

ABSTRACT

LITTLE, AMOS GRAY. Vermicompost-Mediated Host Plant Resistance to Insects. (Under the direction of Dr. Yasmin J. Cardoza).

Vermicompost increases plant growth and productivity as well as plant resistance to arthropod, nematode and pathogen pests but the mechanism responsible for this resistance is yet to be determined. Therefore the effects of vermicompost grown plants on preference and performance of generalist and specialist lepidopteran (*Helicoverpa zea* and *Pieris rapae*) and aphid (*Myzus persicae* and *Brevicoryne brassicae*) pests were evaluated. Effects of vermicompost grown plants on tri-trophic interactions were also tested. For the lepidopteran pests, preference was evaluated in larval leaf disc feeding and whole plant oviposition choice assays. Larval feeding experiments were conducted using discs from the second oldest and second youngest leaves. After 24 h of feeding, *H. zea* larvae showed no preference for any vermicompost treatment, regardless of leaf age, whereas; *P. rapae* larvae consumed more of younger leaves indiscriminate of vermicompost treatments. Contrastingly, *P. rapae* adults oviposited significantly more on plants grown on 60% vermicompost. Antibiosis was also evaluated in no-choice larval development assays. No significant treatment effects were found for *H. zea* development; however, vermicompost treatments had a significant negative effect on *P. rapae* survival. Vermicompost treatments did not affect attraction to *H. zea* damaged plants or development in *H. zea* larvae by the parasitoid *C. marginiventris*.

Aphid preference was evaluated with leaf disc (apterous) and whole plant (alate) choice assays. After 24 h of feeding there was no significant negative effect on the

feeding preference for apterae of either species on any of the treatments tested. On the other hand, alate *M. persicae* preferred alighting on control plants over vermicompost-grown plants, but *B. brassicae* showed no preference towards any of the treatments tested. Interestingly both aphid species deposited significantly more nymphs on control plants than on those grown in 20% vermicompost. Furthermore, plants grown in soil amended with 20% vermicompost significantly suppressed mass accumulation, as well as, numbers of adults and nymphs of both aphid species compared to controls.

Results from these studies show that vermicompost differentially affects insects of varying feeding habits and that intra species effects are stage-dependent. These data also show that vermicompost mediated resistance effects are not dose-dependent and that lower concentrations have a greater effect on insect preference and performance than higher concentrations. Furthermore, vermicompost-mediated resistance to insects appears to be caused by factors other than nutrient levels in plant.

Vermicompost-Mediated Host Plant Resistance to Insects

by
Amos Gray Little

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APPROVED BY

Dr. David Orr

Dr. George Kennedy

Dr. Yasmin Cardoza
Chair of Advisory Committee

DEDICATION

To all my family and friends past, present and future; I wouldn't be here without you.

BIOGRAPHY

Amos Gray Little was born on March 1st, 1983 to Silas Little III and Lisa Gray Millimet in their home in Temple, NH. He was raised in the woods of southern New Hampshire and spent much time roaming around in the woods accompanied by his older brother Wheaton and various family dogs. He graduated from Northfield Mount Hermon School in Northfield Massachusetts in June of 2001, and received a BS from Warren Wilson College in 2006. He plans on continuing on to get a PhD in forest entomology working in pest management.

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INTRODUCTION

Importance of soil in agricultural production

Soil health is closely tied to agricultural productivity; the higher quality of the soil the higher crop yield and the longer the land can be farmed (Pimentel et al., 1995; Naylor, 1996). Soil is important to plants because it provides support for them to grow as well as essential nutrients, such as nitrogen, potassium, phosphorous, and calcium, needed for growth (Pimentel et al., 1995). Increases in agriculture production since the early 1900's have been attributed to increased yields and not to the amount of land used for agriculture. In fact, most of the yield increases are due to application of chemical inputs such as fertilizers and pesticides (Naylor, 1996). However, due to our reliance on high input farming to fulfill the demand for food for the ever-growing human population, nitrogen use in agriculture increased at a rate of approximately 6%/year from 1961 to 1991(Naylor, 1996). Thus, conventional agriculture is ever more dependent on synthetic inputs that are energetically-costly, which makes current food production unsustainable.

Environmental consequences of unsustainable conventional agriculture production practices

Soil loss due to erosion leads to higher demands for pesticides, water and fertilizers utilized in modern agriculture, but even with higher input application there is still yield loss on eroded sites (Pimentel et al., 1995). Soil quality deterioration due to erosion and contamination reduces soil fertility and water retention capabilities which in turn affect soil biota and soil organic matter content. Pesticide use has been linked to a variety issues

including human health, reduction of beneficial insect populations, pest resistance, water pollution and, in some cases lowered crop yields (Pimentel et al., 1992). In a recent study on Parkinson's disease individuals exposed to pesticides were found to have higher occurrences (up to 70%) of Parkinson's disease than individuals who were not (Ascherio et al., 2006). Organophosphates exposure has been linked to Autism Spectrum Disorders (ASD) (Roberts et al., 2007) as well as mental and pervasive developmental disorders (Eskanzi et al., 2007). Contamination of clean water, algal blooms, lower dissolved oxygen, and increased mortality of fish and other aquatic organisms are just a few problems attributed to fertilizers and pesticide runoff caused by their excessive application and production processes (Carpenter et al., 1998).

Therefore, maintaining soil quality and fertility present major challenges for sustainable food production. Soil fertility and quality is greatly influenced by the amount of organic matter present. Moisture and available nutrient content, activity of soil dwelling organisms, and soil fertility and productivity all increase as soil organic matter increases (Reganold et al., 1987). Some soil dwelling organisms (such as earthworms and mycorrhizal fungi) can help recycle nutrients and organic matter back into the soil and can promote soil fertility and stabilization through aggregate formation (Pimentel et al., 1995). It has been estimated that up to 50% of aggregates in the surface layers of soil are formed by earthworms; these aggregates are readily mixed into the soil due to earthworm movement through the soil layers (Edwards, 2004). Nitrogen fixing bacteria are able to increase soil fertility but, unfortunately, not at levels needed to sustain current agricultural yield demand (Naylor, 1996).

Project justification and goals

Due to concerns over public health, environmental pollution and agricultural sustainability there is currently a spike in interest on the development, research and implementation of low input alternatives for sustainable agricultural production. Langdale et al. (1992) showed that conservation tillage increased carbon in the soil as well as nitrogen, stable aggregates and water infiltration. Soil organic matter is a main factor in soil aggregate stabilization, which is in turn correlated to nitrogen content, water holding capacity and cation exchange in soil (Chaney and Swift, 1984). Another area of interest in sustainable agriculture is the use of organic soil amendments to reduce reliance on synthetic agricultural inputs while enhancing plant growth, yield, health and resistance to both pathogen and arthropod pests.

Vermicompost is the result of earthworm feeding activity leading to the composting of organic wastes such as food waste, livestock manure and paper by-products which can then be incorporated into agricultural fields. For example, as of 1997 a composting site in Wilson North Carolina recycled 5 tons of pig manure every week using earthworms to produce vermicompost (Sinha et al., 2008). Municipal waste water can also be treated using earthworms which increases nutrient availability and removes pathogenic organisms thus, creating nutrient rich water that can be used for irrigation of landscape plants, lawns and agricultural crops (Sinha et al., 2008).

Vermicomposts are high in available nutrients, microbial activity and humic substances, which have been linked to increased plant growth (Arancon et al., 2006; Atiyeh et al., 2000b). Amending commercial peat-based potting mixes with small amounts of

vermicompost ranging from 10 to 20% improves germination and growth of marigolds, tomatoes, peppers and lettuce seedlings (Atiyeh et al., 2000a; Atiyeh et al., 2000b; Zaller, 2006). This clearly demonstrates that vermicompost is a viable alternative to peat as a plant growth medium. Harvesting of peat moss for potting mixes can be harmful to sensitive and sometimes endangered wetland ecosystems (Zaller, 2006) thus; vermicompost would provide a more environmentally-friendly alternative for potting mixes. Vermicomposts have also been shown to improve the growth and yield of crossandra (*Crossandra undulaefolia* Salisb.), tomatoes (*Solanum lycopersicum* L.), peppers (*Capsicum annuum* L.), strawberries (*Fragaria x ananassa* L.), lettuce (*Lactuca sativa* L.), marigold (*Tagetes patula* L.) and spinach (*Spinacea oleracea* L.) (Atiyeh et al., 2000a; Arancon et al., 2002; Arancon et al., 2003; Arancon et al., 2004a; Arancon et al., 2004b; Gajalakshmi and Abbasi, 2002; Gutiérrez-Miceli et al., 2007; Peyvast et al., 2007; Zaller, 2007).

Not only has vermicompost been shown to increase plant growth and yield but, several studies have reported increased plant resistance to pathogen, nematode and arthropod pests as well. Case in point, vermicompost has been shown to suppress the plant pathogens *Pythium*, *Verticillum*, *Rhizoctonia*, and *Fusarium* (Szczecz, 1999; Chaoui et al., 2002) and plant parasitic nematodes (Edwards et al., 2007). Populations of, and damage by, arthropod pests such as jassids (*Empoasca kerri*), aphids (*Myzus persicae* and *Aphis craccivora*), spider mites (*Tetranychus urticae*), mealy bugs (*Planococcus citri*), and caterpillars have been suppressed by vermicomposts on crops such as peanut (*Arachis hypogaea* L.), peppers (*Capsicum annuum*), tomatoes (*Lysopersicon esculentum*), cabbages (*Brassicae oleracea*), and cucumbers (*Cucumis sativa*) (Arancon et al., 2005; Arancon et al., 2007; Edwards et al.,

2010; Rao, 2002).

Altogether these studies suggest that vermicomposts may be a viable alternative to synthetic fertilizers and may also reduce the need for synthetic pesticides, while promoting plant growth and productivity. This environmentally-friendly soil amendment can be easily adopted and incorporated into pest management programs for certain crops. In doing so, the use of vermicompost could result in lower inputs of synthetic chemicals in the form of pesticides and fertilizers for agricultural production. Lower synthetic inputs would in turn translate into less pollution of our environment. Increased compost use may lead to more sustainable waste management programs by turning organic wastes such as food, paper, and manures into nutrient rich soil amendments. Furthermore, vermicomposting food and livestock wastes can reduce organic wastes and create agricultural inputs that are more sustainable than their synthetic counterparts.

The objectives of this study are to evaluate the effects on the preference and performance of insect pests on vermicompost grown plants. Specifically, the research was undertaken to characterize the mechanism (or mechanisms) of defense that is (are) responsible for the decreases in pest populations previously documented on vermicompost-grown plants (Arancon et al., 2005; Edwards et al., 2010; Rao, 2002). To determine vermicompost effect on plant defenses to insects with a variety of feeding habits and preferences, *Brassica oleracea* L. var. Capitata was picked for these experiments. This species was chosen to serve as a model plant not because it is a high value cash crop, but because the Brassicaceae family has: a) a wide cohort of herbivorous species associated with them, b) well characterized herbivore-defense response arsenal and c) one of the best studied

model systems, *Arabidopsis thaliana*, which will provide a plethora of molecular tools to facilitate evaluation of vermicompost plant responses in future studies. Furthermore; by utilizing these plants we can use the extensive knowledge gained through previous Brassicaceae research to help identify how vermicompost influences plant defenses and how these translate into insect behavior and performance. Testing the effects of vermicompost-grown plants on generalist vs. specialist pests and pests with chewing mouthparts (caterpillars) vs. pests with piercing sucking mouthparts (aphids) will allow a more complete assessment of the nature of the vermicompost-mediated defense responses within the plant. As mentioned above, *B. oleracea* being related to the most versatile model plant, *Arabidopsis thaliana*, should facilitate future exploitation of the myriad of molecular tools available to discern vermicompost-plant-insect interactions.

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Vermicompost-Mediated Host Plant Effects on Generalist and
Specialist Lepidopterous Cabbage Pests and a Larval
Parasitoid

A. G. Little

Department of Entomology, North Carolina State University,
Campus Box 7613, Raleigh, NC 27695

Introduction

Organic composts, such as vermicomposts, which is the product of earthworm digestion, are rich in available nutrients and therefore, are viable alternatives to synthetic inputs for crop production. Vermicomposts contain increased microbial activity and nutritional content, which promote plant growth and health (Atiyeh et al., 2000; Gajalakshmi and Abbasi, 2002; Arancon et al., 2004b, Arancon et al., 2005; Gutiérrez-Miceli et al., 2007; Peyvast et al., 2007; Zaller, 2007). As a result of the earthworms' digestive process, vermicompost also contains humic acids which are believed to increase plant growth, flowering and fruit yields (Arancon et al., 2006). Potting soil amended with 10% cattle manure vermicompost significantly increased the leaf area, nutrient and mineral contents of spinach, *Spinacia oleracea* L. (Peyvast et al., 2007). Pepper, *Capsicum annuum* L., tomato, *Solanum lycopersicum* L., and strawberry, *Fragaria × ananassa* Duchesne yields were also increased by amending soil with vermicompost (Arancon et al., 2004a, Arancon et al., 2004b, Arancon et al., 2006, and Gutierrez-Miceli et al., 2007).

In addition to increasing yield, vermicompost can increase plant resistance to pests. For example, vermicompost was shown to inhibit *Fusarium* wilt in tomatoes and it is suggested that this resistance is due to biotic factors (Szczecz, 1999). Also, resistance to arthropod pests such as spider mites, *Tetranychus urticae* C. L. Koch, mealybugs, *Pseudococcus* sp., and green peach aphids, *M. persicae* Sulzer, has been reported on the basis of population and damage reduction when plants were grown in soil amended with vermicompost (Arancon et al., 2005; Arancon et al., 2007; Edwards et al., 2010).

The mechanisms responsible for herbivore resistance mediated by vermicompost are not yet known. Therefore, this study was undertaken to determine if vermicompost-mediated plant resistance to herbivores is due to antixenosis and/or antibiosis effects. To accomplish this, we tested the effects of plants grown in various vermicompost concentrations on the preference and performance of generalist (*Helicoverpa zea* Bod.) and specialist (*Pieris rapae* L.) lepidopterous cabbage pests. To test for antixenosis and antibiosis effects we evaluated direct plant resistance based on the feeding preference and performance of the aforementioned pests on vermicompost-grown plants.

Plant resistance to herbivory not only has an effect on pests but their natural enemies as well, and such effects can be either positive and negative (Braman et al. 2004, Francis et al. 2001, Sarfraz et al. 2009). For example, there is the possibility of vermicompost influencing indirect plant defenses (i. e., volatile emissions) leading to attraction of the pests' natural enemies. Induction of direct plant defenses against herbivory has been shown to have negative effects on predators: Thaler (2002) found that induced tomato plants (*Lycopersicon esculentum*) had fewer syrphid fly eggs laid on them, due to smaller pest populations, as well as negative effects on the parasitism rate by *Hyposoter exiguae*. Similarly, development of the endoparasitoid *Glyptapanteles flavicoxis* on its host *Lymantria dispar* was reduced by resistance induction in *Populus nigra* (Havill and Raffa 2000). On the other hand, vermicompost may increase the nutrients available to the plant and this may increase parasitoid performance. Moon et al. (2000) found that *Anagrus armatus* parasitized more *Pissonotus quadripustulatus* eggs on fertilized than on unfertilized plants. Similarly, another parasitoid species, *Diadegma insulare*, performed better on their host *Plutella xylostella*

when this fed on *Brassica napus* plants that had received 3× as much fertilizer as the controls (Safraz et al. 2009). Members of the family Brassicaceae, including *Arabidopsis thaliana* and *Brassica oleracea*, emit volatile compounds in response to insect damage that attract predators and parasitoids (Van Poecke et al., 2001 and Mattiacci et al., 1994). *Cotesia marginiventris* have been shown to be attracted to plants damaged by their hosts (Hoballah et al., 2002) Therefore, vermicompost could potentially influence parasitoid response and performance by altering direct (non-volatile) and indirect (volatile) plant responses to caterpillar herbivory.

For all the above reasons, this study will also investigate the potential affect of vermicompost on the searching behavior of *C. marginiventris* (Hymenoptera:Bracconidae), a parasitoid of caterpillars of the family Noctuidae on vermicompost-grown plants damaged by *H. zea* larvae. In addition, potential detrimental effects of vermicompost-mediated resistance on parasitism rate, survival and development of the wasp will be evaluated.

From previous literature we hypothesize that vermicompost will positively affect the preference and performance of *P. rapae* and negatively affect the preference and performance of *H. zea*. We also expect that vermicompost treatment will negatively affect development , but positively effect searching behavior of *C. marginiventris*.

Materials and methods

Plant and insect rearing

Cabbage, *Brassica oleracea* var. *capitata* 'Early Jersey' seeds were planted in 6-inch pots in Sun-Gro Redi-Earth (Sun Gro Horticulture) potting mix amended with 0 (control) or up to 60% food based vermicompost (Oregon Soil Corporation, Portland, OR). Plants were grown in environmentally-controlled chambers with a 10: 14 h Light: Dark cycle and maintained at 22: 20 °C, respectively. Plants were watered with 100ml of deionized water every day; all plants received 100 ml of nutrient solution instead of water once a week. The nutritional breakdown for all the substrates and fertilizers used in this project is given in Table 1. Plants were used for the experiments when they reached their 8th or 10th true-leaf stage.

Corn earworms, *Helicoverpa zea* Boddie, and imported cabbageworms, *Pieris rapae* L. were raised individually on artificial diet in an incubator maintained at 25 ± 2 °C and 10: 14 h L:D until needed for the experiments at either their 3rd instar or adult stages.

Cotesia marginiventris were originally obtained from a colony maintained by Dr. John Ruberson's laboratory at the Department of Entomology (University of Georgia, Athens, GA) and were subsequently reared on *H. zea* larvae from the colony mentioned above. Adult wasps were kept in BugDorm insect rearing cages (catalog #1452, Bioquip, Rancho Dominguez, CA); adults were supplied with ten percent sugar water and deionized water. One hundred fifty 1st to 2nd instar larvae were placed on cabbage leaves inside the cage

for the adult wasps to oviposit upon every 48 h., after two days larvae were taken out of the cage and placed on artificial diet (5 per cup). Cocoons produced from each batch were transferred from diet cups to a wasp emergence cage as they were formed. Wasps and parasitized caterpillars were kept in a room maintained at 25 ± 2 °C and 10: 14 h L:D.

Larval feeding choice assays

Immature feeding adult oviposition assays were performed to determine if vermicompost-mediated host plant resistance is due to antixenosis effects. Vermicompost treatments used for these experiments were 0 (control), 20, 40 and 60% vermicompost. Immature feeding preference for vermicompost treatment was evaluated using a four-choice leaf disc (2.5 cm diam) assay. Plants used to obtain the discs were representative from each treatment and leaf discs were taken from either the second oldest (Old) or second youngest (Young) leaves from plants at the 8th true-leaf stage. discs were weighed, and placed atop two layers of #1, 9 cm Whatman filter paper (Fisher Scientific, Pittsburgh, PA) moistened with 4 ml of deionized water in a plastic Petri dish (14 cm diam). Four leaf discs, one from each of the treatments, were randomly placed in 1 of 4 positions, equidistant from each other and the center of the dish. Care was taken so that each plate arena received discs from either young or old leaves from all treatments, discs from different age leaves were never mixed. Two third instar caterpillars (either *H. zea* or *P. rapae*) were placed in the center of the dish; the dish was covered and placed in an incubator where insects were kept. Larvae were allowed to feed freely on the discs for 24 h. Leaf discs were weighed before and after insect

feeding to determine percent tissue consumed for each leaf disc. These experiments were set up using complete randomized block design containing four randomly spaced 4-way choice blocks (to control for any potential positional effects within the growth chamber) and were repeated over time in trials using new sets of plants and insects to obtain a total of 24 replicates for each insect species. Each block was made up of 1 Petri dish containing 4 leaf discs, 1 replicate from each treatment. As mentioned before, all leaf discs within a plate were from same age leaves, but plates containing discs from young or old leaves were tested at the same time.

Adult oviposition choice assays

Adult oviposition assays were performed to determine if vermicompost-mediated host plant resistance is due to antixenosis effects. Two mated pairs of 2-3 d old *H. zea* or *P. rapae* adults were released into a 3ft × 3ft × 3ft PVC-framed cage covered with a translucent chiffon mesh. Each cage contained one plant (10 true leaves) from each of the treatments (4 plants total). Plants were randomly assigned to each corner of the cage. Four replicates were conducted in each of 3 trials (different dates) for a total of 12 replicates per insect species. The experiment was run under greenhouse conditions, 14:10 L:D and 25°C ± 5°C. Adults were allowed to oviposit for 48 hours after which, plants were collected and the number of eggs on each plant were counted and recorded. Experiments for each insect species were conducted separately.

No choice larval performance assays

The main aim of these assays was to determine if vermicompost-mediated resistance is due to antibiosis (deleterious compound) effects on the immature insects. For this we used plants with 10 true leaves grown in 0 (control), 5, 10, 15, 20, 40 or 60% vermicompost. Plants were infested with one 3rd instar larva of one insect species. Larvae were confined to a single plant by a chiffon sleeve, which was supported by a wire loop buried in the soil and sealed around the pot with a large rubber band. The sleeve was tied off at the top. Plants were kept in environmental growth chambers; under the same conditions at which they were grown. Caterpillars were weighed and then allowed to feed and develop on the plants until they either pupated or died. Data were collected on time to death, time to pupation and weight at pupation. Due to space and logistic constraints, 20, 40 and 60% vermicompost treatments were tested first with each of the insect species. Experiments for *H. zea* and *P. rapae* were also conducted separately. Since 20% vermicompost was found to have a negative effect on *P. rapae* performance, but not *H. zea*, 5, 10 and 15% vermicompost treatments were only tested with *P. rapae*. In each concentration range plants grown in unamended soil (0% vermicompost) were included for direct comparison. Since performance of *P. rapae* did not differ on the control plants, data from obtained from the two vermicompost concentration ranges were analyzed collectively. These experiments were set up using complete four-way choice randomized block design containing 1 replicate of each vermicompost concentration and 1 control (blocking was to control for any variation in environmental conditions within the growth chamber). Trials containing 1-4 replicates

(based on plant availability) were set up at any given time and 4 trials were set up to yield a total of 16 replicates for each species for vermicompost concentrations of 20-60% and 20 replicates for *P. rapae* on vermicompost concentrations of 5-15%.

Generalist performance on young vs old leaves

While conducting experiments for the previous section, it was observed that *P. rapae* preferentially fed on younger plant tissue (upper canopy); whereas, *H. zea* fed indiscriminately. Therefore, we decided to investigate if this preference might play a role in attenuating vermicompost-mediated resistance against the generalist *H. zea*. For this we used plants with 10 true leaves grown in 0 (control), 20, 40 or 60% vermicompost. This was investigated by confining 3rd instar *H. zea* larvae to just the upper canopy, consisting of four young, not yet fully expanded leaves when plants had 12 true leaves. Insects were weighed and allowed to feed and develop until death or pupation. Experimental design and data collected were as in the previous section for a total of 17 replicates for each treatment.

Vermicompost effects on parasitoid searching behavior and performance

Parasitoid searching behavior: To evaluate potential indirect effects of vermicompost-mediated plant resistance on tri-trophic interactions, cabbage plants were individually infested with 8 2nd instar *H. zea* larvae for 48 hours. Larvae were confined to the plants using chiffon sleeves as in the herbivore performance assays. After this period, chiffon sleeves and larvae were removed and one plant from each treatment was then randomly assigned its own

corner of a 61 cm³ cage. The cage frame was made from 1.27cm diam PVC pipe and covered with organza mesh. Ten 3-5 day old mated adult female wasps were released in the center of the cage, above canopy level. Adult wasps were allowed to acclimate to their new environment for 15 minutes and then observed for 15 minutes. Discovery time (time to first landing on a plant), total number of landings and total time spent per treatment were recorded. The experiment was set-up in single replicates consisting of 4-way choice assays containing one plant from the control and 3 vermicompost concentrations (20, 40 and 60%). This experiment was repeated over time with new sets of plants and insects to obtain a total of 20 replicates.

Parasitoid performance: To evaluate possible negative host-mediated effects of vermicompost grown plants on parasitoid development, sixteen 1st-2nd instar *H. zea* larvae were placed in a 30-ml. clear plastic cup (product #9051, Bio-Serv, Frenchtown NJ) with 3-5 day old mated female wasps for one hour. Four of the exposed larvae were then placed on one plant (10 true leaves, approx. 1 month old) from each treatment and confined to the plant with chiffon sleeves. Parasitoid time to pupation and emergence, percent host parasitism and cocoon weight were recorded. This experiment was set-up in completely randomized blocks containing one replicate of each treatment per block and four blocks per trial (blocking was used to control for variation in any environmental effects within the growth chambers). Five trials were run over time to obtain a total of 20 replicates per treatment.

Vermicompost effects on soil and plant growth and tissue nutritional composition

We also obtained data to determine if host-mediated effects on the insects are due to the nutrition composition in vermicompost-grown plants. First, nutritional analyses of cabbage tissue grown in 0, 20, 40 and 60% vermicompost were performed at the North Carolina Department of Agriculture (NCDA) Agronomics Division, Raleigh, NC. For these analyses, plants were grown as above but were not exposed to insect damage. A total of 6 composite replicates (2-3 8 true-leaf plants each) of plant tissue were placed in paper bags and dried in a 60 °C oven for 7-10 d prior to the analyses. Data obtained from these analyses were used to estimate C:N ratios and to compare levels of the various nutrients in plants across vermicompost treatments.

To determine if insect preference and performance was affected by differences in plant size across treatments, leaf area measurements were obtained for plants grown in the various vermicompost treatments and compared to those from the unamended controls. Plants were grown as described for the insect experiments but were not exposed to insect damage. Once the 10th true leaf was 5cm long, leaves were removed from the plant and the total leaf area was recorded using a canon RE 350 video vizualizer and Computer Imaging Analysis Software (C.I.A.S). Leaves for total of 10 replicates for each treatment were measured and mean leaf area were calculated and compared.

Additionally, the amount of nutrients (N:P:K) provided by the potting mix (Sun-Gro Redi-Earth) and vermicompost substrates and the Hoagland's fertilizer solution (NCSU Phytotron) were also obtained and are provided in Table 1.1.

Statistical analyses

All data was analyzed with SAS version 9.1.3 (SAS Institute, Cary, NC, 2006).

Data for assays with each insect species were analyzed separately.

Larval feeding choice assay data was analyzed with PROC MIXED where "response" (leaf disc percent eaten) to "treatment" (vermicompost concentration) was modeled following a linear mixed model, with "treatments" considered as fixed-effect factor and "random" considered as random effects (residual variation) and "time interval" considered as repeated measures. Significant effects were then followed by Tukey's mean separation tests $\alpha \leq 0.05$.

Data obtained for wasp preference choice assays were subjected to analysis of variance (ANOVA, proc GLM) for possible effects of treatment on discovery time, number of landings, and total time spent on each treatment. Data for all other herbivore and parasitoid preference and performance assays were also analyzed using ANOVAs to test for effects of trial, block, treatment and their interactions on each of the variables of interest. All significant ANOVA effects were followed by Tukey's mean separation tests at $\alpha \leq 0.05$. Even though experiments were conducted at separate times, *P. rapae* larval performance on control plants for each of the vermicompost ranges tested did not differ, therefore these data were analyzed collectively.

Additionally, estimates for intercepts and slopes for leaf area, C/N ratios and percent tissue nitrogen content in response to vermicompost concentration were obtained using regression analysis (proc Reg)..

Results

Effect of trial, block and their interactions with vermicompost treatment were not found to be significant in any of the experiments. Amending potting soil with vermicompost had no negative effect on immature feeding preference by *P. rapae*. However, it was observed that *P. rapae* larvae tended to consume more of the tissue when given discs from younger leaves, as evident by an overall higher percent leaf consumption of the younger leaf discs compared to older leaf discs (Table 1.2). On the other hand, *H. zea* larvae preferentially fed on young leaf discs from plants grown in 60% vermicompost compared to young leaf discs from control plants ($t = -2.67$; $df = 3, 16$; $P = 0.0166$) (Table 1.2). Despite repeated attempts, *H. zea* did not oviposit on the cabbage plants. This was not the case for *P. rapae*, which laid significantly more eggs on plants grown in 60% vermicompost compared to all other treatments ($F = 56.73$; $df = 3, 44$; $P < .0001$) (Figure 1.1). Thus, vermicompost did not have a non-preference effect against *P. rapae* or *H. zea* larvae.

No differences were observed in the survival or development of *H. zea* larvae among treatments in the no-choice antibiosis experiments (Table 1.3). However, there was a consistent significant decrease in the survival rate of *P. rapae* on plants grown in soil amended with as little as 20% vermicompost and up to 60% ($F = 5.48$, $df = 6, 121$, $P = <0.0001$) (Figure 1.2).

Since a feeding preference was observed with *P. rapae* for young compared to old leaves in our leaf disc feeding assays, we conducted a follow-up experiment exploring the potential role of feeding behavior in the lack of resistance observed against the generalist, *H. zea*, which did not discriminate between leaf tissues of different ages. Results from this

experiment show that *H. zea* performed equally well when confined to feeding on young (top canopy) or old (bottom canopy). Thus, differential performance could not be explained by the difference in larval feeding preferences observed between the two species (Table 1.4).

Contrary to what we expected, vermicompost treatments had no effect on attraction of the parasitic wasp *C. marginiventris* to infested cabbage plants (Table 1.4). In fact, number of landings, time to discovery or total time spent on plant were not statistically different for any of the treatments tested (Table 1.5). Vermicompost treatments also had no effect on any of the wasp development parameters measured (Table 1.5).

Vermicompost treatment was found to not be correlated with C:N ratios of plant tissue ($R^2 = -0.0208$; $P = 0.4376$) (Figure 1.4) but was weakly correlated with percent nitrogen within plant tissue ($R^2 = 0.1200$; $P = 0.0542$;) (Figure 1.5). Furthermore, nutrient levels in plant tissues increased with increasing vermicompost concentration (Table 1.6) but, except for manganese, differences in the levels of tissue nutrients between the control and 20% vermicompost treatment were not statistically significant (Table 1.6). Regression analyses for vermicompost treatment and tissue nutrient levels were also not significant. . There was a slight trend for the leaf area to increase as amount of vermicompost added to the growing media increased and a weak positive correlation ($R^2 = 0.1395$; $P = 0.0585$), however treatment effects on mean leaf area were not statistically significant for any of the vermicompost treatments tested (Figure 1.3).

Discussion

Results from the present study show that vermicompost soil amendments at concentrations $\geq 20\%$ confer resistance against *P. rapae* that does not affect insect feeding preference, but reduces the performance of the larvae on cabbage. This resistance is not effective against *H. zea*, in fact, we found that *H. zea* larvae preferred to feed leaf discs from plants grown in 60% vermicompost compared to those grown in control soil, when offered young leaf tissue. Moreover, our evaluation of the potential deleterious effects of vermicompost-mediated resistance on the parasitoid *C. marginiventris*, yielded no significant differences in responses or performance on the vermicompost treatments tested.

Agrawal (2000b) found that both specialists *P. rapae* and *Plutella xylostella* and the generalist *Spodoptera exigua* induced resistance in wild radish plants (*Raphanus sativus*), but the generalist *Trichoplusia ni* did not. It is then possible that the lack of observable vermicompost-mediated resistance in *B. oleracea* against *H. zea* could be due to larval feeding not inducing a plant defensive response. Musser et al (2002) found that ablation of the spinnerets of *H. zea* caterpillars reduced its weight and survival on tobacco; whereas survival and weight gain of caterpillars with intact spinnerets were not significantly reduced. These authors found that spinneret secretions contained enzymes that interfered with defense induction in tobacco plants. A similar phenomenon may be happening with *H. zea* where caterpillars could produce salivary compounds that prevent defensive response elicitation in cabbage. If this were the case, it would mean vermicompost-mediated resistance is due to inducible rather than constitutive compounds, something we did not ascertain in this study. The exact mechanism for the lack of vermicompost-mediated

resistance in cabbage to *H. zea* is a subject that merits attention in future research.

We found no antixenosis effects on larval feeding and adult oviposition assays conducted with either species. To the contrary, *H. zea* larvae significantly preferred feeding on young leaf discs of plants grown in 60% vermicompost over those from plants grown in control soil. This could potentially be due to the increased nitrogen content we found in leaf tissue of vermicompost-grown plants. In a similar manner, *P. rapae* adults showed a strong oviposition preference for plants grown in 60% vermicompost over all other treatments. Insect host preference is mediated by visual, tactile and chemical factors. For example, it has been shown that larger plants are more attractive to females of *P. rapae* than smaller plants (Ives, 1978). We found that plants grown in soil amended with 60% vermicompost tended to be have larger leaves than those grown in control and 20% treatments. Although this difference was not statistically significant, it could partly account for the *P. rapae* oviposition preference observed. *Pieris rapae* adults have sensitive chemoreceptors on their tarsal pads used for detecting the chemical composition in the leaves (Renwick et al., 1992, Stadler et al., 1998). Given the trend of lower C:N ratios (reflective of higher nitrogen concentrations in plant tissues) and larger leaf area obtained of vermicompost-grown plants, oviposition preference by *P. rapae* could be due to size and nutrient levels in these plants. Secondary plant compounds such as glucosinolates can also affect host selection and preference in insects, particularly specialists (Renwick, 2002). Although we did not measure levels of secondary compounds in these plants, these have been shown to be regulated by nutrient levels in the plants, especially nitrogen (Halkier and Gershenzon, 2006). Therefore, increased defense compound levels could have led to the insect behavioral responses

documented herein.

Interestingly and in contrast to most other studies, where *Brassica* resistance due to defensive compounds is more effective against generalists (Agrawal, 2000a, Agrawal, 2000b), our data show strong antibiosis effects of vermicompost-grown plants against the specialist *P. rapae*, while no negative effect was observed on the generalist *H. zea*. These antibiosis effects led to poorer performance of the specialist *P. rapae* on plants grown in vermicompost treatments compared to those grown in control unamended soil. Interestingly, significant vermicompost-mediated resistance against *P. rapae* was only observed for treatments containing 20% vermicompost and did not increase significantly with higher vermicompost concentrations. Our findings are in accord with Agrawal (2000b) who reported induction of secondary defense compounds in wild radish effective against caterpillars of the specialist *P. rapae* and *Plutella xylostella* L, but not the generalist *Trichoplusia ni*. Even further, these effects could not be explained by glucosinolate levels, as Poelman et al. (2008) showed that, even when exposed to increased levels of glucosinolates, the performance of the specialist *P. xylostella* was similar to the performance of the generalist *M. brassicae* on cabbage plants. These results suggest that specialist defenses in *Brassica* plants may be caused by factors other than glucosinolate up-regulation.

Since no direct negative effects of vermicompost treatments upon *H. zea* were observed, we thought an indirect effect through enhanced recruitment of natural enemies might still be possible. However, we found no effects of vermicompost treatment on parasitoid responses to *H. zea* damaged host plants or performance on *H. zea* fed on vermicompost-grown cabbage. Previous research reported increased searching ability,

but lowered parasitoid performance on herbivore-induced plants (Havill and Raffa 2000, Thaler 2002). Since we did not see any effects of vermicompost treatments on the *C. marginiventris*, we presume this to be due to lack of host response elicitation by *H. zea* feeding upon cabbage plants, but this is something yet to be confirmed.

Although vermicompost is high in nutrients it does not appear that elevated nutrient levels in the substrate or within plant tissue are responsible for the increased plant resistance found in our system. Except for manganese, there were no differences in nutrient content between plants grown in 0% compared to 20% vermicompost. Yet, there was significantly lower survival of *P. rapae*, starting with 20% vermicompost amendments to the soil. We are not aware of any scientific studies linking manganese levels with plant resistance to pests.

Results obtained for this study show that the vermicompost mediates antibiosis resistance against specialist caterpillars and that this resistance is not due to plant size or nutrient availability within plant tissue. Furthermore, vermicompost applications do not appear to have a negative effect on the tri-trophic interaction tested, but this may change if evaluated with a different host-parasitoid system, since vermicompost did not appear to induce resistance against *H. zea* in cabbage plants. Future studies need to investigate if vermicompost-mediates resistance against sucking insects, such as aphids, and if the mechanisms involved are the same as those effective against caterpillars. Furthermore, the factors in vermicompost responsible for mediating resistance against insect pests also remain to be identified.

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Table 1.1. Amounts of major nutrient (NPK) provided by each of the substrates used to grow plants in each plant treatment. Values represent means for 3 substrate samples.

Medium (100 g)	Main nutrients (g)		
	Nitrogen	Phosphorous	Potassium
Potting Mix	0.92	0.14	1.22
OSC vermicompost	1.30	0.29	0.82
Hoglands	1.06	0.14	1.11
Vermicompost treatments (%)			
5	0.16	0.035	0.18
10	0.31	0.067	0.27
15	0.42	0.093	0.27
20	0.52	0.11	0.32
40	0.84	0.19	0.53
60	1.06	0.24	0.67

Table 1.2. Larval choice feeding assay; vermicompost treatments had no affect on the feeding preference of *Pieris rapae*. However, when offered young leaf tissue, *H. zea* larvae preferred leaf discs from plants grown in 60% vermicompost compared to the control. Values represent mean percent of leaf disc eaten \pm standard error from 24 replicates. Means within rows with the same letters are not significantly different at $\alpha \equiv 0.05$.

Species	Leaf age	Treatment			
		Control	20	40	60
<i>H. zea</i>	Young	5.6 \pm 1.38a	9.8 \pm 2.60ab	7.5 \pm 1.76ab	15.8 \pm 3.85b
	Old	4.6 \pm 1.50a	5.7 \pm 0.91a	13.6 \pm 5.19a	4.6 \pm 0.98a
<i>P. rapae</i>	Young	25.2 \pm 7.33a	15.5 \pm 3.53a	13.2 \pm 3.59a	18.5 \pm 5.13a
	Old	7 \pm 1.22a	7.3 \pm 1.28a	8.9 \pm 1.90a	8.2 \pm 1.76a

Table 1.3. Performance of *H. zea* larvae in no choice assay. Vermicompost treatment did not significantly affect immature performance. Values represent means \pm standard errors from 48 replicates.

Variable	Treatment			
	Control	20	40	60
Weight Gain (mg)	143.3 \pm 43.91	132.1 \pm 39.19	162.5 \pm 48.76	148.4 \pm 44.02
Time to pupation (d)	19.4 \pm 0.92	18.4 \pm 1.25	20 \pm 1.44	17.4 \pm 1.72
Insect survival (%)	63.6 \pm 15.21	58.3 \pm 14.86	50 \pm 15.08	58.3 \pm 14.86

Table 1.4. *Helicoverpa zea* performance in no choice assay on young (top canopy) leaves: Vermicompost treatment did not significantly affect immature performance. Values represent means \pm standard errors from 44 replicates.

Variable	Treatment			
	Control	20	40	60
Weight Gain (mg)	250.3 \pm 16.77	241.1 \pm 8.43	269.3 \pm 26.06	257 \pm 13.95
Time to pupation (d)	20 \pm 2.54	18.4 \pm 1.25	21.3 \pm 1.27	19 \pm 1.42
Insect survival (%)	60 \pm 13.09	43.8 \pm 12.81	56.3 \pm 12.81	62.5 \pm 12.50

Table 1.5. *Cotesia marginiventris* preference for *H. zea*-damaged, and performance on *H. zea* fed on, vermicompost-grown cabbage. Vermicompost had no effect on wasp attraction to plants or performance. Values represent means \pm standard errors from 20 replicates.

Treatment (%)	No. landings	Total Time on Plant (min)	Time to Discovery (min)	Pupation (d)	Emergence (d)	Survival (%)	Weight (mg)
0							
	3.75 \pm 0.33	3.60 \pm 0.43	3.11 \pm 0.35	11.09 \pm 0.29	16.35 \pm 0.40	58.75 \pm 6.09	284.35 \pm 7.99
20							
	3.8 \pm 0.44	3.50 \pm 0.30	3.65 \pm 0.31	10.95 \pm 0.24	16.00 \pm 0.28	52.50 \pm 5.99	280.47 \pm 8.55
40							
	4.25 \pm 0.38	3.27 \pm 0.33	3.41 \pm 0.46	11.19 \pm 0.26	16.40 \pm 0.34	56.25 \pm 4.76	281.15 \pm 8.67
60							
	3.7 \pm 0.46	2.95 \pm 0.29	3.85 \pm 0.58	11.01 \pm 0.27	16.09 \pm 0.36	46.25 \pm 5.22	275.00 \pm 7.40

Table 1.6. Nutrient analysis of plant tissue from plants grown under various vermicompost treatments. Means within columns followed by the same letter are not significantly different. (Tukey's mean separation test, $P < 0.05$).

Nutrient	Treatment			
	0	20	40	60
B	31.18a	31.30a	35.58a	37.82a
Ca	1.79a	1.74a	1.85a	1.92a
Cu	5.83a	5.17a	5.60a	5.68a
Fe	167.43a	87.68a	101.92a	73.23a
K	3.43a	3.06a	3.66a	4.46a
Mg	0.70a	0.54a	0.55a	0.50a
Mn	98.55a	20.53b	22.20b	21.92b
Mo	0.64a	0.74a	0.76a	0.65a
N	2.68a	2.31a	2.78a	3.71a
Na	0.09b	0.09b	0.13ab	0.18a
P	0.56a	0.52a	0.57a	0.62a
S	1.26a	1.02a	1.05a	0.99a
Zn	30.60b	31.37b	37.42ab	46.05a

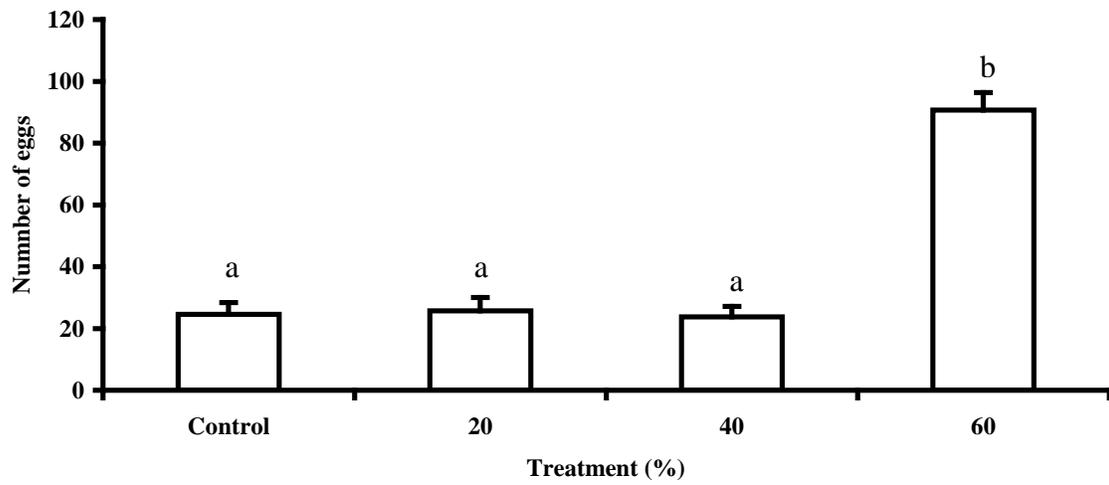


Figure 1.1 *Pieris rapae* oviposition preference on cabbage plants grown in varying vermicompost treatments. Adults showed a significantly higher oviposition preference for plants grown in 60% vermicompost over all other treatments. Bars headed with the same letter are not significantly different, Tukey's mean separation test ($P < 0.05$), $n=12$.

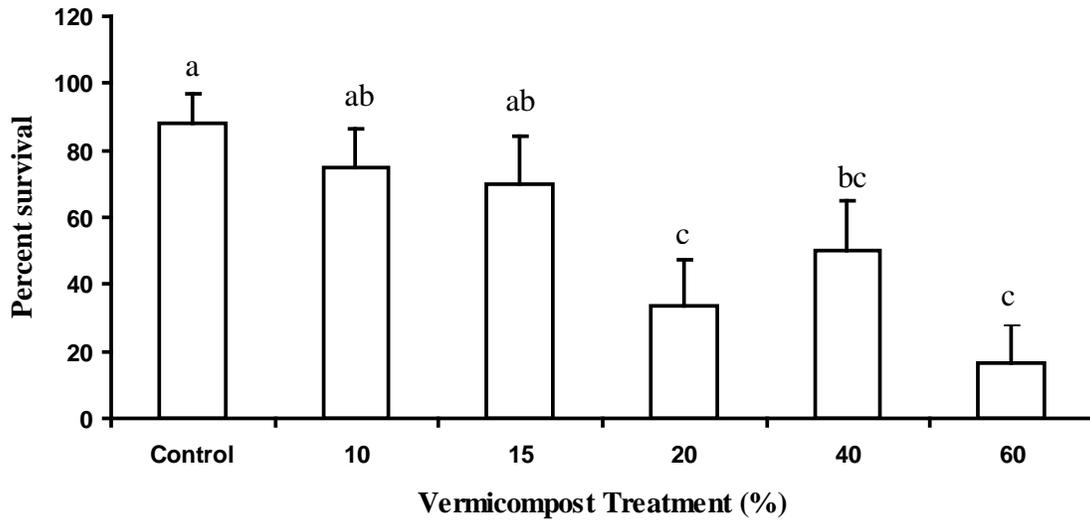


Figure 1.2. *Pieris rapae* mean percent immature survival (3rd instar to pupa) on cabbage grown in soil containing various concentrations of vermicompost. Bars headed with the same letter are not significantly different, Tukey's mean separation test ($P \geq 0.05$), 12 replicates for 20-60% vermicompost and a control group and 20 replicates for 5-15% vermicompost and a control group.

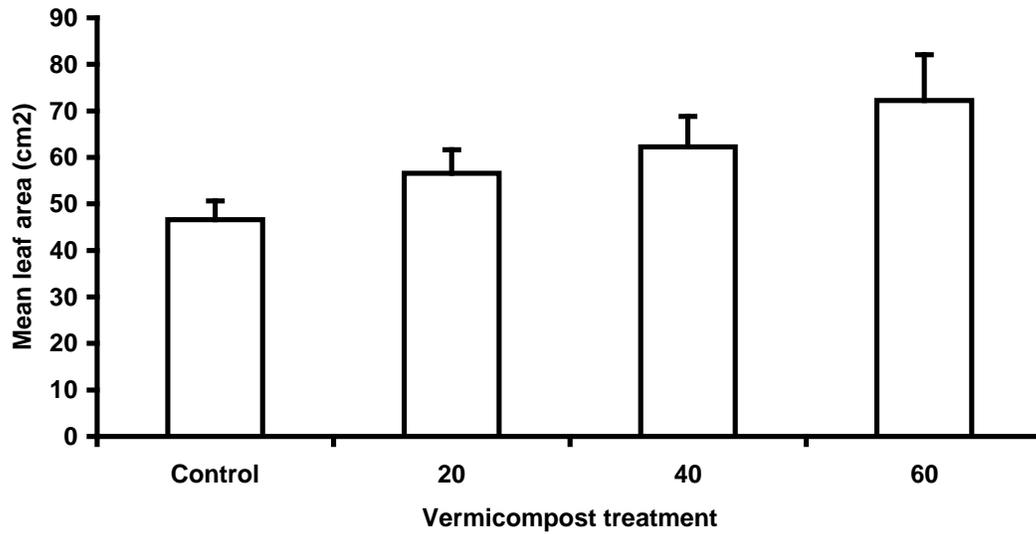


Figure 1.3. Mean leaf area per plant: There were no significant differences among any of the treatments at $\alpha = 0.05$. There was no significant correlation between leaf area and vermicompost treatment.

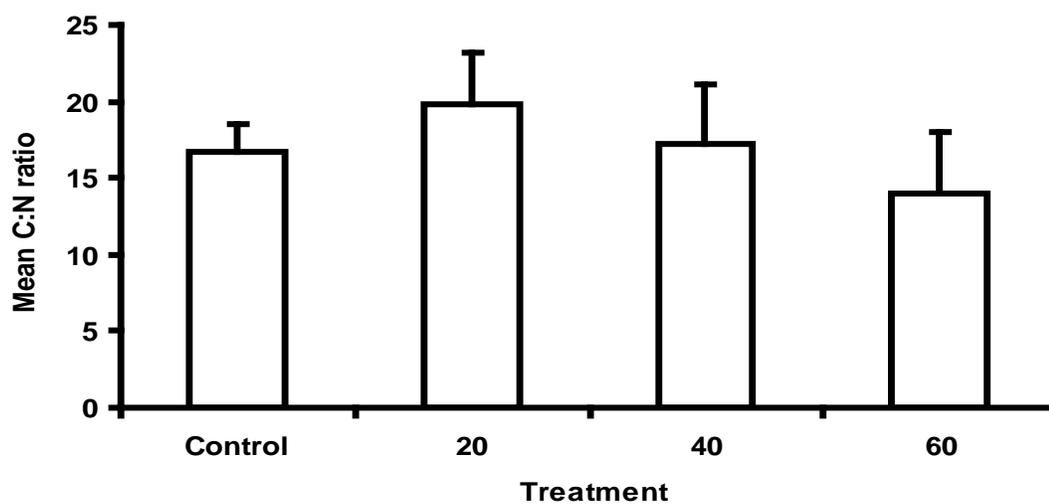


Figure 1.4. Mean tissue Carbon:Nitrogen ratio for cabbage plants grown under various vermicompost treatments: Vermicompost had no effect on C:N ratio of cabbage plants at $\alpha = 0.05$. No significant correlation between C:N ratio and vermicompost treatment was found.

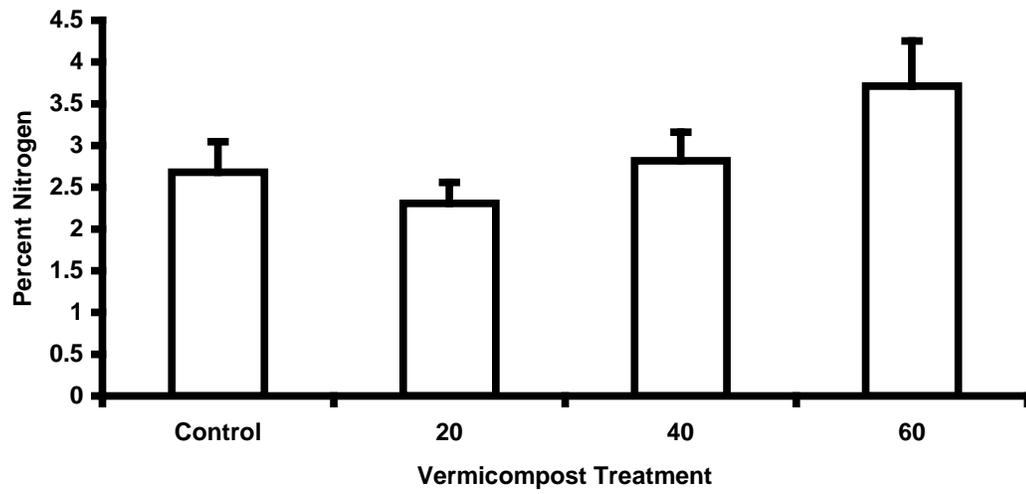


Figure 1.5. Mean percent nitrogen in leaf tissue; percent nitrogen was weakly correlated with vermincompost treatment ($R^2 = 0.1200$; $P = 0.0542$).

Chapter II: Bottom-up effects mediated by an organic soil amendment on the cabbage aphid
pests *Myzus persicae* and *Brevicoryne brassicae*

A. G. Little

Department of Entomology, North Carolina State University,
Campus Box 7613, Raleigh NC 27695

Introduction

Addition of low concentrations of manure- or food-based vermicompost to soil or potting mixes have been shown to increase plant growth (Atiyeh et al. 2000a), as well as, germination, growth and yield (Arancon et al., 2003; Arancon et al., 2004a; Arancon et al., 2004b; Arancon et al., 2006; Atiyeh et al., 2000a; Atiyeh et al., 2000b; Gajalakshmi and Abbasi, 2002; Gutierrez-Miceli et al., 2007; Peyvast et al., 2007; Zaller, 2007). These beneficial effects have been documented for marigold (*Tagetes patula* L.), tomatoes (*Solanum lycopersicum* L.), peppers (*Capsicum annuum* L.), lettuce (*Lactuca sativa* L.), spinach (*Spinacea oleracea* L.), crossandra (*Crossandra undulaefolia* Salisb.), and strawberries (*Fragaria x ananassa* L.).

Vermicomposts are high in microbial diversity (Edwards et al., 2010; Fracchia et al., 2006; Vivas et al., 2009) and available plant nutrients (Edwards and Fletcher, 1988). Furthermore, vermicompost has been found to contain considerable amounts of humic substances, which are believed to increase plant growth (Arancon et al., 2006). More importantly for the purpose of this study, vermicompost has been shown to increase host plant resistance to pests such as pathogens (Chaoui et al., 2002; Szczech, 1999), plant parasitic nematodes (Arancon et al., 2002; Edwards et al., 2010) and a variety of arthropods including jassids (*Empoasca kerri* Pruthi), aphids (*Myzus persicae* L. and *Aphis craccivora* Koch), spider mites (*Tetranychus urticae* Koch), mealy bugs (*Planococcus citri* Risso), and caterpillars (*Pieris rapae* L.) (Arancon et al., 2005; Arancon et al., 2007; Edwards et al., 2010; Rao, 2002). Earlier studies in our laboratory using lepidopterous pests (chapter 1) showed that vermicompost can induce plant antibiosis resistance against specialist (*Pieris*

rapae), but not generalist (*Helicoverpa. zea*) caterpillars.

This study was undertaken to evaluate the effect of varying vermicompost concentrations on the preference and performance of two aphid pests of cabbage: the generalist *Myzus persicae* and the specialist *Brevicoryne brassicae* on cabbage (*Brassica oleracea*). Since aphids pierce and suck to feed on phloem within sieve elements of their host plants, the damage they inflict affects plants differently than damage from chewing mouthparts and; therefore, plant defenses activated by, and effective against, these insects are expected to be different than those effective against pests with chewing mouthparts, such as lepidopterous larvae (Howe and Jander, 2008; Smith and Boyko, 2007). Based on previous literature we hypothesize that vermicompost will positively affect the preference and performance of *B. brassicae* and negatively affect the preference and performance of *M. persicae*.

Materials and methods

Plant and insect rearing

Cabbage, *Brassica oleracea* var. *capitata* Linnaeus ‘Early Jersey’(Wyatt-Quarles seed company, Burke Brothers Hardware, Raleigh NC) seeds were planted in 6-inch pots in Sun-Gro Redi-Earth (Sun Gro Horticulture, Bellevue, Washington) potting mix amended with 0 (control), 20, 40, or 60% food based vermicompost (Oregon Soil Corporation, Philomath, Oregon). Plants were grown in environmentally-controlled chambers with a 10:

14 h Light: Dark cycle and maintained at 22: 20 °C, correspondingly. Plants were watered with 100 mL de-ionized water as needed, except for one day a week when all plants received 100 mL of Hoagland's nutrient solution (Chapter 1, Table1) instead of water. Plants were used for the experiments after they reached their 8th or 10th true-leaf stage for preference or performance experiments, respectively.

Cabbage aphids, *Brevicoryne brassicae* L., were field collected from infested broccoli, *Brassica oleracea* L., var Italica plants in Raleigh NC in April of 2008, and green peach aphids, *Myzus persicae*, originated from colonies maintained in Dr. George Jander's laboratory at Cornell University, Ithaca, NY. Colonies of both aphid species were established and maintained on 3 to 4 week old cabbage plants grown in unamended commercial potting mix (Sun-Gro Redi-Earth). Colonies were kept in an incubator under the same conditions described for the plants above. Experimental insects were obtained from colonies established by transferring 10-20 apterous adults on to new host plants weekly.

Feeding preference choice assays

These experiments were designed to evaluate potential negative effects of vermicompost treatments on the host preference of apterous and alate morphs of *M. persicae* and *B. brassicae*. Data from experiments for each insect species were obtained and analyzed separately. Feeding preference of apterous adults for vermicompost treatment was evaluated using a four-choice leaf disc (2.5 cm diam) assay. Leaf discs were taken from the 2nd youngest leaves from plants at the 8th true-leaf stage. Leaf discs from younger leaves were

selected because both species were observed feeding more on young leaves in their respective colonies (AGL, personal observation). One leaf disc was taken from each treatment (0, 20, 40, and 60%) and placed atop one layer of #1 Whatman filter paper (Fisher Scientific, Pittsburgh, PA) over a water-agar slab (to maintain humidity) in a plastic standard Petri dish (9 cm diam). Leaf discs were randomly assigned to one of four positions, using a random number generator, equidistant from each other and from the center of the dish. Experiments for each insect species were performed at different times. Ten newly molted (<24hr old) apterae adults (*M. persicae* or *B. brassicae*) were placed in the center of the dish, the dish was covered, placed in the incubator where colonies were kept and the adults were allowed to move and feed freely among the discs for 24 hours. Aphid numbers on each disc were recorded at 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 h. This experiment was set up in completely randomized blocks containing one replicate of each of the vermicompost treatments in a 4-way choice arrangement (1 block). The experiment was set up in trials containing 4 blocks each and repeated over time using new sets of plants and insects to obtain a total of 24 replicates for each insect species/treatment combination.

Alate alighting and feeding preference were evaluated using a four-way whole plant choice assay. One 10th true leaf plant from each treatment was randomly assigned to a different corner of a 61 cm³ cage. The cage frame was made from 1.27cm diam PVC pipe and covered with organza mesh. Ten newly-molted (<24 h old) alate adults were released in the center of the cage, above plant canopy level, and allowed to land, settle, feed and deposit nymphs for 24 hours. Alate numbers on each of the treatment plants were recorded at 1, 3, 6, 12, and 24 h after the initiation of the experiment. In addition, total number of nymphs on

each treatment plant was recorded at the end of the experiment (24 h). Experiments for each insect species were performed at different times. These experiments were set up in completely randomized blocks containing one replicate of each vermicompost treatment arranged in a 4-way choice set-up. Four such blocks were set up at any given time and the experiment was repeated over time (trials) using new sets of plants and insects to obtain a total of 24 replicates for each insect species/treatment combination.

No choice apterae colonization assays

The main aim of these assays was to determine if vermicompost-mediated resistance is due to detrimental effects on the performance and survival of the selected aphid species. For this we used plants at the 10 true leaf stage to evaluate colonization ability of apterous adults on plants under the various treatments. Plants were infested with 5 newly molted (<24 h) apterous adults of either species. Aphids were confined to a single plant with a chiffon sleeve that was tied off at the top, supported by a wire loop buried in the soil at either side of the plant, and held snugly around the pot with a large rubber band (Figure 1). Plants were kept in environmental growth chambers under the conditions mentioned previously. Adults assigned to each plant were collectively weighed at the start of the experiment and then allowed to feed and reproduce on the plants for two weeks. At the end of the two week period, all insects were removed from individual plants and final mass and number of adults and nymphs were counted and recorded and the number of nymphs produced per adult was calculated for each treatment. This experiment set up with completely randomized blocks containing one replicate of each vermicompost treatment. The experiment was repeated over

time in trials of 4-5 blocks each to obtain a total of 17 replicates for *M. persicae* and 21 replicates for *B. brassicae*. Experiments for each insect species were performed at different times.

Statistical analyses

All data were analyzed using SAS version 9.1.3 (SAS Institute, 2006) and data for each insect species were analyzed separately. Apterous and alate choice assay data were analyzed with Proc MIXED where "response" (number of aphids per leaf disc) was modeled following a linear mixed model, with "treatments" (vermicompost concentration) considered as fixed-effect factor and "random" (random variables) considered as random effects (residual variation) and "repeated factor" (time intervals) considered as repeated measures. Significant effects were then followed by Tukey's mean separation tests at $\alpha \leq 0.05$. Treatment and treatment by trial effects on numbers of apterous aphids per leaf-disc or alate numbers per plant were tested for each time interval separately. Similarly, effects of treatment and treatment by trial (block) effects were tested for the number of nymphs deposited on each of the treatments in the alate choice tests.

All other data were analyzed using analyses of variance using proc GLM. For aphid performance in no-choice assay the effects of trial, block and treatment on biomass, number of adults and nymphs were tested. All significant effects were followed by Tukey's mean separation tests at $\alpha \leq 0.05$.

Results

Vermicompost treatment had no negative effect on the feeding preference of the apterous phase of either aphid species (Table 2.1). To the contrary, the specialist *B. brassicae* settled and fed more on 20% vermicompost treatment discs at 3 h ($t = -3.78$; $df = 3, 15$; $P = 0.0088$) and 5 h ($t = -2.94$; $df = 3, 15$; $P = 0.0447$) and on 40% vermicompost treatment discs at time 6 h ($t = -3.36$; $df = 3, 15$; $P = 0.0199$) compared to the control discs (Table 2.1). Contrastingly, *M. persicae* appeared to prefer the control and 20% discs over those of 40 and 60% vermicompost treatments, but differences were not statistically significant (Table 2.1).

While vermicompost had a significant negative effect on the alighting preference of *M. persicae*, it did not have the same effect on *B. brassicae* at any of the time intervals observed (Table 2.1). On average *M. persicae* preferentially alighted on control plants over vermicompost grown plants (Table 2.1). On the other hand, nymph deposition by alate morphs of both species was significantly and negatively affected by vermicompost treatments (figures 2.2 and 2.3). *Myzus persicae* alates deposited significantly higher numbers of nymphs on control than on vermicompost-grown plants ($F = 10.89$; $df = 3, 92$; $P < .0001$) (Figure 2.2). As mentioned previously, *B. brassicae* alates showed no alighting preference towards any treatment (Table 2.1), but deposited significantly lower numbers of nymphs on plants grown in 20% vermicompost compared to control plants ($F = 3.16$; $df = 3, 92$; $P = 0.0285$) (Figure 2.3).

Results from our apterae colonization assays revealed that plants grown in all three vermicompost treatments yielded significantly lower *M. persicae* insect biomass ($F = 6.71$; df

= 3, 64; $P = 0.0005$) and fewer adults ($F = 6.18$; $df = 3, 64$; $P = 0.0009$) and nymphs ($F = 6.05$; $df = 3, 64$; $P = 0.0011$) than control plants (Figure 2.4). Contrastingly, only vermicompost treatments of 20% had significantly lower *B. brassicae* biomass ($F = 4.88$; $df = 3, 80$; $P = 0.0036$) and numbers of adults ($F = 4.90$; $df = 3, 80$; $P = 0.0035$) and nymphs ($F = 3.42$; $df = 3, 80$; $P = 0.0212$) when compared to those of control plants. Interestingly, performance of *B. brassicae* on 40% and 60% were equivalent to those of insects on control plants (Figure 2.5). Moreover, the number of nymphs produced per adult did not differ significantly across treatments regardless of insect species (Table 2.2).

Discussion

Results from this study show that cabbage grown in soil amended with varying concentrations of vermicompost does not negatively affect feeding preference by apterae of either aphid species tested, but significantly reduces alighting preference by the generalist, *M. persicae*, and nymph deposition by alates of both species.

Amendments of 20% vermicompost reduced the colonization ability of apterae of both species as measured by biomass accumulation and numbers of adults and nymphs compared to the control. The poorer insect performance on vermicompost treatments does not seem to be due to lower fecundity of aphids since the nymph to adult ratio is not statistically different across treatments for either species (Table 2.2). Thus, we suspect that development time is slower or mortality rate is higher for insects on the vermicompost treatments, but this remains to be determined.

Some specialist insect species use secondary metabolites as cues in host selection

(Powell et al., 2006). For example, application of the glucosinolate sinigrin to the non host plant *Vicia faba* kept the specialist *B. brassicae* on the plant after initial probing (Powell et al., 2006). Hence, the observed feeding preference of *B. brassicae* apterae for plants in the vermicompost treatments over the controls could be due to increased glucosinolate content in the vermicompost-grown plants. The chemical compounds responsible for the resistance against these aphid species observed in vermicompost-grown plants are not known, but given the detrimental effects on both species, it is also possible that novel types of, or compounds other than, glucosinolates are involved.

The chemical factors in vermicompost mediating resistance to insect pests also remain to be determined. In a recent study done by Edwards et al. (2010) it is suggested that vermicompost-mediated plant resistance to insects is due to phenolic compounds present in the vermicompost, which are taken up by plants. However, we believe this not to be the case in our system because if so, non-preference effects should have been observed in our apterae feeding assays, since phenolic compounds transported in the plants' phloem would have had a deterrent effect upon the insects. The insect feeding deterrent effects of phenolic compounds have been widely studied and reported. For example, aphid feeding deterrence of wheat was found to be due to the phenolic content fraction in these plants (Dreyer and Jones, 1981) and a number of phenolic compounds, including some commonly found in plants, proved deterrent against *M. persicae*, *Acyrtosiphon ptsum* and *Schizaphis graminum* when fed on artificial diet membranes (Jones and Klocke, 1987). Phenolic compounds were also shown to result in decreased growth and increased neonate mortality of *Helicoverpa zea* (Summers and Felton, 1994), and were also

correlated with either the development or survival of *Epirrita autumnata* (Haviola et al., 2007). There is also the fact that there was no difference in *B. brassicae* performance between the control and the 40% and 60% vermicompost-grown plants in no-choice antibiosis assays. If resistance was due to phenol-uptake by the plants we should have seen lower feeding leading to lower insect masses and numbers on the higher vermicompost treatments, and not only on the 20% treatment.

Another factor that could be responsible for the increased insect resistance observed is the increased nutrients availability to the cabbage plants grown in the vermicompost treatments. Our nutritional analyses of vermicompost showed this to be a rich source of valuable plant nutrients. Several studies have linked a reduction in pest populations due to nutrients from organic sources (Altieri and Nicholls, 2003; Morales et al., 2001; Yardim and Edwards, 2003). Therefore the increase in *B. brassicae* performance on vermicompost treatments above 20% could be due to increased amounts of nutrients such as nitrogen in the plants (Chapter 1, figure 1.4). This is especially so because nitrogen has been shown to positively influence aphid performance (Jahn et al., 2005; Patriquin et al., 1988; van Emden and Bashford, 1969). However, nutrients do not appear to play a major role in the documented vermicompost-mediated resistance because nutrient content, including nitrogen, of cabbage tissue was not significantly different between control plants and those grown in 20% vermicompost (Chapter 1, Figure 1.4). Yet, plants grown in the 20% vermicompost treatment consistently had significantly negative effects on performance and nymph deposition for both aphid species, as well as, *M. persicae* alate host preference.

As mentioned previously, aphids do not feed by chewing up plant material, as do herbivorous caterpillars, and instead tap into sieve tube elements in the vascular tissue of plants. Therefore, different chemical factors can be expected to confer host plant resistance against these two types of insect pests (Howe and Jander, 2008). One way plants respond to aphid attack is through activation of *R* genes, which are responsible for the production of resistance proteins in plants effective against pathogens, nematodes and some arthropod pests (Howe and Jander, 2008). Vermicompost is rich in microbial activity due to the non-thermophilic conditions during the composting process through earthworm digestion (Fracchia et al., 2006). As such, vermicomposts may contain microorganisms with the ability to interact with the plants' rhizosphere and predispose or "prime" plants to activate defense responses against microbial pathogens, with cross-activity against herbivorous arthropod pests.

Plant defense priming by beneficial soil microbes is a well-documented phenomenon. For example, Timmusk and Wagner (1999) showed that inoculating *Arabidopsis thaliana* with plant growth promoting rhizobacteria (PGPR) increased resistance to pathogens. Since this type of resistance is effective in other parts of the plant away from the site of induction, it is referred to as either systemic acquired resistance, (SAR, induced by virulent, avirulent, non pathogenic microbes and chemical elicitors) or induced systemic resistance (ISR, caused by plant-growth promoting rhizobacteria), and both can be effective against species different from the inducer and can last the whole life of the plant (Durrant and Dong, 2004; Vallad and Goodman, 2004). ISR can be induced by species of non-pathogenic soil-borne bacteria in several different genera including *Pseudomonas*, *Bacillus*, *Serratia*, and *Flavomonas* (Vallad

and Goodman, 2004). For example, *Pseudomonas fluorescens* strain WCS417r has been shown to improve resistance to bacterial leaf, fungal root and leaf-infecting oomycete pathogens in carnation, radish, tomato and *Arabidopsis* (Hoffland et al., 1996).

Pseudomonas and *Bacillus* species have been isolated from vermicomposts (Vaz-Moreira et al., 2008; Gopal et al., 2009). It is then possible that PGPR are present in vermicompost and these microbes are priming plants thereby increasing resistance to insect pests. However, this is an issue that needs to be investigated in future research.

This study shows that vermicompost has an effect on both preference and performance of *M. persicae* and *B. brassicae*; however the species, and even morphs within a single species, are affected differently. Although vermicompost did not affect the preference of the alate morphs of *B. brassicae* directly, the lower numbers of nymphs deposited on plants grown in 20% vermicompost compared to control demonstrates that the alate either spent less time on the plant or these plants were less suitable for colonization after initial probing. Insect colonization encompasses settling, feeding, reproduction and survival. Our data demonstrate lower settling and reproduction on 20% plants compared to control plants and therefore suitability of plants in this treatment for colonization by aphids is lower. Since aphid numbers on the different treatments did not vary significantly over time, yet nymph deposition was significantly affected, we conclude that alate aphids did not perceive vermicompost-grown plants as suitable hosts for themselves and their offspring.

This study is the first to show how vermicompost effects plant resistance to aphid pests and that this resistance is not dose-dependent. Furthermore, we show that vermicompost-mediated host plant effects on aphids are dependent on the morph and species

tested. Although we suggest that PGPR may be responsible for the increased resistance observed in this study, this will need to be tested, along with other feasible hypotheses, in subsequent research.

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Table 2.1. Settling and alighting preference of apterae (leaf discs) and alate (whole plants) morphs of *M. persicae* and *B. brassicae* on cabbage plants grown under various vermicompost regimes.

Species ^c	Morph	Time (h)	Vermicompost treatment (%) ^{a, b}			
			0 (Control)	20	40	60
<i>M. persicae</i>	Apterae	1	1.8 ± 0.24a	1.8 ± 0.42a	2.0 ± 0.21a	2.0 ± 0.26a
		2	2.0 ± 0.29a	2.0 ± 0.39a	1.9 ± 0.24a	1.9 ± 0.31a
		3	2.1 ± 0.30a	2.3 ± 0.28a	1.8 ± 0.24a	2.2 ± 0.35a
		4	2.4 ± 0.26a	2.3 ± 0.32a	2.0 ± 0.24a	2.3 ± 0.38a
		5	2.5 ± 0.36a	2.2 ± 0.32a	2.3 ± 0.25a	2.1 ± 0.33a
		6	2.7 ± 0.36a	2.5 ± 0.35a	2.3 ± 0.26a	2.0 ± 0.31a
		8	2.5 ± 0.39a	2.5 ± 0.35a	2.1 ± 0.25a	2.0 ± 0.32a
		10	2.5 ± 0.36a	2.5 ± 0.26a	2.2 ± 0.25a	2.0 ± 0.32a
		12	2.5 ± 0.32a	2.3 ± 0.22a	2.1 ± 0.27a	2.0 ± 0.32a
		18	2.4 ± 0.36a	2.1 ± 0.29a	2.3 ± 0.34a	2.2 ± 0.33a
		24	2.5 ± 0.40a	2.0 ± 0.28a	2.2 ± 0.33a	2.2 ± 0.32a
		<i>B. brassicae</i>		1	1.8 ± 0.34a	2.7 ± 0.42a
2	1.8 ± 0.29a			3.0 ± 0.39a	2.4 ± 0.44a	2.7 ± 0.36a
3	1.6 ± 0.26a			2.8 ± 0.28b	2.6 ± 0.41ab	2.5 ± 0.35ab
4	1.6 ± 0.29a			2.6 ± 0.32a	2.7 ± 0.37a	2.6 ± 0.40a
5	1.5 ± 0.31a			2.7 ± 0.32b	2.8 ± 0.36ab	2.5 ± 0.36ab
6	1.3 ± 0.37a			2.4 ± 0.35ab	3.0 ± 0.42b	2.4 ± 0.40ab
8	1.5 ± 0.40a			2.4 ± 0.35a	2.9 ± 0.46a	2.4 ± 0.40a
10	1.8 ± 0.46a			2.3 ± 0.33a	2.6 ± 0.43a	2.7 ± 0.39a
12	1.6 ± 0.46a			2.4 ± 0.33a	2.7 ± 0.47a	2.2 ± 0.34a
18	1.4 ± 0.35a			2.2 ± 0.29a	2.8 ± 0.43a	2.4 ± 0.43a
24	1.3 ± 0.32a			2.3 ± 0.37a	2.8 ± 0.48a	2.4 ± 0.42a
<i>M. persicae</i>	Alate			1	1.7 ± 0.25a	0.8 ± 0.16a
		3	2.3 ± 0.19a	0.7 ± 0.18b	1.3 ± 0.21b	1.3 ± 0.14b
		6	2.5 ± 0.18a	0.7 ± 0.18b	1.4 ± 0.25b	1.5 ± 0.19b
		12	2.9 ± 0.19a	1.0 ± 0.15b	1.4 ± 0.20b	1.4 ± 0.19b
		24	3.0 ± 0.25a	1.0 ± 0.19b	1.2 ± 0.23b	1.2 ± 0.18b
<i>B. brassicae</i>		1	1.3 ± 0.19a	1.2 ± 0.19a	1.1 ± 0.21a	1.0 ± 0.20a
		3	1.5 ± 0.20a	1.3 ± 0.22a	1.5 ± 0.19a	1.5 ± 0.24a
		6	2.1 ± 0.22a	1.4 ± 0.22a	1.6 ± 0.20a	1.7 ± 0.24a
		12	2.3 ± 0.24a	1.6 ± 0.22a	1.8 ± 0.24a	1.8 ± 0.21a
		24	2.3 ± 0.25a	1.5 ± 0.20a	1.9 ± 0.25a	2.0 ± 0.25a

^aValues represent mean number of aphids/disc (apterae) or plant (alate) ± SE for each of the treatments

^bMeans within rows followed by the same letter are not statistically different (Tukey's mean separation test, P<0.05)

^cExperiments for each species and morphs within species were conducted separately.

Table 2.2. Mean number of nymphs per adult deposited on cabbage plants under various vermicompost treatments in no-choice apterae colonization assays. Both aphid species produced comparable numbers of nymphs regardless of plant treatment. Values represent means \pm standard errors from 17 replicates for *M. persicae* and 21 replicates for *B. brassicae*.

	Vermicompost Treatment (%)			
	0 (Control)	20	40	60
<i>Myzus persicae</i>	7.8 \pm 0.86	8.9 \pm 1.13	8.4 \pm 0.74	7.4 \pm 0.57
<i>Brevicoryne brassicae</i>	11.8 \pm 1.0	12.5 \pm 2.24	10.8 \pm 1.71	10.0 \pm 1.00



Figure 2.1. No choice apterae colonization experimental setup. Insects were confined to a single plant for 2 weeks by means of a chiffon sleeve which was tied around the pot with a large rubber band and held upright over the plant canopy with an electrical wire loop.

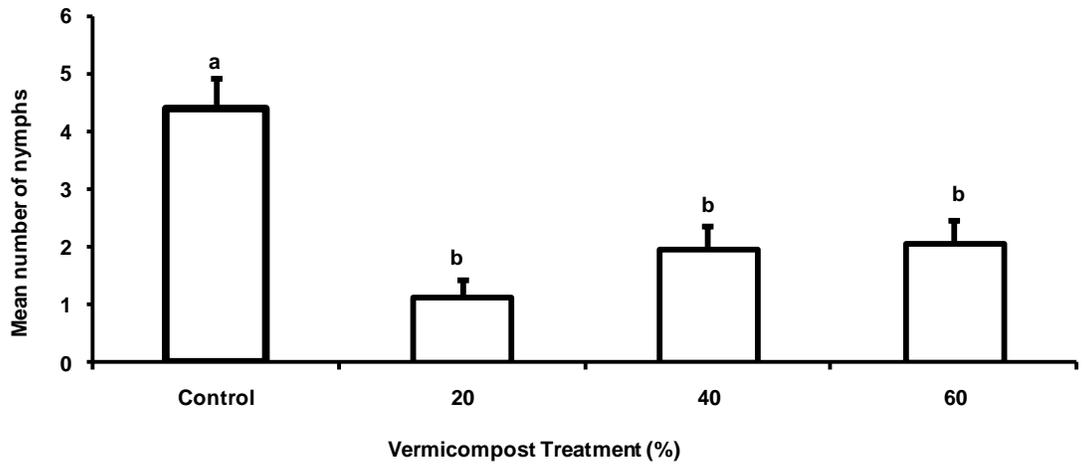


Figure 2.2. Mean number of nymphs deposited within a 24 h period by *M. persicae* alates in 4-way choice assays: All three vermicompost treatments had significantly fewer nymphs than the control. Bars headed with the same letter are not significantly different, Tukey's mean separation tests ($P \geq 0.05$). Error bars denote 1 SE.

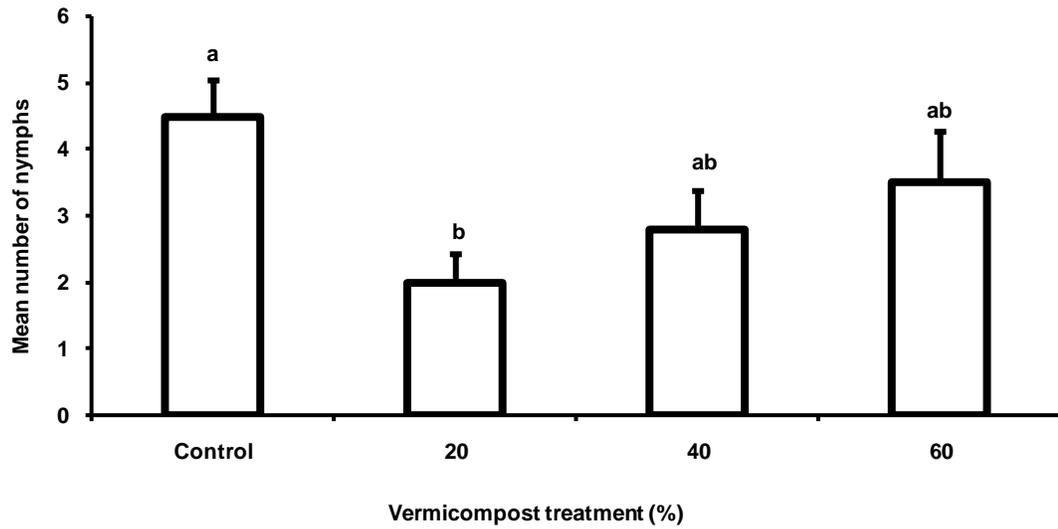
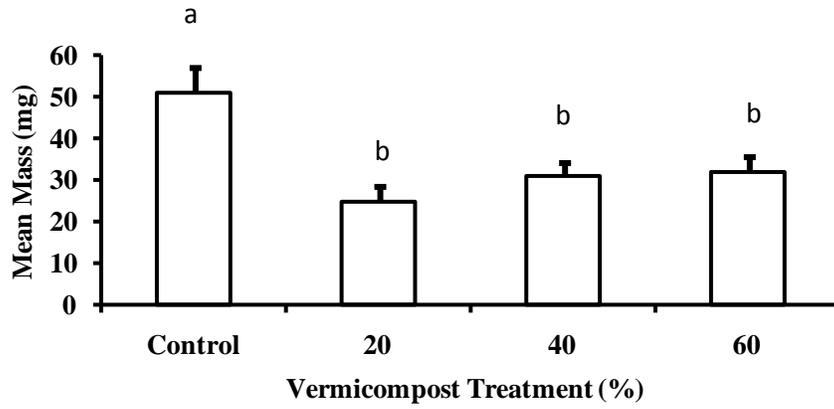


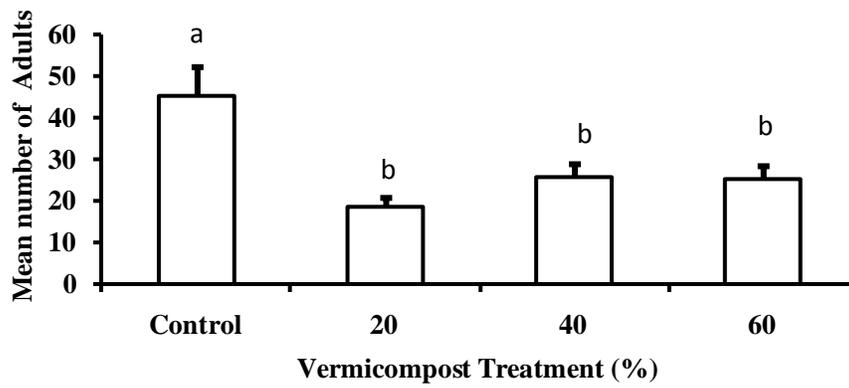
Figure 2.3. Mean number of nymphs deposited within a 24 h period by *B. brassicae* alates in 4-way choice assays: Plants grown in 20% vermicompost had significantly lower numbers of nymphs compared to the control. Bars headed with the same letter are not significantly different, Tukey's mean separation tests ($P \geq 0.05$). Error bars denote 1 SE.

Figure 2.4. *Myzus persicae* mass accumulation in no choice apterae colonization assays: All three vermicompost treatments had significantly lower masses (A), lower numbers of adults (B) and nymphs (C) than controls. Bars headed with the same letter are not significantly different, Tukey's mean separation tests ($P \geq 0.05$). Error bars denote 1 SE.

(A)



(B)



(C)

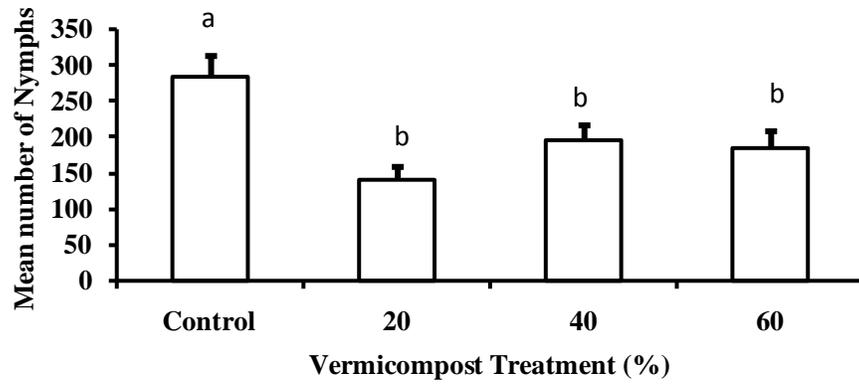
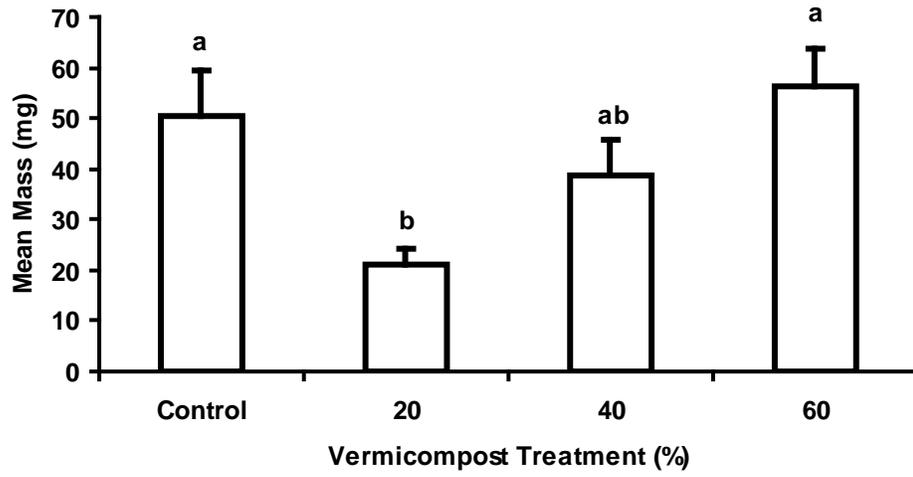
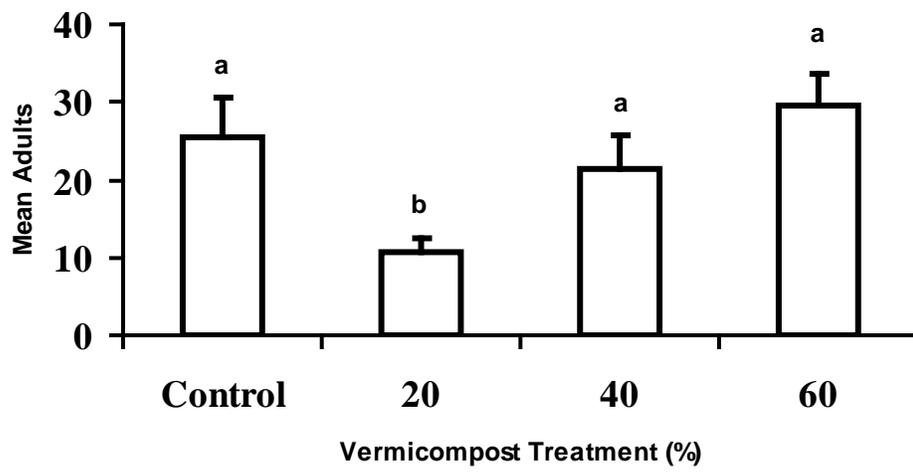


Figure 2.5. *Brevicoryne brassicae* mass accumulation in no choice apterae colonization assays: Plants grown in 20% vermicompost had significantly lower masses (A), lower numbers of adults (B), and nymphs (C) than controls. Bars headed with the same letter are not significantly different, Tukey's mean separation tests ($P \geq 0.05$). Error bars denote 1 SE.

(A)



(B)



(C)

