Effect of Black Soldier Fly Larvae and Food Substrates on Weed Seed Emergence

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ABSTRACT. Black soldier fly larvae (*Hermetia illucens*; BSFL) composting is biotechnology used for organic waste management and an alternative to traditional composting. We designed a two-phase experiment to evaluate the effect of BSFL composting on the emergence of the following six weed species: barnyardgrass (*Echinochloa crus-galli*), common ragweed (*Ambrosia artemisiifolia*), giant foxtail (*Setaria faberi*), ivyleaf morningglory (*Ipomoea hederacea*), redroot pigweed (*Amaranthus retroflexus*), and velvetleaf (*Abutilon theophrasti*). The first experiment phase was in the laboratory (laboratory composting phase), which consisted of 100 seeds of each weed species subjected to five composting treatments [two controls (nontreated and standard Gainesville diet alone) and three types of substrates (standard Gainesville diet, vegetable waste, food waste) + BSFL]. Live pupa weighed 179 mg with the standard Gainesville diet + BSFL and 205 mg with the food waste diet + BSFL. Dry pupa weighed 68 mg and 70 mg, respectively. The BSFL in the vegetable waste + BSFL treatment did not pupate. During the second experiment phase, the composting treatments were placed in a greenhouse to evaluate weed emergence. Emergence in the nontreated control was 62% for barnyardgrass, 38% for common ragweed, 26% for giant foxtail, 66% for ivyleaf morningglory, 3% for redroot pigweed, and 69% for velvetleaf. Compared with the nontreated control, all treatments with BSFL reduced the emergence of each weed species to ≤1%, except for velvetleaf. This study suggests that BSFL composting may effectively reduce weed seed emergence of many weed species and could be a safe alternative to conventional composting processes to minimize weed pressure in compost. However, efficacy may vary by weed species and may be dependent on seed characteristics, such as an impermeable seedcoat.

Black soldier flies (BSF; *Hermetia illucens*) belong to the Stratiomyidae family and are native to the neotropics of North America (Kaya et al. 2021). They are holometabolous insects that develop through complete metamorphosis consisting of four morphologically distinct phases: eggs, larvae (with six instar stages; the sixth instar is called the prepupal stage), pupae, and adults (Diclaro II and Kaufman 2009). Their larvae develop on various organic materials. Black soldier fly larvae (BSFL) composting is a "conversion of organic refuse by saprophages" biotechnology (Diener and Zurbrügg 2008) used for organic waste management (da Silva and Hesselberg 2020; Diener et al. 2011; Liu et al. 2019; Miranda et al. 2021). Depending on the organic waste source, Diener et al. (2009) estimated that one BSFL can consume 61 to 178 mg of feed per day. Consequently, BSFL grown on seven types of substrates had variable growth rates (0.2–7.02 mg per day) and conversion efficiencies (0.14–0.46 g of weight gain per gram of dry matter ingested) (Veldkamp et al. 2021).

Applying BSFL to composting organic wastes is a practical conversion of organic refuse by saprophages biotechnology that can produce animal feed in the form of insect protein, biogas, biofuel, and frass fertilizer as value-added byproducts from the waste conversion, thus providing economic products through insect recycling (Beesigamukama et al. 2022; Fu et al. 2022; Liu et al. 2022). Moreover, BSFL composting is self-sustainable, cost-effective, and profitable. For instance, larval feeding aerates the compost (Mertenat et al. 2019), which can maintain aerobic conditions for other beneficial microorganisms, thus eliminating the need for forced aeration. Compared with traditional compost, BSFL compost can also facilitate stronger metabolically functional bacteria groups, most likely because of the gut microbiome of BSFL influencing the community (Jiang et al. 2019). These bacteria can enhance decomposition and eliminate the need for microbial inoculation. Moreover, farmers can safely use the frass fertilizer byproduct in their vegetable crop production (Quilliam et al. 2020; Terrell 2022), which can reduce costs (Beesigamukama et al. 2022). For example, Beesigamukama et al. (2022) reported 30% to 232% more income when BSFL compost was used on small farms compared with a commercial composted fertilizer (Beesigamukama et al. 2022).

Ideally, when found in composted fertilizer, pathogens should be inactive and weed seeds should be nonviable. To suppress pathogens and weed seed viability, conventional compost must reach at least 55 °C for minimums of 3 d for in-vessel or static aerated pile systems and 15 d for a windrow system, during which time the windrow must turned at least five times (US Environmental Protection Agency 1994). The average BSFL compost temperature only reaches 30.8 °C in the interior (Leist and Dusenbury 2016), but there is evidence that BSFL compost can suppress zoological pathogenic bacteria, such as *Salmonella* species (Lalander et al. 2013; Zhang et al. 2021) and *Staphylococcus aureus* (Gorrens et al. 2021). Thus, there must be other factors besides the temperature contributing to pathogen suppression in BSFL compost. One hypothesis is that the pathogens are ingested along with the substrate and killed in the BSFL gut because of strong acidity and enzymatic activity (Bonelli et al. 2019). Although there is evidence of pathogen suppression, there is no reported information regarding the effect of BSFL composting on weed seeds. Our goal was to determine whether BSFL composting on various substrates can reduce weed seed emergence.

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Materials and methods

A two-phase study (laboratory composting phase and greenhouse emergence phase) was conducted at Purdue University from 2021 to 2023 in West Lafayette, IN, USA. During the laboratory composting phase, the experimental unit consisted of transparent plastic containers (13 inches long × 9.1 inches wide × 5 inches high) containing 100 seeds each of six common weed species. On 4 Oct 2021, weed seeds were placed into each container alone (nontreated control) or followed by 190 g of one of the following three substrates: a standard Gainesville diet [30% alfalfa (*Medicago sativa*) meal, 50% wheat (*Triticum aestivum*) bran, 20% corn (*Zea mays*) meal] (Hogsette 1992), vegetable waste, and food waste. After the weed seeds and substrates were added to the containers, ∼2000 BSFL (PopWorms!, College Station, TX, USA) were placed in each container, except for the nontreated control, which contained weed seeds alone, and the standard Gainesville diet control, which only contained weed seeds plus the standard Gainesville diet (Table 1, Fig. 1).

Weed species. Weed species included barnyardgrass (*Echinochloa crus-galli*), common ragweed (*Ambrosia artemisiifolia*), giant foxtail (*Setaria faberi*), ivyleaf morningglory (*Ipomoea hederacea*), redroot pigweed (*Amaranthus retroflexus*), and velvetleaf (*Abutilon theophrasti*). These species were chosen because ragweed (*Ambrosia* spp.), pigweed (*Amaranthus* spp.), morningglory (*Ipomoea* spp.), and foxtail (*Setaria* spp.) species are ranked among the top five most common and troublesome weed species in US vegetable production systems (Van Wychen 2022), and velvetleaf and barnyardgrass are abundant in these systems as well. Redroot pigweed seeds were collected on 2 Sep 2021, at Meigs Horticulture Research Farm, Lafayette, IN, USA, and stored at room temperature until the initiation of the trial. All other weed seeds were purchased (Azlin Seed Service, Leland, MS, USA) and stored in a refrigerator or freezer. Common ragweed seeds were subjected to a priming procedure, which consisted of placing seeds in a cheesecloth and suspending them in a refrigerated beaker of water, which was replaced several times per week for 1 month. After 1 month, the seeds were dried and refrigerated.

BSFL diet substrates. Water was added to the Gainesville diet + BSFL treatment to follow common practices used when rearing BSFL to prevent larvae desiccation and increase feeding (Hogsette 1992; Myers et al. 2008). Vegetable waste consisted of a mix of summer squash (*Cucurbita pepo*), bell pepper (*Capsicum annuum*), watermelon (*Citrullus lanatus*), and cucumber (*Cucumis sativus*) obtained from a local farm (Meyers' Produce and Plants, LLC, West Lafayette, IN, USA). Standing water from the vegetable waste was removed from the vegetable waste + BSFL treatment as needed. Food waste consisted of 20% spent coffee (*Coffea arabica*) grounds (Copper Moon Coffee, Lafayette, IN, USA) and 80% food scraps (Wiley Dining Court, Purdue University, West Lafayette, IN, USA) (weight/weight).

Substrates and BSFL were observed daily and feeding was maintained until larvae started pupating in the Gainesville diet + BSFL and food waste + BSFL treatments (29 Oct 2021), totaling 850 g of standard Gainesville diet, 2742 g of vegetable waste, and 1800 g of food waste. One experimental block was maintained in the Horticulture Building (temperature 23 °C ± 0.004 °C and relative humidity 46% ± 0.06%), and the other was maintained in Smith Hall (temperature 22.7 °C ± 0.02 °C and relative humidity 43.4% ± 0.21%). The experiment design was a randomized complete block that included four replicates (i.e., experimental units or containers) per experimental block (location) that totaled eight replicates.

Pupal weight. At 6 weeks after trial initiation, live pupae were counted and harvested from each container and weighed en mass. Only individuals that were completely black and had stopped feeding were considered to be in the pupal stage. Then, they were placed in craft bags and stored in a freezer before they were oven-dried at 38 °C for 2 d. Dried pupae were counted and weighed. The live and dry weights were then divided by the number of pupae harvested to obtain the average live and dry pupal weight per individual.

Weed seed emergence. Throughout the laboratory composting phase, some ivyleaf morningglory seeds germinated and rotted; therefore, these seedlings were counted and removed from the composting containers (Fig. 2). After harvesting the pupae, the plastic containers were placed in a −20 °C freezer for 2 weeks to kill any remaining BSFL or prepupae to prevent adult BSF from emerging during the greenhouse phase of the trial. To start the greenhouse emergence phase, BSFL containers were removed from the freezer and allowed to warm to room temperature on 8 Dec 2021. A base layer of potting media (Berger BM2 Seed Germination Mix; Hummert International, Earth City, MO, USA) was placed into the bottom of standard 2-inch-deep plastic flats with drainage holes. The entire contents of each BSFL composting container from the laboratory composting phase were evenly distributed across the surface of the base layer of potting media and covered with a thin layer of potting media. Each flat contained the contents of a single composting container from the laboratory composting phase of the experiment. The flats were placed in the greenhouse (temperature, 71.6 °F; 14-h photoperiod) and gently watered overhead. A 2-inch-deep tray without drainage holes was placed below each flat. The potting media and BSFL composts were kept moist after the initial overhead watering by filling the lower tray with water and allowing the water to wick into the media. Weed seed emergence was recorded by species 9, 19, 29, and 37 d later (Fig. 3). Successful emergence was defined as weeds with fully expanded and fully formed cotyledons. Immediately after emergence was recorded, weeds were hand-removed from the flat.

After 1 week without new weed emergence, flats were subjected to a cold stratification treatment by placing them in a cooler (40 °F) for 3 months. Cold stratification can be used to break primary or secondary dormancy of barnyardgrass, common ragweed, foxtail species, and pigweed species (Mohler et al. 2021). Afterward, the seed trays were moved back to the greenhouse (temperature, 73.4 °F; 14-h photoperiod) and irrigated overhead to maintain even moisture. Weed emergence was recorded 10, 20, 31, and 41 d later, and weeds were removed as previously described. After 1 week without new weed emergence, flats were subjected to a cold stratification treatment again by placing them in a cooler (40 °F) for 14 months. Afterward, the seed trays were moved back to the greenhouse (temperature, 76.2 °F; 14-h photoperiod) and

Table 1. **Description of black soldier fly larvae (BSFL) composting treatments used during a laboratory-based composting study conducted in West Lafayette, IN, USA in 2021.**

Composting treatment	Contents
Nontreated control	Weed seeds alone[i]
Standard Gainesville diet[ii] control	Weed seeds + standard Gainesville diet
Standard Gainesville diet + BSFL	Weed seeds + standard Gainesville diet + ~2000 BSFL
Vegetable waste[iii] + BSFL	Weed seeds + vegetable waste + ~2000 BSFL
Food waste[iv] + BSFL	Weed seeds + food waste + ~2000 BSFL

[i] One hundred seeds from the following six weed species: barnyardgrass, common ragweed, giant foxtail, ivyleaf morningglory, redroot pigweed, and velvetleaf.
[ii] Diet comprising 30% alfalfa meal, 50% wheat bran, 20% corn meal.
[iii] A mix of summer squash, bell pepper, watermelon, and cucumber.
[iv] Waste comprising 20% spent coffee grounds and 80% food scraps from a local dining court.

irrigated overhead to maintain even moisture. Weed emergence was recorded 13, 20, 30, and 41 d later, and weeds were removed as previously described. The study was concluded when no additional weed emergence occurred consecutively for 7 d .

Data analysis. Data were subjected to a statistical analysis using R software (RStudio®; PBC, Boston, MA, USA). Data were analyzed with *lm()*, *glmer()*, or *lmer()* functions and then subjected to an analysis of variance (ANOVA). The explanatory variable was the composting treatment (fixed effect). The block was included as a random effect only with the *glmr()* and *lmer()* functions. Response variables were average live and dry pupal weights and the sum of emerged seeds for each weed species. The nontreated and standard Gainesville diet control treatments were excluded from the average live and dry pupal weight analyses because these treatments did not contain BSFL. The vegetable waste + BSFL treatment was excluded from the average live and dry pupal weight analyses because the BSFL did not pupate during this treatment. Average live and dry pupal weight data were subjected to the *lm()* function.

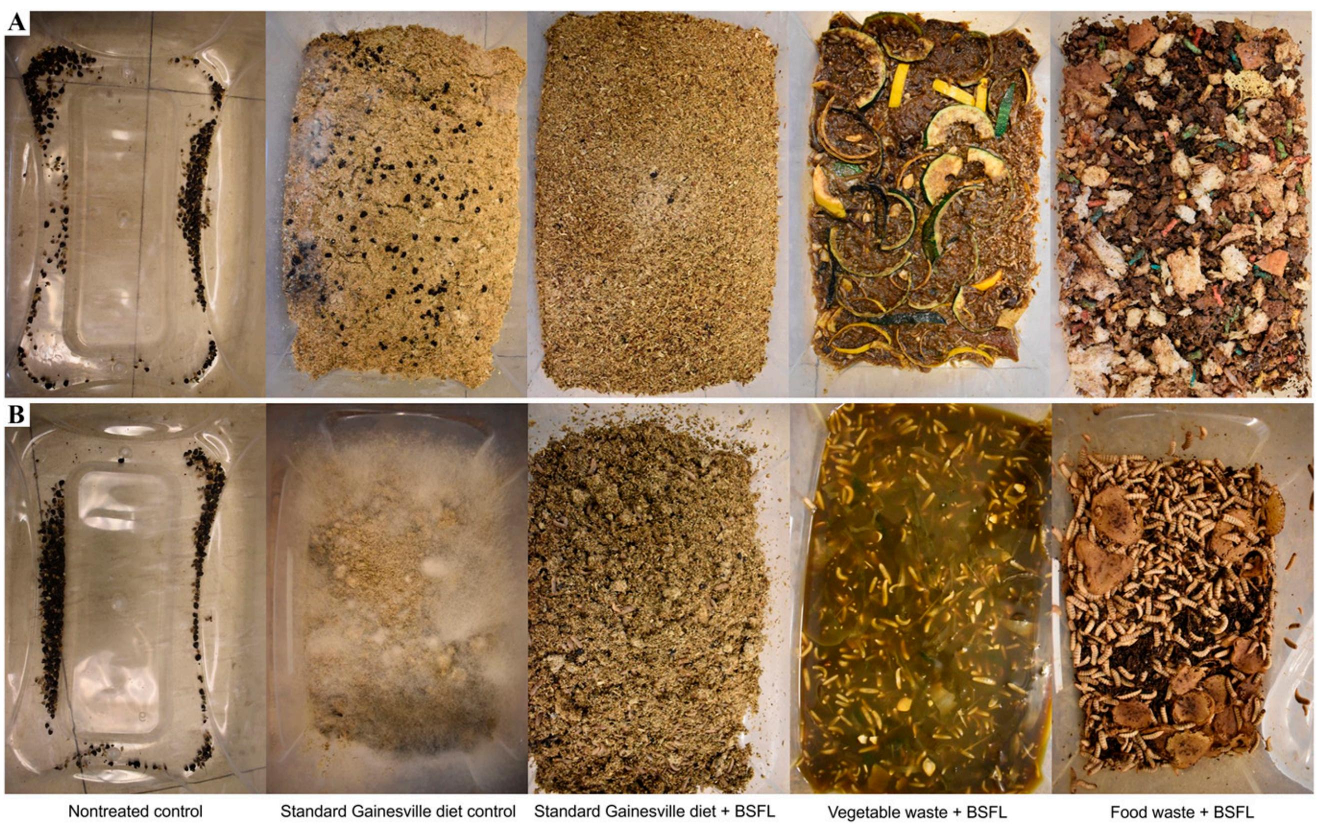

Fig. 1. Photos of composting treatments at 2 (A) and 3 (B) weeks after initiating laboratory-based black soldier fly larvae (BSFL) composting trials in West Lafayette, IN, USA, in 2021. One hundred seeds from six weed species (barnyardgrass, common ragweed, giant foxtail, ivyleaf morningglory, redroot pigweed, and velvetleaf) were placed into composting containers containing BSFL and either Gainesville diet (30% alfalfa meal, 50% wheat bran, 20% corn meal), vegetable waste (a mix of summer squash, bell pepper, watermelon, and cucumber), or food waste (20% spent coffee grounds and 80% food scraps from a local dining court). The nontreated control treatment did not contain a substrate or BSFL. With the standard Gainesville diet control treatment, weed seeds were placed into a composting bin containing Gainesville diet but no BSFL.

Fig. 2. Photo of ivyleaf morningglory seeds germinating in a composting treatment containing standard Gainesville diet (30% alfalfa meal, 50% wheat bran, 20% corn meal) plus black soldier fly larvae (BSFL) at 3 weeks after initiating a laboratory-based BSLF composting study in West Lafayette, IN, USA, in 2021.

Velvetleaf emergence data were subjected to the *glmer()* function with a Poisson family because the Poisson distribution is commonly used to model count variables (Inouye et al. 2017). Emergence data for all weed species except velvetleaf were first subjected to a zero-inflated model because the count data exhibited an excess of zeros. However, because of a lack of convergence, data transformation was performed. Emergence data for all weed species except velvetleaf were arcsine-transformed to normalize the data and then subjected to the *lmer()* function. Then, original velvetleaf emergence data and transformed emergence data for all the other weed species were subjected to a Tukey's honest significant difference (HSD) test to determine pairwise differences among treatments. To facilitate the interpretation of results, original emergence data are reported as the percent emergence in the Results and Discussion section.

Results and discussion

Pupal weight. The BSFL in the vegetable waste + BSFL treatment did not pupate. No live or dry pupal weight differences existed between the standard Gainesville diet + BSFL and the food waste + BSFL treatments (Table 2). The average live and dry pupal weights for the standard Gainesville diet + BSFL treatment pooled across both blocks were 179 and 68 mg, respectively. The average live and dry pupal weights for the food waste + BSFL treatments pooled across both blocks were 205 mg and 70 mg, respectively.

The development of BSFL varies based on the food source, and it typically takes longer when BSFL are fed fruits and vegetables compared with formulated feeds (Seyedalmoosavi et al. 2022). During previous studies, BSFL fed with fruits and vegetables pupated at 37 d (Jucker et al. 2017) and 42 d (Nguyen et al. 2013), and BSFL fed only with vegetables pupated at 48 d (Jucker et al. 2017) according to previous reports. This may explain why we observed prepupae and pupae in our Gainesville diet + BSFL and food waste + BSFL treatments, but not in the vegetable waste + BSFL treatment. Similar to our results, Nguyen et al. (2013) reported that BSFL pupated earlier when fed poultry feed (control treatment) and kitchen waste (restaurant leftovers; 32 d and 33 d, respectively) than when fed with fruit and vegetable waste (42 d). The authors credited the delayed maturation to the lower protein, calorie, fat, and carbohydrate contents in the fruit and vegetable waste (Nguyen et al. 2013). Moreover, Lalander et al. (2020) demonstrated that substrates with a high water content reduced larvae survival. Fruit and vegetable waste has a high water content (Edwiges et al. 2018; Wang et al. 2014) (Fig. 1B), which was observed with the vegetable waste + BSFL treatment. The substrate treatments consisting of the Gainesville diet and food waste resulted in mature pupae with similar sizes. This finding supports the results of previous studies that documented similar development sizes and performance when BSFL were reared on diets with a higher nutritional content (Georgescu et al. 2020; Nguyen et al. 2013), which were likely attributable to an increased protein content. In practice, if vegetable waste is

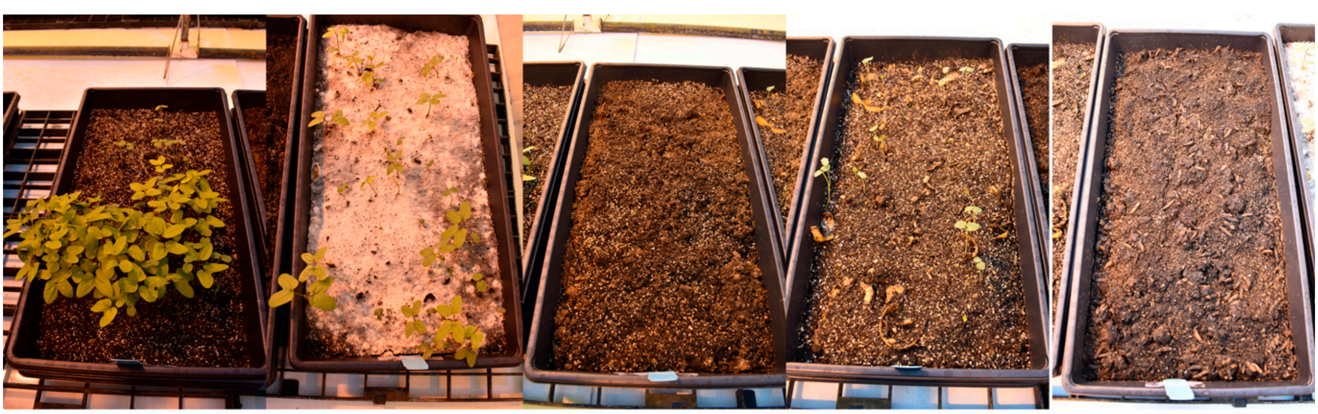

Fig. 3. Emerged weeds at 9 d after placing black soldier fly larvae (BSFL) compost contents into a greenhouse in West Lafayette, IN, USA, in 2021. The greenhouse emergence phase was the second phase during a two-phase experiment that evaluated the effect of BSLF composting and food substrates on the emergence of six weed species. During the first phase, one hundred seeds from six weed species (barnyardgrass, common ragweed, giant foxtail, ivyleaf morningglory, redroot pigweed, and velvetleaf) were placed into composting containers containing BSFL and Gainesville diet (30% alfalfa meal, 50% wheat bran, 20% corn meal), vegetable waste (a mix of summer squash, bell pepper, watermelon, and cucumber), or food waste (20% spent coffee grounds and 80% food scraps from a local dining court). The nontreated control treatment did not contain a substrate or BSFL. With the standard Gainesville diet control treatment, weed seeds were placed into a composting bin containing Gainesville diet but no BSFL. In this figure, weeds that emerged with the nontreated and standard Gainesville diet control treatments are mainly ivyleaf morningglory; weeds that emerged with the vegetable waste + BSFL treatment are mainly velvetleaf.

Table 2. Live and dry pupal weight from black soldier fly pupae harvested 6 weeks after initiating a laboratory-based black soldier fly larvae (BSFL) composting experiment in West Lafayette, IN, USA, in 2021.

	Pupal wt	
	Live	Dry[i]
Composting treatment	mg[ii] per pupa	
Standard Gainesville diet[iii] + BSFL	179 ± 19	68 ± 7
Food waste[iv] + BSFL	205 ± 13	70 ± 2
P value[v]	0.275	0.794
Model information	$F_{1,14} = 1.291$	$F_{1,14} = 0.071$

[i] Dry weight was recorded after oven-drying at 38 °C for 2 d.
[ii] 1 mg = 3.5274×10^{-5} oz.
[iii] Diet comprising 30% alfalfa meal, 50% wheat bran, and 20% corn meal.
[iv] Waste comprising 20% spent coffee grounds and 80% food scraps from a local dining court.
[v] $P > 0.05$ indicates that there are no statistical differences between composting treatments for each column.

used as a substrate, then more protein should be added in the form of another waste product to remove excess moisture and maximize BSFL development.

Weed seed emergence in the absence of BSFL. Total emergence rates for the nontreated control were 62% for barnyardgrass, 38% for common ragweed, 26% for giant foxtail, 66% for ivyleaf morningglory, 3% for redroot pigweed, and 69% for velvetleaf. Emergence rates with the standard Gainesville diet control treatment were 19% for barnyardgrass, 20% for common ragweed, 4% for giant foxtail, 17% for ivyleaf morningglory, 0% for redroot pigweed, and 36% for velvetleaf. Maheswari and Arthanari (2017) applied corn flour to 20-cm-diameter pots containing weed and crop seeds and reported that monkeybush (*Abutilon indicum*) germination was reduced from 92% with the nontreated control to 80% and 6% with pots receiving 10 to 40 g of corn flour, respectively. Similarly, slender amaranth (*Amaranthus viridis*) germination was reduced from 98% with the nontreated control to 90% and 5% with pots receiving 10 to 40 g of corn flour, respectively. Yu and Morishita (2014) reported that corn gluten meal reduced weed seedling emergence of numerous weeds, including redroot pigweed, green foxtail (*Setaria viridis*), and barnyardgrass. The documented ability of corn flour and corn gluten meal to reduce weed seed germination could explain why the standard Gainesville diet control treatment, which has 20% corn meal, reduced weed seed emergence.

Weed seed emergence in the presence of BSFL. Composting treatments with BSFL greatly reduced the emergence of most weeds to ≤1%, except for velvetleaf, which had 54% emergence with the vegetable waste + BSFL, 29% emergence with the standard Gainesville diet + BSFL, and 17% emergence with the food waste + BSFL treatments. Redroot pigweed had 0% emergence in all the composting treatments; however, the nontreated control had only 3% emergence, which we attributed to seed dormancy and insufficient temperature. Pigweed species seeds produced at the end of the season are usually more dormant than those produced first on a plant (Mohler et al. 2021), which is most likely the case because we collected these seeds in September. Additionally, pigweed species seed germination is stimulated by soil temperatures of 86 to 104 °F (Mohler et al. 2021). Although pigweed seeds are capable of germinating at lower soil temperatures as they age, the mean temperature of 71.6 to 76.2 °F observed during the greenhouse phase of the study was likely insufficient. In addition, an average of 24% ivyleaf morningglories emerged and rotted throughout the composting process during the laboratory composting phase with the standard Gainesville diet + BSFL treatment only (data not shown) (Fig. 2), contributing to the reduction of ivyleaf morningglory seed emergence in the greenhouse emergence phase.

We believe that the main factor contributing to reduced weed seed emergence in all of our substrates + BSFL treatments could be related to the moisture content during the composting process (vegetable waste + BSFL) (Fig. 1B). When water was added to a windrow composting process, seed viability was lost faster than when no water was added (Eghball and Lesoing 2000). Water was added to the Gainesville diet + BSFL treatment to prevent desiccation of the larvae, thus keeping the compost moist. Wang et al. (2014) reported water contents of 92% for fruit and vegetable waste and 78% for kitchen waste. Although we did not measure the water contents in our substrates, it is likely that we experienced similar levels. An additional possible factor contributing to reduced weed seed emergence in all of our substrate + BSFL treatments was ammonia generation during the composting process. BSFL composting generates ammonia (Parodi et al. 2020), which can decrease germination (Haden et al. 2011).

Despite the BSFL composting reducing weed seed emergence, some seeds still emerged (Figs. 3 and 4). The internal temperature of the BSFL compost is most likely the primary reason why weed seed emergence was not completely reduced. Although we did not measure the internal temperature of the BSFL compost, we believe that BSFL composting does not reach sufficient temperatures to completely control weed seeds through this mechanism alone because the maximum internal temperature that has been reported for BSFL composting is 30.8 °C (Leist and Dusenbury 2016). Grundy et al. (1998) reported that exposing a compost containing seeds of rosebay willowherb (*Chamaenerion angustifolium*), pineappleweed (*Matricaria discoidea*), annual meadowgrass (*Poa annua*), black nightshade (*Solanum nigrum*), spiney sowthistle (*Sonchus asper*), common chickweed (*Stellaria media*), white clover (*Trifolium repens*), and common field-speedwell (*Veronica persica*) to 45 °C for 3 d reduced germination and viability of most weed species, and that 55 °C for 3 d reduced viability and germination of all species. Typically, it is understood that optimum temperatures (55 to 70 °C) and moisture are both needed to kill seeds during the composting processes (Tompkins et al. 1998). However, Eghball and Lesoing (2000) stated that moist conditions reduced weed seed viability much more than the high temperature. Therefore, we surmised that excess moisture was the leading driver of reduced weed seed emergence during our study.

Velvetleaf emergence was relatively high compared with all other weed species evaluated and likely directly related to physical seed characteristics. The

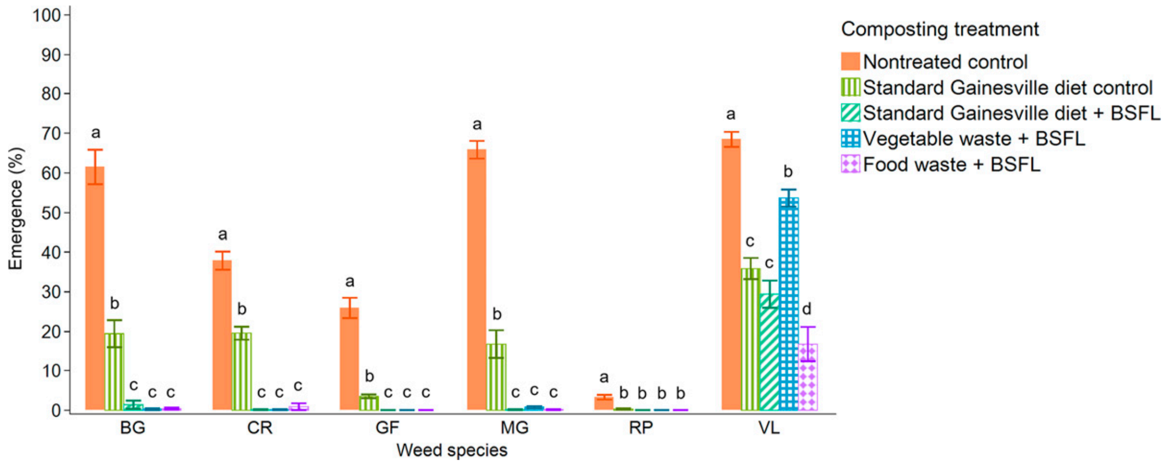

Fig. 4. Percent emergence of weed species calculated as the aggregate of emerged weeds before and after two different cold stratification events in West Lafayette, IN, USA, from 2021 to 2023. Calculations were performed based on 100 seeds each of six species [barnyardgrass (BG), common ragweed (CR), giant foxtail (GF), ivyleaf morningglory (MG), redroot pigweed (RP), and velvetleaf (VL)] originally placed into composting containers containing black soldier fly larvae (BSFL) and Gainesville diet (30% alfalfa meal, 50% wheat bran, 20% corn meal), vegetable waste (a mix of summer squash, bell pepper, watermelon, and cucumber), or food waste (20% spent coffee grounds and 80% food scraps from a local dining court) in West Lafayette, IN, USA, in 2021. The nontreated control treatment did not contain a substrate or BSFL. With the standard Gainesville diet control treatment, weed seeds were placed into a composting bin containing Gainesville diet but no BSFL. Compost contents were placed into flats and emerged weeds were counted and then hand-removed for 44 d, followed by cold stratification [40 °F (4.4 °C)] for 3 months, counted and then hand-removed for 48 d, followed by cold stratification for 14 months, and counted and then hand-removed for 48 d. *P* value and model information: BG ($P < 0.00l$; $F_{4,35} = 114.1$); CR ($P < 0.001$; $F_{4,35} = 192.2$); GF ($P < 0.001$; $F_{4,34} = 239.9$); MG ($P < 0.001$; $F_{4,34} = 233.8$); RP ($P < 0.001$; $F_{4,35} = 52.4$); and VL ($P < 0.001$; $\chi^2 = 333.6$, degrees of freedom = 4). Bars are the mean of eight replicates and *SE* bars are 1 *SE* from the mean. Different letters represent significant differences among means within a weed species based on Tukey's honest significant difference test ($P \leq 0.05$).

thick seedcoat of velvetleaf seeds is an impermeable physical barrier that may reduce water intake (Cardina and Sparrow 1997; Davis et al. 2008). The optimum and ceiling germination temperatures of velvetleaf seeds were estimated at 35 °C and 48 °C, respectively (Sadeghloo et al. 2013). Comparable to our results, Eghball and Lesoing (2000) reported that 14% of velvetleaf seeds were still viable after subjecting them to dairy manure composting windrows for 4 to 5 months because the temperature was below 55 °C.

The Indiana Department of Environmental Management (2021) recommends avoiding composting weed seeds. However, it is practically inevitable that these seeds will make their way into our compost, and there is still a need for the destruction of this plant material in a sustainable way. Thus, these results suggest that BSFL composting may be a sustainable and effective way to reduce weed seed emergence in compost, but that not all weed species may be controlled equally or to an acceptable level. Future research should investigate the mechanisms of BSFL composting that contribute to reduced weed seed emergence and how to integrate BSFL composting on farms to recycle organic wastes and improve weed management.

Conclusions

BSFL composting reduced weed seed emergence of the six weed species tested. Although we hypothesize that substrate water content and ammonia are contributing factors to the reduced weed seed emergence after BSFL composting, additional research is needed to test this hypothesis. The water content of the substrates used to feed BSFL likely contributed to the reduction of weed seed emergence, but an extremely high water content can negatively affect the development of the BSFL. Furthermore, velvetleaf, the species least impacted by the BSFL composting process, demonstrated greater emergence under the treatment with the most water content (vegetable waste). A direct effect of BSFL on the seeds, such as feeding damage, was not studied, but it is hypothesized to contribute to reduced emergence. Future experiments should be performed in which the seeds are retrieved from the substrates to determine BSFL feeding damage.

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