Research Article

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# Trap–treat–release: horizontal transfer of fipronil in field colonies of black carpenter ants, *Camponotus pennsylvanicus*

Grzegorz Buczkowski*

## Abstract

**BACKGROUND: Horizontal insecticide transfer is thought to play an important role in controlling a wide range of urban pests including ants, bed bugs, cockroaches, and termites. Despite decades of research and numerous laboratory studies, horizontal transfer has never been demonstrated in the field. As a result, the importance of horizontal transfer (and the resulting secondary kill) for practical pest management remains unknown. The goal of this study was to provide the first experimental examination of horizontal transfer under field conditions. The specific objective was to investigate horizontal transfer of fipronil in field colonies of black carpenter ants, *Camponotus pennsylvanicus*.**

**RESULTS: Laboratory experiments demonstrated that fipronil is effectively transferred from treated donors to untreated recipients and causes significant secondary mortality. Fipronil was effectively vectored to untreated ants from donors exposed via residual and direct spray applications, and 100% mortality was achieved with both exposure routes. Furthermore, horizontal transfer continued beyond secondary mortality and resulted in significant tertiary mortality, which has not been previously demonstrated in ants. Field experiments utilized a novel, three-step control method consisting of trap–treat–release and demonstrated that fipronil is effectively transferred when foraging workers are trapped, treated, and subsequently released back into their colonies.**

**CONCLUSION: The current study is the first field demonstration of the importance of horizontal transfer for the control of pest ants. The trap–treat–release method may be an effective alternative to broadcast spray applications and could help alleviate problems such as insecticide run-off, environmental contamination, and non-target effects. This method has the potential to provide effective management of invasive and pest ants and should be further tested across a wider range of ant species, habitats, and active ingredients.**


**Keywords:** black carpenter ant; *Camponotus pennsylvanicus*; horizontal transfer; invasive ants; fipronil; secondary kill

## 1 INTRODUCTION

Horizontal transfer of insecticides occurs when active ingredients contained within insecticide formulations are transferred among individuals within an insect population. Active individuals, most often foraging adults, acquire the insecticide at the point of application and inadvertently transfer it to other members of the population through various direct and indirect mechanisms. Subsequently, horizontal transfer may result in secondary mortality in situations in which a lethal dose of the active ingredient is transferred from exposed donors to unexposed recipients.

The concept of horizontal transfer dates back to the early days of urban entomology and observations that pesticide applications were effective beyond their application range.[1] Horizontal transfer was first documented in eusocial insects which live in complex societies and engage in constant interactions that promote horizontal transfer. These studies documented the successful transfer of hydramethylnon in fire ant colonies[2] and the transfer of bait formulations in subterranean termites.[3] Subsequently, Silverman *et al.*[4] demonstrated horizontal transfer in insects that were not eusocial. German cockroaches feeding on hydramethylnon bait excreted the active ingredient in their feces and the feces were toxic to other individuals via coprophagy. Over the past three decades, the principles of horizontal transfer have advanced tremendously and now figure prominently in the field of urban entomology. Horizontal transfer has been investigated in numerous laboratory studies and has been demonstrated to occur in a wide range of urban pests including ants,[5,6] cockroaches,[7,8] termites,[9–11] and more recently bed bugs.[12–14] Furthermore, horizontal transfer has been documented to occur with a number of different chemistries and pest control products including sprays, baits, and dusts.[5,7,13]

In social insects, such as ants, horizontal transfer is thought to be essential for effective pest control to deliver the insecticide to individuals that either cannot feed independently (i.e. larvae) or do not feed independently (i.e. reproductives). Ant management exploits eusociality to deliver the insecticide from the site where it is applied to the numerous and often far-away sites where ants

* *Correspondence to: G Buczkowski, Department of Entomology, Purdue University, West Lafayette, IN 47907, USA. E-mail: gbuczkow@purdue.edu*

*Department of Entomology, Purdue University, West Lafayette, IN, USA*

 

nest. Horizontal transfer usually involves interactions in which both the donors and the recipients are alive. These interactions include direct contact and mutual grooming, which facilitate the spread of spray insecticides,[5,6,17] and trophallaxis, which facilitates the spread of bait insecticides.[18] Additionally, carrying individuals that died from insecticide exposure (necrophoresis) has been shown to play an important role.[6]

Despite decades of research and numerous laboratory studies demonstrating horizontal transfer in a wide range of urban pests, horizontal transfer has never been demonstrated in the field. To date, all experiments on horizontal transfer have been performed under carefully controlled laboratory conditions. As a result, the importance of horizontal transfer (and the resulting secondary kill) for practical pest control remains unknown. Specifically, the relative role of direct exposure (obtaining insecticide directly from product applications) *versus* horizontal transfer (obtaining insecticide from individuals that have become exposed to product applications) for urban pest control is still largely unknown and a subject of debate.

The goal of the current study was to provide the first experimental examination of horizontal transfer under field conditions. The specific objective was to investigate the horizontal transfer of fipronil in field colonies of black carpenter ants, *Camponotus pennsylvanicus*. The first objective was to perform laboratory studies to generate quantitative information on factors affecting horizontal transfer, specifically the number of treated donor ants required to kill a certain number of untreated recipient ants. The effects of donor : recipient ratio and fipronil delivery method to the donors (topical *versus* residual) were considered. The second objective was to utilize information obtained in laboratory experiments to examine horizontal transfer under field conditions.

## 2 MATERIALS AND METHODS

### 2.1 Horizontal fipronil transfer – laboratory study

Colonies of black carpenter ants, *C. pennsylvanicus*, were collected on the campus of Purdue University, West Lafayette, Indiana and transported to the laboratory. Colonies were maintained on a diet of 15% sugar water provided *ad libitum* and freshly killed German cockroaches once a week. A colony fragment consisting of 50 workers (recipients) was placed inside a 20 × 14 × 5 cm high Fluon-coated plastic box and allowed to colonize an artificial nest. The nest consisted of a 12 cm diameter by 2 cm high plastic Petri dish, half-way filled with dental plaster. The outside surface of the dish was painted using black spray paint to keep the nest dark. Ants were provided with drinking water and allowed to acclimate to the nest for 48 h. Food consisting of 15% sugar water was provided during the acclimation period and during the test. At the end of the acclimation period, one or five workers (donors) obtained from stock colonies were introduced into recipient colonies consisting of 50 workers. To differentiate donors from recipients, the tarsal segment on the left middle leg was clipped off in the donors right before insecticide treatment, which did not visually impair their behavior or ability to walk. The donors were treated with fipronil using either direct spray or residual exposure. The goal was to compare the level of mortality that can be achieved in the recipients when the donors obtain fipronil in direct spray *versus* residual exposures. In the direct spray treatment, donors were sprayed with Termidor SC (9.1% fipronil, BASF Corp., Research Triangle Park, NC, USA). The spray solution was prepared by mixing 6.3 mL of Termidor with 1 L of water resulting in 0.06% fipronil solution. The 0.06% concentration is the label rate recommended for controlling pest ants. To prepare the donors, ten worker ants were placed inside a plastic Petri dish (9 cm diameter). The inner side of the dish was coated with Fluon to keep the ants from escaping. A piece of filter paper (Whatman #1; 9 cm diameter) was placed in each dish to absorb any excess spray material. The ants were directly sprayed with the insecticide solution. The spray solution was delivered using a fine mister (atomizer). The atomizer was a 70 mL glass bottle with a hand-pump sprayer (Specialty Bottle, Seattle, WA, USA). Two pumps from the atomizer were delivered for each dish so that all the ants were uniformly coated with a thin layer of the spray solution. Each pump from the atomizer delivers 130 μL of spray solution (± 5%). Therefore, a total of 260 μL of diluted Termidor preparation (0.27 g) was applied for each dish. This is equivalent to 1 L of solution per 25 $m^2$ (label rate for general perimeter application of Termidor). Ants were held in the treatment dish for 5 min to calm down after being sprayed and were then transferred to nest boxes containing the recipients. Control tests consisted of workers sprayed with water. In the residual treatment, a glazed ceramic tile, 15 × 15 cm, was treated with 0.96 mL of 0.06% fipronil, equivalent to the label rate of 1 L per 25 $m^2$. Control tests consisted of tiles treated with water alone. The dilution was pipetted onto the tile and was spread uniformly over the tile using a disposable L-shaped cell spreader. The tile was allowed to dry overnight. The following day, ten ants were placed on the treated tile. A plastic, Fluon-coated ring restricted the ants to the treated surface. Ants were exposed to the tile continuously for 1 h and were subsequently transferred to recipient colonies. Five replications were performed for each donor : recipient ratio. Mortality in the donors and the recipients was determined at 4, 8 and 12 h, and then daily until all donors and recipients died. All tests were performed at 27 ± 2 °C, 50 ± 10% relative humidity, and a 14:10 h light/dark photoperiod(artificial lights, no sunlight).

### 2.2 Horizontal fipronil transfer – role of tertiary mortality

Ants live in dense societies and engage in a variety of behaviors (direct contact, trophallaxis, mutual grooming, necrophoresis) that generate a high probability of insecticide transfer. Horizontal transfer of insecticides from treated to untreated ants has been demonstrated in a number of ant species.[5,6,17] However, the idea that horizontal transfer may continue beyond secondary mortality and involve higher levels such as tertiary mortality has not been previously examined in ants. Previously, tertiary mortality has been demonstrated in German cockroaches feeding on indoxacarb bait.[8] A single bait-fed adult cockroach (the donor) transferred indoxacarb to numerous primary recipients (secondary mortality), which then became secondary donors and induced mortality in other members of the aggregation, causing tertiary mortality.[8] The current objective was to examine tertiary mortality in ant colonies using carpenter ants as a model system. The importance of tertiary mortality in the horizontal transfer of fipronil was investigated in a two-part study. Part one examined the number of worker ants that can be killed by one or five donor workers (secondary mortality). A colony fragment consisting of 50 workers (recipients) was placed inside a plastic box and allowed to colonize an artificial nest (as in Section 2.1 above). After acclimation, one or five donor workers marked by tarsal clipping were introduced into the recipient colony. The donor worker was topically treated with 0.06% Termidor SC (as in Section 2.1 above). Control tests consisted of donor workers treated with water. Five replications were performed for each donor : recipient ratio. Mortality in donors and recipients was determined at 4, 8 and 12 h, and then at 1, 2, 3 and 4 days after the donors were introduced. In part two of the experiment, 20 recipient workers (secondary donors) that died in part one were transferred to a new cohort of 50 workers 24 h after being treated to examine tertiary mortality. In control tests ($n = 5$), 20 workers killed by freezing (10 min at −20 °C) were introduced. Mortality was recorded in the recipient population at 4, 8 and 12 h, and then at 1, 2, 3 and 4 days after the secondary donors were introduced.

### 2.3 Horizontal fipronil transfer – field study

Trees selected for the study were located on the campus of Purdue University, West Lafayette, Indiana. The study site was a managed urban landscape that included mature trees interspersed among various campus buildings.[19] Visual inspections were performed to identify trees occupied by black carpenter ants. Individual trees served as plots (experimental units) for evaluating the horizontal transfer of fipronil. Fipronil transfer was examined in single nest colonies and all trees were inspected to assure monodomy. Trees selected for the study were not connected by trails to any other tree. To estimate initial colony sizes, ant activity was sampled 1 day before the addition of treated donor ants. The total number of ants present on the trunk, the main branches, and the ground 1.5 m around the tree was recorded. Carpenter ants are nocturnal, and all inspections were performed with the aid of a flashlight starting 1 h after sunset and continued through the night until all trees were inspected. Trees with significant ant activity (> 25 ants per count) were tagged with numbered aluminum tags and subsequently used in the study. Previous research indicates that ∼35% of all trees within the study site are colonized by carpenter ants and the average number of workers observed on the trees is $45 \pm 5$.[19] In urban habitats, carpenter ants typically nest inside of live trees and colony size varies from 8000 to 12 000 individuals.[20] The exact number of workers within each test tree (colony) was unknown, but it is assumed that the number of active foragers observed on the tree corresponds to colony size. To investigate the horizontal transfer of fipronil in the field, foraging workers were collected from the trees at night using a trapping technique consisting of brushing foraging workers off a tree into a Fluon-coated box, immediately transporting the ants to the laboratory to be treated with fipronil, and subsequently releasing the treated ants on the trees they were collected from that same night. The number of workers collected from each tree was equivalent to three times the colony count. For example, if 40 workers were observed on the tree during the initial inspection, 120 workers were collected to serve as donors. This assignment is based on preliminary field tests which indicated that the times three ratio is sufficient to achieve effective transfer and satisfactory (> 90%) control. For each colony, the workers were collected into a Fluon-coated plastic box and the tree number was recorded on each box to track colony identity. The ants were transported to the laboratory and treated with 0.06% fipronil. The ants were placed inside a 15 cm diameter by 2 cm high Fluon-coated Petri dish and sprayed topically as in the laboratory study above. Six pumps from the atomizer were delivered for each dish so that all the ants were uniformly coated with a thin layer of the spray solution. Six pumps is equivalent to 780 μL of spray solution and is the recommended label rate of 1 L solution per 25 $m^2$. The treated workers were returned to the field within 1 h of collection, released at the base of the tree they were collected from, and allowed to reunite with their colony. The experiment was replicated eight times. The study was conducted from June to August when the colonies are most active. Following the addition of the treated workers, ant activity was again sampled by re-inspecting ant activity at 1, 3, 7, 14, and 28 days.

### 2.4 Effect of colony spatial structure on horizontal transfer

Mature carpenter ant colonies are frequently polydomous and are partitioned into parent and satellite nests.[20] A previous study examined 1113 trees within the current study site and demonstrated that the average number of trees (nests) per colony was 1.95 (range 1–4); 32% of colonies nested in single trees, 45% nested in two trees, 22% nested in three trees, and 1% nested in four trees.[19] In the current objective, the horizontal transfer of fipronil was investigated in colonies that comprised two nests – primary parent nest and secondary satellite nest. Foraging worker counts were recorded for both trees as above. The tree with the higher worker count was assumed to be the main nest. This assumption was verified by observing worker foraging patterns – workers typically foraged on the satellite tree and returned with food items to the main tree. Workers were collected from the main tree (three the initial count), immediately transported to the laboratory, and treated with fipronil as above. They were then released at the main tree within 1 h of collection and workers activity on both trees was monitored on days 1, 3, 7, 14, and 28. The distance between trees was recorded to test for the potential effect of distance on the level of secondary mortality.

### 2.5 Statistical analysis

All data analyses were performed using Statistica 13.3 statistical software.[21] Multivariate repeated measure analysis tests were performed on results of laboratory and field tests to examine the influence of treatment (fipronil), time, donor: recipient ratio, and colony structure on recipient survival in interactions with donor ants. Each analysis of variance (ANOVA) was followed by Tukey's honest significant difference (HSD) test for significant differences between means. The level of significance was set at $\alpha = 0.05$.

## 3 RESULTS

### 3.1 Horizontal transfer of fipronil – laboratory study

Horizontal transfer was highly efficient and mortality in the recipients reached 100% regardless of donor exposure method (residual *versus* direct spray) or the number of donors (Table 1). In tests involving donors treated via residual treatment (exposure to treated tiles), the effects of treatment ($F = 646.7$, df = 2, 15, $P < 0.001$), time ($F = 344.4$, df = 4, 60, $P < 0.001$), and time × treatment interaction ($F = 81.4$, df = 8, 60, $P < 0.001$) were highly significant. In tests involving donors treated via topical treatment (direct spray application), the effects of treatment ($F = 350.9$, df = 2, 15, $P < 0.001$), time ($F = 416.1$, df = 4, 60, $P < 0.001$), and time × treatment interaction ($F = 105.3$, df = 8, 60, $P < 0.001$) were also highly significant. The rate of recipient mortality was significantly faster in tests involving donors treated via the topical exposure ($F = 160.3$, df = 5, 60, $P < 0.01$), presumably because direct spray applications delivered more fipronil to the donors relative to residual exposure. Furthermore, the rate of recipient mortality in tests involving five donors was significantly faster than recipient mortality in tests with a single donor ($F = 24.9$, df = 1, 10, $P < 0.001$). In both direct spray and residual assays, a single donor exposed to 0.06% fipronil was capable of killing 50 recipients, highlighting fipronil's toxicity and potential for transfer. Recipient mortality in control tests was $4 \pm 4\%$.

### 3.2 Horizontal fipronil transfer – role of tertiary mortality

In the test involving the transfer of fipronil from donors to primary recipients (secondary mortality), recipient mortality reached 100%

  

**Table 1.** Mean cumulative percent mortality (± SEM) in *Camponotus pennsylvanicus* workers exposed to donor nestmates treated with fipronil

| | | Time (h) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Donor treatment | No. of donors | 4 | 8 | 12 | 24 | 48 | 72 | 96 |
| Residual | 1 | 0 ± 0a | 0 ± 0a | 5 ± 4a | 21 ± 7b | 45 ± 14c | 80 ± 11e | 100 ± 0f |
| | 5 | 0 ± 0a | 0 ± 0a | 11 ± 5ab | 61 ± 7d | 79 ± 10e | 97 ± 4f | 100 ± 0f |
| | Control | 0 ± 0a | 0 ± 0a | 0 ± 1a | 1 ± 2a | 2 ± 3a | 4 ± 5a | 5 ± 5a |
| Topical | 1 | 0 ± 0a | 0 ± 0a | 17 ± 9b | 74 ± 13c | 91 ± 8de | 100 ± 0e | 100 ± 0e |
| | 5 | 0 ± 0a | 0 ± 0a | 22 ± 7b | 84 ± 13cd | 100 ± 0e | 100 ± 0e | 100 ± 0e |
| | Control | 0 ± 0a | 0 ± 0a | 1 ± 2a | 2 ± 4a | 3 ± 4a | 3 ± 4a | 3 ± 4a |

Within each treatment (residual *versus* topical) means followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$).

**Table 2.** Secondary and tertiary mortality in *Camponotus pennsylvanicus* workers exposed to nestmates treated topically with fipronil

| | | Time (h) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Level of horizontal transfer | Ratio primary donors to recipients | 4 | 8 | 12 | 24 | 48 | 72 | 96 |
| Secondary (1 or 5 primary donors) | 1 : 50 | 0 ± 0a | 0 ± 0a | 12 ± 8b | 68 ± 9c | 81 ± 12de | 100 ± 0e | 100 ± 0e |
| | 5 : 50 | 0 ± 0a | 0 ± 0a | 31 ± 13b | 81 ± 13cd | 100 ± 0e | 100 ± 0e | 100 ± 0e |
| | Control | 0 ± 0a | 0 ± 0a | 0 ± 0a | 1 ± 2a | 1 ± 2a | 2 ± 2a | 3 ± 4a |
| Tertiary (20 secondary donors) | 1 : 50 | 0 ± 0a | 0 ± 0a | 3 ± 4a | 24 ± 11b | 41 ± 21de | 74 ± 11fh | 90 ± 9*g* |
| | 5 : 50 | 0 ± 0a | 0 ± 0a | 5 ± 4a | 33 ± 14bc | 49 ± 15e | 75 ± 13fg | 94 ± 6h |
| | Control | 0 ± 0a | 0 ± 0a | 0 ± 0a | 1 ± 2a | 2 ± 3a | 3 ± 2a | 5 ± 4a |

Values show mean cumulative percent mortality (± SEM). Means followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$).

in 72 h in colonies provided with one donor and 48 h in colonies provided with five donors. The rate of secondary mortality was significantly faster when the recipients were provided with five donors ($F = 642.5$, df = 2, 15, $P < 0.001$). However, a single donor ant was still capable of killing 50 recipient ants. The transfer of fipronil continued beyond secondary mortality and resulted in significant tertiary mortality (Table 2). When 50 new workers were exposed to 20 workers that died when exposed to a single primary donor, 90 ± 9% of the workers died in 96 h. Similarly, 94 ± 6% of the workers died in 96 h when exposed to 20 workers that died by having contact with five primary donors ($F = 75.4$, df = 2, 15, $P < 0.001$). Recipient mortality in control tests was 5 ± 4%.

### 3.3 Horizontal fipronil transfer – field study and effect of colony spatial structure on horizontal transfer

Fipronil was efficiently transferred under field conditions (Table 3). In monodomous (single-tree) colonies, ant counts declined by 97 ± 4% within 7 days and a 100% decline in ant activity was achieved in 14 days. It should be noted that a zero count in ant activity does not necessarily equate to 100% mortality in the colony. By contrast, ant counts in control experiments increased by 54 ± 37% due to colony growth as the season progressed. Complete colony elimination was achieved on all eight trees utilized in the study. The initial ant counts on the trees ranged from 36 to 98 workers (mean 61 ± 20 workers), suggesting that fipronil is efficiently transferred in colonies ranging from small to relatively large.

In polydomous (two-tree) colonies, ant counts declined by 93 ± 7% within 7 days on the main tree and a 100% decline in ant activity was achieved in 14 days, not significantly different from single-tree colonies ($F = 5.3$, df = 1, 14, $P = 0.03$). Decline in activity on satellite trees was 93 ± 10% at 14 days, not significantly lower than single-tree colonies ($F = 7.9$, df = 1, 14 $P = 0.04$). Complete absence of ants at both main and satellite trees, was achieved on five of the eight (63%) tree pairs utilized in the study. In control experiments, ant counts increased by 30 ± 21% on main trees and 56 ± 32% on satellite trees as the season progressed.

## 4 DISCUSSION

Ant control has changed dramatically over the years and continues to evolve. One of the most important goals is to develop effective ant control strategies while minimizing negative environmental impact. Recent developments in this area include hydrogel baits,[22–24] prey-baiting based on the use of poisoned prey,[15,16] and pheromone-assisted baiting.[25,26] The current study evaluated a novel, target-specific approach for managing pest ants based on a three-step method of trap–treat–release.

Laboratory experiments demonstrated that fipronil is effectively transferred from treated donor ants to untreated recipients and causes significant secondary mortality. Fipronil was effectively vectored to untreated ants from donors exposed via residual and direct spray applications, and 100% mortality was achieved with both exposure routes. However, direct spray applications resulted in significantly faster mortality in the donors, suggesting that treating ant trails may be important for practical ant control, not just in carpenter ants, but other pest ants as well. The transfer of fipronil continued beyond secondary mortality and resulted in significant tertiary mortality. Tertiary mortality was first described in aggregations of the German cockroach where a single donor fed indoxacarb bait transferred the insecticide

  

**Table 3.** Ant activity in field colonies of *Camponotus pennsylvanicus* provisioned with nestmates treated with fipronil

| Colony type | Treatment | Nest type | Time (days) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | 1 | 3 | 7 | 14 | 28 |
| Monodomous (1 tree) | Fipronil | Main | 61 ± 20 | 19 ± 11 (69 ± 14) e | 9 ± 6 (87 ± 10) ae | 2 ± 3 (97 ± 4) ae | 0 ± 0 (100 ± 0) a | 0 ± 0 (100 ± 0) a |
| | Control | Main | 45 ± 23 | 45 ± 18 (−4 ± 19) d | 52 ± 23 (−20 ± 40) cd | 53 ± 23 (−26 ± 41) bcd | 62 ± 29 (−43 ± 43) bc | 65 ± 23 (−54 ± 37) b |
| Polydomous(2 trees) | Fipronil | Main | 91 ± 22 | 43 ± 19 (54 ± 13) fghi | 19 ± 13 (80 ± 10) ghijk | 8 ± 8 (93 ± 7) jk | 0 ± 0 (100 ± 0) k | 0 ± 0 (100 ± 0) k |
| | Fipronil | Satellite | 42 ± 9 | 28 ± 9 (33 ± 14) ef | 20 ± 14 (55 ± 26) fgj | 16 ± 11 (63 ± 25) ghjk | 5 ± 8 (89 ± 16) hik | 4 ± 5 (93 ± 10) i |
| | Control | Main | 83 ± 21 | 79 ± 34 (9 ± 21) de | 92 ± 30 (−9 ± 16) bcd | 89 ± 20 (−8 ± 17) bcd | 100 ± 32 (−18 ± 24) abcd | 108 ± 26 (−30 ± 21) abc |
| | Control | Satellite | 47 ± 15 | 46 ± 25 (7 ± 30) ce | 56 ± 29 (−14 ± 34) bcd | 63 ± 29 (−30 ± 26) a | 73 ± 31 (−54 ± 35) a | 73 ± 23 (−56 ± 32) a |

Values in parentheses are mean percent change in worker counts (negative values indicate increases). Within each treatment (one *versus* two trees) mean worker counts followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$). Values show mean worker counts on trees (± SEM).

to numerous primary recipients (secondary mortality), which then became secondary donors and transferred indoxacarb to other members of the aggregation resulting in tertiary mortality.[8] However, tertiary mortality has not been previously investigated in other urban pests and this is the first demonstration that tertiary mortality occurs in ant colonies. Various behavioral mechanisms may have contributed to the efficient transfer of fipronil. In tests involving secondary mortality, recipients interacted with live and/or symptomatic donors. Fipronil transfer was likely due to a number of behaviors including direct contact, mutual grooming, and possibly trophallaxis of any fipronil that may have been accidentally ingested while grooming. Ant behaviors such as mutual grooming, trophallaxis, and necrophoresis have been shown to be important factors in the transfer of fipronil within ant colonies.[5] In tests involving tertiary mortality, the donor ants were dead and necrophoresis (carrying of dead nestmates) was likely the major behavior contributing to transfer.

To date, all experiments on horizontal transfer in urban pests have been performed under laboratory conditions and no study has examined horizontal transfer in the field. Horizontal transfer had been assumed to play a major role in ant management because colonies often nest in inaccessible locations and are not treated directly. Instead, pesticide applications are typically made to areas where ants are expected to forage, and the foraging workers subsequently translocate the pesticide to the rest of the colony. The current study is the first field demonstration of the importance of horizontal transfer for controlling pest ants. Experiments demonstrated that fipronil is effectively transferred when foraging workers are trapped, treated, and subsequently released back into their colonies. In monodomous (single-tree) colonies, a 100% decline in worker activity was achieved in 7–14 days. On average, 182 ± 60 workers were treated and released per colony. Based on laboratory studies, a single treated worker is capable of delivering a lethal dose of fipronil to at least 50 untreated workers. This suggests that 182 workers may be capable of affecting a colony comprised of at least 9100 individuals. Mature colonies of *C. pennsylvanicus* range from 8000 to 12 000 individuals,[20] validating the results obtained in the field experiment. However, the results of transfer tests in confined laboratory settings may not directly translate to the field. Horizontal transfer was also highly efficient in polydomous (two-tree) colonies. This demonstrates that fipronil has the potential to affect satellite nests located away from the main nest. The process by which mortality was achieved in the satellite nests remains unclear. It is possible that some of the treated workers migrated to the satellite nest. Alternatively, workers from the satellite nest may have visited the main nest and encountered treated workers that were either dead or alive. The movement of treated donor workers, including direction and distance travelled, could be further investigated by marking workers and tracking their dispersal from the release point to the various satellite nests. Overall, results suggest that the trap–treat–release approach may be an effective alternative to broadcast spray applications and could help alleviate problems such as insecticide run-off, environmental contamination, and non-target effects.

The effective management of invasive pest ants is constrained by a number of factors, many relating to their social and spatial structure.[27] Many species reach extremely high population levels and colonies often nest in inaccessible places (e.g. in soil, under concrete slabs, inside trees). Furthermore, many ant species are highly polydomous, comprised of multiple, spatially dispersed nests further complicating efforts to find and effectively treat all nests. Locating nests prior to treatment is a costly and time-consuming process and not practical in most situations. Instead, the insecticide is typically applied in areas where ants are expected to nest and/or forage. Foraging workers subsequently visit the treated areas and translocate the insecticide to other members of the colony, especially individuals that do not or cannot forage independently such as queens and larvae. However, most spray insecticide treatments deployed for ant control result in only a few foraging workers being killed directly, and control is often incomplete, and resurgences are common. Additionally, the majority of pesticides applied for ant control never reach their target. It is estimated that less than 0.1% of pesticides applied for pest control reach their target species.[28] The rest remain in the environment often resulting in environmental pollution and non-target effects. The trap–treat–release approach evaluated in the current study has a potential to alleviate many of these issues and offers numerous benefits including significantly reduced pesticide use, greatly increased target specificity, ability to target multiple nests directly, no concerns over product acceptance (frequent issue with toxic baits), and potential cost savings due to reduced pesticide use and time saved in pre-treatment inspections.

The development of non-repellent, slow-acting insecticides such as fipronil revolutionized ant control and made it possible to exploit natural ant behaviors to maximize treatment efficacy while minimizing negative environmental impact. Fipronil has a number of attributes that contribute to its efficacy: it is toxic in ultra-low (ng) amounts, non-repellent, highly lipophilic, effective by feeding and contact, and readily transferable. Fipronil is non-repellent to ants[17] and baits containing fipronil are highly effective against ants.[18] More recently, fipronil has been used in a prey-baiting approach. Results from field studies demonstrate that prey-baiting using fipronil-treated prey is highly effective in controlling invasive ants such as Asian needle ants[15] and Argentine ants.[16] Fipronil is also readily transferred among ants because of its non-repellency, relatively slow speed of action, and delayed toxicity.[5] Necrophoresis appears to be the primary mechanism by which untreated ants contact insecticide-contaminated nestmates.[6] Furthermore, Choe and Rust[6] tested eight insecticides used in ant control and determined that fipronil was most effectively transferred. Only fipronil was horizontally transferred among nestmates and provided significant secondary kill in Argentine ant colonies.[6]

Future studies should assess the feasibility of the transfer approach evaluated in this study for controlling other pest ants. The approach might be particularly useful for controlling invasive ants in natural habitats where broadcast pesticide use is not possible due to concerns over non-target impacts. The approach might be particularly effective against unicolonial invasive species such as Argentine ants (*Linepithema humile*), yellow crazy ants (*Anoplolepis gracilipes*), little fire ants (*Wasmannia auropunctata*), and others. The target species could potentially be mass-trapped using techniques such trap-mulching,[29] exposed to the insecticide directly in the field (via direct spray or contact with a treated surface), and subsequently released at the site of capture. It is hypothesized that the treated ants would then naturally disperse over a wide network of nests and deliver the insecticide directly to nestmates over a large geographic area. Such an approach would precisely deliver the insecticide to the target species and would completely eliminate the need for direct insecticide applications into the environment.

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