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The Lethal Effects of Herbicides and Herbicide Residues on the Agriculturally Important Wolf Spider Pardosa milvina

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Abstract

Herbicides are applied to commercial crops with increasing frequency and diversity yet are rarely tested for acute or chronic toxicity effects on beneficial non-target species such as spiders. We measured the lethal effects of chronic exposure to field-relevant doses of herbicide-treated soil on an agriculturally abundant wolf spider, *Pardosa milvina*. We tested six herbicides including atrazine, S-metolachlor, mesotrione, glyphosate, 2,4-D, and dicamba. We also tested a mixture of all six herbicides and a distilled water control. Spiders were housed individually in containers with topsoil previously sprayed with a recommended herbicide dosage or water control group. To test for herbicide residue effects, we reared spiders under herbicides exposed to three aging treatments: freshly applied herbicides, herbicides aged for 69 days under room-temperature laboratory conditions (indoor aged) or aged for 69 days in a greenhouse with variable temperature, humidity, light exposure, and evaporative cycling (outdoor aged) (N=960; n= 40 spiders across 24 treatments). Spiders were maintained on these treated substrates for 48 days and fed crickets (*Gryllodes sigillatus*). We recorded daily mortality across all spider treatments during the testing period. Mesotrione and combined herbicide treated spiders showed very high mortality within two weeks of exposure among both freshly applied and indoor-aged soil treatments while mortality was modest across outdoor-aged herbicide treatments. Our results indicate that some herbicides are arachnicides but require chronic and prolonged exposure to produce lethal effects. Further, soil bacterial communities alone were insufficient to break down herbicides or reduce their toxicity while photodegradation, bacterial action, temperature-variation and evaporation cycles were sufficient to dramatically reduce toxicity during chronic exposure.

Introduction

Commercial herbicide use has increased in quantity and diversity due to increases in herbicide-resistant weeds, increases in no-till farming practices, and increasing reliance on herbicide-resistant crop variants (Fernandez-Cornejo and Caswell 2006). Most herbicides are sprayed pre-emergence or post-harvest when there is no crop canopy. This practice can lead to unintentional but direct spraying of non-target ground arthropods such as wolf spiders. Wolf spiders are likely important predators of crop pests since they are frequently agrobionts that occur year-round within commercial crops and tolerate high levels of disturbance (Symondson et al. 2002). The wolf spider *Pardosa milvina* is one of the most common ground spiders within crop systems of the Eastern United State (Marshall et al. 2002). These spiders may attain densities greater than 16/m^2 and consume an estimated half a million insects/acre per week. *Pardosa* is the second largest genus of spider comprising over 600 species and they occur in virtually every agricultural system on six continents. The effects of single herbicides on wolf spiders have not been studied extensively and the effects of multiple herbicides or herbicide breakdown products have been studied even less (reviewed in Pekár, 2012). Given the possible pest-control benefits of these spiders and potential negative effects of herbicide applications on them, excessive herbicide use may be counterproductive to integrated pest management systems by reducing the abundance and diversity of these important generalist predators.

We tested the lethal effects of chronic exposure to six commonly used herbicides on juvenile *Pardosa milvina*. We also tested the lethal effects of freshly applied, indoor aged, or outdoor-aged herbicide residues on these spiders over a 49-day exposure period. Based on previous studies, we hypothesized that exposure to an herbicide cocktail would have more lethal effects than single herbicides and that freshly applied herbicides would result in higher mortality than exposure to aged herbicide residues.

Questions

- **What are the lethal effects of common herbicides on juvenile *Pardosa milvina?***
- **How does the combined application of herbicides affect their toxicity?**
- **What are the residue effects of common herbicides that have been aged under different conditions?**

Methods

Collection & Maintenance

- Juvenile *Pardosa milvina* were collected from a non-sprayed fallow agricultural field near Susquehanna University, Selinsgrove, PA.
- Three soil age treatments were used: freshly sprayed, indoor-aged, and outdoor-aged.
- Indoor-aged soil treatment was aged for 69 days under room-temperature indoor lab conditions.
- Outdoor-aged soil treatment was aged for 69 days in a greenhouse and received 16mL of water every week. This treatment was exposed to light, temperature, evaporative water cycles, and varying humidity

Herbicide Exposure

Spiders were housed in small souffle cups treated with freshly-sprayed herbicide or one of two aged soil conditions.

Measuring Mortality

Spiders were fed the same diet at regular intervals. Lethal effects were assayed by measuring the mortality across treatments every day over the course of the 49-day trial period. Each day post-exposure we recorded if the spiders were dead, alive or paralyzed. Paralyzed spiders later confirmed dead were recorded dead on the date of the initial paralysis recording.

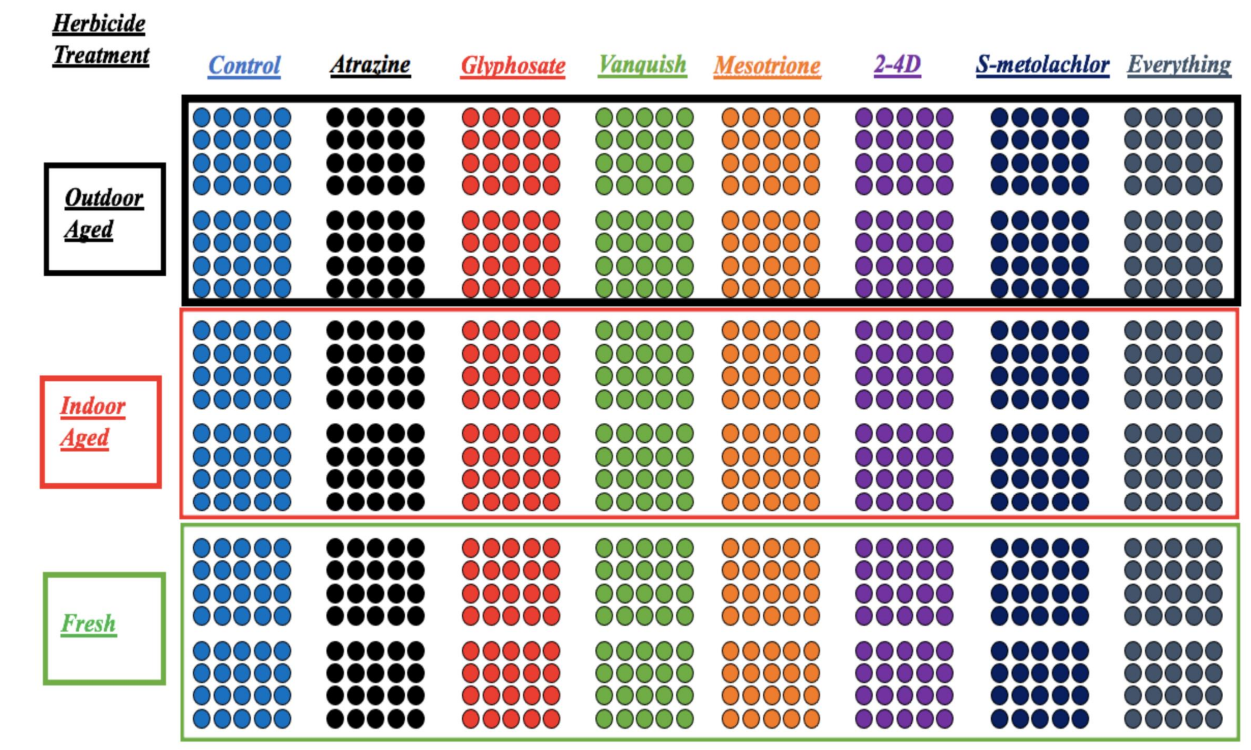


Figure 1: Graphic representation of the eight herbicide treatments across three soil age treatments (N=960, n=40)

Results

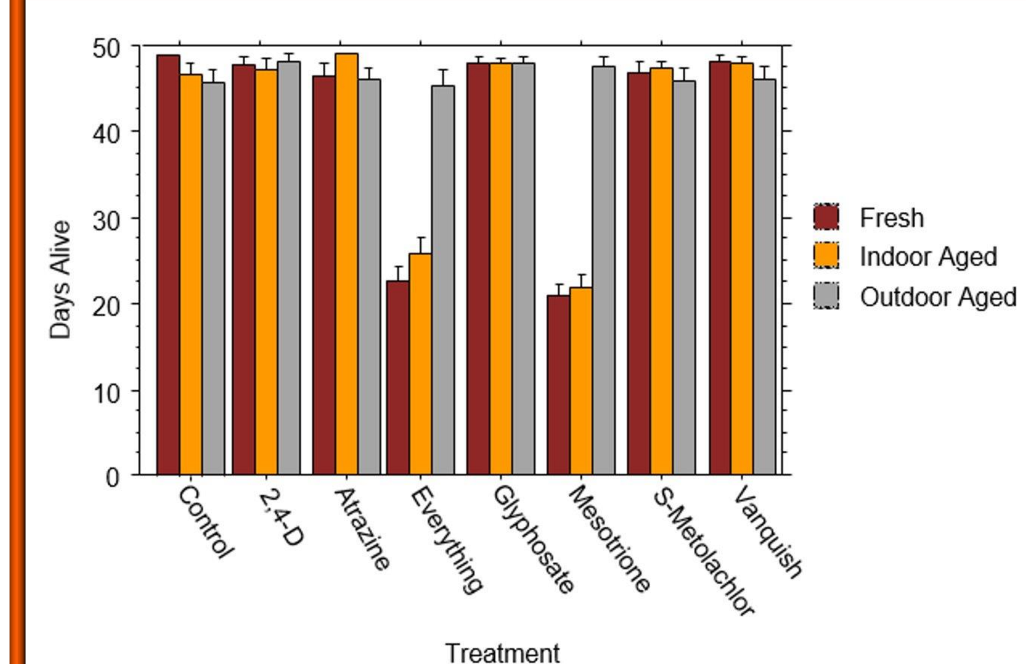
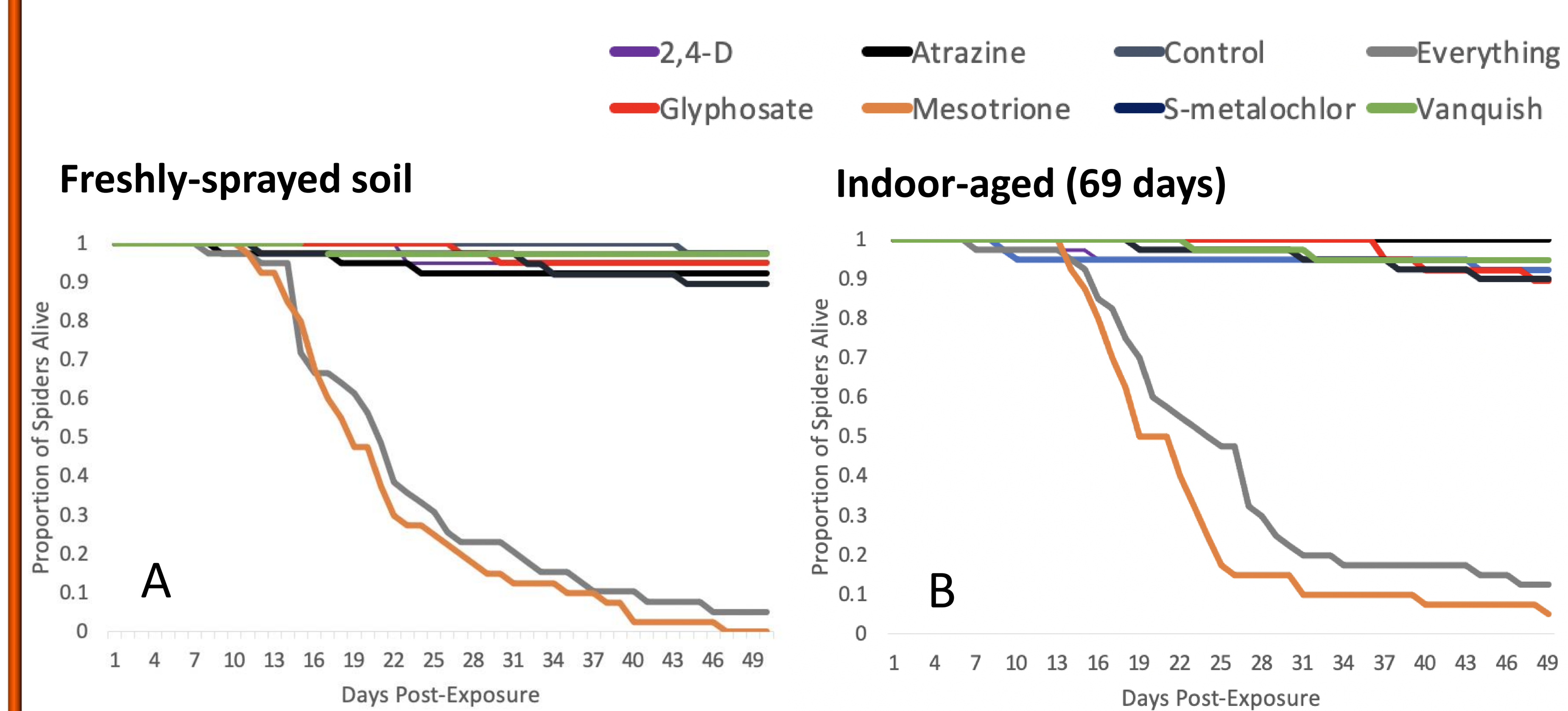


Figure 2: Mean days alive post-exposure by herbicide and soil age over a 49-day period ± S.E. (N=960, n=40).

Table 1: Two-way ANOVA of mean days alive across all herbicide and soil age treatments. (N=960, n=40)

Treatment	F	P-value
Herbicide	118.725	<0.0001*
Soil Age	47.970	<0.0001*
Herbicide * Soil Age	30.043	<0.0001*



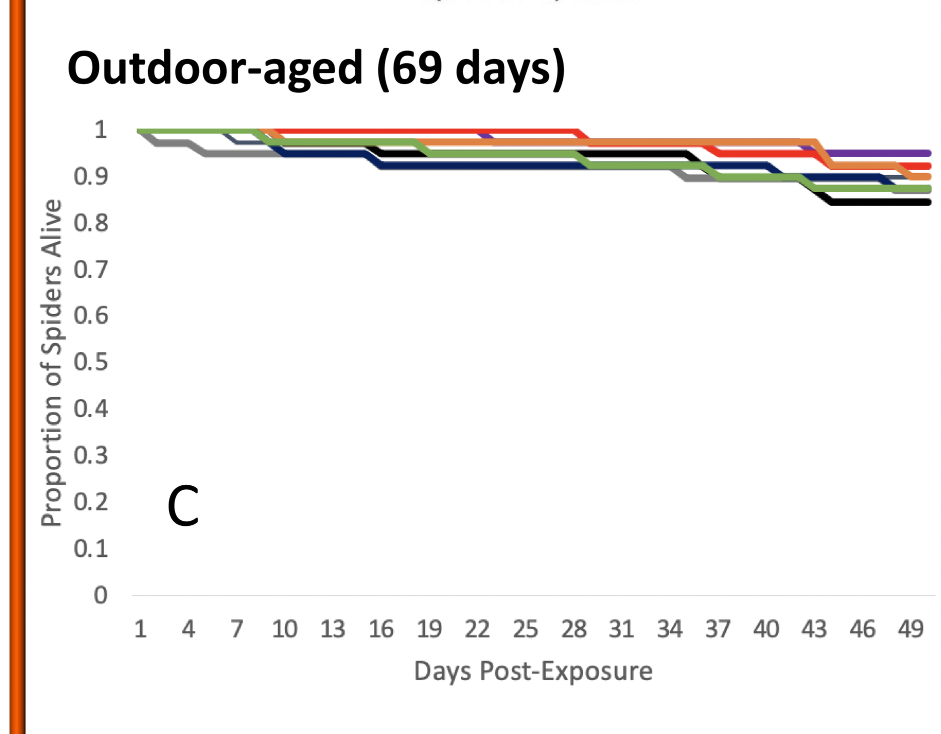


Figure 3: *Pardosa milvina* mortality curves for eight herbicide treatments across three soil treatments: (A) Freshly-sprayed (n=320), (B) indoor-aged (n=320), and (C) Outdoor (greenhouse) aged. All spiders were housed on these substrates continuously for 49 days. Mesotrione and everything treatments suffered significantly higher mortality than all other herbicide treatments for both freshly applied (Mantel-Cox test, X^2=394, p<0.0001) and indoor-aged soil treatments (Mantel-Cox test, X^2=292;p<0.0001). We also found a significant difference between mesotrione and the everything treatment within the indoor-aged soil that didn't occur in the freshly-sprayed treatment (Mantel Cox Test, X^2=3.98; p=0.046). No difference in mortality occurred across outdoor-aged treatments. (Mantel Cox Test, X^2=3.14; p=0.87).

Summary and Conclusions

- **Mesotrione caused significantly higher mortality in juvenile *Pardosa milvina* in the freshly-sprayed and indoor-aged treatments**
- Since mesotrione showed significantly higher mortality than the combined herbicide treatment suggests that there may be antagonistic effects between these herbicides but this was only seen in the indoor-aged treatment. Mesotrione is a powerful arachnicide.
- Atrazine, glyphosate, S-metolachor, 2-4D, and vanquish did not significantly differ from the control group, or affect the mean days alive, in any of the soil treatments
- Previous studies indicated that atrazine, glyphosate, and S-metolachor had a significantly higher survival rate than the control group in adult *Pardosa milvina* (Ward et al. in prep). Ward et al. (in prep.) suggested that these herbicides may be beneficial to *Pardosa milvina* yet our study suggests that any such survival benefits are restricted to adults.
- **Laboratory aging was not sufficient to break down herbicide residues into non-toxic by-products**

References & Acknowledgements

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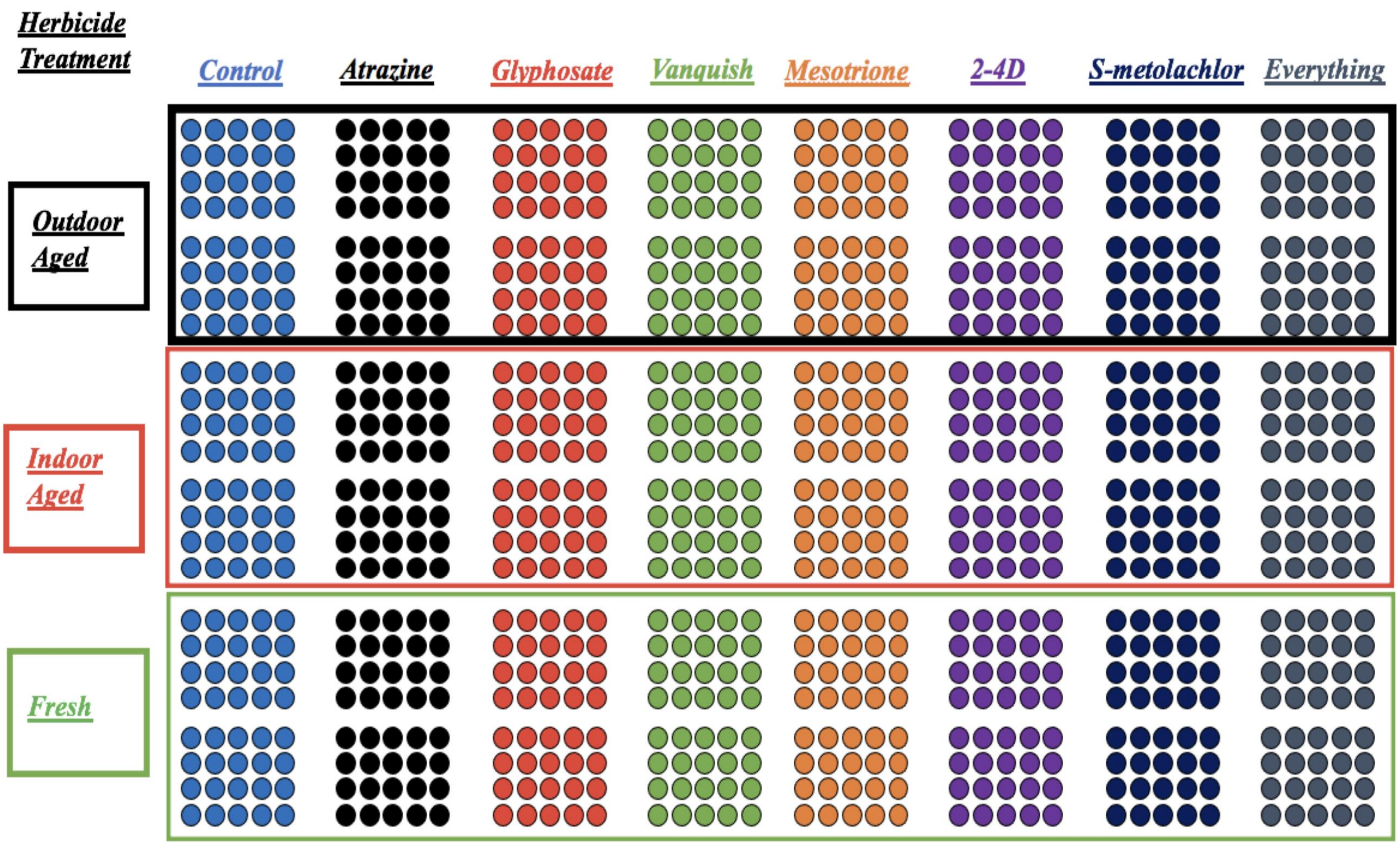

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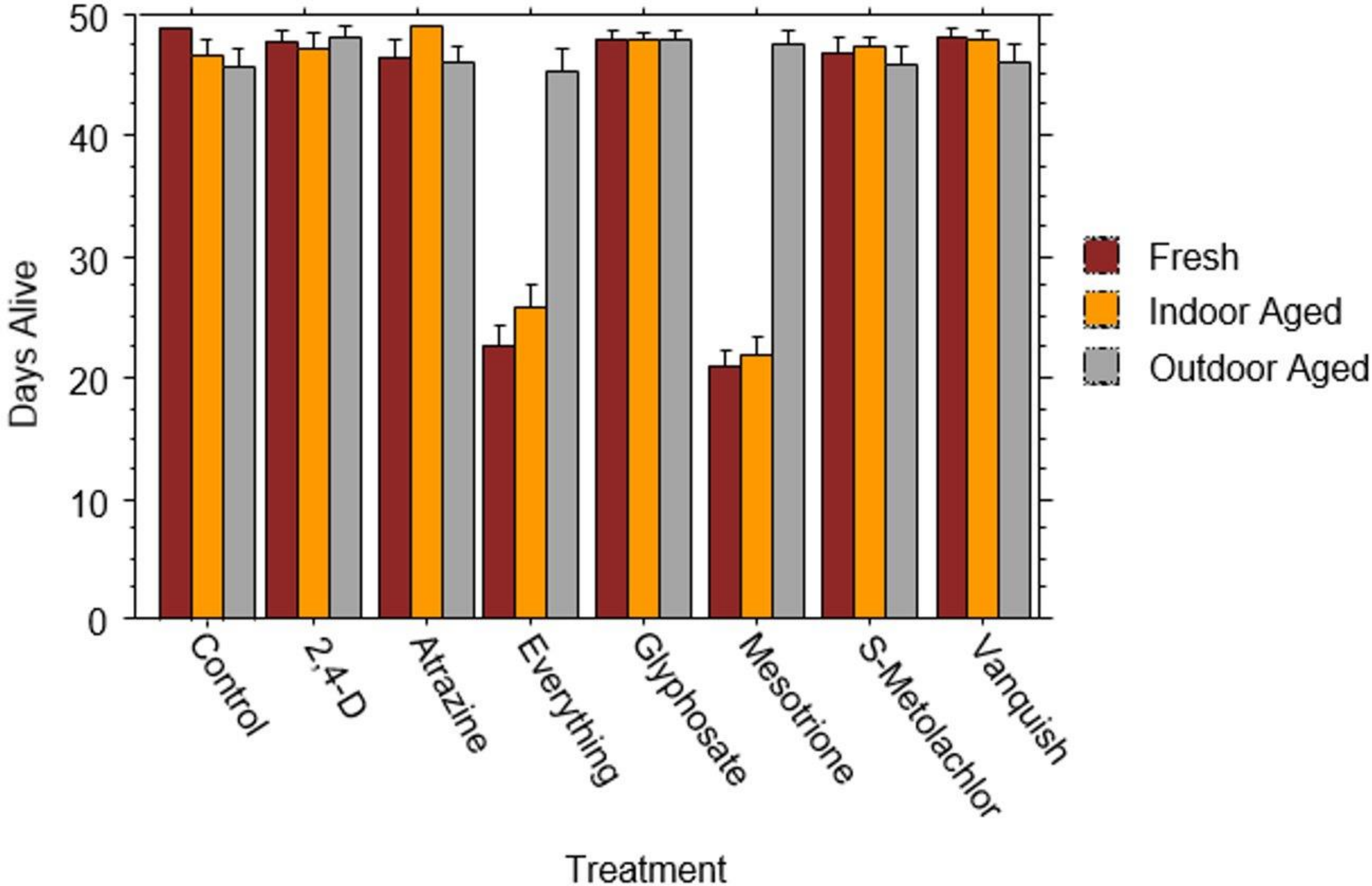

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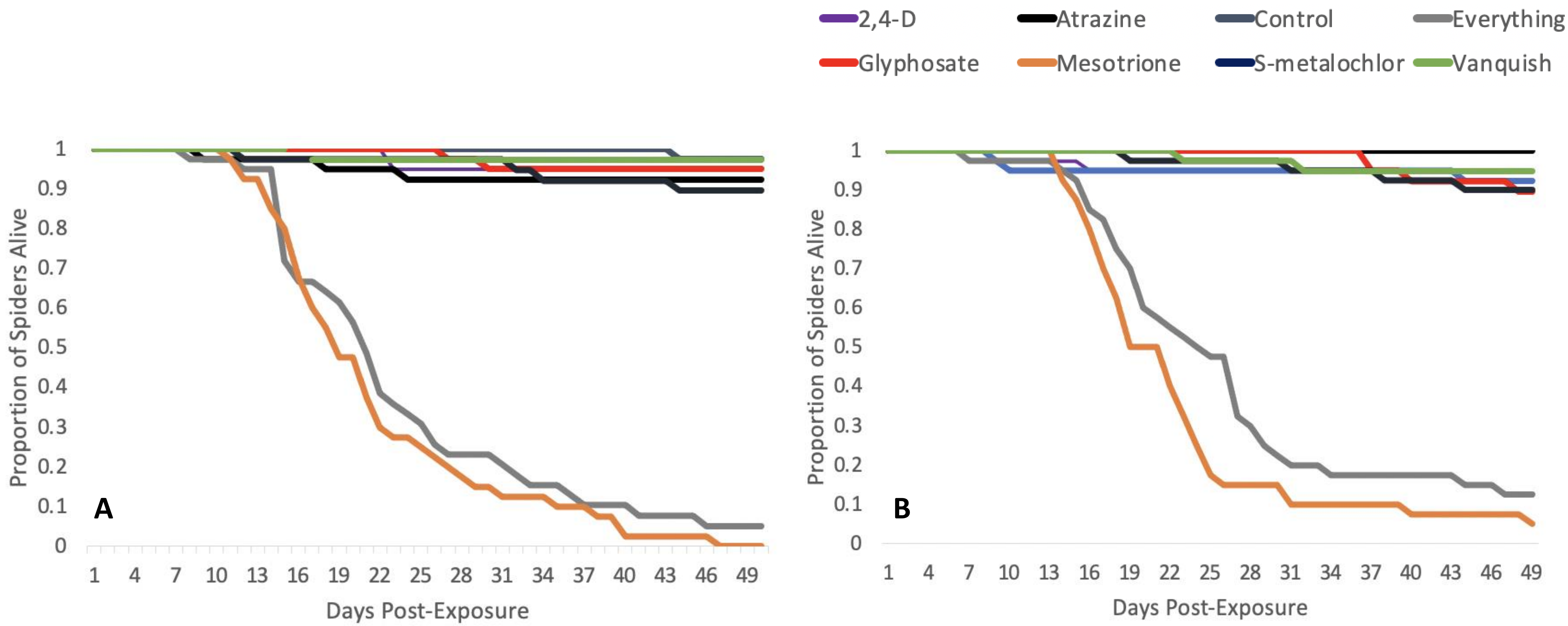

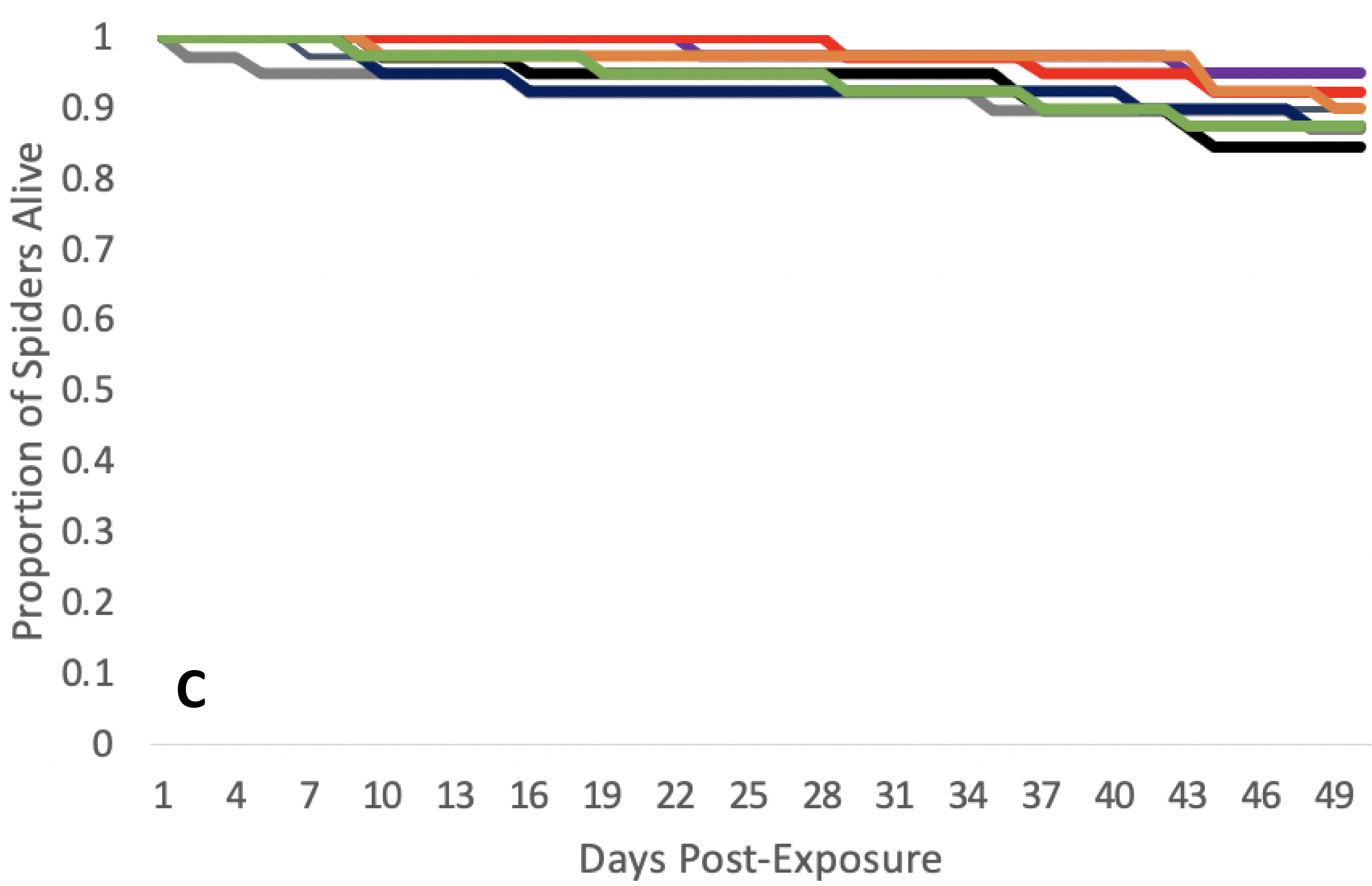

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