The Ceres Trust 2013 Graduate Student Organic Research Grant:
Final Report

Project Title: *Transforming waste: rearing black soldier flies as a source of compost and entomopathogenic nematodes.*

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Accomplishments by Objectives:
Objective 1: *Determine black soldier fly composting rate and quality for plant and animal based farm waste and the number and quality of larvae produced on these substrates.*

Methods and materials:

We completed two experimental trials to address this objective. One trial took place at Swallowtail Farms in Mason, MI and the second took place in a heated greenhouse at the MSU Student Organic Farm. We assigned three BioPods Plus® (Fig. 1A) to one of two treatments: (1) food waste only, and (2) 50:50 mixture of food waste and chicken bedding (Fig. 1B). We collected chicken bedding from Swallowtail Farms. It was straw based and contained manure and spilt chicken feed. Food wastes consisted of rotting fruits and vegetables from the MSU Student Organic Farm and Horticulture Farm, pre-consumer waste from a MSU cafeteria, and coffee grounds from a local coffee shop.

Trial 1 at Swallowtail Farm:

We set-up the BioPods® with 5 gal of material and approximately 700 young black soldier fly larvae. An additional 5 gal of material was added 17 days later, followed by an additional 2 gal 18 days later. Mature black soldier fly larvae (i.e. prepupae) self-harvested by crawling up the built-it ramp of the BioPod® and dropping into the collection basket, which was filled with aspen wood chips. We collected, counted, and weighed black soldier fly larvae found in the collection basket. A sample of the digestate – remaining material that the larvae did not eat – from each BioPod® was submitted to A & L Great Lakes Laboratories, Inc. for nutrient analysis.

Trial 2 in heated greenhouse at MSU Student Organic Farm:

Throughout this Trial I collaborated with Jason Matlock, a graduate student in the MSU department of Horticulture. Feed stocks were mixed in advanced and stored in 27 gal plastic tubs in a walk-in-cooler until needed. BioPods were initial stocked with 2000 early instar black soldier flies and 5 gal (7-8 kg) of feedstock. Additional feedstock in was added into the BioPods on three additional dates at 1 week intervals, 5 gal per week. Prepupae were collected, counted, and weighed twice a week for 5 weeks. Prepupae, digestate, and leachate were collected from each BioPod and submitted for nutrient analysis at A & L Great Lakes Laboratories, Inc.

Jason Matlock and other students in the Organic Pest Management class conducted a germination test with aqueous digestate extracts. They use radish and lettuce seeds and four concentrations of the extracts to test the plant toxicity of using black soldier fly digestate as a soil fertility amendment.

Results:

Trial 1 at Swallowtail Farm:

One very noticeable thing about was that the black soldier fly larvae did not eat the straw found in the chicken bedding or other similar tough plant structures that contained cellulose (Fig. 1B). Even though more black soldier flies (203.3 ± 79.2) self-harvested from the food waste treatment it was not significantly more than the black soldier flies (78.0 ± 32.1) harvested from the mixture of chicken bedding and food waste ($W = 8$, $p = 0.2$) (Fig. 2A). The average mass of the black soldier fly prepupae collected from the food waste (0.163 ± 0.016 g) was not significantly different than the larvae that feed on food waste and chicken bedding (0.169 ± 0.004 g) ($W = 4$, $p = 1$) (Fig. 2B).

A second fly species was also collected from the BioPods. It was identified as *Ptecticus trivittatus* Say (Diptera: Stratiomyidae) which is a close relative of the black soldier fly and commonly found in MI. *Ptecticus trivittatus* prepupae (0.042 to 0.058 g) are much smaller than black soldier fly prepuae (0.135 to 0.191 g) and they have two cream-colored parallel stripes running the length of their bodies both on the dorsal and ventral sides. Significantly more *P. trivittatus* prepupae were harvested from the BioPods that received only food waste than the BioPods with chicken bedding and food waste mixture (138.0 ± 86.2 and 5.3 ± 0.7, respectively) ($W = 9$, $p = 0.038$) (Fig. 3A). The *P. trivittatus* harvested from the chicken bedding and food waste mixture were significantly larger than the prepupae from the treatment with only food waste (0.053 ± 0.002 g and 0.045 ± 0.001 g, respectively) ($W = 9$, $p = 0.038$) (Fig. 3B).

The addition of the chicken bedding to the food waste affected the abundance of some the nutrients in nutrients in the digestate. Phosophorus, calcium, zinc and manganese were higher in the digestate contianing chicken bedding; whereas, potassium was higher in the digestae without chicken bedding (Table 1).

Trial 2 in heated greenhouse at MSU Student Organic Farm:

One very apparent visual observation was that the straw included in the feedstocks was still intact after the black soldier fly had fed, as observed in the first trial. We also noticed that seedlings were sprouting in the BioPods, indicating that the black soldier fly did not feed on them. Not all of the black soldier fly prepupae self-harvested. Many remained in the feeding chamber of BioPod, pupated, and emerged as adults. We observed large mites on the adult black soldier flies that emerged. Adult flies that emerged earlier had a higher mite load than flies that emerged later. Mites were not identified, but we presumed that they came from the chicken bedding and were facultative parasites, attaching to the adults flies in hopes of dispersing. *Ptecticus trivittatus* was not observed in the BioPods during this trial.

The number of prepupae that self-harvested did not different between the two treatments, 225.3 ± 39.8 for food waste and 220.7 ± 9.0 for the chicken bedding and food waste mixture ($W = 6$, $p = 0.7$) (Fig. 4A). Likewise, the prepupae did not differ in size, 0.142 ± 0.004 g and 0.137 ± 0.004 g, respectively ($W = 7$, $p = 0.4$) (Fig. 4B). The black soldier flies

reduced the mass of the feedstock by 35% in the control and 27% in the chicken bedding treatment. Certain nutrients were more concentrated in the digestate from the chicken bedding and food waste mixture than the control (Table 2); however, the prepupe did not differ in nutrient values (Table 3A). They were able to sequester only a small amount of the nutrients from feedstock and this did not differ by feedstock treatment (Table 3B). A minimal amount of nutrients leached from the digestate over the course of the experiment and did not differ in nutrient concentrations between the two treatments (Table 4).

Seedling germination rates were not affected by the aqueous digestate extracts; however, seedling length was shortest at the highest concentration level of 1:1 for both radish and lettuce (Fig. 5). This was probably a result of osmotic stress caused by the high concentration of dissolved solids. At lower concentration levels, seedling length did not significantly differ from the water control for both radish and lettuce.

Objective 2: *Develop an optimized entomopathogenic nematode rearing system based on black soldier fly larvae.*

Methods and Materials

Invertebrate colonies

We reared larvae in batches of 300 individuals in 946 ml plastic deli containers covered with a brown paper towel held in place with a rubber band. We fed larvae 10 g of Gainesville house fly diet (5:3:2 ratio of wheat bran, alfalfa meal and cornmeal) and 17-20 ml of water 5 days a week at 25°C, 80% relative humidity, and in the dark.

We reared four species of entomopathogenic nematodes – *Heterorhabitis bacteriophora*, *Steinernema carpocapsae*, *S. feltiae*, and *S. riobrave* – on the host *Galleria mellonella* in inverted Petri dishes. Harvested infective juveniles were stored in tissue culture flasks in laboratory drawers at ambient room temperature. All infective juvenile used in the experiments described below were less than 14 days old.

Experiment 1a – black soldier fly susceptibility by larval stage

We set-up a 5x5 two-way factorial experiment to test the susceptibility of black soldier fly instars to multiple species of entomopathogenic nematodes. The levels of the first factor were multiple instars (2, 4, 5, 6) of black soldier fly plus the positive control – Galleria. The levels of the second factor were four species of nematodes (*H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*) plus a negative control –water without nematodes. There was a total of 25 treatment combinations.

We washed and dried larvae before distributing them to inverted 60 mm diameter Petri dishes containing one piece of No. 1 Whatman filter paper, one larva per dish. Using a micropipette, we applied 100 infective juveniles in 500 µl of deionized water to the filter paper. We prepared 25 replicates for each treatment and placed in a growth chamber (20.4 ± 0.6°C, 70.4 ± 10.4% relative humidity, no light). We assessed insect mortality daily for 8 days. On the 8th day, we froze the larvae at -20°C to store for later dissection. We dissected cadavers in deionized water and counted adult nematodes under a dissecting microscope.

Experiment 1b – black soldier fly susceptibility at pupal stage

A similar, but separate experiment set-up was used to test if the pupal stage of black soldier fly were susceptible to entomopathogenic nematodes. A 2x5 factorial experiment was set-up. The levels of the first factor were black soldier fly pupae and the positive control –Galleria. The levels of the second factor were four species of nematodes (*H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*) plus a negative control –water without nematodes. There was a total of 10 treatment combinations.

Experiment 2 – injuring larvae

Based on the results from the previous experiment, 5th instar were selected for all following experiments. We set-up a 3x5 two-way factorial experiment to test whether injuring the black soldier fly larvae affects nematode infectivity. The levels of the first factor where injured black soldier fly larvae, non-injured black soldier fly larvae, and late instar Galleria. Galleria were used as a positive control to insure that nematodes were indeed infective. The levels of the second factor were four species of entomopathogenic nematodes (*H. bacteriophora*, *S. carpocapsae*, *S feltiae*, and *S. riobrave*) and water control. There were a total 15 treatment combinations. We prepared 40 replicates for each treatment. The experiment took place in a growth chamber at 25°C, 100% humidity and blocked by space.

Petri dish infection arenas were prepared by inserting 60 mm diameter No. 1 Whatman filter paper circles into 60 mm diameter inverted Petri dishes. Infective juveniles (1000) were applied to the filter paper in 500 µl. Controls (no nematodes) received just 500 µl of water.

Meanwhile, black soldier fly larvae were separated from feeding material and washed in a tub of lukewarm tap water. Under a dissecting microscope, we used a size 0 insect pin to puncture two holes in the cuticle on the ventral lateral edges of nine segments – the mesothorax, metathorax, and first seven abdominal segments. We punctured a total of 18 holes into the larva. After the puncturing operation, larvae were immediately transferred onto the prepare Petri dishes. Insect pins were sterilized by boiling in water for 1 min and storing in 80% ethanol. Non-injured larvae and Galleria were placed into appropriate Petri dishes at the same time. One block of replicates was completed before proceeding onto the next block.

Insect mortality was assessed as in experiment 1. Any larvae that were previously recorded as dead and then alive were revaluated daily. On the fifth day of mortality assessment, we froze all dead individuals plus at least one replicate for each block of the controls. We placed larvae into the freezer (-20°C) to arrest nematode development until dissection could occur.

Cadavers were washed in water to remove exterior nematodes. Cadavers were dissected in Ringers solution under a dissecting microscope. We counted the adult nematodes. One fifth of the negative controls (no nematodes) were dissected to verify no nematode contamination. Any negative controls that died were also dissected to verify no nematode contamination.

Results

Experiment 1a – black soldier fly susceptibility by larval stage

We found that insect mortality was stage-dependent (p-value < 0.001) and there was an interaction between nematode species and instar (p-value = 0.032). We found that

mortality was not significantly different among instars when infected with *H. bacteriophora* or *S. feltiae* (Fig. 6). Second instars (92% ± 5%) and 74% ± 11% of fourth instars were killed by *S. carpocapsae*, which was significantly more than fifth and sixth instars, 13% ± 8% and 17% ± 8%, respectively (Fig. 6). Larvae treated with *S. riobrave,* 71% ± 8% of the second instars and 69% ± 12% of the fourth instars died, which was significantly more than sixth instars (8% ± 5%), but not significantly different than fifth instars (53% ± 9%) (Fig. 6). Second instars were most susceptible to *S. carpocapsae* (92% ± 5%), intermediate susceptible to *S. feltiae* and *S. riobrave* (57% ± 15% and 71% ± 8%, respectively), and less susceptible to *H. bacteriophora* (28% ± 10%). Fourth thru sixth instar susceptibility did not vary by nematode species.

We recovered nematodes from only a few black soldier fly cadavers (Table 5). No *H. bacteriophora* nematodes were recovered from the second instars. We did find nematodes in the fourth through sixth instars, but the mean was four or less nematodes from one or two cadavers. For *S. carpocapsae,* we recovered on average six nematodes from eight second instars, which was significantly more than the two nematodes recovered from three fourth instars and two sixth instars. No *S. carpocapsae* nematodes were recovered from fifth instars. For *S. feltiae*, only one nematode was recovered from three individual second instars, two nematodes from one fourth instar, and a mean of 1.7 nematodes from three sixth instars. No nematodes were found in the fifth instars. For *S. riobrave*, we recovered a mean of five nematodes from three second instars, which was not significantly different from the one nematode in one fourth instar and one nematode each in two fifth instars. We did not recover any nematodes from sixth instars. *Heterorhabditis bacteriophora* infected 56% of the Galleria, *S. carpocapsae* infected 96% of the Galleria, *S. feltiae* infected 48% of the Galleria, and *S. riobrave* infected 72% of the Galleria. We did not recover any nematodes from the untreated controls.

Experiment 1b – black soldier fly susceptibility at pupal stage

We did not recover any nematodes from the pupae.

Experiment 2 – injuring larvae

Injuring the black soldier fly larvae increased the mortality rate when infected by *Steinernema spp.* but not *H. bacteriophora* (Fig. 7). For *H. bacteriophora,* the injured larvae did not die any faster than the non-injured larvae (Fig. 7A). On day 5, all of the injured larvae were dead and all but one non-injured larvae were dead (Fig. 7A). For *S. carpocapsae*, all of the injured larvae were dead by day 2 whereas only five of the non-injured larvae were dead (Fig. 7B). On day 5, 32 of the non-injured larvae were dead (Fig. 7B). A similar pattern was observed for *S. feltiae*. Only one injured larvae was still alive on day 2 whereas 30 non-injured larvae were still alive (Fig. 7C). All but two non-injured larvae died by day 5 (Fig. 7C). For *S. riobrave*, 31 injured larvae and 5 non-injured larvae were dead on day 2 and 40 injured larvae and 39 non-injured larvae were dead on day 5 (Fig. 7D). Only two injured larvae and none of the non-injured larvae died by the fifth day (Fig. 7E). All of the Galleria treated with nematodes were dead on day 2, and only five Galleria died in the untreated control.

Injuring black soldier fly larvae did significantly increase the infectivity of *S. carpocapsae* and *S. feltiae*, but not *H. bacteriophora* or *S. riobrave* (Fig. 8). Significantly more *S. carpocapsae* enter the injured host than the non-injured host (Fig. 9). The number of

infective juveniles that entered the host was not affected by injuring the host for the other three nematode species (Fig. 9).

Objective 3: *Demonstrate black soldier fly composting on organic farms and deliver project findings to organic farmers.*

We purchased a large bioreactor and set-up it up at the MSU Student Organic Farm for student farmers, faculty, and farm visitors to observe black soldier flies devouring organic wastes. We are feeding the black soldier flies post consumer wastes from the MSU cafeterias.

Jason Matlock and I presented a poster at the 2014 annual MOSES conference in LaCrosse, WI discussing our second trial with composting food wastes and chicken bedding with black soldier flies.

I also participated in a grower extension meeting in Saginaw, MI where I instructed a group of growers how to rear their own nematodes using wax worms.

Conclusions:

Black soldier flies can be used to compost various sources of organic matter, except tough plant structures that contain cellulose. They quickly break down organic matter and greatly reduce the amount of biomass with a few short weeks. Black soldier fly compost is safe to use as soil amendment when diluted, but be careful about the presence of any seeds. We think that after black soldier flies have finished feeding, red worms could be added to finish breaking down the compost into a more stable state.

Black soldier fly larvae are not readily susceptible to infection by entomopathogenic nematodes. Injuring them does increase their susceptibility to *Steinernema spp.* but not *H. bacteriophora*.

Figure 1. (A) BioPods at Swallowtail Farms, Mason, MI. (B) Feedstock feed to the black soldier fly larvae, left is chicken bedding and food waste mixture, right is just food waste.

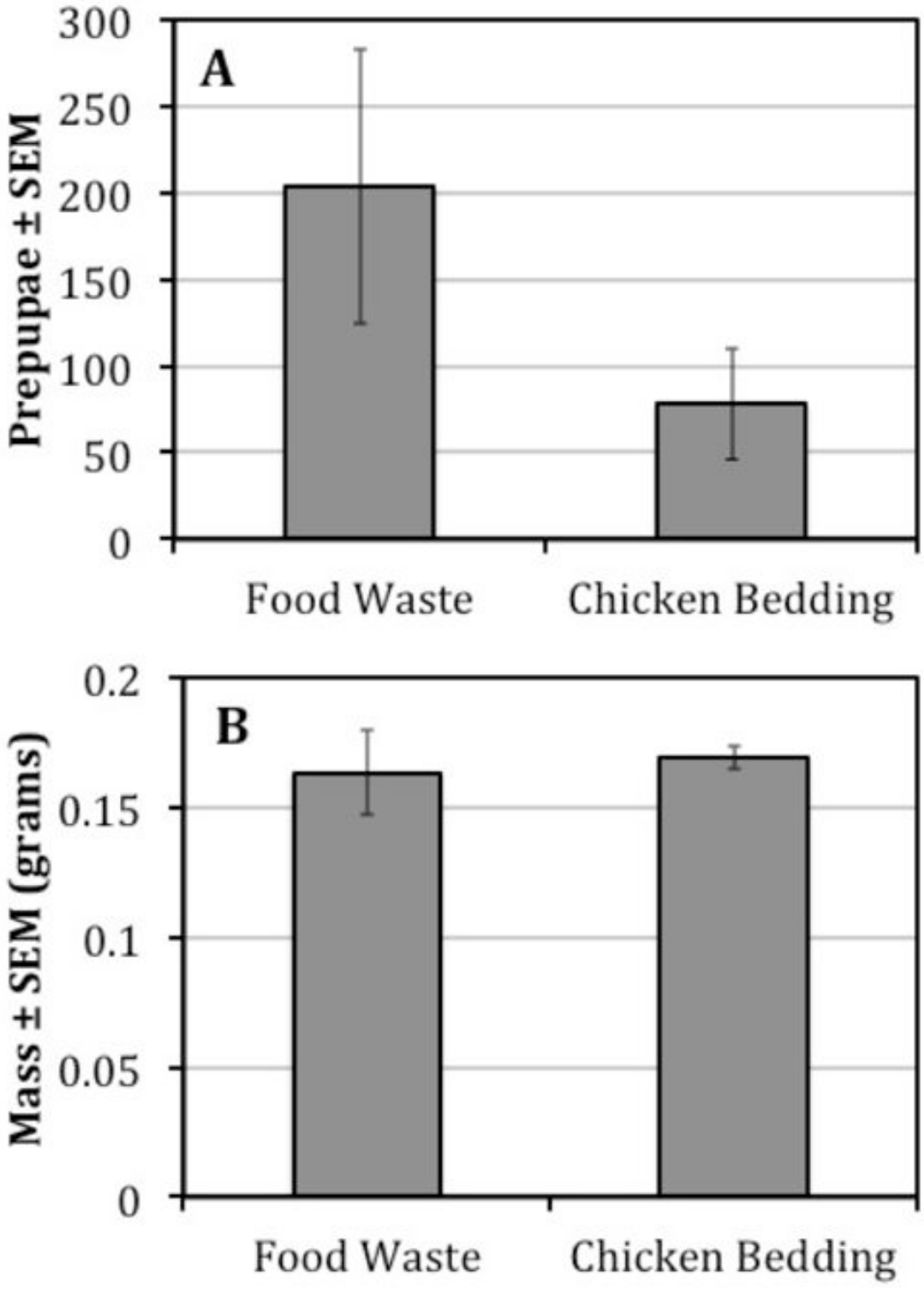

Figure 2. Trial 1. Black soldier fly (*Hermetia illucens*) prepupae, (A) self-harvested, (B) mass.

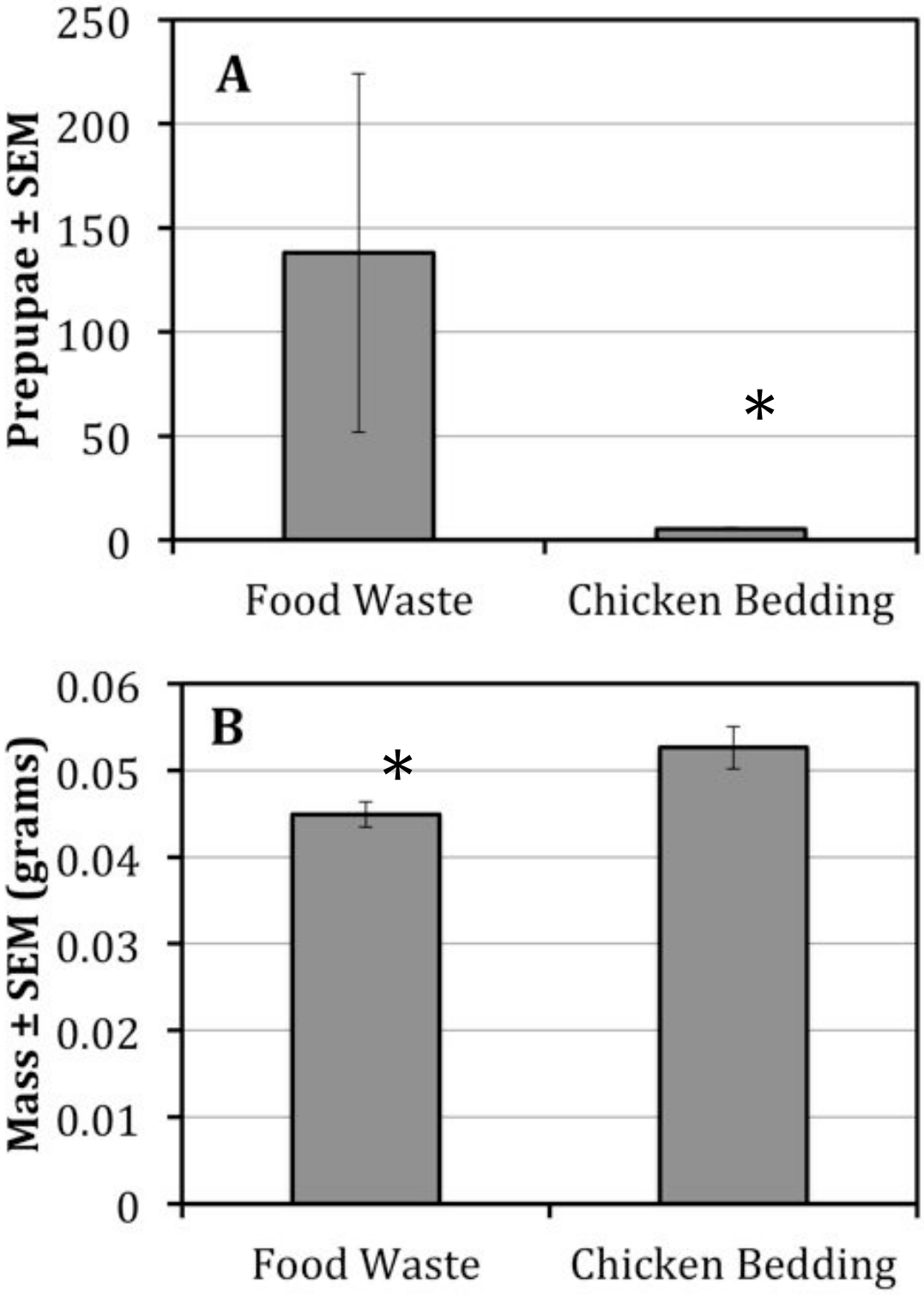

Figure 3. Trial 1. *Ptecticus trivittatus* prepupae, (A) self-harvested, (B) mass.

Table 1. Nutrient analysis of digestate from Trail 1. Means are given for each element and the SEM is given below in parentheses.

	percent (%)							ppm					
	N	**P**	**K**	**S**	**Mg**	**Ca**	**Na**	**B**	**Zn**	**Mn**	**Fe**	**Cu**	**Al**
Food Waste	1.62 (0.10)	0.41 (0.03)	2.27 (0.20)	0.34 (0.03)	0.50 (0.01)	1.35 (0.07)	0.57 (0.23)	17.7 (2.0)	57.3 (4.3)	269.7 (15.2)	3662.3 (351.7)	23.7 (3.3)	2303.3 (265.3)
Chicken Bedding	1.37 (0.10)	0.83 (0.06)	1.50 (0.07)	0.33 (0.01)	0.48 (0.03)	7.96 (0.34)	0.15 (0.01)	18.0 (0.6)	305.7 (13.1)	442.3 (21.9)	4012.3 (330.2)	32.3 (0.7)	2310.3 (52.1)
p-value*	0.15	>0.01	0.02	0.77	0.64	>0.01	0.15	0.88	>0.01	>0.01	0.51	0.06	0.98

* two-tailed t-test

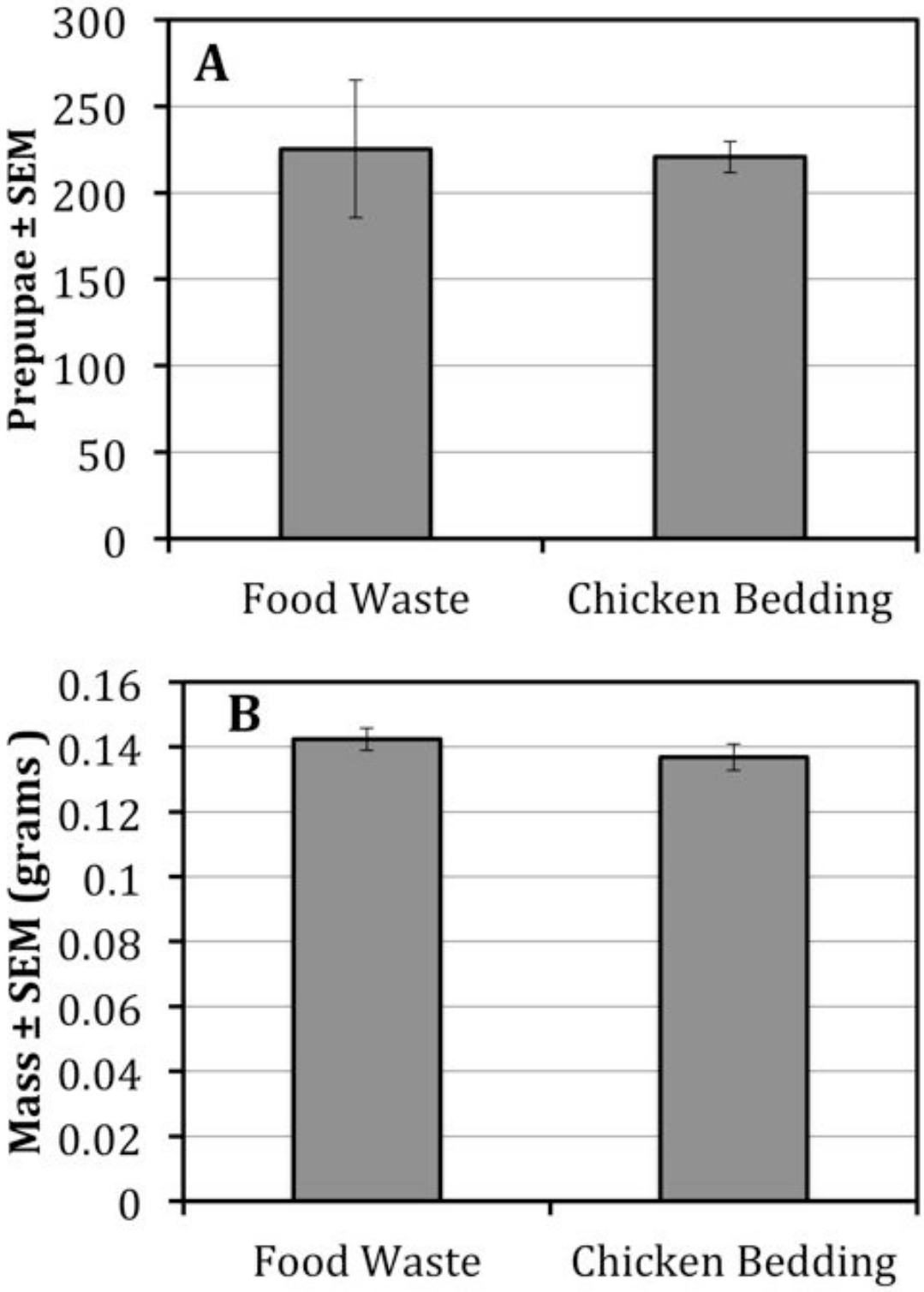

Figure 4. Trial 2. Black soldier fly (*Hermetia illucens*) prepupae (A) self-harvested, (B) mass.

Table 2. Nutrient analysis of digestate from Trial 2. Means are given for each element and the SEM is given below in parentheses.

	percent									mg/kg			percent	
	N	**P**	**K**	**S**	**Mg**	**Ca**	**Na**	**Fe**	**Al**	**Cu**	**Mn**	**Zn**	**Organic Matter**	**Organic C**
Food Waste	4.80 (0.13)	0.57 (0.03)	2.82 (0.11)	0.49 (0.02)	0.55 (0.02)	2.10 (0.08)	0.30 (0.01)	0.27 (0.01)	0.16 (0.01)	50.7 (0.9)	219.3 (13.3)	89.7 (2.9)	74.17 (3.15)	37.08 (1.57)
Chicken Bedding	4.80 (0.26)	1.01 (0.09)	3.27 (0.22)	0.58 (0.03)	0.64 (0.02)	3.35 (0.24)	0.34 (0.02)	0.38 (0.02)	0.20 (0.01)	58.7 (2.7)	340.0 (20.1)	173.3 (17.0)	74.18 (3.30)	37.09 (1.65)
p-value*	0.50	0.01	0.07	0.06	0.01	>0.01	0.04	>0.01	0.01	0.02	>0.01	>0.01	0.50	0.50

* one-tailed t-test

Table 3A. Nutreint Analysis of self-harvested black soldier fly prepupae. Means are given for each element and the SEM is given below in parentheses.

	percent									mg/kg			percent	
	N	**P**	**K**	**S**	**Mg**	**Ca**	**Na**	**Fe**	**Al**	**Cu**	**Mn**	**Zn**	**Organic Matter**	**Organic C**
Food Waste	5.36 (0.13)	0.57 (0.04)	0.80 (0.02)	0.31 (0.01)	0.31 (0.02)	6.33 (0.16)	0.07 (0.00)	0.02 (0.00)	0.01 (0.00)	9.67 (0.67)	773.3 (32.8)	60.3 (6.3)	80.58 (0.47)	40.29 (0.24)
Chicken Bedding	5.33 (0.10)	0.55 (0.01)	0.81 (0.01)	0.31 (0.00)	0.30 (0.01)	6.59 (0.24)	0.07 (0.00)	0.02 (0.00)	0.01 (0.00)	9.0 (0.0)	824.7 (32.1)	61.3 (1.5)	80.16 (0.14)	40.08 (0.07)
p-value*	0.85	0.59	0.61	0.80	0.67	0.40	1.00	0.37	0.37	0.37	0.33	0.89	0.44	0.43

*two-tailed t-test

Table 3B. Percent of nutrients black soldier fly sequestered from the feedstock. Means are given for each element and the SEM is given below in parentheses.

	N	**P**	**K**	**S**	**Mg**	**Ca**	**Na**	**Fe**	**Al**	**Cu**	**Mn**	**Zn**	**Organic Matter**
Food Waste	0.26	0.26	0.08	0.16	0.13	0.82	0.08	0.02	0.01	0.04	0.78	0.16	0.18
Chicken Bedding	0.25	0.18	0.06	0.13	0.16	0.68	0.06	0.02	0.02	0.05	0.70	0.11	0.16
p-value*	0.83	0.13	0.36	0.37	0.37	0.48	0.33	0.15	0.21	0.22	0.56	0.10	0.67

*two-tailed t-test

Table 4A. Nutrient Analysis of Leachate. Means are given for each element and the SEM is given below in parentheses.

	ppm (mg/L)												
	N	**P**	**K**	**S**	**Mg**	**Ca**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**	**pH**	**Electrical Conductivity**
Food Waste	630.0 (132.3)	96.37 (9.72)	1236.7 (497.1)	160.0 (23.0)	86.7 (13.7)	159.0 (12.5)	697.3 (76.9)	25.48 (4.91)	0.22 (0.03)	1.62 (0.31)	0.42 (0.06)	7.70 (0.10)	4.08 (0.44)
Chicken Bedding	473.3 (59.3)	88.10 (4.19)	1691.3 (49.5)	161.0 (10.5)	65.7 (5.5)	120.7 (9.9)	624.3 (29.8)	14.33 (1.67)	0.17 (0.02)	1.02 (0.11)	0.43 (0.07)	7.77 (0.03)	3.84 (0.08)
p-value*	0.34	0.48	0.41	0.97	0.23	0.07	0.43	0.10	0.25	0.14	0.89	0.56	0.62

*two-tailed t-test

Table 4B. Percent nutrients that leached from the feedstock over the course of the 5-week experiment.

	N	**P**	**K**	**S**	**Mg**	**Ca**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
Food Waste	0.21	0.31	0.82	0.57	0.26	0.14	5.21	0.14	0.06	0.11	0.08
Chicken Bedding	0.18	0.24	1.11	0.55	0.28	0.10	4.26	0.13	0.08	0.07	0.07
p-value*	0.64	0.28	0.44	0.91	0.74	0.19	0.40	0.90	0.45	0.21	0.56

*two-tailed t-test

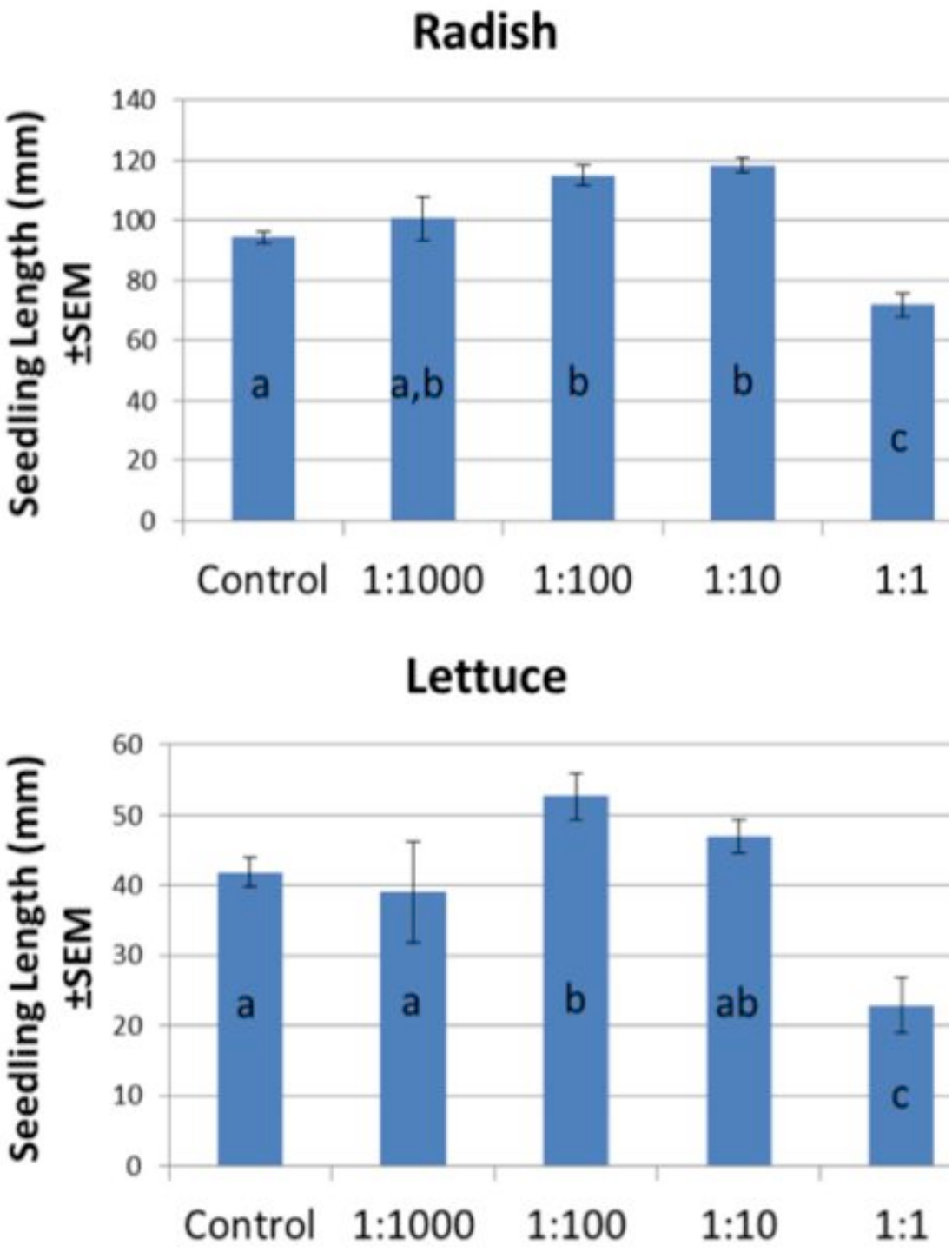

Figure 5. Germination study using aqueous digestate extracts from food waste composted by black soldier fly larvae.

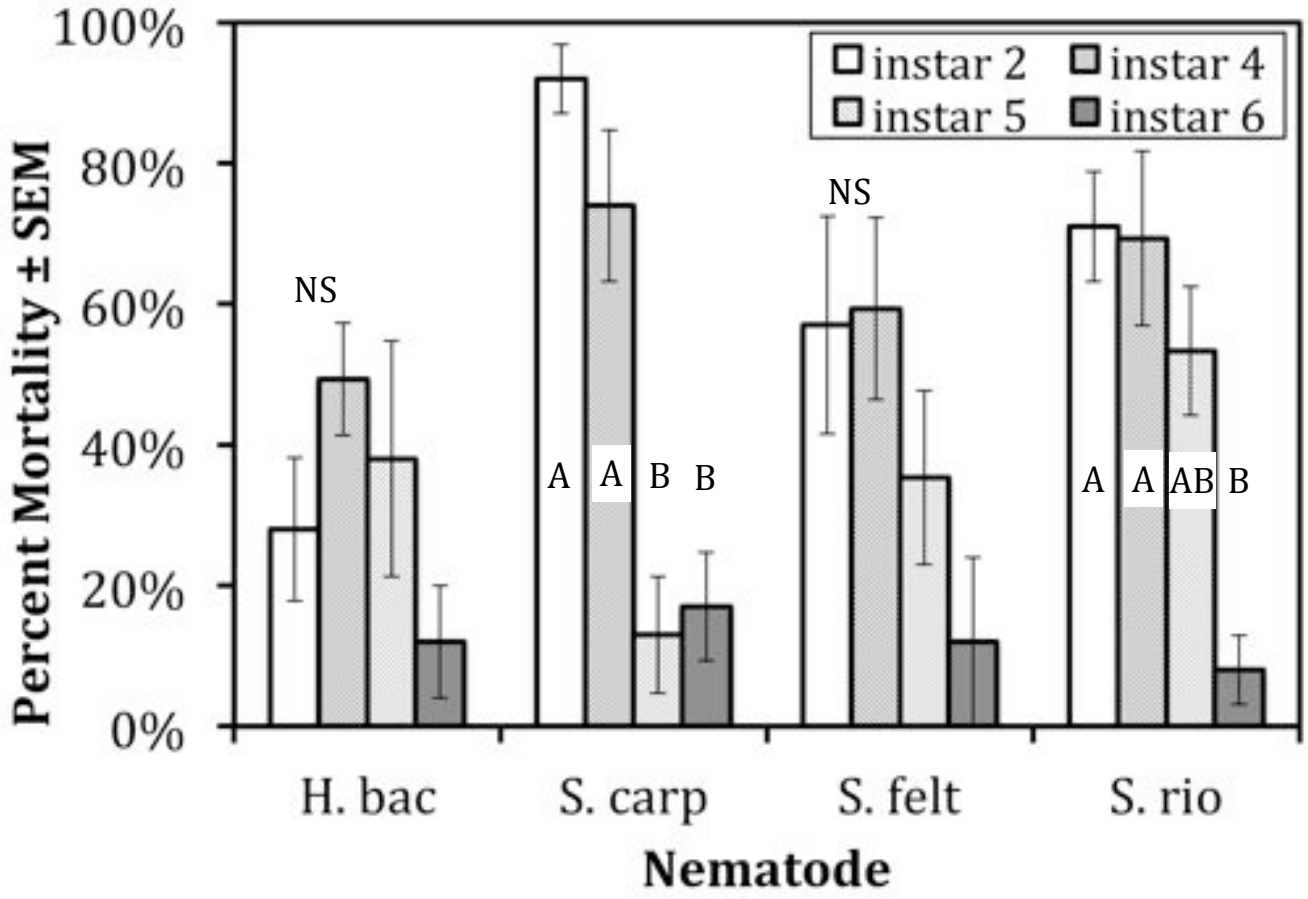

Figure 6. Percent corrected mortality of black soldier fly larvae. 100 juveniles applied per host at 20°C. Mortality for the second instar is from day 7 since there was high mortality on day 8 in the control. Galleria mortality was >95% for all nematode species and 24 ± 10% for the control on day 5 (data not shown). Bars with different letters indicate statistical difference (ANOVA, Tukey HSD, $p < 0.05$). NS = no statistical difference.

Table 5. Adult nematodes recovered from four black soldier fly instars (n=25 per treatment combination). 100 juveniles applied per host at 20°C. None of the controls (no nematodes) were infected. Data not shown for Galleria.

Nematode	Host instar	Percent Infected ± SEM	Nematodes ± SEM
H. bacteriophora	2	0.0 ± 0.0 ns	0.0 ± 0.0ns
	4	8.0 ± 4.9	2.5 ± 0.4
	5	4.0 ± 4.0	4.0 ± 0.0
	6	8.0 ± 8.0	2.0 ± 0.7
S. carpocapsae	2	32.0 ± 4.9a	6.0 ± 0.7ns
	4	12.0 ± 4.9a	1.7 ± 0.4
	5	0.0 ± 0.0b	0.0 ± 0.0
	6	8.0 ± 4.9a	2.0 ± 0.0
S. feltiae	2	12.0 ± 4.9ns	1.0 ± 0.0ns
	4	4.0 ± 4.0	2.0 ± 0.0
	5	0.0 ± 0.0	0.0 ± 0.0
	6	12.0 ± 4.9	1.7 ± 0.2
S. riobrave	2	12.0 ± 8.0ns	5.0 ± 2.0ns
	4	4.0 ± 4.0	1.0 ± 0.0
	5	8.0 ± 4.9	1.0 ± 0.0
	6	0.0 ± 0.0	0.0 ± 0.0

* Kruskal-Wallis test, different letters indicate significant differences, ns= no significant difference.

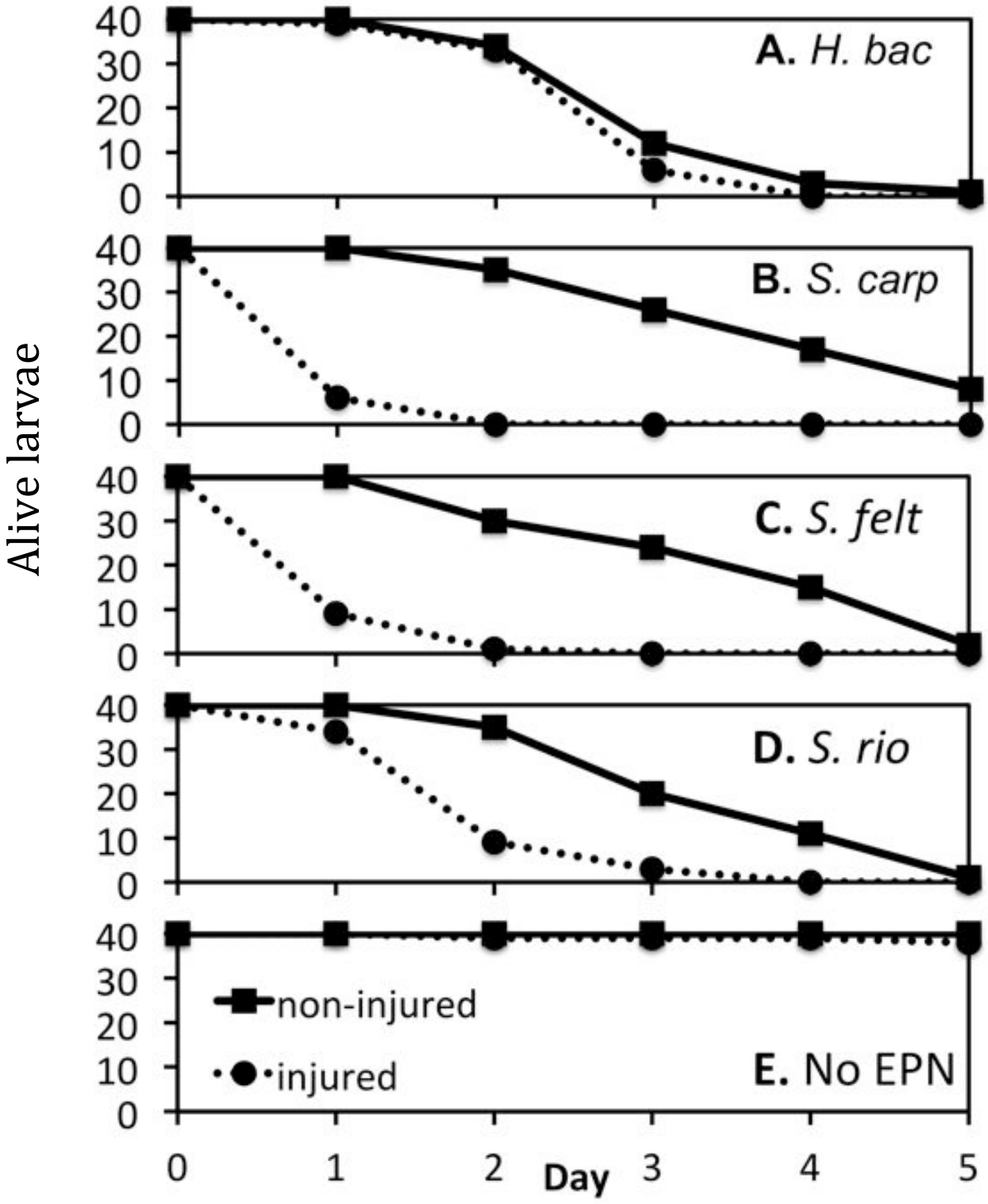

Figure 7. Survival curves for injured (dotted line) and non-injured (solid line) black soldier fly larvae for each nematode species: A) *H. bacteriophora*, B) *S. carpocapsae*, C) *S. feltiae*, D) *S. riobrave*, and E) control (no nematodes). 1000 juveniles applied per host at 25°C. All Galleria treated with nematodes were dead on day 2 and only 5 Galleria died in the control treatment (data not shown).

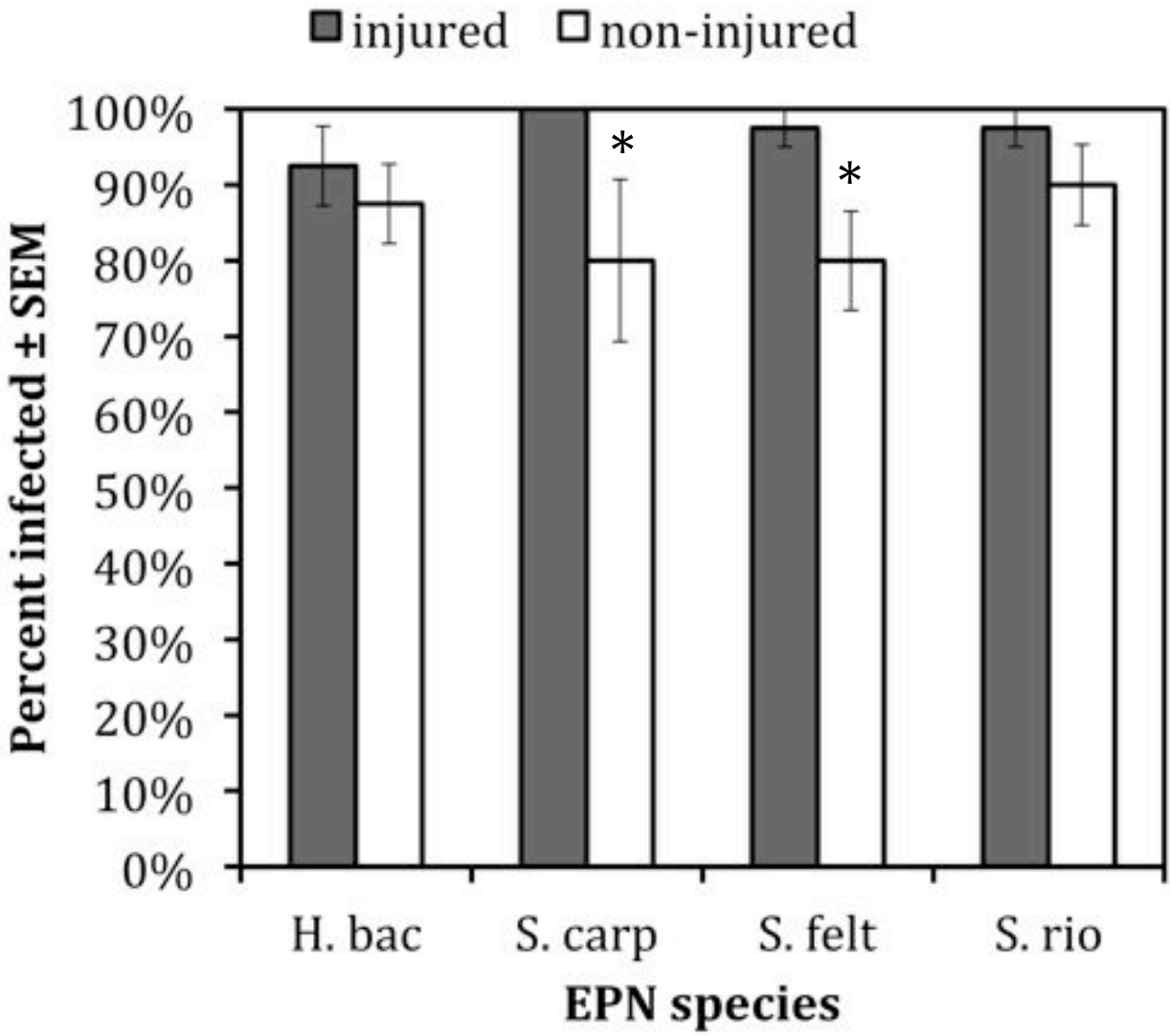

Figure 8. Percent black soldier fly larvae infected with entomopathogenic nematodes (n=40). Infective juveniles (1000) applied per host at 25°C. Asterisk (*) denotes significant difference ($p < 0.05$).

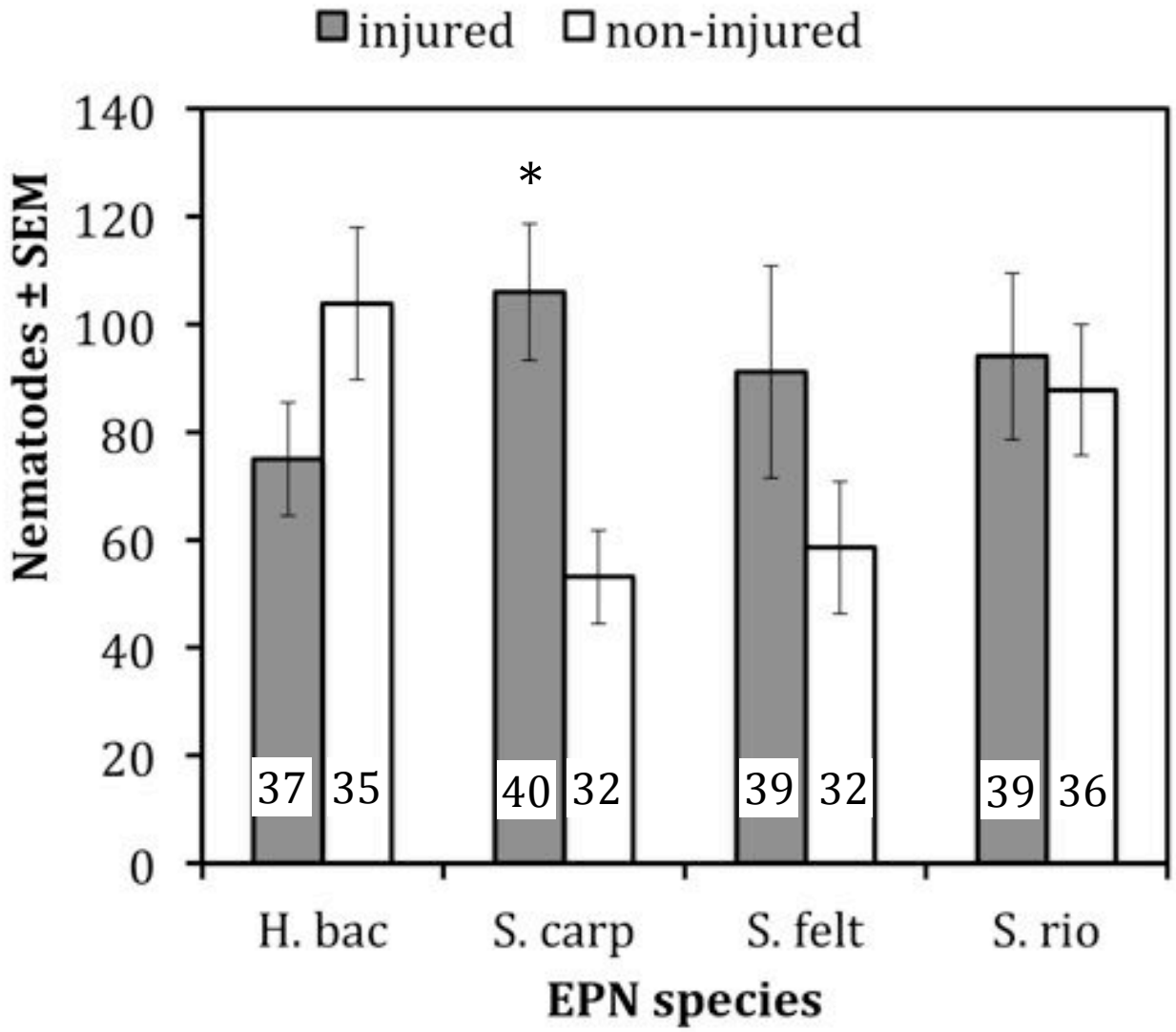

Figure 9. Mean number of entomopathogenic nematodes (first generation adults and juveniles) recovered from black soldier fly cadavers. 1000 juveniles applied per host at 25°C. Numbers within the bars indicate number of infected larvae (max = 40). Asterisk (*) denotes significant difference ($P < 0.05$).