Evaluating the role of biological residues in the horizontal transfer of insecticides in carpenter ants

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Introduction

Horizontal transfer is the process in which insecticides and other chemicals are transferred between individuals of a species. Pest control companies take advantage of this phenomenon by putting insecticide in bait, causing the individuals who consume the bait(**Donors**) to spread the insecticide among the colony (**Recipients**). When observed in ants, it is commonly associated with trophallaxis (the mouth-to-mouth exchange of fluids), grooming, and other forms of direct contact. There is little research done on the exact methods of horizontal transfer in ants. Currently, we do not know if frass, oral excretions, or other biological residues facilitate horizontal transfer.

Objectives

- Assess the level of mortality incurred by exposure of recipients to residues left by donors
- Compare mortality between types of initial donor exposure (bait vs direct contact)
- Compare mortality between different donor-recipient ratios (1:10, 5:10, 10:10)

Figure 1: Donors and their associated residues 48 hours after exposure to bait

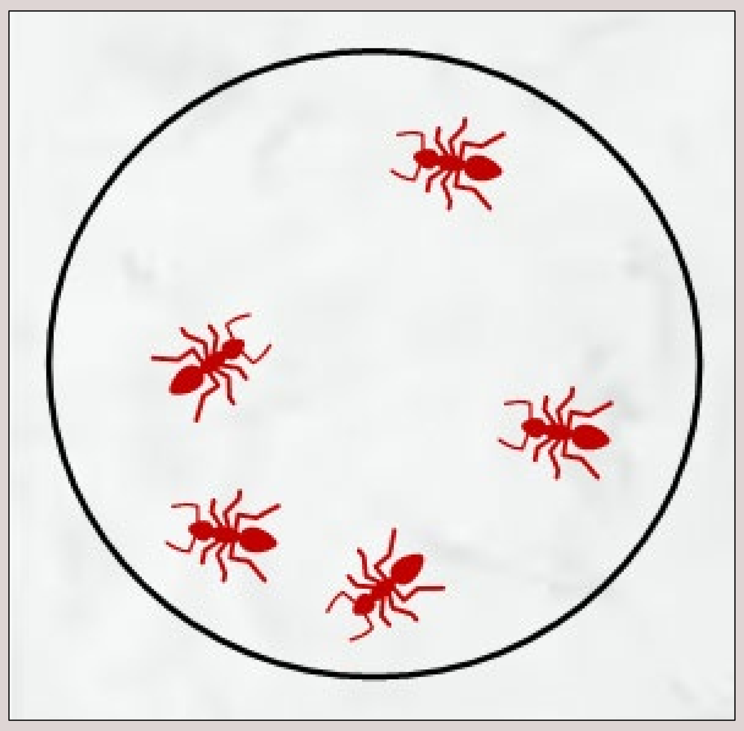

Figure 2: Donor exposure method used in contact bioassays. Donors exposed directly to treated ceramic tile, enclosed by fluon-treated plastic ring

Methods

Two-step laboratory bioassays were used to measure recipient secondary mortality in response to the residues of treated donors. 3 different donor-to-recipient ratios were tested: 1:10. 5:10, and 10:10 (low, medium, and high, respectively). We conducted two types of bioassays; one exposing the donors to fipronil through a liquid bait and one via direct contact. In each, the donors would contaminate the enclosure with biological residues (excrement). Recipient ants would then replace the donors to assess the affects of the residues on mortality.

Bait Bioassays

Donor ants were placed in their enclosure (*figure 1*) and given 0.06% fipronil mixed with sugar water as a bait. Donor mortality was monitored for 48 hours, then all donors were removed, dead or alive. Immediately after, recipient ants were placed in the same enclosure, and the fipronil bait was removed and replaced with pure sugar water. Mortality was recorded every 24 hours for 168 hours.

Contact Bioassays

Donor ants were first placed on a ceramic tile treated with 0.05% fipronil (figure 2). They remained there for 1 hour and were then moved to the enclosure for 24 hours, where there was no bait insecticide, only sugar water. Donors were then removed and replaced with recipient ants, whose mortality was recorded every 24 hours for 168 hours.

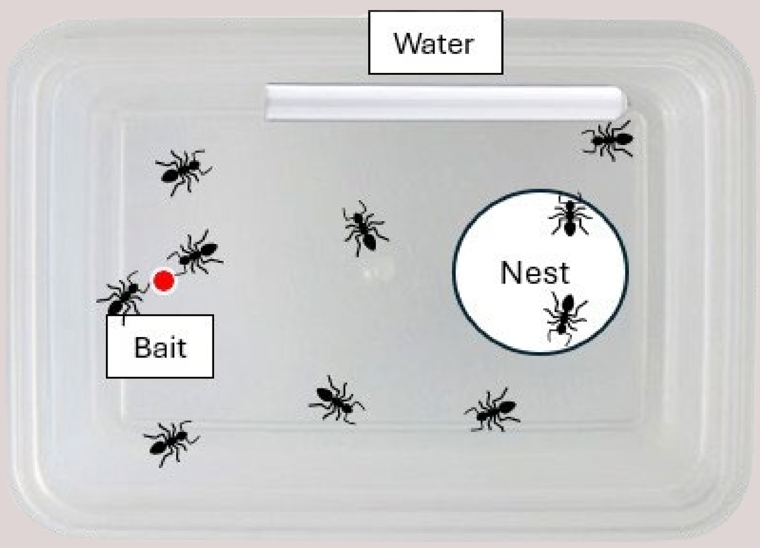

Figure 3: Generalized distribution of *C. pennsylvanicus* within bait bioassay enclosures

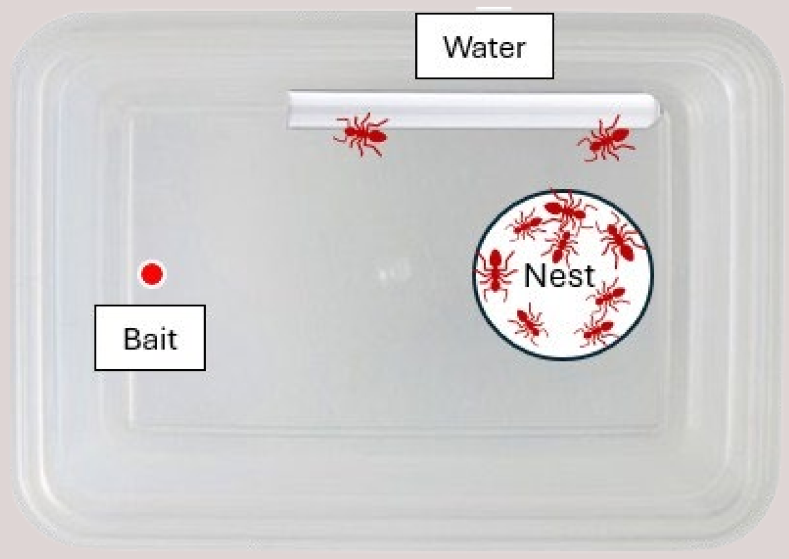

Figure 4: Generalized distribution of *C. floridanus* within bait bioassay enclosures

Results

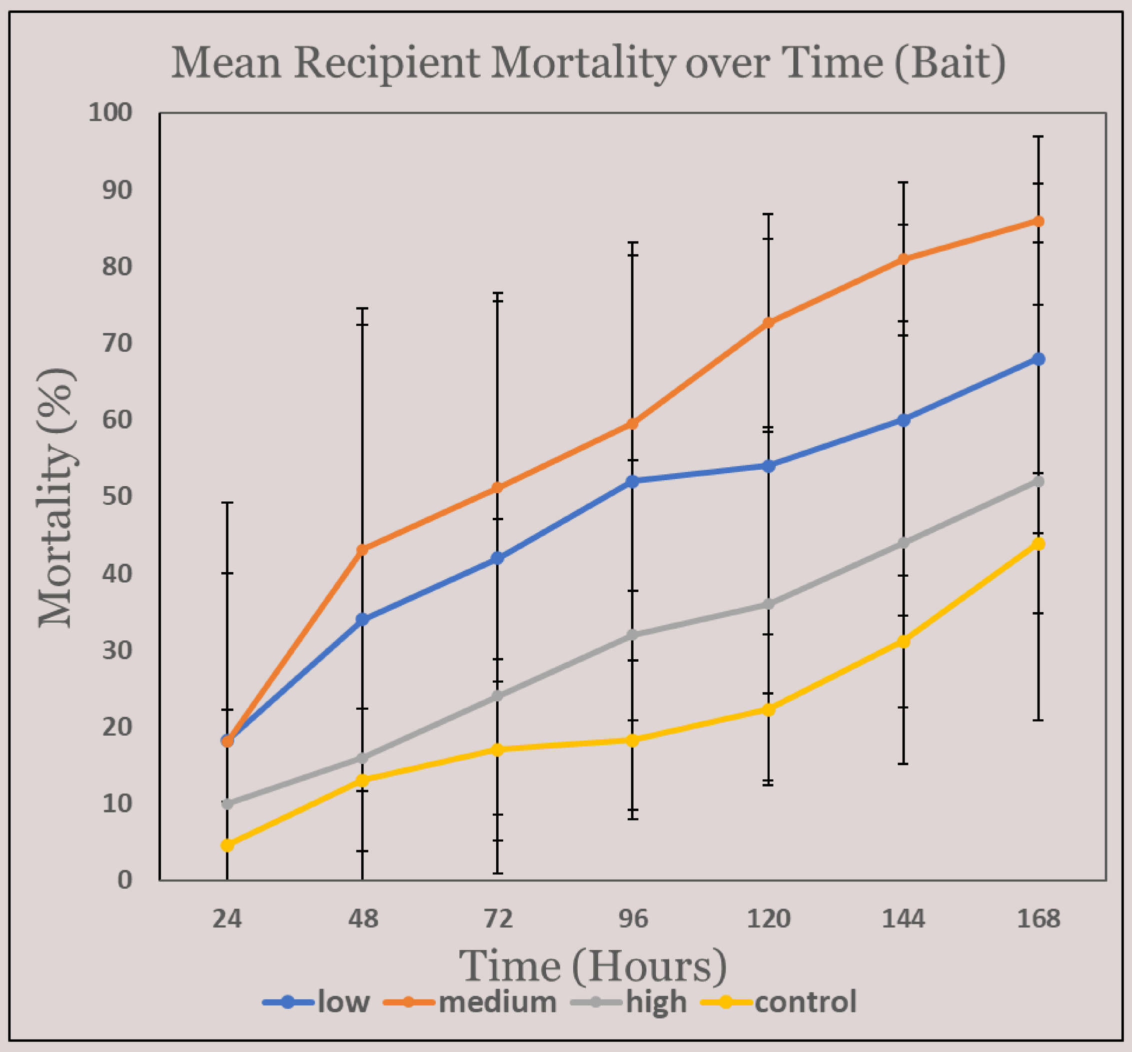

Figure 5: Graph of mean recipient mortality for each bait treatment over time. Error bars indicate standard deviation.

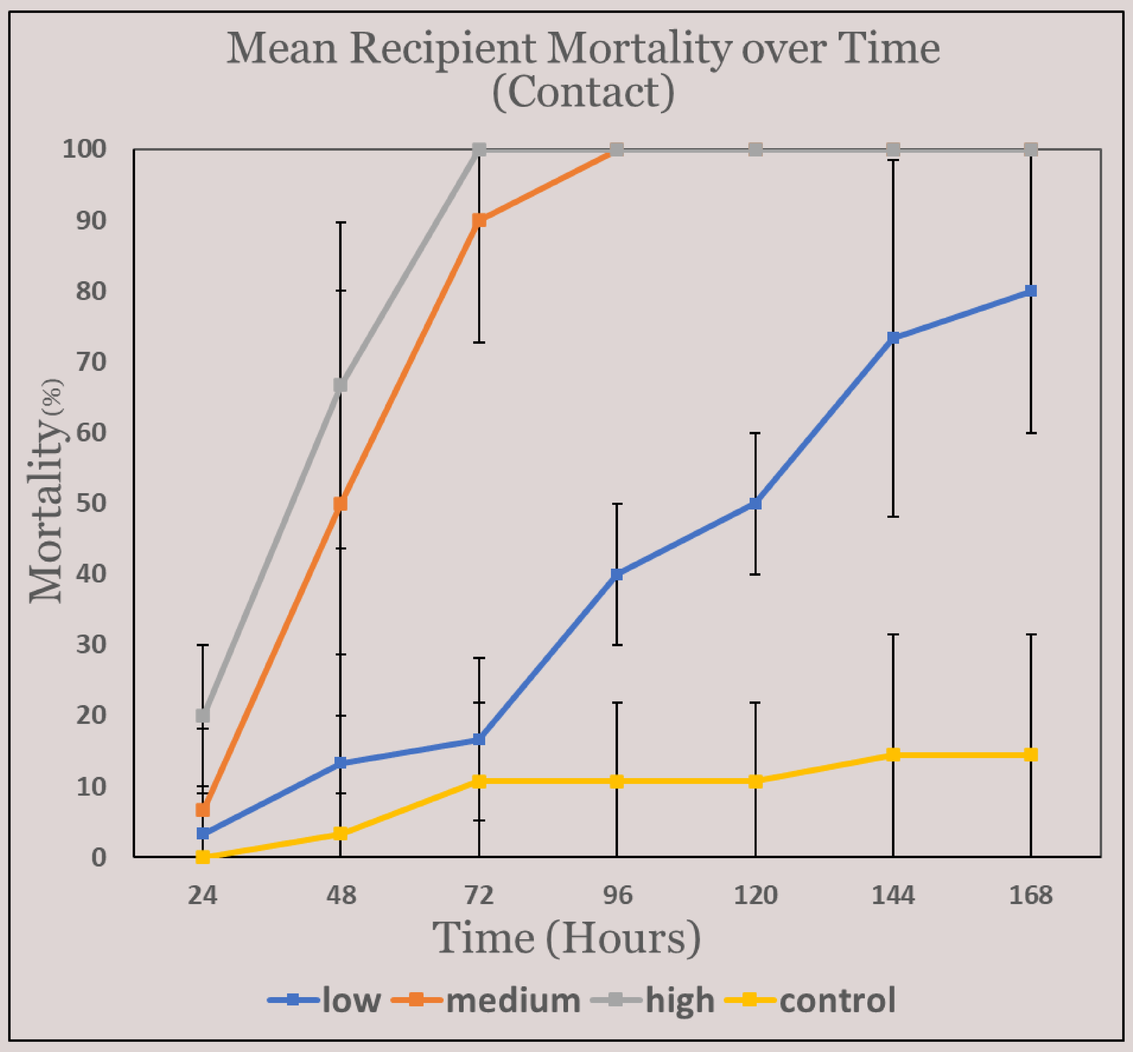

Figure 5: Graph of mean recipient mortality for each contact treatment over time. Error bars indicate standard deviation.

Analysis of Variance (Camponatus_Mortality _Data_April_2)
Marked effects are significant at p < .05000

Variable	SS (Effect)	df (Effect)	MS (Effect)	SS (Error)	df (Error)	MS (Error)	F	p
Hour 24	631.66	3	210.55	5993.80	15	399.5867	0.526925	0.670496
Hour 48	2825.45	3	941.82	10550.44	15	703.3626	1.339021	0.299079
Hour 72	3405.96	3	1135.32	9076.32	15	605.0877	1.876292	0.176961
Hour 96	4924.23	3	1641.41	7826.18	15	521.7455	3.145997	0.056314
Hour 120	6456.57	3	2152.19	6636.20	15	442.4135	4.864661	0.014731
Hour 144	6156.19	3	2052.06	6514.03	15	434.2689	4.725325	0.016294
Hour 168	4603.83	3	1534.61	6652.53	15	443.502	3.460206	0.043332

Table 1: One-way ANOVA of donor-recipient ratios and mortality for bait bioassays. Red text indicates statistical significance between treatments

Analysis of Variance (Camponatus_Mortality _Data_2023)
Marked effects are significant at p < .05000

Variable	SS (Effect)	df (Effect)	MS (Effect)	SS (Error)	df (Error)	MS (Error)	F	p
Hour 24	691.67	3	230.556	533.333	8	66.66667	3.458333	0.071278
Hour 48	8066.67	3	2688.889	3400.000	8	425	6.326797	0.016606
Hour 72	20029.94	3	6676.646	1114.403	8	139.3004	47.92984	1.86E-05
Hour 96	17992.90	3	5997.634	447.737	8	55.96708	107.1636	8.47E-07
Hour 120	16856.79	3	5618.930	447.737	8	55.96708	100.3971	1.09E-06
Hour 144	14647.22	3	4882.407	1851.852	8	231.4815	21.092	0.000373
Hour 168	14802.78	3	4934.259	1385.185	8	173.1481	28.49733	0.000127

Table 2: One-way ANOVA of donor-recipient ratios and mortality for contact bioassays. Red text indicates statistical significance between treatments.

Discussion

Both Bioassays displayed significant levels of secondary mortality in response to biological residues. As expected, contact bioassays displayed more pronounced and immediate results than the bait bioassays. We also observed significant differences between donor-recipient ratios in both bioassays, though it took 120 hours to reach that point in the bait bioassay (*table 1*). Unfortunately, unexpected variables skewed our data in the bait bioassay. Due to seasonal temperature drops, we lost access to *Camponotus pennsylvanicus* colonies to use for the bioassays and began to collect and use *Camponotus floridanus* from Orlando, FL to use instead. This resulted in *C. floridanus* being used in the 10:10 (high) ratio, and their aggregation behavior (figure 4) resulted in much lower mortality than even the low concentration. This aggregation resulted in less individuals exposing themselves to the bait, and less residues being spread across the enclosure.

Conclusions

First and foremost, we can confirm that biological residues *do* facilitate horizontal transfer. Secondary mortality among recipients in both types of bioassays differed significantly from controls. From this data we can also confirm that the ratio of donors to recipients plays a significant role in the magnitude and speed of mortality amongst recipient populations. We observed a trend in which donor-recipient ratios with higher concentrations of donors have higher mortality- though this trend was disturbed in the bait bioassays due to unforeseen experimental conditions. Additionally, it can be concluded that secondary mortality derived from bait-exposed donors is less immediately effective than contact-derived donors. This knowledge has the potential to alter many existing pest management strategies that rely on horizontal transfer. Finally, we can conclude that these residues likely played a role in previous studies on horizontal transfer among ants and potentially other insects as well. As such, we can no longer assume that trophallaxis and other forms of direct contact are the sole vector of insecticide transmission between individuals. This leaves the door open for a variety of future research on other specific methods of horizontal transfer and their associated impact on mortality in eusocial insects.

References

Buczkowski, G. 2019. Trap–treat–release: Horizontal transfer of Fipronil in field colonies of black carpenter ants, *camponotus pennsylvanicus*. Pest Management Science. 75: 2195–2201.