Research Article

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Catch and release: controlling eastern yellowjacket *Vespula maculifrons* colonies using horizontal insecticide transfer

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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. Trap–treat–release is an effective experimental approach that has been used to successfully manage populations of invasive ants in field applications. Trap–treat–release is based on the principles of horizontal transfer. Individuals are captured, treated with the toxicant and released back into the environment. The treated individuals then return to the colony and transfer the toxicant to other members of the population resulting in secondary mortality. The goal of the current study was to evaluate the efficacy of the trap–treat–release technique for controlling field populations of the eastern yellowjacket, *Vespula maculifrons*.

RESULTS: Laboratory experiments demonstrated that fipronil was highly toxic against *V. maculifrons* across a wide range of concentrations. Furthermore, fipronil was efficiently transferred from treated donors to untreated recipients and caused significant secondary mortality. A field experiment utilized trap–treat–release and demonstrated that fipronil was effectively transferred when foraging worker wasps are trapped, treated, released and allowed to return to their respective colonies.

CONCLUSION: The trap–treat–release method may be an effective alternative to direct nest treatments and could help alleviate problems such as insecticide runoff, environmental contamination, and non-target effects. This method has the potential to provide effective management of social wasps.

Keywords: fipronil; horizontal transfer; social wasps; secondary kill

1 INTRODUCTION

Social insects including ants,[1] termites,[2,3] and wasps[4] rank as the most widespread and damaging invaders and are difficult to control at the population level.[5] While most research attention has focused on the management of ants and termites, information and innovation on the management of social wasps is lacking. Invasive wasps have a high economic, ecological and human health impact,[4,5] and various *Vespula* species including yellowjackets and hornets are continuing to spread around the globe.[4,6]

Most notably, three species of social wasps, the German yellowjacket (*Vespula germanica*), the western yellowjacket (*V. pensylvanica*) and the common wasp (*V. vulgaris*) have spread beyond their native range and have become pests worldwide.[4,6]

Effective tools are needed to control the spread and impact of social *Vespula*, in particular yellowjackets, but control options are limited and often ineffective, especially at large spatial scales.[7] Current control options for pest yellowjackets include direct nest sprays, lure traps or toxic baiting.[4,8,9] Nest sprays involve the application of residual spray insecticides directly into the subterranean nests. Nest treatments can be effective, but a major disadvantage is the need to locate the nests, which are highly cryptic and often located in inaccessible locations such as wall voids, hollow trees or difficult terrain. Locating individual nests over large areas is time-consuming and costly, making direct nest treatments impractical for attempting to eradicate yellowjackets over large areas. An alternative approach is lure traps containing various chemicals that attract yellowjackets; the chemicals used include heptyl butyrate, acetic acid and/or 2-methyl-1-butanol. A major disadvantage of lure traps is that they reduce the number of localized foraging workers, but do not eliminate large populations and are therefore unsuitable for areawide population reduction. Other disadvantages include the frequent need to refill and empty the traps, high species specificity, high cost relative to spray treatments, and the need for placement in close proximity to foraging colonies. Toxic baiting with insecticide-laced foods is perhaps the most widely used control method and has proven effective for areawide control of yellowjacket populations.[9–13] Toxic baiting exploits the recruitment and food-sharing behavior of yellowjackets and is often used as an alternative to nest sprays. Baits for yellowjacket control are comprised of processed or fresh meats (e.g. canned chicken, fish or ground beef) mixed with the toxicant.

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However, toxic baits achieve only localized and temporary abatement, are not always competitive with natural foods, and because of regulatory decisions in the early 2000s virtually all reliable bait products were taken off the market and are no longer available. As a result, no 'ready-to-use' bait products are available in the United States and all studies on yellowjacket baiting have utilized experimental rather than commercial baits. Such handcrafted baits are suitable for experimental studies under research permits, but otherwise illegal for yellowjacket control. Given that yellowjackets are nuisance pests of global economic and ecological importance alternative approaches for yellowjacket management are needed.

Trap–treat–release is an effective experimental approach that has been used to successfully manage populations of invasive and pest ants in field applications.[14,15] Trap–treat–release is based on the principle of horizontal transfer. Active individuals, most often foraging workers, are captured, treated with a spray application of the toxicant, and released back into the environment. The treated individuals then return to the colony and inadvertently transfer the toxicant 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 toxicant is transferred from treated donors to untreated recipients. In a field trial with black carpenter ants, *Camponotus pennsylvanicus*, foraging workers leaving individual colonies were captured, topically treated with 0.06% fipronil, and released in close proximity to their respective colonies.[14] Fipronil was efficiently transferred and ant counts declined by 97% within 7 days; 100% colony elimination was achieved within 14 days. A field trial utilizing trap–treat–release in a nature reserve in South Africa demonstrated effective control of invasive Argentine ants.[15] Foraging workers were collected, treated with 0.06% fipronil, and released in experimental plots invaded by Argentine ants. The release of fipronil-treated ants reduced Argentine ant counts by >90% within 24 h. In both cases, the trap–treat–release approach was highly effective, relatively fast and utilized significantly less toxicant relative to standard treatment methods such as baiting or broadcast spraying.

In social insects, including yellowjackets, horizontal transfer is thought to be essential to deliver the insecticide to stationary individuals that either cannot feed independently (larvae) or do not feed independently (reproductives). Therefore, wasp management can exploit eusociality to deliver the insecticide to the numerous and often far-away sites where yellowjackets nest. Previous studies to control yellowjackets have utilized the concept of horizontal insecticide transfer by placing a fipronil-treated metal screen cage over the entrance to the nest.[16,17] Wasps flying out of the nest were trapped inside the cage and obtained fipronil by contact with the cage. The cage was removed after 15–20 min and the contaminated wasps allowed to return to the colony. Colony mortality was observed within 24 h. The goal of the current study was to evaluate the trap–treat–release technique for controlling field populations of the eastern yellowjacket, *Vespula maculifrons*. 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 and perform a field trial to assess the efficacy of the trap–treat–release technique. Finally, an analytical study using gas chromatography tandem mass spectrometry (GC–MS/MS) was performed to obtain quantitative information on the amounts of fipronil necessary for efficient transfer and behavioral mechanisms responsible for horizontal transfer.

2 MATERIALS AND METHODS

2.1 Dose–response laboratory study

The toxicity of fipronil (Termidor SC, 9.1% fipronil, BASF Corporation, Research Triangle Park, NC, USA) was evaluated in direct spray applications against workers of the eastern yellowjacket (*Vespula maculifrons*). Eastern yellowjackets (hereafter referred to as wasps) nest almost exclusively in the ground and are particularly prevalent in lawns in parks and other recreational areas.[18] In Indiana, colonies reach maturity by late September to early October and may contain 1000 workers. Their widespread distribution and often very high population densities in residential and recreational areas make them important urban pests. Wasps were field collected in West Lafayette, Indiana during September 2023. Species identity was confirmed by abdominal maculation patterns on worker specimens.[18,19] To collect the wasps, an insect rearing cage (30 × 30 × 30 cm with wire mesh netting) was placed on the ground over the entrance to the nest. Wasps leaving the nest were then trapped inside the cage. When a sufficient number of wasps had been captured (~20 wasps) the lid of the cage was closed and the wasps were immediately transported to the laboratory and used in assays within 30 min of collecting. The toxicity of three concentrations of fipronil were tested, 0.005%, 0.05% and 0.5%. The goal was to perform a dose–response study and determine the length of time the treated wasps remain alive and able to fly. Individual wasps were seized directly from the cage using fine forceps and immediately treated with the insecticide. The treatment was applied directly to the wasps using a fine mist sprayer (atomizer). The sprayer was a 30-mL glass bottle with a fingertip applicator (Premium Vials SKU B1017). A single pump from the sprayer was delivered for each wasp from 3 cm away to minimize overspray and assure that the wasps were uniformly and thoroughly covered with the spray solution. Each pump from the atomizer delivers 130 µL of liquid. Control wasps were sprayed with 130 µL of water. Immediately after treatment the wasps were placed in individual recovery containers, (475 mL plastic deli cups). Wasps were provided with a cotton wick soaked in 20% sugar water for hydration and nutrition. For each treatment, four wasps from each of four different colonies were tested for a total of 16 wasps per treatment. The condition of the wasps was recorded every 30 min for 4 h post-treatment. At each time point, the condition of the wasps was scored as either alive or dead (subject had no movement when probed). All tests were performed at 27 ± 2°C, 50 ± 10% relative humidity and a 14:10 h light/dark photoperiod.

2.2 Trap–treat–release field study

A field study was performed to evaluate the trap–treat–release technique for the management of eastern yellowjacket (*Vespula maculifrons*) colonies. The goal was to investigate the potential of treated donor wasps to return to the colony and deliver a lethal dose of fipronil to untreated recipients. Wasp colonies were located in West Lafayette, Indiana by scouting urban green areas for wasp nests. Eight sites were selected for the study (Table 1). Two colonies were located at each site for a total of 16 colonies. At each site, the colonies were separated by at least 500 m. Colonies from the first six sites were assigned to the 0.5% fipronil treatment ($n = 12$) and colonies from the remaining two sites were assigned to the untreated control treatment ($n = 4$). The 0.5% fipronil concentration was selected for the field study based on the results of laboratory dose–response tests, which revealed that wasps treated with 0.5% concentration fipronil remained

Table 1. Summary of experimental treatments, study sites and environmental conditions during the field trial. Colony size is the number of workers recovered from fipronil-treated colonies during nest excavation. The condition of the queen was scored as dead or alive. Control colonies were not excavated

Colony no.	Treatment	Location	Treatment date	Air temperature (°C)	Relative humidity (%)	Weather	Wind speed (km/h)	No. workers recovered	No. queens recovered/ condition
1	Fipronil	Horticulture park	14 September 2023	23	32	Sunny	8	457	1/dead
2	Fipronil	Horticulture park	20 September 2023	26	41	Sunny	13	634	1/dead
3	Fipronil	Celery bog nature area	14 September 2023	23	32	Sunny	8	528	1/dead
4	Fipronil	Celery bog nature area	29 September 2023	24	53	Sunny	13	417	1/dead
5	Fipronil	Pickett Memorial Park	16 September 2023	27	45	Sunny	11	492	1/dead
6	Fipronil	Pickett Memorial Park	16 September 2023	27	45	Sunny	11	534	1/dead
7	Fipronil	WL Golf and Country Club	20 September 2023	26	41	Sunny	13	679	1/dead
8	Fipronil	WL Golf and Country Club	16 September 2023	27	45	Sunny	11	473	1/dead
9	Fipronil	Ackerman-Allen Golf Course	29 September 2023	24	53	Sunny	13	580	1/dead
10	Fipronil	Ackerman-Allen Golf Course	6 October 2023	18	39	Sunny	14	610	1/dead
11	Fipronil	Kampen-Cosler Golf Course	6 October 2023	18	39	Sunny	14	536	1/dead
12	Fipronil	Kampen-Cosler Golf Course	6 October 2023	18	39	Sunny	14	573	1/dead
13	Control	Birck Boilermaker Golf Complex	14 September 2023	23	32	Sunny	8		
14	Control	Birck Boilermaker Golf Complex	20 September 2023	26	41	Sunny	13		
15	Control	Coyote Crossing Golf Club	16 September 2023	27	45	Sunny	11		
16	Control	Coyote Crossing Golf Club	16 September 2023	27	45	Sunny	11		

asymptomatic and able for fly for at least 90–120 min, sufficient time for wasps to fly back to the colony. The 0.5% concentration was selected to maximize the amount of fipronil being delivered to the colonies by the donor workers. The location of all colonies was marked with flagging tape and waypoints were marked using a GPS. Experimental replicates consisted of individual, fully established and mature colonies of *V. maculifrons* nesting in the ground. Wasp activity in each nest was quantified pretreatment (day 0) by three 1-min counts of individuals entering the nest and three 1-min counts of individuals leaving the nest. All counts were 5–10 min apart. To collect donor wasps, an insect rearing cage was placed on the ground over the entrance to the colony and wasps leaving the colony were trapped inside the cage. Individual wasps were seized directly from the cage using fine forceps and immediately treated with the insecticide. The donors were sprayed with 0.5% fipronil, which was prepared by mixing 5.15 mL of Termidor SC with 100 mL of water. The treatment was applied directly to the wasps using a fine mist sprayer (atomizer). A single pump from the sprayer was delivered for each wasp (~130 μL) from 3 cm away to minimize overspray and assure that the wasp was uniformly and thoroughly covered with the spray solution. The treatment was performed inside a cardboard box to assure that any overspray was retained in the box and did not contaminate areas where wasps might be foraging. To differentiate donors from recipients, donors were marked with a small dot of acrylic paint (Testors Craft, Rust-Oleum Corp, Vernon Hills, IL, USA) on the abdomen. Control wasps from control colonies were sprayed with 130 μL of water and marked with paint. Immediately after, treated wasps were released and allowed to fly back to the colony. All wasps were treated and released ~15 m from the nest entrance. A total of 35 donor wasps were treated for each colony, with this number based on preliminary tests that revealed that a mature colony of *V. maculifrons* contains 520 ± 97 worker wasps, thereby giving an estimated ratio of 1 treated donor to 15 untreated recipients used in this experiment. All colonies were treated around 3 p.m. (± 1 h), during sunny weather conditions when the air temperature was >18°C, relative humidity was >30%, and the wind speed was <15 km/h (Table 1). The study was conducted from September to November 2023 when the colonies are at their largest and most active. Following release of the treated donors, wasp activity in all treatment and control colonies was monitored daily for 5 days and consisted of 1-min counts of individuals entering and leaving the nest (as above). On day 5, all treated colonies ($n = 12$) were excavated and visually inspected for the presence of any remaining live wasps. All workers present in the nest were recovered, brought back to the laboratory, and counted to determine colony size. In addition, the queen was recovered from all treated nests and the condition of the queen (dead or alive) was assessed.

2.3 Yellowjacket fipronil analysis: analytical study using liquid chromatography tandem mass spectrometry

In the field study, donor wasps were prepared by spraying each wasp with 130 μL of 0.5% fipronil (a single pump from the sprayer). However, because of overspray and other factors, including slight variations in wasp body size, the exact amount of fipronil applied to each wasp was unknown. To estimate the amount of fipronil present on the donors, 15 wasps were individually treated with 130 μL of 0.5% fipronil each, placed in individual vials and kept at −20°C in a freezer until analysis. In addition, the amount of fipronil transferred from donors to recipients was estimated. This was accomplished by collecting 8 dead recipient wasps (lacking paint mark) from 5 of the 12 treated colonies (colonies 2, 6, 9, and 11 in Table 1). The dead wasps were collected on day 5 during nest excavation, placed in individual vials and frozen at −20°C until analysis. To determine the potential mechanisms of horizontal transfer (contact *versus* ingestion), the amount of fipronil present inside *versus* outside the body of the recipient wasps was determined. Externally present fipronil would suggest transfer via contact and mutual grooming, whereas internally present fipronil would suggest transfer via ingestion and trophallaxis. To obtain external samples, individual wasps were placed in 5-mL centrifuge tubes containing 3 mL of room temperature acetone. The tube was gently shaken for 2 min. The wasp was removed using clean tweezers and the washate was stored at −20°C until analysis. Preliminary analysis using three consecutive acetone washes revealed that the first wash removed >98% of externally present fipronil. Therefore, for all subsequent analyses, a single wash was performed. To obtain internal samples, externally washed wasp bodies were processed using the Precellys CK14 Lysing Kit (Bertin Corp., Rockville, MD, USA). Individual wasps were placed inside 2-mL Precellys CK14 tubes containing zirconium oxide beads and 1 mL of cold acetone. Wasp bodies were pulverized using a homogenization cycle (3 × 30 s, 5000–6000 rpm). The extract was transferred to a clean 1.7-mL centrifuge tube and the tube was centrifuged at 94,640× g for 10 min. The supernatant was collected and dried in a vacuum concentrator (Speedvac). The pellet was resuspended in 0.1 mL of acetone for analysis. All extracts were analyzed using GC–MS/MS at the Metabolite Profiling Facility at Bindley Bioscience Center at Purdue University. For GC–MS/MS analysis, each sample was placed into a 1.7-mL centrifuge tube and spiked with an appropriate amount of $^{13}C_4$-fipronil internal standard (Sigma Aldrich, St. Louis, MO, USA). An Agilent 1200 Rapid Resolution gas chromatography (GC) system coupled to an Agilent 6460 series QQQ mass spectrometer (MS) was used to analyze fipronil in each sample. An Agilent Zorbax SB-Phenyl 2.1 × 100 mm, 3.5 μm column was used for GC separation (Agilent Technologies, Santa Clara, CA, USA). The buffers were: (A) water + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid. The linear LC gradient was as follows: time 0 min, 10% B; time 0.5 min, 10% B; time 8 min, 100% B; time 10 min, 100% B; time 11 min, 10% B; time 15 min, 10% B. The flow rate was 0.3 mL/min. Multiple reaction monitoring was used for MS analysis. The jet stream electrospray ionization interface had a gas temperature of 325 °C, gas flow rate of 7 L/min, nebulizer pressure of 45 psi, sheath gas temperature of 250 °C, sheath gas flow rate of 7 L/min, capillary voltage of 3500 V in negative mode, and nozzle voltage of 500 V. The ΔEMV voltage was 400. All data were analyzed with Agilent Masshunter Quantitative Analysis (version B.06.00). A linear calibration curve was prepared from 0.2 to 1500 ng/mL for fipronil and $^{13}C_4$-fipronil was used as internal standard and for absolute quantitation. The retention time of fipronil was 8.97 min. The 434.7→329.9 (fipronil)/438.7→333.9 *m/z* ($^{13}C_4$-fipronil) transition was used for quantitation purposes.

2.4 Statistical analysis

All data analyses were performed using Statistica 13.3 statistical software.[20] Multivariate repeated measures analysis of variance tests were performed on results of laboratory and field tests to examine the influence of treatment (fipronil), and time on colony survival following interactions with donor wasps. Each analysis of variance was followed by Tukey's Honest Significant Difference test for significant differences between means. A dependent *t*-test was used to compare fipronil amounts present internally *vs*. externally. The level of significance was set at $\alpha = 0.05$.

3 RESULTS

3.1 Dose–response laboratory study

Results demonstrate that fipronil is highly toxic to *V. maculifrons*. Mortality in wasps treated with fipronil was significantly greater relative to mortality in control tests: time × treatment interaction ($F = 16.2$, $df = 18$, $P < 0.001$). All wasps appeared normal (asymptomatic) for 90 min (Fig. 1). After 90 min, mortality increased gradually for all concentrations. The rate and level of mortality were similar across the three concentrations and no significant dose–response relationship was detected despite a 100-fold increase in fipronil concentration ($F = 0.86$, $df = 12$, $P = 0.59$). These results suggest that the lowest concentration, 0.005%, is above the threshold necessary to produce 100% mortality. Complete mortality was reached with all three concentrations at 210 min, demonstrating that fipronil-treated *V. maculifrons* remain alive for up to 3.5 h. No wasps died in the control tests.

3.2 Trap–treat–release field study

Fipronil was efficiently transferred under field conditions (Table 1 and Fig. 2). The effects of treatment ($F = 529.8$, $df = 1, 15$, $P < 0.001$), time ($F = 55.0$, $df = 5, 75$, $P < 0.001$), and the time × treatment interaction ($F = 57.5$, $df = 5, 75$, $P < 0.001$) were highly significant. Wasp counts declined by 91% ± 4% within 1 day of releasing the treated donor wasps and a 100% decline in wasp activity was achieved in 5 days. By contrast, wasp counts in control experiments remained steady throughout the study or increased slightly as the season progressed. Colony elimination was achieved on all 12 colonies provisioned with fipronil-treated individuals. The number of colonies where 100% mortality was achieved doubled approximately every 24 h. Complete mortality was achieved in 1 colony at 1 day post-treatment (8%), 2 colonies at 2 days (17%), 4 colonies at 3 days (33%), 7 colonies at 4 days (58%), and all 12 colonies at 5 days (100%). To confirm that zero counts in wasp activity equate to 100% mortality in the colony, all treated colonies were excavated to assess colony size and the condition of individuals within the nest. The mean number of workers recovered was 543 ± 77 individuals per colony (range 417–679; Table 1). The great majority of individuals were dead (no movement when probed) and some were knocked down or moribund (individuals not able to initiate directional movement, but appendages moving slightly and/or infrequently with or without physical probing). A single queen was recovered from each of the treated colonies demonstrating that *V. maculifrons* are monogyne (Table 1). All 12 queens were dead demonstrating efficient transfer of fipronil from workers to queens.

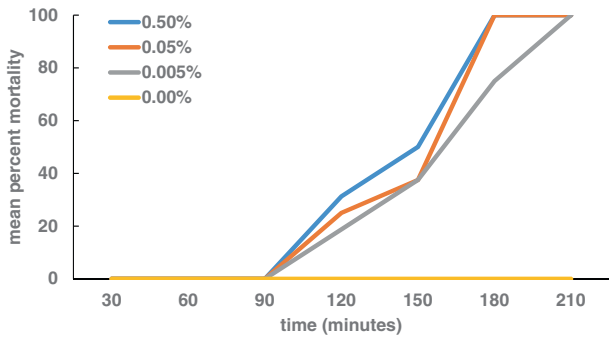

Figure 1. Mean cumulative percent mortality in *Vespula maculifrons* workers treated with topical applications of fipronil in laboratory assays. Standard errors omitted for clarity.

3.3 Yellowjacket fipronil analysis: analytical study using liquid chromatography tandem mass spectrometry

The mean amount of fipronil detected on the treated donors was 12 914 ± 2127 ng (range 9439–15 398 ng). This amount is low relative to the amount delivered by the spray bottle (atomizer). A single pump from the atomizer was applied to each wasp, equivalent to 130 μL of liquid containing 643 500 ng of fipronil. On average, only 12 914 ng was retained on each wasp, equivalent to 2.0% of the total amount sprayed. This suggests that during the application process most fipronil is lost because of overspray, runoff from the wax layer on the cuticle, and potentially other factors such as contact with surfaces after the treatment and sample handling and processing.

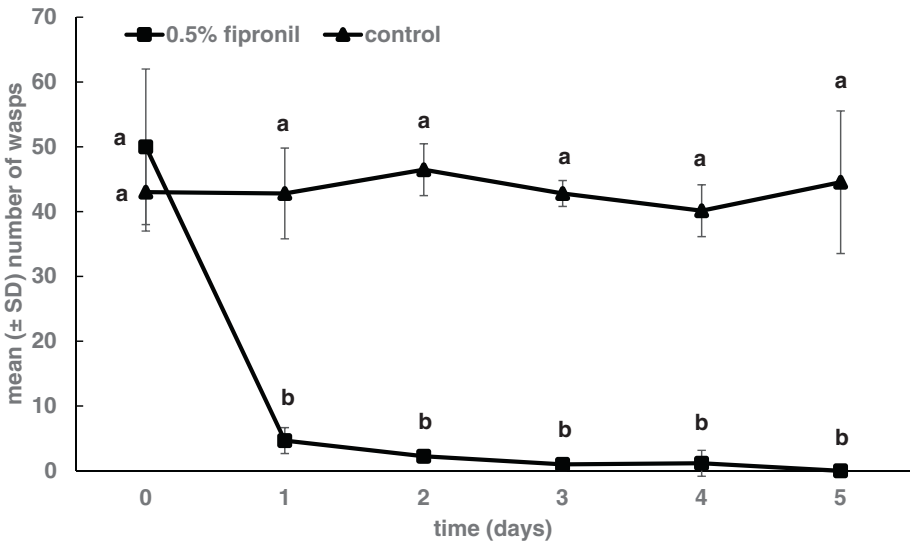

Figure 2. Mean (±SD) number of *Vespula maculifrons* workers detected at experimental colonies provided with fipronil-treated or control wasps. Letters indicate pairwise differences in wasp abundance at each assessment time between fipronil-treated and control plots based on Tukey's Honest Significant Difference test.

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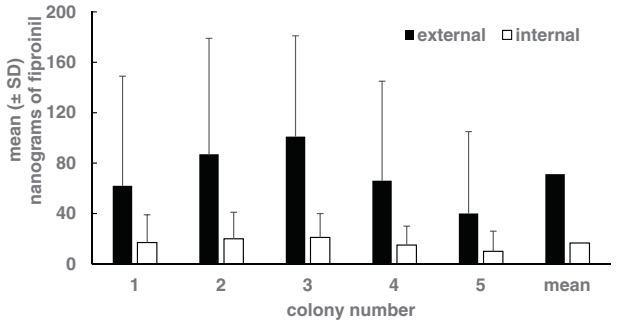

Figure 3. Mean (±SD) amount of fipronil (ng) detected internally *versus* externally on *Vespula maculifrons* wasps.

The mean total (external and internal) amount of fipronil present on the recipients was 87 ± 97 ng (range 7–258 ng) (Fig. 3). This demonstrates that a single donor wasp carrying an average of 12 914 ng of fipronil is capable of delivering a lethal dose to ~148 untreated recipient wasps. As expected, the mean amount of fipronil present on the donors was significantly higher than the amount detected on the recipients (*t*-test, $t = -23.3$, $df = 24$, $P < 0.0001$). The amount of fipronil detected externally was 71 ± 80 ng (range 5–205 ng, 82% of total) and the amount detected internally was 16 ± 18 ng (range 2–60 ng, 18% of total) (*t*-test, $t = 5.5$, $df = 39$, $P < 0.001$).

4 DISCUSSION

The current study evaluated a novel, target-specific approach for managing pest wasps based on a three-step method of trap–treat–release. Results of laboratory tests demonstrated that fipronil is highly toxic to *V. maculifrons* yet relatively slow-acting, making it an ideal candidate for trap–treat–release. The field trial demonstrated that fipronil is effectively transferred from treated donor wasps to untreated recipients and causes significant secondary mortality. All fipronil-treated wasps appeared normal over 90 min and remained alive for up to 3.5 h. This allowed the wasps ample time to return to the nests and engage in social behaviors with other members of the colony. The treated wasps were released ~15 m from the nest entrance and observations indicate that the great majority flew directly towards the nest while some landed on nearby vegetation to dry off their wings. A total of 35 treated donor wasps were released for each experimental colony, a number sufficient to cause 100% mortality in colonies comprised of an average of 543 individuals. The first dead wasps were observed ~6–8 h after releasing the treated wasps. This is similar to 0.1% fipronil bait which required ~6 h to provide a major reduction in yellowjacket colony activity.[11]

Although horizontal insecticide transfer and the resulting secondary mortality are generally viewed as highly advantageous for the efficacy of contact and bait insecticides, horizontal transfer may occasionally have negative consequences when beneficial insects are concerned. In a field trial to control of Argentine ants, hydrogel bait containing 0.05% fipronil was dispersed aerially using a drone.[21] The dispersal process generated aerosol that was toxic to honeybees upwind and several hundred meters from the area being treated. Bees visiting the treated zone became contaminated with fipronil, returned to their colonies, and transferred a lethal dose of fipronil to nestmates, including queens and brood.[21] The study demonstrated that ultralow levels of fipronil are extremely toxic to social Hymenoptera and that insecticides, in particular fipronil, should be used with caution in areawide management programs. Contrary to aerial dispersal, which generated a toxic aerosol, ground placements of hydrogel bait were low risk to pollinators including honeybees.[22] The trap–treat–release method evaluated in the current study may be particularly applicable for reducing non-target effects because it is highly species-specific and involves minute amounts of insecticide applied directly to the target species.

Previous research demonstrates that fipronil has been used successfully as a bait toxicant for *Vespula* wasp control in Argentina,[11] New Zealand,[23] Hawaii[12] and California.[10] Fipronil is a popular choice because it is highly effective and has a number of attributes that contribute to its high efficacy. It is toxic in ultralow (ng) amounts, non-repellent, highly lipophilic, effective by feeding and contact, and readily transferable. Fipronil is also readily transferred among individuals within social insect colonies because of its non-repellency, relatively slow speed of action, and delayed toxicity.[17,24,25]

Interestingly, *V. maculifrons* colonies weakened or killed by fipronil became targets for other social Hymenoptera that prey on wasp larvae and/or scavenge for dead insects. During nest excavations, pavement ants (*Tetramorium immigrans*) were discovered raiding brood in affected *V. maculifrons* colonies. Many of the ants were dead or affected, demonstrating that the transfer of fipronil continued beyond secondary mortality and resulted in tertiary mortality. A previous study evaluated the trap–treat–release method for controlling carpenter ants (*Camponotus pennsylvanicus*) and demonstrated that tertiary mortality played an important role in the horizontal transfer of fipronil within carpenter ant colonies.[14] Treated donors transferred fipronil to numerous primary recipients (secondary mortality), which then became secondary donors and transferred the insecticide to other member of the colony resulting in tertiary mortality. In addition, field ants (*Formica subsericea*) were observed scavenging dead wasps at the nest entrance and carrying them back to their colonies. It is likely that feeding on fipronil-contaminated prey affected colonies of field ants. Previous studies evaluated a prey-baiting approach based on fipronil-treated termite prey to control invasive Asian needle ants[26] and Argentine ants.[27] Fipronil-treated termite prey were scattered in areas invaded by the ants, the ants readily attacked the termites and carried them back to the colonies where they were subsequently consumed, resulting in horizontal transfer and secondary mortality. It is likely that similar effects occurred in the current study, whereby dead wasps carrying fipronil became prey for non-target taxa.

Horizontal transfer is thought to play a major role in pest management because colonies often nest in inaccessible locations and are not treated directly. Horizontal transfer has been demonstrated to play an important role in the management of a wide range of urban pests including ants,[26,28] cockroaches,[29,30] termites[31–33] and bed bugs.[34–36] The current study demonstrated that fipronil is effectively transferred when foraging wasps are trapped, treated and subsequently released back into the environment. Based on laboratory studies, a single treated wasp is capable of delivering a lethal dose of fipronil to at least 148 untreated wasps. Various behavioral mechanisms may have contributed to efficient transfer of fipronil including direct contact, mutual grooming, and possibly trophallaxis of any fipronil that may have been accidentally ingested while grooming. In other social insects, most notably ants, behaviors such as mutual grooming, trophallaxis and necrophoresis have been shown to be important factors in the transfer of insecticides within

colonies.[24,28,37] In the current study, the use of GC–MS/MS allowed quantification of the amounts of fipronil involved in the transfer and identified the behavioral mechanisms responsible for the transfer. Of the total amount of fipronil detected on recipient wasps, 82% was present externally and 18% internally. This suggests that the main route for fipronil transfer is direct contact between donor and recipient wasps or between recipients and the various surfaces contaminated by the donors (e.g. nest entrance and nest material). It is likely that necrophoresis (carrying of dead nestmates) also contributed to transfer. Dead recipient wasps (lacking paint mark) were frequently found outside the nest. Wasps that died inside the nest were carried outside the nest by healthy wasps as part of colony hygienic behavior and wasps performing the cleaning behaviors likely obtained fipronil from the primary recipients and subsequently died as a result of tertiary transfer. Previous studies have demonstrated that necrophoresis is a major behavior contributing to transfer of insecticides in ants.[14,24,28] A relatively small proportion of fipronil was present internally suggesting that trophallaxis plays a minor role. Fipronil was most likely ingested during self-grooming and/or allogrooming. Grooming, specifically antennal cleaning, has been shown to be an important behavior for dispersing insecticides in ant colonies[25] and cockroach aggregations.[29,38]

The effective management of social wasps including yellowjackets and hornets is constrained by a number of factors, many relating to their social and spatial structure.[4] Many invasive species undergo post-introduction phenotypic changes in life history such as shifting from small annual colonies to large perennial colonies, which intensifies their ecological and economic impacts.[39] Many species nest in inaccessible places (e.g. underground burrows, inside trees) or remote and difficult terrain, and locating nests prior to treatment is costly, time-consuming and not practical in most situations. The trap–treat–release approach has a potential to alleviate many of these issues and offers numerous benefits including significantly reduced pesticide use, greatly increased target specificity, the ability to target nests directly, no concerns over product acceptance (frequent issue with toxic baits), and potential cost savings because of reduced pesticide use and time saved in pretreatment inspections. The amount of fipronil used per colony is extremely low compared with sprays or baits. A total of 35 treated wasps were released for each experimental colony and each wasp carried ~13 000 ng of fipronil, equivalent to 0.0005 g of fipronil per colony. Toxic baiting is typically performed with baits containing 0.1% fipronil[11,12] and a single colony may consume >100 g of bait or 0.1 g of fipronil. Direct nest treatments with liquid sprays typically require ~4 L of 0.05% fipronil, equivalent to 2.3 g of fipronil per colony. Overall, results suggest that the trap–treat–release approach may be an effective alternative to direct nest treatments and could help alleviate problems such as insecticide runoff, environmental contamination and non-target effects. Non-target effects are a significant concern with toxic wasp baiting[11,12] as is competition for bait among different wasp colonies.[9,10]

The trap–treat–release method utilizing topical spray applications of 0.5% fipronil is currently being used for areawide control of invasive yellow legged hornets (YLH, *Vespa velutina*) in Georgia, USA (Suiter *et al*., personal communication). The YLH is native to subtropical Southeast Asia and was first detected in the United States by a beekeeper near Savannah, Georgia in August 2023. YLH is a top predator and poses a threat to the honeybee industry and native pollinators, similar to the northern giant hornet (*Vespa mandarina*) in British Columbia, Canada and Washington, USA.[40,41] Because YLH nests are constructed in trees, often in difficult terrain such as swampy areas, it is impractical to locate and treat nests directly over large areas. Effective trapping methods have not yet been developed and toxic baiting is not practical because YLH primarily hunt insects and preferentially feed on honeybees.[42] Given the potential negative impact and capacity for spread, an extensive monitoring and eradication effort was initiated by researchers and apiculturists in the state of Georgia in 2023. The trap–treat–release method[14,15] is being used in an attempt to control YLH in invaded areas. YLH arriving at bee hives are captured, topically sprayed with 0.5% fipronil, and released. The efficacy of this approach cannot be directly quantified, but it is expected that the treated hornets will return to their colonies and transfer a lethal dose of fipronil to other members of the colony, ultimately resulting in colony demise. In conclusion, our results demonstrated that the trap–treat–release technique has high efficacy and environmental benefits compared with other methods and it is envisaged that this method will increasingly be used against a large number of target taxa.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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