Youth Movement

Purdue University researcher Grzegorz Buczkowski is making a name for himself thanks to an intriguing study documenting tertiary kill in German cockroaches.

In a tiny, unassuming office in an out-of-the-way corner of Smith Hall on the campus of Purdue University, entomologist Dr. Grzegorz Buczkowski does what generations of young scientists have done before him — painstakingly document and interpret hundreds of lab tests on cockroaches, ants and other insects, attempting to better understand the biology, behavior and control of household pests. Although only 35 years old, he's come a long way from his days as a youth growing up in Brzeg, Poland, a small town in the southwestern part of the country, thousands of miles and a world away from the Purdue University campus.

Buczkowski came to the United States in 1989 when his father accepted a position as a visiting scientist at North Carolina State University (NCSU). "I grew up in an academic environment where everyone seemed to have a Ph.D., so being surrounded by scientists seemed very normal to me," Buczkowski said. After graduating from high school and earning a degree in Zoology from NCSU in 1995, Buczkowski (nicknamed "Grzesiek") took a few years off before going to graduate school. During that time he worked for Rhône-Poulenc, where he performed some of the early work on fipronil bait for fire ant control. "We looked at mortality. We looked at trophallaxis. That's where I really got interested in entomology."

And he couldn't have been in a better place to pursue his passion for insects. Dr. Coby Schal's NCSU lab was virtually in his backyard. While impressed with Buczkowski's interest in entomology, Schal wasn't convinced the young scientist had what it took to succeed academically. "I am rather embarrassed to admit that I had some early trepidation about Grzesiek as 'graduate-student material' based on his undergraduate grades and performance on standardized graduate school tests," Schal said. However, those initial reservations quickly disappeared as Schal observed the young graduate student in his laboratory. "Grzesiek was a ball of fire in the lab and in the field, had incredible energy and 'smarts'; and he went on to complete a superb Ph.D. dissertation with (Professor) Jules Silverman, who had just joined NCSU," Schal said. "His Ph.D. project was an exciting combination of behavioral ecology, population genetics and chemical ecology directed at understanding colony organization of Argentine ants."

Buczkowski, who currently heads the Industrial Affiliates Program at Purdue University's Center for Urban and Industrial Pest Management, also was "a great citizen of NCSU's entomology department," according to Schal, where he played an active role in promoting the science of entomology and participated in several professional meetings, including the International Conference on Urban Entomology in the Czech Republic and the National Conference on Urban Entomology in Ft. Lauderdale, Fla. "Grzesiek came through one of the strongest research programs in the country, so he's been a perfect fit to lead the Industrial Affiliates Program at Purdue," said Purdue's Dr. Gary Bennett.

Buczkowski's recent work focuses on a diverse range of subjects, including caste differentiation in termites, colony structure and foraging behavior in ants and termites, and insecticide transfer in cockroaches, a topic he's particularly well-versed in, according to Schal. "When Grzesiek joined my lab, Robert Kopanic (now with SC Johnson) had just completed an exciting Ph.D. project on coprophagy (consumption of fecal material) in cockroaches," Schal said. "He showed that cockroaches — particularly first instar nymphs — benefit tremendously from engaging in coprophagy, even to the point of not needing to forage for food.

Grzesiek Buczkowski (shown above) is originally from Brzeg, Poland.

The behavior of German cockroaches makes them susceptible to secondary kill.

German cockroach nymphs benefit from engaging in coprophagy.

Dr. Grzegorz Buczkowski spends much of his time in the entomology lab at Purdue University. (Photos by Vincent P. Walter)

"Because fipronil was the first major insecticide that was effective both by ingestion and contact, Grzesiek followed-up on Bob's work and evaluated the effects of various pathways for introducing fipronil into insects (topical, residual and oral) and the transfer of fipronil from treated (donor) to untreated (recipient) cockroaches by coprophagy, trophallaxis and necrophagy," Schal observed. "He identified a novel mechanism of translocation involving oral secretions, and through an ingenious combination of radiotracer techniques and time-lapse video analysis, Grzesiek showed that small nymphs are 'fatally attracted' to the oral secretions of cockroaches that ingested fipronil. This fit beautifully with Kopanic's observations that first instars do things differently from other life stages. Grzesiek's research resulted in an excellent Master's thesis from which he published three refereed publications, two in the *Journal of Economic Entomology* and one in *Pesticide Biochemistry and Physiology*."

After a brief post-doctoral research stint at Ohio State University, Buczkowski eventually joined Purdue University, where he's led a number of research projects, including recent work on insecticide transfer with Advion® Cockroach Gel Bait from DuPont Professional Products. "As we started to hear back from PCOs about the field performance of the product (after it was introduced)," observed Dr. Clay Scherer, global development product manager, DuPont, "we decided to study it a bit further. But we weren't in the best position internally to evaluate the transfer phenomenon, so we asked ourselves who in the academic community could help us, and based on Grzesiek's past work he stood out."

In his role as director of Purdue's Industrial Affiliates Program, as well as his personal interest in the subject, Buczkowski was more than willing to lend his expertise to the project when approached by Scherer. "Secondary kill has been illustrated both in the lab and in the field with cockroaches and other insects," Buczkowski said, "but we wondered if you could take it a step further. Could we take dead and dying cockroaches, place them in an arena of untreated cockroaches, and have them transfer the insecticide from the donor to the untreated recipient population, resulting in *tertiary* kill. It's very similar to secondary kill, but you're taking it a step further. People hadn't looked at that before." The design for the series of experiments *(see related story on page 90)* took about a month. "After we got the methods worked out we actually ran the formal experiment without too much difficulty. I ran the experiment one time (replicated seven to 10 times)," Buczkowski said. "Then I ran the same experiment again to ensure that what we saw the first time was real."

DOSSIER: Dr. Grzegorz Buczkowski

Research Assistant Professor and Director of Industrial Affiliates Program
Purdue University, West Lafayette, Ind.

Education
- Bachelor's Degree: North Carolina State University, 1995
- Master's Degree: North Carolina State University, 2000
- Ph.D.: North Carolina State University, 2003

Hometown
Brzeg, Poland

Current Research Interests
- Use of immunomarking techniques to study nutrient distribution and foraging behavior in ants and termites.
- Spatial colony structure and foraging ecology in the black carpenter ant, *Camponotus pennsylvanicus*.
- Genomic investigation of caste differentiation in termites.
- Interspecific competition and nutritional ecology in the odorous house ant, *Tapinoma sessile*.
- Sponsored (applied) research in the lab and field on termites and ants.

Publication Credits
Environmental Entomology, *Insect Molecular Biology*, *Insectes Sociaux*, *Animal Behaviour*, *Journal of Economic Entomology*, *Journal of Chemical Ecology*, *Molecular Ecology*, *Pesticide Biochemistry and Physiology, Journal of Insect Behavior and Biological Invasions.*

While tertiary kill has been validated in the lab thanks to Buczkowski's research, it has not been shown in the field, in part because too many variables are at play. "When you do this work in the lab you have a controlled environment," he said. "In the field you don't know how many cockroaches you're dealing with and residents are often using a variety of over-the-counter products that would invalidate the study. It's difficult to design a study that shows secondary or tertiary kill in the field because there are so many outside variables, but it (tertiary kill) is not just hype. These tests have been replicated and repeated in the lab several different times, so what we've observed is real."

Dr. Phil Koehler, professor of entomology, University of Florida, is convinced of the benefits of both secondary and tertiary kill. "The concept of secondary kill — and now tertiary kill — is a concept that goes way back," he said. "It's something researchers always talked about, but research techniques have become refined enough now that you can show it in the lab." Ultimately, however, Koehler said the pest management professional just wants to see results, regardless of how the cockroach acquires a lethal dose of the active ingredient. "It doesn't matter to him how the active ingredient got into those roaches, he just wants them dead."

Nonetheless, Dr. Clay Scherer, a former graduate student of Koehler's, is pleased to have been involved in the research project. "It's been really fun and exciting to work with Grzesiek and Purdue on this research. The scientific paper based on the research (to be published in an upcoming issue of the *Journal of Economic Entomology*) will be part of the history of cockroach baits, and that's exciting *and* gratifying," he said.

Tertiary Mortality
In The German Cockroach

Purdue University research provides new insights about the impact of horizontal transfer on roaches.

By Grzegorz Buczkowski, Ph.D., Gary W. Bennett, Ph.D., and Clay W. Scherer, Ph.D.

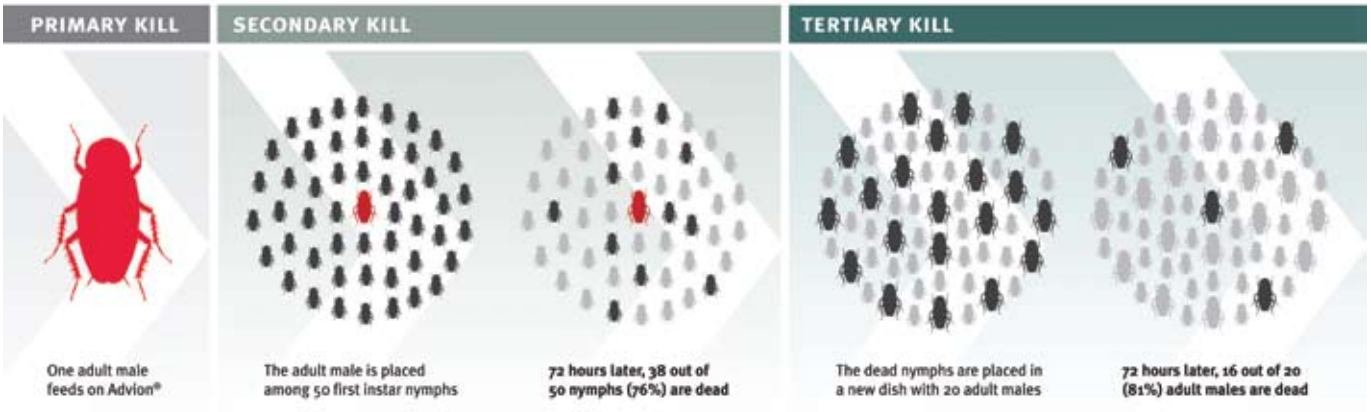
We've all heard that cockroaches are able to reproduce in exponential numbers. The German cockroach's rapid reproductive cycle allows the population to double every two weeks. Under optimal conditions, a single female cockroach and her offspring can produce more than 500,000 cockroaches in one year. Such impressive reproductive potential gives the German cockroach a unique ecological advantage and is most likely the primary factor responsible for its enormous success as a household pest.

Traditionally, cockroach infestations have been fought with various chemical and non-chemical tools, often with mixed results. Recent research conducted at Purdue University by entomologist Dr. Grzegorz Buczkowski shows that there is a new tool that may assist pest management professionals in countering the German cockroach's remarkable reproductive potential. True Exponential Control™ (a term coined by DuPont Professional Products) refers to a unique process of horizontal transfer whereby a single donor cockroach fed Advion® Cockroach Gel is capable of transferring the bait's active ingredient, indoxacarb, to a large number of recipient cockroaches, thus killing them. The process is unique because it involves two levels: *secondary kill* and *tertiary kill*. While secondary kill has been demonstrated with many cockroach baits, tertiary kill has not been previously demonstrated with any insecticide. Thus, we demonstrate that the high killing potential of indoxacarb effectively fights the high reproductive potential of the German cockroach.

HORIZONTAL TRANSFER. Horizontal transfer of insecticides occurs when active ingredients contained within baits or liquid spray insecticides are passed among individuals within an insect population. Active members of a population, most often foraging adults, acquire the active ingredient,

Tertiary Kill: HOW IT WORKS

A graphic representation of Dr. Grzegorz Buczkowski's research demonstrating tertiary kill appears below. The precise definition of the term tertiary – pronounced tûr´shē-ĕr´ē – is "third in order, degree or rank," according to *The American Heritage Dictionary*. "When an active ingredient is transferred successfully from a donor cockroach to a recipient cockroach, resulting in death, that's considered horizontal transfer, which is commonly referred to as secondary kill," Buczkowski said. "What we've shown in our research at Purdue is a third level of control with indoxacarb we're calling tertiary kill. With each additional step, the amount of active ingredient that is available to the insect declines which explains why we didn't see any evidence of quarternary kill in our studies."



return to the nest or an aggregation, and subsequently share the insecticide with more sedentary members of the population which often cannot feed or do not feed independently. The recipients may obtain the insecticide directly from the donors (e.g., mutual grooming, direct contact) or by consuming and/or contacting insecticide-containing excretions deposited by the donors (e.g., coprophagy, emetophagy).

In the German cockroach, *Blattella germanica*, three mechanisms have been shown to facilitate the horizontal transfer of insecticides: coprophagy, necrophagy and emetophagy. Coprophagy, or the ingestion of feces, is especially important in targeting first instars, which represent a significant proportion of the total population under normal conditions, yet may be the most difficult to reach with baits because they do not forage independently, spend the majority of time within the shelter and rely on adult feces for nutrition. Thus, adult cockroaches that feed on the bait directly can be used to deliver the bait to more sedentary stages such as young nymphs. Coprophagy is most effective with slow-acting insecticides, primarily hydramethylnon, which are more likely to be transferred because cockroaches that feed on the bait have plenty of time to return to their shelter and produce insecticide-laden feces before they die. Necrophagy, or ingestion of dead conspecifics, also plays an important

Figure 1. Nymphs feeding on excretions from the oral and anal regions of a dying cockroach.

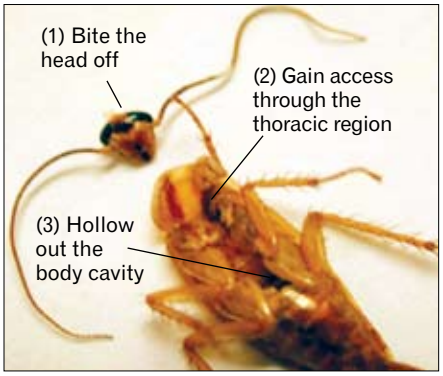


Figure 2. An example of a cannibalized adult cockroach that has consumed indoxacarb.

Figure 3. An adult roach cannibalized by young nymphs serves as a "walking bait arena."

role in the transfer of bait toxicants and has been demonstrated with several commercial baits. Finally, emetophagy, or the ingestion of insecticide-induced regurgitate, has been shown to be an important mechanism by which fast-acting, emetogenic insecticides (e.g., fipronil) are disseminated within cockroach aggregations.

All three mechanisms have been shown to contribute significantly to the overall efficacy of baits by causing secondary kill within cockroach populations. However, the proposition that horizontal transfer may continue beyond secondary mortality and may involve higher levels such as tertiary or quaternary mortality has never been examined. Thus, the goal of our project was to investigate tertiary kill with indoxacarb, a novel neurotoxic oxadiazine insecticide discovered by DuPont Professional Products.

STUDY DESIGN. In a two-part study, we first examined the horizontal transfer of indoxacarb from adult males to first instar nymphs (secondary mortality). We then investigated the transfer of indoxacarb from first instar nymphs (killed via secondary kill) to adult male recipients (tertiary mortality).

The objective for the first phase of our study was to determine the number of nymphs that can be killed by excretions produced by a single adult male donor. We placed 50 first instars in a small Petri dish, provided them with water and harborage, and allowed them to acclimate to the dish for 24 hours without food *(see box on page 90)*. A single bait-fed and symptomatic male was added to the dish at the end of the acclimation period. Mortality in the nymphs was examined at 72 hours.

To examine tertiary mortality we investigated the horizontal transfer of indoxacarb from first instar nymphs that died by feeding on excretions from a bait-fed adult male to adult male recipients. We determined that excretions from a single adult male are capable of killing 38 first instar nymphs. Thus, we examined tertiary transfer of indoxacarb from

Want to Learn More?

Dr. Grzegorz Buczkowski's ground-breaking work on tertiary kill will be featured in an upcoming issue of the *Journal of Economic Entomology*. The bi-monthly publication publishes articles on the economic significance of insects on a wide range of topics including ecology and behavior, veterinary entomology, stored product pests, medical entomology, and household and structural insects. The editor-in-chief of the journal is John Trumble, Department of Entomology, University of California, Riverside. The journal's subject and associate editors, also members of the Entomological Society of America, volunteer their time reviewing manuscripts for possible publication. To access a copy of Dr. Buczkowski's article when it is published, or to learn more about the *Journal of Economic Entomology*, visit www.entsoc.org/pubs/periodicals/jee/index.htm.

38 first instars to 20 adult male recipients. We first set up dishes as described previously (single donor male plus 50 first instars) to obtain the secondary donors. We allowed the first instars to interact with the symptomatic male for 12 hours and then transferred 38 symptomatic nymphs to a clean dish that was provisioned with water and harborage and contained 20 recipient males which had been starved for 24 hours.

RESEARCH RESULTS. Our results indicate that indoxacarb is effectively transferred from adult donor males and results in significant secondary mortality of first instar nymphs. In the test involving the transfer of indoxacarb from a single adult male to 50 first instars, 38 of 50 (76 percent) nymphs died within 72 hours after contacting the symptomatic male. Our results indicate that the transfer of indoxacarb continues beyond secondary mortality and results in significant tertiary mortality. When 20 adult males were exposed to 38 first instar nymphs that died by having contact with the primary donors (bait-fed adults), 16 of 20 (81 percent) adult males died in 72 hours.

Behavioral observations indicated that the mechanisms involved in the horizontal transfer of indoxacarb may include coprophagy, necrophagy and emetophagy. Coprophagy was evident as the recipients were attracted to the rectal region of dying cockroaches and appeared to feed on excretions and/or secretions from the posterior end *(see Figure 1, page 92)*. Necrophagy also appeared to play an important role as we frequently discovered cadavers that had their heads detached and abdomens hollowed out, indicating feeding on the corpses *(see Figures 2 and 3 on page 92)*. Even more apparent was emetophagy, or feeding on indoxacarb-induced regurgitate. We observed that cockroaches dying of indoxacarb poisoning exuded liquid excretions that were attractive and lethal to conspecifics. The exudates were highly attractive to recipient cockroaches, especially first instars. The nymphs aggregated around the symptomatic donors and horizontal transfer of indoxacarb appeared to occur by contact with and/or ingestion of the regurgitated bait.

CONCLUSIONS. Our study demonstrates a unique trophic cascade that leads to tertiary mortality. We describe a chain reaction in which a primary donor transfers insecticide to primary recipients, which then become secondary donors. We show that a single donor delivers a lethal dose of indoxacarb to a considerable number of young nymphs (secondary mortality). These recipients subsequently become donors to other cockroaches and are capable of causing significant mortality in other members of the aggregation (tertiary kill). Although other insecticides have been shown to exhibit secondary mortality, indoxacarb is the first insecticide proven to cause multiple steps of transfer leading to tertiary mortality.

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To learn more about Purdue University's Department of Entomology, visit www.entm.purdue.edu.