The Recruitment Biology and Facultative Recruitment Nature of Conyza canadensis.

by

Eric Tozzi

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ABSTRACT

THE RECRUITMENT BIOLOGY AND FACULTATIVE RECRUITMENT NATURE OF CONYZA CANADENSIS.

Eric Tozzi
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Advisor:
Rene Van Acker

*Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. *canadensis*) is a surface-germinating ruderal facultative winter annual. The ruderal nature is a key adaptive characteristic that implicates emergence timing as an important recruitment factor. Experiments were established at three sites in southern Ontario, Canada from 2009 to 2012 and at the Thermogradient Plate at the University of Saskatchewan in 2011. Experiments in Ontario aimed to determine the possible effect of emergence timing of *Conyza canadensis* on survival, fecundity, and flowering timing in populations emerged seed sourced from Spain, United Kingdom, Ontario, and Iran as well as determine the effects of warming spell timing on survival, fecundity, aboveground biomass, and flowering timing for a population sourced from Ontario. Experiments at the Thermogradient Plate at the University of Saskatchewan aimed to determine a base germination temperature range and the amount of GGDs to 50% germination in seed sourced from populations in Spain, United Kingdom, Ontario, and Iran. Results indicated that seed shed timing parsed out into early/late spring or early/late fall helped observe differences between experimental factors. Overall, early fall and early spring
emerging plants had significantly greater survival and fecundity, as well as shorter times to flowering when compared to late fall and late spring emerging cohorts in all populations. Results also indicated that warming spells closer to the spring season had a significantly larger negative impact on survival, fecundity, aboveground biomass, and flowering timing compared to warming spells occurring earlier in the winter season. Results conducted on the thermogradient plate indicated significant differences in base germination temperature range and the amount of GGDs to 50% germination in seed sourced from populations in Spain, United Kingdom, Ontario, and Iran, suggesting a genetic and therefore evolutionary difference between the international populations. Overall the findings emerging from this research suggest emergence timing plays a critical role in the population dynamics of Conyza canadensis and that this species has the potential to rapidly adapt to new climatic areas. These findings are especially important in a changing climate and may be exploited for better management approaches as herbicide resistant populations increase in frequency.
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# Table of Contents

Title Page  
Abstract  
Acknowledgements  
Table of Contents  
List of Tables  
List of Figures  
1.0 Introduction  
2.0 Literature Review  
  2.1 Biology  
  2.2 Overwinter Survival and Seed Persistence  
  2.3 Recruitment  
    2.3.1 Emergence  
    2.3.2 Spring vs. Fall Recruitment  
  2.4 Evolutionary and Geographic Adaptation  
  2.5 *Conyza canadensis* as a Problem Weed  
  2.6 Conclusion  
3.0 Effects of seedling emergence timing on the population dynamics of *Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. canadensis).  
  3.1 Introduction  
  3.2 Materials and Methods  
    3.2.1 Emergence Timing  
    3.2.2 Cohorts  
    3.2.3 Statistical Analysis  
  3.3 Results  
  3.4 Discussion  
    3.4.1 Disturbance  
4.0 Seed germination response to temperature for of a range of international populations of *Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. canadensis).  
  4.1 Introduction
4.2 Materials and Methods

4.2.1 Statistical Analysis

4.3 Results

4.4 Discussion

4.5 Conclusion

5.0 The effect of recruitment timing on the dynamics of a range of international populations of Conyza canadensis (Conyza canadensis (L.) Cronq. var. canadensis).

5.1 Introduction

5.2 Materials and Methods

5.2.1 Populations

5.2.2 Statistical analysis

5.3 Results

5.3.1 Effect of Recruitment Timing

5.3.2 Differences in Response Between Populations

5.4 Discussion

5.4.1 Effect of Seedling Recruitment Timing

5.4.2 Population Comparisons

5.4.2.1 Ontario

5.4.2.1 Iran

5.4.2.1 United Kingdom

5.4.2.1 Spain

5.5 Conclusion

6.0 The effect of simulated winter warming spells on Conyza canadensis (Conyza canadensis (L.) Cronq. var. canadensis) seeds and plants.

6.1 Introduction

6.2 Materials and Methods

6.2.1 Fall Rosettes

6.2.2 Seeds and Seedlings

6.2.3 Statistical analysis

6.3 Results

6.3.1 Rosettes
6.3.2 Seeds 99
6.3.3 Seedlings 100
6.4 Discussion 101
6.5 Conclusion 105
7.0 General Discussion 106
7.1 Summary of Contributions to Knowledge 107
  7.1.1 Population Model 108
7.2 General Discussion and Critique of Thesis Research and Approaches 110
  7.2.1 Extent of Studies in this Thesis 110
  7.2.2 Comparisons among international populations 111
  7.2.3. Higher Time Scale Resolution for Studying Fall vs. Spring Cohorts 111
  7.2.4 Effect of Disturbance 112
  7.2.5 Genotype 114
7.3 Implications of Thesis Results in the Context of Climate Change 115
7.4 Conclusions 118
8.0 Literature Cited 119
List of Tables

Table 3.1.............................................................................................................................................34

The effect of emergence timing on flowering timing (Julian calendar week when first seed shed occurred) of *Conyza canadensis*. Results represent pooled data for either tilled or not-tilled plots and for three sites in southern Ontario, Canada collected within three individual seasons (2009-2010, 2010-2011, 2011-2012).

Table 3.2.............................................................................................................................................35

The effect of emergence timing (cohort) on the fecundity of *Conyza canadensis*. Results represent pooled data for either tilled or not-tilled plots and three sites in southern Ontario, Canada collected within three individual seasons (2009-2010, 2010-2011, 2011-2012).

Table 3.3.............................................................................................................................................36

The effect of emergence timing on the survival (%) of *Conyza canadensis* plants in August. Results represent data pooled for tilled and not-tilled plots and for three sites in southern Ontario, Canada.

Table 4.1.............................................................................................................................................50


Table 4.2.............................................................................................................................................51

Gompertz model parameter estimates for models fit to the relationship between cumulative germination (%) and temperature (C) for seeds sourced from plants grown in a common garden from seeds, in turn, sourced from Shiraz, Iran, southern Ontario, Canada, Málaga, Spain and Hertfordshire, United Kingdom. For the model parameters, a represents asymptote, b represents growth rate, and c represents inflection point. Values in parentheses are standard errors of the parameter estimates. Individual population models are considered significantly different according to lack of fit F test at p = 0.05.

Table 4.3.............................................................................................................................................52

Results from the ANOVA (linear mixed model) assessing the effect of temperature (°C), seed source (source), experiment run (run), and replicate (rep) on the growing degree days (GDDs) required to reach 50% germination.
Table 4.4

Mean growing degree days (GDD $T_{\text{base}} 0^\circ\text{C}$) required to reach 50% germination for seeds of *Conyza canadensis* sourced from Málaga, Spain, Hertfordshire, United Kingdom, southern Ontario, Canada, and Shiraz, Iran. Means followed by the same letter are considered not significantly different at $p<0.05$.

Table 4.5

Lowest temperature range ($^\circ\text{C}$) for which seed germination occurred for seed of *Conyza canadensis* sourced from populations in Málaga, Spain, Hertfordshire, United Kingdom, southern Ontario, Canada, and Shiraz, Iran.

Table 5.1

Survival of *Conyza canadensis* plants as affected by recruitment timing (cohort) for experiments conducted in Ontario, Canada using seed sourced from one local and three international populations.

Table 5.2

Fecundity (capitula/plant) of *Conyza canadensis* plants as affected by recruitment timing (cohort) for experiments conducted in Ontario, Canada using seed sourced from one local and three international populations.

Table 5.3

Flowering timing of *Conyza canadensis* plants as affected by recruitment timing (cohort) for experiments conducted using seed sourced from one local and three international populations.

Table 6.1

Conditions in growth cabinets for three day simulated warming spell treatments imposed on *Conyza canadensis* seeds, seedlings and rosettes. Refer to material and methods for explanation of determination of warming spell treatment conditions.

Table 6.2

Effect of simulated warming spell dates (WSD) in January, February, and March on fecundity (capitula/plant), above-ground biomass (g at flowering stage), survival (% at bolting stage), and flowering date (first seed shed) for *Conyza canadensis* rosettes established in the fall of 2009 and 2010 at three sites in southern Ontario, Canada. Means within columns followed by different letters denote significant differences at $p<0.05$.

Table 6.3

Average total germination (per 100 seeds) in fall or spring and total percent germination of *Conyza canadensis* seed, seeded either early in fall (September) or late (October) either on the soil surface or just below the surface (0.5 cm) in 2009 and 2010 at three sites.
in southern Ontario, Canada. Means within columns followed by different letters denote significant differences at p<0.05.

Table 6.4…………………………………………………………………………………………..96

Effect of simulated warming spell dates (WSD) in January, February, and March and seeding depth (cm) on seedling survival (% at bolting stage) and flowering date (first seed shed) for seedlings recruiting in the fall from Conyza canadensis seeds (from southern Ontario populations) seeded outside at three sites in southern Ontario in the fall of 2009 and 2010. Means within columns followed by different letters denote significant differences at p<0.05.

Table 6.5…………………………………………………………………………………………..97

Effect of simulated warming spell dates (WSD) in January, February, and March and seeding depth (cm) on seedling survival (% at bolting stage) and flowering date (first seed shed) for seedlings recruiting in the spring from Conyza canadensis seeds (from southern Ontario populations) seeded outside at three sites in southern Ontario in the fall of 2009 and 2010. Means within columns followed by different letters denote significant differences at p<0.05.
List of Figures

Figure 3.1

Observed emerging timing for Conyza canadensis seedlings in tilled or no-tilled plots over three winter annual seasons in southern Ontario, Canada. Data points represent means and error bars represent 1 standard error of the mean.

Figure 4.1

Cumulative germination (%) of Conyza canadensis seed as affected by temperature (°C) for seeds sourced from plants grown in a common garden from seeds, in turn, sourced from A) Shiraz, Iran, B) southern Ontario, Canada, C) Málaga, Spain and D) Hertfordshire, United Kingdom. For model parameters see Table 2.

Figure 7.1

Population dynamics model of Ontario populations of Conyza canadensis from 2009-2012 pooled over three sites in Ontario. Dotted line represents survival (%), solid line represent fecundity (number of capitula/plant), and dashed line represents time to flowering (mean Julian week of first flowering).
CHAPTER 1

1. Introduction

*Conyza canadensis* [(L.) Cronq. var. canadensis] is a weedy winter annual member of the Asteraceae family. Common names include Canada fleabane, horseweed, and mare’s tail, with a previous scientific name of *Erigeron canadensis* (Weaver 2001). Native to North America, *Conyza canadensis* has become fully naturalized internationally, especially in Europe (Frankton and Mulligan 1987). The life history of facultative winter annual weeds is important for determining weediness, adaptiveness, recruitment, and management approaches in species like *Conyza canadensis*. Knowledge of weed recruitment biology can be used to influence management practices, such as herbicide application timing (Hacault and Van Acker 2006). Emergence periods for this species group can vary substantively among species within the group, increasing the importance of understanding emergence timing in relation to management. Facultative winter annual weeds can recruit mostly in the fall, mostly in the spring, or equally in both seasons (Cici and Van Acker 2009). *Conyza canadensis* is a surface-germinating ruderal species with non-dormant seeds that germinate in either fall or spring. These inherent properties make this species an ideal model plant to study factors affecting germination and emergence (recruitment) timing.

Facultative winter annual weeds are becoming more prevalent and possibly even more so under future aspects of climate change. Management techniques may need to adapt to changes in potential negative impacts caused by weedy species. Recent evolution of herbicide resistant biotypes furthers the impact *Conyza canadensis* has on agriculture in the context of current herbicide-centric management practices. This is of concern to the agricultural industry, as facultative winter annuals, such as *Conyza*
canadensis, can be adaptive through their facultative nature and further investigation into their recruitment nature is needed to assist in evolving management approaches. Results relating to aspects affecting recruitment of Conyza canadensis would be critically important in determining impacts on population dynamics of this species, which in turn, may also lead to improvements in management practices.

The focus of this thesis was to investigate and gain insight into the facultative recruitment nature of Conyza canadensis. Specifically, research in this thesis concentrated on what drove emergence timing for this species and the influence emergence timing had on population dynamic factors including fecundity, plant density, survival, above-ground biomass, and flowering timing. Additionally, populations sourced from differing global climatic regions were compared in terms of emergence timing and performance on the basis of emergence timing to provide insight into the adaptiveness of this species with respect to both recruitment timing and performance. The primary null hypothesis was that emergence timing would not have a significant effect on the population dynamics of Conyza canadensis where plants that emerged earlier would not have higher fecundity, plant density, survival, above-ground biomass, nor the shortest time to flowering when compared to plants that emerged later in the season.

Key questions addressed in each chapter:

1. Do warming spells and the timing of these have an effect on the fecundity, aboveground biomass, flowering timing, and overwintering survival of Conyza canadensis?

   a. Hypothesis – Earlier warming spells will have a significantly negative effect on fecundity, above-ground biomass, flowering timing, and
overwintering survival in *Conyza canadensis* when compared to later warming spells.

2. Does the emergence timing of *Conyza canadensis* have any effect on fecundity, plant density, and flowering timing?
   a. Hypothesis - Plants emerging at the earlier emergence periods will have significantly greater fecundity, plant density, and significantly shorter time to flowering compared to later emerged plants.

3. Does germination response to temperature differ among international populations of *Conyza canadensis*?
   a. Hypothesis - Populations sourced from different climates will have different germination responses to temperature.

4. Does the effect of emergence timing on the fecundity, plant density, and flowering timing of *Conyza canadensis* differ among international populations?
   a. Hypothesis - Local adaptation will favour performance in the local climate.

Pursuit of answers to these questions will provide insight into the recruitment nature of *Conyza canadensis* and the impact that recruitment timing has on the performance and population dynamics of this species. Specifically, pursuit of answers to question one will provide awareness into the impact of one element of climate change specifically (winter warming spells) on the adaptive performance of the Ontario population of *Conyza canadensis*. Pursuit of answers to question two will provide insight into *Conyza canadensis* emergence timing effects within Ontario populations over a period of 4 years (3 winter annual seasons). No previous studies have followed
populations of *Conyza canadensis* for this length of time recording emergence throughout the entire growing season. Answers to question two will also provide some understanding into the role *Conyza canadensis* plays in natural succession. Pursuit of answers to questions three and four will provide understanding into the genetic differences (genotype) among international populations in terms of base germination temperatures and germination rates as well as insights into the adaptiveness of *Conyza canadensis*. Pursuit of answers to question four will also provide insights into the effect of genotypic differences with respect to growth and reproduction performance and climate adaptation between international populations. Collectively, the pursuit of answers to the four core questions in this thesis will help provide awareness into the population dynamics, recruitment nature, and biology of *Conyza canadensis*, which in turn will provide some new insight into the nature of winter annual weeds in general. This thesis represents the first study of base germination temperatures of a range of international populations of *Conyza canadensis*, the first study to record emergence throughout the growing season for a substantive period of time (four years), and the first study to explore the effects of one aspect of climate change on the performance of *Conyza canadensis*. 
CHAPTER 2

2.0 Literature Review

2.1 Biology

Winter annual weeds can be categorized as constitutive winter annuals that only germinate in the fall or facultative winter annuals that have the ability to germinate as long as microsite conditions are favourable (Baskin and Baskin 1988). Cici and Van Acker (2008) found that all of the significant winter annual weeds in Canadian agricultural systems are facultative, with the majority of species emerging at two peak periods, April-May and September-October. The ability to germinate across multiple time periods throughout the year compensates for offspring mortality (Evans and Dennehy 2005) and highlights why facultative winter annuals are an important group of species to study, since they have a number of potentially competitive advantages over summer annual species. Cold tolerance and rosette formation are excellent examples of beneficial adaptations to environment over summer annuals. With some factors of climate change, such as warming spells, predicted to increase in severity, frequency, and duration, winter annuals may have inherent competitive advantages and the rapid adaptability required to acclimatize to changes in local climate (Shabbar and Bonsal 2003).

Recruitment timing is a critically important factor in determining plant success, especially in facultative winter annuals (Harper 1977), where large changes in soil surface temperatures are experienced during the recruitment periods. Differing climates can influence adaptation and alter life strategies in plant species to favour spring or fall emergence (Masuda and Washitani 1992). Grime (1977) developed a model to help
categorized plants life strategies. The CSR model positions plants into three separate life strategies based on their level of stress, amount of disturbance, and competition.

Stressful environments are ones that are low on nutrients, light, and water or exhibit too hot or cold temperature for optimum plant growth. Highly disturbed environments are ones where physical movement of the growth medium occurs frequently, such as any modern agricultural system including tillage. A competitive environment is one where there is competition for life and life supporting factors.

Competitors (C) are plants that are found in environments with moderate stress, high competition, and low levels of disturbance. Stress-tolerators (S) are plants that are found in environments with moderate disturbance, high stress, and low competition. Ruderals (R) are plants that are found in environments with high disturbance, low stress, and moderate competition. Ruderals have high productivity and apply most of their energy into reproductive growth. Conyza canadensis is an excellent example of a ruderal species as well as a prominent winter annual species in Ontario (Weaver 2001).

Conyza canadensis is a surface-germinating ruderal facultative winter annual with recruitment that is highly susceptible to changes in microsite conditions (Grime 1977; Regehr and Bazzaz 1979; Buhler and Owen 1997; Nandula et al. 2006; Main et al. 2006).

Conyza canadensis belongs to the Conyza genus in the family Asteraceae. Plants within the Conyza genus may be annual or perennial flowering plants growing to 1-2m in height with alternate leaf organization. After emergence the cotyledons of Conyza canadensis are oblong and hairless being 2 to 3.5mm long and 1 to 2mm wide (Royer and Dickinson 2006). Conyza canadensis leaves are alternate, simple, and linear with somewhat toothed edges ranging from 2 to 10cm in length and have a light carrot-like
scent (Royer and Dickinson 2006). The leaves grow directly off the stem, which does not contain any petioles. Most plants are unbranched and grow one large stem, but some plants may contain multiple stems with some branching. Multiple stemmed plants grow most often when the rosette has been damaged. Herbicide and mowing may cause multiple stemmed Conyza canadensis (Weaver 2001). Rosette size also plays a small role in multiple stemming; larger rosettes are more likely to contain multiple stems (Weaver 2001). Rosettes are only formed by some plants with most fall emerging plants passing through a rosette stage, and most spring emerging plants not passing through a rosette stage. Rosettes are morphologically distinct with several rings of dark green leaves organized radially with each leaf ranging from 0.5 to 5cm in width and 2.5 to 15cm long (Frankton and Mulligan 1987). Each rosette also forms a short taproot (Frankton and Mulligan 1987). Bolting from the rosette occurs in the spring, followed by desiccation of the rosette as the plants mature (Bhowmik and Bekech 1993).

Conyza canadensis flowers and sets seed in late summer, with a portion of the shed seed germinating and forming an overwintering rosette in the fall, and other seed persisting and germinating in the spring of the following year (Regehr and Bazzaz 1979). For a number of Knoxville, Tennessee populations, it was estimated that Conyza canadensis seed has a base germination temperature of 13°C (Steinmaus et al. 2000). Peak seedling establishment of Conyza canadensis generally occurs in early autumn only weeks after peak seed set (Regehr and Bazzaz 1979). Previous studies suggest the seed does not contain any inherent dormancy, which suggests that microsite conditions play a role in the persistence and emergence timing of Conyza canadensis (Regehr and Bazzaz 1979). The ability to germinate in spring or fall is a key weedy trait for this species.
The florets of *Conyza canadensis* are 0.5 to 1 cm long and can be white, yellow, or pink and mainly found in the top portion of the plant. Most florets contain 30 to 50 seeds (oblong and flattened) each with each plant producing upwards of 200,000 seeds. Each seed contain a pappus, which is fan-like structure used to help seed dispersal by wind. The pappus increases the surface area of the seed facilitating wind dispersal to the extent that some seeds may reach the planetary boundary layer and travel globally (Shields et al. 2006). The seeds are quite small and range in size from 1 to 4 mm long, excluding the pappus. *Conyza canadensis* is primarily self-pollinated with a small percentage of outcrossing occurring due to entomophily or wind dispersal. Pollen is mainly released while the capitula are opening allowing for selfing to occur in this self-compatible diploid species. *Conyza canadensis* also has one of the smallest weedy plant genomes (~335 Mb) (Peng 2013).

Currently, *Conyza canadensis* is found globally within latitudes of N 55 to S 45. Potentially native to the southern United States, *Conyza canadensis* has since spread internationally and is now found as far afield as China. *Conyza canadensis* was likely first introduced to the United Kingdom in the 17th century. The seed was reportedly used as stuffing by taxidermists in North America, who took advantage of the quantity of fluffy seed available from each plant, perhaps expediting global dispersal (Salisbury 1961).

**2.2. Overwinter Survival and Seed Persistence**

Overwintering survival and persistence of seed are important factors in the recruitment nature of *Conyza canadensis*. Previous studies have suggested that the winter survival of *Conyza canadensis* is positively correlated to the size of the rosette, until a
certain size is met after which the benefits of a large rosette are outweighed by limited nutrient supply (Regehr and Bazzaz 1979; Main et al. 2006). Early studies suggested that larger rosettes had the greatest winter survival. This observation was largely uncontested until almost 30 years later when it was rosettes larger than 9cm in diameter were observed to have lower winter survival due to relatively lower levels of root reserves, compared to the size of the rosette (Main et al. 2006). The formation of a winter rosette allows the plant to obtain necessary reserves while most other competitors are non-existent or dormant. Rosette survival over winter is also affected by frost heaving, which occurs most commonly in the silt-loam soils that lead to optimal growth for Conyza canadensis (Regehr and Bazzaz 1979). Several factors including soil type, soil moisture levels, and temperature cycles can alter the frequency of frost-heaving events. Frost heaving accounted for up to 86% mortality in a naturally established Conyza canadensis population in Urbana, Illinois, and frost heaving affected smaller rosettes more than larger rosettes (Regehr and Bazzaz 1979).

Plants that survive through the winter may either be photosynthetically active or photosynthetically dormant throughout the winter. Some plant species have no capacity for photosynthetic activity during the entire winter season, and then readily photosynthesize when the conditions are suitable (Regehr and Bazzaz 1976). Other plant species may hold the capacity to photosynthesize until suitable micro-conditions are reached. The latter is representative of the physiological nature of Conyza canadensis. Conyza canadensis has several physiological characteristics that help increase survival during the winter season. A significant photosynthetic response to light occurred faster than 14 minutes, even at 5°C (Regehr and Bazzaz 1976). Maximum photosynthetic rate
was reached after 30 minutes by all experimental plants. During the experiment, the
temperature of the roots was at 0°C due to the near freezing soil in the experimental plots.
For Conyza canadensis, optimal CO₂ uptake occurs at 15°C in the fall and 28°C in the
summer. Internal leaf temperatures in this species can reach upwards of 10°C higher than
ambient temperatures, even in winter (Regehr and Bazzaz 1976). The relatively high root
and leaf temperature in winter suggests that this species can store and metabolize
available nutrients within the rosette itself. Larger rosettes have a greater capacity to
photosynthesize when the conditions are optimal (Regehr and Bazzaz 1976). This notion
ties in with the findings that larger rosettes (to a point) have a greater chance winter of
survival.

The ecological strategy of Conyza canadensis, as expected, appears to be to
maximize the amount of seed dispersal. For this species, seed production is proportional
to plant height, but reproductive effort is inversely proportional to plant height (Regehr
and Bazzaz 1979). This means that Conyza canadensis places more effort into
maximizing plant height (and dispersal) than seed production. This physiological trait is
both surprising and expected from a ruderal species, which typically allocates energy to
maximize generational continuance through seed (Grime 1977). Even if the shed seed is
of a lower quality, the quantity of the seed and height of the plant may compensate, in
terms of achieving progeny spread, for initial progeny losses that would come from low
quality seed. However, because Conyza canadensis seed is so light and plumulated, it can
be transported on airstreams and can reach an altitude of 140m, entering the Planetary
Boundary Layer and potentially traveling up to 500km. The majority of seed, however, is
found within 100m of mother plants (Shields et al. 2006; Dauer et al. 2007; Dauer et al. 2008).

Populations of *Conyza canadensis* are composed of plants germinating from both new and old seed. Laboratory and field tests have shown that *Conyza canadensis* seed may persist for 2 to 3 years with 1% viability after 3 years if seed is stored properly at 4°C and low humidity (Thebaud et al. 1996). Survival of the seed was also found to be only 9 months if submerged in water (Thebaud et al. 1996). Davis et al. (2009b) completed a study on the influence of winter wheat cover crops, crop rotation, and preplant herbicides on the population dynamics of glyphosate resistant *Conyza canadensis*. Seed bank densities were recorded and they found on average a 76% decrease in seed bank density within the first 10 months. Only a small percentage of seed remained viable within the seed bank for at least 2 years. Low persistence and viability after even just one year shows how important it is for *Conyza canadensis* to overwinter as a rosette. On average, the seed is not persistent enough to germinate one year later in the fall, and may or may not be persistent enough to germinate in the spring following shed the previous fall. The amount of viable seed after the overwintering period is something that has yet to be observed *in situ*. Low seed bank density also highlights how quickly *Conyza canadensis* can evolve since each year (by in large) a new population sets seed with only a small percentage of the population being represented by the previous year’s seed (Davis et al 2009a). Some *Conyza canadensis* seed does remain viable for at least 2 years and the full extent of how long it can remain viable has yet to be tested (Thebaud et al. 1996). If *in situ* seed were found to be viable after more than 2 years it would show that the persistence of *Conyza canadensis* has been underestimated and management
strategies may need to be longer term. In addition, the persistence of seed over winter is essential to spring emergence and the nature of *Conyza canadensis* seed persistence is part of the mechanism behind its facultative nature.

Low temperatures keep *Conyza canadensis* rosettes from bolting and seed from germinating throughout the winter period. The frequency of frost heaving increases with fluctuating temperatures in late fall or early spring (Davis et al. 2009). Short periods of warm temperatures, or warming spells, may affect the survival of both the rosette and seed over winter. Experiments on the effect of winter warm spells on emergence timing, winter survival, and reproduction of *Conyza canadensis* have not been conducted to-date.

### 2.3 Recruitment

#### 2.3.1 Emergence

As a ruderal species, *Conyza canadensis* relies heavily on large amounts of seed dispersal to increase the chances of recruitment. Previous studies have shown that microsite conditions do indeed play a large role in seed germination for this species (Regehr and Bazzaz 1979; Nandula et al. 2006; Main et al. 2006). For *Conyza canadensis* populations, disturbance can create a variety of microsites that are suitable for germination. Nandula et al. (2006) conducted a large study on the effects of different treatments on the germination of *Conyza canadensis*. This study provided insight into the potential mechanism of *Conyza canadensis* emergence in relation to microsite conditions. They subjected *Conyza canadensis* seeds to various temperatures, light levels, pH levels, salt levels, and osmotic potentials and they found that optimal germination occurred at 24°C/20°C day-night cycles with 13hr day-length periods while peak germination
occurred in neutral to slightly alkaline solutions. The study further suggest that *Conyza canadensis* is somewhat salt tolerant and can accommodate some germination under water stress (2 to 25% at -0.8MPa). Other weedy species such as *Campsis radicans* L. (Chachalis and Reddy 2000) and *Brunnichia ovata* L. (Shaw et al. 1991) were sensitive to water stress at less than 0.2MPa. *Conyza canadensis* seed germinates best on the soil surface and has limited survival if sown deeper than 0.5cm, although some seed has survived (3% viability) when buried at 10cm after 3 years (Nandula et al. 2006; Thebaud et al. 1996). The quick decline in germination from seed sown below the surface, further suggest that microsite conditions play a large role in *Conyza canadensis* recruitment.

Several studies have shown that *Conyza canadensis* has the greatest emergence in neutral silt-loam soils after daytime temperatures reach above 13°C (Main et al. 2006). Peak establishment occurs 15 days after seed set (Regehr and Bazzaz 1979).

Temperature and light play the largest roles in germination and emergence levels of *Conyza canadensis*. In one study, seed germinated at a higher percent in light and favourable temperature treatments rather than light and favourable moisture conditions (Baskin and Baskin 1988). The result from a study by Nandula et al. (2006) suggests that the quality and quantity of light the seeds of *Conyza canadensis* receive is critical to germination and emergence. They found, for example, that germination was 0 to 15% under dark conditions compared to 0 to 61% in light conditions. In the same study, the authors found that soil temperature, air temperature, and rainfall were not significantly correlated with *Conyza canadensis* emergence, suggesting that the quantity and quality of light received by the seed is most important to germination. This study also showed *Conyza canadensis* to be highly surface germinating with emergence levels highest for
seed sown onto the soil surface with little to no seedlings emerging from seed sown at a depth greater than 0.5cm (Nandula et al. 2006). The extremely shallow recruitment depth may be a product of the light requirements for *Conyza canadensis* germination (Nandula et al. 2006; Cici and Van Acker 2009). All of these results suggest an evolutionary mechanism selecting for biotypes that can germinate in early fall, since there is a longer photoperiod, higher temperatures, and less moisture at that time compared to other germination times of the year.

Crop residue has been shown to have a negative effect on the emergence of *Conyza canadensis*. One study by Nandula et al. (2006) seeded *Conyza canadensis* in several plots of corn, cotton, and soybean. The crop residue was measured after harvesting and the *Conyza canadensis* was allowed to emerge. It was reported that up to 79% fewer *Conyza canadensis* seedlings emerged when crop residue was present suggesting a detrimental effect on emergence due perhaps to the influence of crop residue on microsite conditions. Crop residue can hinder the amount of light available to seed that may have remained on the soil surface. Crop residue can also block seed from reaching the soil surface, preventing any chance of germination and therefore may play a role in management of this species.

2.3.2 Spring vs. Fall Recruitment

Spring emergence for *Conyza canadensis* occurs only 5 to 32% of the time and the spring cohort does not form a rosette (Buhler and Owen 1997). In the study conducted by Buhler and Owen (1997) low spring emergence was observed in naturally occurring plots of *Conyza canadensis* in agricultural fields in Iowa. In that study, the recording of spring emergence began in late April and continued into June, emergence
was recorded if the cotyledons were present and they compared the emergence timing and survival of fall versus spring cohorts. Spring emergence recording did not continue past June 8 although their data suggested that emergence might continue throughout the summer. Observations on the emergence timing of Conyza canadensis have shown that it may emerge at almost any time as long as recruitment conditions are suitable (Buhler and Owen 1997), but to-date, no study has recorded emergence data throughout the entire growth season. The notion of continual emergence of Conyza canadensis falls in line with the findings suggesting that the seed is non-dormant or conditionally dormant. This idea has not been fully explored or tested either in situ or in the laboratory.

For spring emerging Conyza canadensis the lack of a rosette suggests that the plant has a mechanism, most likely based on temperature or daylength, to control rosette formation (Regehr and Bazzaz 1979). Dauer et al. (2009) found that the spring cohort also had less survival (70%) compared to the fall cohort (86%) further suggesting a tradeoff between reproductive effort and plant growth with overwintering plants having potentially more initial nutrients available to them in the spring allowing them to bolt quickly while the spring seedlings are just emerging. Spring emergence may also be negatively correlated with the density of other plants within the emergence area. This supports the characterization of Conyza canadensis as a ruderal or “first-colonizer” (Regehr and Bazzaz 1979; Weaver 2001). Low-density areas may reduce competition and increase the number of available viable microsites, and gap exploitation makes sense for a species that requires high light levels for germination.

Emergence timing can play a significant role in the population dynamics of all plant species, including winter annuals (Donohue et al. 2005). Winter annual species,
especially weedy ones, may be specifically sensitive to emergence timing due to high levels of interspecific and intraspecific competition. Rees et al. (1996), for example, showed that for winter annual species, in the majority of scenarios, population size would increase by a factor of 1.5 if interactions between individuals were minimized. Van Acker and Cici (2012) found that in a comparison of spring vs. fall emerging stinkweed (*Thlaspi arvense* L.) and shepherd’s-purse (*Capsella bursa-pastoris* L.) cohorts, spring cohorts produced earlier flowering plants. Donohue et al. (2005) found that emergence timing can be a critical adaptation to forming genotypes in new locations and that the rate at which species expand their geographic range may be strongly influenced by the rate of evolution of emergence timing in winter annuals. Therefore, seed shed timing may play a critical role in the population dynamics of *Conyza canadensis*. Seed shed timing and its impact on recruitment or recruitment timing in *Conyza canadensis* has not been the focus of any studies in the literature.

*Conyza canadensis* flowers and sets seed during an extended period from late-August to mid-November. Some seed is shed when the ambient temperatures are less than the base germination temperatures preventing any further germination in the fall. This same seed may be responsible for the emergence of the spring cohort when temperatures rise above base germination temperatures once again. This mechanism would suggest that selective pressure to lengthen flowering period would produce plants that have a higher fitness. Their fitness may be elevated because they have an increased number of viable offspring in both seasons. Studying closely related species might provide insight into the development of the facultative nature of *Conyza canadensis*. 
When compared with related species such as *Conyza bonariensis* (L.) Cronquist, *Conyza canadensis* seed is shown to germinate at higher temperatures (Karlsson and Milberg 2007). This suggests an evolution of recruitment mechanisms in *Conyza canadensis* that prevents fatal germination and promotes some spring recruitment. However, germination response to a range of temperatures for *Conyza canadensis* has not been studied to any extent. It is not known to what extent key germination characteristics of *Conyza canadensis* populations differ and what that can tell us about the nature and adaptiveness of this species.

Seed dormancy is defined as the ability of a seed to not germinate in conditions that are suitable for germination. A classification system for seed dormancy was suggested by Baskin and Baskin (2004) and contains five classes: morphological dormancy (MD), physiological dormancy (PD), morphophysiological dormancy (MPD), physical dormancy (PY) and combinational dormancy (PY + PD). Physiological dormancy is most common in species within the Asteraceae family of which *Conyza canadensis* is a part. PD is further divided into levels and types of dormancy. The most common level of PD is non-deep physiological dormancy. Seeds that have non-deep PD are prone to changes in dormancy status driven by microsite conditions. Therefore, small changes in the environment around the seed can change the dormancy properties of the seed. Karlsson and Milberg (2008) suggested that the seed of *Conyza canadensis* has non-deep PD due to its short after-ripening time and the amount of germination of fresh seed in suitable environments. This suggests that *Conyza canadensis* seed may have the capacity for some dormancy, but the effects of storage and non-natural experimental conditions may decrease the already weak dormancy further.
Conyza canadensis seed that overwinters and germinates in the spring develops a plant that does not pass through the rosette stage. This suggests that the seeds are either physiologically different than when they first were shed in the fall or may be capable of detecting spring microsite conditions which somehow alter their development to exclude rosette formation after germination. As mentioned earlier, the overwintering process may alter the dormancy of the seed as well as its physiology. This effect may be one of the possible reasons for the non-formation of a rosette in the spring.

2.4 Evolutionary and Geographic Adaptation of Conyza canadensis

Differences between spring and fall cohorts of Conyza canadensis beg several questions about the evolution of Conyza canadensis. Originally from North America, Conyza canadensis has adapted to many other global locations. These local adaptations have allowed for the development of location specific ecotypes. Although Conyza canadensis is considered a winter annual in Ontario, other areas may classify it as a biennial (Alcorta et al. 2011) or summer annual (Davis et al. 2009b). Clements and DiTommaso (2011) predicted that range expansion of several weed species would outpace current climate change models that do not factor in rapid local adaptation. Traits such as high growth rate, wide climatic tolerance, short generation time, prolific or consistent reproduction, small seed size, adequate dispersal, uniparental reproduction capacity, no specialized germination requirements, high competitive abilities, and effective defences against natural enemies were suggested to be beneficial for fitness in current climate change models. Conyza canadensis displays most, if not all, of these traits (Weaver 2001) making it a formidable candidate for rapid range expansion. Conyza canadensis is well suited for both summer and winter climate conditions allowing for
survival, and potential range expansion, in areas where climate change may alter annual temperatures. McDonald et al. (2009) predicted that northern parts of the United States would experience a more dramatic shift in ecosystem composition when it comes to weedy species compared to other parts of the country. Models predict large changes in climate in this region and more southerly species will begin to expand into this region (Macdonald et al. 2009). Although already present in these areas, *Conyza canadensis* can be quite plastic in terms of growth in the short term (season) to benefit survival, and can have rapid evolution in the longer term (season to season) to benefit fitness (Weaver 2001).

### 2.5 *Conyza canadensis* as Problem Weed

*Conyza canadensis* is a problem weed in agricultural systems (Weaver 2001; Davis et al. 2009a; Main et al. 2006; Brown and Whitwell 1988). Its ability to quickly colonize an area along with rapid growth makes it an effective weed in agricultural settings. *Conyza canadensis* has become a large problem within conservation tillage systems (Keeling et al. 1989). Presence of up to 100 plants/m² has been shown to reduce crop yield by 70 to 90% in no-till soybean (Bruce and Kells 1990). Even in competition with soybean (*Glycine max* (L.) Merrill), *Conyza canadensis* may still produce up to ~70,000 seeds/plant and disperse them as high as 50cm above the crop canopy. Other no-till or limited till crops have similar yield losses with up to 64% loss is sugar beet (*Beta vulgaris* L.) and up to 28% loss in grapes (*Vitis vinifera* L.) (Holm et al. 1997).

*Conyza canadensis* can directly compete with tree saplings for light, water, and nutrients. In some cases, the presence of *Conyza canadensis* can decrease tree height by over 30cm (Weaver 2001). In general, *Conyza canadensis* can grow virtually unnoticed underneath
any crop canopy, and bolt when the timing is right. This presents many different and difficult challenges to harvesting and overall crop maintenance. For example, the presence of *Conyza canadensis* in a crop of grapes can prove rather cumbersome to the entire process of collecting and selling the grapes for consumers. The presence of *Conyza canadensis* can reduce both grape harvest efficiency and yield. During the harvesting process some grapes are damaged and release juices that become sticky when they dry. The grapes must also be thoroughly separated from any other materials such as plant matter; so post-harvesting processing efficiency is also reduced (Holm et al. 1997). *Conyza canadensis* seed is very light and small and is disturbed by the harvesting process, which may lead to some seed adhering to the grapes. The adhered seed can remain on the grapes long enough to germinate, especially if the grapes are to be used to make raisins. The large folds on each raisin can trap *Conyza canadensis* seed making them unfit for consumers, and therefore causing some amount of profit loss (Holm et al. 1997).

Not only does *Conyza canadensis* itself reduce crop yield and harvesting efficiency, but it can also promote and harbor a number of agricultural pests. *Conyza canadensis* can be a host to the glassy-winged sharp shooter (*Homalodisca vitripennis* (Germar)), which is an insect most commonly found in vineyards. It may also be a host to the tarnished plant bug (*Lygus lineolaris* (Palisot de Beauvois)), which is a pest of cotton fields (Main et al. 2006).

Mechanical control has been shown to be effective for managing *Conyza canadensis* since tillage affects recruitment by reducing potential germination microsites, burying seed to depths beyond which germination is possible for this species, or
removing any plants that have emerged (Buhler and Owen 1997; Cici and Van Acker 2009; Main et al. 2006). Fall and spring tillage have been shown to reduce *Conyza canadensis* population densities by 42% and 34%, respectively (Brown and Whitwell 1988). Tilling may not be an appropriate option in agricultural systems with high levels of erosion (Davis et al. 2009b); therefore herbicide use may be required.

Management techniques so far suggest fall tilling is the best non-chemical control method for this weed, but herbicides such as glyphosate and 2,4-D are also currently used to manage *Conyza canadensis* (Brown and Whitwell 1988; Kruger et al. 2008). *Conyza canadensis* can be difficult to manage given that emergence can occur after herbicide application (Buhler and Owen 1997; Davis et al. 2009). Residual herbicides may provide significantly greater control of this species (Davis et al. 2009). Large rosette or plants taller than 10cm have been found to be especially difficult to control with herbicides, most likely due to crop canopy size reducing herbicide coverage (Stougaard et al. 1984; Keeling et al. 1989). The most effective means of managing *Conyza canadensis* with herbicides is to use a combination of glyphosate, 2,4-D, chlorimuron, and/or cloransulam (Weaver 2001). The use of multiple herbicides can be a costly management approach, however. Fall tilling can also control the growth and development of *Conyza canadensis* before the winter as well as in the spring (Davis et al. 2009). Residual herbicides such as chlorimuron can help prevent spring emergence if applied late in the fall, but some spring emergence can still occur. Crop fields should be sprayed with multiple pre-emergence herbicides, much like the fall application, to reduce spring emergence. Byker et al. (2013) found that post-emergence applications of dicamba provided 91 to 100% control of escaped plants from previous herbicide treatments in soybean. Plants taller than 15 to
30cm were found to be extremely difficult to control with common herbicides including cyanazine, 2,4-D, paraquat, chlorimuron, metribuzin, linuron, and imazaquin (Moseley and Hagood 1990). At this stage, the plant is bolting and will continue to gain height quickly. The application of multiple herbicides is most effective on plants smaller than 15cm in height (Weaver 2001). The success rate of control is much lower on larger plants and resistant biotypes will continue to bolt reaching heights of up to 2m. Such large Conyza canadensis plants can seriously affect crop yield and harvesting efficiency (Leroux et al. 1996).

Repeated and consistent application of herbicides to control this species create a high risk for development of resistance in particular given the characteristics of Conyza canadensis including its rapid growth, high fecundity, and rapid turnover of seedbank. Populations of Conyza canadensis have independently evolved resistance to several different herbicides over the past 30 years. In 1980, it was first reported that some populations in Mississippi evolved resistance to paraquat (Heap 2013). In 1989, researchers in Belgium found some populations that were resistant to atrazine growing in cornfields and nearby roadsides and since then, several populations in southern Michigan were found to be resistant to atrazine (Heap 2013). In 1999, several populations in Indiana and Ohio evolved resistant to a range of ALS-inhibiting herbicides. Conyza canadensis evolved resistance to chlorsulfuron, cloransulam, and chlorimuron, all of which are ALS-inhibiting herbicides (Heap 2013). In 2000, it was found that populations grown in soybean fields in Delaware, U.S.A. had evolved resistance to glyphosate (VanGessel 2001). Glyphosate resistant populations have since been found in 24 States in the U.S.A., as well as Brazil, Israel, Spain, China, and the Czech Republic (Heap
Recently, glyphosate resistant *Conyza canadensis* was found in the province of Ontario, in Canada (Byker 2013). The occurrence of herbicide resistant biotypes of *Conyza canadensis* continues to rise given the herbicide intensity of common cropping systems around the world.

A recent study has shown that the ratio of glyphosate resistant *Conyza canadensis* to non-resistant can change in response to different herbicide treatments (Davis et al. 2009b). Post-emergence and residual herbicides were applied to a no-till field in Indiana that contained resistant and non-resistant *Conyza canadensis*. The field was rotated between soybean, corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.) and chlorimuron (spring residual), cloransulam (post-emergence) and glyphosate (fall and spring emergence) were applied. It was found that spring applied preplant herbicides were most effective at reducing *Conyza canadensis* emergence, but a change in the ratio of glyphosate resistant (R) to non-resistant (NR) plants in the *Conyza canadensis* population was seen as the ratio shifted from 3(R):1(NR) to 6(R):1(NR) over 4 years when a preplant herbicide was followed by non-glyphosate post-emergence herbicides. The study shows that *Conyza canadensis* is clearly capable of rapid adaptation to selection pressure.

### 2.6 Conclusion

Facultative winter annual weeds are a common problem in agricultural fields and they are also an invasive threat. The key unique characteristic of these species is their ability to germinate and emerge over a wide range of conditions in both fall and spring. *Conyza canadensis* is a prominent winter annual weed because it is broadly distributed across North America and the rest of the world. In addition, there is evolved resistance to
many common herbicides in many populations of this species. Studies to date on *Conyza canadensis* have not focused on its facultative winter annual nature, yet gaining a better understanding of why *Conyza canadensis* recruits when it does can provide insight into its weedy and invasive capability. In addition, in the context of climate change, facultative winter annuals may be particularly adaptive because of their facultative nature. The purpose of this thesis is to gain insight into the facultative recruitment nature of *Conyza canadensis*. 
Effects of seedling emergence timing on the population dynamics of *Conyza canadensis* (L.) Cronq. var. *canadensis*.

E. Tozzi and R. C. Van Acker
Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada, N1G 2W1

Nomenclature: *Conyza canadensis*, *Conyza canadensis* (L.) Cronq. var. *canadensis*
Keywords: Recruitment, facultative winter annuals, germination timing

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Tables and Figures Included within text.
CHAPTER 3

Effects of seedling emergence timing on the population dynamics of *Conyza canadensis* (L.) Cronq. var. *canadensis*.

*Conyza canadensis* (L.) Cronq. var. *canadensis* is a surface-germinating ruderal facultative winter annual. The ruderal nature is a key adaptive characteristic that implicates emergence timing as an important recruitment factor. Experiments were established at three sites in southern Ontario, Canada from 2009 to 2012 to determine the possible effect of emergence timing of *Conyza canadensis* on plant number, fecundity, and flowering timing. Emerged seedlings were tagged in 0.25m$^2$ plots in five, two-week cohorts in the fall and spring of each experimental season. Each plot was followed though until the plants contained within each plot completed their life cycle. Generally, spring emerging plants were found to flower earlier than fall emerging plants, but with fall emergence there were higher plant densities come August each season compared to spring emergence. Overall, there was no difference in fecundity between spring or fall emerging cohorts, but when cohorts were parsed beyond just spring or fall emergence we found that early fall and early spring emerging plants were more fecund and flowered earlier than late fall and late spring emerging plants. Disturbance (tilled versus not-tilled) significantly affected emergence levels but not emergence timing. The differences in performance among emergence cohorts are likely due to spatial or temporal density-dependent growth advantages. These results show that spring emerging cohorts of *Conyza canadensis*, especially early spring emerging cohorts, should not be discounted when considering the weediness of this species, and this may hold true for other facultative winter annual weeds as well.
3.1 Introduction

Understanding the population dynamics of facultative winter annual weeds, such as *Conyza canadensis* can provide insight into their recruitment nature and guide management approaches. Facultative winter annual weeds can emerge mostly in the fall, mostly in the spring, or equally in both seasons (Cici and Van Acker 2009). *Conyza canadensis* is a surface-germinating ruderal facultative winter annual with recruitment that is highly susceptible to changes in microsite conditions (Grime, 1977; Regehr and Bazzaz, 1979; Buhler and Owen, 1997; Nandula *et al.* 2006; Main *et al.* 2006).

*Conyza canadensis* flowers and sets seed in late summer, with some seed germinating and forming an overwintering rosette, and other seed persisting and germinating in the spring of the following year (Regehr and Bazzaz 1979, Weaver 2001). The lack of dormancy within the seed suggests that microsite conditions play a significant role in the persistence and emergence timing of *Conyza canadensis* (Regehr and Bazzaz 1979, Weaver 2001). The ability to germinate in spring or fall highlights how important our understanding of microsite factors are to the relative success of this species, its competitiveness in certain farming systems (e.g. tilled vs. no-till systems), and to approaches and timing for management.

*Conyza canadensis* plants may produce thousands of florets with most florets containing 30-50 seeds each (Regehr and Bazzaz 1979; Weaver 2001). Each seed has a pappus, a fan-like structure used to help seed dispersal by wind. The seed morphology impacts the population dynamics of *Conyza canadensis* since wind dispersal mechanisms have promoted the recruitment of seeds both locally and globally (Dauer *et al.* 2007).
Observations on the emergence timing of *Conyza canadensis* have shown that it may emerge at any time in a season so long as recruitment conditions are suitable (Nandula et al. 2006). The continual emergence of *Conyza canadensis* is possible because of the lack of seed dormancy (Nandula et al. 2006).

Effects of emergence timing on individual plant performance affect density-dependent population growth given that intraspecific and interspecific interactions play a significant role in the population dynamics of all plant species including winter annuals (Donohue et al. 2005). Rees et al. (1996), for example, showed that for winter annual species, in the majority of scenarios, population size would increase by a factor of 1.5 if interactions between individuals were minimized. Van Acker and Cici (2012) found that in a comparison of spring vs. fall emerging stinkweed (*Thlaspi arvense* L.) and shepherd’s-purse (*Capsella bursa-pastoris* L.), spring emerging cohorts produced earlier flowering plants. Donohue et al. (2005) found that germination timing can be a critical adaptation driving the adaptive evolution of genotypes in new locations and that the rate at which species expand their geographic range may be strongly influenced by the rate of evolution of emergence timing in winter annuals.

Differences in performance between spring and fall emerging cohorts of *Conyza canadensis* have been studied to a very limited extent. Studies to-date suggest that spring emergence occurs for less than one third of shed seeds and that the spring cohort of seedlings do not form rosettes (Buhler and Owen 1997). Regehr and Bazzaz (1979) are the only ones to have published research on this. They suggested that spring emerging *Conyza canadensis* may not form a rosette and they reported that for *Conyza canadensis* populations from the US mid-west, spring emerging plants produce less seed and seed
with lower longevity while seed from fall emerging plants experiences higher mortality but individual fall emerging plants produce more seed per plant.

Time of emergence may play a significant role in the population dynamics of *Conyza canadensis* yet the influence of emergence timing on the performance of *Conyza canadensis* has been investigated to a very limited extent. The objective of this study is to explore the effect of emergence timing of *Conyza canadensis* on fecundity, plant density, and flowering timing. This study will allow us to gain a deeper understanding of the population dynamics, recruitment nature, and biology of winter annuals in general, and *Conyza canadensis* specifically. The results of this work will also provide information to help us to better manage this important weed species.

**3.2 Materials Methods**

Experiments were established at three sites and followed for three winter annual (late summer through to following summer) growing seasons (2009-2010, 2010-2011, 2011-2012) in a northern region of North America; south central Ontario, Canada (sites were located near the towns of Woodstock, Simcoe and Guelph). The Woodstock site was situated on a Guelph Loam series soil (Gray Brown Luvisol) containing 35% sand, 52% silt, 13% clay, and 3.6% organic matter, with a pH of 6.4. The Simcoe site was situated on a Berrien sandy-loam soil containing 55% sand, 30% silt, and 15% clay, 1.93% organic matter, with a pH of 6.8. The Guelph site was situated on a very fine sandy loam soil containing 55 to 60% sand, 28 to 34% silt, and 10 to 11% clay with a neutral pH (7). The *Conyza canadensis* infestations at each of these sites existed previously and were not augmented for this study.
3.2.1 Emergence Timing

At each site, ten 0.25m² quadrats were marked as observational plots. To characterize emergence timing we counted seedlings at each site each week from Julian week 34 (August 26-September 8) to Julian week 47 ((November 19-November 25) in the fall and from Julian week 15 (April 9-April 16) to Julian week 24 (June 4-June 17) in the spring. No seedlings were removed once counted. The same plots were followed through all three seasons in order to monitor changes in the populations over time.

To investigate the role disturbance would play in affecting emergence, half (5) of the plots at each site were disturbed by hand tillage and the other half were left undisturbed. Disturbance treatments occurred once each season in Julian week 33 (August 13-August 19) just as new Conyza canadensis seeds for the given year were starting to be shed and just before emergence counts started for a given season. For the disturbance treatments we used a hand rake to till each plot (3-4 rakings in each of two directions in each plot) to a depth of 3-4 cm. Disturbance treatments were aggressive enough to eliminate existing vegetation in each plot each season.

3.2.2 Cohorts

To facilitate data analysis and interpretation, we classified the emergence data into emergence timing cohorts. We classified a total of 10 cohorts per winter annual season and each emergence cohort covered a two-week emergence period. The 5 fall cohorts ran from August 26-September 8, September 9-September 22, September 23-October 6, October 7-October 20, October 21-November 3. The 5 spring cohorts ran from April 9-April 22, April 23-May 6, May 7-May 20, May 21-June 3, June 4-June 17.
The key performance measures we included in the study were; survival of emerged seedlings, flowering timing, and fecundity. Survival (plant number/plot) was the number of *Conyza canadensis* plants at any stage of development (rosettes, seedlings, bolting etc.) present in each plot in August of the following year. Given the indeterminate nature of *Conyza canadensis*, flowering timing was recorded as Julian week of first seed shed for 5 plants at each site for each cohort (plants were chosen randomly from among the plots at each site and tagged using colored paper clips with a specific color designating a particular cohort). Fecundity was measured by multiplying the average number of seeds per flower by the number of flower heads on each tagged plant (these counts were done just prior to tillage treatments). Average number of seeds per flower was determined by counting the number of seeds per capitula in 50 capitula from 10 surviving plants chosen at random at each site each year. For this study, the average number of seeds per flower was 46.

### 3.2.3 Statistical Analysis

Statistical analysis of data was conducted using JMP 10.0.2 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, SAS Institute, Inc.). All data were subjected to an ANOVA using a repeated measures linear mixed effects model with year (random), site (random), season (fall versus spring) (random), cohort (random), and till/no-till (fixed) as factors, and with replication nested in site for fecundity (capitula/plant), plant number (in each 0.25m\(^2\) plot), and flowering date (Julian week of first seed shed). On the basis of an examination of residual plots, data were deemed to
meet the assumptions of ANOVA including homogeneity of variance. In all cases, means were considered to be significantly different on the basis of \( P<0.05 \).
Figure 3.1 Observed emerging timing for *Conyza canadensis* seedlings in tilled or no-tilled plots over three winter annual seasons in southern Ontario, Canada. Data points represent means and error bars represent 1 standard error of the mean.
The effect of emergence timing on flowering timing (Julian calendar week when first seed shed occurred) of *Conyza canadensis*. Results represent pooled data for either tilled or not-tilled plots and for three sites in southern Ontario, Canada collected within three individual seasons (2009-2010, 2010-2011, 2011-2012).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Flowering Timing&lt;sup&gt;b&lt;/sup&gt; (Julian week)</th>
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<tbody>
<tr>
<td>5 (June 4-June 17)</td>
<td>38.6 e</td>
</tr>
<tr>
<td>5 (Oct 20-Nov 3)</td>
<td>38.4 e</td>
</tr>
<tr>
<td>4 (May 21-June 3)</td>
<td>37.6 d</td>
</tr>
<tr>
<td>4 (Oct 6-Oct 20)</td>
<td>37.5 d</td>
</tr>
<tr>
<td>3 (May 7-May 20)</td>
<td>35.4 c</td>
</tr>
<tr>
<td>3 (Sept 22-Oct 6)</td>
<td>35.5 c</td>
</tr>
<tr>
<td>2 (April 23-May 6)</td>
<td>35.1 bc</td>
</tr>
<tr>
<td>1 (April 9-April 22)</td>
<td>35.0 abc</td>
</tr>
<tr>
<td>2 (Sept 8-Sept 22)</td>
<td>34.6 ab</td>
</tr>
<tr>
<td>1 (Aug 26-Sept 8)</td>
<td>34.5 a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers represent numbered cohort order for each season

<sup>b</sup> Means within columns followed by different letters denote significant differences at p<0.05 according to Tukey’s HSD.
Table 3.2 The effect of emergence timing (cohort) on the fecundity of *Conyza canadensis*. Results represent pooled data for either tilled or not-tilled plots and three sites in southern Ontario, Canada collected within three individual seasons (2009-2010, 2010-2011, 2011-2012).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Fecundity (capitula/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (Oct 20-Nov 3)</td>
<td>635.8 a</td>
</tr>
<tr>
<td>5 (June 4-June 17)</td>
<td>644.9 a</td>
</tr>
<tr>
<td>4 (Oct 6-Oct 20)</td>
<td>754.4 b</td>
</tr>
<tr>
<td>4 (May 21-June 3)</td>
<td>771.2 b</td>
</tr>
<tr>
<td>3 (May 7-May 20)</td>
<td>888.06 c</td>
</tr>
<tr>
<td>3 (Sept 22-Oct 6)</td>
<td>906.42 cd</td>
</tr>
<tr>
<td>2 (April 23-May 6)</td>
<td>982.5 cde</td>
</tr>
<tr>
<td>2 (Sept 8-Sept 22)</td>
<td>995.6 de</td>
</tr>
<tr>
<td>1 (April 9-April 22)</td>
<td>996.0 de</td>
</tr>
<tr>
<td>1 (Aug 26-Sept 8)</td>
<td>1008.8 e</td>
</tr>
</tbody>
</table>

*a Numbers represent numbered cohort order for each season

*b Means within columns followed by different letters denote significant differences at p<0.05 according to Tukey’s HSD.
Table 3.3 The effect of emergence timing on the survival (%) of Conyza canadensis plants in August. Results represent data pooled for tilled and not-tilled plots and for three sites in southern Ontario, Canada.

<table>
<thead>
<tr>
<th>Cohort*</th>
<th>Survivala (Mean %)</th>
<th>Cohort</th>
<th>Survival (Mean %)</th>
<th>Cohort</th>
<th>Survival (Mean %)</th>
<th>Cohort</th>
<th>Survival (Mean %)</th>
<th>Cohort</th>
<th>Survival (Mean %)</th>
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</thead>
<tbody>
<tr>
<td>1 (Aug 26-Sept 8)</td>
<td>94 a</td>
<td>1 (Aug 26-Sept 8)</td>
<td>90 a</td>
<td>1 (Aug 26-Sept 8)</td>
<td>90 a</td>
<td>1 (Aug 26-Sept 8)</td>
<td>94.6 a</td>
<td>1 (Aug 26-Sept 8)</td>
<td>87.2 a</td>
</tr>
<tr>
<td>2 (April 23-May 6)</td>
<td>90.6 ab</td>
<td>2 (Sept 22)</td>
<td>82 ab</td>
<td>2 (Sept 22)</td>
<td>88 ab</td>
<td>5 (Oct 20-Nov 5)</td>
<td>90.6 ab</td>
<td>5 (Oct 20-Nov 5)</td>
<td>86.6 a</td>
</tr>
<tr>
<td>2 (Sept 8-Sept 22)</td>
<td>90.6 ab</td>
<td>3 (Sept 22-Oct 6)</td>
<td>78.6 ab</td>
<td>2 (Sept 22-Oct 6)</td>
<td>71.2 bc</td>
<td>2 (Sept 8-Sept 22)</td>
<td>89.2 abc</td>
<td>2 (Sept 8-Sept 22)</td>
<td>84.6 a</td>
</tr>
<tr>
<td>3 (Sept 22-Oct 6)</td>
<td>86.6 ab</td>
<td>2 (April 23-May 6)</td>
<td>77.2 ab</td>
<td>2 (April 23-May 6)</td>
<td>70 c</td>
<td>1 (April 9-April 22)</td>
<td>88.6 abc</td>
<td>2 (April 23-May 6)</td>
<td>83.2 a</td>
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<td>1 (April 9-April 22)</td>
<td>84.6 a</td>
<td>1 (April 9-April 22)</td>
<td>70.6 b</td>
<td>1 (April 9-April 22)</td>
<td>69.2 c</td>
<td>2 (April 23-May 6)</td>
<td>87.2 abc</td>
<td>3 (May 7-May 20)</td>
<td>78 ab</td>
</tr>
<tr>
<td>3 (May 7-May 20)</td>
<td>82.6 a</td>
<td>3 (May 7-May 20)</td>
<td>68.6 b</td>
<td>3 (May 7-May 20)</td>
<td>54.6 cd</td>
<td>3 (Sept 22-Oct 6)</td>
<td>86.6 abc</td>
<td>1 (April 9-4)</td>
<td>77.2 ab</td>
</tr>
<tr>
<td>4 (May 21-June 3)</td>
<td>56.6 b</td>
<td>4 (Oct 4-Oct 20)</td>
<td>47.2 c</td>
<td>4 (Oct 4-Oct 20)</td>
<td>48.6 d</td>
<td>3 (May 7-May 20)</td>
<td>78 bc</td>
<td>3 (Sept 22-Oct 6)</td>
<td>75.2 abc</td>
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<tr>
<td>4 (Oct 6-Oct 20)</td>
<td>56.6 b</td>
<td>4 (May 21-June 3)</td>
<td>44 c</td>
<td>4 (May 21-June 3)</td>
<td>41.2 d</td>
<td>4 (Oct 6-Oct 20)</td>
<td>75.2 cd</td>
<td>5 (June 4-June 17)</td>
<td>71.2 abc</td>
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<tr>
<td>5 (June 4-June 17)</td>
<td>14.6 c</td>
<td>5 (Oct 5-Oct 20-Nov 3)</td>
<td>13.2 d</td>
<td>5 (June 21-June 3)</td>
<td>17.2 e</td>
<td>4 (May 21-June 3)</td>
<td>64 d</td>
<td>4 (Oct 6-Oct 20)</td>
<td>64.6 bc</td>
</tr>
<tr>
<td>5 (Oct 20-Nov 3)</td>
<td>9.6 c</td>
<td>5 (June 4-June 17)</td>
<td>12 d</td>
<td>5 (June 4-June 17)</td>
<td>16.6 e</td>
<td>5 (June 21-June 3)</td>
<td>50 e</td>
<td>4 (May 21-June 3)</td>
<td>59.2 c</td>
</tr>
</tbody>
</table>

aNumbers represent numbered cohort order for each season

bMeans within columns followed by different letters denote significant differences at p<0.05 according to Tukey’s HSD.
3.3 Results

When comparing spring versus fall emergence cohorts, spring emerging *Conyza canadensis* plants flowered significantly earlier than fall emerging plants (ANOVA, F ratio = 5.39, P < 0.0203, df = 1, n = 1699) (Table 3.1). Spring emerging plants less commonly pass through a rosette phase like their fall emerging counterparts resulting in less time and energy spent in the seedling stage (Buhler and Owen 1997). This ability enables a shorter time to bolting and flowering; however, per plant fecundity was not significantly different between spring and fall emerging plants (ANOVA, F ratio = 0.099, P < 0.7529, df = 1, n = 1699, Table 3.2).

When emergence timing was parsed beyond spring or fall we found that *Conyza canadensis* plants emerging in early fall and early spring were more fecund and flowered earlier than plants emerging in late fall and late spring. This effect was consistent in all three seasons, at all sites and under both tilled and not-tilled conditions (Table 3.1 and Table 3.2). Early emerging plants, either fall or spring emerging, were visibly larger and taller than plants that emerged later (either in fall or spring) (personal observation). Previous studies have shown that plant height may be exponentially related to fecundity in *Conyza canadensis* (Regehr and Bazzaz 1979; Dauer et al. 2008). Due to the single stalk morphology and ruderal nature of *Conyza canadensis*, adaptations for optimization of high dispersal and high fecundity in a relatively short lifespan directly correlate plant height with biomass (Dauer et al. 2008). These results show that *Conyza canadensis* plants that have an early start, either in spring or fall, are more likely to produce more seed. For fall emerging plants, late fall emergence may not allow for the formation of robust rosettes.
3.4 Discussion

The formation of a rosette helps reduce overwintering mortality in *Conyza canadensis* come spring time (Regehr and Bazzaz 1979; Main et al. 2006). Late fall and spring emerging plants are also subjected to more competition including intraspecific competition which can lead to reduced fecundity and greater mortality (Grime 1977; Main et al. 2006). In this study we also found that earlier emerging cohorts of *Conyza canadensis* had significantly higher survival (Table 3.3). This effect may be related to density-dependent competition as well as accumulated biomass.

The shortened time to flowering for late emerging plants (either fall or spring) may indicate a set time in each season for flowering if growth requirements are met *ad libitum*. A 4-6 month period after the beginning of bolting has been observed as the time it takes to reach full reproductive maturity for *Conyza canadensis* (Regehr and Bazzaz 1979), but maximum flowering timing, in general, for *Conyza canadensis* in southern Ontario is early August (personal observation). Other potential factors influencing the relationship between emergence timing and flowering timing may be lack of nutrients required to maximize growth or reproduction. In this case, most plants have physiological mechanisms designed to optimize and promote flowering to maximize fitness. Some *Conyza canadensis* plants, for instance, have been observed to flower at a height of only 10cm reinforcing the notion of resource limiting induction of reproduction (personal observation). Day length and light intensity may also play a factor in time to flowering although no studies have been conducted on the effect of daylength or light quantity on the flowering timing of *Conyza canadensis*. Late fall and late spring emerging cohorts of *Conyza canadensis* may be experiencing either the set time to
flowering or resource limitations as both cohorts reach bolting stage at later dates than
their earlier emerging counterparts. Some studies suggest bolting in this species is not
directly related to daylength (e.g. Nandula et al. 2006). If this were the case, most plants
would bolt around the same period each year. Instead, we observed that plants bolted
after ~6 weeks of growth, regardless of rosette or seedling size. Our results suggest that
Conyza canadensis plants that emerge earlier have a greater chance of bolting earlier
leading to possibly significant competitive advantages.

In the sites we used for this study, more Conyza canadensis seedlings emerged in
the fall versus the spring (Figure 3.1). Over all factors, peak plant emergence occurred
between August 27 and September 9 (112.1 plants/0.25m²), and May 14 and May 27
(10.44 plants/0.25m²) of each season (Figure 3.1). This result represents a significant
(order of magnitude) difference in the proportionality of Conyza canadensis emergence
when comparing spring vs. fall emergence levels and compares to previous findings of 5
to 32% spring emergence (Buhler and Own 1997).

3.4.1 Disturbance

Disturbance (tilled versus not-tilled) significantly affected emergence levels but
not emergence timing (Figure 3.1, Table 3.4). In the tilled plots emergence levels were
very similar (almost identical) among the three seasons while in the plots that were not
tilled the total emergence density dropped significantly in seasons 2 and 3 (2010-11 and
2011-12) versus season 1 (2009-10). This result may be due to the early effects of
succession and competition. Without tillage, other competitive plants (including mature
Conyza canadensis plants) likely reduced the recruitment opportunities for Conyza
canadensis. *Conyza canadensis* is a ruderal species and therefore is inherently a first colonizer to new available microsites favourable to germination (Weaver 2001). Over time, *Conyza canadensis* can lose favourable recruitment microsites to other weeds including hardier winter annuals and perennials including chickweed (*Stellaria media* L.) and Canada thistle (*Cirsium arvense* L.), respectively (personal observation). Even with the formation of a rosette in the winter, to create a competitive advantage come springtime, *Conyza canadensis* is eventually subjected to high density-dependent competition factors and recruitment is subsequently reduced. In the tilled plots the tillage removed other competing plants each season, reducing barriers to recruitment. In addition, tillage creates favorable recruitment microsites for ruderal species (Brown and Whitwell 1988).

The lack of differences in overall fecundity between fall and spring emerging plants, but the significant differences between early and late emerging plants within spring or fall cohorts, is a unique and important result not only for *Conyza canadensis* but perhaps for other facultative winter annual weeds. It confirms that emergence timing is an important factor affecting the performance of *Conyza canadensis*, but it also shows that fall emerging plants do not necessarily outperform spring emerging plants. This may have broader implications in studies that have chosen to compare only fall vs. spring emerging cohorts of facultative winter annual weeds (Regehr and Bazzaz 1979; Buhler and Owen; Dauer et al. 2007) and it has implications for the characterization, modeling, and prediction of population performance for *Conyza canadensis* and perhaps other facultative winter annuals.
Acknowledgements

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Seed germination response to temperature for a range of international populations of *Conyza canadensis* (L.) Cronq. var. canadensis.


*Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada, N1G 2W1, †Agriculture and Agri-Food Canada (AAFC), 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2, ‡Instituto de Agricultura Sostenible (C.S.I.C.), Alameda del Obispo, Aptdo. 4080, 14080 Córdoba, Spain, and §Rothamsted Research, West Common, Harpenden, Hertfordshire, AL5 2JQ

Nomenclature: *Conyza canadensis, Conyza canadensis* (L.) Cronq. var. canadensis

Keywords: germination response, base germination, facultative winter annuals, thermogradient plate

Manuscript-Chapter Differences:
Tables and Figures Included within text.
CHAPTER 4

Seed germination response to temperature for a range of international populations of *Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. *canadensis*).

*Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. *canadensis*) is a surface-germinating ruderal facultative winter annual with recruitment that is highly susceptible to changes in microsite conditions. A key adaptive characteristic for a facultative winter annual species, like *Conyza canadensis*, is germination response to temperature. The objective of this study was to determine the germination response to temperature for *Conyza canadensis* seed sourced from regions around the world with differing climates, and by doing so, gain insight into the role that seed germination biology plays in the adaptiveness and weediness of facultative winter annual weeds. Seed was sourced from populations in Málaga, Spain, Hertfordshire, UK, Shiraz, Iran, and Southern Ontario, Canada, and grown out in a common garden under controlled conditions to produce seed for this study. These seeds were then subjected to temperatures from 6.5 to 20\(^\circ\)C at 1.5\(^\circ\)C increments using a thermo-gradient plate (TGP). Cumulative daily germination counts for 30 days were recorded. Results indicated that temperature and source location had a significant effect on germination response. Estimated base germination temperature ranges were significantly different amongst the populations (Ontario (8 to 9.5\(^\circ\)C), Iran (9.5 to 11\(^\circ\)C), Spain (12.5 to 14\(^\circ\)C), UK (11 to 12.5\(^\circ\)C) as were accumulated growing degree days (GDDs) required to reach 50% germination. For three of the four populations estimated base germination temperature range values were below those previously reported in the literature. These differences are likely evolutionary adaptations to local
climate and highlight the important role recruitment biology plays in adaptation to
climate in the context of agriculture and the particular adaptiveness of *Conyza canadensis*
in this respect.

### 4.1 Introduction

*Conyza canadensis* (*Conyza canadensis (L.) Cronq. var. canadensis*) is a surface-
germinating ruderal facultative winter annual with recruitment that is highly susceptible
to changes in microsite conditions (Grime 1977; Regehr and Bazzaz 1979; Buhler and
Owen 1997; Nandula *et al.* 2006; Main *et al.* 2006). Recently disturbed moist soil with
light availability is the ideal condition for *Conyza canadensis* seedling recruitment
(Nandula *et al.* 2006). Any alteration to these conditions has an impact on successful
recruitment. The lack of dormancy within the seed and indeterminate flowering period
for *Conyza canadensis* suggest that microsite conditions play a large role in the
persistence, seed germination, and recruitment timing of this species (Regehr and Bazzaz
1979). With seed that can travel large distances, *Conyza canadensis* can experience a
diverse variety of microsite conditions (Dauer *et al.* 2007; Dauer *et al.* 2008). The ability
of this species to germinate in spring or fall highlights how important our understanding
of these factors is to the relative success of this species as a weed and to potential
approaches for management.

It has been estimated that *Conyza canadensis* seed has a base germination
temperature of $13^\circ C$ (Steinmaus *et al.* 2000). Peak seedling establishment of *Conyza
canadensis* generally occurs in early autumn only weeks after peak seed set (Regehr and
Bazzaz 1979). *Conyza canadensis* flowers and sets seed during an extended period from
late-August to mid-November (Regehr and Bazzaz 1979). Some seed is shed when the ambient temperatures are less than the base germination temperatures preventing germination of this seed in the fall. This same seed is likely responsible for the emergence of spring cohorts when temperatures rise above base germination temperatures in the spring. For facultative winter annuals like *Conyza canadensis*, germination response to temperature is an important characteristic influencing both fall and spring recruitment timing, duration, and proportion. Regional adaptation of weed species, and in particular facultative winter annuals, may be very much a function of germination response to temperature. In addition, germination response to temperature may impact the flexibility of a given population in relation to variations in climate and adaptation to climate change.

When compared with related species such as *Conyza bonariensis* (L.) Cronquist, *Conyza canadensis* seed is shown to germinate at higher temperatures (Karlsson and Milberg 2007). This suggests an evolution of mechanisms in *Conyza canadensis* that prevents fatal germination and promotes some spring recruitment. However, germination response to a range of temperatures for *Conyza canadensis* has not been studied to any extent. The ability of *Conyza canadensis* seed to enter the planetary boundary layer and travel globally results in populations of *Conyza canadensis* around the world (Shields et al. 2006). It is not known to what extent key germination characteristics of these populations differ and what that can tell us about the nature and adaptiveness of this species.

To-date, studies on different populations of *Conyza canadensis* have been focused on populations that are resistant to herbicides (Davis et al. 2009; VanGessel et al.
2009; González-Torralva et al. 2010; Nol et al. 2012). Attention to other adaptations of *Conyza canadensis* is also important in terms of its weediness. A key adaptive characteristic for a facultative winter annual species like *Conyza canadensis* is germination response to temperature. The objective of this study, therefore, was to determine the germination response to temperature for *Conyza canadensis* seed sourced from regions around the world with differing climates, and by doing so, to gain insight into the role that seed germination biology plays in the adaptiveness and weediness of facultative winter annual weeds and *Conyza canadensis* specifically.

### 4.2 Material and Methods

In the spring of 2011, *Conyza canadensis* seed was sourced from collaborating weed scientists in Málaga, Spain, Hertfordshire, United Kingdom, and Shiraz, Iran. Each of these scientists collected seed from a number of plants from a number of populations near these cities. In the late summer of 2011, we also collected seed from populations near the towns of Guelph, Woodstock and Simcoe, Ontario that were pooled to create a southern, Ontario population. The average climate for these locations is provided in Table 4.1.

Once received or collected, all seed was stored at 4 °C until the fall of 2011 when 200 to 300 seeds of each population were germinated in petri dishes. Resulting seedlings were transplanted into pots (30cm diameter) containing a commercial potting soil mixture. One plant was grown per pot to ensure the highest quantity and quality of seed and there were 20 plants (pots) for each population. Plants were grown in a growth room at 24 °C/16 °C day/night temperatures with 16 h days until the plants flowered and
produced seed (approximately 4 to 6 months). Plants were bagged with micro-perforated glycine bags to allow seed to fully mature and shed. Shed seed was collected in these bags, transferred to paper bags, and stored at 4°C until January 2012. This common garden procedure was used to equilibrate seed relative to maternal environment effects.

The germination response of seeds from the various populations to temperature was investigated using Thermo-Gradient Plate (TGP) apparatus at the University of Saskatchewan in Saskatoon, SK, Canada. There are two TGPs at this facility with 96 and 176 individually controlled cells, respectively. Individual plates deliver temperatures accurate within 0.1°C with a potential range of 0°C to 40°C. A thermoelectric pump controls the temperature in each cell (McLaughlin et al. 1985). For each Conyza canadensis population, there were two replicates of each treatment (temperature regime) and each experiment was run twice. One 100mm petri dish lined with filter paper dampened using de-ionized water was placed in each TGP cell and 100 seeds were used in each petri dish. Filter paper was kept moist throughout the experiment.

Seeds were subjected to ten constant temperatures ranging from 6.5 to 20°C at 1.5°C increments (i.e. 6.5°C, 8°C, 9.5°C, 11°C, 12.5°C, 14°C, 15.5°C, 17°C, 18.5°C, 20°C). The temperature range was chosen to bracket the current estimated base germination temperature of 13°C for Conyza canadensis (Steinmaus et al. 2000). The temperature range in this experiment accurately buffers a lower or higher base germination temperature while simultaneously allowing the number of experimental units to fit the available number of cells with each TGP machine. The experiment was organized in a complete randomized design. Germination counts were conducted for each cell each day for 30 days, which was long enough to allow for at least several days
of no further germination in the lowest temperature treatments. Seeds were deemed to have germinated when the radicle or cotyledons were visible.

For each population, we made a special note of cells (and their set temperatures) where there was no germinated seed at the end of each experiment run. For each and all populations the lowest temperatures where there was still some germination in given cells was consistent between replicates and between runs (no variation) for a given population. We noted this temperature for each population and estimated that the base germination for the given population would have been between that temperature and the next lower temperature in the range. We recorded these in Table 5, and given that there was no variation in these mean ranges within a population we did not include any statistics or mean separation information with these data.

Given that TGP cells were kept at constant temperature, the Growing Degree Day (GDD) for a given TGP cell was calculated by subtracting \( T_{\text{base}} \) from the given cell temperature. A \( T_{\text{base}} \) of 0°C was used in the GDD calculation in order to provide a common base for all populations. At the end of each experiment run, GDDs to 50% germination was determined for each cell by first determining the cumulative germination for each cell by day and then determining the day (date) of 50% germination and equating this to the corresponding GDD.

4.2.1 Statistical Analysis

Statistical analysis of data was conducted using JMP 10.0.2 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, SAS Institute, Inc.). For the GDDs to 50% germination data, an ANOVA was conducted using a linear mixed effects model
with population (random) and temperature (fixed) as factors, and with replication nested in population. On the basis of an examination of residual plots, data were deemed to meet the assumptions of ANOVA including homogeneity of variance. In all cases, means were considered significantly different on the basis of \( P < 0.05 \) using Tukey’s HSD.

A non-linear three-parameter Gompertz sigmoidal model was fit to the data representing the relationship between temperature and germination. The germination response model was:

\[
y(t) = ae^{be^{ct}}
\]

Where \( y \) is percent germination, \( t \) is temperature, \( a \) the asymptote, \( b \) the rate of change in germination and \( c \) the inflection point. Models were fit for each population separately and these models were compared for common parameter values using a lack of fit F test at \( p=0.05 \) similar to the methods described in Lawson et al. (2006).

<table>
<thead>
<tr>
<th>Month</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Daily mean °C</td>
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<td>-6.4</td>
<td>-1.2</td>
<td>5.8</td>
<td>12.5</td>
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<td>19.8</td>
<td>18.7</td>
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<td>78.3</td>
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<td>65.6</td>
<td>82.7</td>
<td>73.6</td>
<td>907.9</td>
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</tr>
<tr>
<td>Daily mean °C</td>
<td>5.3</td>
<td>7.7</td>
<td>11.8</td>
<td>16.2</td>
<td>22.5</td>
<td>27.7</td>
<td>29.8</td>
<td>28.7</td>
<td>24.5</td>
<td>18.4</td>
<td>11.7</td>
<td>6.8</td>
<td>17.9</td>
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<tr>
<td>Precipitation mm</td>
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<td>48.4</td>
<td>30.6</td>
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<td>0.0</td>
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<td>19</td>
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<td>13</td>
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<td>4</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Precipitation mm</td>
<td>69.3</td>
<td>59.4</td>
<td>46.5</td>
<td>70.1</td>
<td>58.1</td>
<td>58.9</td>
<td>46.0</td>
<td>68.9</td>
<td>51.7</td>
<td>84.3</td>
<td>93.9</td>
<td>80.9</td>
<td>788.0</td>
</tr>
<tr>
<td>Málaga, Spain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily mean °C</td>
<td>12.0</td>
<td>12.8</td>
<td>14.1</td>
<td>15.6</td>
<td>18.7</td>
<td>22.2</td>
<td>24.8</td>
<td>25.4</td>
<td>23.1</td>
<td>19.0</td>
<td>15.4</td>
<td>12.9</td>
<td>18.0</td>
</tr>
<tr>
<td>Precipitation mm</td>
<td>81</td>
<td>55</td>
<td>49</td>
<td>41</td>
<td>25</td>
<td>12</td>
<td>2</td>
<td>6</td>
<td>16</td>
<td>56</td>
<td>95</td>
<td>88</td>
<td>526</td>
</tr>
</tbody>
</table>
Table 4.2 Gompertz model parameter estimates for models fit to the relationship between cumulative germination (%) and temperature (C) for seeds sourced from plants grown in a common garden from seeds, in turn, sourced from Shiraz, Iran, southern Ontario, Canada, Málaga, Spain and Hertfordshire, United Kingdom. For the model parameters, a represents asymptote, b represents growth rate, and c represents inflection point. Values in parentheses are standard errors of the parameter estimates. Individual population models are considered significantly different according to lack of fit F test at p = 0.05.

<table>
<thead>
<tr>
<th>Population</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran</td>
<td>98.5 (1.24)</td>
<td>0.6 (0.03)</td>
<td>12.4 (0.05)</td>
<td>0.994</td>
</tr>
<tr>
<td>Ontario</td>
<td>96.7 (0.89)</td>
<td>0.7 (0.03)</td>
<td>11.0 (0.05)</td>
<td>0.994</td>
</tr>
<tr>
<td>Spain</td>
<td>92.9 (1.14)</td>
<td>1.53 (0.175)</td>
<td>13.3 (0.076)</td>
<td>0.991</td>
</tr>
<tr>
<td>U.K.</td>
<td>96.0 (0.772)</td>
<td>0.7 (0.07)</td>
<td>13.5 (0.094)</td>
<td>0.983</td>
</tr>
</tbody>
</table>
Table 4.3 Results from the ANOVA (linear mixed model) assessing the effect of temperature (°C), seed source (source), experiment run (run), and replicate (rep) on the growing degree days (GDDs) required to reach 50% germination.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Experimental factor</th>
<th>DF</th>
<th>F-value</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDDs to 50% Germination</td>
<td>Temperature (°C)</td>
<td>9</td>
<td>336.1472</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td>Source</td>
<td>3</td>
<td>21.5543</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td>Run</td>
<td>1</td>
<td>0.0726</td>
<td>0.7880</td>
</tr>
<tr>
<td></td>
<td>Rep</td>
<td>1</td>
<td>0.1771</td>
<td>0.6745</td>
</tr>
</tbody>
</table>

DF, degrees of freedom; F-value, treatment mean square/error mean square; P > F, significance probability value.
Table 4.4 Mean growing degree days (GDD $T_{\text{base}} 0^\circ$C) required to reach 50% germination for seeds of *Conyza canadensis* sourced from Málaga, Spain, Hertfordshire, United Kingdom, southern Ontario, Canada, and Shiraz, Iran. Means followed by the same letter are considered not significantly different at $p<0.05$.

<table>
<thead>
<tr>
<th>Population</th>
<th>GDDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran</td>
<td>141.6 a</td>
</tr>
<tr>
<td>U.K.</td>
<td>120.1 ab</td>
</tr>
<tr>
<td>Spain</td>
<td>105.8 bc</td>
</tr>
<tr>
<td>Ontario</td>
<td>94.3 c</td>
</tr>
</tbody>
</table>
Table 4.5 Lowest temperature range (°C) for which seed germination occurred for seed of *Conyza canadensis* sourced from populations in Málaga, Spain, Hertfordshire, United Kingdom, southern Ontario, Canada, and Shiraz, Iran.

<table>
<thead>
<tr>
<th>Population</th>
<th>Temperature Range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontario</td>
<td>8-9.5</td>
</tr>
<tr>
<td>Iran</td>
<td>9.5-11</td>
</tr>
<tr>
<td>U.K.</td>
<td>11-12.5</td>
</tr>
<tr>
<td>Spain</td>
<td>12.5-14</td>
</tr>
</tbody>
</table>
Figure 4.1 Cumulative germination (%) of *Conyza canadensis* seed as affected by temperature (°C) for seeds sourced from plants grown in a common garden from seeds, in turn, sourced from A) Shiraz, Iran, B) southern Ontario, Canada, C) Málaga, Spain and D) Hertfordshire, United Kingdom. For model parameters see Table 2.
4.3 Results

For each of the source locations, collaborating experts confirmed the plants from which the seed was sourced was *Conyza canadensis*. However, when growing these four populations in a common garden for seed production we observed and recorded some differences in morphologies amongst the populations. Plants from the Ontario population exhibited a typical North American morphology for fleabane (Weaver 2001) with one large bolting stem, medium size (~1.5cm wide) linear lobate leaves, medium size (~0.5cm) white florets, and reaching a height of ~180cm. Plants from the U.K. population shared a similar morphology but with reduced height in all plants of ~150cm. The Spanish population exhibited a “thinner” form with more than one thin bolting stem, small (~0.5cm wide) linear lobate-ciliate leaves, small size (~0.25cm) white florets, and a height of ~150cm. Plants from the Iranian population were the most unique with one large bolting stem branching off into multiple stems near the top of the plant, large size (~2.5cm wide) linear lobate leaves, medium size (~1.5cm) white florets, and reaching a height of ~150cm.

The sigmoidal Gompertz model described well the relationship between temperature and seed germination for all four fleabane populations ($R^2$ values ranging between 0.98 and 0.99, Table 2) but there were significant differences in the models among the populations (Figure 4.1, Table 4.2). The Spanish population had the most unique model with a noticeably longer lag period and a very steep germination growth rate beyond a certain threshold temperature. The responses for the other three populations were more similar but still statistically significantly different from each other. The Spanish, U.K. and Ontario populations all had very similar germination rates, but the
inflection points differed significantly with the Ontario population inflecting at the lowest temperature followed by the Iranian, then the Spanish and U.K. populations.

There were significant relationships between temperature and seed source, and accumulated growing degree days (GDDs) required to reach 50% germination (Table 4.3). There were no significant relationships between run and rep, and accumulated GDDs required to reach 50% germination (Table 4.3). There were significant differences among the four populations in accumulated GDDs required to reach 50% germination with the southern Ontario population requiring the least, (94 at $T_{\text{base}} 0^\circ C$, Table 4.4) followed by the Spanish (106) and U.K. (120) populations, with the Iranian population requiring the most (142). In this study we observed the lowest temperature range for which we recorded germination in the TGP plates (Table 4.5) but this was not a determination of $T_{\text{base}}$ per se. However, our approach in this regard did allow us to make comparisons among the populations, which was the intent of this study. The lowest germination temperature ranges differed among the four populations and for three of the four populations (Ontario, Iran and U.K.) the lowest germination temperature ranges were below the average $T_{\text{base}}$ value presented in the literature ($13^\circ C$, Steinmaus et al. 2000). The Ontario population germinated at the lowest temperature range (8 to 9.5°C), while Spanish populations had the highest base germination temperature range (12.5 to 14°C). For the Iranian and U.K. populations, the base germination temperature ranges were between the other two populations (9.5 to 11°C and 11 to 12.5°C, respectively). The rankings of the Iranian, U.K., and Spanish populations, according to these base germination temperature ranges, were not the same as the rankings for these populations according to GDDs required for 50% germination. The Ontario population required the
least accumulated GDDs to reach 50% germination and it had the lowest estimated base germination temperature range.

4.4 Discussion

Given that the seeds used in this study were from a common garden, the differences among the populations we observed in terms of estimated base germination temperature range and GDDs required to reach 50% germination are likely inherent (genetic) and the result of regional adaptation due to climatic selection pressures (Clements and DiTommaso 2011). Amongst the four populations, the Ontario population was the only one that would have regularly been subjected to temperatures below 0°C during the winter (Table 1). Freezing temperatures repeated over seasons would likely encourage the adaptation of Conyza canadensis populations in this region, or moving into this region, to a winter annual habit. Ideally, Conyza canadensis seeds that recruit in the fall to form a rosette would have a competitive advantage over summer annual crops and spring emerging weeds (Regehr and Bazzaz 1979). This competitive advantage could result in greater fecundity. The selection pressure of consistently freezing winter temperatures would continue to encourage this adaptation. Interestingly, the low base germination temperature range for the Ontario population may play a substantive role in the population dynamics of this species in this region. Typically in southern Ontario, temperatures do not dip below 9.5 °C until mid-October (Table 4.1), but seed shed continues until mid-November (personal observation). This can lead to a large number of un-germinated seeds left to overwinter. Any viable seed that survives the winter period, given little or no dormancy in the seed, could recruit in the spring. In this fashion, base
germination temperature and seed-shed timing together impact the recruitment timing of facultative winter annual species like *Conyza canadensis*. The specific base germination temperature range of the southern Ontario population we studied is therefore a function of its fitness over time; driven by selection for a base germination temperature range that is high enough to halt fall germination before it impacts overwinter ability, and low enough to allow for spring germination that is early enough to provide competitive seedlings in a summer annual scenario. This is important in the context of our finding that for three of the four populations the base germination temperature ranges we estimated were below what had been previously reported in the literature. This highlights the importance of seed germination response to temperature in adaptation but also in weediness. The scenario above demonstrates the importance that base germination temperature range adaptation plays in weedy adaptation in the context of given agricultural scenarios.

The Iranian population was sourced from the Shiraz region where the moderate climate keeps mean temperatures above 6°C in the winter (Table 4.1). This region rarely experiences freezing temperatures although it does experience the second coldest winters compared to other regions included in this study (Table 4.1). This climate may have provided the selection pressure required to produce the second lowest estimated base germination temperature range amongst the populations we studied. It is interesting that although the Iranian population had the second lowest estimate in this regard it had the highest required accumulated GDD to achieve 50% germination. This region is quite dry, however, and the average precipitation for August and September is 0mm and only 5mm for October (Table 4.1). In addition, in November, the average temperature (12°C) hovers just around the base germination temperature range we observed for this population,
although there is some modest precipitation (21mm on average) in this month (Table 4.1). These kinds of conditions could delay fall germination (until November for example) to prevent germination, which would lead to seedling death during typically extended dry conditions (August to October).

The U.K. populations were sourced from Hertfordshire, U.K. where the maritime climate keeps mean temperatures above 6°C in the winter (Table 4.1). Consistent and extended freezing temperatures are uncommon in this region. The germination response to temperature for the UK population was more similar to the Iranian population than it was to the Ontario population and this may be due to similarity in winter temperatures between these regions (Table 4.1). Temperatures in this region fluctuate, on average, 15°C throughout the year leading to a less intense selection pressure for germination at low temperatures (Table 4.1). In these regions, *Conyza canadensis* could almost germinate year round if average monthly high temperatures occurred.

The Spanish populations were sourced from near Málaga where the Mediterranean climate keeps mean temperatures above 12°C in the winter (Table 4.1). This is the only region included in this study where *Conyza canadensis* most likely germinates year round. Figure 4.1 shows the Spanish populations exhibiting a steep rise in germination beyond an extended lag phase and this population had the highest estimated base germination temperature range. These results suggest that the Spanish populations can germinate quickly if microsite conditions are right. The average daily temperatures in this region commonly stay above our estimated base germination temperature range, which would limit selection pressure for germination at lower temperatures.
**Conyza canadensis** is native to North America but not originally to Canada, and it is not native to the other three regions included in this study. **Conyza canadensis** seed can enter the planetary boundary layer and travel globally, but it can also travel via trading routes and man-made transport (Shields et al. 2006). The earliest known populations of *Conyza canadensis* outside of North America were found in the U.K. 200-300 years ago and were speculated to have arrived there via taxidermist’s shipments where *Conyza canadensis* plumes (often containing seeds) were used as animal stuffing (Stace 1997). Given the trading state of England at that time it is not hard to imagine how *Conyza canadensis* seed from the U.K. could have made its way to many other locations around the globe. If this was the case, then the significant differences we found among these populations suggests a relatively rapid rate of regional evolution and adaptation given the moderate level of selection pressure.

The pace of evolution may have been facilitated by presence in agricultural systems. *Conyza canadensis* seed within typical farmed fields has very regular access to favourable microsite conditions for germination including abundant light, moisture, seed-soil contact, and very little ground cover (Brown and Whitwell 1988; Bhowmik and Bekech 1993). This would lead to high levels of germination and large populations in agricultural versus non-agricultural settings favouring more rapid adaptation (Jasieniuk et al. 1996). The population size and selection pressure facilitated by agriculture along with specific characteristics of *Conyza canadensis* including high levels of seed germination allowing for rapid population shifts and its potentially high fecundity would combined to allow populations to evolve relatively rapidly and achieve distinct regional recruitment biology adaptations to climate (Cruden 1976). In the case of the populations considered
in this study, in general, selection pressure to lower base germination temperature would have been greater than selection pressure to increase it. This is due to the fact that a lower base germination temperature is only a disadvantage if higher temperatures bring more survival and fitness (Allendorf and Lundquist 2003).

4.5 Conclusion

This study shows that there are significant differences in the germination response to temperature among disparate international populations of *Conyza canadensis* and that these differences are likely evolutionary adaptation to local climate, which may have been facilitated by agriculture. Both estimated base germination temperature ranges and GDDs required to 50% germination were significantly different among these populations and for three of the four populations, estimated base germination temperature was below levels previously reported in the literature (Steinmaus et al. 2000). These differences highlight the important role that recruitment biology and seed germination response to temperature plays in adaptation to climate in the context of agriculture and the particular adaptiveness of *Conyza canadensis* in this respect.

Acknowledgments

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CHAPTER 5

The effect of recruitment timing on the dynamics of a range of international populations of *Conyza canadensis* (L.) Cronq. var. *canadensis*.

*Conyza canadensis* (L.) Cronq. var. *canadensis* is a surface-germinating ruderal facultative winter annual with recruitment that is highly susceptible to changes in microsite conditions. Rapid potential adaptation to climate is a key adaptive characteristic for a facultative winter annual species like *C. canadensis* (*Conyza canadensis*). The objective of this study was to determine the effect of recruitment timing on survival, fecundity, and time to flowering between a range of international populations. Seed was sourced from populations representing a variety of climates in Málaga, Spain, Hertfordshire, UK, Shiraz, Iran, and Southern Ontario, Canada, and grown out in a common garden under controlled conditions to produce seed for this study. Recruitment timing was represented as eight monthly cohorts with four each in the fall (August-November) and spring (March-June). Ten completely randomized plots (25 cm x 25 cm) were established for each population and each cohort at each site. Five randomly selected plants in each plot were selected for monitoring of survival, fecundity, and time to flowering. Results indicated that plants recruiting in earlier months, in either fall or spring, had significantly higher survival and fecundity as well as earlier flowering. Differences were seen among the populations in terms of these performance measures under Ontario conditions. We suggest that these differences reflect evolutionary adaptations to local climate. The results of this study highlight the key role recruitment timing plays in population performance for facultative winter annuals generally and
Conyza canadensis, specifically. The study also highlights the significant adaptiveness to climate of origin for Conyza canadensis populations and is an indication of the potential of this species to adapt to a changing climate.

5.1 Introduction

Conyza canadensis (L.) Cronq. var. canadensis is a surface-germinating ruderal facultative winter annual weed with recruitment that is highly susceptible to changes in microsite conditions (Grime, 1977; Regehr and Bazzaz, 1979; Buhler and Owen, 1997; Nandula et al. 2006; Main et al. 2006). It is native to North America and has become widely naturalized in Europe due to the seeds ability to enter the planetary boundary layer and travel globally (Frankton and Mulligan 1987, Dauer et al. 2007). The high mobility of seed results in populations of Conyza canadensis establishing around the world including Europe, Israel, China, Iran, and Australia (Shields et al. 2006, Weaver et al. 2001).

The small seed size of this species (2-4mm without pappus) assists in the ability for seed to travel globally and disperse to a variety of microsite conditions (Dauer et al. 2007; Dauer et al. 2008). Recently disturbed moist soil with light availability is the ideal condition for Conyza canadensis seedling germination and emergence (recruitment) (Nandula et al. 2006). With little to no seed dormancy, recruitment can be rapid if a suitable microsite condition is found and germination of seed occurs within 3 days on average (Regehr and Bazzaz, 1979).
It has been estimated that *Conyza canadensis* seed has a base germination temperature of 13°C (Steinmaus et al. 2000) in a Knoxville, Tennessee population, but recent work has shown that base germination temperatures for this biotype of this species may be lower and that base germination temperature depends on the geographic source of the seed (Tozzi et al. in press). *Conyza canadensis* seed shed occurs during an extended period from late August to mid-November (Regehr and Bazzaz, 1979, Weaver et al. 2001). This long seed shed period enables up to ~200,000 seeds/plant to be dispersed per generation (Weaver et al. 2001). High seed numbers along with a long seed shed period mean that *Conyza canadensis* seed can experience a wide variety of summer and fall soil surface temperatures allowing for a variety of germination results in relation to temperature as well as the possibility for some seed to overwinter, resulting in spring seedling recruitment (Nandula et al. 2006). Tozzi et al. (in press) found that under controlled conditions (experiments conducted on a thermal gradient plate apparatus) populations sourced from different climates responded uniquely to temperature in terms of germination level and rate. This study highlighted the relatively rapid adaptation to climate of this weed species and the fact that there is real regional adaptation in terms of seed recruitment characteristics. What this study did not show was how these adaptations affect the performance of this species in terms of survival of seedlings or fecundity, for example, and the recent study by Tozzi et al. (in press) was not done in natural outdoor conditions. These are critical considerations in terms of more broadly assessing the
weediness and invasiveness of this species and perhaps its ability to adapt to a changing climate.

The objective of this study was to determine the effect of seedling recruitment timing on the performance (outdoors) of *Conyza canadensis* populations sourced from four different global climatic locations: central Canada (Ontario), Spain, Iran, and the United Kingdom. With the results of this study we hope to gain insight into the adaptiveness and weediness of facultative winter annual weeds generally and *Conyza canadensis* specifically.

### 5.2 Materials and Methods

Experiments were established at three sites and followed for a single winter annual (late summer through to following summer) growing season (2011-2012) in a region of northern North America; south central Ontario, Canada (sites were located near the towns of Woodstock, Simcoe and Guelph). The Woodstock site was situated on a Guelph Loam series soil (Gray Brown Luvisol) containing 35% sand, 52% silt, 13% clay, and 3.6% organic matter, with a pH of 6.4. The Simcoe site was situated on a Berrien sandy-loam soil containing 55% sand, 30% silt, and 15% clay, 1.93% organic matter, with a pH of 6.8. The Guelph site was situated on a very fine sandy loam soil containing 55 to 60% sand, 28 to 34% silt, and 10 to 11% clay with a neutral pH(7).
5.2.1 Populations

In the spring of 2011, *Conyza canadensis* seed was sourced from collaborating weed scientists in Málaga, Spain; Harpenden, United Kingdom; and Shiraz, Iran. Each of these scientists collected seed from a number of plants (5-10) from a number of populations (3-10) near these cities. In the late summer of 2011, we collected seed from populations near the towns of Guelph, Woodstock and Simcoe, Ontario. For each location, seed samples were pooled to create a combined population seed source from each location. The average climate for each of these locations is provided in Chapter 4 (Table 4.1).

Once received or collected, all seed was stored at 4°C until the late fall of 2011 when a common garden was established for the four populations. Two to three hundred seeds of each population were germinated in petri dishes and the resulting seedlings were transplanted into pots (30cm diameter) containing a commercial potting soil mixture. Once established, seedlings were thinned to one plant per pot to ensure the highest quantity and quality of seed and there were 20 plants (pots) for each population. Plants were grown in a growth room at 24°C/16°C day/night temperatures with 16 h days until the plants flowered and produced seed (approximately 4 to 6 months). Plants were bagged with micro-perforated glycine bags to allow seed to fully mature and shed. Shed seed was collected, transferred to paper bags, and stored at 4°C until use. This common garden procedure was used to equilibrate the seed relative to maternal environment effects.
To facilitate data analysis and interpretation we categorized seed shed timing (or seeding) data into seeding date (seedling recruitment timing) cohorts. We classified a total of 8 recruitment timing treatment cohorts with each cohort corresponding to the month when we seeded the particular cohort. The 4 fall cohorts included August, September, October, and November and they represented potentially fresh seed shed timings for *Conyza canadensis*. The 4 spring cohorts included April, May, June, and July and they represented potential recruitment timing from seed that had overwintered.

Ten completely randomized plots (25 cm x 25 cm) were established for each population and each cohort at each site. 400 seeds for a given population we seeded in each plot. The seeds were evenly distributed on the soil surface. Once a reasonable number of seedlings emerged within a plot, 5 plants were randomly chosen in each plot by placing a grid over the plot and using a random number generator to select 5 grid squares from which to select plants to tag and follow. Seedlings were tagged with coloured paper clips placed around their base.

Within each plot we measured seedling survival, fecundity and flowering timing. For seedling survival we recorded the number of tagged seedlings per plot (out of 5) that successfully flowered. Fecundity was measured by multiplying the average number of seeds per flower by the number of flower heads on each tagged plant (counted in late October to ensure maximum reproductive development). Average number of seeds per flower was determined by counting the number of seeds per capitula in 50 capitula from surviving plants chosen at random from within each of the three experiment sites. For this study the average number of seeds per flower was 46. Given the indeterminate nature of
Conyza canadensis, flowering timing was recorded as Julian week of first seed shed for surviving tagged plants.

5.2.2 Statistical Analysis

Statistical analysis of data was conducted using JMP 10.0.2 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, SAS Institute, Inc.). All data were subjected to an ANOVA using a repeated measures linear mixed effects model with population (fixed), plot (random), site (random), cohort (random) as factors, and with replication nested in site for fecundity (capitula/plant for tagged plants), survival (number of tagged plants still living in each 0.25m² plot in August of the following season), and flowering date (Julian week of first seed shed for tagged plants). On the basis of an examination of residual plots, data were deemed to meet the assumptions of ANOVA including homogeneity of variance. Random effects of site and site by treatment were insignificant and therefore pooled for statistical analysis. In all cases, means were considered to be significantly different on the basis of P<0.05.
Table 5.1 Survival of *Conyza canadensis* plants as affected by recruitment timing (cohort) for experiments conducted in Ontario, Canada using seed sourced from one local and three international populations.

<table>
<thead>
<tr>
<th>Iran Cohort</th>
<th>Survival (%)</th>
<th>Ontario Cohort</th>
<th>Survival (%)</th>
<th>Spain Cohort</th>
<th>Survival (%)</th>
<th>U.K. Cohort</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>86.6 A</td>
<td>September</td>
<td>94.6 A</td>
<td>September</td>
<td>90.6 A</td>
<td>August</td>
<td>86.6 A</td>
</tr>
<tr>
<td>March</td>
<td>84.4 AB</td>
<td>August</td>
<td>94.0 A</td>
<td>August</td>
<td>86.6 A</td>
<td>September</td>
<td>86.6 A</td>
</tr>
<tr>
<td>August</td>
<td>82.0 ABC</td>
<td>March</td>
<td>90.6 AB</td>
<td>March</td>
<td>84.6 AB</td>
<td>March</td>
<td>82.6 AB</td>
</tr>
<tr>
<td>May</td>
<td>78.0 ABC</td>
<td>April</td>
<td>90.0 AB</td>
<td>April</td>
<td>74.0 BC</td>
<td>April</td>
<td>80.0 AB</td>
</tr>
<tr>
<td>September</td>
<td>76.6 BCD</td>
<td>May</td>
<td>89.2 AB</td>
<td>May</td>
<td>70.6 C</td>
<td>May</td>
<td>75.2 B</td>
</tr>
<tr>
<td>November</td>
<td>74.6 CD</td>
<td>October</td>
<td>88.6 AB</td>
<td>June</td>
<td>55.2 D</td>
<td>June</td>
<td>62.6 C</td>
</tr>
<tr>
<td>October</td>
<td>73.2 CD</td>
<td>June</td>
<td>85.2 B</td>
<td>October</td>
<td>52.0 D</td>
<td>October</td>
<td>56.6 C</td>
</tr>
<tr>
<td>June</td>
<td>68.6 D</td>
<td>November</td>
<td>84.6 B</td>
<td>November</td>
<td>37.2 E</td>
<td>November</td>
<td>55.2 C</td>
</tr>
</tbody>
</table>
Table 5.2 Fecundity (capitula/plant) of *Conyza canadensis* plants as affected by recruitment timing (cohort) for experiments conducted in Ontario, Canada using seed sourced from one local and three international populations.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Iran</th>
<th>Ontario</th>
<th>Spain</th>
<th>U.K.</th>
</tr>
</thead>
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<tr>
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<td>625 D</td>
<td>November 703 C</td>
<td>November 726 D</td>
<td>November 651 C</td>
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</table>
Table 5.3 Flowering timing of *Conyza canadensis* plants as affected by recruitment timing (cohort) for experiments conducted using seed sourced from one local and three international populations.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Iran</th>
<th>Ontario</th>
<th>Spain</th>
<th>U.K.</th>
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<td>Julian Week</td>
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<td>37.4 A</td>
<td>June 35.7 A</td>
<td>November 36.4 A</td>
<td>November 37.2 A</td>
</tr>
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<td>November 35.1 AB</td>
<td>May 36.3 A</td>
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<td>37.1 A</td>
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<td>34.3 C</td>
<td>September 34.2 C</td>
<td>September 34.2 C</td>
<td>August 34.3 C</td>
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</tbody>
</table>
5.3 Results

5.3.1 Effect of Recruitment Timing

Generally, recruitment timing had an effect on survival come August for all four populations. Early seedling recruitment in either fall or spring (E.g. August, September, April, and March) resulted in greater survival versus later seedling recruitment (E.g. May, June, October, and November) (Table 5.1). The same general effect was seen for fecundity (Table 5.2) where earlier seedling recruitment in either fall or spring (E.g. August and March) resulted in higher fecundity, generally, compared to later seedling recruitment (E.g. June and November). The same effect was seen for flowering timing where earlier recruitment in either spring or fall (E.g. August, September, April, and March) resulted in earlier flowering in general (Table 5.3). This last result mirrors that reported in Chapter 3 where time of emergence impacted flowering timing and earlier emergence lead to earlier flowering.

5.3.2 Differences in Response Between Populations

In general we found that the Ontario populations had the highest mean survival (89.6%), greatest mean fecundity per plant (935 capitula), and shortest mean time to flowering (Julian week 34.7) when compared to the other populations (Tables 5.1-5.3). The Iranian population displayed the second highest mean survival (78%) but the lowest mean fecundity (801 capitula), and longest mean time to flowering (Julian week 36.1) when compared to the other populations (Tables 5.1-5.3). The U.K. population displayed the second lowest mean survival (73%), second lowest mean fecundity (851 capitula), and second longest mean time to flowering (Julian week 35.3) when compared to the
other populations (Tables 5.1-5.3). The Spanish population displayed the lowest mean survival (66%), second highest mean fecundity (900 capitula), and second shortest mean time to flowering (Julian week 34.9) when compared to the other populations (Tables 5.1-5.3).

Given that we subjected these populations to a common garden before starting the experiment, these results suggest that there is significant genotype based adaptation of these populations to their geographic origin. In Chapter 4 we found that there were differences among these same populations in terms of their time to 50% germination and their apparent base germination temperatures and in chapter 4 we also subjected the populations to a common garden before starting the experiment and so those differences were also genotype-based adaptations. In this experiment, the results may not necessarily be directly related to differences in base temperature given that we corrected for time of emergence by using recruitment timing as treatments but as in Chapter 4, where we document adaptations to response to temperature among these populations in relation to seed germination, the results in this experiment may also reflect adaptations in response to temperature, but in relation to growth and development.
5.4 Discussion

5.4.1 Effect of Seedling Recruitment Timing

Generally, the month in which seedlings recruited had a significant effect on the survival of *Conyza canadensis*. Earlier recruitment in either spring or fall, lead to higher survival suggesting that earlier recruitment provides a survival advantage. Reductions in survival for later emerging seedlings in fall may be due to a reduction in biomass accumulation needed for overwinter survival while in spring the differences may be related to size requirements for inter/intra-specific density dependent competition (Weaver 2001, Main et al. 2006). Plants that emerge earlier in both seasons seem to have a distinct competitive advantage possibly due to the advantages of longer periods of growth and reproduction. Weather conditions are favourable for germination in late spring cohorts (Nandula et al. 2006), which is quite different than the weather conditions experienced by late fall cohorts. Interspecific and intraspecific competition along with differences in time to 50% germination may play a greater role than weather conditions in the recruitment of late spring cohorts due to temperatures remaining above base germination temperature ranges for all populations.

Plants from earlier recruiting cohorts were significantly more fecund than plants from later emerging cohorts, in both fall and spring (Table 5.2). Fecundity has been shown to increase exponentially with plant height in *Conyza canadensis* and this is linked to time of recruitment. Dauer et al (2007) found, for example, that early fall (before October 15th) and early spring (before May 1st) emerging *Conyza canadensis* plants had
greater plant height compared to later emerging cohorts come flowering time. This result also suggest that earlier seed shed, and therefore recruitment, may be responsible, in part, for the results seen in this experiment where Conyza canadensis plants from earlier recruiting cohorts in the fall required less time to flowering than plants from later fall cohorts.

Differences in these factors between populations may also be a result of inherent growth and reproduction differences after germination as a result of local adaptation to climate (McDonald et al, 2009). This effect may play a secondary role after recruitment timing in determining difference between populations. Differences in early cohort survival, fecundity, and time to flowering between populations in both seasons suggest these adaptations to climate have some competitive effects after germination, although larger differences are seen in later cohorts (Tables 5.1-5.3).

5.4.2 Population Comparisons

Rapid local adaptation to climate in the four regions from which the seed was sourced for this study may help to explain the results from this study with respect to differences in response between the populations. Each population has had at least 200 years of selection pressure from local climate (Weaver 2001). The ruderal nature of Conyza canadensis selects for quick growth and high fecundity, which are inherently linked to time of recruitment in this species, resulting in plants that are significantly affected in survival, fecundity, and time to flowering by recruitment timing. This effect is seen within both cohort and season in this experiment (Regehr and Bazzaz 1979).
High fecundity and rapid growth in this species creates a scenario where rapid evolution and adaption can occur (Weaver 2001) (Table 5.1-5.3).

5.4.2.1 Ontario

The mean temperature in Ontario includes regular and extended periods below 0°C during the winter; something unique among the population locations included in this study. This climate would present a significantly distinct selection pressure that would encourage adaptation to a winter climate (Bertrand and Castonguay, 2004). Even though Conyza canadensis is native to North America, previous studies suggest southern North American as the place of origin (Weaver 2001). High survival, high fecundity, and shortest time to flowering for this population, in comparison to the others, was not surprising given that the Ontario population was being grown in its adapted climate.

The estimated base germination temperature range for the Ontario populations is 8-9.5°C (Chapter 4) and local fall soil surface temperatures would typically not reach that temperature range until mid-October. Seed shed after this time would generally have a greatly reduced chance of emergence or take significantly longer time than average to emerge (Chapter 4), most likely resulting in plants with significantly less survival and fecundity, and with a much longer time to flowering (Table 5.1). This is seen in all populations in this experiment (Tables 5.1-5.3). Differences between each population may be directly linked to their base germination temperature range and/or their GDD requirement to reach 50% germination.
In chapter 4 we also saw differences in time to 50% germination where the Ontario population had the shortest time suggesting this population is perhaps most adapted for rapid growth and development (perhaps due to the inherently colder climate of origin), which, in relation to the results in this experiment, may allow for the Ontario population to have greater productivity in a shorter time. Rapid adaptation to local climate may also help expand geographic growth limits for this species, especially as climate change plays a larger role in changes in habitat limits (Parker et al. 2002, Bertrand and Castonguay 2004) Clements and DiTommaso (2010) predicts weed species range expansion will exceed predicted models that do not include evolutionary changes and state climatic changes may alter key adaptive traits such as flowering timing, growth, and fecundity.

5.4.2.2 Iran

Iranian populations displayed the second highest survival, lowest fecundity, and longest time to flowering compared to the other populations in this study (Tables 5.1-5.3). The Iranian population was sourced from the Shiraz region where the moderate climate keeps mean temperatures above 6°C in the winter, which is the second coldest climate of origin for the populations in this study (see Chapter 4). The relatively high survival rate for plants from this population, therefore, may be related to a relative adaptation to cold climate. Certainly in Chapter 4 we saw that seed from the Iranian population had the second lowest base germination temperature. The low fecundity (the lowest in fact) of this population may be related to its adapted growth response to
temperature. Although this population had a relatively low base germination temperature, the time to 50% germination was the longest amongst these four populations suggesting that its growth performance in relation to temperature may be low. This effect may be an adaptation to length of season (Patterson, 1995). Although the Shiraz region was the second coldest climate of origin for the populations in this experiment, it also has a relatively long frost-free period (a long season). A long season may select for a slower growth rate (Masuda and Washitani, 1992). Similarly, the long time to flowering for this population (the longest amongst the 4 populations) may also be an adaptation to long seasons. In addition, time to flowering and fecundity are linked (Dauer et al. 2007), it is likely, therefore, that a long time to flowering reduces fecundity, especially in the shorter Ontario season.

5.4.2.3 United Kingdom

The U.K. population displayed the second lowest mean survival, second lowest mean fecundity, and second longest mean time to flowering when compared to the other populations (Table 1-3). The U.K. populations were sourced from Hertfordshire, U.K. where the maritime climate keeps mean temperatures above 6°C in the winter (Table 4.1) and temperatures in this region fluctuate, on average, only 15°C throughout the year leading to a less intense selection pressure for germination at low temperatures (Table 4.1) and perhaps less selection pressure for aggressive growth response to temperature (Masuda and Washitani, 1992). In Chapter 4, we showed that seed from this population
had the second highest base germination temperature range amongst these populations (Table 4.2). This explanation for the performance of this population follows the explanation for the performance of the Iranian population, the difference being that the UK climate being somewhat more moderate than the Iranian climate (cooler average mean temperatures) perhaps lead to an adapted growth response to temperature that was somewhat more aggressive than for the Iranian population, which in turn would result in somewhat better performance for this population under Ontario conditions.

5.4.2.4 Spain

The Spanish populations displayed the lowest mean survival but the second highest mean fecundity, and the second shortest mean time to flowering when compared to the other populations (Tables 5.1-5.3). The Spanish populations were sourced near Málaga where the Mediterranean climate keeps mean temperatures above 12°C year-round (Table 4.1), allowing the possibility for Conyza canadensis to germinate throughout the year.

The low survival rate for the Spanish population may reflect a lack of adaptation to cold climate. In Chapter 4, we showed that seed from this population has the highest base germination temperature range (12.5-14°C). However, those plants that did survive from this population performed relatively well (second best in terms of fecundity and time to flowering). In Chapter 4 we also showed that seed from the Spanish population had the second shortest time to 50% germination when compared to the other populations.
in this study. Therefore, even though the Spanish population may not be adapted to cold weather survival (given that it came from the warmest climate of origin amongst the populations in this study), it appears to be adapted for a relatively rapid growth response to temperature (second shortest time to 50% germination) and perhaps development as well. This would explain the relatively short time to flowering and the relatively high fecundity for surviving plants. Of course, the shorter time to flowering would also support higher fecundity.

5.5 Conclusion

The results of this study reflect a relationship between the performance of these populations and adaptation to their climate of origin. In general, populations better adapted for colder temperatures had significantly higher survival and fecundity, as well as shorter times to flowering. In later spring months, where temperatures are not a limiting factor, density-dependent inter/intra-specific competition most likely plays a much larger part in determining levels of survival, fecundity, and time to flowering, with base germination temperature ranges heavily influencing these factors. We reported similar results in Chapter 3, where early spring or early fall recruitment produced plants with significantly higher levels of survival and fecundity, and lowest time to flowering. This suggests that differences between spring and fall recruitment play less of a role on population dynamics than time of recruitment in each season.
The results in this study highlight the incredibly rapid level of adaptation to climate of *Conyza canadensis* and highlight the importance of seed shed timing on population dynamics. Differences in fecundity, flowering timing, and survival between each international population underscore genetic differences. These differences, in turn, impact the adaptiveness and weediness of this species in suitable microsites in diverse climates and locations around the world. Special attention to this species’ potential for rapid evolution and naturalization should be paid, especially in a management approach, and especially in a changing global climate. With predictions suggesting large potential shifts in weed species composition and competitiveness due to increased temperature or available CO$_2$ due to climate change (Patterson, 1995, McDonald et al. 2009), winter annuals, such as *Conyza canadensis* may become a larger management issue. Rapid adaptation to changing temperatures, especially changes in base germination temperatures, would significantly alter the competitiveness and population dynamics of *Conyza canadensis*, as seen in this study. Quick growth and high fecundity in this ruderal species give *Conyza canadensis* the potential to adapt promptly to any changes in climate in the foreseeable future, making this species particularly hazardous to agricultural systems.

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The authors would like to thank the Natural Sciences and Engineering Research Council of Canada and The Ontario Ministry of Agriculture and Food for providing
funding for this study and Robert Grohs, Rachel Riddle, and Don Kitchen for technical assistance. We also want to thank Hugh Beckie, Ross Weiss, Jonathan Storkey, Jose Conzolez-Andujar, and Cici Zahra-Hosseini for sourcing seed for this study.
The effect of simulated winter warming spells on *Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. canadensis) seeds and plants.

E. Tozzi, E. M. Lyons, and R. C. Van Acker

Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada, N1G 2W1

Nomenclature: *Conyza canadensis, Conyza canadensis* (L.) Cronq. var. canadensis

Keywords: Recruitment, facultative winter annuals, warming spells, climate change, *Conyza canadensis*

Manuscript-Chapter Differences:
Tables and Figures Included within text.
CHAPTER 6

The effect of simulated winter warming spells on *Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. canadensis) seeds and plants.

Experiments were establish at three sites in southern Ontario, Canada in 2009 and 2010 to determine the possible effect of winter warming spells applied in either January, February or March on seed, seedlings, or rosettes of *Conyza canadensis* including effects on winter survival, fecundity, above-ground biomass, and flowering timing. Warming spells reduced survival of fall-established rosettes and fall established seedlings. Warming spells occurring late in winter (March) had a greater effect where March warming spells reduced the survival of rosettes and seedlings on average by 53% and 80%, respectively. In addition, overwintering *Conyza canadensis* plants (rosettes or seedlings) exposed to warming spells flowered earlier (between 29 and 71 days earlier). This study also confirms that *Conyza canadensis* seed has little or no dormancy and that the great majority of seed recruits (either in fall or spring) within a given season (between 84% and 93%). We also determined that timing of seed shed in the fall significantly affects the proportion of seedlings emerging either in the spring or fall with late shed favoring seed overwintering and spring seedling emergence. The results of this study suggest that winter warming spells, especially later in the winter (into early spring), may limit the success of *Conyza canadensis* and in particular its success as a winter annual.
6.1 Introduction

Understanding the recruitment nature of facultative winter annual weeds such as Conyza canadensis (Conyza canadensis (L.) Cronq. var. canadensis) can provide insight into their population dynamics and management. Facultative winter annual weeds can germinate mostly in the fall, mostly in the spring, or equally in both seasons (Cici and Van Acker 2009). Conyza canadensis is a surface-germinating ruderal facultative winter annual with recruitment that is highly susceptible to changes in microsite conditions (Buhler and Owen 1997; Regehr and Bazzaz 1979; Nandula et al. 2006; Main et al. 2006). The formation of an overwintering rosette allows Conyza canadensis to capture resources (in late fall and early spring) while most other summer annual competitors are non-existent. In Ontario, Canada, fleabane flowers and sets seed in the late summer and fall with seed germinating to form a seedling or rosette, and some seed not germinating. Conyza canadensis seed can also overwinter and germinate in the spring producing seedlings that sometimes do not form a rosette (Regehr and Bazzaz 1979). Nandula et al. (2006) determined that the ideal seed germination conditions for Conyza canadensis were disturbed moist soil with $24^\circ C/20^\circ C$ day-night cycles with 13hr day-length periods and that any changes to these conditions can have an impact on successful seedling recruitment. The lack of seed dormancy and indeterminate flowering period for Conyza canadensis suggest that microsite conditions play a large role in recruitment timing and perhaps overwintering biology (Regehr and Bazzaz 1979). The ability to recruit in either spring or fall highlights how important our understanding of these factors is to the relative success of this species, its competitiveness in certain farming systems and to approaches and timing for management.
Rosette survival over winter is affected by a variety of conditions including frost heaving, which occurs commonly in the silt-loam soils preferred by Conyza canadensis (Regehr and Bazzaz 1979). Frost heaving accounted for up to 86% mortality in a naturally established Conyza canadensis population in Urbana, Illinois, affecting smaller rosettes more than larger rosettes (Regehr and Bazzaz 1979). The frequency of frost heaving increases with fluctuating temperatures in late fall or early spring (Taber 1929) and may be facilitated by warming spells in the winter or spring.

Winter warm spells are defined as a period of consecutive temperatures in the 80th percentile of average temperatures (Shabbar and Bonsal 2003). Accordingly, longer warm spells (>=3 days) in one region of northern North America (southern Ontario, Canada) occur once every 10 years, but the frequency, duration, and severity of warm spells is expected to increase as global climate change progresses (Shabbar and Bonsal 2003). Three days was found to be the minimum time needed for a warming spell to produce influential results on the localized environment (Shabbar and Bonsal 2003). Shorter warm spells (1 or 2 days) are even more frequent and may still affect the winter survival and spring emergence of ruderal winter annual species such as Conyza canadensis given that seed germination is greatly influenced by microsite changes. Warm spells may affect seed microsite conditions and temporarily create sites suitable for seed germination. The influence of warm spells may be especially important for opportunistic facultative winter annual ruderal weed species such as Conyza canadensis.

Low temperatures keep Conyza canadensis rosettes from initiating vertical growth (bolting) and seed from germinating throughout the winter period (Regehr and Bazzaz 1979). Short periods of warm temperatures in the winter, or warming spells,
could affect critical elements of population dynamics including the survival of rosettes and the viability of seed over winter. Warm spells may also affect spring seed germination and spring seedling recruitment which could shift the relative spring versus fall emerging proportions of given Conyza canadensis populations (Regehr and Bazzaz 1979).

The objective of this study was to determine the effect of warming spells on fecundity, above-ground biomass, flowering timing, overwintering survival, and fate of seed in Conyza canadensis. The results of this study will provide insight into the possible impact of one aspect of climate change, warming spells, on the population dynamics of this species and the management implications.

6.1 Materials and Methods

The intent of this study was to explore the effect of warming spells on the whole population dynamics of Conyza canadensis and as such we investigated the effect on rosettes (established in the fall and overwintering), seed (shed late in the fall and overwintering) and seedlings (emerging in the late fall or spring). To provide a robust assessment of effects we measured winter survival (to bolting in the following summer) of fall established rosettes and seedlings and survival to bolting of spring seedlings, fate of seed shed in the fall, and growth, flowering timing and fecundity of overwintering rosettes and of seedlings emerging in either fall or spring. Experiments were established at three sites in each of two seasons (fall through to summer of 2009-2010 and 2010-2011) in a region of south central Ontario, Canada (sites were located near the towns of Woodstock, Simcoe and Guelph). The Woodstock site was situated on a Guelph Loam
series soil (Gray Brown Luvisol) containing 35% sand, 52% silt, 13% clay, and 3.6% organic matter, with a pH of 6.4. The Simcoe site was situated on a Berrien sandy-loam soil containing 55% sand, 30% silt, and 15% clay, 1.93% organic matter, with a pH of 6.8. The Guelph site was situated on a very fine sandy loam soil containing 58% sand, 31% silt, and 10% clay with a neutral pH(7). All experiments were conducted using a pool of seeds collected from these three locations in 2009 and 2010. Seeds were collected from fully mature plants in mid-October each year at all sites. Micro-perforated plastic bags (30cm wide by 100cm long) were placed over each plant before any seed shed occurred to maximize seed capture. When flowering and seed set had ceased, the flowering parts of the plant were cut with the bag still attached and these were placed into large paper bags. Seeds were threshed by banging the flowering stalks against the inside of the paper bag. All seeds were combined each year to mitigate any population-site differences and stored at 4°C until needed.

6.2.1 Fall Rosettes

To investigate the impact of warming spells on fall established rosettes, four warming spell treatments including a control (no warming spell) were used. We also included two seeding date treatments (early and late fall). A quantity of Conyza canadensis seed large enough to provide enough plants for the experiment was germinated indoors in trays containing a potting mix (Pro-Mix) soil under 16hr of daylight at 22°C. One cohort of seeds was germinated in mid-August to simulate early seed shed, while another was germinated in late-September to simulate late seed shed. Rosettes were grown to approximately 8 cm in diameter (5-6 weeks) and were then
transplanted into 96 small rubberized plastic tubs (40cm x 25cm x 30cm) with two rosettes per tub. The plastic tubs ensured ease of removal from the ground when it came time for transfer of plants to growth chambers to simulate warming spells. Thirty-two tubs were used at each of the three sites in each season. The tubs for each site had holes drilled in the bottom for drainage and were filled with soil collected from each respective site. The rosettes were no larger than 9 cm in diameter when they were placed outside. Main et al. (2006) reported that rosettes larger than 9cm had increased mortality.

Trenches measuring 3m long, 0.5m wide, and 0.3m deep were dug into the soil at each site to house the tubs. Tubs containing the early seed shed rosettes (early) were placed outdoors in mid-September, whereas tubs containing the late seed shed rosettes (late) were placed outdoors in mid-October. The tubs were organized in a completely randomized design with four replicates per treatment per site.

To simulate warming spells, eight tubs at each site (representing four replicates of each of the early or late seeded treatments for one warming spell timing) were removed at one of three periods throughout the winter (either mid-January, mid-February or mid-March). At each site, a set of control treatment tubs were left in the ground for the duration of the experiment and not exposed to any warming spell treatment. Different tubs were removed from each site for each warming spell treatment. These tubs were transported by hand to a growth chamber set to represent warming spell conditions. Chamber temperatures for a given warming spell treatment were based on a warming spell definition from Shabbar and Bonsal (2003) and were set as 3 days of consecutive temperatures in the 80th percentile of average temperatures for each site for the days of year for each specific warming spell period (mid-January, mid-February or mid-March).
based on long term weather data for each site (Table 6.1). After each warming spell treatment, tubs were returned to their respective outdoor sites.

   Fecundity was determined by multiplying the average number of seeds per flower by the number of flower heads on each plant (counted late in each season). Average number of seeds per flower was determined by counting the number of seeds per capitula in 50 capitula from surviving plants chosen at random from within each of the three experiment sites each year. Survival was measured as the number of plants that survived to bolting. Above-ground dry biomass was measured by harvesting plants at soil level, drying them at 105°C for 72hrs, and weighing them. Given the indeterminate nature of fleabane, flowering timing was recorded as date of first seed shed on the week of data recording.

6.2.2 Seeds and Seedlings

   To investigate the effect of warming spells on the fate of seed (shed in the fall) and seedlings emerging either in fall or spring, freshly collected Conyza canadensis seed (pooled among collection sites) was placed outside in plastic containers at each site in the fall of 2009 and 2010. Containers measured 15cm long X 10cm wide X 5cm deep (each container was considered an experimental unit). These were filled with soil from each respective site and there were 100 seeds per container. Containers were set outside at each site at two dates, mid-September and mid-October in each year with 24 containers (3 replicates x 4 warming spell treatments x 2 seeding depths) set out at each date at each site in each year. The two seeding depths were soil surface or just below the soil surface (0.5 cm deep). These treatments allowed us to investigate whether shallow seed burial
would affect seed recruitment timing. Containers were arranged in a completely randomized design at each site. At each warming spell date, 6 containers from each site were subjected to a simulated warming spell following the methods described above for the rosette study. Seedling emergence from the containers was recorded in the fall and spring by counting number of seedlings emerged each week in each container from mid-August to mid-November in the fall and from the beginning of April to mid-July in the spring. In the spring, seedlings that emerged late in the fall (not forming a rosette) and overwintered or seedlings that emerged in the spring were monitored through the season to determine survival (survival to bolting) and flowering timing.

6.2.3 Statistical Analysis

Statistical analysis of data was conducted using JMP 9.0.2 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, SAS Institute, Inc.). All data were subjected to an ANOVA using a linear mixed effects model with date of warming spell (fixed), site (random), year (random), and early/late seeding (fixed) as factors, and with replication nested in site for fecundity (capitula/plant), above-ground biomass (g at flowering stage), survival (% at bolting stage), and flowering timing (first seed shed). On the basis of an examination of residual plots, data were deemed to meet the assumptions of ANOVA including homogeneity of variance. For fecundity, above-ground biomass, and survival; site, year, seeding date, and block were all found to not be significant and there were no significant interactions between these factors. As such, data were pooled across these factors for subsequent ANOVA. Similarly, we were able to pool data for flowering date
over site, seeding date, and block. In all cases, means were considered significantly different on the basis of $P < 0.05$ using Tukey’s HSD.
Table 6.1 Conditions in growth cabinets for three day simulated warming spell treatments imposed on *Conyza canadensis* seeds, seedlings and rosettes. Refer to material and methods for explanation of determination of warming spell treatment conditions.

<table>
<thead>
<tr>
<th>Month of Warming Spell</th>
<th>Temperature (°C day/night)</th>
<th>Relative Humidity (%)</th>
<th>Photoperiod (hrs day/night)</th>
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<tr>
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<td>7/5</td>
<td>80</td>
<td>9/15</td>
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<tr>
<td>February</td>
<td>7/5</td>
<td>80</td>
<td>10/14</td>
</tr>
<tr>
<td>March</td>
<td>13/8</td>
<td>80</td>
<td>11/13</td>
</tr>
</tbody>
</table>
Table 6.2  Effect of simulated warming spell dates (WSD) in January, February, and March on fecundity (capitula/plant), above-ground biomass (g at flowering stage), survival (% at bolting stage), and flowering date (first seed shed) for Conyza canadensis rosettes established in the fall of 2009 and 2010 at three sites in southern Ontario, Canada. Means within columns followed by different letters denote significant differences at p<0.05.

<table>
<thead>
<tr>
<th>WSD</th>
<th>Fecundity(^1) (capitula/plant)</th>
<th>Dry Weight(^1) (g)</th>
<th>Survival(^1) (%)</th>
<th>Flowering Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2010</td>
</tr>
<tr>
<td>Control</td>
<td>940 (a)</td>
<td>54.0 (a)</td>
<td>88.5 (a)</td>
<td>246 (a)</td>
</tr>
<tr>
<td>Jan</td>
<td>889 (ab)</td>
<td>51.3 (ab)</td>
<td>82.3 (ab)</td>
<td>229 (ab)</td>
</tr>
<tr>
<td>Feb</td>
<td>777 (bc)</td>
<td>50.4 (ab)</td>
<td>75.0 (b)</td>
<td>212 (b)</td>
</tr>
<tr>
<td>March</td>
<td>739 (c)</td>
<td>48.8 (b)</td>
<td>46.9 (c)</td>
<td>171 (c)</td>
</tr>
</tbody>
</table>

\(^1\)Data pooled over years
Table 6.3 Average total germination (per 100 seeds) in fall or spring and total percent germination of *C. canadensis* seed, seeded either early in fall (September) or late (October) either on the soil surface or just below the surface (0.5cm) in 2009 and 2010 at three sites in southern Ontario, Canada. Means within columns followed by different letters denote significant differences at p<0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2009$^1$</th>
<th>Total</th>
<th>2010$^1$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall</td>
<td>Spring</td>
<td>%</td>
<td>Fall</td>
</tr>
<tr>
<td>Early 0.0cm</td>
<td>52.2 $a$</td>
<td>36.2 $a$</td>
<td>88.3</td>
<td>64.2 $a$</td>
</tr>
<tr>
<td>Early 0.5cm</td>
<td>44.3 $ab$</td>
<td>43.8 $a$</td>
<td>88.2</td>
<td>66.2 $a$</td>
</tr>
<tr>
<td>Late 0.0cm</td>
<td>60.0 $a$</td>
<td>30.3 $a$</td>
<td>90.3</td>
<td>43.0 $b$</td>
</tr>
<tr>
<td>Late 0.5cm</td>
<td>38.4 $b$</td>
<td>45.1 $a$</td>
<td>83.5</td>
<td>37.5 $b$</td>
</tr>
</tbody>
</table>

$^1$Data pooled over sites within year
Table 6.4 Effect of simulated warming spell dates (WSD) in January, February, and March and seeding depth (cm) on seedling survival (% at bolting stage) and flowering date (first seed shed) for seedlings recruiting in the fall from *Conyza canadensis* seeds (from southern Ontario populations) seeded outside at three sites in southern Ontario in the fall of 2009 and 2010. Means within columns followed by different letters denote significant differences at p<0.05.

<table>
<thead>
<tr>
<th>WSD</th>
<th>Flowering Date$^1$</th>
<th>Survival$^1$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0cm</td>
</tr>
<tr>
<td>Control</td>
<td>246 $a$</td>
<td>68.3 $a$</td>
</tr>
<tr>
<td>Jan</td>
<td>244 $ab$</td>
<td>63.4 $ab$</td>
</tr>
<tr>
<td>Feb</td>
<td>217 $bc$</td>
<td>45.8 $b$</td>
</tr>
<tr>
<td>March</td>
<td>175 $c$</td>
<td>20.0 $c$</td>
</tr>
</tbody>
</table>

$^1$Data pooled over years
Table 6.5. Effect of simulated warming spell dates (WSD) in January, February, and March and seeding depth (cm) on seedling survival (% at bolting stage) and flowering date (first seed shed) for seedlings recruiting in the spring from *Conyza canadensis* seeds (from southern Ontario populations) seeded outside at three sites in southern Ontario in the fall of 2009 and 2010. Means within columns followed by different letters denote significant differences at p<0.05.

<table>
<thead>
<tr>
<th>WSD</th>
<th>Flowering Date</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0cm</td>
</tr>
<tr>
<td>Control</td>
<td>247 a</td>
<td>25.8 a</td>
</tr>
<tr>
<td>Jan</td>
<td>240 a</td>
<td>21.7 a</td>
</tr>
<tr>
<td>Feb</td>
<td>242 a</td>
<td>20.0 a</td>
</tr>
<tr>
<td>March</td>
<td>239 a</td>
<td>15.8 a</td>
</tr>
</tbody>
</table>
6.3 Results

6.3.1 Rosettes

For rosettes of *Conyza canadensis* established indoors and placed outside in the fall of 2009 and 2010, simulated warming spells did impact survival (P<0.001), growth (above-ground dry biomass) (P<0.001) and fecundity (P<0.0107). There were significant differences for the means of these factors between the different timings of the warming spells (Table 6.2). There was significantly less survival in both years for rosettes that were subjected to warming spells compared to controls (no imposed warming spells) and among the warming spell timings, rosettes that were exposed to warming spells in March had significantly lower survival levels versus those exposed to warming spells in either January or February (Table 6.2). The March warming spell reduced survival of *Conyza canadensis* rosettes on average by almost 50%.

For rosettes of *Conyza canadensis* established indoors and placed outside in the fall, simulated warming spells did significantly impact flowering date (P<0.0428) (Table 6.2). The rosettes subjected to a March warming spell flowered on average 62 days earlier than rosettes not subjected to any warming spell (the control treatment). Rosettes that were subjected to a February warming spell flowered 37 days earlier on average than rosettes in the control treatment and those subjected to a warming spell in January showed numerical but not significantly earlier flowering timing (Table 6.2).

6.3.2 Seeds

For seeds of *Conyza canadensis* that were placed outside in the fall of 2009 and 2010 but that did not germinate in the fall to produce seedlings (and as such overwintered
as seed) there was no effect of warming spell treatments on the survival (to bolting) and flowering date of seedlings emerging from these seeds in the spring (Table 6.3). However, time of seeding (early vs. late fall) and depth (soil surface vs. 0.5cm) did have significant effects on total (fall plus spring) germination levels (Table 6.3). In the fall, significantly more seed germinated in the early versus the late seeded treatments. In the spring, the opposite was true and more seedlings emerged from the late versus the early seeded treatments. There was some interaction with seeding depth in this respect, wherein 3 of 4 cases, seed burial encouraged greater overwintering seed persistence and subsequent germination in the spring, in particular for the late seeding date (Table 6.3).

Total percent germination of Conyza canadensis seed was very high in this study ranging from 83.5% to 92.9% (Table 6.3) and very little seed did not germinate. This supports the notion that Conyza canadensis supports only a small seed-bank and is a seed limited species (Van Acker 2009) and it corroborates the results of other studies. Davis et al. (2009), for example, measured a 76% decrease in the seed-bank of Conyza canadensis after 10 months and Thebaud et al. (1996) determined that only 1% of Conyza canadensis seed remained viable after 3 years on the soil surface.

6.3.3 Seedlings

For seedlings that were established in the fall from seed placed outside in the fall of 2009 or 2010, timing of warming spell (month) did have a significant negative effect on over-all seedling survival and flowering timing (P<0.0001) (Table 6.4). A March warming spell reduced survival of fall established seedlings 80% coming from surface placed seed and 96% for seedlings coming from seed placed at 0.5cm depth, whereas a
February warming spells reduced survival by 55% for surface placed seed and 88% for seedlings coming from seed at the 0.5cm depth. For January warming spells (37% reduction in survival seedlings from surface placed seed and 66% for seedlings from seed at the 0.5cm depth) seedling survival did not significantly differ from the seedlings in the control treatment (32% and 65% reduction in survival for seedlings from seed at the surface or 0.5cm depth, respectively). There was no significant effect of date of warming spell (month) on flowering date for seedlings emerging in the spring (Table 6.5), showing that the warming spell effect on flowering did not occur when the warming spell was imposed on the seeds. Seedlings emerging in the fall from seeds placed in trays outside in the fall that overwintered and were subjected to a March warming spell, flowered on average 71 days earlier than the control seedlings while similar seedlings exposed to a February warming spell flowered on average only 29 days earlier than the control seedlings (Table 4).

Depth of seeding had a significant effect on seedling survival (P<0.0292) where there was greater seedling survival for seedlings establishing from seeds on the soil surface (Table 6.4).

6.4 Discussion

The effect of warming spells on the survival of Conyza canadensis rosettes may have resulted in part from the effects that warming spells can have on the surrounding environment, including the facilitation of frost heaving. Frost heaving can occur during periods of fluctuating freezing and thawing. Frost heaving can rip the root system of the rosettes apart or uproot the entire plant causing mortality (Jonasson 1986). Regehr and
Bazazz (1979) have previously shown that frost heaving may cause significantly higher levels of mortality in *Conyza canadensis* rosettes. Warming spells can exacerbate frost heaving by providing rapid thawing periods, followed by a return to freezing temperatures once the warming spells has passed. In our study, mortality increased significantly when the warming spell was closer to spring when temperature fluctuations tend to be greater and frost heaving is more likely (Taber 1929).

Warming spells also subjected the rosettes to rapidly increasing and decreasing temperatures over a relatively short period. Both an increase and decrease in external temperatures places stresses on rosettes evoking an acclimatization response (Gilmour and Thomashow 1991; Thomashow 1999; Holt 1995). The acclimatization response period of *Conyza canadensis* to these types of temperature stresses is not known but plants exposed to a cold acclimation period can be more tolerant to freezing than plants that have experienced a cold shock (Guy et al. 1985; Kalberer et al. 2006). In this study, the rosettes exposed to the March warming spells may have begun a spring acclimatization process since March air and soil surface temperatures are significantly higher than in January or February in this region (Holt 1995). A warming spell in March may initiate an acclimatization response at a higher energy cost that may lead to increased mortality when plants are returned to cold temperatures (Dulai et al. 1998; Saarinen et al. 2011). The plants would experience an increase of ~10-15°C during these warming spell periods, depending on the daily weather. In addition, *Conyza canadensis* has been shown to have a remarkably rapid photosynthetic response rate even in the winter if there is sufficient light (Regehr and Bazzaz 1976; 1979). In this study, more than 10cm of snow covered rosettes in January and February, but there was only ~5cm in
March (personal observation) and this may have allowed rosettes to engage in relatively higher levels of photosynthesis before and after the warming spells in March increasing energy cost and susceptibility to cold temperature stress. Snow cover was most likely not a significant impact factor since snow was not fully melted on top of the buckets by the end of the warming spell treatments and relatively similar amounts of snow were placed back on top of the rosettes after the warming spell treatments. The plants or seeds in both the large and small experimental containers expressed a similar response to the warming spell treatments. This result highlights how snow cover or heat capacity of each container likely had little influence on the effects of the warming spell in this experiment since snow cover and heat capacity of each container size were greatly different.

The effect of warming spells on flowering timing may have been due to the triggering of Flowering Locus C (FLC) genes (Rudnoy et al. 2002). Conyza canadensis has FLC genes that block flowering prior to vernalization removing any cold treatment requirement for floral initiation (Regehr and Bazzaz 1979). The results of this study suggest that plants (rosettes or overwintering seedlings) that are subjected to a disruption in vernalization take a significantly shorter time to flower. A cold period could silence the FLC genes, which are responsible for the suppression of flowering inducing hormones (He et al. 2004). Therefore, a vernalization period would promote the production of flowering inducing hormones (Wilczek et al. 2009). This could help to explain why we saw a gradient in flowering timings from latest to earliest for plants exposed to warming spells in January to March, respectively. In this regard, the warming spells reduced the suppression of flowering in all treatments, but March temperatures were potentially high enough after the warming spell for the plants to not return to a
flowering suppression state and plants exposed to March warming spells had the longest cold period with the highest temperatures after the warming spell. These conditions would have created the highest concentrations of flowering-inducing hormones and a period of high enough temperatures after the warming spell to prevent plants from re-entering a dormant (vernalization) state in relation to flowering. This combination of conditions would drive earlier flowering.

Warming spells did not have any significant effects (in terms of survival to bolting, flowering timing or fecundity) on seedlings emerging in the spring from seed set outside in the fall, nor on the timing of recruitment of the seed. This suggests that any stress or triggering caused by the warming spells was effective on plants but not seeds.

This study shows that there is significant variability in the over-winter survival of Conyza canadensis and that the survival of Conyza canadensis plants can potentially be very significantly affected by warming spells. We also showed that the timing and temperature of winter warming spells has a significant impact on level of survival and flowering timing, where the later the warming spell the greater the reduction in survival and the earlier the flowering timing. This could have significant effects on the population dynamics of Conyza canadensis in a changing climate. Since a greater frequency of warming spells is predicted for this region (Shabbar and Bonsal 2003) in the future, this may encourage earlier flowering and seed shed, leading to greater proportional fall germination and also greater mortality for overwintering rosettes. This may also, however, select for spring emerging populations of Conyza canadensis possibly making this weed a greater issue in spring versus fall seeded crops, and as such, perhaps a more common weed problem in spring sown crops. Since in-lab germination rates were near
99% after 72hrs (data not shown) the field results of this study support the notion that *Conyza canadensis* seed has little or no dormancy and that the great majority of seed recruits (either in fall or spring) within a given season, as per other studies (Weaver 2001). Seed burial encouraged greater overwintering seed persistence and germination in the spring, in particular for the late seeding date (Table 6.3). Depth of seeding had a significant effect on seedling survival (P<0.0292) where there was greater seedling survival for seedlings establishing from seeds on the soil surface (Table 6.4). This may have been related to time of emergence in the fall and size of seedlings where emergence was more rapid for the surface placed seed (personal observation) and those seedlings were perhaps more robust than later emerging seedlings.

These results suggest that *Conyza canadensis* seed does have an ability to remain viable over winter and that the later seed is shed in the fall the more likely that seed will germinate in the spring versus the fall. This supports the idea that seed shed timing (and to some extent shallow seed burial) plays a role in determining fall versus spring recruitment of *Conyza canadensis*. As a result, limiting the number of plants setting seed would be an effective tactic for limiting *Conyza canadensis* populations, which is good news for farmers managing *Conyza canadensis*, especially herbicide resistant populations.

6.5 Conclusion

This study highlights the unique recruitment plasticity of facultative winter annual weeds and for *Conyza canadensis*, that there is a strong relationship between seed shed timing and the extent to which this species recruits in the fall or spring. Understanding
the recruitment biology and ecology of a facultative winter annual like *Conyza canadensis* will help us to better manage this species and the data and results from this study represent a start to predicting how this species, and perhaps other facultative winter annuals, will perform as weeds in a changing climate.

**Acknowledgements**

The authors would like to thank the Natural Sciences and Engineering Research Council of Canada and The Ontario Ministry of Agriculture, Food and Rural Affairs for providing funding for this study and Robert Grohs, Rachel Riddle and Don Kitchen for technical assistance.
CHAPTER 7

7. General Discussion

7.1 Summary of Contributions to Knowledge

The primary focus of this thesis was to investigate and gain insight into the facultative recruitment nature of *Conyza canadensis*. The influence recruitment timing had on population dynamic factors including fecundity, plant density, survival, above-ground biomass, and flowering timing were studied. Populations sourced from differing global climatic regions were compared in terms of germination response to temperature, emergence timing, and performance to provide insight into the adaptiveness of this species. In the context of this study, the primary hypothesis that emergence timing would have a significant effect on the population dynamics of *Conyza canadensis*, where plants that emerged earlier would have higher fecundity, plant density, survival, above-ground biomass, and have the shortest time to flowering when compared to plants that emerged later in the season, can be generally accepted.

Considering all results from this thesis, conclusions can be made that flowering timing and therefore seed shed timing is a critical factor in the population dynamics of *Conyza canadensis*. Changes in flowering and seed shed timing produce significant differences in fecundity, aboveground biomass, survival, plant density, and time to flowering the following season. These differences are further highlighted when comparing the performance of populations sourced from differing global climates. It is apparent that *Conyza canadensis* displays a rapid ability to adapt to selection pressures placed upon it and this can present a challenge for management, especially as herbicide resistant populations become more common (Heap 2013) and climate change effects
become more apparent (Clements and DiTommaso 2011). Studies focusing on winter
annual species should make informed decisions on temporal periods of data recording as
not all significant differences were seen in just spring versus fall comparisons in this
thesis. Future studies should focus not only on herbicide resistance in this species, as
many other factors play a role in determining the weediness of this species and the effect
of Conyza canadensis in agricultural settings.

7.1.1 Population model

I created a simple population model to highlight key outcomes of the research in
this thesis. Figure 7.1 represents the culmination of three seasons of data collection on the
population dynamics of Conyza canadensis. The model contains only the Ontario
population data pooled over all three sites. Changes from the use of monthly emergence
cohorts (which was the resolution of our data) to only early/late spring/fall cohorts was
done to simplify the model and to show obvious differences in the effect of recruitment
timing. Generally, the model shows that early fall and early spring emerging plants have
higher fecundity, survival, and shorter time to flowering than plants emerging in late fall
and spring emergence. Also, population density decreases significantly from year to
year in non-disturbed areas compared to disturbed areas. Further detailed discussion of
these results is found below.
Figure 7.1. Population dynamics model of Ontario populations of *Conyza canadensis* from 2009-2012 pooled over three sites in Ontario. Dotted line represents survival (%), solid line represents fecundity (number of capitula/plant), and dashed line represents time to flowering (mean Julian week of first flowering).
7.2 General discussion and critique of thesis research and approaches

7.2.1 Extent of studies in this thesis

The methodology used in this thesis was appropriate to meeting the goals as stated in the introduction but the studies were not exhaustive. Investigations of further generations within each study would have helped to further support arguments relating fitness differences to recruitment timing, such as long-term changes in fecundity. Results within this thesis focused on growth and fecundity, but did not directly focus on fitness, as success of offspring was not measured due to constraints of time, scheduling, and cost.

The methodology in Chapters 5 did not extend the duration of the experiment to multiple years as was done in Chapter 3. Follow-through of the international populations for similar time periods as in Chapter 3 may have provided more insight into the population dynamics of each population, since effects of succession on fecundity, aboveground biomass, survival, and time to flowering are not known for these populations. Differences in response to succession between international populations would not only provide more evidence for evolution to local climate, but also provide further insight into the potential invasiveness and geographical range for this species (Clements and DiTommaso 2011). Theoretically, if one population has higher rates of survival or fecundity than another in following seasons where succession begins to play more of a role, then its competitive ability is greater (Regehr and Bazzaz 1979). This adaptation would represent a significant advantage in terms of competition and may lead to an increased chance of population spread over a given geographic area.

7.2.2 Comparison among international populations
International populations were grown to seed shed within greenhouse settings in this experiment. This methodology provided seed for Chapters 3 and 5 and it removed maternal effect as a factor. In Chapter 5, the seed from all international populations was sown in an Ontario climate. Unsurprisingly, the Ontario populations performed significantly better than the other populations. Comparing native population performance with non-native population performance in each of the local climates (Ontario, U.K., Spain, Iran) would have provided deeper insights into the effect climate plays on performance and adaptation, especially for facultative winter annuals. The part that local climate plays on the experimental factors tested would be much more apparent if all population sites were used. For example, fecundity could be recorded for the Ontario sourced population in 4 sites (Ontario, U.K., Spain, Iran) and averaged to provide a more true representation of the overall performance of the Ontario population. However, this approach was not possible because it would have been costly and/or would have required much greater contributions from our seed source collaborators.

7.2.3. Higher time scale resolution for studying fall versus spring cohorts

Ruderal species, such as *Conyza canadensis*, have life strategies of rapid growth and high fecundity suggesting that time of recruitment may play a role in plant and population dynamics (Grime 1977). Results from this thesis show that recruitment timing can have a significant effect on fecundity, survival, and time to flowering (Table 3.1, 3.2, 3.3, Figure 3.1). Differences in these factors would have not been seen if comparisons between only spring and fall cohorts were chosen. Higher resolution of time scale within fall and spring cohorts, such as month (Chapter 5) or 2-week periods (Chapter 3), led to findings
showing significant differences within fall or spring emergence cohorts and some unexpected results in the performance comparisons between individual emergence cohorts in terms of fecundity, survival, and time to flowering. Results from this thesis suggest making comparisons between fall and spring cohorts alone is neither as effective nor as informative as comparisons made between timing (early or late) within either spring or fall emergence cohorts. This may be important in the context of studies that use only spring and fall as temporal periods (e.g. Buhler and Owen 1997). Future studies on Conyza canadensis seed shed timing should set the resolution of time periods to at least one-month intervals to ensure that the effect of emergence timing on performance is not missed. For example, if data on survival, fecundity, and time to flowering were pooled as only spring and fall cohorts, significant differences between survival and time to flowering would be seen, but not fecundity. At a higher resolution of 2-week cohorts, significant differences were seen between early and late cohorts in each season for survival, fecundity, and time to flowering (Chapter 3).

7.2.4 Effect of disturbance

Results from this thesis have shown that disturbance plays an important role in the population dynamics and adaptability of this species. As a ruderal species, its “first-colonizer” behaviour is inherently linked to recently disturbed and available microsites to maximize germination (Chapter 3). Any areas of disturbed soil in an agricultural setting or otherwise provide beneficial microsite conditions for germination. Main et al. (2006) established that crop residue, which would cover available microsites, decreased germination by 77% suggesting that no-till agricultural systems may have lower levels of Conyza canadensis recruitment than tilled systems. Previous studies have shown that late
summer tilling can significantly reduce a *Conyza canadensis* population through shallow disking (Brown and Whitwell 1988) by destroying any emerged seedlings or rosettes. In this thesis, tilling (disturbance) was carried out in mid-August of each year. Our intent was to consider the effect of disturbance both on emergence during peak seed shed (in mid-August) and on final plant density in August of each year. Annually disturbed plots showed a significant difference in plant density when compared to undisturbed plots (Figure 7.1). After one year of no disturbance, population density was 74% less than the previous year, and 89% less the year after that. When disturbance was practiced the population density was only 2% less than the previous year, and only 7% less the year after that. The effect of disturbance in this study was a combined effect of disturbance on microsite (which would impact levels of recruitment) and the effect of population density on recruitment and the survival of individuals that successfully recruited.

In the no-disturbance plots, the populations of *Conyza canadensis* from the previous year, along with any other weeds from the previous year, were left to continue to grow through the peak recruitment time. The high density of mature plants in these undisturbed plots obviously had a tremendous negative impact on the new recruitment of *Conyza canadensis*. As seen in the population model (Figure 7.1), this result specifically highlights the effect of succession on the population dynamics of *Conyza canadensis* since previous year’s plant matter (both alive and dead) has a large impact on performance. Regehr and Bazzaz (1979) found similar results where they reported a sharp decline in population density after one year.

In terms of significant soil disturbance including tillage, this thesis shows that tilling in August potentially provides adequate microsites to maximize recruitment
(Chapter 6). By this time in the year, seed rain has already begun and tilling can encourage more seed recruitment by producing microsite conditions needed for germination. Brown and Whitwell (1988) showed that fall tillage could reduce population densities by 42% if done in early November. Time of tilling is therefore critical to the management of this species. If possible, tilling before any seed shed occurs in late July or early August may significantly decrease population numbers. Tilling anytime after seed shed begins would not be significantly beneficial until soil surface temperatures were below that of the base germination range, due to the increased potential for volunteer recruitment to occur. In Ontario populations, for example, those temperatures are not reached until late October (Table 4.1). Tilling after this time period would result in a significantly higher spring population due to a large number of ungerminated seed residing on or near the soil surface.

Due to limitations of space for additional treatments, timing of disturbance was not studied in this thesis. Different disturbance timings may have resulted in differences in aboveground biomass, survival, fecundity, and time to flowering in secondary and tertiary generations. Although agriculturally impractical to disturb the soil during crop growth periods, insight into the effects of disturbance on this species may help in the development of better management approaches.

7.2.5. Genotype

Differences between populations from international sources were seen in the results in Figure 3.1 and 4.1. By using a common garden method to produce seed for the experiments in these chapters, the effects we reported we suggested were due to genetic
differences. Our results also suggested that adaptation of this species to local climate was relatively rapid. Rapid growth and high fecundity would be an aid in this regard and *Conyza canadensis* may, therefore, be more adaptive than many other weed species (Weaver 2001). Other weed species have significantly larger genomes: Common lambs quarter (*Chenopodium album* L.) - 2279 Mbp, Large crabgrass (*Digitaria sanguinalis* (L.) Scop.) - 1176 Mbp, and Smooth pigweed (*Amaranthus hybridus* L.) - 686 Mbp (Basu et al. 2004).

The results in Chapters 4 and 5 also show that in differing climates, seed shed timing plays an important role in adaptation given that the risk to individuals in a population, due to stressors such as desiccation or freezing, provide substantive selection pressures (Thomashow 1999). Newly recruited populations from seed not local to a given area would have to adapt quickly to survive multiple seasons. From the results in this thesis it seems that adaptation would likely be hastened by early fall or early spring emergence because these emergence timings favour survival, fecundity, and shortened time to flowering.

### 7.3 Implications of thesis results in the context of climate change

Climate change is predicted to have significant impacts on weedy species (Clements and DiTommaso 2011). In the context of this thesis, one factor (warming spells) of climate change was studied. Results showed a significant impact of warming spell timing on fecundity, aboveground biomass, flowering timing, and overwintering survival in *Conyza canadensis* (Chapter 6). Significant decreases in fecundity, aboveground biomass, survival, and time to flowering were seen in plants subjected to late-winter warming spells (Chapter 6). Specifically, results from this thesis showed a
significantly decreased time to flowering for Ontario populations exposed to warming spells. Similar warming spell studies on the international populations used in this thesis could have provided more insight into climate change effects. The potential for populations adapted to differing climates to have different responses to climate change would provide insight into not only the rate of adaptation, but individual plant plasticity as well. If the trends from the population dynamics model for Ontario (Figure 7.1) are applied to locations in the U.K., Spain, or Iran, the Ontario population may outcompete all natively adapted populations in U.K. and Spain. This may occur due to the availability of water in these regions, or the timing of that availability (rainfall pattern). Iranian populations may still outcompete Ontario populations in local conditions due to the lack of rainfall during the summer and early fall, the period during which Ontario populations would need it most. Climate change may alter climate patterns in one, many, or all of these regions with potentially unknown effects on local plant life in the region, but any changes in selection pressures would most likely result in a swift adaptation from *Conyza canadensis* populations in the region. *Conyza canadensis* is well suited for both summer and winter climate conditions allowing for survival, and potential range expansion, in areas where climate change may decrease annual temperatures (Clements and DiTommaso 2011; Weaver 2001).

Perhaps the most interesting result from our simulated warming spell experiment was that some plants exposed to the experimental warming spells flowered significantly earlier than the controls. The flowering timing was so early (June) that the potential for a second generation within one growth year was plausible. This phenomenon would have a significant impact on management of those species, especially in no-till systems, as the
presence of *Conyza canadensis* plants at higher densities would most likely decrease yield further. Without fall tilling, some plants in a double generation scenario, might flower after crop harvest creating the potential for a very large fall population of seedlings and possibly a higher contribution to the seed bank. In addition, secondary emergence in the fall may then contribute to a larger spring population the following year (Figure 7.1).

A double generation in one year might also have other significant effects on population dynamics in the following growing seasons. If, instead of a full double generation, the second generation did not make it to flowering, most *Conyza canadensis* plants would have high rates of mortality over winter. This would occur if plants had grown past the rosette stage and into bolting, which leads to greater winterkill. In this scenario, population densities would be negatively impacted leading to a potentially smaller populations year on year. If plants did not grow to the bolting stage and instead developed into larger rosettes overwintering mortality would decrease. Larger rosettes (~9 cm) have lower rates of mortality than smaller one (Main et al. 2006) up until a certain size where the cost/benefit ratios to the plant of sustaining such a large rosette are negative. Flowering earlier might allow more rosettes to reach the upper limit of rosette size thus leading to increases in overwinter survival, which in turn, would lead to plants with higher fecundity and earlier flowering as well, as seen in Chapter 4. If applied to the population model (Figure 7.1), subsequent generations that flower earlier, would lead to an increase in survival, fecundity, and a potentially even shorter time to flowering in the following seasons. This would most certainly alter current population dynamics of this species by encouraging earlier and earlier flowering until a point is reached where the
negatives, such as interspecific competition with crops or other plant species, outweigh the benefits of early flowering. The influence of other aspects of climate change, instead of warming spells, on *Conyza canadensis* is specifically currently unknown, but Clements and DiTommaso (2011) predict an increasing in population density and range in weedy species due to climate change.

**7.4 Conclusions**

Major goals in the analysis of recruitment timing effects of *Conyza canadensis* were met. Effects of recruitment timing on fecundity, survival, aboveground biomass, and time to flowering in populations from differing global climates were observed. Base germination temperature ranges and amount of GDDs to 50% germination were also found for four international populations. Effects of warming spells on Ontario populations were also successfully observed. Results from this thesis could assist producers in making educated decisions in management within agricultural systems, especially as herbicide resistant populations become more common. Development of a novel population dynamics model will assist in further understandings about the important role *Conyza canadensis* plays in both natural and agricultural ecosystems. To my knowledge, the model produced in this thesis along with findings of base germination temperature ranges have not been previously published and represent significant contributions to weed ecology.
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