Pheromone Biosynthesis Activating Neuropeptide (PBAN)/Pyrokinin Family of Peptides and Fire Ants, Solenopsis spp.

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ABSTRACT

The fire ant, Solenopsis invicta, is an economically important invasive pest ant, causing over 6 billion dollars in control and repair costs each year in the United States. The fire ant is becoming a global problem increasing its importance and the need for the development of biologically-based control methods. The PBAN/pyrokinin gene is ubiquitous to insects and produces 4-5 neuropeptides that play critical roles in insect development and in reproduction. The most well studied function is regulation of moth pheromone biosynthesis through the Pheromone Biosynthesis Activating Neuropeptide, PBAN. The fire ant is one of the most studied social insects and over the last 50 years a great deal has been learned about the behaviors and chemistry of pheromone communication in this ant. However, virtually nothing is known about the regulation of these pheromone systems. We review here our research to date on the PBAN/pyrokinin gene and the fire ant in preparation for determining the function of the product neuropeptides in brood development and in adults. We discuss the following: a) PBAN/Pyrokinin peptides in fire ants; b) PBAN immunocytochemistry and the fire ant central nervous system (CNS); c) Identification of PBAN/pyrokinin neuropeptides from S. invicta and other Solenopsis species; and d) PBAN/Pyrokinin gene expression in the head, thorax and abdomen of S. invicta. These studies help lay the ground-work for the utilization of the PBAN/pyrokinin gene/peptide product system for novel biologically-based fire ant control.

Key words: fire ant, Solenopsis, PBAN, neuropeptide, gene expression, immunocytochemistry

Introduction

Of the world’s 100 worst invasive alien species, ants comprise 5%, and of the 17 land invertebrates listed, 28% are ants, including the red imported fire ant,

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Solenopsis invicta (Lowe et al., 2000)! In the last decade S. invicta has changed from an invasive pest ant in the United States to a global problem, with infestations occurring in Taiwan (Chen et al., 2006), Australia (Henshaw et al., 2005), mainland China (Zeng et al., 2005; Zhang et al., 2007), Mexico (Sánchez-Peña et al., 2005), and many Caribbean Island countries (Davis et al., 2001).

Solenopsis invicta, is an economically important invasive pest ant species, as has been well documented in the United States. This opportunistic omnivore occurs in very large numbers in its invasive range and prefers disturbed habitats—wherever there are human activities. In the United States the fire ant infests over 321 million acres and over $6 billion per year is spent for control and damage repair. This does not include medical costs or difficult to quantify environmental impacts. The economic sectors affected include: residential households, electric and communication systems, agriculture, golf courses, commercial businesses, schools and medical facilities, and parks and recreational areas (Lard, 2006). Of the chemical methods for controlling fire ants, toxic baits introduce the least amount of insecticide into the environment; however, they are expensive, negatively affect non-target ant species, and most have limited EPA registration. There is a need for biologically-based control alternatives.

Pheromone communication serves a range of functions in social insects, including mating, alarm, recognition, recruitment, orientation, and aggregation. In addition, social insects depend on sophisticated pheromone communication to maintain colony cohesiveness and sociality through releaser and primer pheromones. Wilson (1962) set the stage for fire ant chemical ecology for decades to come with three papers that investigated releaser pheromone communication among workers of the fire ant. Mass foraging (recruitment) and colony emigration were defined and could be fully induced by presenting extracts of Dufour’s glands to workers (Wilson, 1962a, b, c). Alarm behavior (rapid erratic movement of workers) was attributed to a cephalic substance and secondarily to Dufour’s gland components. Since this time other releaser pheromones have been defined behaviorally and chemically, and the probable source determined. The queen-produced recognition pheromone has been at least partly identified (Glancey et al., 1984) and is biosynthesized by the poison gland and released through the sting apparatus onto deposited eggs (Vander Meer et al., 1980; Vander Meer and Morel, 1995). After a long struggle a component of the fire ant alarm pheromone was isolated and identified as a pyrazine derivative (Vander Meer et al., 2010). Not well defined chemically is the chemical ecology of fire ant mating flights. These are characterized by the opening of the normally closed nest tumulus and frenzied activity by workers (sterile) and male and female sexual alates, prior to the alates taking flight. This behavior is mediated by a releaser pheromone linked to the mandibular gland (Obin and Vander Meer, 1994; Alonso and Vander Meer, 1997), but the chemistry remains unknown. Brood pheromones that induce worker care have not been adequately demonstrated in fire ants or other ants (Morel and Vander Meer, 1988). Several fire ant primer pheromones have been described functionally, however, none have been isolated and identified (Vargo, 1998; Vander Meer and Alonso, 2002). The above represents considerable activity in the chemical ecology of fire ants; however, thirty-nine different glands have been described for Formicidae (Billen and Morgan, 1998) and nine major exocrine glands of fire ants have been described including pygidial, metapleural, and propharyngeal glands (Billen, 1990). This suggests that additional pheromone discoveries are yet to be made.
Fire ant pheromone chemistry/behavior examples

There are a number of fire ant releaser pheromones where the behavior and chemistry have been elucidated. Queen recognition pheromones were isolated in a bioassay driven separation of active compounds from large numbers of fire ant queens. One component (dihydroactinidiolide) was later thought to be an artifact of the separation. The remaining two compounds are shown in Figure 1A (Rocca et al., 1983a, b).

The fire ant recruitment pheromone is a model of behavioral and chemical complexity. All components are derived from the Dufour’s gland. Z,E-alpha-farnesene (Fig. 1B) is solely responsible for worker orientation (back and forth movement along the trail (Vander Meer et al., 1981), whereas worker attraction (olfactometer bioassay) requires the farnesene and the homoeudesmane (see Fig. 1B) (Vander Meer et al., 1988). However, non-trailing worker ants are not induced to follow a trail composed of these two components. A third bioassay was developed to measure orientation induction, which indicated that the complex chemistry of the Dufour’s gland had to be reconstructed to elicit activity (12 components gave 85% of the activity from Dufour’s gland extracts; (Vander Meer et al., 1990). The three recruitment sub-categories are differentially activated by the amount of Dufour’s gland material released.

The fire ant alarm pheromone has been a perplexing problem since it was first described almost five decades ago (Wilson, 1962c). The amount of material produced by the fire ant is very small and the active compounds are highly volatile. We have only recently identified one component of the alarm pheromone, 2-ethyl-3,5-dimethylpyrazine (Vander Meer et al., 2010), from mandibular glands. The pheromone is available commercially as a 1:1 mixture with its isomer, 2-ethyl-3,5-
dimethylpyrazine (Fig. 1C).

**Pheromone production and regulation**

Although a variety of fire ant pheromones have been identified, little is known about the regulation of their production and release. Insects are known to use three hormonal signaling molecular classes of compounds to regulate pheromone biosynthesis: juvenile hormones, ecdysteroids, and pheromone biosynthesis activating neuropeptides (PBAN) (Tillman et al., 1999). PBAN is synthesized in the subesophageal ganglion (SG), located near the brain, and released into the hemolymph, where it acts on pheromone glands to stimulate pheromone biosynthesis in moths. It was first identified from the moth, *Helicoverpa* *zea* (Raina et al., 1989). The mechanism of PBAN control over pheromone production is well understood for sex pheromone biosynthesis in a number of lepidopteran moths. However, thus far no other insect group has been shown to regulate pheromone biosynthesis using PBAN.

**PBAN/Pyrokinin peptides in insects**

PBAN is one of five peptides produced by the PBAN/pyrokinin gene that represent a family of peptides that have in common a five C-terminal amino acid sequence, FXPRLamide, that represents the minimal sequence required for activity (Raina and Kempe, 1990, 1992; Fonagy et al., 1992; Kuniyoshi et al., 1992). FXPRLamide peptides will generally stimulate inappropriate sex pheromone synthesis in moths. Insects from a variety of orders have been found to have the PBAN/pyrokinin gene and have peptides with the FXPRLamide motif. Besides pheromone regulation, members of the PBAN/pyrokinin family of peptides have multiple effects on insect development and reproduction, for example: 1) stimulate pheromone biosynthesis in female moths (Raina et al., 1989); 2) induce melanization in moth larvae (Matsumoto et al., 1990; Altstein et al., 1996); 3) induce embryonic diapause in *Bombyx mori* (Suwan et al., 1994); 4) stimulate visceral muscle contraction (Nachman et al., 1986; Predel and Nachman, 2001); 5) accelerate puparium formation in several flies (Zdarek et al., 1997; Verleyen et al., 2004); and 6) terminate pupal diapause in heliothine moths (Sun et al., 2003; Xu and Denlinger, 2003). These data highlight the multifunctional roles for PBAN/pyrokinin peptides identified in insects, as well as possible avenues for novel control.

**Mode of action of PBAN in insect pheromone biosynthesis**

Generally, PBAN is released into the hemolymph to circulate and reach the target pheromone gland. PBAN acts directly on pheromone glands by stimulating pheromone biosynthesis in a number of lepidopteran moths. The signal transduction mechanism for enzyme activation is activated quickly once PBAN binds with the receptor. The pathway remains active for a period of time. Recently, the first G-protein coupled receptor for PBAN has been identified from pheromone glands of *H. zea*, and it was demonstrated that PBAN activates the receptor at low nanomolar concentrations (Choi et al., 2003). Additional PBAN receptors identified from different moth species are similar based on peptide sequence identity (Hull et al., 2004; Rafaeli et al., 2007; Zheng et al., 2007; Kim et al., 2008).

**PBAN/Pyrokinin peptides in fire ants**

Generally, corn earworm, *Helicoverpa* *zea*, female moths do not produce pheromones during the photophase or when decapitated. However, injection of a) decapitated *H. zea* females, or b) females in the photophase
with Br-SG extracts or with *H. zea* PBAN, induces, otherwise inappropriate synthesis of *H. zea* sex pheromone (Raina et al., 1989). If fire ants produced PBAN/Pyrokinin family peptides, injection of these into decapitated *H. zea* females should induce some level of *H. zea* sex pheromone production. The results of injecting 2-day old *H. zea* females, which were decapitated 24-h earlier, with *S. invicta* Br-SG extract are shown in Fig. 2. All female (workers, female alates, and functional queens) and male fire ant Br-SG extracts stimulated the inappropriate biosynthesis of the *H. zea* sex pheromone, (Z)-11-hexadecenal (Fig. 2) significantly greater than the saline control. However, the amount of pheromone production was significantly lower than the result for synthetic *H. zea* PBAN injections for all fire ant samples (Fig. 2). The quantitative differences in pyrokinin/PBAN-like peptides from the fire ant sexual forms might be attributable to differences in production, gene expression related to sexual dimorphism, or functional differences between workers and sexuals (Choi et al., 2009). Moths are the only insects that have been shown to use a PBAN to stimulate pheromone production, although correlations of moth PBAN stimulating some pheromone biosynthesis have been reported in the Hessian fly, *Mayetiola destructor* (Foster et al., 1991).

**PBAN immunocytochemistry and the fire ant central nervous system (CNS)**

Immunocytochemical tools (anti-serum generated against a truncated C-terminal end of *H. zea* PBAN) had been developed to localize PBAN synthesis/accumulation in the central nervous system (CNS). This system was used to visualize PBAN immunoreactivity in dissected Br, SG and ventral nerve cord (VNC) of *S. invicta*, queens, female and male alates, and workers (see Fig. 3 for worker example). The number and location of PBAN-like immunoreactive neurons showed a similar pattern for all sexual forms (Choi et al., 2009). The distribution pattern of PBAN-like immunoreactive materials in the fire ant CNS was similar to that already shown in fly and moth species (Choi et al., 2001; Choi et al., 2004). However, unlike...
Fig. 3. Central nervous system of an adult fire ant worker. Photomicrographs of the brain and subesophageal ganglion (a), thoracic ganglia (b), and abdominal ganglia (c). (d) representation of CNS and PBAN like immunoreactivity in an adult fire ant worker (Br brain, SG subesophageal ganglion, CC corpora cardiaca, CA corpora allata, PSO perisympathetic organ, T1-T3 first to third thoracic ganglia, A1-A8 first to eighth abdominal ganglia). Bar = 50 μm (from Choi et al., 2009).
moths, the last fire ant abdominal ganglion does not contain PBAN immunoreactive neurons (Fig. 3).

Concurrent with the pyrokinin/PBAN studies, an anatomical atlas was constructed of the entire CNS of the fire ant. This was the first report of a complete CNS atlas of an ant species. The ventral nerve cord in ancestral insects has been shown to consist of eight discrete abdominal ganglia. In evolutionarily advanced insects, the number of abdominal ganglia varies. In most cases, the first abdominal and/or the terminal abdominal ganglia are fused with one or more neuromeres; therefore, the number of discrete ganglia is reduced (Niven et al., 2008). The ventral nerve structure of fire ant female alates and queens shows only two thoracic and four abdominal ganglia. The pro-thoracic ganglion (T1) is discrete, but the meso- (T2) and meta- (T3) thoracic ganglia, and the first (A1) and second (A2) abdominal ganglia are fused together to form the second structurally discrete thoracic ganglia, as found in most insects. Of the four discrete abdominal ganglia, A3, A4, and A5 are distinct ganglia; however, the last three fire ant abdominal ganglia may be fused forming the single terminal ganglion, which account for the expected eight abdominal ganglia (Choi et al., 2009). A similar fusion pattern for both thoracic and abdominal neuromeres in the ventral nerve cord has been shown in Lepidoptera and Hymenoptera species (Niven et al., 2008).

Demonstrating the presence of pyrokinin/PBAN neuropeptides and the localization of the immunoreactive neurons in S. invicta provided correlative evidence of physiological roles for these neuropeptides in fire ants.

Identification of PBAN/pyrokinin neuropeptides from S. invicta

Specific PBAN/Pyrokinin gene PCR primers were used to amplify and isolate a 780 bp-long full cDNA sequence that contained the entire open reading frame (ORF) of 531 nucleotides encoding 176 amino acids, including four FXPRL peptide domains. The structures of the four S. invicta FXPRL peptides and their homology with the known moth PBAN/Pyrokinin peptides are: (1) Diapause Hormone (DH) = 15-AA (TSQDIASGMWFGPRL); (2) β-Neuropeptide = 8-AA (QPQFTPRL); (3) PBAN = 26-AA (GSGEDLSYGDAYEVDED DHPLFVPRL); and (4) γ-Neuropeptide = 9-AA (LPWIPSPLR) (Choi and Vander Meer 2009). Unlike the five peptides, DH, α, β, PBAN and γ peptides, encoded from PBAN genes of lepidopteran moths, α-NP is not present in fire ant PBAN gene based on peptide homology. The four S. invicta PBAN/Pyrokinin gene products were synthesized and their pheromonotropic activities measured by their ability to induce inappropriate sex pheromone biosynthesis in decapitated H. zea females (Choi and Vander Meer, 2009). All four synthetic peptides were capable of activating significant sex pheromone biosynthesis in the female moths, showing the expected cross-reactivity of the conserved FXPRL-NH₂ moiety (Fig. 4).

When compared with other PBAN/Pyrokinin genes, the S. invicta PBAN/Pyrokinin cDNA is similar to the honeybee, but is distant from moth and beetle species (Choi and Vander Meer, 2009). Regarding PBAN, S. invicta PBAN (26-AA) is short and has a low degree homology with the honeybee PBAN (33-AA) even though they are both Hymenoptera. Based on amino acid sequences encoded from known insect PBAN/Pyrokinin genes, neuropeptide diversity is consistent with the taxonomic or phylogenetic classification of Insecta (Choi and Vander Meer, 2009). While we now know the amino acid sequences of the S. invicta PBAN/Pyrokinin peptides their function remains to be determined.
Identification of PBAN/pyrokinin neuropeptides from other Solenopsis species

The Solenopsis group is a large genus with 185 described species (Pitts et al., 2005). The genus is difficult taxonomically due to the lack of reliable diagnostic characters. Most recently they have been re-classified into four complexes: S. virulens, S. tridens, S. geminata and S. saevissima (Pitts et al., 2005). Using the same techniques used to isolate and sequence the S. invicta PBAN/Pyrokinin cDNA (Choi and Vander Meer, 2009) the PBAN/Pyrokinin cDNA was sequence for four additional Solenopsis species: S. richteri and a hybrid of S. invicta and S. richteri (Saevissima complex), S. geminata (Geminata complex), S. pergandii and S. carolinensis (members of a large group classified as thief ants that live primarily underground). Solenopsis PBAN genes were divided into two groups of cDNAs translating 176-AA for S. invicta, S. richteri and the hybrid, and 177-AA for S. geminata, S. pergandii and S. carolinensis. The one additional amino acid residue (F) in the later group was associated with the PBAN domain (Choi et al., 2010).

Comparison of the five fire ant PBAN/Pyrokinin genes, showed that S. carolinensis was the most distant from the other species based on nucleotide sequence homology and the Saevissima complex species were separated from the Geminata complex species (Fig. 5). This phylogenetic classification by the neuropeptide sequence is consistent with the morphological cladistic analysis of the Solenopsis genus (Pitts et al., 2005), except that S. pergandii most closely resembled S. geminata, and both were very distant from S. carolinensis, indicating significant evolutionary distance between the two thief ant species (Fig. 5).

![Graph showing pheromonotropic activity](image)

**Fig. 4.** Pheromonotropic activity of synthetic peptides deduced from Soi-PBAN cDNA and Hez-PBAN in Helicoverpa zea female moths (top) and four synthetic peptide sequences (bottom). Bars represent the means SEM of at least 5 replications. Bars with the same letters are not statistically different by analysis of Fisher PLSD (ANOVA) (p < 0.05) (from Choi and Vander Meer, 2009).
**PBAN/Pyrokinin gene expression in the head, thorax and abdomen of *S. invicta***

*Solenopsis invicta* PBAN/Pyrokinin gene transcripts from head, thorax and abdominal tissues of female adults were quantified using reverse transcription (RT)- and quantitative (Q)-PCRs (Choi et al., 2011) (Fig. 6). The head had the strongest transcriptional signal and maximum number of Soi-PBAN gene copies (1990 ± 280) supporting the strong PBAN immunoreactive response detected from the fire ant Br-SG (see Fig. 3) (Choi et al., 2009). PBAN/Pyrokinin mRNA transcription and gene copies in thoracic tissue were lower (149 ± 47 copies); however, abdominal tissue gave a non-detectable transcriptional signal and minimal number of PBAN/Pyrokinin gene copies (6 ± 2) (Fig. 6) (Choi et al., 2011). The latter result was not predicted based on the strong PBAN-like immuno-response detected from abdominal neurons (see Fig. 3). The most likely explanation for this apparent discrepancy is that the immuno-response assay is not specific to the PBAN peptide but responds generally to peptides with a-FXPRLamide sequence at the C-termini. Therefore, there is a gene or genes in the abdomen other than the PBAN/Pyrokinin gene that also produce FXPRL C-terminal peptides. Currently, only two gene families are known to produce FXPRL peptides: the PBAN/Pyrokinin and the capability (CAPA) genes. The CAPA gene encodes one FXPRL neuropeptide with a very conserved motif, WFGPRL at the C-termini (Predel and Wegener, 2006). Many CAPA genes encoding FXPRL peptides have been isolated from the abdominal neurohemal organs of several insect groups (Predel and Wegener, 2006). This result increases the complexity of the FXPRL neuropeptide physiology but extends the opportunities to disrupt the normal functioning of fire ant colonies.

**Conclusion**

The fire ant is an excellent social insect model to determine the role of PBAN and/or similar peptides in the regulation of pheromone biosynthesis and other physiological functions. Several fire ant pheromone systems have already been well defined behaviorally and chemically, thus providing measures for their disruption or enhancement. The key to utilization of the neuropeptides in control of fire ants is the identification of the physiological effect(s) of the fire ant.
PBAN/Pyrokinin peptides. The accumulation of work presented here lays the foundation for the elucidation of these physiological effects in terms of pheromone regulation, as well as the many other essential functions in fire ant development and reproduction.

**References**


**Choi M-Y, Vander Meer RK.** 2009. Identification of a new member of the PBAN family of neuropeptides from the fire ant, *Solenopsis invicta*. Insect


Kim YJ, Nachman RJ, Aimanova K, Gill S, Adams ME. 2008. The pheromone biosynthesis activating neuropeptide (PBAN) receptor of Heliothis virescens:


that regulates sex pheromone production in female moths. Science 244: 796-798.


Wilson EO. 1962b. Chemical communication


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Feromone biosynthesis activating neuropeptide (PBAN/Pyrokinin) gene and Solenopsis species fire ants

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Abstract

Invasive fire ant Solenopsis invicta is an economically significant invasive species that spends millions of dollars annually for fire ant control and eradication in the United States. Fire ants are also a major concern globally, and there is an increasing need to develop biological control. Neuropeptides are widely present in insects, and peptides produced by 4-5 classes of neurons have key roles in the development and reproduction of insects. Although the research on PBAN/Pyrokinin gene regulation of moth feromone biosynthesis is the most advanced, fire ants are one of the most well-studied species in the social insect community, and there is a wealth of research on their behavior and chemical components of feromone communication. However, we do not know how these feromone systems regulate fire ants. In this paper, we review the research conducted to date on PBAN/Pyrokinin genes and fire ants to determine the functional roles of neuropeptide products in larval development and adult behavior. We will discuss the following:

1. Fire ant PBAN/Pyrokinin peptides;
2. PBAN peptide immune cell fluorescent staining and fire ant central nervous system (CNS);
3. Identification of invasive fire ant S. invicta and other fire ant species Solenopsis fire ant PBAN/Pyrokinin peptides;
4. Expression of PBAN/Pyrokinin genes in the head, thorax, and abdomen of invasive fire ant S. invicta.

These studies will help establish the foundation of the PBAN/Pyrokinin gene peptide production system and can be utilized in new fire ant biological control.

Keywords: fire ants, fire ant species, feromone biosynthesis activating neuropeptides, neuropeptides, gene expression, immune cell fluorescent staining.

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