Proceedings of the Eleventh International Conference on Urban Pests 2025
AF-Borgen, Academic Society, Lund, Sweden
William H Robinson, editor

PRECISION TARGETING: CONTROLLING URBAN PEST ANTS USING HORIZONTAL INSECTICIDE TRANSFER.

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ABSTRACT Horizontal insecticide transfer is thought to play an important role in controlling a wide range of urban pests. 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 the current 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*. 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. 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 runoff, environmental contamination, and non-target effects.

Key words Black Carpenter Ant, *Camponotus pennsylvanicus*, horizontal transfer

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 where a lethal dose of the active ingredient is transferred from exposed donors to unexposed recipients. In eusocial insects, such as ants, horizontal transfer is thought to be essential for effective pest control in order 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 nest (Choe and Rust 2008). 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. As a result, the importance of horizontal transfer (and the resulting secondary kill) for practical pest control remains unknown. 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. The second objective was to utilize information obtained in laboratory experiments to examine horizontal transfer under field conditions.

MATERIALS AND METHODS

Horizontal fipronil transfer – laboratory study

Colonies of black carpenter ants, *Camponotus pennsylvanicus*, were collected and transported to the laboratory. A colony fragment consisting of 50 workers (recipients) was placed inside a Fluon-coated plastic box and allowed to colonize an artificial nest. The ants were provided with drinking water and allowed to acclimate to the nest for 48 h. At the end of the acclimation period, 1 or 5 workers (donors) obtained from stock colonies were introduced into recipient colonies consisting of 50 workers. The donors were treated with fipronil using either direct spray or residual exposure. The goal of was to compare the level of mortality that can be achieved in the recipients when the donors obtain fipronil in direct spray vs. residual exposures. In the direct spray treatment, the donors were sprayed with 0.06% fipronil solution. In the residual treatment, a glazed ceramic tile was treated with 0.06% fipronil. Ten ants were placed on the treated tile. A plastic, Fluon-coated ring restricted the ants to the treated surface. The ants were exposed to the tile continuously for 1 hour 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, 12 hours, then daily until all donors and recipients died.

Horizontal fipronil transfer – field study

Trees selected for the study were located on the campus of Purdue University, West Lafayette, Indiana. Individual trees served as plots (experimental units) for evaluating horizontal transfer of fipronil. To estimate initial colony sizes ant activity was sampled 1 day before the addition of treated donor ants. To investigate horizontal transfer of fipronil in the field, foraging workers were collected from the trees. They were transported to the laboratory, treated with 0.06% fipronil, and subsequently released on the trees they were collected from within 1 hour of collection. The experiment was replicated 8 times. 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.

RESULTS

Horizontal transfer of fipronil – laboratory study

Horizontal transfer was highly efficient and mortality in the recipients reached 100% regardless of donor exposure method (residual vs. direct spray) or the number of donors. 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. 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.

Horizontal fipronil transfer – field study

Fipronil was efficiently transferred under field conditions. In monodomous (single tree) colonies, ant counts declined by 97 ± 4% within 7 days and 100% decline in ant activity was achieved in 14 days. 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 8 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 (2 tree) colonies, ant counts declined by 93 ± 7% within 7 days on the main tree and 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 from 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 5 of the 8 (63%) tree pairs utilized in the study.

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 (Buczkowski et al. 2014, Tay et al. 2017), prey-baiting based on the use of poisoned prey (Buczkowski et al. 2018), and pheromone-assisted baiting (Sunamura et al. 2011). 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. The transfer of fipronil continued beyond secondary mortality and resulted in significant tertiary mortality. 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 (Soeprono and Rust 2004). In tests involving tertiary mortality the donor ants were dead and necrophoresis (carrying of dead nestmates) was likely the major behavior contributing to transfer.

The effective management of invasive pest ants is constrained by a number of factors, many relating to their social and spatial structure (Silverman and Brightwell 2008). 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 100% decline in worker activity was achieved in 7-14 days. 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. 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 runoff, environmental contamination, and non-target effects.

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. 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 (Pimentel 2007). The rest remains 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 and greatly increased target specificity.

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