Assessing Invasive Species Seed Viability in Compost

Rachel Brown

An Honours thesis submitted to the Trent School of the Environment in partial fulfillment of the requirements for the degree of Bachelor of Science (Honours) in Environmental and Resource Science

Trent University
Peterborough, Ontario
April 2018

Abstract

Assessing Invasive Species Seed Viability in Compost

Rachel Brown

Invasive plants pose a challenge to land managers because they can reproduce rapidly, compete with desirable vegetation, and alter ecological services in areas where they become established (Mack et al., 2000). Management focuses its resources on developing several removal techniques, which aim at eradicating these species and preventing their spread. Composting has been shown to be effective at inactivating a range of plant species by exposing seeds to elevated temperatures for extended periods of time (Dougherty, 1999; Eghball and Lesoing, 2000). The two main objectives of this study were to 1) determine whether terrestrial invasive plant (i.e. *A. petiolata, F. japonica, Phragmites* sp., *R. cathartica* and *V. rossicum*) seeds and rhizomes (*Phragmites* sp.) remain viable after being incubated in a mushroom compost after 7 and 30 days, and 2) to determine the critical temperature threshold for reducing seed and rhizome viability in a laboratory setting. It was hypothesized that seeds and rhizomes would show a reduction in germination after 7 days, and after 30 days, seeds and rhizomes would become completely inactivated. Results from the study indicate that compost temperatures as low as 21.23^{o}C were needed to eliminate seed viability for *R. cathartica* and reduce *Phragmites* sp. rhizome viability. Oven incubations show that a critical temperature increase from 50-60^{o}C are needed to reduce viability for most species, and that seed viability is greatly reduced within the first 24 and 48 hours of being exposed to elevated temperatures. Compost composition results demonstrate that %organic matter and %moisture content were well above the normal range, suggesting that the compost is immature, ultimately affecting the compost temperature regime. In the future, management should look at minimum depth and temperature requirements to reduce seed viability, along with the effect that moisture content and phytotoxin leachates have on reducing seed viability. This will provide management with a better understanding of how seed viability is effectively inactivated in compost piles

Keywords: compost pile, seed viability, *A. petiolata*, *F. japonica, Phragmites* sp., *R. cathartica*, *V. rossicum* temperature, germination

Acknowledgements

I would like to express my deep gratitude to Eric Sager, my supervisor, for his patient guidance, valuable knowledge and enthusiastic encouragement towards this project. It would not have been made possible without his knowledge and expertise on the subject. Many thanks to Matthew Porter, who provided and turned the compost, along with the Trent Experimental Farm facilities and equipment. I would also like to thank my second reader Catherine Eimers for her supportive advice and assistance in keeping my progress on schedule. Funding for this project was provided by the Ministry of Natural Resources and Forestry, York Region and the Ontario Invasive Plant Council, who also contributed by providing their knowledge and wisdom towards the project. I am especially thankful to Sarah Nelson, Eliza Montgomery and Tanner Liang for their help and eagerness in the collection of over 2000 plant propagules (per species), no matter the weather conditions. Finally, I wish to thank my friends and family for their support and encouragement throughout my project.

Table of Contents

List of Figures

List of Appendices Figures

List of Tables

Chapter 1: Introduction

1.1 Invasive Plant Species in the Environment

Invasive plants pose a challenge to land managers because they can reproduce rapidly, compete with desirable vegetation, and alter ecological services in areas where they become established (Mack et al., 2000). Management efforts have focused on minimizing these impacts through a wide range of control options (Van Rossum and Renz, 2015). Although several options to manage invasive populations exist, many involve physical removal of aboveground portions (i.e. hand pulling, mowing, cutting the stem, biological control agents, and herbicides) (Van Rossum and Renz, 2015; Chilton and Durocher, 2009). Aboveground material that is removed can contain viable propagules (seed or perennial organs) (Van Rossum and Renz, 2015). These propagules can sustain populations if left in controlled areas or lead to further spread if the material is transported off site (Van Rossum and Renz, 2015). Ontario's diverse economy, growing population, and geographic location put the province at the highest risk of species invasions compared to any other Canadian province or territory (Government of Ontario, 2018). Ontario has been and will continue to be susceptible to invasive species arriving and surviving due to the favourable environmental conditions and nature of our society (i.e. industrialized, urbanized and high density), and multiple land and water entry points (Government of Ontario, 2018).

1.3 Terrestrial Invasive Plant Species in Ontario

Alliaria petiolata (garlic mustard), *Fallopia japonica* (Japanese knotweed), invasive *Phragmites* sp., *Rhamnus cathartica* (European buckthorn) and *Vincetoxicum rossicum* (dog-strangling vine) are common invasive plant species found throughout southern Ontario. These plants reproduce via seed, however *Phragmites* and *F. japonica* also reproduce vegetatively through their extensive rhizomatic root system. Their root systems can deter the growth of neighbouring plants, allowing the plants to form dense monocultures and preventing native wildlife from reaching suitable habitat. Seeds can travel short and long distances, and are often dispersed by wind, water, wildlife, and anthropogenic influences (i.e. moving machinery or equipment) (OIPC, n.d.). Each plant has a unique life history, making it an ideal candidate for

influencing habitats in Ontario. These species occur in a wide range of habitats and spread quickly along roadsides, ditches, forest edges and fence lines (OIPC, n.d.).

1.2 Composting as a Management Tool

Compost is best defined as a mixture of various organic substances, in which the natural decaying of organic matter occurs through the biological process wherein microorganism's convert organic material into a nutrient-rich resource (Dougherty, 1999; Urdang and Flexner, 1984). During the composting process, microorganisms consume oxygen from the organic matter and release carbon dioxide (Dougherty, 1999). Active, functional composting produces a large amount of heat, releasing water vapour into the air (Dougherty, 1999). Today, composting is a technique used to accelerate the natural decay process, which can take as long as a year or a little as 14 days, depending upon the amount of human influence (Pennsylvania Department of Environmental Protection [PDEP], 2010). The primary ingredients of compost are carbon and nitrogen, and an ideal carbon to nitrogen ratio of 30:1 is needed for fastest decomposition (Dougherty, 1999). The volume of finished compost is 50% or less of the original volume of raw materials, which makes composting an effective means of waste management and control (Dougherty 1999).

As a waste management system, the composting process has been shown to be effective at inactivating a range of plant species by exposing seeds to elevated temperatures for extended periods of time (Dougherty, 1999; Eghball and Lesoing, 2000). However, the effectiveness of composting varies among plant species (Van Rossum and Renz, 2015). Some exotic plants have been successfully composted in the past, but the composting of invasive species has not been fully investigated (Rogers, 1993). For example, seed viability of *Amaranthus restroflexus* L. (redroot pigweed) was reduced to zero in 4 weeks, but 14% of *Abutilon theophrasti* Medik. (velvetleaf) seeds remained viable after 4-5 months of composting (Tompkins et al. 1998). Although several aquatic invasive plants have been found susceptible to compost (Meier et al. 2014). Currently, there are few studies that have focused on complete inactivation of seed and rhizome viability of invasive plant species in Ontario. Therefore, it is

necessary for current and future research to focus on determining specific terrestrial invasive plants that are inactivated by the composting process.

Ontario has restricted the transport of certain invasive plant species (i.e. *F. japonica, Phragmites* sp. and *V. rossicum*), therefore, making it difficult for municipal residents and workers to dispose of invasive plant propagules in a controlled manner. Municipal compost may be redistributed to various locations, making it important to determine the likelihood of viable plant propagules in redistributed compost. Complete inactivation is critical in Ontario, and many other provinces that regulate the movement of invasive plant propagules (Boyce, 2003). Ultimately, management is interested in knowing the exact temperature at which propagules become inactivated in compost.

1.4 Research Objectives and Hypothesis

The objectives of this study are to 1) determine whether terrestrial invasive plant (i.e. *A. petiolata, F. japonica, Phragmites* sp., *R. cathartica* and *V. rossicum*) seeds and rhizomes (*Phragmites* sp.) remain viable after being incubated in mushroom compost after 7 and 30 days, and 2) to determine the critical temperature threshold for reducing seed and rhizome viability in a laboratory setting. The following research question was addressed: What specific temperature do seeds and rhizomes become inactivated, and how does compost affect viability? It was hypothesized that seeds and rhizomes will show a decrease in germination after 7 days of compost incubation, and at 30 days seeds and rhizomes will be completely inactivated. These plants were chosen because the Invasive Species Acts restricts the movement of some of the species, and they are common invaders found in southern Ontario. These plants produce a high abundance of seeds, and have extensive root systems, making them easily spread by wildlife and humans.

Chapter 2: Methodology

2.1 Field Methodology

2.2 Study Area and Seed Collection

A mushroom compost pile was constructed and managed on the property of Trent University, at the Trent Experimental Farm (44°21'44.99" N 78°16'37.17" W) (Figure 1). The compost was used for all field mortality tests and compost was harvested from this same pile for laboratory mortality tests. Approximately 2000 seeds and rhizomes (i.e. *A. petiolata, F. japonica, Phragmites* sp., *R. cathartica* and *V. rossicum*) were collected in early June to late November (2017) in the City and County of Peterborough. Only mature plants were considered for this study, due to the higher percentage of viable seed on each plant (see Appendix A for an example of a mature plant). Mature plants were based upon colour (i.e. leaves were fully developed), texture (i.e. rough bark or stem) and size (i.e. 1-7 m in height, and/or a diameter breast height (DBH) >25 cm). Fruits (berries/flowers) and seeds pods were harvested from mature plants; mature berries were defined as being blackened in colour, and having a mushy texture, whereas seeds were defined as being black or dark brown in colour, and easily extractable from the pods or plant. Seeds were carefully extracted from seed pods and berries, where they were rinsed in distilled water to remove comma and pulp particulate matter. Seeds were then air dried at room temperature (20°C) in the lab for 24 hours to ensure seeds were devoid of moisture.

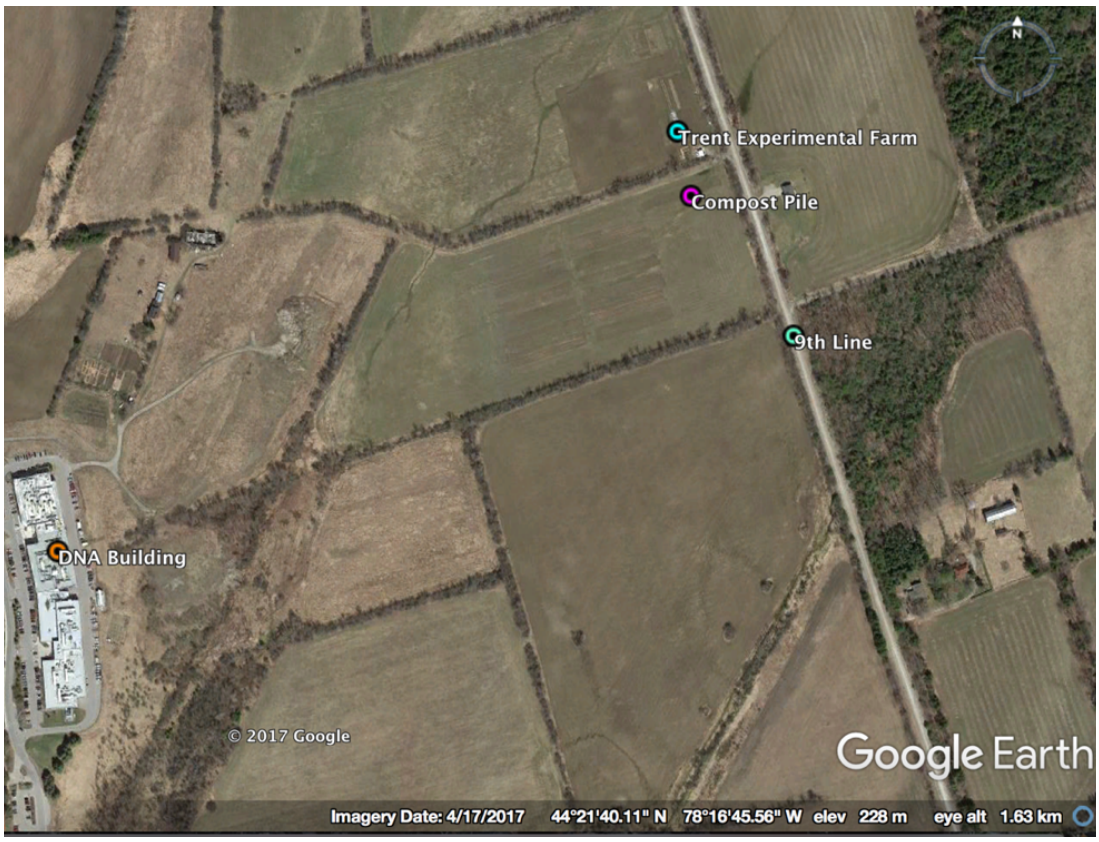

Figure 1: Compost pile location on the property of Trent University (44°21’44.99” N 78°16’37.17” W), Peterborough, ON.

2.3 Compost Mortality Tests

The pile was approximately 3 m tall, 2 m wide at the base and 2 m in length, and was comprised of horse and cow manure, animal bedding and wheat straw. Guidelines from the Ontario Compost Quality Standards were used to determine the appropriate temperature for killing off pathogens. Ontario requires that compost must maintain a minimum temperature of 55°C for at least 15 days (Government of Ontario, 2016). The height and width allowed the pile to become insulated and generate enough heat to kill most pathogens, and maintain a high temperature in winter conditions (Rynk et al. 1992). To ensure adequate oxygenation and mixing of composting materials, the compost was turned every 7-days.

A total of 68 sample bags (6.5 x 5.5 cm) was prepared for the study (Larney and Blackshaw, 2003), sample bags contained 6 replicates of 25 seeds or 4 replicates of 20 rhizomes. The bags were made from a fine nylon-mesh that was able to retain the seeds and

rhizomes while allowing their exposure to temperature and moisture conditions within the pile (Larney and Blackshaw, 2003). A HOBOware data-logger was placed in the packet to record temperature at hourly intervals. Packets were then inserted into the centre (2 ft. deep) of the pile for 7 and 30-day incubation periods (Van Rossum and Renz, 2015). Packets were removed prior to pile turning and then reinserted randomly into the pile (sticking to the centre) to simulate the turning process (Van Rossum and Renz, 2015). Upon removal, the packets were placed in a controlled-environment chamber and assessed for viability.

2.4 Laboratory Methodology

2.5 Oven Mortality Tests

Seeds and rhizomes underwent oven incubations to determine the exact period at which inactivation occurred for each species. Seeds were placed at a depth of 1 cm in 125 ml glass jars that were filled with compost, whereas rhizomes were placed at a depth of 2 cm in 200 ml aluminum baking trays. The compost was moistened when necessary using distilled water (Gorai et al. 2005). Seeds and rhizomes were then incubated in a GCA Mechanical Convection Incubator for a total of 24 and 48 hours at constant temperatures ranging from 40 – 70^{o}C (Vieira, 2010). Compost temperature was monitored using an oven thermometer. Seeds and rhizomes were then assessed for viability in a controlled-environment chamber.

2.6 Stratification and Scarification Treatments

Stratification mimics the seeds overwintering state (i.e. dormancy period), whereas scarification scars the seed coat, allowing the scarification treatment (i.e. boiling water, chemical and mechanical) to penetrate the seed. Kurylo and Endress (2014) found that *A. petiolata, F. japonica, Phragmites* sp., *R. cathartica* and *V. rossicum* seed coats promotes some level of dormancy; it was therefore expected that if seeds were subjected to stratification and scarification treatments, seeds would germinate at a higher quantity. *A. petolata, R. cathartica* and *V. rossicum* seeds were stored in a paper bag in complete darkness under refrigeration (4^{o}C ± 1^{o}C) for 2 months (DiTommaso et al. 2004). *F. japonica* was stored in a paper bag at room temperature (20^{o}C ± 1^{o}C) for a 2-month period (Groeneveld et al. 2014). *Phragmites* sp. seeds and rhizomes were stored 5 cm below the surface in non-mineral soil for 2 months, under

refrigeration (4^{o}C ± 1^{o}C) (Kettenring and Whigham, 2009). After the 2-month period *R. cathartica* seeds were scarified by pouring boiling water over the seeds, followed by immediate placement into the controlled-environment chamber. Seeds were left in the water as it cooled for 24 hours (Dumroese et al. 2008). *A. petiolata* seeds were scarified by rubbing seeds between two sheets of Norton Ultra Fine sandpaper for 3 seconds (Sosnoskie and Cardina, 2009). Seeds were then placed in 10 ml of 2mM of GA_3 (gibberellic acid) for 48 hours in the controlled-environment chamber (Yasin and Andreasen, 2015).

2.7 Seed Viability

To get an estimate of the percentage (%) of viable seed/rhizome, 20 seeds and rhizomes were placed in a non-mineral soil in a controlled-environment chamber, control pots were set-up and seeds and rhizomes were allowed to freely germinate. Temperature was maintained at a constant 20 ± 1^{o}C, with a photo-light period of 16 hours and 8 hours of darkness, which mimicked spring-time conditions (Hotchkiss et al., 2007). Oven and compost incubations were also placed in the chamber for 30 days to test germination viability. Propagules were considered viable when they broke through the surface of the compost (Hotchkiss et al., 2007). *A. petiolata* seeds were placed on a windowsill and allowed to freely germinate. Temperatures reached 22 ± 1^{o}C during the day, and fell to 20 ± 1^{o}C at night. All seeds were waterd using distilled water and rhizomes were watered using Otonabee River water. River water was used for the rhizomes, because they had higher germination percentages when compared to rhizomes watered with distilled water.

2.8 Compost Analysis

The maturity of the compost pile was determined by testing for pH, %organic matter and %moisture content. pH was measured using an Oakton multimeter, in a solution of 1:2.5 soil:solution (RO water) ratio. From there, %organic matter was determined using the loss-on-ignition (LOI) method via a muffle furnace set at 450^{o}C for 10 hours (Matthiessen et al. 2005). %moisture content was determined by wet weight based off an oven (105^{o}C) dried soil sample (Trautmann and Richard, 1996). Moisture was also assessed using the 'feel' test (i.e. squeezing a handful of compost and determining whether it felt like a moist sponge) (Meier et al. 2014).

2.9 Data Analysis

The 30-day compost period was focused on for the data analysis because this time interval suggests the ability to render plant propagules inactive (Van Rossum and Renz, 2015). Temperature data from the study were converted to cumulative degree day (CDD) as described by Larney et al. (2000). The degree day concept was used to integrate temperatures over the composting period (Larney and Blackshaw, 2003). Using a base temperature of 40°C, degree days were calculated for the pile. 40°C was chosen as it represents the lower threshold for thermophilic bacteria common to the composting process (Ryckeboer et al. 2003).

(1)

$$CDD = \frac{(Tmin + Tmax)}{2} - 40$$

This model (1) accounts for the intensity of temperature and duration of the exposure of materials to this temperature (Van Rossum and Renz, 2015). CDDs were calculated by adding the piles' daily maximum (T_{max}) to the daily minimum temperature (T_{min}), dividing the sum by two (daily mean temperature) and subtracting 40°C (Van Rossum and Renz, 2015). If the mean daily temperature was < 40°C, then degree days were 0 (Larney and Blackshaw, 2003). The degree day values were then summed to give CDD for the pile and sampling date (Larney and Blackshaw, 2003). Daily mean compost temperatures were used to determine temperature per day for each of the composting periods.

The percent (%) germination equation (2) was used to determine the viability of plant propagules after compost and oven incubations. %germination was calculated by using the germination percentage (GP), the number of seeds germinated (n), and the total number of seeds (N=150) (Pirasteh-Anosheh and Hamidi, 2013).

(2)

$$GP = \frac{n}{N}\, x\, 100$$

3.0 Statistical Analysis

The effect that time and temperature (i.e. oven and compost incubations) had on %germination values was analyzed by SigmaPlot, using a one-way analysis of variance (ANOVA). Results were considered significant at $p < 0.05$ (α 0.05). Temperature means were calculated using Microsoft Excel 2017.

Chapter 3: Results

3.1 Compost Pile Composition

The results from this study show that %organic matter (85.00%) and %moisture content (59.56%) are considerably higher than what is considered within the normal range of mature mushroom compost, indicating, that the compost is of younger composition (i.e. immature). Despite the above normal results, the pH (7.15) was within the normal range (Table 1). The squeeze test resulted in two drops released from the compost, again indicating the immaturity of the compost.

The pile was unable to reach the ministry required 55^{o}C for 15 consecutive days, as required by the Government of Ontario (Figure 3). The purpose of the required temperature is to ensure pathogens have been killed off effectively, as previously stated. Despite the unachieved desired temperature, both temperature regimes were high enough to ensure full lethality of the seeds, and reduce rhizome viability. Over the 30-day period the compost achieved a maximum temperature of 53.01^{o}C, with a minimum temperature of 21.23^{o}C. The 7-day composting period achieved similar maximum and minimum temperature results (52.23^{o}C and 27.00^{o}C) respectively (Table 2). Mean air temperatures ranged from 4.11 – 0.91^{o}C over the 30-day period in November 2017 (Table 2). Cumulative degree days (CDD) for the compost mortality tests was 52.23 (7-day) and 74.77 (30-day) (Table 2). CDD increased as the length of the compost days increased (Figure 3).

During the first turning session the temperature of the compost fell considerably, which was the lowest it would reach throughout the 30-day period. After the first turning, the internal temperature of the pile slowly began to increase, regardless of the air temperature. Ambient air temperature did not impact the internal temperature of the compost (Figure 2). Towards the end of the 30-day period, the temperature of the compost slowly began to decline, indicating that turning was required, or the pile was not receiving an adequate oxygen supply (Figure 2).

Table 1: Results of Trent University's compost pile composition. The normal range is based off mature spent mushroom compost requirements.

Analyte (Units)	Results	Normal Range
pH	7.15	6.00-8.506.00-8.50 (Landschoot and McNitt, 2018)
Organic matter (%)	85.00	26.00-60.00 (Fidanza et al., 2010)
Moisture content (%)	59.56	40.00-50.00 (Fidanza et al., 2010)

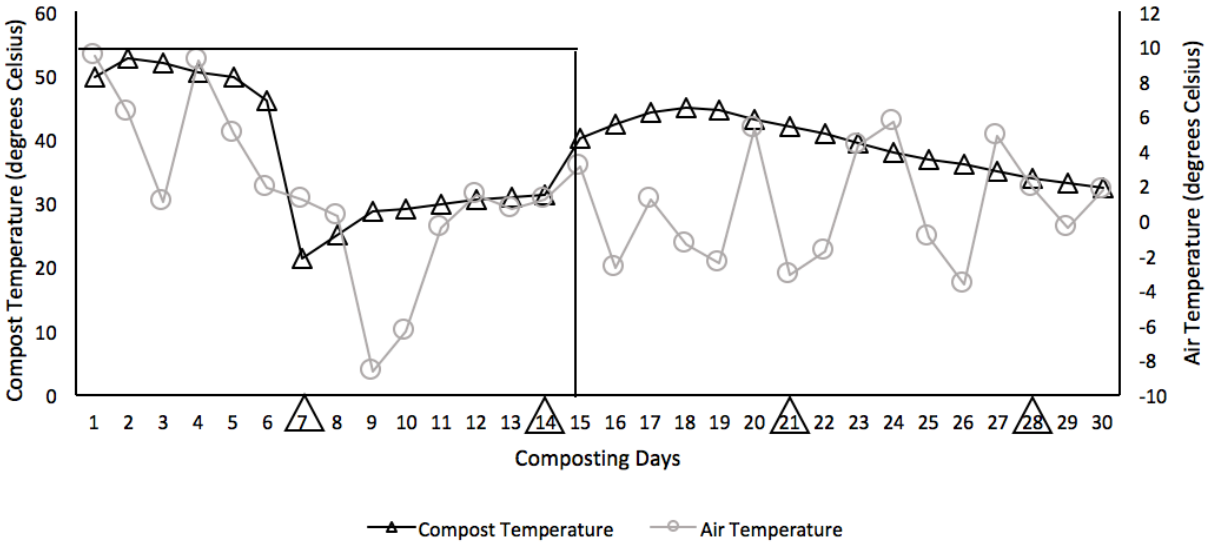

Figure 2: Air temperature (°C) and internal temperature (°C) of the compost. Values are the daily averages taken over the 30-day period (November 2017). The horizontal line at 55°C and vertical line at 15 days represents the limit to reduce pathogens (Ontario Compost, Quality Standards, 2016). The triangles illustrate the compost turning days.
*Climate data was provided by the Trent University Climate Station.

Table 2: Cumulative degree day (CDD), and mean minimum and maximum temperature (^{o}C) for 7 and 30-day incubations during November (2017).
*Climate data was provided by the Trent University Climate Station.

Compost incubation period	Mean air temperature (^{o}C)	Minimum temperature (^{0}C)	Maximum temperature (^{0}C)	CDD
7-day	4.11*	27.00	52.23	52.23
30-day	0.91*	21.23	53.01	74.77

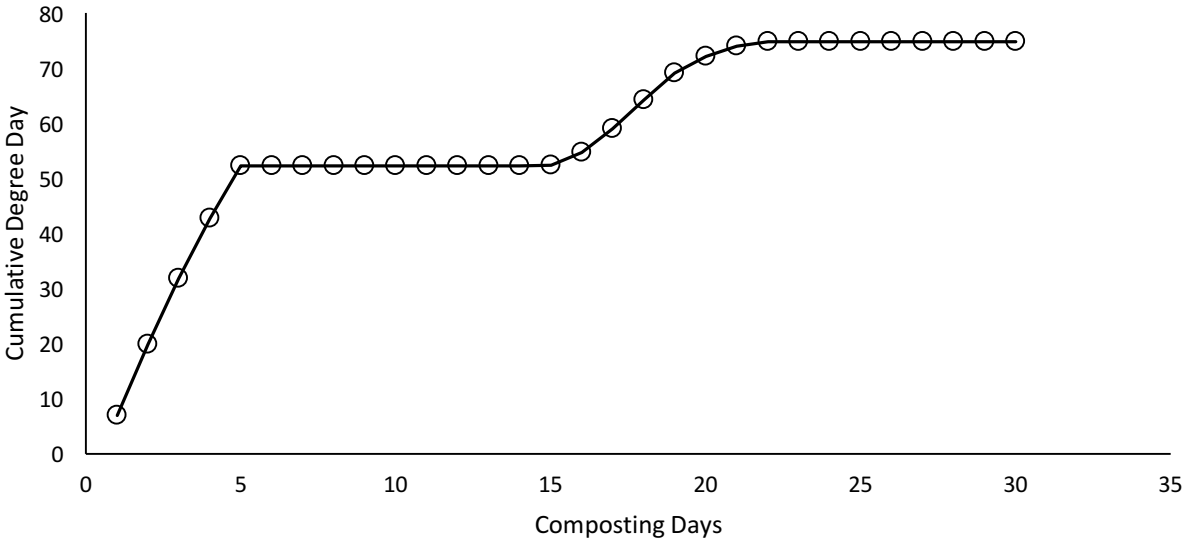

Figure 3: Cumulative degree days (CDD) compared to composting days over a 30-day period.

3.2 Effect of Compost Temperature on Seed Viability

Survivorship for compost mortality tests was reduced to 0 (i.e. 100% mortality) (Table 3). Control %germination (n=20) was adequate for all species, except for *A. petiolate* (15%) and *Phragmites* sp. seeds (0%), despite meeting literature requirements (Table 3). Temperatures as low as 21.23^{o}C proved to be effective at eliminating *R. cathartica* seed viability over the 7-day compost period, whereas *Phragmites* sp. rhizomes had a reduction in germination after 7 days, viability was further reduced after 30 days, but it was not eliminated (Table 4) (see Appendix C and D for compost incubation impacts on rhizomes). These results coincide with the oven

mortality results. Temperatures ranging from 40-70^{o}C at 24-48 hour intervals proved to effective at destroying *R. cathartica* seed viability. *Phragmites* sp. rhizomes did not germinate until the oven temperature reach 70^{o}C at 48 hours, where it reached 8% germination, which was quite surprising (Table 5). *F. japonica* had a %germination value of 5%, at a temperature of 40^{o}C for 24 hours (Table 5). As the temperature and time increased, the %germination value decreased. *F. Japonica* was proven to be not statistically significant (p-value: 0.09) using a one-way ANOVA. Temperatures exceeding 50^{o}C were effective at destroying seed viability for most species. *V. rossicum* preferred an increase in temperature and time [i.e. 40^{o}C (48hr = 2%); 50^{o}C (24hr = 12%) (48hr = 13%)], until 60^{o}C where its viability was eliminated, this was proven to be statistically significant (p-value: 0.03) (Table 5). Overall, higher temperatures and longer time intervals proved to effective at reducing seed viability for most of the species.

Table 3: Control germination results (n=20).

Species	**Germination (%) control**
A. petiolata	15.00
F. Japonica	90.00
Phragmites sp. (rhizome)	100.00
Phragmites sp. (seed)	0.00
R. cathartica	80.00
V. rossicum	100.00

Table 4: Compost mortality results from 7 and 30-day incubations.
*Only *R. cathartica* seeds and *Phragmites* sp. rhizomes were used for compost incubations, due to the other species still undergoing their stratification treatments.

Species	**Group**	**Germination (%)**	**Mortality (%)**
Phragmites sp. (rhizomes)	7-day	13.00	87.00
	30-day	2.00	98.00
R. cathartica (seeds)	7-day	0.00	0.00
	30-day	0.00	0.00

Table 5: The effect of oven temperature on %germination.

Group	Compost temperature (°C)	Species	Germination (%)	Mortality (%)
24-hour	40	*Phragmites* sp., *R. cathartica*, *V. rossicum*	0.00	100.00
		F. japonica	5.00	95.00
	50	*Phragmites* sp., *F. japonica*, *R. cathartica*	0.00	100.00
		V. rossicum	12.00	88.00
	60	*F. japonica*, *Phragmites* sp., *R. cathartica*, *V. rossicum*	0.00	100.00
	70	*F. japonica*, *Phragmites* sp., *R. cathartica*, *V. rossicum*	0.00	100.00
48-hour	40	*F. japonica*, *Phragmites* sp., *R. cathartica*	0.00	0.00
		V. rossicum	2.00	98.00
	50	*F. japonica* *Phragmites* sp., *R. cathartica*	0.00	0.00
		V. rossicum	13.00	87.00
	60	*F. japonica* *Phragmites* sp., *R. cathartica*, *V. rossicum*	0.00	0.00
	70	*F. japonica*, *R. cathartica*, *V. rossicum*	0.00	0.00
		Phragmites sp.	8.00	92.00

Chapter 4: Discussion

4.1 Compost

Compost composition results from the current study indicate that the mushroom compost is at an immature or 'younger' stage, accounting for the extreme %organic matter and %moisture content values. Unstable compost can have a more volatile temperature regime, greatly impacting the consistency of the internal temperature of the pile. The turning method employed (i.e. tractor) for the duration of the study, likely hindered the composts ability to receive adequate oxygen directly in the centre of the compost. On most turning days, the tractor was unable to adequately turn the compost because of the sheer volume and thickness of the pile, which likely influenced the temperature in the pile. High organic matter content is due to the immaturity of the compost, as the compost matures the soil microorganisms will slowly release the nutrients (i.e. N, P, K) over time (Fidanza and Beyer, 2005), decreasing the overall organic matter content. High moisture content in compost has been shown to directly influence the amount of oxygen reaching the pile. Van Rossum and Renz (2015) found that the higher the moisture content, the more it influenced and reduced airflow from entering the pile, which inadvertently reduced compost temperature rate. The high moisture content of the pile likely directly influenced the amount of oxygen reaching the centre, preventing the temperature from consistently reaching above the desired 55^{o}C. One of the goals for this study was to replicate the conditions that occur at compost facilities in Ontario, by utilizing similar compost types and turning regimes. Although this study was not successful, the CDD suggests that the compost temperature was sufficient at deterring seed viability.

4.2 Effects of Compost on Seed Viability

Although Ontario's requirements weren't met, compost temperatures as low as 21.23^{o}C eliminated seed viability for *R. cathartica* seeds in just 7 days, and reduced *Phragmites* sp. rhizome viability, but did not eliminate it. Van Rossum and Renz (2015) also found that 1-day of composting reduced viability by 88% for *R. cathartica*, with predicted 99% inactivation after 3 days for the seeds. The effects of composting proved to be an effective control method for *R. cathartica*, despite the low temperature regime. Van Rossum and Renz (2015) suggest that this is due to the seed coat thickness. Seed scarification further weakened the seed coat

thickness of *R. cathartica* making it more susceptible to the compost temperature regime. Van Rossum and Renz (2015) discovered that when comparing seed coat decay rate, *R. cathartica* seeds were proven to have the highest rate over *A. petiolata* seeds (Van Rossum and Renz, 2015), again suggesting that *R. cathartica* seeds have weak seed coats. Although *A. petiolata* seeds were unable to be used for the compost incubations, it was found that under similar composting conditions as this study; seed viability was reduced to 79% after 1-day of composting, and viability was further reduced to 99% after 3 days of composting (Van Rossum and Renz. 2015). It was found that composting *A. petiolata* for 7 days in a manure-based compost yielded zero viable seeds (Van Rossum and Renz, 2015). This suggests that these seeds would be susceptible to the composting process. *Phragmites* sp. proved to be effective at buffering the composting effect. The rhizomes that accounted for the %germination values were not affected by the microbial activity of the compost. Other studies investigating composting effects on *Phragmites* sp. rhizomes could not be found. However, a similar study using *F. japonica* rhizomes found that rhizomes were more likely to survive in high moisture content compost pile rather than in drier compost (Xian et al. n.d.). Rhizomes were readily inactivated by 45^{o}C after 48 hours, compared to 72 hours required for compost piles with a high moisture content (Xian et al. n.d.). These findings, along with the current study's findings, suggest that rhizomes are susceptible to typical compost process temperatures (Xian et al. n.d.), but can be greatly hindered when the pile has a high moisture content.

4.3 Effects of Temperature on Seed Viability

Oven incubations proved that temperatures as low as 40^{o}C were needed to eliminate seed viability of *R. cathartica* seeds, and further reduce viability of the other test species. However, to fully eradicate seed viability, temperatures >60^{o}C will be needed within the compost pile. This study, along with Egley (1990) found that seed viability rapidly decreases during the first 24 and 48 hours, viability is also further decreased from being exposed to temperatures ranging from 40-70^{o}C. For example, 95% of *F. japonica* seeds were killed within the first 24 hours, and by 48 hours seed viability had been completely eliminated. Only small differences in germination occurred between seeds exposed to lower temperatures but a

critical increase, between 50-60^{o}C prevented germination for most of the species (Thompson et al. 1996). Jones and Blair (1996) found that critical temperatures required to prevent germination have been shown to be in the range of 50-80^{o}C. Furthermore, the peaks of germination among the species at each temperature vary in relation to time (Jones and Blair, 1996). Responses to higher temperatures varied among species, but increasingly higher temperatures and longer treatment reduced the number of surviving seeds (Egley, 1990). As exposure time to the high temperature continues, seeds that are highly susceptible to heat are killed and other seeds are either unaffected or induced to germinate (Egley, 1990). However, seedlings produced from seeds induced to germinate by heat may not survive the intense heat and the net result is reduced seed population without increased weed emergence from the soil surface. Thus, the intense heat and elevated moisture may enhance germination but will kill the seedlings prior to emergence and result in a net decrease in weed stand (Egley, 1990).

4.4 Additional Factors

Additional factors affecting seed viability may be propagule placement and moisture content of the pile, along with the creation of phytotoxin leachates from the composting process. Depending on where the plant propagules end up in the pile, determines the temperature at which it will be exposed to. Propagules located closer to the surface will be unable to attain the elevated temperatures required for reducing viability. Van Rossum and Renz (2015) found that viability was initially influenced by the propagule placement within the compost pile, possibly due to temperature differences within the pile (Daugovish et al. 2007). For this study, the ministry required temperature was not achieved, however, seed and rhizome viability was still greatly reduced. Larney and Blackshaw (2003) have found that high temperatures combined with wet conditions are more effective at killing seeds when compared to high temperatures alone. Certain weed seeds put into compost with a high moisture content have been shown to imbibe the moisture from the compost, encouraging germination (Egley, 1990), however, other weed species put into moist compost have had their seed viability eliminated (Egley, 1990). Variation in seed survival at temperatures obtained through the

composting process has been attributed to seed coat water permeability, seed dormancy, and seed moisture content (Egley, 1990; Nobel et al. 2011).

Eghball and Lesoing (2000) reported that when a compost pile has a high moisture content, weed seed viability may be destroyed even though the critical temperature is not reached, possibly because of compost phytotoxin leachates. Production of water-soluble organic phytotoxins such as short-chain fatty acids (i.e. acetic acid), during composting has been reported (Kirchmann and Widen, 1994). Ozores-Hampton et al. (1999) found that germination of *Ipomoea hederacea* L. (ivyleaf morning glory), *Echinochloa crus-galli* L. (barnyard grass), and *Portulaca oleracea* L. (common purslane) was delayed and decreased by extracts from 3 and 30-day composts as compared with a mature compost extract (1-year old). This was attributed to higher amounts of acetic acid in the immature composts (Larney and Blackshaw, 2003). Phytotoxin leachates, combined with a high moisture content could explain why *R. cathartica* seeds were unable to survive in the compost pile, and why *Phragmites* sp. rhizome viability was severely impacted. Seeds and rhizomes that underwent oven incubations did not show the same amount of damage, as the compost incubations, this possibly attributes to the combination of temperature, moisture content and phytotoxins within the pile.

Chapter 5: Conclusions

5.1 Summary

This study showed that heating seeds and rhizomes in compost reduced germination potential, compared to the unheated control. Temperature required to achieve complete elimination of viability was species-dependent. *R. cathartica* seeds proved to be the most sensitive to compost and oven temperature regimes, while *V. rossicum* preferred warmer temperatures for longer time periods. A critical temperature increase between 50-60^{o}C prevented germination for all species, except for *Phragmites* sp. rhizomes. In the compost pile, the relationship between temperature and viability was not proven to be effective as increasing the amount of time seeds and rhizomes spent in the pile. This suggests that other factors such as phytotoxin leachates and moisture content, may play an important role in reducing overall viability of plant species.

5.2 Study Limitations

This study was limited by several factors. Firstly, each species required their own stratification treatment. Stratification treatments required seeds to undergo long periods of induced dormancy, disallowing most of the species to be used for compost incubation tests, thus, limiting the results for the study. Secondly, seed viability seemed to vary greatly between each species. It took several months for *A. petiolata* to germinate (i.e. 4 months), making it impossible to use in either the compost or oven incubation tests. *Phragmites* sp. seed failed to germinate entirely, again rendering it useless for this study. Thirdly, this study was unable to replicate municipal windrow compost heaps, possibly altering the results that a windrow compost pile may achieve.

5.2 Future Recommendations

Future studies should look at propagule survivability within different depths of the compost, and the effect that moisture content and phytotoxin leachates have on weed seed viability. Studies should continue testing *A. petiolata*, *F. japonica, Phragmites* sp. and *V. rossicum* seeds in compost piles, because of the shortcomings with these species in this study. Additionally, studies should look at the specific temperature and time needed to render

rhizome viability to zero. Further experiments concentrating on lower temperature ranges may be required under both moist and dry compost conditions, with and without turning (Larney and Blackshaw, 2003). Lastly, piles maintained at higher temperatures would reduce the time that each plant propagule spent in the pile, indirectly preventing the propagules from potentially escaping.

References

Boyce, J. (2003). *Invasive species – an emerging issue for mining and reclamation. Pg. 702-707 in proceedings of the 2002 National Meeting of American Society of Mining and Reclamation.* Lexington, KY. American Society of Mining and Reclamation.

Chilton, E., and Durocher, P. (2009). *Aquatic Vegetation Management in Texas: A Guidance Document*. Austin TX: Department Inland Fisheries, Texas Parks and Wildlife.

DiTommaso, A., Brainard, D., and Webster, B. (2004). Seed characteristics of the invasive alien vine *Vincetoxicum rossicum* are affected by site, harvest date, and storage duration. *Canadian Journal of Botany.* 83: 102-110.

Dougherty, M. (1999). *Field Guide to On-farm Composting*. Ithaca, NY: Natural Resources Cooperative Extension, Agriculture, and Engineering Service NRAE114.

Dumroese, K., Luna, T., and Landis, T. (2008). *Nursery manual for native plants: a guide for tribal nurseries* (Vol. 1). United States Department of Agriculture.

Eghball B., and Lesoing G. (2000). Viability of Weed Seeds Following Manure Windrow Composting. *Compost Science and Utilization.* 8: 46-53.

Egley, G. (1990). High-temperature on germination and survival of weed seeds in soil. *Weed Science.* 38 (4): 429-435.

Fidanza, M., and Beyer, D. (2005). *Plant nutrients and fresh mushroom compost.* Retrieved from http://www.mushroomcompost.org/files/theme/NPK2(1).pdf

Fidanza, M., Sanford, D., Beyer, D., and Aurentz, D. (2010). Analysis of fresh mushroom compost. *HortTechnology.* 20 (2): 449-453.

Gorai, M., Vadel, A., and Neffati M. (2006). Seed germination characteristics of *Phragmites communis*: effects of Temperature and Salinity. *Royal Botanical Society of Belgium.* 139 (1): 78-86.

Government of Ontario. (2016). *Ontario compost quality standards.* Retrieved from https://www.ontario.ca/page/ontario-compost-quality-standards

Government of Ontario (2018). *Ontario Invasive Species Strategic Plan.* Retrieved from https://dr6j45jk9xcmk.cloudfront.net/documents/2679/stdprod-097634.pdf

Groeneveld, E., Belzile, F., and Lavoie, C. (2014). Sexual reproduction of Japanese knotweed (*Fallopia japonica* S.L.) at its northern distribution limit: new evidence of the effect of climate warming on an invasive species. *American Journal of Botany.* 101 (3): 459-466.

Hotchkiss, E., DiTommaso, Brainard, D., and Mohler, C. (2008). Survival and performance of the invasive vine *Vincetoxicum rossicum* (Apocynaceae) from seeds of different embryo number under two light environments. *American Journal of Botany.* 95 (4): 447-453

Kettenring, K., and Whigham, D. (2009). Seed Viability and seed dormancy of non-native *Phragmites australis* in suburbanized and forested watersheds of the Chesapeake Bay, USA. *Aquatic Botany.* 91: 199-204.

Kurylo, J., and Endress, A. (2014). Effects of scarification and stratification on germination of common Buckthorn (*Rhamnus cathartica* L.) seeds. *Journal of Biodiversity Management and Forestry.* 4 (3): 1-4.

Lafleur, P. (November 2017). *Trent University Climate Station Data*. Peterborough, ON.

Landschoot, P., and McNitt, A. (2018). *Using spent mushroom substrate (mushroom soil) as a soil amendment to improve turf*. Retrieved from http://plantscience.psu.edu/research/centers/turf/extension/factsheets/mushroom-soil

Larney, F., and Blackshaw, R. (2003). Weed seed viability in composted beef cattle feedlot manure. *Journal of Environmental Quality.* 32: 1105-1113.

Larney, F., Olson, A., Carcamo, A., and Chang, C. (2000). Physical changes during active and passive composting of beef feedlot manure in winter and summer. *Bioresource Technology*. 75: 139-148.

Lhotska, M. (1975). Notes on the ecology of germination of *Alliaria petiolata. Folia Geobot Phytotax,* 10 (2): 179-183.

Mack R., Simberloff D., Lonsdale W., Evans H., Clout M., and Bazzaz F. (2000). Biotic Invasions: Causes, Epidemiology, Global Consequences and Control. *Ecological Applications.* 10: 689-710.

Matthiessen, M., Larney, F., Selinger, B., and Olson, A. (2005). Influence of loss-on-ignition temperature and heating time on ash content of compost and manure. *Communications in Soil Science and Plant Analysis.* 36: 2561-2573.

Meier, E., Waliczek, T., and Abbott, M. (2014). Composting invasive plants in the Rio Grande River. *Invasive Plant Science and Management.* 7 (3): 473-482.

Nobel, R., Dobrovin-Pennington, A., Pietravalle, S., Weekes R., and Henry CM. (2011). Indicator organisms for assessing sanitation during composting of plant wastes. *Waste Management.* 31: 1711-1719.

Ontario Invasive Plant Council. (n.d.). *Best Management Practices.* Retrieved from https://www.ontarioinvasiveplants.ca/resources/best-management-practices/

Ozores-Hampton, M., Stoffella, P., Bedwick, T., Cantliffe, D., and Obreza, T. (1999). Effect of age of cocomposted MSW and biosolids on weed seed germination. *Compost Science and Utilization.* 7 (1): 51-57.

Pennsylvania Department of Environmental Protection [PDEP] (2010). *How to make compost*. Retrieved from http://www.dep.state.pa.us

Pirasteh-Anosheh, H., and Hamidi, R. (2013). Does seed chemical priming improve germination and early growth of oil rapeseed? *International Journal of Agronomy.* 4 (4): 805-808.

Rogers, A., (1983). *Composted Waterhyacinth as a Container Amendment*. Unpublished thesis. Davis, CA: University of California, Davis. 158 p.

Ryckeboer, J., Mergaert, J., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., and Swings, J. (2003). A survey of bacteria and fungi occurring during composting and self-heating process. *Ann Microbial.* 53: 349-419.

Sosnoskie, L., and Cardina, J. (2009). Laboratory methods for breaking dormancy in garlic mustard (*Alliaria petiolata*) seeds. *Invasive Plant Science and Management*. 2 (2): 185-189.

Thompson, A., Jones, N., and Blair, A. (1997). The effect of temperature on viability of imbibed weed seeds. *Association of Applied Biologists.* 130: 123-134.

Tompkins, D., Abiola, A., and Chaw, D. (1998). Effect of windrow composting on weed seed germination and viability. *Compost Science and Utilization*. 6: 30-34.

Trautmann, N., and Richard, T. (1996). *Moisture content.* Retrieved from http://compost.css.cornell.edu/calc/moisture_content.html

Van Rossum, J., and Renz, M. (2013). Evaluation of Composting as a Management Strategy for Garlic Mustard and Common Buckthorn Plant Debris. *University of Wisconsin System Solid Waste Research Program.*

Van Rossum, J., and Renz, M. (2015). Composting Reduces Seed Viability of Garlic Mustard (*Alliaria petiolata*) and Common Buckthorn (*Rhamnus cathartica*). *Invasive plant Science and Management.* 8 (3): 284-291.

Vieira, D., Socolowski, F., and Takaki, M. (2010). Seed germination and seedling emergence of invasive exotic species, *Clausena excavate. Brazilian Journal of Biology.* 70 (4): 1015-1020.

Yasin, M., and Andreasen, C. (2015). Breaking seed dormancy of *Alliaria petiolata* with phytohormones. *Plant Growth Regulation.* 77 (3): 307-315.

Xian, C., Bardos, P., and Robinson, S. (n.d.). *Can composting kill Japanese knotweed.* Retrieved from http://www.organicsrecycling.org.uk/uploads/article2149/Can%20composting%20kill%20Japanese%20Knotweed%20Version%202.pdf

Appendix: A

Figure A1: A digital photograph of a mature *V. rossicum* (dog-strangling vine) plant.

Appendix: B

Figure A2: *Phragmites* sp. rhizomes before entering compost pile. %germination = 100.00%

Appendix: C

Figure A3: *Phragmites* sp. rhizomes after 7 days of being incubated in the compost. %germination = 13.00%

Appendix: D

Figure A4: *Phragmites* sp. rhizomes after 30 days of compost incubation. %germination = 2.00%