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Spent Coffee Grounds Applied as a Top-Dressing or Incorporated into the Soil Can Improve Plant Growth While Reducing Slug Herbivory

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Abstract: Between 8 and 15 million tons of spent coffee grounds (SCG) are produced as global waste each year. To reduce waste to landfill, SCG are proposed as a carbon and nutrient source for degraded soils. SCG contain caffeine and other toxins that inhibit plant growth. However, they also repel slugs and snails. We examined whether partial decomposition can neutralize SCG to promote plant growth while maintaining anti-herbivore properties. We aged SCG for <1 to 14 months and also produced SCG-derived Black Soldier Fly (*Hermetia illucens*) frass. The aged SCG and frass were applied, either incorporated into soil or as a 1 cm top-dressing, to pots with radish and tomato seedlings. SCG treatments were also examined for direct (repellent) and indirect (plant-mediated) effects on four slug species (*Arion ater*, *Deroceras laeve*, *Derocerus reticulatum* and *Lehmannia marginata*). SCG of ≤7 months inhibited plant growth and development and reduced herbivory when incorporated into soil, whereas 14-month-old SCG promoted growth but had no effect on herbivory. When applied as a top-dressing, SCG at 7 months promoted growth and reduced herbivory through repellent and host quality effects—including possible systemic effects. Our results indicate that the benefits of SCG for radish and tomato growth and to reduce slug herbivory can be achieved simultaneously by applying partially decomposed SCG (aged for up to 8 months) as a top-dressing.

Keywords: black soldier fly; circular agriculture; frass; integrated pest management; repellence; systemic defenses

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1. Introduction

In recent decades, considerable attention has been focused on promoting circular economies and, in particular circular agricultural systems, to help meet the UN Sustainable Development Goals [1]. Circular agriculture converts agricultural wastes into useful secondary products to increase resource-use efficiency [2]. For example, several agricultural by-products, including non-harvested organic biomass, manures, and residues from primary processing can be recycled to produce fertilizers, soil amendments and composts that enhance crop or livestock production [2–4]. Organic urban wastes from supermarkets, restaurants or domiciles are also increasingly used in fertilizer and compost production where efficient waste collection permits [5].

Coffee is produced in over 80 countries with a global value of USD 19 billion [6]; however, over 80% of that production occurs in just 10 countries (Brazil, Colombia, Ethiopia, Guatemala, Honduras, India, Indonesia, Mexico, Vietnam and Uganda) [7]. Nevertheless, coffee is consumed as a beverage worldwide with the largest per capita consumption

in Europe and the USA [7]. Wastes from the coffee industry include fruit wastes (pulp, husks and skins) that remain close to production sites, and spent coffee grounds (SCG) that are generated at points of processing for instant coffees and at points of sale—such as coffee shops and restaurants—for filtered coffees [7,8]. Estimates of the annual global production of SCG vary from 8 to 15 million tons, with each ton of coffee beans expected to generate about 650 kg of dry SCG [7,9]. Spent coffee grounds have a high organic content (40–50% carbon) with 1 to 2.5% nitrogen. They are high in hemicelluloses (ca. 38%) and celluloses (9%) and contain a range of polysaccharides, phenolics, and tannins, as well as caffeine (1–2% caffeine) [10]. Most of the world's SCG go to landfill; however, SCG can become an environmental contaminant when caffeine and other components leach into soils and ground waters [8]. Caffeine is a frequent contaminant of water with a range of adverse physiological effects on aquatic vertebrates and invertebrates—even at low concentrations [11].

Since 2010 the number of research publications related to SCG valorization has greatly increased [12,13]. SCG can have several uses including the conversion of oil components to biofuels and conversion of hemicellulose and cellulose components to bioethanol and bio-composite materials. SCG can also be utilized for the synthesis of advanced materials for environmental sustainability, can be pelleted as a dry fuel for burning, or converted to biochar [7,8,13–16]. Because of their relatively high carbon content, SCG have also attracted attention as a possible soil amendment, particularly for carbon-depleted soils [14,17]. Furthermore, at local scales, many large coffee franchises currently promote the use of SCG in urban gardening [12]. Despite these trends there is still little evidence that SCG improve plant growth; indeed, several studies that applied fresh SCG to soils have reported negative effects on plant growth and development. For example, SCG mixed with topsoil have been shown to reduce the growth of broccoli, cress, leek, lettuce, radish, sunflower, and viola, among other plant species [14,17–21].

Because of their growth-inhibiting effects, incorporating SCG with soil has been proposed as a possible method to control weeds in field crops [20–23]. To avoid direct negative effects on the crop while still suppressing weeds, non-composted SCG can be applied as a mulch (top-dressing) after crop-seedling emergence. For example, SCG mulch successfully reduced weed abundance in fields of wheat and soya in Japan with only minor effects on yields in some years [22]. The mechanisms underlying weed control by SCG mulches have not been elucidated; however, these probably include some level of phytotoxicity, as well as possible changes to the physical properties of the soil surface that reduce weed seedling survival. Finally, because caffeine solutions are effective in reducing slug damage to greenhouse crops [24,25], SCG are sometimes recommended for the control of slugs and snails [26,27]. However, there is still little evidence to support the use of SCG to reduce snail and slug herbivory.

In contrast to the use of fresh SCG, a number of studies that applied composted or otherwise partially decomposed SCG to soils have reported improved plant growth that reduces the need for inorganic fertilizers [28–30]. Composing SCG neutralizes the pH, reduces total phenolic and tannin concentrations and increases concentrations of gallic acid while maintaining nitrogen and mineral contents [30–33]. Similarly, during vermicomposing, earthworms can neutralize the pH, increase nitrogen content, and reduce the phenolic and caffeine contents of SCG [27,28]. Furthermore, studies have shown that Black Soldier Fly (BSF: *Hermetia illucens*) larvae can grow and develop on fresh SCG [34,35]. BSF are capable of converting a range of organic wastes to high-protein (40–70% dry weight) insect biomass suitable for animal feeds [36,37] while also producing a rich frass used as fertilizer [38–40].

Whereas SCG-derived composts and bioconversion products can improve plant growth [28,30], the effects of composting SCG on some of their other possible benefits have not been assessed. We hypothesized that the benefits of SCG can be achieved through partial decomposition that promotes plant growth while maintaining some anti-herbivore effects on slugs. Composting might, for example, reduce the anti-herbivore properties

of SCG by reducing phenolic and caffeine concentrations. However, residual toxins in composted or bio-converted SCG might be sufficient to repel herbivores without detriment to developing crop plants, particularly if applied as a mulch. Therefore, the main objective of this study was to compare the effects of SCG of different ages on plant growth and development and determine whether growth enhancement could be achieved while maintaining possible SCG anti-herbivore properties. We aged batches of SCG for 7 to 14 months, and compared the resulting, partially decomposed SCG against relatively fresh SCG for their effects on radish (*Raphanus raphanistrum* ssp. *sativus*) and tomato (*Solanum lycopersicum*) seedlings. We also included SCG-derived BSF frass in our experiments; however, to assess the possibilities for accumulating SCG-derived BSF frass at industrial scales we examined BSF growth and development on SCG. Finally, we examined whether fresh and aged SCG directly or indirectly reduced slug herbivory by testing for repellence effects and possible plant-mediated effects in a series of bioassays with four slug species. To our knowledge, this is the first study to assess the effects of SCG and derived substrates on slug herbivory.

2. Methods

2.1. Survival and Growth of BSF on SCG

2.1.1. Soldier Fly Colony

BSF eggs were obtained from a colony maintained by EcoLaVerna in Kildinan, Ireland, which was initiated two years earlier using ca. 1000 individuals originating from Hexafly, Meath, Ireland. The colony was kept in a heated chamber (275 × 250 × 210 cm (Length × Width × Height)) at 25–28 °C, 60% relative humidity, with augmented lighting. To obtain eggs, trays (20 × 20 × 7 cm (Length × Width × Height)) of moistened (40% *w*/*v*) wheat bran (wholewheat [55%], wheat bran [5%]) were exposed to mated females in the chamber. Each tray had bound strips of corrugated cardboard (22 cm × 2.5 cm (Length × Width)) laid across the top (ca. 1 to 2 cm above the feeding substrate). The females laid their eggs into the corrugated cardboard. The larvae were allowed to hatch and drop down to the feeding substrate. After 3 to 5 days the larvae were transferred to larger plastic feeding boxes (42 × 27 × 35 cm (Length × Width × Height)) supplied with wheat bran (to maintain the colony).

BSF growth experiments were conducted in a second chamber (275 × 80 × 250 cm (Length × Width × Height), 28 ± 1.5 °C, 40% RH). This second chamber was kept in darkness during the experiments. Larvae of the required ages were extracted from the stock colony using a soft paintbrush (early instars) or by sieving (later instars). The rearing boxes were provided *ad libitum* (5 cm deep) with SCG-based substrates (see below). These non-sealed feeding boxes (17.4 × 11.5 × 5 cm (Length × Width × Height) = 1000 mL) were placed inside open skirting trays (42 × 27 × 35 cm (Length × Width × Height)) to collect any escaped larvae, which were returned to the original boxes each day. We used a block design for all experiments with each block positioned at a different shelf height to avoid the effects of possible temperature and humidity gradients inside the chamber.

2.1.2. Effects of BSF Size on Survival and Growth on SCG

BSF larvae of five different size categories and ages were separated from the feeding boxes. These were: >3 dry mg (ca. 3 days old), 6 dry mg (ca. 7 days old), 18 dry mg (ca. 12 days old), 30 dry mg (15 days old) and 40 dry mg (15 days old). Larvae were separated to sizes using mechanical sieves based on body length and a subsample of larvae from the different size categories was dried in an oven and weighed. Fresh SCG were sterilized by emersion in boiling water and were subsequently drained, dried at room temperature and stored at −20 °C. At the start of the experiment 100 g of fresh (< 7 days) SCG (50% water) were placed in each of 28 plastic recipients. Thirty larvae of >3, 6 or 18 dry mg, and 15 larvae of 30 or 40 dry mg were added to the recipients. There were 6 replicates for the 3 smaller size categories and 5 for the larger categories. The SCG were moistened daily using a mister. After 12 days, the recipients were sampled by sieving the contents to

remove all larvae. The larvae were counted, dried in a forced draught oven at 60 °C for 7 days, and weighed.

2.1.3. Effects of SCG on Larval Growth and Development

Three substrates were used in the experiment: 100% fresh SCG (50% water); fresh SCG + bran (1:1; 50% water); and 100% wheat bran (50% water). The mixed substrate was thoroughly homogenized. Portions of each substrate (50 g) were placed in plastic recipients inside larger boxes as described above, and each recipient infested with 30 BSF larvae (8- to 10-days-old) using a fine paintbrush. Larvae were allowed to feed and develop for 12 days, after which the larvae were separated from the remaining substrate. The larvae were counted, dried in a forced draught oven at 60 °C for 7 days, and weighed.

2.2. Effects of SCG and BSF Frass of Plant Growth

2.2.1. Soil Amendments

We used a range of soil amendments in our experiments. The soil base consisted of clay-loam soil mixed with peat moss at a ratio of 2:1 (soil:peat moss). Coffee grounds were collected from coffee shops as SCG. The SCG were collected in staggered batches. Coffee filters and tea bags were extracted from the coffee shop waste and the SCG placed inside black plastic bags (20 kg). Because we wished to examine the effects of aging on SCG specifically, we did not add straw or other vegetation to the original SCG. The plastic bags were perforated on one side and placed as a single layer on the ground, outdoors. The SCG were allowed to age such that SCG of 8 months and 1 month were available at the same time for one group of experiments, and SCG aged for 14 months, 7 months and < 1 month were available at the same time for a second group (see below).

We also applied BSF frass collected after feeding by late instar larvae on moistened, fresh SCG. Because of poor larval growth and development on SCG (see below), we exposed the fresh SCG to high densities of late instar larvae (ca. 30 to 40 dry mg) in plastic boxes (42 × 27 × 35 cm (Length × Width × Height)). The SCG were watered daily until most of the substrate was converted to frass by the larvae (determined by the substrate appearance). After conversion, the frass and remaining residue was air dried and sieved to remove any remaining coffee powder.

The amendments were added to the soil base either by thoroughly mixing amendments into the soil at a 3:1 (soil to amendment) ratio or by placing a layer of amendment over the soil (i.e., mixed and layered, respectively). Where amendments were layered over the soil, the layer thickness was about 1 cm deep over the surface of the entire experimental recipient (i.e., tray, pot or arena).

2.2.2. Effects of Soil Amendments on Growth of Radish

Radish seeds (variety Sparkler [Unwins]) were placed on moistened filter paper in a dark chamber until the seeds germinated. The germinated seeds were placed in plastic pots (20 cm × 20 cm (Height × Diameter)) filled with soil or with the prepared substrate (mixed soil + amendments). Each seed was placed in a depression in the substrate (ca. 1.5 cm deep) and the depression covered with the corresponding substrate. Where amendments were added as layers, the seeds were place in depressions at 0.5 cm deep and were backfilled with the corresponding amendment layered to 1 cm above the soil. Layers were added at the time of seeding. Each pot had 5 seedlings spaced at >3 cm from each other and >2 cm from the edge of the pot. There were 8 pots for each experimental treatment ((4 amendments × 2 mixed/layered + soil control) × 8 = 72 pots).

Pots were randomized in an outdoor plot (temperatures 5 to 15 °C (average = 11 °C)). Each pot was covered with a sealed plastic tube (20 cm × 25 cm (Height × Diameter) to avoid herbivore damage and cool temperatures. The tubes were removed daily for 3 to 5 hours at about midday to avoid condensation and allow watering. The experiment was run for 30 days. At the time of evaluation, the number of surviving plants was counted and a single arbitrarily selected plant from each pot was sampled by gently pulling it

from the pot to keep the roots intact. The roots were rinsed under running water and the following parameters measured: plant height; number of cotyledons and true leaves; cotyledon surface area (based on the largest cotyledon); true leaf surface area (based on the largest leaf); greenness of true leaves (based on a standardized color chart); and the wet weight of the whole plant. Based on the number of cotyledons and true leaves, each plant was categorized according to developmental stage as 1= sprouting, 2 = cotyledons emerging, 3 = cotyledons fully expanded, 4 = first true leaf emerging, 5 = second true leaf emerging, 6 = first and second true leaves fully expanded, 7 = at least a third true leaf has emerged. After evaluating the plants, the pots were left exposed (i.e., without the sealed plastic tube) for 10 days. After 10 days, each plant was assessed for slug herbivory by noting the number of cotyledons and true leaves that were damaged.

2.2.3. Effects of Soil Amendments on Growth of Tomato

Tomato seeds (variety Alicante [Unwins]) were placed on moistened filter paper in a dark chamber until the seeds germinated. The germinated seeds were placed in plastic pots (7 cm × 7 cm (Height × Diameter)) filled with soil or with amended substrate. Each seed was placed in a depression in the substrate (ca. 1.5 cm deep) and the depression covered with the corresponding substrate, or—where amendments were layered—the seeds were place in depressions at 0.5 cm deep and were backfilled with the corresponding amendment layered to 1 cm above the soil. Layers were added at the time of seeding. Each pot had a single seedling placed at the center of the soil surface. The pots were placed in a heated greenhouse (20 °C) and arranged as a randomized block design with 5 blocks ((4 amendments × 2 mixed/layered + soil control) × 5 = 45 pots). After 20 days the tomato plants were destructively sampled by carefully pulling the plants from the soil, ensuring that the roots remained intact. Each plant was measured (plant height) and wet weighed. The number of leaves on each plant were counted and the plants assigned a development category (see Section 2.2.2).

2.3. Effects of SCG on Slug Herbivory

2.3.1. Slug Species

We conducted herbivory bioassays with four slug species: *Arion ater*, *Deroceras laeve*, *Deroceras reticulatum* and *Lehmannia marginata*. The slugs were collected from an Irish grassland meadow in March (*A. ater* and *D. reticulatum*) and September (*D. laeve* and *L. marginata*). Slugs were kept in plastic boxes (separated by species) and fed with lettuce (*Lactuca sativa*) until required for the bioassays. Corresponding bioassays were conducted in April (*A. ater* and *D. reticulatum*) and November (*D. laeve* and *L. marginata*) at 12 to 15 °C and >70% humidity. Slugs of similar wet weights were used during comparative bioassays and were starved for 24 hours before each bioassay.

2.3.2. Repellent Effects of SCG on Slug Herbivory

We examined the repellent effects of soil amendments on slug herbivory in a series of arena bioassays. These included no-choice, multi-choice and binary choice assays with *A. ater* and *D. reticulatum*. For the bioassays, we used 8-month SCG and 1-month SCG either mixed or layered on the soil, and unamended soil as a control (i.e., 5 treatments). All repellence bioassays were initiated at 10:00 am (daytime) and at 21:00 pm (nighttime) and evaluated after 8 (daytime) or 12 (nighttime) hours.

No-choice bioassays were conducted using plastic containers (16.5 × 10.5 × 4.0 cm (Length × Width × Height)), each filled to a height of 3.0 cm with a single soil treatment. The containers with each substrate type were replicated five times for the day and night experiments with each slug species randomly assigned to one of five blocks. Lettuce disks (area = 4.9 cm^2, r = 1.25 cm) were cut using a circular blade. A single disk was placed on the substrate surface in each container. A single slug of either species was placed on top of the soil at the opposite end from the leaf disk and the containers were sealed with plastic lids. At the end of the experiment the proportion of each leaf disk consumed was estimated.

For multichoice bioassays trial arenas were prepared by dividing plastic containers (16.5 × 10.5 × 4.0 cm (Length × Width × Height)) into five separate equal sections (3.3 × 10.5 × 4.0 cm). Each section was then filled with one of five soil types. The order of the soil treatments was randomized to avoid position biases in the bioassays. Lettuce disks (4.9 cm^2) were placed on each soil type in all containers (i.e., each container had five leaf disks). One slug of either species (randomized selection) was then placed on the container lid and sealed onto the container. The slugs were allowed to move and feed freely. There were six replicate containers per slug species. At the end of the experiment, the proportion of each leaf disk consumed was estimated.

In a series of binary choice bioassays, each amendment type was tested against the unamended soil for effects on slug herbivory. For each bioassay (day and night), arenas (16.5 × 10.5 × 4.0 cm (Length × Width × Height)) were divided into two equal compartments (8.25 × 10.5 × 4.0 cm per compartment) and filled with soil in one compartment and one of the four soil amendment types on the other side. Each combination was replicated six times per slug species. A single lettuce disk (4.9 cm^2) was placed in each compartment of the container (i.e., each container had two disks). The slugs of either species were placed in the middle of the containers and allowed to move and feed. At the end of the bioassays the proportion of each leaf disk consumed was estimated.

2.3.3. Effects of Plant Quality of Slug Herbivory

Three sets of bioassays were conducted to examine the effects of soil amendments on slug herbivory as determined by host-plant quality. The different sets of bioassays used different sets of treated soils and different slugs according to the availability of plant materials and slugs at the time of the bioassays. Experiments with *A. ater* and *D. reticulatum* were conducted in early spring, whereas experiments with *L. marginata* and *D. laeve* were conducted in late autumn.

In the first set of bioassays we used *A. ater and D. reticulatum* with radishes grown in unamended soil and in soils amended with 8- and 1-month-old SCG (mixed and layered). To prepare the plants, germinated radish seeds were planted in pots (20 × 20 cm; Height × Diameter), with 12 pots for each substrate type (5 × 12 = 60 pots). The pots were then placed inside a greenhouse during early spring (February) and allowed to grow for 31 days. After the 31 days, the number of surviving plants per pot, plant height, the number of leaves, and leaf greenness were recorded. We conducted no-choice and multichoice bioassays with these materials.

In a second set of multichoice bioassays we used *L. marginata* and *D. laeve* with radishes grown in unamended soil, and in soils amended with, 14-, 7- and <1-month-old SCG (mixed and layered). The plants used in the bioassays were those described in Section 2.2.2. In a further bioassay we used *D. laeve* with tomato plants grown in unamended soil, and soils amended with 14-, 7- and <1-month-old SCG (mixed and layered). The foliage used in the bioassay was from plants as described in Section 2.2.3. These latter bioassays were conducted during late autumn.

For the no-choice bioassay we used pots, each with a single, non-damaged radish plant. The pots were individually covered with a transparent plastic container (20 cm × 25 cm, Height × Diameter). One slug of the test species was placed into each transparent container and allowed to feed overnight on the plant. Pots were replicated six times (5 soil types × 2 slug species × 6 replicates = 60 pots). After 10 hours of exposure, the areas of the cotyledons and true leaves consumed by the slugs were recorded by pulling the plants from the soil, taking an image of the damaged leaves and estimating the missing areas using Image-J. Because the pots contained soil, the bioassays were influenced by both repellent and plant-quality effects.

For the multichoice bioassays, plants grown in each soil type were pulled from the soil, thoroughly washed, dried with paper towels, and placed together in plastic containers (5 or 7 plants per container (see below)) with each plant attached to the container using sticky tape. The relative positions of the plants in each container were randomized. The

containers contained no soil of any type. A single slug of the test species was placed inside each container and allowed to feed for 10 hours. Bioassays were replicated six times for each slug species. At the end of the bioassays, the area and type (cotyledon or true leaves) of each leaf that was consumed was estimated as described above. Because the containers had no soil, we assume that the bioassays were influenced only by host-plant quality effects.

2.4. Statistical Analyses

The effects of BSF larval size categories and substrate type on cohort weight gain, proportional weight gains and the proportion of larvae surviving were analyzed using univariate general linear models (GLMs). For BSF larval size categories we also assessed the best fit models for the cohort weight gain data.

We used univariate GLMs to analyze radish and tomato growth parameters on amended substrates. In each case, we initially included blocks as a random factor (corresponding to outdoor groupings of pots for radish and trays of pots in the greenhouse for tomato plants). In cases where block had no effect, the factor was subsequently removed. Post hoc Tukey tests were applied to identify homogenous treatment groups. Proportional data were arcsine-transformed and residuals were plotted after analyses to test for normality and homogeneity.

The areas of lettuce leaf disks that were consumed by slugs were ranked within blocks for no-choice bioassays and within recipients/arenas for multichoice bioassays. For the multichoice bioassays, ranking was conducted because on non-independence of observations. For the binary choice bioassays, we calculated the differences between areas consumed from control and treated leaves and compared across treatments using GLMs. We also compared areas consumed in the different soil-treatment combinations using paired t-tests. For all analyses, areas consumed during the night and day were standardized for differing bioassay durations. Bioassays with different slug species and conducted during the day or night were analyzed separately.

No-choice and multichoice bioassays with whole plants were analyzed using Multivariate Analysis of Variance (MANOVA) to test for treatment effects on cotyledons and true leaves. In the case of no-choice bioassays, groups or trays of pots (for radishes and tomatoes, respectively) were included as a blocking factor that was subsequently removed where there was no effect. For the multichoice bioassays, damage to cotyledons and true leaves was ranked within recipients/arenas. Post hoc Tukey tests were applied to identify homogenous treatment groups. Proportional data were arcsine-transformed and residuals were plotted after analyses to test for normality and homogeneity.

3. Results

3.1. BSF Larval Survival and Growth on SCG

Larval survival (69 $\pm$ 7%) was lower for the smallest size category but was similar among the larger larvae (89–99%)($F_{4,26} = 6.342$, $p = 0.002$). Cohort weight gains declined with increasing larval size (Figure 1; see also Table S1).

Larvae of 15 to 17 mg (dry weight) had high survival (97%) on all substrates ($F_{2,18} = 0.415$, $p = 0.668$: Table S1); however, absolute cohort weight gains were < 10% of the gains on SCG + bran or on bran alone (Figure 2A: $F_{2,18} = 63.321$, $p < 0.001$) and proportional cohort weight gains on SCG were about 30% of gains on the other substrates (Figure 2B: $F_{2,18} = 46.867$, $p < 0.001$).

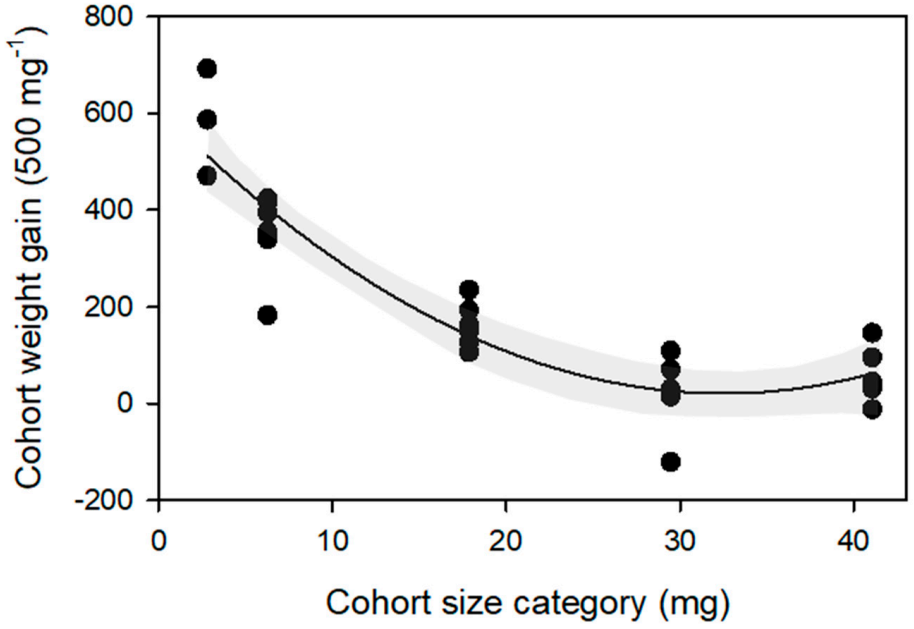

Figure 1. Relationship between average BSF larval size per cohort and absolute weight gain on SCG during 12 days. The curve equation is $y = 609.79 - 36.26x + 0.56x^2$ ($F_{2,24} = 57.375$, $p < 0.001$; $R^2 = 0.84$); 95% confidence intervals are indicated. For further details see Table S1.

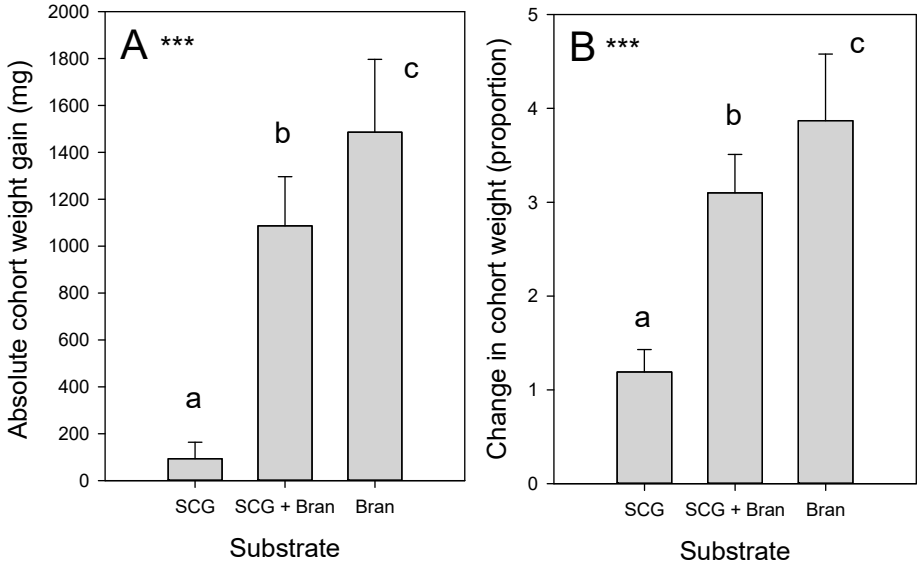

Figure 2. (**A**) Absolute weight gain of BSF larvae on three substrates supplied *ad libitum* during 12 days of feeding with (**B**) proportional changes in cohort weights (SCG = spent coffee grounds). Results of GLMs are indicated as *** = $p < 0.001$; lowercase letters indicate homogenous substrate groups (Tukey $p > 0.05$). Standard errors are indicated (N = 6). For further details see Table S1.

3.2. Effects of Amendments on Radish Growth and Development

Seedling survival declined where soil was mixed or layered with BSF frass, or where 14-month-old SCG were applied as a top layer over the soil (Figure 3A: $F_{8,72} = 8.734$, $p < 0.001$). BSF frass was also associated with delayed plant development (Figure 3B: $F_{8,72} = 9.9383$, $p < 0.001$), yellowing (Figure 3C: $F_{8,72} = 4.873$, $p < 0.001$) and a reduction in plant height (Figure 3D: $F_{8,72} = 8.329$, $p < 0.001$).

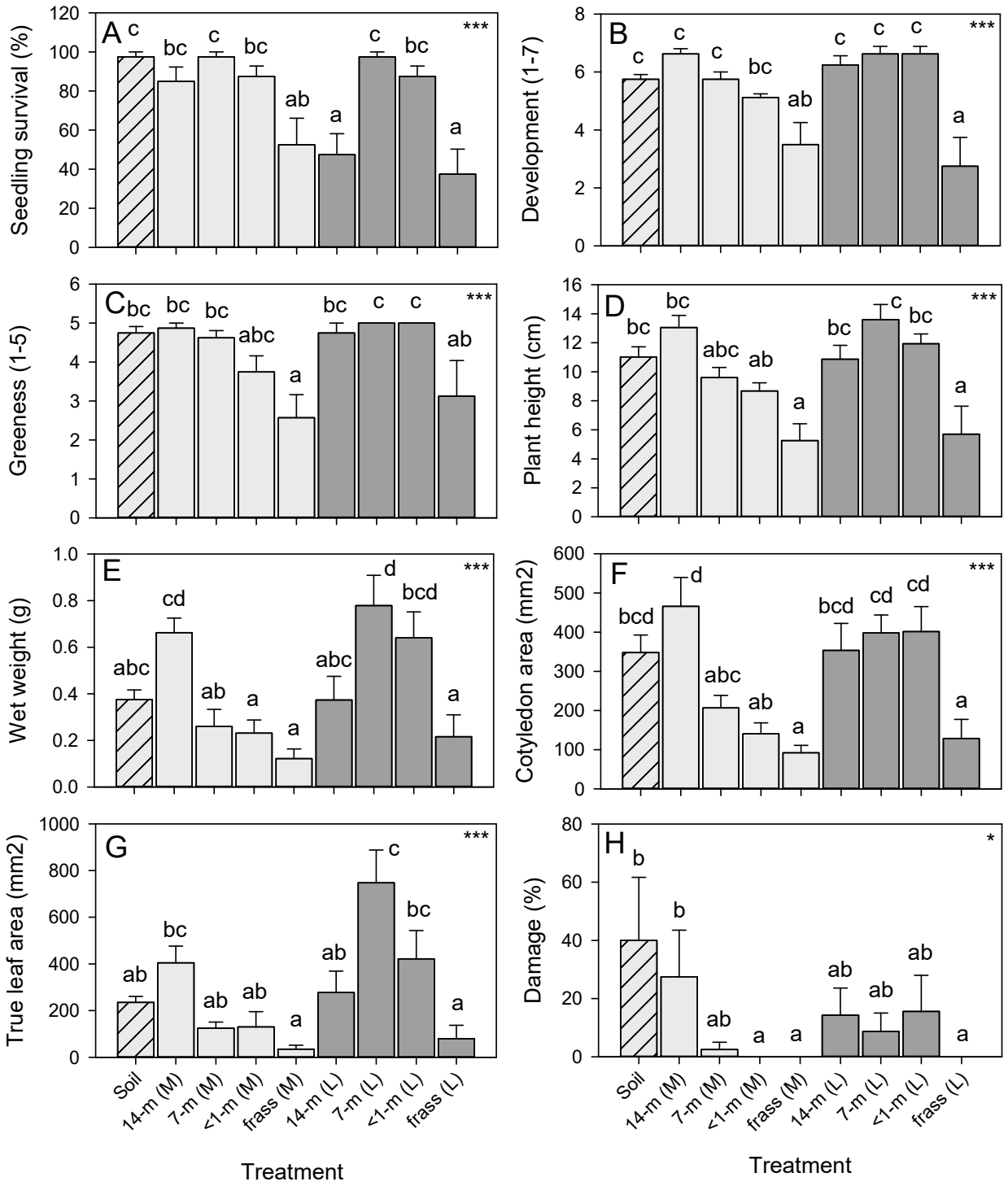

Figure 3. Growth of radish plants in pots with amendments applied as mixed (M = light gray) or layered (L = dark gray) with soil. Control pots with unamended soil (Soil) are indicated by the hatched bars on each plot. Amendments included 14-, 7-, and <1-month-old SCG, as well as frass from BSF larvae reared on SCG. Measured parameters are presented as (**A**) seedling-survival, (**B**) growth stage (related to the number of leaves), (**C**) greenness, (**D**) plant height, (**E**) wet weight of plant, (**F**) cotyledon leaf area, (**G**) leaf area of true leaves, and (**H**) damage to plants during outdoor exposure for 10 days after the experiment. Results of GLMs are indicated as * = $p < 0.05$ and *** = $p < 0.001$. Lowercase letters indicate homogenous substrate groups (Tukey $p > 0.05$). Standard errors are indicated (N = 8).

Plant wet weight (Figure 1E: $F_{8,72} = 7.540$, $p < 0.001$) and the surface areas of cotyledons (Figure 3F: $F_{8,72} = 7.696$, $p < 0.001$) and true leaves (Figure 3G: $F_{8,72} = 7.940$, $p < 0.001$) were greatest where soil had 14-month-old SCG incorporated or where 7- and <1-month-old SCG were applied as a top-dressing.

3.3. Effects of Amendments on Tomato Growth and Development

SCG that were aged for 14 months stimulated the growth of tomato seedlings with development ($F_{8,45} = 8.706$, $p < 0.001$: Figure 4A), plant height ($F_{8,45} = 18.590$, $p < 0.001$: Figure 4B) and wet weight ($F_{8,32} = 8.334$, $p < 0.001$; Figure 4C), declining with increasingly fresher SCG and where soils were amended with BSF frass. Compared with unamended soil, applying the amendments as top layers had no apparent negative effects on any of the parameters (Figure 4A–C).

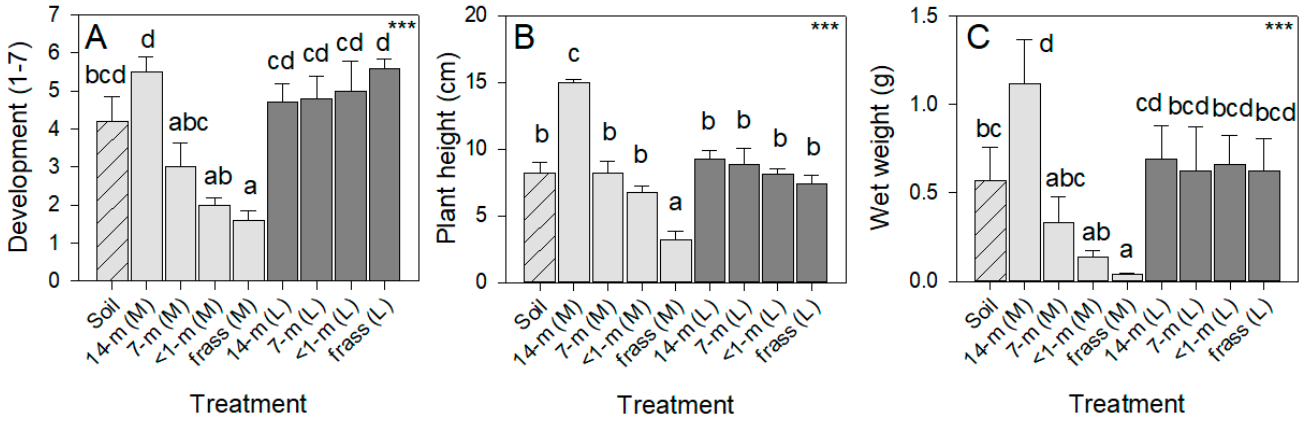

Figure 4. Growth of tomato seedlings in soil with SCG amendments. Results for plant development (**A**), plant height (**B**) and wet weight (**C**) are presented for plants grown in unamended soil (hatched bars) and soil with SCG amendments (solid bars). Amendments included 14-, 7- and <1-month-old SCG, as well as frass from BSF larvae reared on SCG. Each amendment was applied either mixed with the soil (M = light gray bars) or as a layer on top of the soil (L = dark gray bars). Results of GLMs are indicated as *** = $p < 0.001$. Lowercase letters indicate homogenous substrate groups (Tukey $p > 0.05$). Standard errors are indicated (N = 5).

3.4. Repellent Effects of SCG on Slug Herbivory

The results of no-choice, multichoice and binary choice bioassays corresponded well (Tables S2 and S3). Furthermore, the effects of substrates were similar irrespective of observation period (day or night) and were largely independent of slug species. In all cases, the consumption of lettuce leaf disks was significantly reduced where soil was mixed or layered with 1-month-old SCG (Figures 5 and 6). The inhibitory effect of 8-month-old SCG was lower than for 1-month-old SCG; however, in some of the bioassays, 8-month-old SCG layered over the soil had a greater inhibitory effect than when mixed with the soil (i.e., Figure 5C,D—nighttime; Figure 6B).

3.5. Leaf-Quality Effects on Slug Herbivory on Radish

Details of the plants used in these bioassays are presented in Figure S1 and Table S4. The results from no-choice and multichoice bioassays with *A. ater* and *D. reticulatum* were largely consistent. Both slug species preferentially fed on cotyledon leaves (Figure 7). Where true leaves were also damaged, these were mainly from the same plants from which the cotyledons were preferred (i.e., Wilk's lambda = 8.173 (*A. ater*—no-choice); 7.084 (*D. reticulatum*—no-choice); 5.223 (*A. ater*—multichoice); and 2.876 (*D. reticulatum*—multichoice); all *p*-values < 0.01). In the no-choice bioassays, treatments affected herbivory on cotyledons (*A. ater*: $F_{4,30} = 19.573$, $p < 0.001$; *D. reticulatum*: $F_{4,30} = 12.193$, $p < 0.001$) and true leaves (*A. ater*: $F_{4,30} = 4.809$, $p = 0.005$; D. *reticulatum*: $F_{4,30} = 5.308$, $p = 0.003$);

however, in the multichoice bioassays, soil type only affected preferences for cotyledons (cotyledons—*A. ater*: $F_{4,30} = 14.157$, $p < 0.001$; *D. reticulatum*: $F_{4,30} = 3.583$, $p = 0.019$; true leaves—*A. ater*: $F_{4,30} = 0.999$, $p = 0.427$; *D. reticulatum*: $F_{4,30} = 1.050$, $p = 0.193$: Figure 7C,D). Slugs consumed more from leaves grown in unamended soil and from soil that had SCG applied as a top layer (Figure 7A,C,D). However, soil that was mixed and layered with 1-month-old SCG was generally less favored than corresponding soils mixed and layered with 8-month-old SCG (Figure 7A–D).

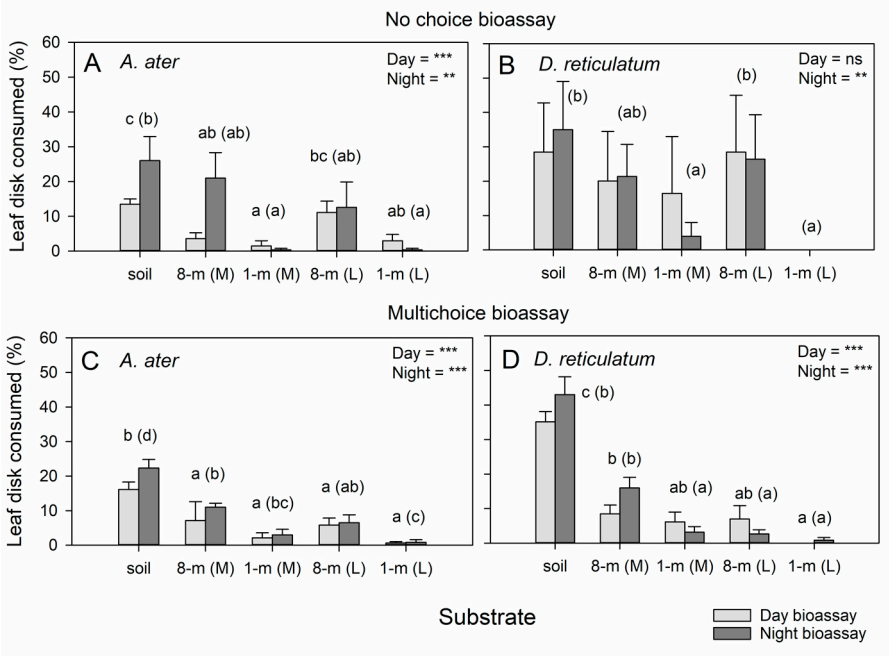

Figure 5. Results of no-choice (**A**,**B**) and multichoice (**C**,**D**) bioassays with *A. ater* (**A**,**C**) and *D. reticulatum* (**B**,**D**). Bioassays were conducted during daytime (light bars) and at nighttime (dark bars). Leaf disks (lettuce) were placed on substrates that included control (unamended) soil, 8-month-old SCG mixed (M) with soil or as a top layer (L) and 1-month-old SCG mixed with soil or as a top layer. Results from GLMs are presented for daytime (day) and nighttime (night) bioassays as ns = $p > 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ (N = 5 for no-choice, N = 6 for multichoice). Lowercase letters indicate homogenous substrate groups for daytime and nighttime (in parentheses) bioassays (Tukey, $p > 0.05$). Standard errors are indicated. (See also Table S2).

In the no-choice bioassays with *D. laeve*, the areas consumed from cotyledons were not affected by treatment; however, slugs consumed more of the true leaves in pots with 14-month-old SCG incorporated than on unamended soil, or soil treated with <1-month-old SCG (Figure 8A: Wilk's lambda = 2.465, $p = 0.012$: cotyledons $F_{6,35} = 1.482$, $p = 0.221$; true leaves: $F_{6,35} = 4.494$, $p = 0.003$). In the no-choice bioassay with *L. marginata*, more area was consumed from cotyledons and true leaves in control, unamended soil, and with 14-month-old SCG incorporated (Figure 8B: Wilk's lambda = 7.265, $p < 0.001$: cotyledons $F_{6,35} = 14.245$, $p < 0.001$; true leaves: $F_{6,35} = 7.251$, $p < 0.001$) and this was greater than feeding from plants in pots with other treatments.

The results from the multichoice bioassays with *L. marginata* and *D. laeve* were largely similar. Both slug species preferentially fed on cotyledon leaves (Figure 8C,D); however, where true leaves were damaged, these were from the same plants (i.e., Wilk's lambda = 5.549 (*L. marginata*); and 5.598 (*D. laeve*); all *p*-values < 0.001). Treatments affected herbivory on cotyledons (*L. marginata*: $F_{6,42} = 5.595$, $p < 0.001$; *D. laeve*: $F_{6,42} = 10.024$, $p = 0.021$) and true leaves (*L. marginata*: $F_{6,42} = 5.833$, $p < 0.001$; *D. leave*: $F_{6,42} = 2.917$, $p = 0.021$). Slugs consumed more from leaves grown in unamended soil and from soil that had 14-, 7- and <1-month-old SCG applied as a top layer (Figure 8C,D). Both *L. marginata* and *D. laeve* consumed relatively large portions of the leaves from radishes grown in soil

mixed with 14-month-old SCG but avoided leaves from plants grown in soil that was mixed with fresher SCG (i.e., 7- and <1-month-old SCG: Figure 8C,D).

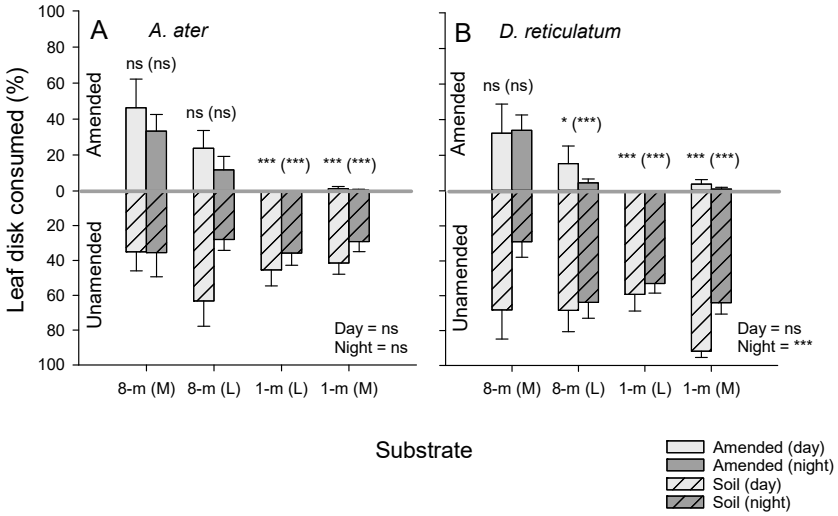

Figure 6. Results from binary-choice bioassays with *A. ater* (**A**) and *D. reticulatum* (**B**). Bioassays were conducted during daytime (light bars) and at nighttime (dark bars). Leaf disks (lettuce) were placed on amended and unamended soil in divided containers. The substrates included 8-month-old SCG mixed (M) with soil or as a top layer (L) and 1-month-old SCG mixed with soil or as a top layer. The percentages of leaf disks consumed over amended substrate (open bars) and unamended soil (hatched bars) are indicated. Results from GLMs based on differences between areas consumed in paired tests are presented for daytime (day) and nighttime (night) bioassays as ns = $p > 0.05$ and *** = $p < 0.001$ (N = 5). The results from paired t-tests for soil-amendment combinations are presented above the bars for daytime and nighttime (in parentheses) bioassays as ns = $p > 0.05$, * = $p < 0.05$ and *** = $p < 0.001$ (N = 5). Standard errors are indicated. (See also Tables S2 and S3).

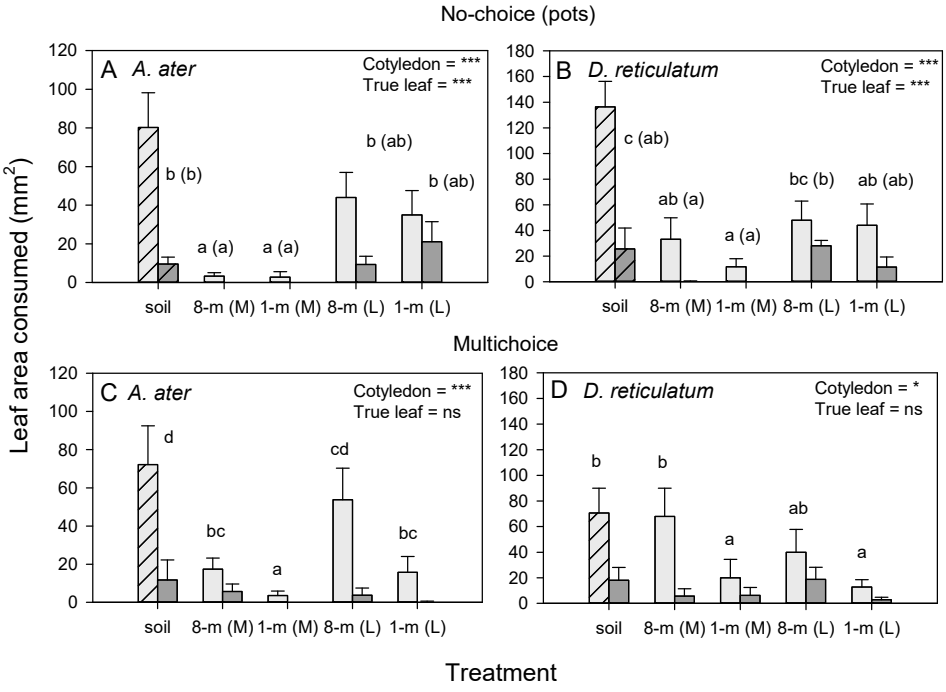

Figure 7. Results from no-choice bioassays with (**A**) *A. ater* and (**B**) *D. reticulatum* on radish plants in pots, and from multichoice bioassays with (**C**) *A. ater*, and (**D**) *D. reticulatum* on radish plants without

soil in arenas. In the no-choice bioassays, radish plants in unamended soil (hatched bars), and soil that had 8- and 1-month-old SCG mixed (M) with soil or added as a layer (L) on top of the soil were exposed to the slugs such that the soil and plant quality affected slug behaviors. In the multichoice bioassays, corresponding plants were exposed without soil. All arenas had both cotyledon (gray bars) and true leaves (dark gray bars). The results of MANOVA are presented for areas consumed from cotyledons and true leaves as * = $p < 0.05$ and *** = $p < 0.001$. Lowercase letters indicate homogenous leaf groups, with groupings for true leaves in parentheses. Standard errors are indicated (N = 6). Plants grown in each soil type as used in the bioassay are described in Figure S1.

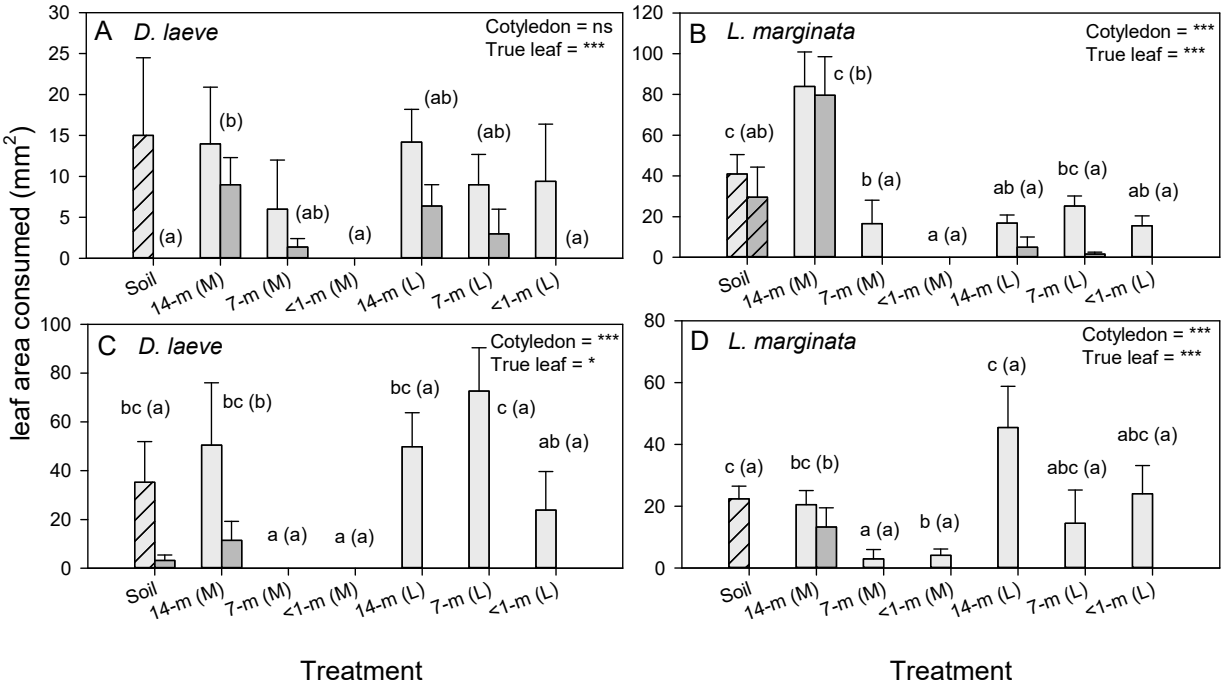

Figure 8. Results from no-choice bioassays with (**A**) *D. laeve* and (**B**) *L. marginata* on radish plants in pots, and from multichoice bioassays with (**C**) *D. laeve* and (**D**) *L. marginata* on radish plants without soil in arenas. In the no-choice bioassays, radish plants in unamended soil (hatched bars), and soil that had 14-, 7- and <1-month-old SCG mixed (M) with soil or added as a layer (L) on top of the soil were exposed to the slugs, such that the soil and plant quality affected slug behaviors. In the multichoice bioassays, corresponding plants were exposed without soil. All plants had both cotyledon (light gray bars) and true leaves (dark gray bars) and were without soil when exposed to slugs. The results of MANOVA are presented for areas consumed from cotyledons and true leaves as ns = $p > 0.05$, * = $p < 0.05$, and *** = $p < 0.001$. Lowercase letters indicate homogenous treatment groups, with groupings for true leaves in parentheses. Standard errors are indicated ((**A**,**B**): N = 5; (**C**,**D**): N = 6). Plants grown in each soil type as used in the bioassay are described in Figure 3.

3.6. Leaf-Quality Effects on Slug Herbivory on Tomato

D. laeve consumed more from cotyledons than true leaves, with trends consistent across soil types (Wilk's lambda: $F_{12,68} = 6.570$, $p < 0.001$: Figure 9). Soil type affected herbivory (cotyledons: $F_{6,42} = 8.412$, $p < 0.001$; true leaves: $F_{6,42} = 7.236$, $p < 0.001$), with more of the cotyledons consumed from tomatoes grown in 14-month-old SCG, and more of the true leaves consumed where tomatoes were grown in unamended soil or soil amended with 14-month-old SCG either mixed with the soil or as a top layer (Figure 9).

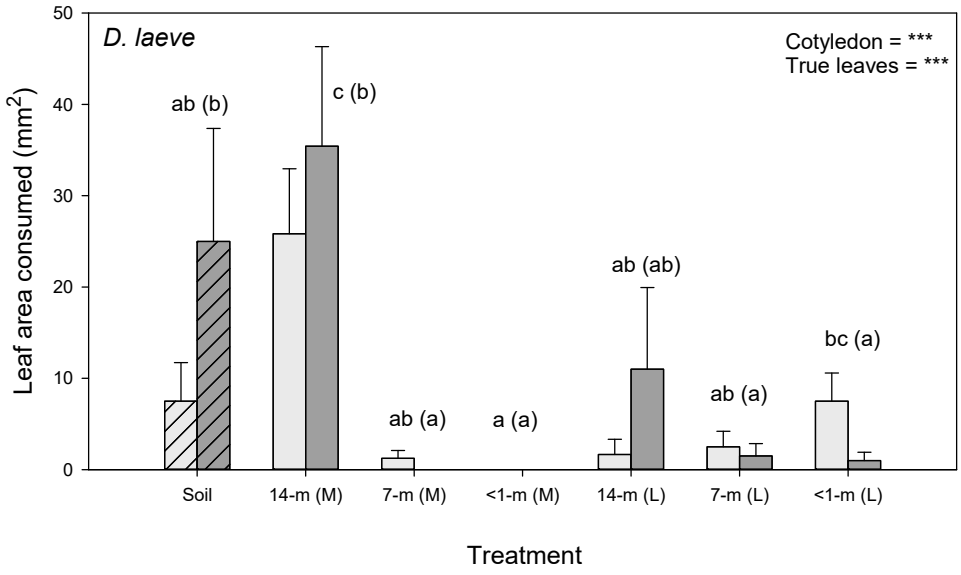

Figure 9. Results from a multichoice bioassay with tomato leaves exposed to *D. laeve*. Tomato plants were grown in unamended soil (hatched bars), and soil that had 14-, 7- and <1-month-old SCG mixed (M) with soil or added as a layer (L) on top of the soil. Cotyledons (light gray bars) and true leaves (dark gray bars) were exposed during the bioassay without soil. The results of MANOVA are presented for areas consumed from cotyledons and true leaves as *** = $p < 0.001$. Lowercase letters indicate homogenous treatment groups, with groupings for true leaves in parentheses. Standard errors are indicated (N = 6). Plants grown in each soil type as used in the bioassay are described in Figure 4.

4. Discussion

We confirmed that SCG reduce the growth and development of radish and tomato plants when applied within one month of coffee percolation. Radish and tomatoes grown in soil mixed with relatively fresh SCG had reduced growth and delayed development (Figure 3, Figure 4 and Figure S1). However, aging the SCG for 8 months reduced these negative effects and aging for 14 months significantly enhanced plant growth (Figure 3, Figure 4 and Figure S1). BSF larvae survived and gained weight on fresh SCG, but growth and development were poor (Figure 2). The resulting frass reduced the growth of radish and tomato plants when incorporated into the soil (Figures 1 and 2). In bioassays with two slug species, SCG reduced feeding on lettuce leaf disks, but the repellence effect declined as SCG aged (Figures 5 and 6). Herbivory was also reduced by an indirect plant-quality effect. This induced effect decreased as SCG aged and was absent for 14-month-old SCG (Figures 8 and 9). These results suggest that any negative, plant-mediated effects on slug herbivory will be lost or significantly reduced by the time that SCG are suitable to promote plant growth. In contrast, 7-month-old and <1-month-old SCG applied as a top dressing successfully repelled slugs while at the same time promoting the growth and development of radish and tomato plants (Figures 2, 3, 7 and 8). Our results indicate that some of the benefits of SCG for plant growth and herbivore suppression could be attained using aged SCG as a mulch, particularly for relatively tolerant plants like tomato. We discuss the effects of SCG on BSF, plant growth and slug herbivory in the following sections.

4.1. Effects of SCG on BSF Larval Growth and Development

Studies have shown that processing by earthworms (e.g., *Eisenia foetida*) or fungi (*Aspergillus* spp., *Penicillium* spp., *Pleutotus ostreatus*) can accelerate the decomposition of SCG and/or reduce caffeine contents to produce effective soil amendments and composts [10,12,41,42]. Decomposition rates by earthworms are relatively slow. In contrast, BSF larvae can rapidly consume large quantities of organic wastes to convert these to insect biomass. Furthermore, BSF larvae have a high protein (40–70% dry weight) content and are

used as animal feeds [36,37]. The larvae also have several other potential industrial uses; for example, BSF larvae are a source of biofuels and other chemical products, including chitins [38,43]. BSF bioconversion rates are high on nutrient-rich substrates such as spent grains, but they are considerably lower on low-nutrient substrates that have high hemicellulose and cellulose contents [44,45]. We therefore expected BSF growth and development to be relatively slow; however, we also assessed whether the BSF larvae could neutralize the toxic effects of fresh SCG to produce a suitable frass fertilizer. In agreement with two previous studies, we observed slow BSF growth rates on SCG (see Permana et al. (2018) [35] and Fischer et al. (2021) [34]). In our comparison of BSF development on SCG and bran, we observed that larval weight gains were ca. 15 times lower than on bran, and 10 times lower than on a SCG and bran mix (Figure 2). Furthermore, all larvae reached prepupal stages on the bran and bran mixed substrates by the end of our experiment, but no prepupae were observed on the SCG substrate. In a similar experiment, and using the same bran as in the present study, Horgan et al. (2023) [46] found that a blend of apple pomace and bran (1:1) resulted in improved BSF growth rates and weight gains than on pomace or bran alone. That this did not occur in the present study suggests that the SCG inhibited growth and were possibly toxic to the developing larvae. This corroborates the results of Hadj-Saadoun et al. (2020) [47], who observed high mortality of 5 to 7-day-old BSF larvae on SCG.

We assessed whether larval age or size at inoculation affected BSF survival and weight gain. We found that earlier instars (smaller larvae) had significantly lower survival than medium-sized or larger larvae; however, these smaller larvae also resulted in the greatest cohort weight gains (Figure 1, Table S1). The relatively small gains in cohort weight (compared to BSF on bran) and the slow development of larvae on SCG would prohibit the use of BSF for SCG bioconversion to insect proteins without mixing the grounds with relatively large amounts of other organic substrates. Furthermore, BSF larvae reared on SCG have low levels of long-chain polyunsaturated fatty acids that are essential in feed produced for aquaculture [34]. Nevertheless, the larvae did produce a consistent frass (i.e., similar granular appearance throughout). Furthermore, Fischer et al. (2021) [34] have shown that BSF frass generated during feeding on SCG has 3.3% nitrogen, which is similar to the nitrogen contents of commercial BSF frass products [39]. Because of the slow bioconversion rates, we generated BSF frass by placing large numbers of late stage BSF on moistened SCG until the substrate was converted to a consistently granular texture. During this process, the BSF cohort would have lost weight because of high intraspecific competition on the low-nutrient substrate [46]. We applied the frass in relatively large quantities to cover the topsoil in our plant growth experiments; however, we found that the top layer of frass inhibited cotyledon and leaf development in radish (Figure 3) and, when incorporated into the soil, frass inhibited radish and tomato growth (Figures 3 and 4). This was probably due to the hydrophobic nature of the frass (personal observation), as well as some low-level toxicity (see Borkent and Hodge (2021) [39]). The BSF gut microbiome has a noted ability to detoxify feeding substrates [48], but toxins may also remain in the frass [40]. Trends in Figures 3 and 4 suggest that BSF frass possibly accumulated caffeine or other toxins to produce similar, but greater, negative effects on radish and tomato growth when compared to fresh SCG, but this idea requires further study. Because of the strong negative effects on plant development, we did not assess the effects of SCG-derived BSF frass on herbivory.

4.2. Effects of SCG on Plant Growth

SCG have several reported benefits when used as a soil amendment, particularly in carbon-poor soils; for example, 10% SCG can increase soil carbon, nitrogen, phosphorus and potassium levels [14]. Furthermore, 10% SCG increases soil porosity and reduces soil bulk density [41]. SCG also reduce pesticide leachate in low carbon soils [49]. SCG applied as a soil amendment have also been associated with a decline in plant diseases and are a suitable substrate for *Trichoderma* spp., which are antagonistic to plant pathogens [18,27].

Furthermore, incorporating SCG into soil as an amendment reduces GHG emissions as well as reducing waste to landfill [14]. Fresh SCG may stimulate seed germination [50], and when mixed with sawdust can be an effective substrate during mushroom production [51]. Despite these benefits of SCG as a soil amendment there are several noted drawbacks.

Our results are in agreement with previous studies that found fresh SCG to inhibit plant growth [14,19,20]. Growth inhibition by SCG has been attributed to the immobilization of nutrients in the soil by microbes (bacteria and fungi) as carbon and other SCG components are broken down, and to improved soil water retention that reduces water to roots and accelerates wilting [14,20,41,52]. Strong phytotoxic effects of caffeine and other phenols in SCG can further inhibit growth. This is observed as increased oxidative stress in the roots and leaves of lettuce plants [53]. For example, 10% SCG can increase total phenolic compounds in soil by 4000× [14]. SCG also reduce soil pH and can reduce the organic nitrogen content and mineral elements of lettuce plants [17,54] (but see Cervera-Mata et al. (2019) [42]). Moderate SCG amendments can increase carotenoid and chlorophyll contents in lettuce, but this declines at higher SCG levels [17]. In our experiments with radish, fresh SCG reduced leaf greenness and often produced bright yellow cotyledons indicative of seedling stress.

In contrast to fresh SCG, incorporation of 14-month-old SCG into the soil improved the growth and development (i.e., number of emerged leaves) of radish and tomato plants (Figures 3 and 4). Ronga et al. (2016) [30] also found SCG to improve growth of basil and tomato after static-pile composting. However, when applied as a top-dressing, 14-month-old SCG significantly reduced radish survival after germination (Figure 3A) and resulted in slower plant development and weight gains compared to 7-month-old and <1-month-old SCG top-dressings (Figure 1B,E–G). This effect was probably due to the physical properties of the older SCG that had a low porosity and tended to remain wetter after watering compared to the other SCG types. This may have reduced water availability to the underlying soil. The same effect was not seen in the experiment with tomato plants (Figure 4), possibly because smaller pots were used in the experiment with tomatoes and water accumulated in the potting trays. Because 14-month-old SCG have no apparent effect on slug herbivory (see below), and because of its water retention properties, then applying such old SCG as a mulch is not recommended. However, we do not know whether such old SCG could reduce the incidence of weeds if applied as a top-dressing after crop emergence.

Our results depict a gradual improvement in SCG for plant growth with increased aging. Previous studies have shown that composting SCG (including vermicomposting) similarly reduced SCG inhibitory effects on other plants, including lettuce. This has been attributed to the limitation of microbial activity, the chelating of caffeine and other toxins, neutralizing pH, and a reduction in C:N ratios [27,28,31,55,56]. Effective composting, to neutralize the negative effects of SCG and enhance potential benefits, can take several months: composting SCG for 100 to 156 days has given good results in previous studies [30,32], but in our system, 7-month-old SCG (i.e., >200 days) still had several negative effects on plant growth (but see Figure S1). The slower neutralization of SCG in our system is probably due to the relatively anaerobic conditions under which we stored the SCG. We did not compost the SCG with straw or other vegetation debris or manures because we wished to evaluate the direct effects of SCG; however, traditional composting methods or methods that employ decomposer fungi [10,29] can accelerate the decomposition process and may further improve SCG properties.

Applying 7- and <1-month-old SCG as top-dressings reduced their negative impacts on radish and tomato growth. Indeed, 7- and <1-month-old SCG as top-dressings had similar beneficial effects on radishes when compared to 14-month-old SCG that were incorporated into the soil (Figures 3 and 4). This suggests that nutrients are leached from the SCG top-dressing to improve plant growth without the movement of toxins deeper into the soil. The positive and neutral effects on radish and tomato, respectively, of SCG top-dressings supports observations by Hirooka et al. (2016, 2017) [22,23] that top-dressings can reduce weed emergence with minimal effects on the main crop species. The results also

suggest that the benefits of SCG in reducing slug herbivory can be maintained if SCG are applied as a top-dressing. However, we caution that our experiments were conducted with seedlings in pots, and experiments at field plot scales are recommended to test whether the benefits of SCG top-dressings for crop plants are maintained until harvest.

4.3. Effects of SCG on Slug Herbivory

Our results from bioassays with leaf disks were clear: leaf damage from *A. ater* and *D. reticulatum* was significantly reduced by 8- and 1-month-old SCG. The effects were greater with fresher SCG and when the SCG were applied as a top-dressing (Figures 5 and 6). Similar results from a series of no-choice and choice bioassays during the day and night verified that our methodology was robust. In a no-choice bioassay with the same slug species confined to potted plants, we noted that damage to radish was lowest when plants were grown with SCG incorporated into the soil. Because we also monitored the growth of radishes across the different treatments (Figure S1), we believe that this reduction in herbivory cannot be attributed to possible effects on the nutrient quality of the radish plants. For example, radish plants growth with 8-month-old SCG incorporated or layered over the soil had similar survival, plant height, greenness and development compared to control plants grown in unamended soil (Figure S1). The relatively higher damage to plants in pots with top-dressings compared to incorporated SCG was partly due to slugs climbing into the plants to feed (Figure 7). In multichoice bioassays without soil, *D. ater*, *L. marginata* and *D. laeve* all consumed more from radishes that were grown in soil with SCG top-dressings, than from radishes with 8-, 7- and <1-month-old SCG incorporated (Figures 7 and 8). In the case of *L. marginata* and *D. laeve*, we cannot reject the possibility that this was due to relatively poor plant nutritional quality; however, the multichoice bioassay with *A. ater* clearly suggests that the radishes may have acquired defenses from the incorporated SCG. Several plants will sequester caffeine from the environment [57,58]; however, it is unknown whether these plants will have subsequently higher defenses against herbivores. Our results suggest that they may have. In the experiment with tomatoes the same trend was apparent; however, *D. laeve* showed a strong preference for tomatoes grown in soil with 14-month-old SCG incorporated (Figure 9).

Our results are in agreement with a small number of previous studies. For example, in laboratory bioassays Hollingsworth et al. (2002) [24] topically applied caffeine solutions to *Zonitoides arboreus* and found that heart rates declined with increasing caffeine concentration; at higher concentrations (0.1, 0.5 and 2% caffeine), snail mortality was high (70–100% in 96 hours). The authors reported 2% caffeine to be more effective than commercial metaldehyde formulations in controlling the snail in greenhouse orchids. Kang et al. (2002) [59] corroborated these findings in bioassays with *Acusta despecta* and *Deroceras varians* on dipped cabbage leaves (damage was reduced to zero at 0.5% caffeine) and further reported that these mollusk species had high mortality when dipped in caffeine solutions (100% at 0.1% caffeine within 1400 and 400 minutes, respectively). Das (2022) [60] described the responses by *Laevicaulis alte* to aqueous tea leaf extracts (that also contain caffeine); the slugs were described as exhibiting profuse sliming and wreathing. Similarly, Carvalho de Brito et al. (2021) [61] reported toxicity of aqueous yerba mate extracts on the aquatic snail *Pomacea canaliculata*. Jeong et al. (2012) [25] examined the effects of caffeine extracts alone and in combination with ethyl alcohol and/or tobacco extracts against *Lehmannia valentiana*. The authors found that 0.5% caffeine mixed with 5 to 7% alcohol repelled the slug from Chinese cabbage, but the solution had some phytotoxic effects on marigolds in a greenhouse study. These studies are generally consistent in reporting that low concentrations of caffeine (0.5 to 2%) have repellent and contact-toxicity effects on a range of snails and slugs. Furthermore, the graded physiological and herbivory reduction responses to incremental increases in caffeine concentrations are similar to our results with progressively fresher SCG. Furthermore, fresh SCG contains about 2% caffeine [10], which is similar to the highest concentrations in solutions that were used in some previous studies. As caffeine is chelated during decomposition concentrations will decline, but concentrations

as low as 0.1% will likely maintain some repellence effects according to Hollingsworth et al. (2002) [24] and Kang et al. (2012) [59].

4.4. Recommendations

Based on previous research, attaining a balance between the benefits and drawbacks of using SCG in agriculture and horticulture will depend on a range of factors including the crop species, the age of SCG, the composting process, the volume applied to the soil, and the method of application, among others [19,20,22,28,30]. Based on our results, we recommend that SCG be aged (including through composting or vericomposting) before application as a soil amendment. SCG do have anti-herbivore properties as suggested anecdotally by several sources; however, to avoid inhibition of plant growth, SCG should be applied as a top-dressing to repel slugs. Further research is required to avoid potential leaching of caffeine from SCG to soil and water—however, our results suggest that some aging of SCG could reduce caffeine contents yet still provide systemic protection against slugs. Metaldehyde, which is used as a commercial molluscicide, is also a major contaminant of water (like caffeine) [62] and has detrimental effects on beneficial invertebrates [63–65]. Substituting commercial molluscicides with SCG to control snails and slugs would probably reduce such negative environmental effects on predatory invertebrates, and through adequate aging or composting would also reduce environmental contamination by caffeine.

5. Conclusions

If properly managed, SCG have several possible benefits for agriculture and horticulture. We found that aging SCG or applying SCG as a top-dressing reduced growth inhibition in radish and tomato. When aged sufficiently (14 months in our study), SCG improved the growth and development of radish and tomato. To our knowledge, this is the first study to demonstrate repellent and possible plant-mediated inhibition of slug herbivory by SCG. However, we also found that there were trade-offs between the possible benefits from aging SCG for plant growth and protection against herbivory. Nevertheless, our results suggest that suitable aging of SCG (i.e., between 8 and 14 months), which is required to avoid inhibition of plant development, will not necessarily eliminate SCG anti-herbivory properties. Therefore, some aging of SCG, together with application as a top-dressing and used with caffeine tolerant, or caffeine sequestering plants, could potentially reduce inputs of pesticides and inorganic fertilizers while contributing to waste reduction, reduced GHG emissions and circular agriculture.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture13020257/s1, Table S1 Results of larval size category and substrate bioassays with Black Soldier Fly larvae; Table S2 Results of univariate GLMs for no-choice, multichoice and binary choice bioassays with two slug species; Table S3 Results from paired t-tests comparing leaf disk damage over soil (controls) and amended soil; Table S4 Results from GLMs for radish growth and development associated with five substrate-types; Figure S1 Growth of radish seedlings in soil with SCG amendments.

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