Illuminating Insect Chemical Ecology for Effective Pest Control

Dr Robert K. Vander Meer

CREDIT: Sanford Porter, retired USDA fire ant Unit Scientist

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Because of growing international trade, increasing numbers of invasive pest insects are being transported throughout the world. If they become established, invasive insects can have enormous impacts on agriculture, human health and natural ecosystems. However, it can be difficult to control them without causing further damage to the surrounding environment. **Dr Robert K. Vander Meer** of the USDA Agricultural Research Service studies the chemistry of pest ants, as it pertains to their behaviour and biological systems, with the aim of identifying efficient novel methods to monitor and control them.

Pest Insects

There are currently 13 insects among the world's 100 most invasive species, which wreak havoc on the delicate native ecosystems they invade. Almost 40% of these destructive invaders are ant species. Imported fire ants, which are native to South America, infest more than 130 million hectares in the southern US, and they continue to spread. In addition to damaging native ecosystems, these destructive pests are responsible for losses of over $6 billion in damage and control costs each year, particularly for households and agriculture.

Currently, the control of insect pests relies heavily on chemical insecticides. However, insecticides can be very damaging to the environment and negatively affect important non-target species, such as bees and other pollinators. Furthermore, there is a risk of pests developing resistance to these chemicals over time.

Therefore, Dr Robert Vander Meer, of the USDA Agricultural Research Service, investigates the biochemical processes of several insect pests, in order to better understand how they function and discover better ways to control their populations. His work is informing safer and more effective biologically-based methods of pest control that may also be species-specific, allowing us to move away from broad-spectrum harmful insecticides, while also protecting natural ecosystems and human food security.

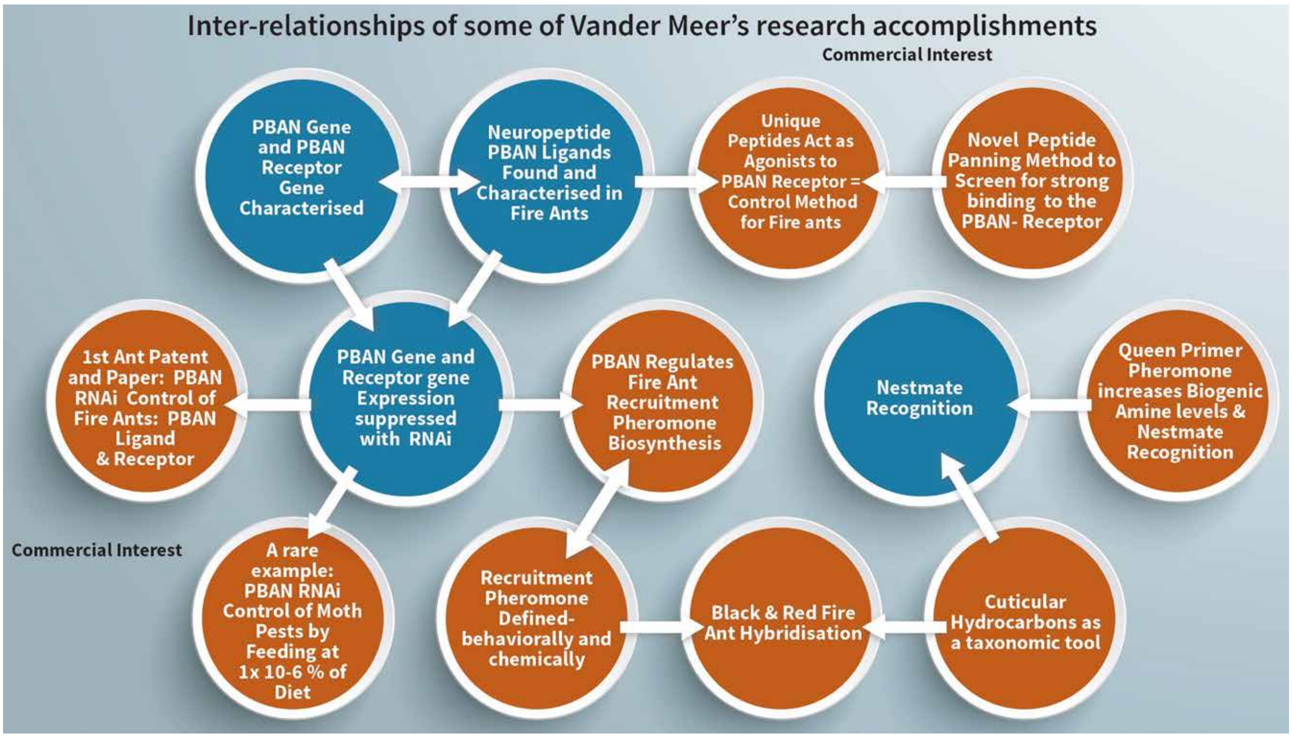
Ant Trails

Ant trail pheromones are one of the most complex forms of chemical communication found in social insects. These chemicals are essential for efficient foraging, as they are used to guide worker ants to the location of discovered food. Forager fire ants release trail pheromones upon food discovery by touching their sting to the ground on their way back to the colony, which guide recruited workers to the food source. They release additional pheromones at the nest, which attracts workers and activates them to follow the trail and collect the food.

Dr Vander Meer and his team were the first to identify the trail pheromone components of the red imported fire ant, and determine their roles in the recruitment process. The ant's Dufour's gland is the source of recruitment pheromones, and the team showed that secretions from this gland have multiple functions. They also found that different chemical combinations trigger different behaviours in worker ants.

By studying red imported fire ants, Dr Vander Meer's group divided the behaviours elicited by trail pheromones into two categories. 'Orientation induction' involves workers at the nest becoming 'activated' to follow the trail, while 'orientation' behaviours allow the workers to find their way along the trail. Each of these two categories is controlled by a different blend of chemicals.

The team discovered that the orientation induction pheromone changes the physiology of the worker ant receiving the signal, in such a way that their sensitivity to stimuli is heightened. If that stimulus is the very weak orientation trail laid by the first foraging ant, they will follow the trail to the food source. This pheromone-induced change in the ant's physiological state is necessary for them to respond to the very low concentration of the orientation component of the pheromone on the



trail. A worker reaching the food at the end of the trail will be stimulated to reinforce the trail on its way back, so the trail becomes stronger and stronger until the food source starts to diminish. As the food diminishes, fewer workers reinforce the trail and the trail becomes weaker until there is no food left. Dr Vander Meer's 10-year project provided the first example of elucidation of a complex recruitment system, leading the way for future research.

Recognising Mates and Intruders

Nestmate recognition is critical for organisation in social insects. It provides a way to maintain territoriality and ensure that parasites and predators cannot access the colony. An ant is able to recognise another ant by touching its antennae to the cuticle of the other, where species-specific chemicals are located. Upon contact with an intruder, the ant can compare the cuticle chemistry of the trespasser with the known scent of its colony and react accordingly.

Dr Vander Meer and his team provided the first experimental evidence that chemicals called hydrocarbons are correlated with nestmate recognition in some species and are causative in others. They found that hydrocarbons significantly modified aggressive behaviour in a species of desert ant called *Cataglyphis niger*, with an elevation in aggressive behaviour when exposed to an unfamiliar odour. This was more pronounced than the reduction in aggressive behaviour on exposure to nestmate odour, suggesting that ants are more sensitive to differences than they are to similarities, perhaps to better recognise threats.

Myrmecophilous beetles live among fire ants and have found a way around the ants' ability to recognise intruders: their cuticles contain hydrocarbons acquired from their hosts. The research team found that when these beetles are removed from the ant colony, they lose these hydrocarbons, leaving a cuticle pattern innate to the beetle. Meanwhile, transferring them to a colony of another species causes the surviving beetles (80% mortality) to acquire the specific hydrocarbons of the new hosts. Dr Vander Meer and his team discovered that they acquire these chemicals through direct contact, grooming behaviour, and by ingesting the larvae of the hosts. This study, which was published in the prestigious journal Science, catalysed the exponential growth of cuticular hydrocarbon research in social insects.

In red imported fire ants, a worker's ability to recognise intruders of their own species decreases if the colony queen is removed. The research team suggested that this is because the queen releases a primer pheromone that upregulates the concentration of octopamine in workers – a chemical known to increase an insect's sensitivity to chemical stimuli, affecting their ability to recognise nestmates. In one study, the researchers observed that, while queen-less workers had reduced brain octopamine levels and their ability to discriminate was impaired, feeding them octopamine increased octopamine levels and recognition acuity. While this does not identify the suspected primer pheromone, it does define the primer pheromone's action on recipient workers (elevated octopamine levels).

Fire Ant Hybrids

While conducting his research on fire ant trail pheromones, Dr Vander Meer serendipitously discovered hybridisation between red and black imported fire ants. His group then applied their knowledge of the species-specificity of fire ant cuticle hydrocarbons and venom compounds to the hybrid question. Upon analysing the hybrid ant's venom and surface hydrocarbons using a technique known as gas chromatography, the team showed that the hybrid produced hydrocarbon and venom compounds unique to red fire ants and others unique to black fire ants, confirming hybridisation. This chemical analysis was necessary to confirm hybridisation, because the hybrid is physically indistinguishable from black fire ants.

For both red and black fire ants, the team found that the response to trail pheromones is species specific, but hybrid workers respond to the Dufour's gland extracts of both. Similarly, the two different species respond to pheromones from the hybrid. The researchers showed that red fire ants respond poorly to the extract of black fire ants and vice versa, showing a distinct behavioural difference, but the response of both parental species to the hybrid's extract is identical to the response they had to their own.

Hybridisation throws into question whether red and black fire ants are indeed separate species and emphasises the importance of using newer methods for modern taxonomy studies. Dr Vander Meer's findings bring us closer to understanding the distribution of these species and their hybrid through the southern US. Vander Meer's publications on hybrids have been cited over 450 times and spawned over 250 additional papers on fire ant hybrids.

PBAN in Fire Ants

Neuropeptides are a group of hormones found in most animals and have important regulatory functions. The pheromone biosynthesis activating neuropeptide (PBAN) is one such hormone, which activates the production of sex pheromones in moths. The system works like a lock and key, where PBAN is the key that opens a lock (the PBAN receptor). When the key opens the lock, the synthesis of a sex pheromone occurs in moths. This process can be turned on and off as the pheromone is needed. For decades, this model was restricted to moths. However, Dr Vander Meer and his team were able to demonstrate that PBAN is also present in red imported fire ants. The team also demonstrated that neural extracts from fire ants stimulated pheromone biosynthesis in moths in the same way that PBAN does. This means that the PBAN-like material found in the central nervous system of ants is very similar to that observed in moths, and could be involved in fire ant pheromone biosynthesis.

Through a series of basic research experiments, Dr Vander Meer's group discovered that the PBAN receptor (lock) is expressed near the Dufour's gland, which produces the fire ant recruitment pheromone. By suppressing the expression of the PBAN (key) gene and the PBAN receptor (lock) gene, the team demonstrated that PBAN controls the biosynthesis of the fire ant recruitment pheromone. For 25 years, PBAN was thought to only control moth pheromone synthesis. This ground-breaking research removes the limits to PBAN/pheromone biosynthesis.

Pest Control Using PBAN

Interestingly, the team found that the PBAN gene is expressed in all fire ant life stages – egg through to adults – suggesting that it has functions other than pheromone production. Disruption of the PBAN gene could have negative impacts during fire ant development, including increased larval and adult mortality.

Dr Vander Meer's group explored these possibilities and discovered that PBAN RNAi (RNA interference) can be ingested by the workers and then transferred to the larvae in the colony, resulting in death of the larvae. They further showed that suppression of the fire ant PBAN receptor gene also resulted in negative effects for fire ants. The team's results revealed that interfering with PBAN and/or PBAN receptor gene expression could be an effective form of pest control, and they recently patented and published their technology.

Sting apparatus: Dufour's and poison gland

Dr Vander Meer's group extended their fire ant PBAN RNAi research technology to 'switch off' the PBAN gene in two economically important moth pest species: the corn earworm and the Tobacco budworm. They found that when moth larvae of these species ingested their specific RNAi, this resulted in delayed larval growth, interference in pupal development, and mortality. This is one of only a few examples of the negative impact that RNAi can have on pest insect species by ingestion, again supporting its possible use in pest management. This technology uses double-stranded RNA (dsRNA), and is an emerging form of pest management. Three patents have been issued covering these areas and there is commercial interest.

Dr Vander Meer's work has shown that PBAN dsRNA can survive gut enzymes following ingestion and still produce lethal effects. This makes RNA interference a promising alternative to current methods of pest control, such as insecticides, which can harm the environment. The use of RNAi is thought to pose no risk of pest insects developing resistance.

Meet the researcher

Dr Robert K. Vander Meer
Imported Fire Ant and Household Insects Research Unit
USDA Agricultural Research Service
Gainesville, FL
USA

Dr Robert K. Vander Meer earned his BSc in Chemistry and Mathematics from Blackburn College, his MSc in Chemistry from John Carroll University, and his PhD in Synthetic Organic Chemistry from the Pennsylvania State University. Since then, he has worked in several prestigious universities, including the University of the South Pacific, Cornell University and is affiliated with the University of Florida. Currently, Dr Vander Meer is the Research Leader of the Imported Fire Ant and Household Insects Research Unit at the USDA Agricultural Research Service (USDA-ARS) and a courtesy faculty member at the University of Florida in the Entomology and Nematology Department. He is working on a number of projects related to chemistry and associated behaviours of several economically important pest ant species, particularly fire ants, and the methods by which they may be better controlled.

CONTACT

E: bob.vandermeer@usda.gov
W: http://www.ars.usda.gov/saa/cmave/ifahi/rkvandermeer

FUNDING

USDA-ARS; CRADAs (Cooperative Research and Development Agreement) with industry; CRADAs associated with SBIR Phase 1 and 2 grants; NRI Competitive grants; Israel-United States Binational Science Foundation grants; USDA/ARS Areawide Project.

FURTHER READING

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