacephala (Big-headed</td>
<td>Africa</td>
<td>10</td>
</tr>
<tr>
<td>ant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomyrnex electus</td>
<td>Brazil</td>
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<tr>
<td>Rhytidoponera metallica</td>
<td>Queensland (Australia)</td>
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<td>Solenopsis aurea</td>
<td>Southern California (US)</td>
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<tr>
<td>Solenopsis geminata (Tropical fire</td>
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<td>ant)</td>
<td></td>
<td></td>
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<tr>
<td>Solenopsis invicta (Red imported</td>
<td>South America</td>
<td>13, 105-107</td>
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<td>fire ant)</td>
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<td></td>
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<tr>
<td>Solenopsis richteri (Black imported</td>
<td>South America</td>
<td>13, 44, 108</td>
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<tr>
<td>fire ant)</td>
<td></td>
<td></td>
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<tr>
<td>Solenopsis saevissima</td>
<td>São Paolo (Brazil)</td>
<td>109, 110</td>
</tr>
<tr>
<td>Solenopsis xyloni</td>
<td>Southwestern states (US), Mexico</td>
<td>13, 44</td>
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<tr>
<td>Tetraponera rufonigra</td>
<td>Sri Lanka, Thailand</td>
<td>7, 18</td>
</tr>
<tr>
<td>Wasmannia auropunctata (Little fire</td>
<td>Central and South America</td>
<td>111, 112</td>
</tr>
<tr>
<td>ant)</td>
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*Origin of the yellow crazy ant is unknown although some studies indicate it is from tropical Asia.

*T. rufonigra* and *O. denticulata* can be done by the body shape, color, and the number of nodes on the petiole segments. *T. rufonigra* has two nodes on the petiole segment while, *O. denticulata* has a single node (Figure 1). Moreover, *O. denticulata* has a narrow node with a small spine on the petiole segment.

**Ant sting and venom isolation**

The tropical fire ant (*S. geminata*) is an aggressive fire ant with a painful sting that is potentially life threatening. Normally, anaphylactic reactions caused by ants occur in patients that come into contact with the ant venom during stinging. In general, the constituents of ant venom responsible for anaphylaxis are composed of spray acid and alkaloids derived from piperidine. The ant venom is usually injected from a poison gland located at the posterior part of the ant (gaster), which secretes various chemicals from different compartments depending on whether the ant is a queen or a worker. The main toxic chemicals from an *S. geminata* queen were identified by gas chromatography–mass spectrometry and included 2-alkyl-6-methylpiperidine alkaloids, δ-lactone, and α-pyrone, which were originally identified as components of the *S. invicta* queen attractant pheromone.

Ant whole body extract (WBE) or the induced secretion of poison from the posterior gland has been used for ant immunotherapy. This isolated venom is collected by a micro-glass tube during induced ant stinging, and might be useful for
immunotherapy or testing the cross-reactivity to other hymenoptera, although it is difficult to isolate and the activity is easily lost.31, 32 Currently, S. invicta WBE is commercially available for skin testing.25, 33, 34

Ant allergens

Ant venoms are composed of various biologically active peptides and protein components with each ant species having a variety of major allergenic proteins. The most frequently studied ant venom components are from fire ants with the venom of the red imported fire ant (S. invicta) being the most extensively investigated.35 Each S. invicta sting transfers 0.04 to 0.11 µL of venom and 10 to 100 ng of proteins.36 The alkaloid part of the S. invicta venom causes a sterile pustule at the sting site, and has cytotoxic and hemolytic properties.37 The protein part is composed of four major allergens, i.e., Sol i 1, Sol i 2, Sol i 3, and Sol i 4.38 Sol i 1 is a 37-kDa protein with phospholipase A1 activity that comprises 2–5% of the total venom protein.39 Sol i 2 is a 26-kDa protein that is also present in the venom of other species but is dissimilar to the other sol allergens. This allergen comprises 67% of the total venom proteins. Sol i 3 is a 24-kDa protein that is a member of the antigen 5 family and comprises 20% of the total venom protein. Sol i 4 has a 13-kDa molecular mass and comprises 9% of the total venom proteins, which are different from the other sol allergens.14

There are still limited data on protein allergens from ants in Asia, especially those in Thailand where T. rufonigra and S. geminata are the most common causative species of ant anaphylaxis.8 T. rufonigra protein allergen was characterized and identified by immunoblot assay and showed a dominant active protein of approximately 120 kDa.7 A study of S. geminata allergen revealed that it was composed of four venom proteins: Sol gem 1, Sol gem 2, Sol gem 3, and Sol gem4 with molecular weights of 37, 28, 26, and 16 kDa, respectively.40 Of these, Sol gem 2 has a dimer form with a molecular weight of 28/15 kDa (under non-reducing and reducing condition). The amino acid sequence analysis of Sol gem 2 exhibited a degree of homology with the Sol i 2 allergen of S. invicta and their allergenic properties are probably similar. A study of Myrmecia ant venom identified many venom allergens with post-translational processing, and found that most allergen activity was caused by disulfide-linked heterodimers.41

Ant venom nomenclature

The International Union of Immunological Studies Allergen Nomenclature Subcommittee (IUIS) has assigned official names to fire ant allergens based on venom proteins: 1 was assigned to phospholipase A1B; 2 was assigned to Sol i 2 homologous proteins; 3 was assigned to antigen 5-related proteins; and 4 was assigned to Sol i 4 homologous proteins.42 A revision of the nomenclature of allergenic components of Myrmecia should be done to conform to the IUIS guidelines.43

Cross-reactivity among Solenopsis species

In addition to S. invicta, four other species of Solenopsis, including S. richteri (black imported fire ant), S. xyloni (southern fire ant), S. aurea (desert fire ant), and S. geminata (tropical fire ant) also cause allergic reactions.44

Regarding the structure of S. richteri allergens, Sol r 1 and Sol r 3 are similar to Sol i 1 and Sol i 3 allergens from S. invicta, respectively, while the Sol r 2 allergen of S. richteri and S. invicta have less homology.44 There is no allergen that is analogous to

(A) Solenopsis geminata  (B) Tetraponera rufonigra  (C) Odontoponera denticulata

Figure 1. Species of ants most often responsible for anaphylaxis in Thai patients.
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Sol i 4 in *S. richteri* even though the venom allergens of *S. richteri* and *S. invicta* appear to be allergenically similar.45

A study of *S. xylopi* allergic reactions showed that serum from patients with reactions to stings by *S. xylopi* were reactive to Sol i 1 and Sol i 3, but were only marginally reactive to Sol i 2.44

Allergic reactions from *S. aurea* stings are reported occasionally, but data is limited. Nonetheless, cross-reactivity between allergens of *S. aurea* and *S. invicta*, especially Sol 1 and Sol 3, have been reported.34

The structures of four *S. geminata* venom proteins appear to be similar to the Sol i 1, Sol i 2, Sol i 3, and Sol i 4 allergens of *S. invicta*. The Sol gem 2 allergen from *S. geminata* venom was characterized and shown to have allergenic properties similar to those of Sol i 2 from *S. invicta*.40 Sol i 2 has 72.3% amino acid sequence homology with Sol gem 2 and 78.2% sequence homology with Sol r 2.46 In summary, there are some sequence differences between the Sol 2 and Sol 4 antigens of all fire ant venoms, but IgE antibodies against fire ant venoms are highly cross-reactive. Indeed, reactions to stings from any fire ant species can cause sensitization to other species.

**Cross-reactivity between Solenopsis species and other ants**

Ants of the genus *Pachycondyla* are a major cause of ant hypersensitivity in Asia and the Middle East.37,48 Studies of cross-reactivity between *Pachycondyla* and imported fire ants are controversial, however. A study from Korea revealed no cross-reactivity,49 but another study from the Middle East showed cross-reactivity between *Pachycondyla sennaarensis* and imported fire ant venom by immunoblot testing.50

Ants of the genus *Myrmecia* are the predominant cause of ant hypersensitivity in Australia.51 Allergens from these ants are complex with highly basic peptides.42, 52 However, no evidence of cross-reactivity between *Myrmecia* and fire ant venom was found in allergic patient sera.46

![Figure 2.](http://www.apjai-journal.org) Morphological outline of the tropical imported fire ant (*Solenopsis geminata*). Tropical fire ant (*S. geminata*) workers are usually used for species identification. *S. geminata* has the sting apparatus at the end of gaster and the number of petiole segments is usually used for species identification. (A) *S. geminata* workers have a 2-segmented petiole and two notches on the head. (B) The ant body is divided into four parts including head, alitrunk, petiole, and gaster.
Cross-reactivity between Solenopsis species and other Hymenoptera

Cross-reactivity among insects and unique ant allergens have been established for some hymenoptera venoms with studies reporting cross-reactivity among major proteins of vespid (yellow jackets) and S. invicta. The Sol i 1 allergen of S. invicta is a phospholipase related to those found in vespid venom with 33–38% amino acid identity. A study reported cross-reactivity between Sol i 1 and vespid phospholipase with clinical significance. Sol i 3 is a member of the antigen 5 family with 43–50% amino acid sequence identity with vespid antigen 5. Sol i 3, however, has few conserved areas in common with vespid antigen 5, and there is a lack of IgE cross-reactivity between the two allergens.

Clinical presentation of ant hypersensitivity

Hymenoptera sting-induced anaphylaxis has been reported worldwide, as well as in Thailand, with studies revealing cases of ant-induced severe hypersensitivity reactions. Reactions to ant hypersensitivity can be divided into immediate- and delayed-type hypersensitivity reactions. Immediate reactions occur within 1–4 hours after ant stings, and can be further divided into normal local reactions (pain, swelling, erythema, heat, and sterile pustules at sting sites), large local reactions (a reaction larger than 10 cm in diameter persisting for longer than 24 hours), generalized cutaneous reactions (pruritus and urticaria), and systemic reactions (anaphylaxis).

There have been many previous reports of imported fire ant-induced hypersensitivity reactions and anaphylaxis. A large study of 20,755 cases with fire ant stings estimated that 413 patients (2%) had anaphylaxis. Another study showed that S. invicta-induced hypersensitivity reactions were the cause of death in 83 of 29,300 patients in a physician survey. Infants and elderly people are at risk of indoor imported fire ant stings. A review of local newspapers in the United States between 1991 and 2004 revealed 10 indoor fire ant stings that were previously unreported and 10 stings that were reported in the medical literature. Six of the 20 patients died within one week after hypersensitivity reactions occurred.

Immediate reactions from the stings of ants other than imported fire ants have also been reported. Ants of the genus Pachycondyla are the most common cause of anaphylaxis in tropical areas of Asia and the Middle East. Pachycondyla sennaarensis was the cause of anaphylaxis in 31 United Arab Emirates patients with positive skin tests and specific IgE antibodies to this ant, and Pachycondyla chinensis induced large local reactions after stings in 1.6% of patients in Korea. Another study showed that 7 of 327 patients (2.1%) had systemic immediate reactions and that four patients had anaphylaxis. Ants of the genus Myrmecia in Australia caused several deaths from anaphylaxis. Fatal anaphylaxis from the southern fire ant or S. xyloni in the United States was also reported. Nonfatal immediate reactions from S. xyloni were also shown in other studies. Other native fire ants (S. aurea and S. geminate) caused immediate hypersensitivity reactions in studies from the United States, as did harvester ants (genus Pogonomyrmex) and oak ants (Pseudomyrmex species). Delayed reactions usually occur more than 4 hours after ant stings and cause dermatitis, arthralgia, and lymphadenopathy from serum sickness, renal abnormalities, and vasculitis, respectively. Serum sickness from fire ant attacks was reported in the United States and nephrotic syndrome occurred in a child two weeks after being stung by a fire ant.

Diagnosis of ant-induced hypersensitivity

Diagnosis of ant-induced hypersensitivity can be performed by documenting a patient history of allergic reaction to ant stings, by physical examination, or through the use of in vivo and/or in vitro tests. A history of the circumstances of the sting, including where it occurred, what the individual was doing, and the nature of the ant habitat, should be asked. Identification of the causative ant should be determined by an entomological specialist. Physical examination for evidence of ant stings should also be completed. Vital signs, as well as respiratory and gastrointestinal signs and symptoms, should be carefully examined for evidence of anaphylaxis. Skin lesions, such as pseudopustules at the sting site within 24 hours after being stung, can also help diagnose imported fire ant stings.

A widely used in vivo method to diagnose fire ant-sting hypersensitivity includes skin testing using imported fire ant WBE. The result is positive when the skin-prick test is performed using a concentration of 1:1000 (w/v) of imported fire ant WBE. If the skin-prick test result is negative, an intradermal skin test using concentrations of 1:1,000,000 to 1:1000 (w/v) of the imported fire ant WBE should be performed.
Serum specific IgE to imported fire ant venom can be used as an in vitro test to diagnose hypersensitivity to imported fire ants. The sensitivity of serum specific IgE is lower than skin testing and therefore, is only recommended when skin testing cannot be done or interpreted. Serum specific IgE testing can also help in diagnosis of imported fire ant allergy in suspected cases with negative skin-test results. Twenty-nine percent of adults with a history of fire ant exposure without systemic reactions had a positive skin-test result. A study revealed that 24% of non-allergic control cases had positive specific-IgE test results for imported fire ant venom. Skin testing and serum specific IgE should not be performed without a clinical history of allergic reactions from imported fire ants because of the high degree of asymptomatic IgE production in an exposed population.

The skin-prick test was used to diagnose *P. chinensis* anaphylaxis in Korea and higher *P. chinensis*-specific IgE levels were observed in patients with anaphylaxis compared with patients with large local reactions or the asymptomatic population.

The ant-sting challenge test is used for patients with a history of ant anaphylaxis but negative skin testing and serum specific IgE results. It has not been used extensively because of the chance of provoking serious allergic reactions. Specialized centers are required for sting challenges.

Management of ant-induced hypersensitivity

Management of ant-induced hypersensitivity can involve either immediate treatment for anaphylaxis or preventive treatment. Immediate treatment for anaphylaxis includes epinephrine at a dosage of 0.01 mg/kg to a maximum of 0.3 mg for children and 0.3 to 0.5 mg for adults. An epinephrine auto-injector should be prescribed for patients with histories of severe allergic reactions. Other medications, including antihistamines and bronchodilators, should be given as symptomatic treatments. Late-phase anaphylaxis can be prevented by corticosteroids. Supportive treatments should follow standard guidelines for anaphylaxis, including fluid resuscitation, oxygen therapy, and placing the individual in a recumbent position. Local reactions can be treated by local wound care, including wound dressing, topical or systemic antibiotics for secondary infection, cold compression to reduce local swelling, and topical corticosteroids to limit the swelling of large local reactions. Pseudopustules from fire ant stings should be kept clean and intact to prevent secondary bacterial infection.

Preventive measures for the treatment of ant anaphylaxis include immunotherapy and re-sting avoidance. Immunotherapy with imported fire ant WBE is considered for adults and children with systemic reactions to ants who have shown positive results by skin testing or specific IgE antibodies. This recommendation is the same as the immunotherapy recommended for hymenoptera hypersensitivity. Children ≤16 years of age with isolated cutaneous systemic reactions from ant stings do not require immunotherapy because of the low risk of subsequent systemic reactions. Nevertheless, children with cutaneous reactions from fire ant stings with a significant exposure risk may be considered for treatment with imported fire ant immunotherapy.

The dosing schedule for imported fire ant immunotherapy is not uniform because of the rapidity of the build-up phase. Most expert recommendation protocols indicate treatment once or twice weekly until a maintenance dose is reached. A maintenance dose of 0.5 ml of 1:100 (w/v) imported fire ant WBE is recommended. If treatment failure occurs, increased doses can be considered. Some recommendations suggest 0.5 ml of 1:10 (w/v) imported fire ant WBE as a maintenance dose. In endemic areas with a high incidence of imported fire ant stings, the rapid increase of the dose protocol or a rush schedule in the maintenance phase is preferable to the conventional schedule. A 2-day rush protocol with imported fire ant immunotherapy showed good safety and efficacy. A 1-day rush immunotherapy protocol in children and adults was also safe and effective.

Imported fire ant immunotherapy can be advanced to the maintenance phase within only 1–2 days by rush protocol. The duration of immunotherapy is usually 3–5 years. For patients at risk of re-exposure, the recommendation for discontinued immunotherapy is when the results of skin testing or serum specific IgE for fire ants are negative.

Currently, there is insufficient data on the efficacy of immunotherapy for ant species other than the imported fire ant, and therefore, exposure avoidance—such as not disturbing ant nests, allowing nests to be removed by trained professionals, avoiding walking barefoot or with sandals, and wearing long pants and long-sleeved shirts when working outdoors—should be
recommended to ant hypersensitivity patients.

Acknowledgments

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