Design and analysis of population demographic experiments for use in environmental risk assessments for genetically modified crops.

by

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Abstract

Genetically modified crops with putative fitness-enhancing traits are being field-tested throughout Canada and the world, but robust methods with which to compare their fitness with conventional cultivars are lacking. Additionally, field analyses of GM crops in non-agricultural areas are complicated because novel GM crops have no naturalized populations and creating sufficiently large populations for study under containment is difficult. Using the tools afforded to us by population matrix modeling, we may estimate and compare the fitness of GM crops with conventional (comparator) cultivars. We present methodology to establish populations of GM crops under confinement and develop suggestions for possible assessment endpoints based on the population growth rate of a GM crop and comparator cultivar (assessment endpoint scenarios). Finally, we used assessment endpoint scenarios and stochastic estimates of population growth to determine that the invasiveness of a hybrid canola cultivar was generally no greater than an open-pollinated variety, and, in fact, was less in some environments.

Preface

Chapters 3 and 4 were co-authored by the candidate, Dr. Linda Hall, Dr. Hugh Beckie, Dr. Marie-Josée Simard, Dr. Robert Nurse, Dr. David Clements, Dr. Dr. Mark Lewis, Dr. Ron-Cai Yang, and Dr. Peter Blenis. The candidate was responsible for conducting trials at St. Albert, compilation of the data, statistical analysis, demographic modeling, and writing of the manuscript. Dr. Hugh Beckie was responsible for conducting trials in Saskatchewan with the help of his technical support staff. Dr. Marie-Josée Simard was responsible for conducting trials in Québec with the help of her technical support staff. Dr. Robert Nurse was responsible for conducting trials in Ontario with the help of his technical support staff. Dr. David Clements was responsible for conducting trials in British Columbia with the help of his technical support staff. Dr. Mark Lewis and his team provided guidance in the construction and analysis of population matrix models. Dr. Rong-Cai Yang provided statistical analysis support in SAS. Dr. Peter Blenis provided statistical analysis and data manipulation support in R. Technical field support in Alberta was provided by Mrs. Judy Irving, Miss Lisa Raatz, Mr. Keith Topinka, Mrs. Breanne Tidemann, Dr. Kim Walsh, Mrs. Jagroop Kahlon, Mr. Dana Sanderson, and Mr. Ryan Low. Dr. Linda Hall was the graduate student supervisor for the candidate and worked with him on the writing of all manuscripts and advised him throughout his program.

No part of this thesis has previously been published.

Dedication

This work is dedicated to my wife and parents, my family and friends,
all of whom have supported me as I worked my way through
a seemingly fruitless endeavor only to start over again.

Many apologies for the trouble.

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List of Abbreviations

ALS	Acetolactate synthase
CFIA	Canadian Food Inspection Agency
EBRA	Environmental biosafety risk assessments
GDD	Growing degree days
GLMM	Generalized linear mixed model
GM	Genetically modified
GMO	Genetically modified organism
i.i.d.	Independent and identically distributed
LLP	Lower level parameters
LTRE	Life table response experiment
PBO	Plant Biosafety Office
PNT	Plants with novel traits
PPT	Precipitation
WRA	Weed risk assessment

Chapter 1: Introduction

1.1 Background

Genetic modification of plants raises concerns regarding increased fitness for the modified plants or wild relative-crop hybrids resulting in weedy or invasive populations (Warwick et al. 2009). Within the regulatory framework, new genetically modified (GM) crops are evaluated on the basis of familiarity (Conner et al. 2003) and an understanding of the inherent properties of the crops, including domestication traits. Proponents of GM crops have been required to characterize morphological and reproductive properties of GM and conventional varieties, usually grown in agricultural fields (Horak et al. 2014). Quantifiable traits include germination, volunteer potential, competitive ability, seed bank longevity and fecundity. There is no evidence that the currently used GM traits (insect and herbicide tolerance) in crops are contributing to invasiveness in natural areas (Crawley et al. 2001). However, there are traits currently under development that may impact fitness and invasiveness including tolerance to abiotic and biotic stresses as well as increased yield (Canadian Food Inspection Agency 2015; USDA/APHIS. United States Department of Agriculture, Animal and Plant Health Inspection Service 2016). Additionally, most crop species have wild or weedy congeners (Hall et al. 2006; Warwick et al. 2009) and assessing the fitness of GM crops and hybrids growing outside of cultivation is challenging (Simard et al. 2005).

A robust method for quantifying and comparing the fitness of GM crops remains undeveloped. Population demographic modeling is widely used to assess fitness in

ecological studies (Crawley et al. 1993; Caswell 2001) and to examine the factors that most influence fitness using survival and fecundity data. However, it is currently not widely used in agriculture. Fitness is defined here as an individual's ability to achieve genetic representation in future generations and is a function of survival and fecundity (hereafter referred to as vital rates or lower level parameters (LLPs)). Focusing on the components of fitness rather than fitness as a whole could lead to erroneous conclusions regarding population demographics (Caswell 2001). Population matrix models quantify population growth using both survival and fecundity, and provide a more direct measure of fitness than either survival or fecundity alone (Caswell 2001).

GM crops or their congeners may have increased fitness in some environments due to modified or inserted genes (Raybould and Gray 1994; Warwick et al. 1999; Ellstrand 2001). Concerns over the release of GM crops are that with increased fitness will come increased invasive potential in the crops or their congeners. New GM crops with traits including stress tolerance and nitrogen use efficiency are being developed to enhance drought, cold, salinity and general stress tolerance (Canadian Food Inspection Agency 2011). Considerable research has addressed whether herbicide-resistant and insect-tolerant crops will be invasive (Cummings and Alexander 2002; Tranel and Wright 2002; Burke and Rieseberg 2003; Snow et al. 2003; Crawley and Brown 2004; Halfhill et al. 2005; Begg et al. 2006; Guadagnuolo et al. 2006). In most instances, methods have not employed trait-based approaches that attempt to assess plant fitness and invasive potential by examining the correlation of some phenotypic traits with invasive potential (Pheloung 2001), but have

addressed the question of relative fitness between GM and conventional (comparator) crops or hybrids. Analysis has focused on comparison of fecundity (Snow et al. 2003; Yang et al. 2012) rather than fitness. Experiments designed to measure the fitness of GM crops are also often conducted in a small subset of environments in which we are interested (agricultural) and are not representative of most natural environments. This is due to increased soil nutrients, soil disturbance, and soil homogeneity in agricultural environments compared to ruderal and natural environments.

1.2 Research Objectives

1.2.1 Develop field-based methods and suggest experimental design considerations and assessment criteria to measure and compare the fitness of GM crops in agricultural and non-agricultural environments.

Newly developed GM crops cannot be sampled from naturally occurring populations as is conventional in most demographic studies. Additionally, GM crop regulations mandate that seed and pollen must be controlled and confined within strict area boundaries. Therefore, deliberately planned populations must be established with enough seed to avoid zero frequency data and sufficient replication to obtain the desired statistical power while keeping the total experimental area as small as possible. We investigate the precision associated with measuring survival, fecundity, and population growth and relate it to the importance of measuring these parameters precisely using prospective and retrospective analyses. We investigate the inherent differences between using null hypothesis tests and equivalence tests and demonstrate why those differences are important. We suggest a scenario-based method for comparing the invasive potential of GM crops that attempts to

simplify hypothesis testing. Finally, we discuss a tiered experimental design for confined release trials.

1.2.2 Assess and compare the invasive potential of two canola cultivars using population matrix modeling while operating under the paradigm of 'substantial equivalence'.

Using population matrix models and stochastic analyses, we compare two cultivars of canola (*Brassica napus* L.), using methods outlined in chapter 3, to determine their expected propensity for being weedy or invasive on their own and relative to each other. We estimate stochastic population growth rates, elasticity of stochastic population growth rates to LLP, probability of quasi-extinction, and time to quasi-extinction to assess their relative risks of invasiveness.

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Chapter 2: Literature Review

Regulatory frameworks for new crops are established by government entities to limit the potential risk posed by releasing novel species or traits. Within these frameworks, environmental biosafety risk assessments provide the criteria for assessing the risk of novel species which includes (but is not limited to) the propensity for being weedy or invasive or causing a naturally-occurring related species to become weedy or invasive (McCammon 2006; Canadian Food Inspection Agency 2013).

2.1 Environmental biosafety risk assessment of GM crops

Risk assessment is the foundation for regulator decision on the unconfined release of GM crops. Risk assessment is a "structured, reasoned approach to identify a GM crop's potential to cause adverse effects (harm) and to characterize the seriousness and likelihood of the potential harm" (Keese et al. 2014). Environmental biosafety risk assessments (EBRA) were first modeled after chemical risk assessment where risk was quantified as a dose response. Unfortunately, plants are unlike chemicals; plants can reproduce, disperse and persist (become weedy or invasive). The EBRA for transgenic crops are now framed within a weed risk assessment (WRA) used to make decisions regarding the prevention and intervention strategies for invasive plants (Keese et al 2014).

In Canada, environmental safety is the responsibility of the Plant Biosafety Office (PBO) of the CFIA. The PBO is also responsible for post-commercialization and monitoring activities of GM crops (Canadian Food Inspection Agency 2011). In Canada, GM crops are classified as plants with novel traits (PNTs). A PNT is defined as "…a plant that contains a

trait that is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health." (Canadian Food Inspection Agency 2013), and includes plants derived from mutagenesis as well as conventional breeding. Environmental safety assessments examine three broad adverse impacts with respect to weedy/invasive potential (Canadian Food Inspection Agency 2011):

- The potential of the plant to become a weed
- The potential of the plant to create a weed by cross-pollinating with another plant
- The potential impact on biodiversity

Risk assessments cannot prove an absence of absolute risk but can quantify relative risk. Therefore, comparative risk assessments to quantify relative risk are necessary and defining the appropriate baseline for comparison and decision is critical. In Canada, the principle of substantial equivalence is employed so that a PNT may be evaluated relative to its conventional (comparator) counterpart (Canadian Food Inspection Agency 2011).

Regulatory decisions are based on scientific data and each assessment is conducted on a case-by-case basis (Conner et al. 2003). Assessments must consider not only the potential risks of a PNT, but the likelihood that harm will result. Risk (r) is therefore defined as a function (f) of hazard (h) and its probability of exposure (e):

$$r = f(h, e) \qquad 2\text{-}1$$

(Wilkinson et al. 2003). The reliability of a risk assessment method can only be determined by empirical validation, derived from comparing predictions with outcomes (Keese et al 2014).

2.1.1 Tiered risk assessments

Tiered risk assessments begin with artificial 'worst case' scenarios and then move to more environmentally realistic ones (Wilkinson et al. 2003; Raybould and Cooper 2005). It follows a non-linear approach so that the most relevant lines of evidence are brought forward (Wolt 2009). Tiered testing is designed to be time and resource efficient and limit the collection of superfluous data (Mallory-Smith et al. 2015)

Tier 0, also known as the 'problem formulation step', focuses on the organism, the trait, and the receiving environment, and it ensures that the conclusions drawn will be appropriate to the decision-making process (Garcia-Alonso et al. 2006). All information gathered is retained and utilized in the synthesis and interpretation of subsequent tiers.

Tier I, the first experimental tier, is conducted under 'worst case' conditions (Raybould and Cooper 2005). The analytical phase of any risk assessment is initiated with tests that conservatively address broad questions using simple experimental designs (Wolt 2009). This assessment is not intended to be realistic, rather to maximize the chance for detection of hazard occurrence and minimize the chance of committing Type II errors. In Tier I, laboratory or greenhouse conditions are generally preferred over field studies, as they remove environmental factors that may complicate observations and results (Garcia-

Alonso et al. 2006). For example, if one is assessing the potential for interspecific hybridization between two species, Tier I may be the emasculation of the pollen receptor followed by manual pollination, and perhaps even embryo rescue of the hybrid (Raybould 2006). If no hybrids are detected (no occurrence of hazard), testing can be terminated based on the assumption that hybrid formation (hazard) will be less likely under more natural (less artificial) conditions and risk can be deemed low. If hazards are detected in Tier I, higher tier tests (Tier II) should be performed to better assess risk (Raybould and Cooper 2005; Wolt 2009).

Tier II experiments are intended to be more realistic. They may include additional laboratory or greenhouse experiments, or progress to small-plot field experiments (Raybould and Cooper 2005). For instance, Tier II may involve small-plot experiments that encourage hybridization events by minimizing the distance between pollen donor and receptor populations. If Tier II results indicate that risk is acceptable or negligible, testing may terminate (Garcia-Alonso et al. 2006). However, if results from Tier II indicate a potential risk, higher tiered tests will be employed.

Tier III tests are designed to mimic more natural conditions, and may be conducted as medium to large-scale field experiments (Raybould and Cooper 2005). These studies are often laborious, and the results provided can be difficult to interpret without the aid of the previous tiers' data (Garcia-Alonso et al. 2006). If the results from Tier III indicate the risk level to be acceptable, no further experiments will be required. If the results from Tier III

confirm sufficient risk, further refinement may be required, or a decision of unacceptable risk may be made.

This systematic scientific tiered approach is iterative, where knowledge obtained in lower tiers directs data collection at higher tiers (Wilkinson et al. 2003; Garcia-Alonso et al. 2006). Risk assessments analyze the probability that harm will occur, the likely magnitude of harm and the uncertainty associated with those predictions (Raybould and Cooper 2005). A tiered approach to risk assessment provides a foundation of knowledge which informs subsequent regulatory decision-making.

2.1.2 Traits, Climate Matching, History of Invasiveness

Trait-based approaches attempt to assess plant fitness and invasive potential by examining the correlation of some phenotypic traits with invasive potential (Pheloung 2001). For example, having a history of weediness or invasiveness is a good predictor of weediness or invasiveness, especially if the receiving environment has a similar climate (Forcella and Wood 1984; Panetta and Mitchell 1991a,b).

Climate matching is based on niche theory. Hutchinson (1957) described the niche as the n-dimensional hypervolume of abiotic and biotic factors required for an organism to survive and reproduce. The fundamental niche area is the total area where an organism could possibly survive and reproduce. In contrast, the realized niche is where the organism currently survives and reproduces. When organisms are predicted to be invasive due to climate matching arguments, the regulators are really saying that the fundamental niche of

the organism extends into the receiving environment even though the receiving environment is not currently part of the realized niche. These assessment methods may not be as useful when assessing the invasive potential of GM crops because novel GM crops have no realized niche and no history of invasiveness.

2.2 Population matrix modeling

The most direct method of assessing the potential for a species to be weedy or invasive is by estimating the population growth rate. There are several tools available to demographers, but we will focus on those relevant to annual species and within the context of an EBRA.

2.2.1 The Population Projection Matrix

Population matrix modeling uses linear algebra to incorporate survival and fecundity (vital rates) data across ages or stages of an organism's lifecycle to estimate the population growth rate (Caswell 2001a). Lifecycle information is used to construct a lifecycle diagram representing the survival, growth, and fecundity transitions that may occur based on the life stages on which the experimenter has chosen to focus. The lifecycle diagram is isomorphic to the population projection matrix $\boldsymbol{A}$ that is used to project a population vector n to some later point in the future (Caswell 2001b). The population vector n contains information regarding the structure of the population based on the stages that have been chosen. The amount of time between the original vector ($n_{(t)}$) and the future vector ($n_{(t+1)}$) is called the time-step and in most studies is equal to one year. The relationship between $n_{(t)}$ and $n_{(t+1)}$ is described by the projection equation

$$n_{(t+1)} = \boldsymbol{A} n_{(t)} \qquad 2\text{-}2$$

where $n_{(t)}$ is a vector describing the population structure at time 't', $n_{(t+1)}$is a vector describing the population at time '$t + 1$', and $\boldsymbol{A}$ is the population projection matrix constructed with some combination of survival, growth and fecundity estimates. We want to find a value λ such that

$$\boldsymbol{A} n = \lambda n \qquad 2\text{-}3$$

where λ is a constant known as an eigenvalue (Caswell 2001c). It is possible for matrices to have multiple eigenvalues. In fact, matrices have a number of eigenvalues equal to the order of the polynomial created when taking the determinant of the characteristic equation

$$\det(\boldsymbol{A} - \lambda \boldsymbol{I}) = 0 \qquad 2\text{-}4$$

The order of the polynomial is equal to the number of population stages in $n_{(t)}$ (the number of rows in the vector $n_{(t)}$). The dominant eigenvalue has the largest magnitude when compared to the other eigenvalues, and is most often the value reported in the literature because it determines, and is the best estimate of, the asymptotic population growth rate. Other eigenvalues are often taken into account when considering the transient behavior of a matrix model. Much of the work that has been done in population matrix modeling has revolved around creating stage-structured or age-structured models. While the lifecycles of annual species can be described as stage or age-structured, it has been suggested that periodic matrices are superior (Caswell 2001d).

2.2.2 Periodic Matrices

Demographic information for annual plants is usually taken on a sub-annual time scale, and for this reason periodic matrices are used instead of stage or age structured matrix models (Caswell 2001d). Periodic matrix models describe the lifecycle of populations in time-varying environments where the projection matrix $\boldsymbol{A}$ is equal to

$$\boldsymbol{A}_h = \boldsymbol{B}_{h-1} * \dots \boldsymbol{B}_1 * \boldsymbol{B}_m \dots \boldsymbol{B}_{h+1} * \boldsymbol{B}_h \qquad 2\text{-}5$$

where

$$h = 1, \dots, m \qquad 2\text{-}6$$

and each sub-matrix $\boldsymbol{B}_i$ $(1 \leq i \leq m)$ is created from survival and fecundity (LLP) estimates and project a population vector from one sub-annual time to another. Within a stage or age-structured matrix, survival and fecundity estimates are often referred to as 'vital rates'. However, in a periodic matrix we will refer to them as 'lower level parameters' (LLP), just as a naming convention, to recognize that the $\boldsymbol{A}$ matrix is composed of $\boldsymbol{B}$ matrices that are themselves populated by LLP. A key property of the eigenvalues of periodic matrices is that they are independent of the value of h. This provides an opportunity to choose h so as to most easily calculate the eigenvalues. Because the lifecycle of an annual plant collapses to a single stage in the fall (seed), multiplication of the $\boldsymbol{B}$ matrices in such a way that the first matrix is of dimensions (1xS) and the last matrix is of dimensions (Rx1) creates a 1x1 $\boldsymbol{A}$ matrix (a scalar) whose value is necessarily equal to the eigenvalue of the matrix because

$$\det(c - \lambda \mathbf{I}) = c - \lambda \qquad 2\text{-}7$$

can be simplified to

$$c = \lambda \qquad 2\text{-}8$$

where $c > 0$.

For the annual species we observed, λ can be expressed explicitly in one linear equation:

$$\boldsymbol{A} = \lambda = \underbrace{(1 - g_f) * (1 - \sigma_w) * \sigma_b}_{Term\ 1} + \underbrace{(1 - g_f) * \sigma_w * \sigma_{sdl} * \Phi}_{Term\ 2} + \underbrace{g_f * \sigma_f * \sigma_{sdl} * \Phi}_{Term\ 3} \qquad 2\text{-}9$$

where g_f is the proportion of seeds that are recruited in the fall, σ_f is the proportion of fall seedlings that survive until the spring, σ_b is the proportion of seeds that survive in the seedbank from spring until the fall, $\sigma_w$is the proportion of seeds that remain in the seedbank in the fall and subsequently germinate in the spring, σ_{sdl} is the proportion of seedlings that survive until maturity, and Φ is the average number of seeds produced per mature plant. Term 1 represents the underground pathway, the method by which a population achieves genetic representation in the future by surviving in the seedbank. Terms 2 and 3 both represent aboveground pathways, the methods by which a population achieves genetic representation in the future by germinating and producing new individuals. Term 2 is the pathway followed by individuals that germinate in the spring and Term 3 is the pathway followed by individuals that germinate in the fall. When no seeds germinate in the fall (g_f= 0) then the above equation simplifies to

$$\lambda = \underbrace{(1-\sigma_w)*\sigma_b}_{Term\ 1} + \underbrace{\sigma_w * \sigma_{sdl} * \Phi}_{Term\ 2} \qquad 2\text{-}10$$

2.2.3 Prospective Analysis

Sensitivity and elasticity are the results of prospective perturbation analyses that predict the change in a population parameter (like λ) with respect to a small change in a LLP while keeping all other vital rates constant (Caswell 2001e). Sensitivity analysis may best be understood as the absolute change in the population parameter with respect to a small, absolute change in a vital rate given by

$$\boldsymbol{S} = \frac{\partial \lambda}{\partial a_{ij}} \qquad 2\text{-}11$$

whereas elasticity analysis is the relative change in a population parameter with respect to a small, relative change in a vital rate given by

$$\boldsymbol{E} = \frac{a_{ij}}{\lambda}\frac{\partial \lambda}{\partial a_{ij}} \qquad 2\text{-}12$$

For periodic models, a modification to the above equations needs to be done through application of the chain rule such that the sensitivity of λ to a LLP is

$$\frac{\boldsymbol{\partial \lambda}}{\boldsymbol{\partial x}} = \sum_{i,j}\frac{\partial \lambda}{\partial a_{ij}}\frac{\partial a_{ij}}{\partial x} \qquad 2\text{-}13$$

and the elasticity is

$$\frac{\boldsymbol{x}}{\boldsymbol{\lambda}}\frac{\boldsymbol{\partial \lambda}}{\boldsymbol{\partial x}} = \frac{x}{\lambda}\sum_{i,j}\frac{\partial \lambda}{\partial a_{ij}}\frac{\partial a_{ij}}{\partial x} \qquad 2\text{-}14$$

(Caswell 2001f). When λ can be expressed as one linear equation in terms of the LLPs, such as when the periodic model collapses to a single stage (equations 2-9, 2-10), the sensitivity of λ to a LLP (σ_w for example) can be calculated by calculating the derivative of λ with respect to the LLP (σ_w) while keeping the other LPPs constant. Elasticity values are most often reported in the literature, especially when the researchers are considering LLPs that naturally differ in scale by orders of magnitude. For example, when comparing survival probabilities with fecundities, the former is necessarily bound between 0 and 1 whereas the latter has only a lower bound of 0 and no upper bound. Caswell (2001e) notes that the issue of scale is not actually resolved by using elasticity instead of sensitivity, it is simply being moved to the perturbation. For example, for a survival probability of 0.05 and a fecundity of 100 seeds plant^{-1}, a 10% perturbation on the scale of the survival probability would be ±0.005 but on the scale of fecundity it would be ±10.

2.2.4 Retrospective Analysis (Life Table Response Experiment)

Prospective analyses are used to determine what changes would occur to a population parameter if an LLP were to change (Caswell 2001g). A core assumption of prospective analysis is that those LLPs are able to change, that there is variation in the values. Retrospective analyses like life table response experiments (LTRE) are used to decompose differences in λ into contributions from the LLP. These contributions are calculated as the product of the differences observed in a vital rate between two populations and the

sensitivity of the population parameter to that vital rate, often evaluated at the mean matrix ($\boldsymbol{A}^{\dagger}$). LTRE are calculated by

$$\lambda^m \approx \lambda^r + \sum_{i,j}\left(a_{ij}^{(m)} - a_{ij}^{(r)}\right)\frac{\partial\lambda}{\partial a_{ij}}\bigg|_{\boldsymbol{A}^{\dagger}} \qquad 2\text{-}15$$

where

$$\boldsymbol{A}^{\dagger} = \frac{\boldsymbol{A}^m + \boldsymbol{A}^r}{2} \qquad 2\text{-}16$$

Here $\boldsymbol{A}^m$ and $\boldsymbol{A}^r$ refer to the treatment and reference matrices, respectively. λ^m and λ^r are the treatment and reference population growth rates, respectively.

2.2.5 Environmental Stochasticity

Over time, the LLP will vary due to environmental fluctuations. Stochastic population growth rates ($\log \lambda_s$)take into account several years of data, and thus environmental variation and variation in the LLP. Environmental stochastic models can be decomposed into three parts: a function that randomly selects environmental states, a function that associates each environmental state with a population projection matrix $\boldsymbol{A}$, and a sequence of population vectors N_t that result from applying the projection matrices to some start vector N_0 (Caswell 2001h).

The simplest description of a stochastic environment treats the environmental states as independent and identically distributed (i.i.d.). If the total number of environmental states is $\boldsymbol{x}$, then at every projection each environmental state has a probability $\frac{1}{\boldsymbol{x}}$ of being

selected. Thus, each environmental state is equally weighted and has an equal chance of being randomly selected regardless of the past environmental state.

The calculation of the stochastic population growth rate ($\log \lambda_s$) is most easily done through simulation by projecting a starting population vector N_0 into the future using randomly selected projection matrices ($\boldsymbol{A}_1, \boldsymbol{A}_2, \boldsymbol{A}_3 \ldots \boldsymbol{A}_T$) through $\boldsymbol{T}$ iterations and applying the equation

$$\widehat{\log \lambda_s} = \frac{1}{T}\sum_{t=0}^{T-1} r_t \qquad 2\text{-}17$$

where

$$r_t = \log(\frac{N_{(t+1)}}{N_{(t)}}) \qquad 2\text{-}18$$

The 95% confidence interval for $\log \lambda_s$ can be calculated by

$$\widehat{\log \lambda_s} \ \pm 1.96 \sqrt{\frac{V(r)}{T}} \qquad 2\text{-}19$$

where $V(r)$ is the variance of the calculated r_t.

The distribution of $\widehat{\log \lambda_s}$ is asymptotically Gaussian due to the Law of Large Numbers, and therefore $\boldsymbol{T}$ is generally chosen to be large (10,000 for example). However, note that the calculation of the confidence interval for $\log \lambda_s$ relies on $\boldsymbol{T}$ and also that they are inversely related. The confidence interval narrows as $\boldsymbol{T}$ increases in magnitude. Therefore, the above calculations will yield increasingly accurate estimates of $\log \lambda_s$ as $\boldsymbol{T}$ increases.

Selecting extremely large values of $\boldsymbol{T}$ increases our confidence in the point estimate of $\log \lambda_s$, but it obscures the variability that was observed in the original data and could lead to inaccurate conclusions regarding the confidence we have in limited datasets to forecast the future. Alternatively, a short-term forecast (Caswell 2001h) that approximates the experimental design might be used to examine the variation around estimates of $\log \lambda_s$Instead of setting $\boldsymbol{T}$ to some large number, a value could be chosen based on the number of study years, or the total number of experimental units. Relating the short-term forecast to the number of experimental years might lead proponents to invest in collecting much larger datasets that span across many years.

Stochastic population calculations generally estimate $\log \lambda_s$ over a large number of iterations (large $\boldsymbol{T}$) and then discard an initial set of iterations to remove transient behavior (Caswell 2001h). For our simple model, discarding iterations may not be necessary as the population vector projected from time t to time $t+1$ is a scalar, and therefore the value of r_t is always going to be equal to $\log(\lambda_t)$ because the matrix $\boldsymbol{A}_t$ and population vectors are also scalar (Equations 2-2 and 2-18). Note as well that because $\boldsymbol{A}_t$ is scalar then $\boldsymbol{A}_t = \log(\lambda_t)$ **.** However, if our population vector was not a scalar, then r_t would not necessarily equal $\log(\lambda_t)$ because $\boldsymbol{A}_t$ would alter the stage distribution of the population vector at each time-step (Caswell 2001h). In addition, $\boldsymbol{A}_t$ would not be a scalar and therefore would also not be equal to λ_t. Additionally, for this model, $\log(\lambda_t)$ is a scalar that is strictly greater than zero, and that means that at no point will the population vector actually reach zero (unless $N(0) = 0$). As an example, if you wanted to calculate the long

term average for a fair six-sided die, then over many iterations the value would approach 3.5. It wouldn't make sense to perform 10,000 iterations (rolls) and then eliminate the first 1,000 to avoid transient behavior because the die does not have a memory of past rolls. Any averaged 10,000 rolls (or any large $\boldsymbol{T}$), as long as they're random, should approximate 3.5 when their values are averaged. While our population vector does have a memory as it is projected forward through time, the value of r_t will always be equal to the value of $\log(\lambda_t)$ for the matrix $\boldsymbol{A}_t$ (for the reasons mentioned above) that was selected in i.i.d. fashion. So any averaged 10,000 values for r_t should approximate $\log \lambda_s$ because the λ's are selected in i.i.d. fashion and because λ and the population vector are both scalar.

2.2.6 Quasi-Extinction

The stochastic model described above may forecast populations that decline over time, however they will never reach zero (Caswell 2001h). Quasi-extinction refers to populations that have dropped below some predetermined fraction of their initial size - an extinction threshold. The extinction threshold is calculated by

$$\theta = \frac{N_q}{N(0)} \qquad 2\text{-}20$$

where $N(0)$ is the initial population size, N_q is the minimum size for a population to be considered extant, and θ is the extinction threshold. To calculate the probability (P_q) of the population dropping below N_q we first modify equation 2-2 to calculate the λ_t

$$\frac{N_{(t+1)}}{N_{(t)}} = \lambda_t \qquad 2\text{-}21$$

Because the λ_t are equal to the $\boldsymbol{A}_t$ we can calculate the growth rate of mean population size as

$$\log u = \log \bar{\lambda} \qquad 2\text{-}22$$

sensu Caswell (2001h). We then calculate the rate at which the variance of $\log N_{(t)}$ grows (σ^2) from

$$\sigma^2 = 2(\log \mu - \log\ \lambda_s) \qquad 2\text{-}23$$

sensu Caswell (2001h). The probability (P_q) of the population dropping below N_q is

$$P_q = \begin{cases} 1 & if\ \log \lambda_s\ \leq 0 \\ \exp(\dfrac{2 \log \lambda_s \log \theta}{\sigma^2}) & if\ \log \lambda_s > 0 \end{cases} \qquad 2\text{-}24$$

The time for a population to go extinct is T_q, and the mean of T_q is given by

$$\frac{-\log\theta}{|\log \lambda_s|} = E(T_q) \qquad 2\text{-}25$$

2.2.7 Stochastic Elasticity

There are at least two ways to calculate the elasticity (e_x) of $\log \lambda_s$ to the LLP. This thesis calculates stochastic elasticity by perturbing a LLP at each time step by 10% (increase and decrease) and then averaging the results of e_x (Claessen et al. 2005). Let e_{x^+} represent the elasticity from an increase in the LLP, let e_{x^-} represent the elasticity from a

decrease in the LLP, and let $e_{x'}$ represent the general notation for either an increase or a decrease. Then

$$e_{x'} = \frac{\log \lambda_s - \log \lambda'_s}{\log x - \log x'} \qquad 2\text{-}26$$

Using 2-26 we calculate both e_{x+} and e_{x-} and take their average to estimate e_x.

2.3 Biology of Investigated Species

2.3.1 *Brassica napus*

Canola (*Brassica napus* L.) is a member of the Brassicaceae family that was differentiated from rapeseed and became a major oilseed crop species through the selection of low glucosinolate, low erucic acid cultivars in Canada in the early 1970s (Gulden et al. 2008). In Canada and Australia, canola is widely grown as an annual crop, but in most of Europe, where it is called oilseed rape, it is grown as a winter annual. GM canola resistant to the herbicides glyphosate and glufosinate were released in 1995, were rapidly adopted (Beckie et al. 2006) in Canada and continue to be a component of the dominant cultivars grown in 2015. Australia has adopted GM herbicide-resistant canola in some states while Europe has not adopted GM crops of any kind but grows oilseed rape resistant to imidazolinone herbicides (Huang et al. 2016).

Canola is a competitive crop in most agricultural fields where grown in Canada (Harker et al. 2013). Canola is also an abundant agricultural weed species (Leeson et al. 2005). Canola seed is lost prior to and during harvest (Gulden et al. 2003). Gulden et al (2003) reported that canola seed bank additions averaged 3,000 viable seeds m^{-2} in a 2-year study

of 35 fields in Saskatchewan, and similar seed loss has been reported in the UK (Lutman et al. 2005). While canola has no primary seed dormancy, it has secondary inducible dormancy that can be initiated by temperature, burial and is influenced by canola genotype (Gulden et al. 2004). Seed banks decline over time. Gulden et al. (2003) reported a maximum persistence of 44, 14 and 0.2% over the first three years,respectively, of the original seed bank. Canola seed banks are depleted by seed predation, fatal germination (germination followed by seedling death), pathogens, and can expire (become energy depleted). Within cropping systems, the vast majority of canola seedlings are recruited in the year following seed dispersal, but canola may persist and emerge at low densities through a 3 or 4-year rotation (Legere et al. 2001; Harker et al. 2005a).

Seedlings emerge early (Boyd and Van Acker 2003; Bullied et al. 2003) and in agricultural fields these 'volunteers' are effectively controlled pre-seeding and in-crop by a wide variety of herbicides in most crops (e.g. cereals), whether or not they are resistant to herbicides (Beckie et al. 2001, 2006, 2013).

Canola seed can be dispersed away from fields as a contaminant in equipment; roadside populations are common in Canada and elsewhere as a result of repeated spillages by trucks. Canola can establish on roadsides and persist for several years (Crawley et al. 1993; Crawley and Brown 1995; Yoshimura et al. 2006; Busi and Powles 2016). There are no reports that canola has become invasive of natural areas in Canada or elsewhere (Beckie and Warwick 2010).

2.3.2 *Kochia scoparia*

Kochia (*Kochia scoparia* L. Schrad.) is a member of the Amaranthaceae family, which contains approximately 2,500 species and is the only kochia species found in Canada (Blackwell Jr et al. 1978). It is native to Eurasia and was introduced to the Americas in the mid to late 1800's as an ornamental but subsequently escaped cultivation and formed naturalized populations (Friesen et al. 2009). The species utilizes the C4 photosynthetic pathway, giving the plant an advantage in water-use efficiency and salinity tolerance as compared to C3 species. It has adapted to dry, hot conditions with reduced leaves with a hairy undersurface, inconspicuous apetalous flowers, and a deep taproot with an extensive lateral root system (Friesen et al. 2009). Kochia can outcross (Stallings et al. 1995), which contributes to the high genetic diversity within and among populations (Mengistu and Messersmith 2009).

Kochia is a secondary successionary species that can invade disturbed sites such as agricultural fields in arid and semiarid regions. However, it is also commonly found in fields, gardens, roadsides, railway right-of-ways, industrial areas and rangeland pastures (Frankton and Mulligan 1993). It was listed as a rare species by Alberta and Saskatchewan weed surveys in 1948, but is now considered the 10th most abundant weed in agricultural areas and can be found in all Canadian provinces, with the exception of Newfoundland and Labrador (Friesen et al. 2009). Although it is found within all eco-regions of the Prairie provinces, it is most common in Mixed Grasslands (Leeson et al. 2003).

A kochia plant may produce between 2,000 and 30,000 seeds per plant (Friesen et al. 2009), depending on environmental conditions and resource availability. As the plant reaches maturity, an abscission zone forms at the base of the plant, and wind speeds of between 40 and 48 km h^{-1} cause the abscission to break at the base of the plant, allowing the mature kochia plant to deposit its seed across large distances as a tumbleweed (Becker 1978).

Kochia has low innate seed dormancy, with seed viability decreasing significantly after 4 months (Zorner et al. 1984) in the soil. Kochia germinates rapidly, within 2 to 3 hours under favorable conditions (Lodhi 1979). Kochia emergence was quantified in fields and emergence began after 50 growing degree days (GDD) with additional germination continuing throughout the growing season (Bullied et al. 2003). Bullied et al. (2003) and Schwinghamer and Van Acker (2008) reported approximately 80% of seedlings emerged before even 10% of the seedlings of other weed species common to the Northern Great Plain typically emerge. They also reported that kochia germination is influenced by seed placement in soil; 74% of exposed kochia seeds on soil germinated compared to 57% of kochia seeds planted at a depth of 3 mm. No seedlings germinated when kochia seed was planted at depths in excess of 40 mm. In the absence of seed return, the germinable end-of-season kochia seed bank is typically less than 10% of the spring seed bank total (Schwinghamer and Van Acker 2008).

Morphologically, mature kochia is very plastic, with the environment playing a large part in its phenotypic characteristics with growth, height, and seed production influenced

by both inter- and intraspecific competition within a field (Becker 1978; Friesen et al. 2009). Increasing plant density decreases plant size, height, seed size, and harvest index (Kumar and Jha 2015). When grown in competition with other plants, kochia is erect and may grow up to 2m tall; when grown without competition, it assumes a more spherical habit and typically grows to approximately 1m tall (Eberlein and Fore 1984) and 1m in diameter.

Kochia has been selected for resistance to herbicides. Beckie *et al.* (2011) reported that 85% of western Canadian kochia populations were resistant to Group 2 acetolactate synthesis (ALS) inhibitors. Kochia has evolved resistance to the glyphosate herbicide, reported initially in 2006 in three fields in Kansas (Waite 2008). As of 2014, glyphosate-resistant kochia has been reported in the American states of Kansas, South Dakota, North Dakota, Nebraska, Montana, Colorado, Oklahoma, Montana, and in the Canadian provinces of Alberta, Saskatchewan, and Manitoba. In 2016, dicamba-resistant kochia was reported in Saskatchewan (Beckie et al. 2015). Kochia is a significant weed of concern and a better understanding of kochia demographics may aid in controlling kochia plants.

2.3.3 *Triticum aestivum L.*

Wheat is the 2nd largest food crop grown in the world, second only to rice. Modern wheat is a product of interspecific hybridization between three diploid species to produce an allopolyploid. Modern bread wheat (hexaploid, 2n=6x=42) is derived from three species

contributing three genomes (AABBDD). The hybridization of wild Einkorn (*Triticum urartu*) (AA) and *Aegilops speltoides* (BB) produced wild Emmer (*Triticum dicoccoides*) and cultivated Emmer (*Triticum dicoccon*) (AABB). The introgression of *Aegilops tauschaii* (DD) resulted in spelt wheat (*Triticum spelta*), which is the precursor to modern hexaploid wheat (Hegde and Waines 2004). The D genome is shared with many weedy relatives that occur in North America such as jointed goatgrass (*Aegilops cylindrica* Host.) and has the potential to outcross with cultivated wheat (Morrison et al 2002). For the purpose of this brief review, we will narrow our description to common spring wheat as grown in Western Canada.

Wheat has some primary seed dormancy (after ripening) that has been selected to reduce sprouting in heads (sprouting resistance). They have no specialized mechanism for dispersal, and wheat has been selected for reduced seed dispersal so seeds are held in the head prior to harvest. Seed movement is primarily due to intentional seeding, seed source contamination and anthropogenic seed dispersal from equipment or during transport. Seed loss prior to and at harvest has been rarely quantified. Clarke (1985) reported that harvest losses in western Canada were variable, from 35 to more than 800 seeds m^{-2}. This is similar to reports by Anderson and Soper (2009) who reported harvest losses average 2% of yield or approximately 240 seeds m^{-2}. However, these estimates should be treated with caution because data includes winter wheat and estimates from regions with higher yields. Seed loss occurs as both seed heads and as threshed seed. The longevity of seeds in unthreshed

ears is known to be greater than that of loose seeds in the soil (Komatsuzaki and Endo 1996).

Volunteer wheat emerges early, but expressed as the proportion of seeds broadcast, emergence was low, averaging 4.3% (De Corby et al. 2007). The presence of wheat volunteers has been determined following in crop controls in the Western Canada weed survey (Leeson et al 2005). As expected due to the large number of acres of wheat planted, volunteer wheat is the 12th most abundant weed, with average densities in field of 5.9 plants m^{-2}. However, wheat volunteers are underestimated in field surveys because volunteers cannot be differentiated from crop plants. The fecundity of volunteer wheat in crop fields has not be adequately quantified.

Seed persistence in artificial seed banks was reported by Nielson et al. (2008) for western Canada. Seed viability declined exponentially with time. Seed on the soil surface persisted longer than buried seed, but there were no differences between cultivars. Volunteer populations are much smaller than the seed bank initiated in fall (> 5 plants m^{-2}) (Harker et al. 2005b), and do not persist greater than 3 years in the absence of seed return by volunteer plants. Seeds are removed from the seed bank by fatal germination of wheat prior to winter due to the absence of seed dormancy, by seed predation, pathogens or physical damage. The presence of feral wheat has not been reported.

2.3.4 *Camelina sativa*

Camelina (*Camelina sativa* L.) is a hexaploid member of the Brassicaceae family that was used as an oilseed crop since the iron age (Zubr 1997). This 'abandoned crop' has received recent interest as an alternative oilseed in Canada and the USA and has been genetically modified for several purposes (Bansal and Durrett 2016). Camelina reached North America first as a weed, primarily as a seed contaminant in crop seed in the early 19th century (Francis and Warwick 2009). Camelina is an annual oilseed crop with has a relatively short growing season (85–100 days) grown for food and for biodiesel or other industrial uses (Bansal and Durrett 2016). Both winter and spring varieties are grown. Camelia has tolerance to cold and germinates in the fall and may persist in Western Canada as a rosette.

In this small-seeded crop, seed losses at harvest ranged from 1,200 to 43,430 viable seeds m^{-2} (12.0 to 434.3 kg ha^{-1}) (Walsh et al. 2013). Camelina, like most crops, has no primary or secondary seed dormancy and has limited seed bank persistence (Walsh et al 2013). In a seed bank study viable seed persisted less than 15 months at all seed depths. Seeds persisted longer on the soil surface, presumably because the drier surface of the soil afforded fewer opportunities for germination, while seed buried at 3 and 10 cm depleted more rapidly. Seed bank depletion was dependent on site and environmental conditions.

Volunteers of camelina emerge in fall and early spring and have been reported as being numerous. In a study of 11 commercial fields planted to camelina, Walsh et al. (2013) reported variable densities (9 to 4,839 plants m^{-2}). However, populations sharply declined

over time and were nearly extinct after 2 years under conventional production practices. While camelina has a high fecundity and large seed losses at harvest, it has limited seed bank persistence.

Camelina has no specialized mechanism for seed dispersal, and seed movement is primarily anthropogenic. Camelina has been reported as an occasional species of roadsides (Francis and Warwick 2009). Limited cropping acres and consequent seed movement may be limiting the presence of camelina on roadsides.

2.4 Statistics and Experimental Design

2.4.1 Hypothesis testing and environmental safety

Frequentist statistical methods use hypothesis testing as one of the main tools for answering questions regarding random samples of data taken from populations (Zar 2010). Hypothesis testing takes on the form of stating a null hypothesis (H_0) and an alternate hypothesis (H_A). H_0 is stated clearly and succinctly, and allows us to determine the probability of the data (or more extreme data) given H_0. H_A is often stated in terms that cover all the possibilities that H_0 does not. For example, a simple set of hypotheses could be:

$$H_0: The\ populations\ are\ equal. \qquad 2\text{-}27$$

$$H_A: The\ populations\ are\ not\ equal.$$

Or in terms of a summary statistic, like the mean 'u':

$$H_0: u_A - u_B = 0, u_A = u_B \qquad 2\text{-}28$$

$$H_A: u_A - u_B \neq 0, u_A \neq u_B$$

In the event that the sample data are decided to be unlikely given H_0 then H_A is assumed to be true. Failing to prove that the sample data are unlikely given H_0 is not equivalent to proving that H_0 is true, and is also not equivalent to proving that H_A is false. The analysis is instead likely to be inconclusive with regards to the correctness of either hypothesis (Parkhurst 2001).

Statistical error associated with hypothesis testing revolves around Type I and Type II (Zar 2010). Type I error is the rejection of H_0 when H_0 is in fact correct. Type II error is the failure to reject H_0 when H_0 is in fact false. The probability of a Type I error is α, set by the researcher (often arbitrarily at 0.05). The probability of a Type II error is β and cannot be directly set by the researcher. Power (1-β) is the probability of correctly determining that the data are unlikely given H_0 when H_0 is in fact false and is affected by absolute effect size (absolute difference between population means, the signal), variation (the noise), and chosen α value (Zar 2010). Some indices of effect size conflate the absolute effect size with a measure of variation (Cohen's d, for example) (Sullivan 2012), but for the purpose of this document effect size is the absolute effect size. Small effect size, small sample size, high variation, and low α are all causes of low experimental power and therefore increase the probability of making a Type II error (Zar 2010). The consequences of Type I and Type II errors depend on the specification of the hypotheses.

Specification of the hypotheses is important for EBRA's, especially when low experimental power is likely due to small effect size, small sample size, high variation, or low α. The classical 1-tailed hypothesis for testing whether a population value is less than a pre-determined assessment endpoint 'x' is:

$$H_1: u_A \geq x \qquad \text{2-29}$$

$$H_2: u_A < x$$

The consequences of Type I and Type II errors for this hypothesis are as follows:

Type I error consequence	**Type II error consequence**
u_A is incorrectly determined to be less than x. Potentially invasive GM crop is released, possibly resulting in environmental problems.	The analysis fails to show that u_A is less than x. Potentially safe GM crop is not released, possibly resulting in financial losses.

Neither consequence is desirable. Failing to show that a crop is safe may mean that a perfectly safe crop is not being released. Failing to show that a crop is harmful may mean negative environmental effects. Within the context of an EBRA, the goal is ultimately to protect the environment, and therefore in the case above we might use a low value of α. This hypothesis test assumes that the product has a negative environmental effect until proven otherwise, which is consistent with the precautionary principle.

Alternatively, the research question may be whether two populations are different, or whether one population has a larger population mean than the other. The first case is a two-tailed hypothesis that tests whether the two populations are different. The second case is a one-tailed hypothesis that tests whether the two populations are different by some amount equal to x.

$$H_0: u_A - u_B = 0 \qquad \text{2-30}$$

$$H_A: u_A - u_B \neq 0$$

$$H_1: u_A - u_B \geq x \qquad \text{2-31}$$

$$H_2: u_A - u_B < x$$

Equation 2-31 is really no different from what has been described in equation 2-29. For equation 2-30, a Type I error would be determining that the populations are different when they are not. A Type II error would be failing to determine that the populations are different when they are. Essentially, for equation 2-30, the burden of proof is on proving that the populations are different from each other, and is made difficult if experimental power is low. An unfortunate consequence of failing to reject H_0 is that this is sometimes interpreted proof for H_0 being true (the populations are equal), which is not at all the case. Failing to reject H_0 does not prove that population A and B are equivalent ($u_A - u_B = 0$).

An equivalence test is more appropriate when the goal is to prove that two populations are equivalent. We restructure the hypotheses such that we can prove that it is likely that

$\frac{u_A}{u_B}$ falls between some set of predetermined values. For example, if we state that we will accept that population A and B are the same as long as $\frac{u_A}{u_B}$ is between $\frac{1}{1+x}$ and $1 + x$ then the hypotheses are:

$$H_1: \frac{u_A}{u_B} \leq \frac{1}{1 + x\%} \; or \; \frac{u_A}{u_B} \geq 1 + x\% \qquad 2\text{-}32$$

$$H_2: \frac{1}{1 + x\%} < \frac{u_A}{u_B} < 1 + x\%$$

A 1-2α confidence interval that lies between $\frac{1}{1+x}$ and $1 + x$ allows us to reject H_1 at the level of α (Schuirmann 1987). This is because the equivalence test takes on the form of two one-tailed tests at the level of α that must both pass in order to declare the populations equivalent.

Statistically significant differences and substantially important differences are not the same thing. In this document, statistically significant differences are differences between populations for which the probability of observing the data, or more extreme data, given the null or primary hypothesis is less than some α. Substantially important differences are differences that are biologically important. It is possible, due to low power, that biologically important differences between populations are not found to be statistically significantly different or that, due to extremely high power, statistically significant differences are found that are not biologically important. The biological importance of the measured effect size must be considered in spite of whether or not it has statistical significance. For example, a statistical test may not determine whether a population has 1% survival or has 2%

survival. However, if λ is highly elastic to survival, then the difference is biologically important as it represents a doubling in an important LLP.

2.4.2 Generalized Linear Mixed Models

Generalized linear mixed models (GLMMs) are a relatively new form of statistical analysis that relax the traditional ANOVA assumptions for distribution and allow for both fixed and random effects (Bolker et al. 2008; Stroup 2014). Instead of assuming a normal distribution, or transforming data to produce a normal distribution, GLMMs allow the user to choose an appropriate link function and distribution that best fits the data. Poisson, negative binomial and binomial family distributions are available in SAS (GLIMMIX) and R (lme4). GLIMMIX uses the Satterthwaite approximation (which we used) as a default method for calculating P-values, however other more computationally intensive methods like the Monte Carlo Markov Chain (MCMC) are also available.

2.4.3 Bootstrap

The bootstrap is a resampling procedure capable of estimating confidence intervals and standard error from data whose distribution is unknown (Efron and Tibshirani 1986). A core assumption is that the data are representative of the population. The data are resampled many times, with replacement, following as closely as possible the original experimental design. Confidence intervals may be calculated from the resultant bootstrap distribution as percentile or bias corrected depending on the skewness of the distribution.

2.4.4 Bias and Variance

Bias is the difference between an estimated parameter and the true population parameter. It is useful in demographic analysis to have a population act as a reference or standard when estimating population parameters like λ, but it is not possible to reliably determine how biased the estimates may actually be. That is to say we can only ever estimate population means from our sample data but we can't ever know the true population means without a census of the statistical population. Historical data regarding the propensity of a reference species to be weedy or invasive could be useful for corroborating the accuracy of experimental estimates. Variance is represents the spread of the observed data and can be quantified empirically in a demographic analysis.

2.4.5 Seed Sample Size Calculation

Given a probability x for some event (germination or survival) to occur, we can calculate the number of individuals (seeds or plants) that will be required for us to witness at least one event with probability P.

$$1 - x = probability\ of\ some\ event\ (death\ in\ this\ situation)$$

$$x = probability\ of\ survival$$

$$n = number\ of\ seeds$$

$$1 - P = probability\ of\ observing\ all\ seeds\ dying$$

$$P = probability\ of\ seeing\ at\ least\ one\ seed\ survive$$

$$1 - P = (1 - x)^n \qquad 2\text{-}33$$

We can solve for *n* with the following equation

$$\frac{\log(1 - P)}{\log(1 - x)} = n \qquad 2\text{-}34$$

P is a value chosen by the researcher and x can be estimated from the literature, calculated using a pilot study, or simply assumed to be some value. For example, if we want a 95% chance of seeing at least one seed survive, and we believe that the survival rate is 1% then we would want to plant:

$$\frac{\log(1 - 0.95)}{\log(1 - 0.01)} = 298.07\ seeds$$

In general, if a survival rate is $\frac{1}{s}$ then we should plant approximately $s * 3$ seeds if we want a 95% chance of observing at least one seed survive.

2.4.6 Confidence Interval Calculation for Zero Frequency Proportions

In cases where the observed proportion is equal to zero, confidence intervals can still be calculated using 'the rule of three'(Eypasch 1995; Hanley and Lippman-Hand 1983). The rule of three states that a reasonable approximation to the upper 95% confidence interval can be calculated as $\frac{3}{n}$ for zero-value proportions as long as $n > 30$.

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Chapter 3: Experimental design and analysis considerations when using population matrix modeling to assess and compare the invasive potential of genetically modified crops with conventional cultivars in agricultural and non-agricultural environments.

3.1 Introduction

Prior to the release of genetically modified (GM) crops, Canada, like other countries, mandates that crop fitness (weediness, invasiveness) must be quantified relative to conventional (comparator) cultivars to determine if they are substantially equivalent (Canadian Food Inspection Agency 2013). Traits currently under development that may impact fitness and invasiveness include tolerance to abiotic and biotic stresses as well as increased yield (Canadian Food Inspection Agency 2015; USDA/APHIS. United States Department of Agriculture, Animal and Plant Health Inspection Service 2016). Field trials to assess the fitness effects of new traits must be conducted under confinement where seed and pollen-mediated gene flow from experimental GM crops is reduced to near zero (Canadian Food Inspection Agency 2011). Only isolated, small-scale experiments are permitted to limit the risk of inadvertent escape. Following release, mandated post release monitoring is minimal (Beckie et al. 2010); after release, GM crops may rapidly become widely distributed, affording high propagule pressure (Colautti et al. 2006). Costs of eradication of GM crops are estimated to be high (Ryan and Smyth 2012), and would be expected to increase if the GM crops prove to be invasive. Methodology to assess GM crop fitness must be sufficiently rigorous to compare crop fitness in different environments, including agricultural fields, ruderal and natural areas where repeated planting or

inadvertent seed spillage may occur, in all agro-climatic regions of Canada, and account for annual stochasticity that could affect invasiveness. Our goal is to develop field-based methods, suggest experimental design considerations and assessment-endpoint criteria to measure and compare the fitness of GM crops in agricultural and non-agricultural environments.

Population demographic modeling is an established method of assessing and comparing population fitness by estimating population growth rates (λ) (Caswell 2001a). Population fitness can be estimated by the deterministic population growth rate, which is a function of survival and fecundity, parameters that hereafter may be referred to as 'lower level parameters' (LLP) (Caswell 2001b). In addition, demographic modeling provides the analytical tools of prospective (Caswell 2001c) and retrospective (Caswell 2001d) analysis that are crucial in identifying LLP with the potential to have a large influence on λ and that are also responsible for the observed variability in λ. In ecology, demographic models are used to help make informed decisions with regards to pest management and conservation problems (Davis 2006; Matlaga and Davis 2013; Servanty et al. 2014). A demographic approach has been used to evaluate canola invasiveness in Europe (Claessen et al. 2005; Crawley et al. 1993; Garnier et al. 2014; Lavigne et al. 2004), and demographic modeling has been proposed as a method of examining persistence and invasiveness of GM crops (European Food Safety Authority 2014), as part of a tiered risk assessment (Garcia-Alonso et al. 2006; Raybould 2007; Raybould and Cooper 2005).

For demographic modeling to be useful in environmental risk assessment there should be an explicit value that determine the safety of the GM crop: an assessment endpoint (Wolt et al. 2010). An agreed upon hypothesis structure for assessing and comparing population parameters is needed. An assessment endpoint of $\lambda < 1$ ($log\lambda < 0$) indicates a decreasing population while $\lambda \geq 1$ ($log\lambda \geq 0$) indicates a persistent or increasing population, a prerequisite for population growth and invasiveness. In this case using the classic 1-tailed hypothesis structure of

$$H_0\colon\ log\lambda\ \geq 0 \qquad \text{3-1}$$

$$H_A\colon log\lambda < 0$$

is consistent with the precautionary principle. If the 1-2α confidence interval overlaps with 0 or any value greater than 0, then we fail to reject the null hypothesis that the species has the potential to be weedy or invasive at α (Zar 2010). If the 1-2α confidence interval does not overlap with 0 or any value greater than 0, then we may reject the null hypothesis and accept the alternative as being true at the α level. Rejecting the null hypothesis when it's true is a Type I error, and is controlled directly by the analyst (α). Failing to reject the null hypothesis when the null hypothesis is false is a Type II error. The consequence of a Type I error would be incorrectly determining that a species has a negative population growth rate, in other words a species may be deemed to lack the capacity to be weedy or invasive when in fact the species has the potential to be weedy or invasive. Because we exert direct control over the Type I error rate through our choice of α value, if we consider the consequence of Type I error to be unacceptable we may lower α. A Type II error would be

failing to determine that the species has a negative population growth rate (not weedy/invasive) resulting in a delayed release for the species or never releasing the species at all (financial loss/production loss). After data has been collected, the only tool available to us for reducing Type II error is to increase α, thereby increasing Type I error. Most tools for decreasing Type II error lie in experimental design (planning for one-tailed tests, increasing replication, using designs that increase power like paired experiments or blocking). The burden of scientific proof lies on proving that the species is safe, and insufficient experimental power will make this more difficult due to highly-variable parameter estimates.

Comparing two populations to determine if they are substantially equivalent is more difficult because the assessment endpoint is not as clear, especially under the guideline of 'substantial equivalence'. Typically the hypotheses for population comparisons are set up as

$$H_0: \lambda_1 - \lambda_2 = 0 \qquad \text{3-2}$$

$$H_A: \lambda_1 - \lambda_2 \neq 0$$

In equation 3-2, if the 1- α confidence intervals for the difference of the means overlaps with zero, then we fail to reject the null hypothesis at the level of α. Failing to reject the null hypothesis does not mean that the null hypothesis is true, and does not mean that the alternative hypothesis is false. Instead it could indicate a lack of experimental power stemming from a small absolute effect size, large variation in the sample, low α, and/or low

experimental replication resulting in wide confidence intervals. In equation 3-2, the consequence of a Type I error is incorrectly determining that $\lambda_1 \neq \lambda_2$, and therefore incorrectly determining that λ_1 is less than or greater than λ_2. The consequence of a Type II error is incorrectly determining that $\lambda_1 = \lambda_2$, that there is no difference between the two populations when in fact a difference does exist. The burden of scientific proof lies in proving that the species are different from each other (rather than equal) and will be more difficult if there was low experimental power. Since the proponents who conduct the research may have a vested interest in release of a GM crop, this approach could encourage superficial investigation and erode public confidence.

Equation 3-2 is inconsistent with the precautionary principle, and there are two ways to approach this issue: First, absolute effect size should be the primary consideration, especially when precision is low (Sullivan and Feinn 2012). While the P-value is a useful tool in hypothesis testing, it is not the be all and end all of statistical analysis, and says nothing about the probability of a hypothesis being true. The absolute effect size, that is the actual observed difference between the population means, should be analyzed within the context of biological importance whether considering differences in λ or LLP. For example, if two species differ in their fecundity by 50% but the P-value is not significant at some predetermined α, then we should still investigate if the difference in fecundity is substantial enough to be biologically important regardless of whether the result was statistically significant. The elasticity of λ to fecundity should be considered in addition to the observed difference in fecundity. Alternatively, we can test for equivalence:

$$H_1: \frac{\lambda_1}{\lambda_2} \leq \frac{1}{1+x} \; or \; \frac{\lambda_1}{\lambda_2} \geq 1 + x \qquad 3\text{-}3$$

$$H_2: \frac{1}{1+x} < \frac{\lambda_1}{\lambda_2} < 1 + x$$

In equation 3-3, H_1 assumes that the two populations are not equal, that they are different by $\pm\, x$. A Type I error in this example would be incorrectly determining that the difference between the two populations is less than x. A Type II error would be failing to reject H_1, that the two populations differ by at least x when in fact they differ by less than x. This is on par with the precautionary principle: the populations are assumed to be different by some amount until proven otherwise; the burden of scientific proof lies in proving equality rather than difference. For equivalence tests, if a $1 - 2\alpha$ confidence interval lies entirely between $\frac{1}{1+x}$ and $1 + x$,then we may reject H_1 in favor of H_2 at the level of α (Schuirmann 1987). One could argue that a weakness of this approach is that it requires some acceptable assessment endpoint; an acceptable maximum difference between the populations must be determined *a priori*. It's true that this is a weakness, but it's not a weakness that's unique to equivalence testing. A sample size calculation should be required *a priori* to determine the number of replicates necessary to achieve the desired level of experimental power given an expected or biologically important absolute effect size. And even if a power analysis is not done *a priori,* the confidence interval for the difference of the means must still be considered post hoc within the context of effect size and precision. A 95% confidence interval for the difference between population means with a lower bound of -0.1 and an upper bound of 100 technically fails to reject the null hypothesis in equation 3-2, but is

such a confidence interval really narrow enough (precise enough) to make important management decisions? What is an acceptable effect size?

In an attempt to simplify the issue, we list the possible assessment endpoint scenarios based on the population growth rates for a comparator and GM cultivar (Table 3-1). In scenario 1, we would have evidence that both populations have negative population growth. Any difference between the populations would result in a difference in persistence, but both populations are predicted to be ephemeral and therefore low risk. Nevertheless, if an increase in persistence is considered a problem, then an assessment endpoint based on time to extinction could be used, and desired number of years until extinction should be agreed upon. Quasi-extinction estimates are suitable for estimating time until extinction and are part of a stochastic analysis (Caswell 2001e). In scenario 2, the GM cultivar has a positive population growth rate ($\log \lambda > 0$)whereas the comparator cultivar has a negative population growth rate ($\log \lambda < 0$). This is sufficient information to delay or halt the release of the GM cultivar: the available data have failed to prove substantial equivalence regardless of what a statistical test may suggest due to the biological significance of shifting from negative to positive population growth, and therefore the GM crop should be classified as high risk. In the third scenario, the comparator cultivar has a positive population growth rate whereas the GM cultivar has a negative population growth rate. Like in scenario 2, the data would fail to prove equivalence. However, the data would support the hypothesis that the GM cultivar is less weedy than the comparator, and therefore low risk. In the fourth scenario, both cultivars would have a positive population

growth rate. This is a particularly difficult scenario and may require more complex considerations to determine a reasonable resolution to the problem, especially under the paradigm of substantial equivalence. Lenient guidelines may dictate that the GM cultivar must be equivalent to the comparator cultivar within $x\%$, however determining an acceptable level of x may not be straightforward. Much stricter guidelines might propose that the GM cultivar may not have a greater population growth rate than the comparator,

$$H_0: \lambda_1 - \lambda_2 \geq 0 \qquad \text{3-4}$$

$$H_A: \lambda_1 - \lambda_2 < 0$$

leading to a much easier 1-tailed hypothesis test (equation 3-4) with a clear assessment endpoint at the expense of substantial equivalence.

Comparisons of population growth rates answer questions relating to whether population growth rate is different, but do not address why the differences occur or which LLP might influence population growth in the future. Elasticity and LTRE analyses may be used to further investigate LLP that can cause (elasticity) (Caswell 2001c) or did cause (LTRE) (Caswell 2001d) differences in population growth rates. If the population growth rate is highly elastic to a LLP, then large changes made to the LLP with cause proportionally large changes to the population growth rate. If a crop or weedy relative is highly elastic to changes in a particular LLP, then genetic modifications that affect that LLP may have predictably large effects on the population growth rate. It's often the case that vital rates or LLP to which population growth rate is highly elastic are also not extremely variable in

nature (de Kroon et al. 2000), however, the tools of genetic modification may introduce new variability. Alternatively, LTRE analysis is used to investigate differences in population growth rates between different populations or treatments and decompose those differences into contributions from the LLP. Used in conjunction, these analytical tools can help identify differences in the contributions made to population growth between a GM and comparator cultivar and then use these contributions to estimate effects in other species or environments as long as the elasticities are known.

Experiments using GM crops have constraints that limit their size and scope. Pre-release GM crops can only be grown outside under confinement protocols that limit the size and location of experimental plots and thus the number of seeds/individuals that can be assessed. Secondly, populations of pre-release GM crops do not exist in ruderal or natural areas and may be difficult to establish. These constraints generate labor-intensive experiments that, as a result, have minimal treatment replications and low precision for the estimated population parameters. Therefore, to test our experimental design, we used a selection of species that are both important in agriculture and offer a wide range of survival and fecundity. We chose three crop species, canola (*Brassica napus* L.), camelina (*Camelina sativa* L.), wheat (*Triticum aestivum* L.) and the weed species kochia (*Kochia scoparia* L. Schrad.) that ranged in ability to increase and persist in agricultural, ruderal and natural environments.

Canola is an annual crop, genetically modified for herbicide resistance and modified oil, grown in 7.7 million ha in western Canada in 2014. It was the 14th most abundant weed in

western Canada (Leeson et al. 2005), and weediness was influenced by high fecundity, pre- and post-harvest seed loss (>8,000 seed m^{-2} (Haile et al. 2014)), and secondary inducible dormancy (Gulden et al. 2003). Due to seed spillage, canola is frequently found along roadsides (Yoshimura et al. 2006), but is not known to be invasive of native areas (Beckie and Owen 2007). In the current study, an open-pollinated (OP) and hybrid variety were compared; open-pollinated varieties produce fewer and smaller seeds, are generally less robust (Elliott et al. 2008) and are less competitive as seedlings than hybrid cultivars (Brandt et al. 2007).

Camelina is a small-seeded oilseed with high fecundity (Table 3-2) and little seed dormancy (Francis and Warwick 2009; Walsh et al. 2013) that is being developed as a genetically modified crop (Iskandarov et al. 2014). Volunteers are abundant in fields following production, but limited in persistence (Elliott et al. 2008; Walsh et al. 2013). Davis et al. (2006) reported that camelina had a low population growth rate in rangeland areas and was unlikely to be invasive. However, camelina has been documented as occurring as a ruderal species (SL Martin, personal communication).

Wheat has relatively low fecundity (Table 3-2) compared to the other species, has transient primary dormancy (after ripening) (Gerjets et al. 2010) and limited seed persistence (Nielson et al. 2008). It is also a common weed in agricultural fields following cultivation, being the 12th most abundant weed in western Canada (Leeson et al. 2005). Despite being widely distributed through seed spillage, wheat has not been considered a weed of concern in ruderal or natural areas.

The weedy species kochia is common in western Canada with high potential seed production (Table 3-2) widely adapted to agricultural and ruderal land, and is invasive in native areas (Friesen et al. 2009). It is cold tolerant as a seedling, emerges early in spring (Al-Ahmadi and Kafi 2007), is highly competitive and tolerant of drought and salinity. Invasiveness is aided by wind-mediated seed dispersal (Friesen et al. 2009). Kochia has increased in area over the last 30 years to become the 10th most abundant weed in agricultural fields (Leeson et al. 2005) and is common in ruderal areas.

Our goal is to develop field-based methods and suggest experimental design considerations and assessment criteria to measure and compare the fitness of GM crops in agricultural and non-agricultural environments. We estimated and compared the LLPs of four species and two cultivars at as single site (Alberta), grown at two densities and in three disturbance regimes. We describe the growth rate λ, elasticity of λ to the LLPs, and contributions to λ from the LLP (hybrid and OP canola). We discuss the inherent differences between using null hypothesis tests and equivalence tests, and demonstrate why those differences are important for EBRA. We provide a comparison example using a scenario-based assessment method that attempts to simplify the process of hypothesis testing. Finally, we discuss the utility of experimental design for confined release trials and consider appropriate seed sample sizes (seeds per replicate) based on survival, germination, and fecundity.

3.2 Materials and Methods

3.2.1 Site choice and establishment

Experiments were conducted in 2011-2014 in five Canadian locations chosen in key agro-ecological regions (Figure 3-1), and were established in three adjacent disturbance regimes at each location: high disturbance (agricultural); intermediate disturbance (ruderal); and low disturbance (natural), appropriate for each region. The St. Albert, AB site belongs to the subhumid Parkland region, and is prairie (Ecological Stratification Working Group 1995). The Kenaston, SK site belongs to the semiarid Grassland region, and is prairie (Ecological Stratification Working Group 1995). The J.C. Chapais, QB site belongs to the St. Lawrence Lowlands, and is Mixedwood plains (Ecological Stratification Working Group 1995). The Harrow, ON site belongs to the Lake Erie Lowland, and is Mixedwood plains (Ecological Stratification Working Group 1995). The British Columbia site is not included in the analysis to do experimental problems. Within a disturbance regime 4 blocks were setup in a split-plot design. Each block contained both cultivars (two main plots within each block). Density was a sub-plot within the cultivar plots. Separate blocks were established for the spring and fall experiments. Soil was sampled at 15 cm at each site and analyzed for organic matter, pH, electrical conductivity, and texture (Table 3-3); however the study described in this chapter only includes the Alberta site. Average monthly precipitation and temperature data was acquired for the closest weather station (Table 3-4, 3-5).

Four annual species, canola (*Brassica napus,* cultivars Barrier and Dekalb 7345), camelina (*Camelina sativa,* cultivar Calena), and wheat (*Triticum aestivum,* cultivar AC Superb) and one weedy species kochia (*Kochia scoparia*) were included in the study to provide a wide range of survival, fecundity, and population growth values. The biology of these species is familiar (Nap et al. 2003). Two cultivars of canola were used, an OP variety (VT Barrier) and hybrid variety (Dekalb 7345), both contained the Roundup Ready® trait. Seed increases for canola (VT Barrier), camelina and wheat were conducted annually at the Ellerslie research station (Edmonton, AB). Seed for kochia was grown at Scott, Saskatchewan to provide uniform seed quality for all locations. Seed for Dekalb 7345 was provided annually by Monsanto Canada Inc. (900 Research Road, Winnipeg, Manitoba).

To ensure sufficient individuals for evaluation through the annual life cycles, two experiments were established per year at each location and disturbance regime, one initiated in the fall to assess fall germination (g_f), fall seedling survival overwinter (σ_f), and over winter seed survival and subsequent recruitment (σ_w), and one in the spring to assess other seedling survival to maturity (σ_{sdl}) and fecundity (Φ) (Table 3-6). Prior to fall seeding, the high disturbance treatment received fall tillage. Prior to spring seeding plots, the agricultural disturbance areas received a glyphosate application, the ruderal disturbance area was lightly scraped to expose soil, and natural disturbance areas were mowed to facilitate seed placement. The agricultural disturbance treatment was hand-weeded to maintain a low-competition environment. Sites were protected with snow fencing or exclusion cages to limit destruction due to wildlife.

To enable precision planting, all seeds but wheat were glued to plastic toothpicks using Elmer's water-soluble white glue. Seeds were planted using drilled plexi-glass templates to ensure uniformity. Plots were $0.5m^2$, however the total area planted within the plots was $0.125m^2$. High-density plots contained 80 seeds (640 seeds m^{-2}) and low-density plots contained 24 seeds (192 seeds m^{-2}). Fall-initiated experiments were only seeded at the high-density (assuming that seed germination and emergence were not density-dependent), and the seeds were placed on the soil surface to emulate dispersal at harvest. Seeds were planted at two densities in the spring and were inserted 1 cm below the soil surface except for wheat that was planted at a depth of 2 cm.

Seedlings were counted in fall-seeded plots weekly after initiation until being covered by snow and then again in the spring every week until new seedlings stopped emerging for two consecutive weeks. New seedlings were counted in the spring-initiated plots every week for 1 month and then biweekly until harvest. Individual plants were harvested and seeds threshed and counted to determine seed production per plant. Seed viability tests were performed on seed, pooled by sub-plots using 3 replicates of 100 seeds per sub-plot, or as much as was available if there was less than 300 seeds within a sub-plot. In both experiments in Alberta cohorts of seedlings emerging in the same week were identified using coloured rings placed around the seedlings. The rings were removed upon plant death.

3.2.2 Estimation of Lower Level Parameters: Survival and Fecundity

Calculations for LLP are available in Table 3-7. Fall recruitment was measured as the proportion of seeds planted in the fall that were recruited in the fall (g_f). Overwinter seedling survival (σ_f) was measured as the proportion of fall seedlings that survived until the spring. The overwinter survival rate (σ_w) was measured as the proportion of seeds remaining in the seedbank after fall recruitment that were recruited in the spring. Fecundity (Φ) was calculated as the average number of seeds produced per mature plant per plot. Seedling survival (σ_{sdl}) is the proportion of seedlings in the spring that survive until reproductive maturity in the fall. Seed survival from the spring to the fall within the seed bank (σ_b) is the proportion of viable seeds in the spring and survive until the fall. σ_b was not measured experimentally so appropriate values were selected based on the literature (Table 3-2). Primary and secondary seed dormancy, along with recruitment influences the ability of a species to survive in the summer seed bank. None of the species had primary dormancy, and only canola has inducible secondary dormancy.

3.2.3 Matrix Model

The life cycle of an organism is depicted by a life cycle graph that is isomorphic to the population projection matrix $\boldsymbol{A}$ (Caswell 2001f). Demographic modeling is based on the difference equation

$$n_{t+1} = \boldsymbol{A} n_t \qquad 3\text{-}5$$

where $\boldsymbol{A}$ is the population projection matrix of size $i\ x\ i$ populated by vital rate (survival and fecundity) data, n_t is the population vector of size $i\ x\ 1$ at time t and n_{t+1} is the population vector of size $i\ x\ 1$ at time $t+1$

For annual species, periodic matrix models are recommended because they can include as much within-year data as is available as well as a seed bank to explore between-year dynamics (Caswell 2001g). A periodic matrix $\boldsymbol{A}$ is made from submatrices $\boldsymbol{B}$ that divide a one year time step into as many steps as are desired.

$$n_{t+1} = (\boldsymbol{B}_{(h-1)} \dots \boldsymbol{B}_1 \boldsymbol{B}_m \dots \boldsymbol{B}_{h+1} \boldsymbol{B}_h) n_t \qquad 3\text{-}6$$

where

$$h = 1, \dots, m \qquad 3\text{-}7$$

and each sub-matrix $\boldsymbol{B}_i$ $(1 \leq i \leq m)$. The $\boldsymbol{B}$ submatrices were populated by LLP, which were measured from field and harvest counts and take the form proportions (survival) and rates (fecundity). The LLP are equivalent to the vital rates of a stage-structured $\boldsymbol{A}$ matrix. The matrix $\boldsymbol{A}$ discussed here is simple and is made up of $\boldsymbol{B}$ submatrices that can be multiplied together in such an order that we are able to express λ explicitly with a single equation

$$\boldsymbol{A} = \lambda = \underbrace{(1-g_f) * (1-\sigma_w) * \sigma_b}_{Term\ 1} + \underbrace{(1-g_f) * \sigma_w * \sigma_{sdl} * \Phi}_{Term\ 2} + \underbrace{g_f * \sigma_f * \sigma_{sdl} * \Phi}_{Term\ 3} \qquad 3\text{-}8$$

For Alberta this equation can further be simplified

$$\lambda = \underbrace{(1-\sigma_w)*\sigma_b}_{Term\ 1} + \underbrace{\sigma_w * \sigma_{sdl} * \Phi}_{Term\ 2} \qquad 3\text{-}9$$

because we did not observe any fall recruitment in Alberta. For this paper we will deal with equation 3-9. Term 1 and Term 2 in equation 3-9 represent two pathways by which individuals can achieve genetic representation in future years. Term 1 is the underground pathway and is characterized by seeds surviving in the seedbank from one year to the next. Term 2 is the above ground pathway characterized by the recruitment of seedlings in the spring and fecundity in the fall. This equation differs from that used by Davis and Liebman (2003) and Davis et al. (2003) because σ_w in this study conflates overwinter survival and subsequent spring seedling recruitment.

3.2.4 Elasticity analysis

Elasticity analysis is a type of prospective analysis that calculates the relative change in a population parameter (like λ) with respect to a small, relative change in a vital rate and is given by

$$\boldsymbol{E} = \frac{a_{ij}}{\lambda}\frac{\partial \lambda}{\partial a_{ij}} \qquad 3\text{-}10$$

(Caswell 2001c). Through application of the chain rule elasticity of the population parameter to a LLP may be calculated as

$$\frac{\boldsymbol{x}}{\boldsymbol{\lambda}}\frac{\boldsymbol{\partial \lambda}}{\boldsymbol{\partial x}} = \frac{x}{\lambda}\sum_{i,j}\frac{\partial \lambda}{\partial a_{ij}}\frac{\partial a_{ij}}{\partial x} \qquad 3\text{-}11$$

3.2.5 Life Table Response Experiment

The role of a Life Table Response Experiment (LTRE) is to decompose the differences observed in a population parameter into contributions from the LLP (Caswell 2001d). Contributions are a function of the difference between LLP and the sensitivity of the population parameter to those LLP.

$$\lambda^m \approx \lambda^r + \sum_{i,j}\left(a_{ij}^{(m)} - a_{ij}^{(r)}\right)\frac{\partial\lambda}{\partial a_{ij}} \mid \boldsymbol{A}^{\dagger} \qquad 3\text{-}12$$

where

$$\boldsymbol{A}^{\dagger} = \frac{\boldsymbol{A}^m + \boldsymbol{A}^r}{2} \qquad 3\text{-}13$$

and $\boldsymbol{A}^{\boldsymbol{r}}$ is a reference matrix calculated as the average of all the treatment matrices (Caswell 2001d).

3.2.6 Data Analysis

The analysis of the LLP was conducted using PROC GLIMMIX in SAS (ver. 9.4 SAS Institute, Inc, Cary, NC). Survival values were estimated using a logit link function and assuming that the response variable (survival values) followed a binomial distribution (family=binomial). Fecundity values were estimated using a log link function and assuming that the fecundity response variable followed a negative binomial distribution (family=negative binomial). We modeled the fixed effects as

$$y \sim intercept + species + density + species * density + error \quad 3\text{-}14$$

for the spring experiment values and

$$y \sim intercept + species + error \quad 3\text{-}15$$

for the fall experiment. For the fall survival values 'block' was the only random effect included. For the spring survival values 'block' and 'block*species' were both included as random effects to account for the density effect being nested within the species effect. For the survival values the total number of individuals per plot at time t+1 was taken as the numerator and the total number of individuals per plot at time t was taken as the denominator. For the fecundity analysis the number of seeds produced per plot was used as the response variable and the number of mature plants within the plot (log transformed) was used as an offset. Demographic information is most useful when it is site-specific, especially when the sites have different climates. Demographic information averaged over sites is potentially misleading, a species may have an overall low population growth rate but still be weedy or invasive in one or more locations. Therefore, data were analyzed within site-years. Multiple comparisons tests were done using the lsmeans function in SAS with a Bonferroni correction.

Estimates for population growth, elasticity, and LTRE contributions were calculated directly from the data, and 95% confidence intervals were produced using a bootstrap procedure in R. A population of 16 replicates (4 fall, 4 spring) was created for the bootstrap sampling procedure and each bootstrap replicate consisted of drawing four of the

population replicates (with replacement), and taking the average of the plant/seed count values to calculate population growth rates and elasticities. 2000 bootstrap replicates were generated in this fashion, and percentile 95% confidence intervals were calculated. Bias-corrected confidence intervals did not seem necessary as the differences between the mean and median were not large, as estimated from the $\boldsymbol{z}$ score (data not shown) (Caswell 2001h).

Differences between population growth rates between hybrid canola and OP canola were estimated in a similar fashion as above, but are different in a few aspects. First, a data frame was created by column-merging hybrid canola and OP canola data by year, site, disturbance and block, thus creating a population of 64 replicates. This was done to ensure that the blocking factor remained intact so as to faithfully follow the field design. Each bootstrap replicate consisted of drawing four of the population replicates (with replacement) and then calculating the difference and the ratio of the population growth rates for hybrid and OP canola. 2000 bootstrap replicates were generated in this fashion, and percentile 95% confidence intervals were calculated.

3.3 Results and Discussion

Sites were chosen to maximize differences in climate in agricultural areas across Canada, and therefore LLPs were analyzed within site. LLPs were analyzed within sites and years due to heterogeneous variances and within disturbance due to a lack of replication of disturbance within sites. Data availability for LLPs differed between sites, years and disturbance regimes ostensibly because the true population values for σ_w and σ_{sdl} were

lower than what could be reasonably detected given the number of seeds planted. The Alberta site had the most consistent and complete data acquisition of LLPs and, for the purpose of critically evaluating methodology, is described in detail below.

3.3.1 Overwinter survival (σ_w), Alberta

σ_w was measured (non-zero values in at least one replicate) in 12 of 15 treatment combinations (species and year) for the high (agricultural) disturbance regime, 6 of 15 treatment combinations for the medium (ruderal) disturbance regime, and 13 of 15 treatment combinations for the low (pasture) disturbance regime (Table 3-8). In the high disturbance regime, 'species' was a significant source of variation in 2011 (P<0.001) and 2012 (P=0.011) but not in 2013 (P=0.60). In 2011, camelina σ_w was approximately 10-fold higher (0.47) than the average of other species (0.045). In 2012, hybrid canola and wheat (σ_w 0.05) were not significantly larger than camelina and kochia (σ_w 0.015). However, OP canola (σ_w 0.10) was approximately 6.6-fold higher than that of camelina and kochia. In 2013, only OP canola and camelina had non-zero values and were not significantly different from each other (P=0.60).

In the medium disturbance regime, species were significantly different in 2011 (P<0.001) and in 2012 (P=0.037), but not in 2013 (P=0.67). In 2011 camelina (σ_w 0.32) was approximately 7-fold higher than kochia and wheat (σ_w 0.05). Hybrid canola and OP canola failed to germinate in all replicates. In 2012, OP canola (σ_w 0.06) was approximately 6-fold larger than wheat (σ_w 0.01). Hybrid canola, camelina and kochia failed to germinate in all

replicates. In 2013, hybrid canola and OP canola were not significantly different, with the remaining species failed to germinate.

In the low disturbance regime, species were significantly different in 2011 (P<0.001), 2012 (P=0.026), and in 2013 (P<0.001). In 2011, camelina (σ_w0.51) was approximately 13-fold larger than the average all the other species (σ_w0.04). In 2012, hybrid canola, camelina and wheat were not statistically different from kochia or OP canola, but OP canola was approximately 6-fold larger than kochia. In 2013, camelina $\sigma_w$was 9.5-fold larger than that of hybrid canola and kochia. OP canola and wheat failed to germinate.

Factors that affect $\sigma_w$include seedling death (fatal germination of seedlings that failed to survive to spring, disease, loss of viability, and seed predation) and failure to germinate due to dormancy or microsite limitations. At the Alberta site, no fall-germinating seeds were observed in any year. A delay in germination may have been influenced by seed attachment by glue to toothpicks. In greenhouse trials for all species, glued seeds had a significantly larger time to 50% germination (t_{50}). While we assumed germination/emergence was not density-dependent, the presence of plant competitors may have influenced moisture and light regimes and thus recruitment. Seed predation was not measured directly and has been poorly quantified in Alberta. However, seed predation is a likely cause of the differences in overwintering survival between the high and medium/low disturbances regimes.

Most species had a low σ_w,with the exception of camelina, which had σ_w< 0.20 in only 4 of 15 treatment combinations in Alberta. Camelina seedlings have cold tolerance and in

some regions can survive as a winter annual, suggesting that camelina seedlings are less susceptible to cold than the other species that had overwinter survival (σ_w) of less the 0.10 in all treatments. Hybrid canola and OP canola $\sigma_w$were never significantly different from each other, but there was a trend that OP canola was greater than hybrid canola by 1.5-2 fold and this may be biologically significant.

3.3.2 Seedling survival (σ_{sdl}), Alberta

Seedling survival to maturity was measured for all treatment combinations, including high and low density in the high disturbance regime, 24/30 treatment combinations in the medium disturbance regime, and 19/30 treatment combinations in the low disturbance regime (Table 3-8). In the high disturbance regime, species was significant in 2011 (P<0.01) and 2013 (P<0.01) but not in 2012 (P=0.27). Density was significant in 2011 (P<0.001) and 2013 (P=0.035) but not in 2012 (P=0.13). There were no species-density interactions in 2011 (P=0.08) or 2013 (P=0.30), but there were in 2012 (P<0.01). In 2011, hybrid canola and kochia σ_{sdl}were not significantly different from camelina, wheat and OP canola, but camelina and wheat $\sigma_{sdl}$was 57% greater than OP canola. For all species, the high density treatment $\sigma_{sdl}$was 24% higher than the low density. There were larger differences in σ_{sdl}between the densities for wheat and hybrid canola than for the other species, but the interaction was not significant at P=0.05. In 2012, high density camelina and OP canola $\sigma_{sdl}$was higher than their low density counterparts. However, for the other species, the low density treatments showed higher survival. High density camelina $\sigma_{sdl}$was significantly larger than low density camelina (P<0.001). There were no other significant

differences. In 2013, OP canola, hybrid canola and kochia σ_{sdl}were not statistically different from wheat or camelina, but wheat survival was 4.5-fold higher than camelina. For all species, high density survival was 26% higher than low density survival.

In the medium disturbance regime, species was not significant in 2011 (P=0.16) but was significant in 2012 (P<0.001) and 2013 (P<0.001). Density was not significant in 2011 (P=0.65) or in 2012 (P=0.98), but was significant in 2013 (P<0.001). A species-density interaction was not significant in 2011 (P=0.95), 2012 (P=0.45), or 2013 (P=0.22). In 2011, hybrid canola and OP canola seedlings failed to survive to maturity in all replicates. In 2012, wheat and kochia $\sigma_{sdl}$was approximately 7-fold higher than other species. In 2013, OP canola and camelina $\sigma_{sdl}$was essentially zero, and there was no significant difference between the remaining species. When OP canola and camelina were removed from the analysis, there were no differences in σ_{sdl}between high and low density treatments.

In the low disturbance, regime species was not a fixed effect in 2011 because wheat was the only species whose seedlings survived to maturity. Species was not significant in 2012 (P=0.41), but it was significant in 2013 (P=0.018). Density was not significant in 2011 (P=0.085), 2012 (P=0.74), or in 2013 (P=0.99). There were no significant species-density interactions in any year. In 2013, wheat survival (σ_{sdl}0.23) was approximately 23-fold higher than hybrid canola (σ_{sdl}0.01). Low density camelina seedlings failed to survive to maturity.

Seedling survival to maturity excludes loss prior to recruitment and is influenced by microsites, seedling disease, predation, and inter- and intra-specific competition. In the

agricultural field, this stage is frequently influenced by herbicide and crop competition, excluded from these experiments. In the high and medium disturbance regimes with limited competition, all species were much less likely to survive from seed to seedling (σ_w) than from seedling to maturity (σ_{sdl}). However, in the low disturbance regime, species generally had the lowest σ_{sdl}, and only wheat survived until maturity in all years and densities.

3.3.3 Fecundity (ϕ), Alberta

Seed production was measured for all treatment combinations in the high disturbance regime, 17/26 treatment combinations in the medium disturbance regime where seedlings survived to maturity, and 13/19 treatment combinations in the low disturbance regime where seedlings survived to maturity (Table 3-8). In the high disturbance regime, species was a significant effect in 2011 (P<0.001), 2012 (P<0.001), and 2013 (P<0.001). Density was significant in 2011 (P<0.001), but not in 2012 (P=0.26) or 2013 (P=0.11). There was no species-density interaction in 2011 (P=0.74) or 2012 (P=0.35), but there was in 2013 (P=0.02). In 2011 kochia ϕ was 3688, 4.4-fold higher than OP canola (ϕ 1227) and camelina (ϕ 473) and 47-fold higher than wheat (ϕ 87). Hybrid canola fecundity (ϕ 1489) was 3-fold higher than camelina and 22-fold higher than wheat. OP canola and camelina fecundity was 11-fold higher than wheat. There was no significant difference between kochia and hybrid canola, hybrid canola and OP canola, and OP canola and camelina. Low density fecundity was 2.4-fold higher than ϕ in high density plots. In 2012, camelina fecundity (ϕ 1904) was 8-fold higher than kochia (ϕ 176), and 43-fold higher than wheat

(ϕ 53). Hybrid canola, OP canola and kochia fecundity was 11-fold higher than wheat. In 2013, camelina fecundity (ϕ 322) was 6-fold higher than all other species but kochia (ϕ 12). Camelina fecundity was not significantly different than kochia fecundity. All species but camelina had higher fecundities in the low density treatment than the high density treatment.

In the medium disturbance regime, species was not significant in 2011 (P=0.99), but was significant in 2012 (P=0.026) and 2013 (P=0.014). Density was not significant in any year. In 2012, camelina (ϕ 621) had 7.3-fold higher fecundity than hybrid canola (ϕ 24), and there were no other significant differences between the species. In 2013, wheat fecundity was essentially zero and there was no statistical difference between hybrid or OP canola.

In the low disturbance regime, fecundity was low in all years. In 2011, wheat was the only species to form viable seeds. Species was significant in 2012 (P=0.014), but was not significant in 2013 (P=0.97). Density was not significant in 2011 (P=0.45) or 2013 (P=0.98), but was significant in 2012 (P=0.012). Species-density interaction was not significant in any year. In 2012, hybrid and OP canola were not significantly different from camelina or wheat, but camelina was 2.3-fold higher than wheat. Overall, low density fecundity was 1.7-fold higher than high density fecundity.

Fecundity is influenced by resource availability throughout the plant life cycle. It was expected that fecundity varied by species in the high disturbance regime, as the maximum seed production of these species varies (Table 3-2). High disturbance areas had high

resource availability and were less limited by inter-specific competition. Density influences intra-specific resource availability, and density significantly affected fecundity in 2011, and there was a significant species-density interaction in 2013 where ϕ was higher for all species at low density, except for camelina. In medium and low disturbances, intra-specific competition becomes a more important limitation to ϕ. Camelina produced viable seed in the medium disturbance regime in 4/6 density years, consistent with reports of camelina populations persisting in ruderal locations in Alberta and Saskatchewan (SL Martin, personal communication).

3.3.4 Elasticity, Precision, and LTRE

Generally speaking, λ was highly and positively elastic to σ_w, σ_{sdl}, and ϕ (above ground parameters) in the high disturbance regime, while in the medium and low disturbance regimes, λ was highly and positively elastic to σ_b(below ground parameter). In the medium and low disturbance regimes, in some cases, λ was negatively elastic to σ_w, meaning that an increase in σ_w would result in a decrease in λ. Negative elasticities to σ_w imply that recruitment into the above ground pathway leads to a reduction in population due to low survival or fecundity in the above ground pathway. More specifically, λ was more elastic to the above ground parameters in highly productive environments (high disturbance) and years (2011, 2012), whereas λ was highly elastic to σ_b in low production environments (medium disturbance, low disturbance), and years (2013). In highly productive environments or years, each seed that germinates has a high probability of contributing many new individuals to the next population, so the best method of ensuring population

survival and growth is to germinate and reproduce. In terms of individual fitness, the best way for a seed to gain genetic representation in future generations is to germinate and produce new seed during highly productive years. Above ground LLP values for the high disturbance regime are largest in 2011, slightly smaller in 2012, and substantially smaller in 2013, with a corresponding decrease in their elasticity values demonstrating that the return on investment for germinating in the spring decreased from 2011 to 2013. In low production environments and years λ, is most elastic to σ_b, which represents the population's ability to persist in the seedbank to increase the probability that individuals will survive and experience a higher productivity year. In low production environments and years, the best way for an individual to gain genetic representation in future generations is to survive until a high production year; germinating during a low production year is the least likely way to achieve genetic representation in future generations. Therefore, traits that increase above ground LLP are predicted to cause small proportional increases in λ in low production environments if there is no corresponding decrease in σ_b, and traits that increase σ_b should cause proportionally large increases in λ in low production environments. Additionally, the interval between high production years within low production environments must be short enough that the population does not extinguish in the interim.

Statistical significance should not be conflated with biological significance, and in the case of LLPs, it is much more important to consider effect size in combination with elasticity to address substantial biological differences. The effect sizes between species LLP

were not always statistically significant even though they are relatively large and have high elasticity values. For example, in the high disturbance regime, OP canola consistently had higher σ_w values than hybrid canola (1.5-2 fold in 2011 and 2012), and additionally λ is highly elastic to changes in σ_w in those years (0.99). An elasticity of 1 (or close to 1) means that a 10% change in σ_w results in a 10% change in λ. A doubling in σ_w would result in an approximate doubling of λ. Highly elastic parameters measured with low precision may have a large potential influence on the estimated variability in λ,therefore, precise estimates of LLP to which λ is highly elastic could aid in reducing the variability in estimates of λ. For highly productivity environments and years, increasing the precision in measurements of σ_w, σ_{sdl}, and ϕ could increase the precision in the estimate of λ, while in low productive environments and years, increasing the precision in measurements of σ_b could increase the precision in the estimate of λ.

An LTRE analysis examining the LLP contributions to differences in λ between hybrid and OP canola reveals trends in contributions from σ_w and ϕ, but not σ_{sdl} (Figure 3-2). Differences in σ_w contributed more to the population growth rate of OP canola than hybrid canola in 6 of the 8 cases. These contributions were also generally quite large in comparison to the contributions from ϕ and σ_{sdl}. The exceptions were the low disturbance regime in 2011 where the overall differences were very slight, and 2012. A result of evaluating the sensitivities at $\boldsymbol{A}^{\dagger}$ occurred in the low disturbance regime in 2012: the contributions for hybrid canola and OP canola were both positive. While hybrid canola had a smaller value for σ_w than OP canola, the sensitivity of population growth to σ_w was

negative for hybrid canola but positive for OP canola, resulting in positive contributions for both cultivars. In the high disturbance regime ϕ contributed more to λ for hybrid canola than for OP canola, however the contributions were not as large in magnitude as those from σ_w and it isn't clear if this extends to the medium and low disturbance regimes. Lower σ_w and higher ϕ contributions may represent the effects of hybrid canola having larger seeds and more robust seedlings/plants than OP canola. Larger seeds may be more susceptible to predation over winter while more robust seedlings/plants leads to increased fecundity. Confidence intervals are intentionally left out because of their large size.

3.3.5 Population growth rate (λ), Alberta

Population growth rate was generally highest in the high disturbance regime and in 2011 while lowest in the low disturbance regime and 2013 for all species and densities (Table 3-9). Hypothesis testing followed the structure of equation 3-1 and the null hypothesis was tested using bootstrapped 95% confidence intervals. Failing to reject H_0 indicates that a species is potentially weedy or invasive at the planting density and within the disturbance regime. Populations in the high disturbance regime were much more likely to be positive ($log\lambda \geq 0$) than the medium and low disturbance regimes. All species-density combinations have positive population growth in the high disturbance regime in 2011 and 2012. In 2013, we failed to reject the null hypothesis that camelina and OP canola (both densities) had $log\lambda \geq 0$. In the medium disturbance, most species had population growth rates significantly lower than zero except for high and low density camelina and high density kochia in 2011, high density kochia in 2012, and high and low density hybrid

canola and high density OP canola in 2013. No species had positive population growth in the low disturbance regime. Camelina and kochia had the highest overall λ values, OP and hybrid canola had intermediate λ values and wheat had the lowest λ value.

3.3.6 Comparison assessment endpoints: An example using Hybrid and OP canola

Hypothesis test comparisons of λ between two populations is difficult because the assessment endpoint is not as clear. An acceptable absolute effect size has not been established. For a null hypothesis to be effective, an *a priori* power analysis should be done to estimate the number of replicates required to measure a biologically important absolute effect size with probability P. How do you calculate power without first determining the absolute effect size that you want to detect? Making determinations from an equivalence test requires that a value for x (relative difference) needs to be chosen. Table 3-10 shows bootstrap comparisons of hybrid canola and OP canola. Because the 95% confidence interval for the difference of the means (equation 3-2) always overlaps with 0, we fail to prove that the two populations are significantly different in any case, a conclusion that could be misinterpreted as meaning that the populations are equal. In many cases the variation was quite high (confidence intervals are large), suggesting that the experimental power was low. Alternatively, the hypothesis structure in equation 3-3, where x is the desired % equivalence, establishes a level of precision with which we might be comfortable and provides different, more nuanced results than the null hypothesis test (equation 3-2). The ratios of hybrid canola/OP canola show that the hybrid canola population growth rate is greater than the OP canola population growth rate in only 6 of 18 comparisons and

usually not by much. Of those 6 comparisons, hybrid canola λ is greater than OP canola λ by more than 50% in only 2 comparisons. Only 1 of those 2 comparisons (2013, medium disturbance, low density) occurs in a non-agricultural environment. Equivalence testing provides much more nuanced comparison, but both null hypothesis tests and equivalence tests must still consider absolute effect size and variance.

Alternatively, we can use Table 3-1 to classify hybrid and OP canola. If we treat the hybrid canola as our GM crop and OP canola as our comparator then the majority of comparisons are placed within scenario 1 (10 of 18, both populations are decreasing) or scenario 3 (2 of 18, GM is decreasing and comparator is increasing). While placement in scenario 1 does not prove substantial equivalence, it does suggest that the cultivars have a low risk of invasive potential in those environments. Placement in scenario 3 suggests that the GM may be lower risk but this would have to be verified with more data. 5 of 18 comparisons belong to scenario 4 (both populations are increasing), a higher risk category. However, 4 of these comparisons occur in the agricultural regime, which could be considered low risk. There are only two comparisons that might cause concern, and both occurred in the medium disturbance regime in 2013. Of those two comparisons, only one (2013, medium disturbance, low density) shows that hybrid canola has a larger λ than OP canola and is concerning because it falls into scenario 2 (GM population is increasing, comparator population is decreasing). Further analysis is required to determine the stochastic impact of this result. Overall, with the exception of the 2013 medium disturbance, low density treatment hybrid canola does not seem to be more

weedy/invasive than OP canola. However, our analysis falls short of pronouncing hybrid canola to be safe due to a high-risk result occurring in a non-agricultural environment (medium disturbance regime) in 2013.

3.3.7 Experimental design recommendations

GM crop confinement limits the methods by which we can achieve reasonably precise data while minimizing labor associated with data collection and containment because there are regulations that govern the total amount of seed that can be used as well as the locations and conditions under which the seed may be planted. Using the experimental methods described in this paper, it would be expensive and laborious to increase the number of experimental units. To increase the precision, we suggest a tiered approach wherein Tier 0 is a literature review of conventional crop invasiveness, and an initial dataset (Tier I) is collected from naturally occurring populations of non-GM plants, and then enhanced by comparative experiments (Tier II) using GM crops in confined areas. Naturally occurring populations of frequently grown crops are common, and an initial dataset for survival and fecundity can be populated by observations of these naturally occurring populations, be they weedy-relatives or crops (Tier III). From that dataset, elasticities may be calculated and important LLPs identified so that experiments using GM crops may focus limited resources on measuring LLPs to which population growth is highly elastic. Combining the elasticity information gained from the naturally occurring species with the comparative information in the confined agricultural experiments we could make estimates as to the population growth rate of the GM crops in the ruderal and natural areas.

Based upon the results of these experiments, there may be enough information to help support a proposal to move to unconfined environmental release. Finally, should initial results prove ambiguous, GM populations could be established and their population growth rate estimated (Tier IV).

In environmental biosafety risk assessments, increasing experimental power and decreasing the consequence of Type II errors should be a priority in the design of experiments regardless of whether you employ null hypothesis testing or equivalence testing. Based on Tier I and II information, the precision and assessment endpoint may be negotiated with regulators prior to confined field trials. Increasing the precision in the LLP will increase precision in estimates of λ, but if there is time and money to increase precision in only a few LLP, then it is sensible to focus on the LLP to which λ is highly elastic. Increasing confidence in the LLP would require an increase in the number of experimental units sampled, increasing α, and/or by using blocking factors.

The costs involved in conducting such a demographic experiment might be reduced by reducing the number of times populations are censused each year. Experiments initiated in spring could be simplified by identifying seedlings once and then revisiting at maturity. Methods should be supplemented with seed burial experiments established in the fall to measure overwinter survival and seed bank survival from the spring until the fall. Germination can be estimated by estimating the number of seeds per unit area in the seedbank in the spring and by recording the total number of seedlings per unit area. Selecting new individuals at each stage *i* and recording survival or fecundity at some later

stage *j* is better than following the same individuals through multiple stages because it can help ensure balanced sampling with less missing data. In the event that all of the individuals die before reaching their final stage *j*, there will be some measurements that cannot be taken.

In many cases, too few seeds were used to estimate survival parameters, especially σ_w, which caused problems for both the estimability of the parameter as well as in the variation of λ. In cases where we observed values of zero for σ_w in all replications within a treatment combination, we cannot say for certain that the value of σ_w was actually measured. For example, if we assume that the true value of σ_w for some species is 0.01, and we also assume that each seed germinates independently from one another, then the total number of seeds that we should plant to have a 95% chance of observing at least one seed germinate is:

$$(1 - P) = (1 - x)^n \qquad 3\text{-}12$$

$$\frac{\log(1 - P)}{\log(1 - x)} = n \qquad 3\text{-}13$$

$$\frac{\log(0.05)}{\log(0.99)} \sim 298 \qquad 3\text{-}14$$

Seed sample size estimates for the species in this experiment can be found in Table 3-11. Where x is the probability of not observing recruitment, and P is the probability of not observing recruitment after n seeds have been planted. In this example, it would require ~300 seeds to have a 95% chance of observing at least 1 germinate rather than the 80

seeds per replicate used in this study. Additionally, for a species like wheat, that has fecundities around 10^2 seeds per plant (Table 3-2), assuming a minimum σ_w value of 0.01 is reasonable: anything much lower than 0.01 should result in a negative $\log \lambda$ and therefore limited resources might be better used measuring other LLPs. However, for species like canola, camelina, and kochia that can have fecundities in the range of $10^3 - 10^4$ (Table 3-2) we would have to consider the possibility that σ_w could be much lower than 0.01 and yet generate populations with a positive $\log \lambda$. Zero values for σ_w in some, but not all replicates, also increases the variability in the measured LLP that in turn increases the variability in λ (simulated in R, data not shown). Use of the above equation could help reduce the chance of observing zero values, and therefore reduce the variability in λ.

Under the frequentist paradigm, it is difficult to discuss the probability of hypotheses, and therefore difficult to assess the probability of a hypothesis being true that states a species is either invasive or non-invasive. The 95% confidence intervals do not indicate that there is a 95% chance that the true population parameters are bound between the lower and upper limits. Instead, if we were to repeat this experiment very many, then 95% of our 95% confidence intervals would be bound around the true population parameter. As a result, low frequency events such as invasions are difficult to predict and involve unpredictable interactions between the receiving environment and the invading organism. If we are unlucky enough to measure population parameters in low production years and to miss the high production years, then it would be easy to miss a potentially invasive species. Instead, the receiving environment must be considered in terms of susceptibility to

invasion (invasibility) as well as intrinsic value. An environment rich with biodiversity is harder to invade than one with low biodiversity, and natural environments and environments containing rare species may be considered to have higher value than agricultural environments. A species that is weedy in agricultural environments might be considered less of a risk than one that is weedy in natural environments due to the intrinsic value placed on natural environments as well as the difference in control options between agricultural and natural environments. The risk of invasion must always be considered alongside the risk of invasibility. Actual prediction of invasions and invasive plants may fall under the Bayesian rather than the frequentist paradigm.

Our goal was to develop field-based methods and suggest experimental design considerations and assessment criteria to measure and compare the fitness of GM crops in agricultural and non-agricultural environments. To that end, we have described small-scale experimental methodology capable of measuring demographic data for GM crops. We suggested improvements to the design that should increase the ease of collecting data as well as the precision of the results. We demonstrated the differences between null hypothesis testing and equivalence hypothesis testing and discussed why those differences are important in the context of an EBRA. We introduced and used assessment endpoint scenarios and used it to show that hybrid canola is generally low risk relative to OP canola. However, stochastic simulations are required to supplement this analysis due to a single result that placed hybrid canola in a high-risk category.

Table 3-1. Four possible assessment endpoint scenarios based on the $\boldsymbol{log\lambda}$ of the GM crop and that of the comparator cultivar.

Comparator (λ_1)\GM(λ_2)	$\log\lambda_2 < 0$	$\log\lambda_2 \geq 0$
$\log\lambda_1 < 0$	1. Neither cultivar has a positive population growth rate. Differences will result in different rates of persistence but both populations are ephemeral.	2. The GM cultivar has a positive population growth rate whereas the comparator cultivar does not. This should be enough evidence to delay or halt the release of the GM cultivar, the effect is biologically significant.
$\log\lambda_1 \geq 0$	3. The comparator cultivar has a positive population growth rate whereas the GM cultivar has a negative population growth rate. This may not be sufficient evidence to prove that the GM crop is safe. However, it is certainly not evidence that the GM crop is more invasive than the comparator.	4. Both cultivars have positive population growth rates. How much more or less positive is the GM population growth rate? Is there an acceptable assessment endpoint? Can we allow the GM cultivar to have a higher population growth rate than the common cultivar?

Table 3-2. Lower-level parameter estimates/ranges based on literature values.

Species	Cultivar	Maximum fecundity (seeds/plant)	Seed dormancy	Over summer survival %	σ_b used	Over winter survival %	References
Brassica napus	Barrier	3000	No primary dormancy	~0 (at 1cm depth)	0.3	40-70%	Gruber and Claupein 2007; OECD 1999)
	Dekalb 7345	3,480 (730)	Inducible secondary dormancy	~20-30% at 10cm depth)		(depends on snow cover)	
Camelina sativa	Calena	~3000 (Hall, unpublished data)	No primary or secondary dormancy reported	20% max (Edmonton AB)	0.2	10% max (Edmonton AB)	([CFIA] Canadian Food Inspection Agency 2011; Francis and Warwick 2009; Robinson 1987; Walsh et al. 2013)
Triticum aestivum	Superb	Poorly reported on an individual plant basis	Transient primary dormancy No secondary dormancy	47% max (AB)	0.5	30% max (AB)	(Nielson et al. 2008; Nielson et al. 2008; OECD 1999; Townley-Smith et al. 2010; Townley-Smith et al. 2010; Willenborg and Van Acker 2008)
Kochia scoparia		30,000	No primary or secondary dormancy	22%	0.25		(Friesen et al. 2009; Schwinghamer and Van Acker 2008)

Table 3-3. Soil characteristics for Alberta disturbance regimes in 2011, 2012, and 2013.

Location	Year	Disturbance level	Soil texture	Soil OM	Soil pH	EC
				%		dS m^{-1}
St. Albert, AB	2011	Agricultural/High	Clay	11	6.9	0.45
St. Albert, AB	2011	Ruderal/Medium	Clay Loam	11.4	7	0.23
St. Albert, AB	2011	Natural/Low	Clay	10.4	7.1	0.49
St. Albert, AB	2012	Agricultural/High	Clay	9.2	7	0.28
St. Albert, AB	2012	Ruderal/Medium	Clay Loam	11.9	6.7	0.23
St. Albert, AB	2012	Natural/Low	Clay	10.9	7.4	0.29
St. Albert, AB	2013	Agricultural/High	Silty Clay	11.6	7.4	0.7
St. Albert, AB	2013	Ruderal/Medium	Clay	11.1	7.5	0.54
St. Albert, AB	2013	Natural/Low	Clay Loam	9.6	6.7	0.28

Table 3-4. Precipitation for St. Albert in 2011, 2012, 2013, and 2014.

		Precipitation mm (% of Long term average)											
Site	Year	January	February	March	April	May	June	July	August	September	October	November	December
St. Albert, AB	2011	34(237)	7(89)	12(83)	10(37)	12(27)	140(241)	114(124)	20(43)	14(49)	15(90)	8(70)	6(50)
St. Albert, AB	2012	8(51)	6(70)	8(54)	42(164)	46(108)	28(47)	135(147)	28(60)	14(46)	20(119)	22(202)	15(138)
St. Albert, AB	2013	14(96)	10(127)	22(163)	21(81)	36(85)	124(214)	87(95)	109(229)	9(31)	13(78)	18(164)	20(179)
St. Albert, AB	2014	13(89)	4(51)	7(51)	35(135)	56(133)	61(105)	114(124)	22(45)	18(61)	8(45)	20(183)	4(37)

Long term averages calculated over 2000-2015 except Harrow which was 2001-2014.

Table 3-5. Air temperature in St. Albert in 2011, 2012, 2013, and 2014.

		Air Temperature Degrees Celcius (Long term average)											
Site	Year	January	February	March	April	May	June	July	August	September	October	November	December
St. Albert,	2011	-11 (-9)	-11 (-8)	-9 (-4)	3 (5)	13 (11)	15 (16)	17 (19)	17 (17)	14 (12)	7 (5)	-4 (-3)	-3 (-10)
St. Albert,	2012	-7 (-9)	-6 (-8)	-1 (-4)	5 (5)	12 (11)	17 (16)	20 (19)	18 (17)	14 (12)	2 (5)	-7 (-3)	-14 (-10)
St. Albert,	2013	-9 (-9)	-4 (-8)	-6 (-4)	1 (5)	14 (11)	15 (16)	17 (19)	18 (17)	14 (12)	6 (5)	-6 (-3)	-13 (-10)
St. Albert,	2014	-6 (-9)	-15 (-8)	-7 (-4)	4 (5)	10 (11)	15 (16)	20 (19)	18 (17)	12 (12)	8 (5)	-7 (-3)	-7 (-10)

Long term averages calculated over 2000-2015 except Harrow which was 2001-2014.

Table 3-6. Experiment timings (initiation, harvest, end date) for Alberta

Location	Year	Disturbance level	Planting date		Harvest date	End date
			Spring Experiment	Fall Experiment	(Spring)	(Fall, follwing year)
St. Albert, AB	2011	Agricultural/High	May 19	Sep 22	Oct 05	Sep 06
St. Albert, AB	2011	Ruderal/Medium	May 19	Sep 22	Oct 05	Sep 06
St. Albert, AB	2011	Natural/Low	May 19	Sep 22	Oct 05	Sep 06
St. Albert, AB	2012	Agricultural/High	May 03	Oct 12	Oct 11	Jul 18
St. Albert, AB	2012	Ruderal/Medium	May 03	Oct 12	Oct 11	Jul 18
St. Albert, AB	2012	Natural/Low	May 03	Oct 12	Oct 11	Jul 18
St. Albert, AB	2013	Agricultural/High	May 09	Oct 15	Oct 10	May 13
St. Albert, AB	2013	Ruderal/Medium	May 09	Oct 15	Oct 10	May 13
St. Albert, AB	2013	Natural/Low	May 09	Oct 15	Oct 10	May 13

Table 3-7. Lower-level parameter calculations from count data.

LLP	LLP calculation	Experiment
g_f	(#seedlings recruited in the fall) / (#seeds planted in the fall)	Fall
σ_w	(#seedlings recruited in the spring) / (#seeds remaining in the seedbank after fall recruitment)	Fall
σ_f	(#seedlings that overwinter as seedlings) / (#seedlings recruited in the fall)	NA
σ_b	(#seeds alive in the fall) / (#seeds remaining in the seedbank after spring recruitment))	Spring
σ_{sdl}	(#mature plants in the fall) / (#seedlings recruited in the spring)	Spring
ϕ	(#seeds produced) / (#mature plants in the fall)	Spring

Table 3-8. Estimates, standard error, and 95% confidence intervals for the lower level parameters for Alberta in all disturbance regimes, years, species, and densities.

			Lower Level Pararameters																											
			σ_w									σ_{sdl}									ϕ									
			2011			2012			2013			2011			2012			2013			2011			2012			2013		
Disturbance	Density	Species	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.
High Disturbance	High	Hybrid Canola	0.04 (0.02) b	0.02	0.09	0.05 (0.02) ab	0.03	0.10	0	0	0	0.90 (0.04) ab	0.79	0.96	0.48 (0.09) ab	0.31	0.65	0.48 (0.11) ab	0.27	0.70	1489 (392) bc	850	2609	1431 (688) ab	533	3848	33 (13) bc	15	72
		OP Canola	0.07 (0.03) b	0.04	0.14	0.10 (0.03) a	0.06	0.17	0.02 (0.01) a	0.01	0.12	0.65 (0.08) ab	0.47	0.79	0.42 (0.09) ab	0.25	0.62	0.61 (0.11) ab	0.38	0.80	1227 (324) bc	700	2151	868 (417) abc	323	2333	15 (6) c	7	34
		Camelina	0.47 (0.08) a	0.32	0.63	0.02 (0.01) b	0.01	0.04	0.01 (0.01) a	0.01	0.08	0.96 (0.02) a	0.91	0.99	0.76 (0.06) a	0.62	0.86	0.25 (0.09) ab	0.11	0.46	473 (125) dc	270	828	1904 (915) ab	708	5119	322 (121) a	145	716
		Kochia	0.05 (0.02) b	0.03	0.11	0.01 (0.01) b	0.01	0.04	0	0	0	0.79 (0.07) ab	0.64	0.89	0.51 (0.09) ab	0.34	0.69	0.36 (0.10) ab	0.18	0.58	3688 (969) ab	2108	6455	176 (85) bcd	66	472	57 (22) abc	26	126
		Wheat	0.02 (0.01) b	0.01	0.05	0.05 (0.02) ab	0.02	0.09	0	0	0	0.96 (0.02) a	0.91	0.99	0.57 (0.08) ab	0.40	0.72	0.78 (0.08) a	0.57	0.90	87 (23) e	50	152	53 (26) d	20	141	12 (5) c	6	25
	Low	Hybrid Canola										0.64 (0.13) ab	0.36	0.85	0.64 (0.13) ab	0.36	0.86	0.29 (0.11) ab	0.12	0.55	4157 (1092) ab	2375	7276	1088 (523) ab	405	2926	37 (14) bc	17	82
		OP Canola										0.53 (0.11) b	0.32	0.73	0.26 (0.12) ab	0.09	0.55	0.62 (0.13) ab	0.35	0.83	2395 (639) ab	1356	4229	499 (340) abcd	124	2023	51 (21) abc	22	120
		Camelina										0.89 (0.05) ab	0.76	0.96	0.24 (0.09) b	0.11	0.46	0.12 (0.07) b	0.04	0.33	1186 (318) bc	670	2100	4346 (2953) a	1073	17606	134 (53) ab	59	308
		Kochia										0.70 (0.10) ab	0.48	0.86	0.55 (0.16) ab	0.25	0.83	0.31 (0.11) ab	0.14	0.57	7834 (2070) a	4462	13755	759 (421) abcd	242	2377	63 (24) abc	29	141
		Wheat										0.75 (0.07) ab	0.58	0.87	0.66 (0.10) ab	0.43	0.83	0.77 (0.10) ab	0.52	0.92	241 (64) d	138	422	86 (42) cd	32	232	31 (12) bc	14	69
Medium Disturbance	High	Hybrid Canola	0	0	0	0	0	0	0.01 (0.01)	0.01	0.07	0	0	0	0.05 (0.03) b	0.02	0.13	0.13 (0.16) a	0.01	0.76	.	.	.	24 (13) a	8	74	145 (227) a	1	21142
		OP Canola	0	0	0	0.06 (0.02) a	0.03	0.12	0.01 (0.01)	0.01	0.10	0	0	0	0.05 (0.03) b	0.02	0.15	0.02 (0.03) a	0.01	0.40	.	.	.	28 (26) a	4	195	44 (79) a	1	13735
		Camelina	0.32 (0.04) a	0.25	0.40	0	0	0	0	0	0	0.69 (0.31) a	0.09	0.99	0.13 (0.05) ab	0.07	0.25	0.01 (0.01) a	0.01	0.12	381 (548) a	15	9897	44 (21) a	17	116	0	0	0
		Kochia	0.07 (0.02) b	0.04	0.11	0	0	0	0	0	0	0.16 (0.17) a	0.02	0.75	0.67 (0.08) a	0.49	0.81	0.20 (0.22) a	0.02	0.83	391 (563) a	16	10159	621 (291) a	235	1639	0	0	0
		Wheat	0.02 (0.01) b	0.01	0.04	0.01 (0.01) b	0.01	0.04	0	0	0	0.54 (0.28) a	0.10	0.94	0.62 (0.09) a	0.44	0.78	0.06 (0.08) a	0.01	0.55	1 (1) a	1	4	29 (14) a	11	76	1 (1) a	1	76
	Low	Hybrid Canola										0	0	0	0.04 (0.03) b	0.01	0.16	0.20 (0.23) a	0.01	0.85	.	.	.	4 (3) a	1	18	267 (432) a	2	46219
		OP Canola										0	0	0	0.09 (0.06) ab	0.03	0.31	0	0	0	.	.	.	9 (6) a	3	36	.	.	.
		Camelina										0.69 (0.35) a	0.06	0.99	0.17 (0.07) ab	0.08	0.34	0	0	0	77 (136) a	2	4181	100 (47) a	38	263	.	.	.
		Kochia										0.10 (0.14) a	0.01	0.81	0.52 (0.11) ab	0.30	0.73	0.14 (0.17) a	0.01	0.79	0	0	0	0	0	0	0	0	0
		Wheat										0.46 (0.28) a	0.06	0.92	0.59 (0.1) a	0.39	0.77	0.03 (0.04) a	0.01	0.43	0	0	0	22 (11) a	9	58	0	0	0
Low Disturbance	High	Hybrid Canola	0.04 (0.02) b	0.02	0.10	0.02 (0.02) ab	0.01	0.08	0.01 (0.01) b	0.01	0.04	0	0	0	0.07 (0.04) a	0.03	0.20	0.01 (0.01) a	0.01	0.09	.	.	.	3 (1) ab	2	5	0	0	0
		OP Canola	0.06 (0.03) b	0.03	0.13	0.06 (0.04) a	0.02	0.19	0	0	0	0	0	0	0.04 (0.03) a	0.01	0.15	0.04 (0.04) a	0.01	0.24	.	.	.	4 (2) ab	2	8	1 (1)	1	2
		Camelina	0.51 (0.1) a	0.33	0.69	0.03 (0.02) ab	0.01	0.09	0.19 (0.07) a	0.08	0.38	0	0	0	0.18 (0.07) a	0.07	0.38	0.02 (0.02) a	0.01	0.17	.	.	.	9 (2) a	6	13	0	0	0
		Kochia	0.02 (0.01) b	0.01	0.05	0.01 (0.01) b	0.01	0.03	0.03 (0.02) b	0.01	0.09	0	0	0	0	0	0	0.02 (0.02) a	0.01	0.16	.	.	.	.	.	.	0	0	0
		Wheat	0.04 (0.02) b	0.02	0.10	0.03 (0.02) ab	0.01	0.11	0	0	0	0.33 (0.07) a	0.17	0.56	0.16 (0.07) a	0.06	0.37	0.49 (0.24) a	0.11	0.88	1 (1) a	1	2	2 (1) b	2	4	1 (1)	1	1
	Low	Hybrid Canola										0	0	0	0.06 (0.04) a	0.02	0.22	0.01 (0.01) a	0.01	0.13	.	.	.	6 (2) ab	3	12	0	0	0
		OP Canola										0	0	0	0.10 (0.07) a	0.03	0.34	0.03 (0.03) a	0.01	0.25	.	.	.	6 (2) ab	4	11	0	0	0
		Camelina										0	0	0	0.13 (0.07) a	0.04	0.36	0	0	0	.	.	.	7 (2) a	5	11	.	.	.
		Kochia										0	0	0	0	0	0	0.02 (0.03) a	0.01	0.23	.	.	.	.	.	.	0	0	0
		Wheat										0.51 (0.09) a	0.26	0.76	0.14 (0.08) a	0.04	0.37	0.39 (0.24) a	0.08	0.85	2 (1) a	1	3	6 (2) ab	4	11	1 (1)	1	1

A value of zero indicates that we observed no individuals in any replicate for a treatment combination at the sample size used.

A "." indicates that values could not be measured due to zero survival at a previous transition.

Mean (Standard Error)

ilink backtransformed 95% Confidence Intervals (L.C.I. and U.C.I)

Letters within columns and disturbance levels indicate statistically different means

Table 3-9. Population growth rate and elasticity estimates with 95% confidence intervals for Alberta in all disturbance regimes, years, species, and densities.

			λ									Elasticity of λ to lower level parameters																				
												σ_w									σ_{sdl}, ϕ								σ_b								
			2011			2012			2013			2011			2012			2013			2011			2012			2013			2011			2012			2013		
Disturbance	Density	Species	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I
High Disturbance	High	Hybrid Canola	48.60	22.91	103.30	43.36	21.77	65.41	0.81	0.54	1.13	0.99	0.99	1.00	0.99	0.99	1.00	0.63	0.44	0.73	0.99	0.99	1.00	0.99	0.99	1.00	0.64	0.46	0.74	0.01	0.00	0.01	0.01	0.00	0.01	0.36	0.26	0.54
		OP Canola	55.63	40.50	77.28	35.28	10.02	70.60	0.50	0.30	1.07	0.99	0.99	1.00	0.99	0.97	1.00	0.40	0.00	0.72	0.99	0.99	1.00	0.99	0.97	1.00	0.41	0.00	0.74	0.01	0.00	0.01	0.01	0.00	0.03	0.59	0.26	1.00
		Camelina	231.54	143.14	332.45	16.66	0.20	43.43	0.84	0.41	1.46	1.00	1.00	1.00	0.99	0.00	1.00	0.76	0.52	0.86	1.00	1.00	1.00	0.99	0.00	1.00	0.76	0.52	0.86	0.00	0.00	0.00	0.01	0.00	1.00	0.24	0.14	0.48
		Kochia	151.08	15.99	305.50	1.11	0.25	2.35	1.05	0.62	1.45	1.00	0.98	1.00	0.77	0.00	0.89	0.76	0.60	0.83	1.00	0.98	1.00	0.78	0.00	0.90	0.77	0.61	0.83	0.00	0.00	0.02	0.22	0.10	1.00	0.23	0.17	0.39
		Wheat	1.75	0.50	3.65	1.66	0.98	2.94	0.78	0.66	0.89	0.71	0.00	0.86	0.70	0.49	0.83	0.36	0.24	0.44	0.72	0.00	0.87	0.71	0.51	0.84	0.39	0.27	0.46	0.28	0.13	1.00	0.29	0.16	0.49	0.61	0.54	0.73
	Low	Hybrid Canola	89.56	36.66	267.66	65.60	5.26	121.29	0.69	0.42	1.35	1.00	0.99	1.00	1.00	0.94	1.00	0.56	0.29	0.78	1.00	0.99	1.00	1.00	0.95	1.00	0.58	0.32	0.79	0.00	0.00	0.01	0.00	0.00	0.05	0.42	0.21	0.68
		OP Canola	96.60	28.72	209.46	15.99	0.27	34.70	1.10	0.30	2.87	1.00	0.99	1.00	0.98	-0.13	0.99	0.73	0.00	0.90	1.00	0.99	1.00	0.98	0.00	0.99	0.73	0.00	0.90	0.00	0.00	0.01	0.02	0.01	1.00	0.27	0.10	1.00
		Camelina	669.72	214.97	1359.97	13.78	0.20	38.69	0.41	0.23	1.07	1.00	1.00	1.00	0.99	-0.01	0.99	0.52	0.12	0.81	1.00	1.00	1.00	0.99	0.00	0.99	0.52	0.13	0.81	0.00	0.00	0.00	0.01	0.01	1.00	0.48	0.19	0.87
		Kochia	294.51	25.08	655.78	5.17	0.25	21.53	1.22	0.49	2.19	1.00	0.99	1.00	0.95	-0.01	0.99	0.80	0.49	0.89	1.00	0.99	1.00	0.95	0.00	0.99	0.80	0.51	0.89	0.00	0.00	0.01	0.05	0.01	1.00	0.20	0.11	0.49
		Wheat	3.10	0.50	6.85	2.07	1.25	3.20	1.22	1.09	1.47	0.84	0.00	0.93	0.76	0.60	0.84	0.59	0.54	0.66	0.84	0.00	0.93	0.77	0.61	0.86	0.61	0.56	0.67	0.16	0.07	1.00	0.23	0.14	0.39	0.39	0.33	0.44
Medium Disturbance	High	Hybrid Canola	0.29	0.29	0.29	0.31	0.30	0.32	0.62	0.30	1.45	-0.04	-0.04	-0.04	0.02	0.00	0.06	0.52	0.00	0.79	0.00	0.00	0.00	0.02	0.00	0.07	0.52	0.00	0.79	1.00	1.00	1.00	0.98	0.93	1.00	0.48	0.21	1.00
		OP Canola	0.29	0.29	0.29	0.39	0.28	0.50	1.34	0.30	5.44	-0.04	-0.04	-0.04	0.22	-0.09	0.40	0.78	-0.01	0.94	0.00	0.00	0.00	0.27	0.00	0.44	0.78	0.00	0.95	1.00	1.00	1.00	0.73	0.56	1.00	0.22	0.05	1.00
		Camelina	34.10	5.06	186.77	0.22	0.20	0.28	0.19	0.19	0.19	0.99	0.96	1.00	0.09	0.00	0.29	-0.04	-0.04	-0.04	1.00	0.97	1.00	0.10	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.03	0.90	0.70	1.00	1.00	1.00	1.00
		Kochia	17.91	0.23	45.07	1.75	0.25	5.60	0.24	0.24	0.24	0.99	-0.11	0.99	0.86	0.00	0.96	-0.04	-0.04	-0.04	0.99	0.00	0.99	0.86	0.00	0.96	0.00	0.00	0.00	0.01	0.01	1.00	0.14	0.04	1.00	1.00	1.00	1.00
		Wheat	0.49	0.48	0.50	0.62	0.50	0.77	0.49	0.48	0.51	-0.01	-0.03	0.00	0.19	0.00	0.35	-0.01	-0.04	0.03	0.00	0.00	0.01	0.20	0.00	0.36	0.02	0.00	0.06	1.00	0.99	1.00	0.80	0.64	1.00	0.98	0.94	1.00
	Low	Hybrid Canola	0.29	0.29	0.29	0.30	0.30	0.30	0.64	0.30	1.41	-0.04	-0.04	-0.04	0.00	-0.01	0.00	0.53	0.00	0.79	0.00	0.00	0.00	0.00	0.00	0.01	0.54	0.00	0.79	1.00	1.00	1.00	1.00	0.99	1.00	0.46	0.21	1.00
		OP Canola	0.29	0.29	0.29	0.34	0.28	0.38	0.30	0.29	0.30	-0.04	-0.04	-0.04	0.11	-0.07	0.21	-0.01	-0.02	0.00	0.00	0.00	0.00	0.16	0.00	0.27	0.00	0.00	0.00	1.00	1.00	1.00	0.84	0.73	1.00	1.00	1.00	1.00
		Camelina	9.95	0.13	33.62	0.25	0.20	0.38	0.19	0.19	0.19	0.98	-0.54	0.99	0.21	0.00	0.47	-0.04	-0.04	-0.04	0.99	0.00	1.00	0.21	0.00	0.47	0.00	0.00	0.00	0.01	0.00	1.00	0.79	0.53	1.00	1.00	1.00	1.00
		Kochia	0.23	0.23	0.24	0.25	0.25	0.25	0.24	0.24	0.24	-0.07	-0.11	-0.03	0.00	-0.01	0.00	-0.04	-0.04	-0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Wheat	0.49	0.48	0.50	0.62	0.50	0.78	0.48	0.48	0.48	-0.01	-0.04	0.00	0.20	0.00	0.36	-0.04	-0.04	-0.04	0.00	0.00	0.00	0.21	0.00	0.37	0.00	0.00	0.00	1.00	1.00	1.00	0.79	0.63	1.00	1.00	1.00	1.00
Low Disturbance	High	Hybrid Canola	0.29	0.26	0.30	0.30	0.29	0.30	0.30	0.30	0.30	-0.05	-0.14	0.00	-0.01	-0.05	0.01	-0.01	-0.01	0.00	0.01	0.00	0.05	0.02	0.00	0.07	0.00	0.00	0.00	0.99	0.95	1.00	0.98	0.93	1.00	1.00	1.00	1.00
		OP Canola	0.28	0.27	0.29	0.29	0.26	0.31	0.29	0.29	0.29	-0.07	-0.10	-0.03	-0.04	-0.14	0.03	-0.04	-0.04	-0.03	0.00	0.00	0.00	0.05	0.00	0.15	0.00	0.00	0.01	1.00	1.00	1.00	0.95	0.85	1.00	1.00	0.99	1.00
		Camelina	0.10	0.09	0.11	0.25	0.21	0.32	0.16	0.14	0.18	-1.03	-1.32	-0.78	0.21	0.04	0.37	-0.26	-0.48	-0.10	0.00	0.00	0.00	0.24	0.06	0.40	0.00	0.00	0.00	1.00	1.00	1.00	0.76	0.59	0.94	1.00	1.00	1.00
		Kochia	0.25	0.24	0.25	0.25	0.25	0.25	0.24	0.24	0.25	-0.02	-0.02	-0.01	-0.01	-0.02	0.00	-0.03	-0.06	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Wheat	0.49	0.48	0.50	0.49	0.47	0.53	0.48	0.48	0.48	-0.02	-0.04	0.00	-0.01	-0.06	0.05	-0.04	-0.04	-0.03	0.03	0.01	0.06	0.04	0.00	0.12	0.00	0.00	0.01	0.97	0.94	0.99	0.96	0.88	1.00	1.00	0.99	1.00
	Low	Hybrid Canola	0.28	0.26	0.30	0.30	0.29	0.32	0.30	0.30	0.30	-0.06	-0.17	0.00	0.01	-0.04	0.06	-0.01	-0.01	0.00	0.00	0.00	0.00	0.04	0.00	0.12	0.00	0.00	0.00	1.00	1.00	1.00	0.96	0.88	1.00	1.00	1.00	1.00
		OP Canola	0.28	0.27	0.29	0.33	0.30	0.37	0.29	0.29	0.29	-0.07	-0.10	-0.03	0.10	0.00	0.19	-0.04	-0.04	-0.04	0.00	0.00	0.00	0.18	0.04	0.32	0.00	0.00	0.00	1.00	1.00	1.00	0.82	0.68	0.96	1.00	1.00	1.00
		Camelina	0.10	0.09	0.11	0.23	0.20	0.30	0.16	0.14	0.18	-1.03	-1.32	-0.78	0.14	0.00	0.32	-0.26	-0.48	-0.10	0.00	0.00	0.00	0.17	0.03	0.35	0.00	0.00	0.00	1.00	1.00	1.00	0.83	0.64	0.97	1.00	1.00	1.00
		Kochia	0.25	0.24	0.25	0.25	0.25	0.25	0.24	0.24	0.25	-0.02	-0.02	-0.01	-0.01	-0.02	0.00	-0.03	-0.06	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Wheat	0.51	0.48	0.54	0.54	0.46	0.66	0.48	0.48	0.48	0.02	-0.03	0.07	0.08	-0.08	0.24	-0.04	-0.04	-0.03	0.06	0.01	0.13	0.12	0.00	0.30	0.00	0.00	0.01	0.94	0.87	0.99	0.88	0.70	1.00	1.00	0.99	1.00

When OW survival is zero in all replicates an estimate of 0.0375 is used instead

Table 3-10. Bootstrapped differences and ratios (hybrid – OP; hybrid / OP) for canola demonstrating the benefit of using equivalence hypothesis tests over null hypothesis tests in precautionary principle scenarios.

										Null Hypothesis Test	Equivalence test				
										H_0 is rejected (-), or H_0 is not rejected (+)	H_1 is not rejected (+) or H_1 is rejected (-)				
										H_0: $\lambda_1 - \lambda_2 = 0$	H_1: $\lambda_1 / \lambda_2 \leq 1/(1+x)$ or $\lambda_1 / \lambda_2 \geq (1+x)$				
										H_A: $\lambda_1 - \lambda_2 \neq 0$	H_2: $1/(1+x) < \lambda_1 / \lambda_2 < (1+x)$				
			Differences ($\lambda_{Hybrid} - \lambda_{OP}$)			Ratios ($\lambda_{Hybrid} / \lambda_{OP}$)				95% CI	90% CI, x varies from 1% - 50%				
Year	Disturbance	Density	estimate	U.C.I.	L.C.I	estimate	U.C.I.	L.C.I	Quadrant		1%	5%	10%	20%	50%
2011	High	High	-7.02	-46.39	50.92	0.87	0.40	1.91	4	+	-	-	-	-	-
		Low	-7.04	-168.22	195.36	0.93	0.25	4.58	4	+	-	-	-	-	-
	Medium	High	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
		Low	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
	Low	High	0.01	-0.02	0.02	1.02	0.95	1.08	1	+	-	-	+	+	+
		Low	0.00	-0.02	0.02	1.01	0.93	1.08	1	+	-	-	+	+	+
2012	High	High	8.08	-32.54	39.65	1.23	0.59	3.20	4	+	-	-	-	-	-
		Low	49.61	-17.35	106.90	4.10	0.54	79.94	4	+	-	-	-	-	-
	Medium	High	-0.08	-0.19	0.02	0.79	0.65	1.08	1	+	-	-	-	-	-
		Low	-0.04	-0.08	0.02	0.89	0.81	1.04	1	+	-	-	-	-	+
	Low	High	0.01	-0.01	0.04	1.02	0.97	1.11	1	+	-	-	-	+	+
		Low	-0.03	-0.07	0.00	0.91	0.83	0.99	1	+	-	-	-	-	+
2013	High	High	-0.20	-0.77	0.00	0.60	0.32	1.00	3	+	-	-	-	-	-
		Low	-0.80	-2.57	0.00	0.27	0.12	1.00	3	+	-	-	-	-	-
	Medium	High	-0.72	-4.73	1.08	0.46	0.11	3.89	4	+	-	-	-	-	-
		Low	0.35	0.00	1.18	2.16	1.00	4.71	2	+	-	-	-	-	-
	Low	High	0.00	0.00	0.00	0.99	0.99	1.00	1	+	-	+	+	+	+
		Low	0.00	0.00	0.00	0.99	0.99	1.00	1	+	-	+	+	+	+

For the null hypothesis test, a failure to reject the null hypothesis is a failure to prove that there is a difference.

For the equivalence test, a fauluer to reject the primary hypothesis is a failure to prove that the populations are equal at the level of 'x'.

The interpretation of '+' and '-' is not exactly equal between the null hypothesis test and the equivalence test.

When σ_w survival is zero a value of 0.001 is used instead.

The equivalence test uses a 90% CI because it represents two one tailed tests.

A 1-2α confidence interval tests at the α level for an equivalence test.

The Classical hypothesis test uses a 95% CI to test at the level of α.

Table 3-11. Number of individuals required for a 95% chance to see at least one survive to the next stage based on lower level parameter estimates.

			$\sigma_{w(n)}$			$\sigma_{sdl(n)}$		
Disturbance	Density	Species	2011	2012	2013	2011	2012	2013
High Disturbance	High	Hybrid Canola	74	59	NA	2	5	5
		OP Canola	42	29	149	3	6	4
		Camelina	5	149	299	1	3	11
		Kochia	59	299	NA	2	5	7
		Wheat	149	59	NA	1	4	2
	Low	Hybrid Canola				3	3	9
		OP Canola				4	10	4
		Camelina				2	11	24
		Kochia				3	4	9
		Wheat				3	3	3
Medium Disturbance	High	Hybrid Canola	NA	NA	299	NA	59	22
		OP Canola	NA	49	299	NA	59	149
		Camelina	8	NA	NA	3	22	299
		Kochia	42	NA	NA	18	3	14
		Wheat	149	299	NA	4	4	49
	Low	Hybrid Canola				NA	74	14
		OP Canola				NA	32	NA
		Camelina				3	17	NA
		Kochia				29	5	20
		Wheat				5	4	99
Low Disturbance	High	Hybrid Canola	74	149	299	NA	42	299
		OP Canola	49	49	NA	NA	74	74
		Camelina	5	99	15	NA	16	149
		Kochia	149	299	99	NA	NA	149
		Wheat	74	99	NA	8	18	5
	Low	Hybrid Canola				NA	49	299
		OP Canola				NA	29	99
		Camelina				NA	22	NA
		Kochia				NA	NA	149
		Wheat				5	20	7

A value of 'NA' indicates that we observed no seedlings emerge in any of the replicates.

Figure 3-1. Experiments were initiated in five important agro-ecological regions of Canada.

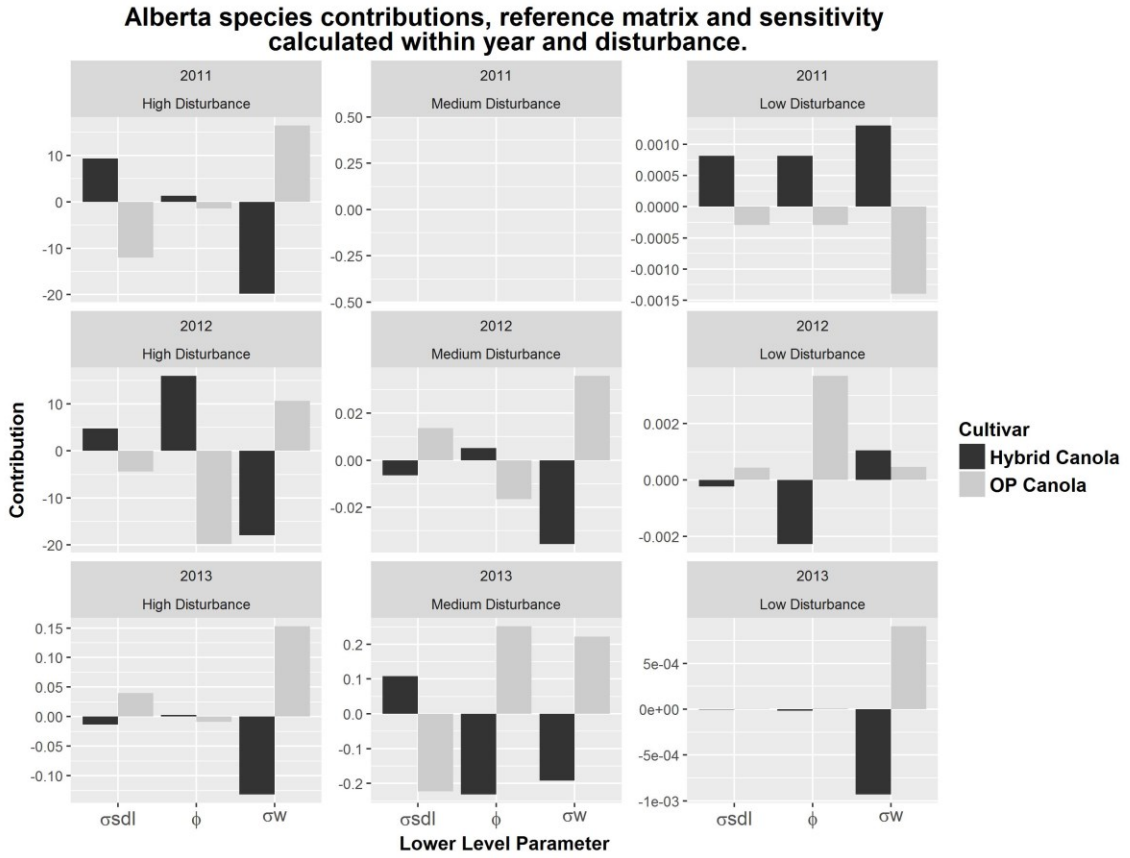

Figure 3-2. The contributions of lower level parameters to the difference in λ between the hybrid and OP canola cultivars for all year and disturbance combinations in Alberta. Contributions are a product of the difference between LLP and the sensitivity of λ to the LLP.

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Chapter 4: Persistence and invasiveness of genetically modified canola (*Brassica napus*) in Canada: a stochastic analysis comparing the invasive potential of hybrid and OP cultivars.

4.1 Introduction

To date, GM crops have been produced with moderate changes, insufficient to allow them to overcome domestication. New GM crops with traits including stress tolerance, increased yield, and nitrogen use efficiency are being developed that may enhance their ability to withstand drought, cold, salinity, and general stress (Canadian Food Inspection Agency 2015). Whether these traits are sufficient to overcome the constraints of domestication is not known. Prior to the release of GM crops, Canada, like other countries, mandates that their invasive potential must be quantified in relation to comparator crops to determine if they are substantially equivalent (Canadian Food Inspection Agency 2013). Field trials must be conducted under confinement where seed and pollen-mediated gene flow from experimental GM crops is reduced to near zero. Only small-scale experiments are permitted to limit the risk of inadvertent escape. Experimental methodology must be sufficiently rigorous to measure invasive potential in different environments, including agricultural, ruderal, and natural areas. Because seed moves freely in Canada after release, invasive potential must be considered in all agro-climatic regions of Canada, and account for annual weather stochasticity. Our goal is to compare the invasive potential of hybrid and OP canola cultivars using stochastic analyses afforded to us by population matrix modeling.

Of the most widely grown GM crops (canola, corn, soybean, and cotton), canola (*Brassica napus* L.) is the most weed-like. In addition to being a major oilseed crop in Canada, canola is an abundant agricultural weed, and commonly occurs in ruderal sites (roadsides and waste areas). Haile et al. (2014) surveyed 25 Canadian canola fields (2010 to 2012) and reported an average seed loss of >8000 seed m^{-2}, others report seed loss of similar magnitude (Cavalieri et al. 2014; Gulden et al. 2003a; Pari et al. 2013). Volunteer canola populations resulting from seed loss are numerous: volunteer canola ranked 14th in weed abundance in Western Canada (Leeson et al. 2005). Unlike many domesticated crops, canola seed has inducible secondary dormancy (Pekrun et al. 1997) that varies with genotype, time of harvest and environment (Gulden et al. 2003b; Gulden et al. 2004; Haile and Shirtliffe 2014). While most seed germinates in the first year following seed loss (Baker and Preston 2008; Harker et al. 2006), some persists in the seed bank and germinates in the following years (Chadoeuf et al. 1998). Canola seed is inadvertently dispersed along roadsides and seed processing/storage areas which results in feral populations. These have been widely documented in Europe (Crawley et al. 1993, 2001; Crawley and Brown 1995a; Hecht et al. 2014), USA (Pessel et al. 2001), Australia (Busi and Powles 2016), New Zealand (Meffin et al. 2015), and Canada (Yoshimura et al. 2006).

Matrix projection models are widely used in ecology to quantify the fitness of rare or invasive species because they are tractable and use LLP (survival and fecundity) from the entire lifecycle (Caswell 2001a). Prospective analyses (elasticity) highlight LLP that would have a large effect on λ if perturbed. (Caswell 2001b). The population growth rate (λ) is

equivalent to a currency of fitness (Salguero-Gómez and De Kroon 2010). $\lambda < 1$ ($\log \lambda < 0$) indicates a decreasing population while $\lambda \geq 1$ ($\log \lambda \geq 0$) indicates a persistent or increasing population, a prerequisite for population growth and invasiveness. A demographic approach using λ as an assessment endpoint (Crawley et al. 1993) has been used to evaluate canola invasiveness in the USA and Canada (Parker and Kareiva 1996) and Europe (Claessen et al. 2005b; Garnier and Lecomte 2006; Hooftman et al. 2015a; Lavigne et al. 2004). Demographic models have been proposed as a method of examining persistence and invasiveness of GM plants (European Food Safety Authority 2014).

Demographic models have been used extensively in Europe to quantify canola invasiveness (ability of canola to increase and spread) and persistence (the length of time a population remains extant) in ruderal and field conditions (Begg et al. 2007; Claessen et al. 2005a; Claessen et al. 2005b; Garnier and Lecomte 2006; Garnier et al. 2014; Hooftman et al. 2015b). Canola populations are generally ephemeral; they persist primarily through longevity in the seed bank (Claessen et al. 2005b; Hooftman et al. 2015a) and through continual anthropogenic seed input that founds new populations (Crawley and Brown 1995b). It is not possible to directly compare the demographics of winter oilseed rape, which is planted in the fall, may overwinter as a rosette, and flowers/produces seed in the following summer, growing in European environments with spring canola, which is planted in spring with flowering and seed production in the same year, grown in Canada. In most places in Canada, seed that germinated in fall following harvest cannot survive the winter, with exception of some environments in Québec (Simard et al. 2002). Demographic

parameters are influenced by time of harvest and seed loss, seedling emergence, the presence of overwintering rosettes and seed bank characteristics that differ in the two agronomic systems.

Conventional and transgenic breeding of canola cultivars has increased seed size, yield (fecundity) and possibly fitness. Most cultivars sold in Canada are hybrids, resistant to either glufosinate or glyphosate herbicides. Compared to open-pollinated (OP) varieties, hybrids have greater seed weight, (Elliott et al. 2008), greater seedling vigor and higher maximum yield. Hybrids were reported to have a greater ability to tolerate stress imposed by flea beetles and higher seedling biomass from large seeds increases competitive ability (Harker et al. 2015). Volunteers of herbicide resistant varieties are reported to be as easy to control as conventional canola in agricultural systems (Beckie et al. 2001), however, hybrid canola may have a different population growth rate, or be more persistent or invasive than OP canola.

Our goal was to assess and compare the invasive potential of a hybrid and an OP canola in four key agro-ecological regions of Canada, three disturbance regimes (agricultural fields in the absence of weed control measures; ruderal areas, emulating roadsides and semi-natural areas; and natural areas), and two planting densities using a stochastic analysis and assessment endpoint scenarios (Table 4-1) discussed in sections 3.1 and 3.3.5.

4.2 Materials and Methods

The experimental design was described in detail in section 3.2. Sites were established in five agro-ecological regions of Canada in high disturbance (agricultural), medium

disturbance (ruderal), and low disturbance (natural) areas to compare the demography of hybrid and OP canola seeded at two different planting densities (Table 4-1). Due to experimental difficulties the British Columbia site is not included in this analysis. Soil was sampled at 15 cm at each site to characterize the soils and analyzed for OM, pH, EC and texture (Table 4-2). Average monthly precipitation and temperature data was acquired for the closest weather station (Table 4-3, 4-4). The canola varieties were Round-up Ready® Hybrid Dekalb 7345 (hybrid canola) and Round-up Ready® OP VT Barrier (OP canola). Experiments were initiated twice each year: fall to assess overwinter survival and emergence; and spring to measure seedling survival and fecundity (Table 4-5). Because the hybrid cultivar is known to have increased seed weight and seedling vigor, it plays the role of the GM crop in this analysis while OP canola is the common cultivar. Experiments were repeated over 3 years to account for environmental stochasticity.

4.2.1 Estimation of Lower Level Parameters: Survival and Fecundity

Lower level parameters were estimated according to Table 4-6. The overwinter survival rate (σ_w) was measured as the proportion of viable seeds planted in the fall that were recruited and survived in the following spring. Fecundity (Φ) was calculated as the average number of seeds produced per mature plant per plot. Seedling survival (σ_{sdl}) is the proportion of seedlings that survive until reproductive maturity and (σ_b) is the proportion of viable seeds in the spring seed bank that survive until the fall. σ_b was not measured experimentally but was estimated from the literature (Table 4-7).

4.2.2 Matrix Model

The full model was described in section 3.2.3.

4.2.3 Environmental Stochasticity

LLP will vary over time due to environmental fluctuations. The goal of a stochastic model is to randomly generate $\boldsymbol{A}$ matrices based on observed environmental states and use these $\boldsymbol{A}$ matrices to project a population vector N forward through time (Caswell 2001c). We treat our environmental states as independent and identically distributed (i.i.d.), meaning that each environment has an equal chance of being selected regardless of the past environment.

The calculation of the stochastic population growth rate ($\log \lambda_s$) was done through simulation by projecting a starting population vector N_0 using randomly selected projection matrices through $\boldsymbol{T}$ iterations and applying the equation

$$\widehat{\log \lambda_s} = \frac{1}{T}\sum_{t=0}^{T-1} r_t \qquad 4\text{-}1$$

where

$$r_t = \log(\frac{N_{t+1}}{N_t}) \qquad 4\text{-}2$$

The 95% confidence interval for $log\lambda_s$can be calculated by

$$\widehat{log\lambda_s} \pm 1.96\sqrt{\frac{V(r)}{T}} \qquad 4\text{-}3$$

4.2.4 Quasi-Extinction

Quasi-extinction calculations determine the probability (P_q) and time (T_q) to quasi-extinction for a given population (Caswell 2001c). To become quasi-extinct, a simulated population must drop below some predetermined size. The extinction threshold is θ

$$\theta = \frac{N_q}{N(0)} \qquad 4\text{-}4$$

where $N(0)$ is the initial population size and N_q is the size of the extinct population. The probability (P_q)of the population reaching size N_q is

$$P_q = \begin{cases} 1 & if\ log\lambda_s \leq 0 \\ \exp(\frac{2log\lambda_s\ log\theta}{\sigma^2}) & if\ log\lambda_s > 0 \end{cases} \qquad 4\text{-}5$$

and the time for a population to reach N_q is T_q, and the mean of T_q is given by

$$\frac{-log\theta}{|\log\lambda_s|} = E(T_q) \qquad 4\text{-}6$$

(*sensu* Caswell 2001c). For this paper we use $\theta = 0.01$. Lower values of θ will increase P_q,and vice versa. $E(T_q)$ is inversely related to $\log\lambda_s$, meaning that large values of $\log\lambda_s$ (either positive or negative) will result in small estimates of $E(T_q)$. When $\log\lambda_s$ is negative, this makes intuitive sense: populations with large, negative growth rates will extinguish very quickly. When $\log\lambda_s$ is positive, you must also take into account P_q. With a large,

positive $\log \lambda_s$ there is a very small probability of quasi-extinction. However, for a population with such a growth rate to go extinct, it must occur very quickly (Caswell 2001c).

4.2.5 Stochastic Elasticity

We calculate stochastic elasticity by perturbing a LLP at each time step in $\boldsymbol{T}$ by 10% (increase and decrease) and then averaging the results (Claessen et al., 2005b). Let e_{x+} represent the elasticity from an increase in the LLP, let e_{x-} represent the elasticity from a decrease in the LLP, and let $e_{x\prime}$ represent the general notation for either an increase or a decrease. Then

$$e_{x\prime} = \frac{\log \lambda_s - \log \lambda\prime_s}{\log x - \log x\prime} \qquad 4\text{-}7$$

Using 4-7we calculate both e_{x+} and e_{x-} and take their average to estimate e_x.

4.2.6 Data Analysis

The analysis of the LLP described in 3.2.6.

Estimates for stochastic population growth, stochastic elasticity, P_q, and T_q were calculated directly from the data using the equations above and setting $\boldsymbol{T}$ at 10,000. We intentionally did not discard any iterations. 95% confidence intervals were produced using a bootstrap resampling procedure to simulate a short-term forecast. A population of 16 replicates (4 fall, 4 spring) was created for the bootstrap sampling procedure, and each bootstrap replicate consisted of drawing four of the population replicates (within a year

and with replacement) for 3 years ($\boldsymbol{T}$ = 3) and calculating stochastic population growth, stochastic elasticity, P_q and T_q. We created 10,000 bootstrap replicates and calculated percentile confidence intervals.

Differences between population growth rates between hybrid canola and OP canola were estimated in a similar fashion as above, but are different in a few aspects. First, a data frame was created by column-merging hybrid canola and OP canola data by year, site, disturbance and block, thus creating a population of 64 replicates. This was done to ensure that the blocking factor and years remained intact so as to faithfully follow the field design. Each bootstrap replicate was calculated as above and then we computed the difference and the ratios of the population growth rates for hybrid and OP canola. We created 10,000 bootstrap replicates and calculated percentile confidence intervals.

4.3 Results and Discussion

4.3.1 Lower level parameters

4.3.1.1 Overwinter survival (σ_w), Alberta

σ_w was measured (non-zero values in at least one replicate) in 5 of 6 treatment combinations (species and year) for the high (agricultural) disturbance regime, 3 of 6 treatment combinations for the medium (ruderal) disturbance regime, and 5 of 6 treatment combinations for the low (pasture) disturbance regime (Table 4-8). In the high disturbance regime, cultivars were not significantly different in 2011 (P=0.17) or 2012 (P=0.15) although OP canola σ_w was consistently 2-fold greater than hybrid canola σ_w. In 2013, we failed to measure σ_w for hybrid canola because no seeds survived. In the medium

disturbance regime, we failed to measure σ_w for both cultivars in 2011 and for hybrid canola in 2012. In 2013, hybrid canola and OP canola did not have significantly different estimates for σ_w. In the low disturbance regime, differences were not statistically significant in 2011(P=0.42) or 2012 (P=0.16). In 2013, we failed to measure σ_w for OP canola; no individuals survived.

4.3.1.2 Overwinter survival (σ_w), Saskatchewan

In Saskatchewan, the fall experiment was only initiated in 2011 and 2012. σ_w was measured (non-zero values in at least one replicate) in 1 of 4 treatment combinations (species and year) for the high (agricultural) disturbance regime, 0 of 6 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 6 treatment combinations for the low (pasture) disturbance regime (Table 4-8). σ_w was measured for OP canola in the agricultural regime in 2011.

4.3.1.3 Overwinter survival (σ_w), Ontario

σ_w was measured (non-zero values in at least one replicate) in 6 of 6 treatment combinations (species and year) for the high (agricultural) disturbance regime, 6 of 6 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 6 treatment combinations for the low (pasture) disturbance regime (Table 4-8). In the high disturbance regime, the cultivars were not significantly different in 2011 (P=0.12) but were significantly different in 2012 (P=0.005) and 2013 (P<0.001). In the medium disturbance regime, the cultivars were not significantly different in 2011 (P=0.13), but were significantly different in 2012 (P=0.04) and 2013 (P=0.001). We failed to measure σ_w in the

low disturbance regime. As in Alberta, the values for OP canola were consistently larger than those for the hybrid variety.

4.3.1.4 Overwinter survival (σ_w), Québec

In Québec, fall experiment was only initiated in 2011. σ_w was measured (non-zero values in at least one replicate) in 1 of 2 treatment combinations (species and year) for the high (agricultural) disturbance regime, 0 of 2 treatment combinations for the medium (ruderal) disturbance regime, and 2 of 2 treatment combinations for the low (pasture) disturbance regime (Table 4-8). In the high disturbance regime, we failed to measure σ_w for hybrid canola. In the medium disturbance regime, we failed to measure σ_w for both cultivars. In the low disturbance regime, the cultivars were not significantly different (P=0.10).

Overwinter survival σ_w in the high disturbance regimes was low and this life stage represents a major limiting factor in agricultural areas where seed deposition can be extreme. σ_w in most locations was less than 10%, but was highest in the more mesic Ontario site, where nearly 25% of the seeds survived until spring. This suggests that traits that might increase overwinter survival such as frost tolerance and abiotic stress may alter the weediness of canola in agricultural areas.

4.3.1.5 Fall germination (g_f), Ontario

g_f was measured (non-zero values in at least one replicate) in 4 of 6 treatment combinations (species and year) for the high (agricultural) disturbance regime, 6 of 6 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 6 treatment

combinations for the low (pasture) disturbance regime (Table 4-8). In the high disturbance regime, hybrid canola g_f was significantly greater than OP canola g_f in 2011 (P<0.001) and 2012 (P=0.016). In the medium disturbance regime, hybrid canola g_f was significantly higher than OP canola g_f in 2011 (P=0.003), 2012 (P=0.002), and 2013 (P=0.001).

4.3.1.6 Fall germination (g_f), Québec

g_f was measured (non-zero values in at least one replicate) in 2 of 2 treatment combinations (species and year) for the high (agricultural) disturbance regime, 2 of 2 treatment combinations for the medium (ruderal) disturbance regime, and 1 of 2 treatment combinations for the low (pasture) disturbance regime (Table 4-8). In the high disturbance regime hybrid canola g_f was significantly higher than OP canola in 2011 (P<0.001). In the medium disturbance regime the two cultivars were not statistically different. In the low disturbance regime, we failed to measure g_f for OP canola.

4.3.1.7 Fall seedling overwinter (σ_f), Ontario

σ_f was measured (non-zero values in at least one replicate) in 4 of 6 treatment combinations (species and year) for the high (agricultural) disturbance regime, 2 of 6 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 6 treatment combinations for the low (pasture) disturbance regime (Table 4-8). In the high disturbance regime, the cultivars were not significantly different in 2011 (P=0.55) or 2012 (P=0.35). In the medium disturbance regime, the cultivars were not significantly different in 2011 (P=0.38).

4.3.1.8 Fall seedling overwinter (σ_f), Québec

σ_f was measured (non-zero values in at least one replicate) in 1 of 2 treatment combinations (species and year) for the high (agricultural) disturbance regime, 1 of 2 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 2 treatment combinations for the low (pasture) disturbance regime (Table 4-8). We did not observe hybrid canola seedlings survive overwinter.

Fall seedling germination and overwintering were measured only in the more mesic sites of Ontario and Quebec, but σ_f was only substantive (>0.1) in one year in Ontario. Given the seed density in agricultural and ruderal areas following seed spill, fall seedling overwintering should be carefully monitored in GM crops with stress tolerance.

4.3.1.9 Spring seedling survival to maturity (σ_{sdl}), Alberta

σ_{sdl} was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 7 of 12 treatment combinations for the medium (ruderal) disturbance regime, and 8 of 12 treatment combinations for the low (pasture) disturbance regime (Table 4-9). In the high disturbance regime, species was not a significant effect in 2011 (P=0.053), 2012 (P=0.098), or 2013 (P=0.15). Density was not a significant effect in 2012 (P=0.94) or 2013 (P=0.11), but was it was in 2011 (P=0.009). There was no significant species-density interaction in 2011 (P=0.20), 2012 or 2013 (P=0.10), but there was in 2012 (P=0.35). Multiple comparisons tests revealed that high density hybrid canola σ_{sdl} was significantly larger

than both densities of OP canola in 2011, but was not significantly larger than the low density hybrid canola.

In the medium disturbance regime, we failed to measure σ_{sdl} in 2011 and there was not a significant species effect in 2012 (P=0.45), but there was in 2013 (P<0.001). There was no significant density effect in 2012 (P=0.75), but there was in 2013 (P<0.001). There was no significant species-density interaction in 2012 (P=0.35), but there was in 2013 (P<0.01). Multiple comparisons tests revealed that none of the treatment combinations were significantly different from each other in 2011, but in 2012, low density OP canola was significantly less than the other treatment combinations.

In the low disturbance regime, we failed to measure σ_{sdl} in 2011 and there was not a significant species effect in 2012 (P=0.94) or 2013 (P=0.29). Density was not a significant effect in 2012 (P=0.37) or 2013 (P=0.81). There was no significant species-density interaction in 2012 (P=0.22) or in 2013 (P=0.81). Multiple comparisons tests revealed that the treatment combinations were not significantly different from each other in 2012 or 2013.

4.3.1.10 Spring seedling survival to maturity (σ_{sdl}), Saskatchewan

σ_{sdl} was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 4 of 12 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 12 treatment combinations for the low (pasture) disturbance regime (Table 4-9). In the high disturbance regime, the species effect was not significant in 2011 (P=0.43), 2012 (P=0.91),

or 2013 (0.61). The density effect was significant in 2011 (P=0.01) and 2013 (P=0.01), but not 2012 (P=0.09). There was a significant species-density interaction in 2011 (P<0.01), but not in 2012 (P=0.19) or 2013 (P=0.76). Multiple comparisons tests revealed that none of the treatment combinations were statistically different from each other in any year.

In the medium disturbance regime, species was not a significant effect in 2011 (P=0.91). Density was not a significant effect in 2011 (P=0.09). There was no species-density interaction in 2011 (P=0.27).

4.3.1.11 Spring seedling survival to maturity (σ_{sdl}), Ontario

σ_{sdl} was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 0 of 12 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 12 treatment combinations for the low (pasture) disturbance regime (Table 4-9). In the high disturbance regime, species was not a significant effect in 2011 (P=0.19), 2012 (P=0.82), or 2013 (P=0.14). Density was not a significant effect in 2011 (P=91) or 2012 (P=0.11), but it was in 2013 (P=0.007). There was no species-density interaction effect in 2011 (P=0.64), 2012 (P=0.17), or 2013 (P=0.50). Multiple comparisons tests revealed that there were no significant differences between the treatment combinations in 2011 or 2012, but in 2013 high density OP canola was significantly lower than low density hybrid canola and low density OP canola, but not statistically different from high density hybrid canola.

4.3.1.12 Spring seedling survival to maturity (σ_{sdl}), Québec

σ_{sdl} was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 10 of 12 treatment combinations for the medium (ruderal) disturbance regime, and 8 of 12 treatment combinations for the low (pasture) disturbance regime (Table 4-9). In the high disturbance regime, species was not a significant effect in 2011 (P=0.52), 2012 (P=0.25), or 2013 (P=0.20). Density was not a significant effect in 2011 (P=0.14), but it was in 2012 (P=0.003) and 2013 (P=0.007). There was no species-density interaction in 2011 (P=0.08) or 2012 (P=0.43), but there was in 2013 (P<0.001). Multiple comparisons tests revealed that there were no statistically significant differences between the treatment combinations in 2011.However, in 2012 and 2013, low density and high density OP canola were significantly different from each other, but not significantly different from the other treatment combinations. In 2012, low density OP canola was significantly higher than high density OP canola, and in 2013 the reverse was true.

In the medium disturbance regime, species was not a significant effect in 2011 (P=0.26) or 2013 (P=0.09), and in 2012 no OP canola seedlings survived to maturity. Density was a significant effect in 2011 (P=0.02), but not in 2012 (P=0.71) or 2013 (P=0.60). There was no significant species-density interaction in 2011 (P=0.42), but there was in 2013 (P=0.03). Multiple comparisons tests revealed that none of the treatment combinations were significantly different in any year.

In the low disturbance regime, species was not a significant effect in 2011 (P=0.16) or in 2013 (P=0.69), and in 2012 no seedlings survived until maturity. Density was not a significant effect in 2011 (P=0.43) or in 2013 (P=0.88). There was no significant species-density interaction in 2011 (P=0.96) but there was in 2013 (P=0.009). Multiple comparisons tests revealed that none of the treatment combinations were significantly different in any year.

Spring seedling survival was always substantive in the agricultural sites, often in excess of 0.9, and also important in some ruderal areas of Alberta, Saskatchewan and Ontario and in all regimes in Québec.

4.3.1.13 Fecundity (ϕ), Alberta

ϕ was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 7 of 7 treatment combinations for the medium (ruderal) disturbance regime, and 5 of 8 treatment combinations for the low (pasture) disturbance regime (Table 4-9). In the high disturbance regime, the species effect was not significant in 2011 (P=0.23), 2012 (P=0.24), or 2013 (P=0.61). The density effect was significant in 2011 (P<0.001) and 2013 (P=0.04), but not in 2012 (P=0.45). There was no significant species-density interaction in 2011 (0.67), 2012 (P=0.62), or 2013 (P=0.21). Multiple comparisons tests revealed that in 2011 low density hybrid canola had significantly higher fecundity than any other treatment combination. In 2012, there were no significant differences between the treatment combinations. In 2013, low density OP canola fecundity was significantly higher than high density OP canola, and

neither of these two treatment combinations were different from the hybrid canola combinations.

In the medium disturbance regime, the species effect was not significant in 2012 (P=0.49) or in 2013 (0.89). In 2013, the data for low density OP canola was missing because zero seedling survived to maturity, so in 2013 the species effect was gauged by the high density hybrid canola – high density OP canola comparison test. Density was not a significant effect in 2012 (P=0.05), or in 2013 (P=0.65). In 2013, the density effect was gauged using the high density hybrid canola – low density hybrid canola comparison test. There was no significant species-density interaction in 2012 (P=0.44), and was not measurable in 2013 because of missing data. Multiple comparisons tests revealed that there were no significant differences between the treatment combinations in 2012 or 2013.

In the low disturbance regime, species was not a significant effect in 2012 (P=0.58) and was not measurable in 2013. Density was a significant effect in 2012 (P=0.046) and was not measurable in 2013. There was no significant species-density interaction in 2012 (P=0.30). Multiple comparisons tests revealed that none of the treatment combinations were significantly different from each other.

4.3.1.14 Fecundity (ϕ), Saskatchewan

ϕ was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 2 of 4 treatment combinations for the medium (ruderal) disturbance regime, and no seedlings survived to maturity in the low (pasture) disturbance regime (Table 4-9). In the high

disturbance regime, the species effect was significant in 2011 (P=0.001), but not in 2012 (P=0.81) or 2013 (P=0.24). The density effect was not significant in 2011 (P=0.11), 2012 (P=0.98), or 2013 (P=0.26). There was no species-density interaction in 2011 (P=0.91), 2012 (P=0.18), but it was significant in 2013 (P<0.001). Multiple comparisons test revealed that in 2011, low density hybrid canola was significant larger than high density OP canola low density OP canola, but no different than high density hybrid canola.

In the medium disturbance regime the species effect was not measurable because OP canola did not survive until maturity. High density hybrid canola was significantly smaller than low density hybrid canola (P=0.003).

4.3.1.15 Fecundity (ϕ), Ontario

ϕ was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, and seedlings did not survive to maturity in the medium or low disturbance regimes (Table 4-9). In the high disturbance regime, the species effect was not significant in 2011 (P=0.20), 2012 (P=0.45), or in 2013 (P=0.53). The density effect was significant in 2011 (P<0.001) and in 2012 (P=0.03), but not in 2013 (P=0.24). There was no significant species-density interaction in 2011 (P=0.74) or 2013 (P=0.93), but there was in 2012 (P=0.04). Multiple comparisons test revealed that high density hybrid and OP canola were significantly smaller than low density hybrid canola and OP canola in 2011, but there were no other significant differences in any year.

4.3.1.16 Fecundity (ϕ), Québec

ϕ was measured (non-zero values in at least one replicate) in 12 of 12 treatment combinations (species and year) for the high (agricultural) disturbance regime, 5 of 10 treatment combinations for the medium (ruderal) disturbance regime, and 0 of 8 treatment combinations for the low (pasture) disturbance regime (Table 4-9). In the high disturbance regime, there was no significant species effect in 2011 (P=0.28) or in 2012 (P=0.67) but there was in 2013 (P=0.007). Density was not significant in 2011 (P=0.88), 2012 (P=0.06), or 2013 (P=0.06). There were no significant species-density interactions in 2011 (P=0.18), 2012 (P=0.25), or 2013 (P=0.50). Multiple comparisons tests revealed that there were no significant differences between the treatment combinations in 2011 or 2012, but in 2013 low density OP canola was significantly larger than high density hybrid canola, but no different from the other treatment combinations.

In the medium disturbance regime, the species effect could not be assessed in 2012, and it was not significant in 2013 (P=0.97). The density effect was not significant in 2012 (P=0.08) or 2013 (P=0.98). There was a significant species-density interaction in 2013 (P=0.04).

ϕ was always substantive in agricultural regimes, but variable, which is not unexpected. Volunteer plants in the absence of control and competition may be highly fecund. As has been previously reported (Crawley et al. 1993; Busi and Powles 2016), seed set is rare in ruderal and natural areas.

4.3.2 Stochastic growth rate, elasticity, probability of and time to quasi-extinction

In the high disturbance regime, both cultivars had positive growth rates at the Alberta and Ontario sites, (λ_s 8.68 to 11.15 and 30.21 to 235.55, respectively), while the OP canola has a positive growth rate in Québec (λ_s 1.94 to 3.38) and neither cultivar has a positive growth rate in Saskatchewan (Table 4-10). A much lower ϕ was observed in Saskatchewan and Quebec, as well as fatal fall germination in Québec that may have resulted in lower values of λ_s. Additionally, overwinter survival data was limited for Saskatchewan and Quebec.

In the high disturbance regime, the probability of extinction varied from <0.01% to 100%. Growth rates were highly elastic to LLP that contributed to above ground pathways ($\sigma_w, \sigma_{sdl}$and ϕ) (Table 4-10). The years to extinction (T_q) were highly variable, from a low of 1 in Alberta to 106 in Saskatchewan. Positive population growth for crops within agricultural regimes should surprise no one, and could be considered a low-risk scenario. In the absence of control in the agricultural environment, canola may persist because it is adapted to high resource environments. Simard et al. (2002) reported the survival of canola populations 5 years following a canola crop. Similarly, Beckie and Warwick (2010) conducted a survey of canola populations in fields following the removal of a canola type and reported that canola volunteers were present 7 years after the cultivar was withdrawn from cultivation. While it is difficult to compare European persistence studies with Canadian, Lutman et al. (2003) reported oilseed rape persistence in fields for 11 years while Belter (2016) reported persistence of GM canola in field where released

experimentally for up to 15 years following cultivation. In non-agricultural environments, T_q will vary based on the extinction threshold chosen as well as σ_b.

In the Alberta high disturbance regime, hybrid canola had a slightly higher stochastic population growth rate than OP canola, but the absolute effect size was slight (Table 4-10). In Ontario and Québec, OP canola had a larger stochastic population growth rate than hybrid canola, and the differences were much more substantial than in Alberta.

In the low and medium disturbance regimes, stochastic population growth rates reveal that neither cultivar has a positive population growth rate in any site, except for OP canola in the Alberta medium disturbance regime, high density (Table 4-10). The data forecast a 100% probability that populations in the medium and low disturbance regimes (with the exception of the aforementioned high density OP canola in Alberta) will extinguish in time (P_q), (Table 4-10). The predicted average time to extinction for populations in the medium and low disturbance regimes (T_q) is generally between 4-5 years, however some populations have upper-bound estimates of 10 or 45 years. These values are similar to those predicted by Garnier et al. (2006) for feral population in France; persistence up to 5 years in the absence of seed addition, and up to 10 years if there were a low level of seed addition. Busi and Powles (2016) reported canola populations in Australia persisting at least 3 years on a roadside following a spill close to a collection facility, but not in natural areas. The stochastic population growth rate was highly elastic to σ_b. Seed dormancy and the ability to develop a seedbank appear to be a key trait for feral species including canola. Models developed to predict the persistence of feral populations demonstrate that seed

persistence is a key trait driving population persistence (Crawley et al. 1993; Garnier and Lecomte 2006), especially when environmental stochasticity is considered (Claessen et al. 2005a, 2005b).

Genetic changes that alter seedbank survival may have large effects on the stochastic growth rate in the medium and low disturbance regimes, and future studies on roadsides populations should focus on seed bank dynamics.

4.3.3 Assessment and comparison of hybrid and OP canola

One-tailed hypothesis tests place each hybrid-OP comparisons within 1 of 4 assessment endpoint scenarios (Table 4-11). Our estimates place the majority of comparisons within the 1st scenario: both cultivars are expected to extinguish. This is evidence supporting the notion that the hybrid cultivar is low risk as it will extinguish eventually. The T_q is generally quite similar between hybrid and OP cultivars, around 4-5 years in most sites, disturbances and densities. In most cases, OP canola has a higher (sometimes substantially higher) T_q upper bound. In fact, in only 1 of the 16 quadrant 1 cases does hybrid canola have a larger T_q upper bound than OP canola (Alberta, medium disturbance, low density), even though the mean T_q values are equal.

A handful of comparisons fall into the 3rd scenario: the OP cultivar has a positive growth rate whereas the hybrid cultivar is negative. Given the biological importance of this difference, the hybrid cultivar should be considered low risk in these scenarios.

Finally, in the high disturbance regime in Alberta and Ontario we find that the comparisons fall into the 4th quadrant: both populations have positive growth rates. Given the nature of the high disturbance regime (agricultural land), we argue that positive population growth rates within this regime are low risk. Nevertheless, the cultivars can be evaluated based on effect size and equivalence testing. In Alberta, in the high disturbance regime, high-density hybrid canola has a smaller growth rate than high-density OP canola, and vice versa for the low density treatment. Neither the high nor the low density combinations are equivalent at 50% due to a substantial amount of variation in the estimates. In Ontario, hybrid canola has a much more negative population growth rate than OP canola in both the high and low density, and the combinations are not equivalent at 50%.

We did not observe any combinations that fell into the 2nd quadrant, the high risk quadrant. The controversial result from chapter 3 (2013 medium disturbance, low density) seems to have disappeared in the stochastic analysis. Data sets spanning many more years would help determine how common controversial scenario 2 results are, and could affect stochastic estimates.

Because we only ever observed positive stochastic growth rates for hybrid canola in the agricultural environment, and because in these cases OP canola had a larger stochastic growth rate in 3 of 4 cases, we determine that the risk of hybrid canola becoming weedy or invasive is relatively low, and certainly no more than OP canola.

4.3.4 Null hypothesis testing vs. equivalence testing

The structure of the null hypothesis influences the decision outcome when comparing the growth rates for the two cultivars (Table 4-11). Hybrid and OP canola are different cultivars with different survival and fecundity rates. The purpose of comparing them is not to prove that they are different, but rather to prove that their stochastic population growth rates are similar enough. In some cases, the result of hypothesis testing may be the same between the null hypothesis testing, in which means and confidence intervals are compared, and equivalence testing, where the level of precision if established. In 18 of the 24 combinations, the null hypothesis test failed to detect a difference between the cultivars, possibly due to the high amount of variation in most cases. However, the equivalence test is much more discerning and is able to determine at what percentage the cultivars could be considered equivalent. Equivalence testing provides much more nuanced comparison, but both null and equivalence tests must still consider effect size and precision. Increased experimental replication, and therefore increased precision, should narrow the confidence intervals for both hypotheses and result in higher confidence in the results of hypothesis tests.

For a short-term forecast, we recommend making $\boldsymbol{T}$ a function of the number of study years. Relating the short-term forecast to the number of experimental years might lead proponents to invest in collecting much larger datasets that span across many years. In this analysis we use $\boldsymbol{T} = 3$ to calculate confidence intervals for $log\lambda_s$, elasticities, probability of quasi-extinction (P_q), and time to quasi-extinction (T_q). While we are not staunch advocates

for setting $\boldsymbol{T} = \#study\ years$, we do feel that it's important to set $\boldsymbol{T}$ as a function of the number of study years.

As with any experiment, the results may be considered predictive under the context of the sites, disturbances, and years in which the data was collected. To extend the results to sites, disturbances, or years for which we did not take data requires the assumption that those sites, environments, and years are similar enough to those in which the data was collected. Stochastic calculations of population growth cannot extend beyond the data that was actually observed. Therefore, we can conclude two things: First, datasets spanning many years and environments will be more robust than those that span just a few, and second, climate change may cause datasets to become obsolete.

Our goal was to compare the invasiveness potential of hybrid and OP canola cultivars using a stochastic analysis. The threshold for substantial equivalence should be established *a priori*. However, we find that in most cases (14 of 24) hybrid canola and OP canola are substantially equivalent at the 50% level, and in 8 of 24 cases they are substantially equivalent at the 1% level. In only 4 of 24 cases does hybrid canola have a positive stochastic population growth rate, and these cases are all in agricultural regimes, leading us to assess it as low risk. In all other cases, hybrid canola has a negative population growth rate and a comparable T_q to OP canola. We suggest that while population growth rate is useful for determining the potential for invasiveness, the assessment endpoint of substantial equivalence may require a more strict definition to be useful.

Table 4-1. Four possible assessment endpoint scenarios based on the $\boldsymbol{log\lambda}$ of the GM crop and that of the comparator cultivar.

Comparator $(\lambda_1)\backslash GM(\lambda_2)$	$log\lambda_2 < 0$	$log\lambda_2 \geq 0$
$log\lambda_1 < 0$	1. Neither cultivar has a positive population growth rate. Differences will result in different rates of persistence but both populations are ephemeral.	2. The GM cultivar has a positive population growth rate whereas the comparator cultivar does not. This should be enough evidence to delay or halt the release of the GM cultivar; the effect is biologically significant.
$log\lambda_1 \geq 0$	3. The comparator cultivar has a positive population growth rate whereas the GM cultivar has a negative population growth rate. This may not be sufficient evidence to prove that the GM crop is safe. However, it is certainly not evidence that the GM crop is more invasive than the comparator.	4. Both cultivars have positive population growth rates. How much more or less positive is the GM population growth rate? Is there an acceptable assessment endpoint? Can we allow the GM cultivar to have a higher population growth rate than the common cultivar?

Table 4-2. Soil characteristics for Alberta, Saskatchewan, Ontario, and Québec disturbance regimes in 2011, 2012, and 2013.

Location	Year	Disturbance level	Soil texture	Soil OM %	Soil pH	EC dS m^{-1}
St. Albert, AB	2011	Agricultural/High	Clay	11	6.9	0.45
		Ruderal/Medium	Clay Loam	11.4	7	0.23
		Natural/Low	Clay	10.4	7.1	0.49
	2012	Agricultural/High	Clay	9.2	7	0.28
		Ruderal/Medium	Clay Loam	11.9	6.7	0.23
		Natural/Low	Clay	10.9	7.4	0.29
	2013	Agricultural/High	Silty Clay	11.6	7.4	0.7
		Ruderal/Medium	Clay	11.1	7.5	0.54
		Natural/Low	Clay Loam	9.6	6.7	0.28
Kenaston, SK[a]	2011	Agricultural/High	Loam	2.4	7.6	0.22
		Ruderal/Medium	Clay Loam	1.5	8.5	0.22
		Natural/Low	Loam	3.4	7	0.11
	2012	Agricultural/High	Loam	2.4	7.6	0.22
		Ruderal/Medium	Clay Loam	1.5	8.5	0.22
		Natural/Low	Loam	3.4	7	0.11
	2013	Agricultural/High	Loam	2.4	7.6	0.22
		Ruderal/Medium	Clay Loam	1.5	8.5	0.22
		Natural/Low	Loam	3.4	7	0.11
J. C. Chapais, QB[b]	2011	Agricultural/High	Sandy clay loam	4.71	5.41	68
		Ruderal/Medium	.	.	.	.
		Natural/Low	.	.	.	.
	2012	Agricultural/High	Sandy clay loam	4.71	5.41	68
		Ruderal/Medium	.	.	.	.
		Natural/Low	.	.	.	.
	2013	Agricultural/High	Sandy clay loam	4.71	5.41	68
		Ruderal/Medium	.	.	.	.
		Natural/Low	.	.	.	.
Harrow, ON[b]	2011	Agricultural/High	Sandy loam	2.6	6.4	.
		Ruderal/Medium	.	.	.	.
		Natural/Low	.	.	.	.
	2012	Agricultural/High	Sandy loam	2.6	6.4	.
		Ruderal/Medium	.	.	.	.
		Natural/Low	.	.	.	.
	2013	Agricultural/High	Sandy loam	2.6	6.4	.
		Ruderal/Medium	.	.	.	.
		Natural/Low	.	.	.	.

[a] Data only taken one year

[b] Data only taken one year and only in the Agricultural/High disturbance

Table 4-3. Precipitation for Alberta, Saskatchewan, Ontario, and Québec in 2011, 2012, 2013, and 2014.

		Precipitation mm (% of Long term average)											
Site	Year	January	February	March	April	May	June	July	August	September	October	November	December
St. Albert, AB	2011	34(237)	7(89)	12(83)	10(37)	12(27)	140(241)	114(124)	20(43)	14(49)	15(90)	8(70)	6(50)
	2012	8(51)	6(70)	8(54)	42(164)	46(108)	28(47)	135(147)	28(60)	14(46)	20(119)	22(202)	15(138)
	2013	14(96)	10(127)	22(163)	21(81)	36(85)	124(214)	87(95)	109(229)	9(31)	13(78)	18(164)	20(179)
	2014	13(89)	4(51)	7(51)	35(135)	56(133)	61(105)	114(124)	22(45)	18(61)	8(45)	20(183)	4(37)
Kenaston, SK	2011	19(172)	8(114)	5(36)	11(35)	59(129)	136(164)	54(94)	53(87)	17(65)	24(98)	8(59)	14(126)
	2012	3(30)	2(23)	8(54)	45(144)	100(220)	27(33)	61(108)	18(30)	0(2)	16(66)	28(200)	12(104)
	2013	15(136)	10(130)	15(112)	11(34)	11(24)	72(88)	42(74)	24(40)	42(162)	4(18)	11(79)	10(93)
	2014	6(50)	3(35)	14(100)	62(198)	37(82)	208(252)	20(35)	135(222)	31(119)	23(94)	19(131)	5(45)
Harrow, ON	2011	84(160)	109(190)	100(175)	132(196)	177(240)	72(112)	142(204)	107(147)	176(263)	96(163)	191(291)	90(127)
	2012	53(101)	46(79)	54(95)	27(40)	75(101)	35(55)	74(106)	87(119)	48(71)	57(97)	16(24)	51(72)
	2013	103(195)	72(125)	13(22)	106(158)	57(77)	162(250)	214(307)	74(101)	64(96)	81(138)	36(55)	65(92)
	2014	47(89)	76(132)	39(68)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
J. C. Chapais, QB	2011	29(45)	94(157)	173(247)	110(119)	130(137)	87(73)	131(115)	171(160)	107(96)	78(73)	63(75)	114(112)
	2012	96(149)	62(103)	59(85)	69(74)	122(128)	179(151)	94(82)	66(62)	71(64)	146(136)	13(16)	129(126)
	2013	73(113)	68(113)	66(94)	73(79)	260(273)	112(95)	125(110)	92(86)	66(59)	102(95)	118(140)	91(89)
	2014	93(144)	55(92)	76(108)	132(142)	72(76)	106(90)	138(121)	169(158)	105(94)	127(118)	80(96)	108(106)

Long term averages calculated over 2000-2015 except Harrow which was 2001-2014.

Table 4-4. Air temperature in Alberta, Saskatchewan, Ontario, and Québec in 2011, 2012, 2013, and 2014.

		Air Temperature Degrees Celcius (Long term average)											
Site	Year	January	February	March	April	May	June	July	August	September	October	November	December
St. Albert, AB	2011	-11 (-9)	-11 (-8)	-9 (-4)	3 (5)	13 (11)	15 (16)	17 (19)	17 (17)	14 (12)	7 (5)	-4 (-3)	-3 (-10)
	2012	-7 (-9)	-6 (-8)	-1 (-4)	5 (5)	12 (11)	17 (16)	20 (19)	18 (17)	14 (12)	2 (5)	-7 (-3)	-14 (-10)
	2013	-9 (-9)	-4 (-8)	-6 (-4)	1 (5)	14 (11)	15 (16)	17 (19)	18 (17)	14 (12)	6 (5)	-6 (-3)	-13 (-10)
	2014	-6 (-9)	-15 (-8)	-7 (-4)	4 (5)	10 (11)	15 (16)	20 (19)	18 (17)	12 (12)	8 (5)	-7 (-3)	-7 (-10)
Kenaston, SK	2011	-6 (-4)	-5 (-3)	1 (2)	8 (9)	15 (14)	21 (20)	25 (23)	22 (21)	17 (18)	11 (11)	7 (5)	2 (-1)
	2012	-1 (-4)	0 (-3)	8 (2)	8 (9)	17 (14)	21 (20)	23 (23)	21 (21)	17 (18)	11 (11)	4 (5)	2 (-1)
	2013	-2 (-4)	-3 (-3)	1 (2)	7 (9)	16 (14)	20 (20)	22 (23)	21 (21)	17 (18)	12 (11)	3 (5)	-2 (-1)
Harrow, ON	2011	-10 (-12)	-10 (-10)	-4 (-4)	3 (4)	11 (12)	17 (17)	21 (20)	19 (18)	16 (14)	8 (7)	3 (1)	-6 (-8)
	2012	-11 (-12)	-9 (-10)	-1 (-4)	5 (4)	13 (12)	17 (17)	20 (20)	20 (18)	14 (14)	8 (7)	-1 (1)	-6 (-8)
	2013	-12 (-12)	-9 (-10)	-2 (-4)	4 (4)	12 (12)	15 (17)	20 (20)	18 (18)	13 (14)	8 (7)	-1 (1)	-12 (-8)
	2014	-13 (-12)	-13 (-10)	-11 (-4)	2 (4)	12 (12)	17 (17)	19 (20)	18 (18)	13 (14)	9 (7)	-1 (1)	-6 (-8)
J. C. Chapais, QB	2011	-15 (-14)	-15 (-14)	-10 (-6)	3 (4)	11 (10)	15 (15)	20 (19)	19 (18)	14 (13)	7 (4)	-5 (-5)	-6 (-13)
	2012	-10 (-14)	-8 (-14)	1 (-6)	5 (4)	11 (10)	17 (15)	21 (19)	18 (18)	13 (13)	2 (4)	-7 (-5)	-16 (-13)
	2013	-15 (-14)	-10 (-14)	-12 (-6)	-4 (4)	13 (10)	16 (15)	18 (19)	19 (18)	15 (13)	4 (4)	-7 (-5)	-18 (-13)
	2014	-15 (-14)	-20 (-14)	-11 (-6)	2 (4)	11 (10)	14 (15)	18 (19)	18 (18)	12 (13)	6 (4)	-8 (-5)	-9 (-13)

Long term averages calculated over 2000-2015 except Harrow which was 2001-2014.

Table 4-5. Experiment timings (initiation, harvest, end date) for Alberta, Saskatchewan, Ontario, and Québec.

			Planting date		Harvest date	End date
Location	Year	Disturbance level	Spring Experiment	Fall Experiment	(Spring)	(Fall, follwing year)
St. Albert, AB	2011	Agricultural/High	May 19	Sep 22	Oct 05	Sep 06
St. Albert, AB	2011	Ruderal/Medium	May 19	Sep 22	Oct 05	Sep 06
St. Albert, AB	2011	Natural/Low	May 19	Sep 22	Oct 05	Sep 06
St. Albert, AB	2012	Agricultural/High	May 03	Oct 12	Oct 11	Jul 18
St. Albert, AB	2012	Ruderal/Medium	May 03	Oct 12	Oct 11	Jul 18
St. Albert, AB	2012	Natural/Low	May 03	Oct 12	Oct 11	Jul 18
St. Albert, AB	2013	Agricultural/High	May 09	Oct 15	Oct 10	May 13
St. Albert, AB	2013	Ruderal/Medium	May 09	Oct 15	Oct 10	May 13
St. Albert, AB	2013	Natural/Low	May 09	Oct 15	Oct 10	May 13
Kenaston, SK[a]	2011	Agricultural/High	May 09	Oct 13	Aug 01	May 30
Kenaston, SK[a]	2011	Ruderal/Medium	May 09	Oct 13	Aug 01	May 30
Kenaston, SK[a]	2011	Natural/Low	May 09	Oct 13	Aug 01	May 30
Kenaston, SK[a]	2012	Agricultural/High	May 23	Oct 13	Aug 25	*
Kenaston, SK[a]	2012	Ruderal/Medium	May 23	Oct 13	Aug 25	*
Kenaston, SK[a]	2012	Natural/Low	May 23	Oct 13	Aug 25	*
Kenaston, SK[a]	2013	Agricultural/High	Apr 30	-	Jul 18	-
Kenaston, SK[a]	2013	Ruderal/Medium	Apr 30	-	Jul 18	-
Kenaston, SK[a]	2013	Natural/Low	Apr 30	-	Jul 18	-
Harrow, ON	2011	Agricultural/High	Jun 13	Oct 25	Aug 08	Apr 13
Harrow, ON	2011	Ruderal/Medium	May 12	Oct 25	Aug 04	Apr 13
Harrow, ON	2011	Natural/Low	May 13	-	Aug 05	-
Harrow, ON	2012	Agricultural/High	May 02	Nov 06	Jul 25	May 13
Harrow, ON	2012	Ruderal/Medium	May 02	Nov 07	Jul 25	May 06
Harrow, ON	2012	Natural/Low	May 02	-	Jul 25	-
Harrow, ON	2013	Agricultural/High	May 13	Oct 31	Jul 25	May 29
Harrow, ON	2013	Ruderal/Medium	May 13	Oct 31	Jul 25	May 29
Harrow, ON	2013	Natural/Low	May 13	-	Jul 25	-
J. C. Chapais, QB	2011	Agricultural/High	May 04	Nov 02	Oct 04	Apr 30
J. C. Chapais, QB	2011	Ruderal/Medium	May 04	Nov 02	Oct 04	Apr 30
J. C. Chapais, QB	2011	Natural/Low	May 04	Nov 02	Oct 04	Apr 30
J. C. Chapais, QB	2012	Agricultural/High	May 10	-	Oct 02	-
J. C. Chapais, QB	2012	Ruderal/Medium	May 10	-	Oct 02	-
J. C. Chapais, QB	2012	Natural/Low	May 10	-	Oct 02	-
J. C. Chapais, QB	2013	Agricultural/High	May 08	-	Oct 07	-
J. C. Chapais, QB	2013	Ruderal/Medium	May 08	-	Oct 07	-
J. C. Chapais, QB	2013	Natural/Low	May 08	-	Oct 07	-

*' indicates that dataset comprised only of zeros

-' indicates that data was not taken

Table 4-6. Lower-level parameter calculations from count data.

LLP	*LLP calculation*	*Experiment*
g_f	(#seedlings recruited in the fall) / (#seeds planted in the fall)	Fall
σ_w	(#seedlings recruited in the spring) / (#seeds remaining in the seedbank after fall recruitment)	Fall
σ_f	(#seedlings that overwinter as seedlings) / (#seedlings recruited in the fall)	NA
σ_b	(#seeds alive in the fall) / (#seeds remaining in the seedbank after spring recruitment))	Spring
σ_{sdl}	(#mature plants in the fall) / (#seedlings recruited in the spring)	Spring
φ	(#seeds produced) / (#mature plants in the fall)	Spring

Table 4-7. Lower-level parameter estimates/ranges based on literature values.

Species	*Cultivar*	*Maximum fecundity*	*Seed dormancy*	*Over summer survival*	σ_b *used*	*Over winter survival*	*References*
		(seeds/plant)		%		%	
Brassica napus	Barrier	3000	No primary dormancy	~0 (at 1cm depth)	0.3	40-70%	Gruber and Claupein 2007; OECD 1999)
	Dekalb 7345	3,480 (730)	Inducible secondary dormancy	~20-30% at 10cm depth)		(depends on snow cover)	

Table 4-8. Estimates, standard error, and 95% confidence intervals for the fall experiment lower level parameters for Alberta, Saskatchewan, Ontario, and Québec in all disturbance regimes, years, species, and densities.

					LowerLevel Parameters																							
					σ_w									g_f									σ_f					
					2011			2012			2013			2011			2012			2013			2011			2012			2013		
Site	Disturbance	Density		Species	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.
Alberta	High	High		Hybrid Canola	0.04 (0.02) a	0.02	0.09	0.05 (0.02) a	0.03	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0.07 (0.03) a	0.04	0.14	0.10 (0.03) a	0.06	0.17	0.02 (0.01) a	0.01	0.12	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola																											
				OP Canola																											
	Medium	High		Hybrid Canola	0	0	0	0	0	0	0.01 (0.01)	0.01	0.07	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0.06 (0.02)	0.03	0.12	0.01 (0.01)	0.01	0.1	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola																											
				OP Canola																											
	Low	High		Hybrid Canola	0.04 (0.02) a	0.02	0.1	0.02 (0.02) a	0.01	0.08	0.01 (0.01) a	0.01	0.04	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0.06 (0.03) a	0.03	0.13	0.06 (0.04) a	0.02	0.19	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola																											
				OP Canola																											
Saskatchewan	High	High		Hybrid Canola	0	0	0	0	0	0	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	.	.	.	NA	NA	NA	NA	NA	NA
				OP Canola	0.02 (0.01)	0.01	0.06	0	0	0	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	.	.	.	NA	NA	NA	NA	NA	NA
			Low	Hybrid Canola																											
				OP Canola																											
	Medium	High		Hybrid Canola	0	0	0	0	0	0	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	.	.	.	NA	NA	NA	NA	NA	NA
				OP Canola	0	0	0	0	0	0	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	.	.	.	NA	NA	NA	NA	NA	NA
			Low	Hybrid Canola																											
				OP Canola																											
	Low	High		Hybrid Canola	0	0	0	0	0	0	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	.	.	.	NA	NA	NA	NA	NA	NA
				OP Canola	0	0	0	0	0	0	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	.	.	.	NA	NA	NA	NA	NA	NA
			Low	Hybrid Canola																											
				OP Canola																											
Ontario	High	High		Hybrid Canola	0.05 (0.02) a	0.02	0.12	0.14 (0.03) b	0.1	0.19	0.04 (0.01) b	0.02	0.07	0.33 (0.03) a	0.28	0.39	0.23 (0.03) a	0.19	0.29	0	0	0	0.39 (0.05) a	0.29	0.5	0.03 (0.02) a	0.01	0.12	.	.	.
				OP Canola	0.10 (0.04) a	0.06	0.2	0.25 (0.03) a	0.2	0.31	0.21 (0.03) a	0.17	0.26	0.15 (0.02) b	0.12	0.2	0.15 (0.02) b	0.11	0.2	0	0	0	0.44 (0.08) a	0.3	0.6	0.07 (0.04) a	0.02	0.21	.	.	.
			Low	Hybrid Canola																											
				OP Canola																											
	Medium	High		Hybrid Canola	0.01 (0.01) a	0.01	0.04	0.04 (0.02) b	0.02	0.07	0.02 (0.01) b	0.01	0.04	0.18 (0.03) a	0.14	0.23	0.23 (0.03) a	0.18	0.28	0.14 (0.02) b	0.1	0.19	0.13 (0.05) a	0.06	0.26	0	0	0	0	0	0
				OP Canola	0.03 (0.02) a	0.02	0.07	0.09 (0.02) a	0.06	0.13	0.07 (0.02) a	0.05	0.12	0.08 (0.02) b	0.06	0.12	0.11 (0.02) b	0.08	0.16	0.27 (0.03) a	0.22	0.33	0.20 (0.08) a	0.08	0.43	0	0	0	0	0	0
			Low	Hybrid Canola																											
				OP Canola																											
	Low	High		Hybrid Canola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola																											
				OP Canola																											
Quebec	High	High		Hybrid Canola	0	0	0	NA	NA	NA	NA	NA	NA	0.39 (0.03) a	0.33	0.45	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA
				OP Canola	0.03 (0.01)	0.01	0.06	NA	NA	NA	NA	NA	NA	0.05 (0.02) b	0.03	0.08	NA	NA	NA	NA	NA	NA	0.08 (0.08)	0.01	0.55	NA	NA	NA	NA	NA	NA
			Low	Hybrid Canola																											
				OP Canola																											
	Medium	High		Hybrid Canola	0	0	0	NA	NA	NA	NA	NA	NA	0.02 (0.01) a	0.01	0.05	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA
				OP Canola	0	0	0	NA	NA	NA	NA	NA	NA	0.02 (0.01) a	0.01	0.04	NA	NA	NA	NA	NA	NA	1	1	1	NA	NA	NA	NA	NA	NA
			Low	Hybrid Canola																											
				OP Canola																											
	Low	High		Hybrid Canola	0.01 (0.01) a	0.01	0.03	NA	NA	NA	NA	NA	NA	0.01 (0.01)	0.01	0.04	NA	NA	NA	NA	NA	NA				NA	NA	NA	NA	NA	NA
				OP Canola	0.03 (0.01) a	0.02	0.06	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA				NA	NA	NA	NA	NA	NA
			Low	Hybrid Canola																											
				OP Canola																											

A value of zero indicates that we observed no individuals in any replicate for a treatment combination at the sample size used.
A "." indicates that values could not be measured due to zero survival at a previous transition.
Mean (Standard Error)
ilink backtransformed 95% Confidence Intervals (L.C.I. and U.C.I)
Letters within columns, sites, and disturbance levels indicate statistically different means

Table 4-9. Estimates, standard error, and 95% confidence intervals for the spring experiment lower level parameters for Alberta, Saskatchewan, Ontario, and Québec in all disturbance regimes, years, species, and densities.

					LowerLevel Parameters															
					σ_{sdl}									ϕ						
					2011			2012			2013			2011			2012			2013		
Site	Disturbance	Density		Species	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.	mean (se)	L.C.I.	U.C.I.
Alberta	High	High		Hybrid Canola	0.90 (0.04) a	0.79	0.96	0.48 (0.09) a	0.32	0.65	0.48 (0.11) a	0.27	0.7	1489 (392) a	850	2609	1431 (688) a	533	3848	33 (13) ab	15	72
				OP Canola	0.64 (0.08) b	0.46	0.79	0.42 (0.09) a	0.25	0.62	0.61 (0.11) a	0.38	0.8	1227 (324) a	700	2151	868 (417) a	323	2333	15 (6) a	7	34
			Low	Hybrid Canola	0.65 (0.13) ab	0.36	0.85	0.65 (0.13) a	0.37	0.86	0.29 (0.11) a	0.12	0.55	4157 (1092) b	2375	7276	1088 (523) a	405	2926	37 (14) ab	17	82
				OP Canola	0.53 (0.11) b	0.31	0.73	0.26 (0.12) a	0.09	0.55	0.62 (0.13) a	0.35	0.83	2395 (639) a	1356	4229	499 (340) a	124	2023	51 (21) b	22	120
	Medium	High		Hybrid Canola	0	0	0	0.05 (0.03) a	0.02	0.13	0.13 (0.16) a	0.01	0.76	.	.	.	24 (13) a	8	74	145 (227) a	1	21142
				OP Canola	0	0	0	0.05 (0.03) a	0.02	0.16	0.02 (0.03) a	0.01	0.4	.	.	.	28 (26) a	4	195	44 (79) a	1	13735
			Low	Hybrid Canola	0	0	0	0.04 (0.03) a	0.02	0.16	0.20 (0.23) a	0.01	0.85	.	.	.	4 (3) a	1	18	267 (432) a	2	46219
				OP Canola	0	0	0	0.10 (0.06) a	0.03	0.32	0	0	0	.	.	.	9 (6) a	3	36	.	.	.
	Low	High		Hybrid Canola	0	0	0	0.07 (0.04) a	0.03	0.2	0.01 (0.01) a	0.01	0.09	.	.	.	3 (1) a	2	5	0	0	0
				OP Canola	0	0	0	0.04 (0.03) a	0.01	0.15	0.04 (0.04) a	0.01	0.24	.	.	.	4 (2) a	2	8	1 (1)	1	2
			Low	Hybrid Canola	0	0	0	0.06 (0.04) a	0.02	0.22	0.01 (0.01) a	0.01	0.13	.	.	.	6 (2) a	3	12	0	0	0
				OP Canola	0	0	0	0.10 (0.07) a	0.03	0.34	0.03 (0.03) a	0.01	0.25	.	.	.	6 (2) a	4	11	0	0	0
Saskatchewan	High	High		Hybrid Canola	0.89 (0.07) a	0.68	0.97	0.58 (0.18) a	0.23	0.87	0.93 (0.07) a	0.65	0.99	76 (34) ab	31	187	224 (107) a	84	598	50 (35) a	12	206
				OP Canola	0.35 (0.14) b	0.13	0.66	0.71 (0.15) a	0.35	0.92	0.96 (0.04) a	0.76	1	16 (8) a	6	44	88 (42) a	33	234	29 (20) a	7	120
			Low	Hybrid Canola	0.84 (0.10) ab	0.54	0.96	0.82 (0.14) a	0.41	0.97	0.77 (0.18) a	0.31	0.96	171 (75) b	70	419	99 (48) a	37	265	145 (102) a	35	605
				OP Canola	0.95 (0.04) a	0.78	0.99	0.77 (0.15) a	0.38	0.95	0.87 (0.11) a	0.47	0.99	32 (14) a	13	77	202 (84) a	86	473	48 (34) a	12	199
	Medium	High		Hybrid Canola	0.14 (0.11) a	0.03	0.52	0	0	0	0	0	0	1 (1) b	1	1	.	.	.	.	.	.
				OP Canola	0.08 (0.07) a	0.02	0.39	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.
			Low	Hybrid Canola	0.04 (0.04) a	0.01	0.28	0	0	0	0	0	0	3 (2) a	1	7	.	.	.	.	.	.
				OP Canola	0.05 (0.05) a	0.01	0.36	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.
	Low	High		Hybrid Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
Ontario	High	High		Hybrid Canola	0.85 (0.03) a	0.78	0.89	0.90 (0.04) a	0.81	0.95	0.84 (0.05) ab	0.72	0.92	443 (70) b	314	625	91 (41) a	36	227	2079 (988) a	780	5543
				OP Canola	0.81 (0.04) a	0.73	0.87	0.81 (0.05) a	0.71	0.89	0.64 (0.07) b	0.49	0.77	615 (98) b	436	867	164 (74) a	66	411	2857 (1358) a	1072	7616
			Low	Hybrid Canola	0.87 (0.04) a	0.76	0.93	0.91 (0.05) a	0.76	0.97	0.94 (0.04) a	0.8	0.99	1337 (212) a	948	1887	313 (141) a	125	785	3728 (1771) a	1399	9938
				OP Canola	0.79 (0.06) a	0.66	0.89	0.94 (0.04) a	0.82	0.98	0.91 (0.06) a	0.73	0.97	1639 (260) a	1162	2314	346 (156) a	138	868	4966 (2360) a	1863	13238
	Medium	High		Hybrid Canola	0	0	0	0	0	0	0	0	0									
				OP Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
	Low	High		Hybrid Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
			Low	Hybrid Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
				OP Canola	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.
Quebec	High	High		Hybrid Canola	0.70 (0.14) a	0.36	0.91	0.48 (0.08) ab	0.33	0.64	0.85 (0.06) ab	0.69	0.94	266 (163) a	76	941	257 (102) a	113	581	27 (13) a	11	69
				OP Canola	0.46 (0.17) a	0.17	0.79	0.32 (0.07) b	0.19	0.47	0.86 (0.06) a	0.7	0.94	134 (82) a	38	471	465 (185) a	205	1052	78 (36) ab	30	200
			Low	Hybrid Canola	0.72 (0.15) a	0.36	0.93	0.63 (0.09) ab	0.43	0.79	0.86 (0.07) ab	0.66	0.95	240 (147) a	68	849	860 (341) a	380	1949	49 (23) ab	19	126
				OP Canola	0.68 (0.17) a	0.3	0.92	0.55 (0.10) a	0.35	0.73	0.52 (0.12) b	0.29	0.75	123 (76) a	35	435	665 (264) a	294	1506	256 (118) b	99	661
	Medium	High		Hybrid Canola	0.26 (0.16) a	0.05	0.7	0.06 (0.04)	0.01	0.29	0.62 (0.04) a	0.55	0.68	0	0	0	2 (2)	1	13	1 (1) a	1	2
				OP Canola	0.05 (0.04) a	0.01	0.28	0	0	0	0.64 (0.04) a	0.57	0.7	0	0	0	.	.	.	1 (1) a	1	1
			Low	Hybrid Canola	0.40 (0.22) a	0.08	0.84	0.08 (0.09)	0.01	0.64	0.74 (0.06) a	0.62	0.84	0	0	0	25 (24)	3	241	2 (1) a	1	6
				OP Canola	0.23 (0.16) a	0.04	0.7	0	0	0	0.55 (0.07) a	0.42	0.68	0	0	0	.	.	.	0	0	0
	Low	High		Hybrid Canola	0.12 (0.06) a	0.04	0.31	0	0	0	0.21 (0.04) a	0.14	0.3	0	0	0	.	.	.	0	0	0
				OP Canola	0.30 (0.10) a	0.13	0.56	0	0	0	0.12 (0.04) a	0.07	0.2	0	0	0	.	.	.	0	0	0
			Low	Hybrid Canola	0.17 (0.09) a	0.05	0.46	0	0	0	0.11 (0.05) a	0.05	0.24	0	0	0	.	.	.	0	0	0
				OP Canola	0.34 (0.12) a	0.14	0.63	0	0	0	0.24 (0.06) a	0.15	0.37	0	0	0	.	.	.	0	0	0

A value of zero indicates that we observed no individuals in any replicate for a treatment combination at the sample size used.
A "." indicates that values could not be measured due to zero survival at a previous transition.
Mean (Standard Error)
ilink backtransformed 95% Confidence Intervals (L.C.I. and U.C.I)
Letters within columns, sites, and disturbance levels indicate statistically different means

Table 4-10. Population growth rate and elasticity estimates with 95% confidence intervals for Alberta, Saskatchewan, Ontario, and Québec in all disturbance regimes, years, species, and densities.

					$\log_e(\lambda_s)$			λ_s			Elasticity of λ_s to lower level parameters														P_q			T_q		
											g_f			σ_f			σ_W			σ_{sdl}, ϕ			σ_b			%			#Years		
Site	Disturbance	Density		Species	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.	mean	L.C.I.	U.C.I.
Alberta	High	High		Hybrid Canola	2.16	-1.14	4.07	8.68	0.32	58.47	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.06	0.99	0.68	0.06	0.99	0.32	0.01	0.94	0.05	0.03	100	2	1	13
				OP Canola	2.27	-0.67	4.05	9.70	0.51	57.61	0.00	0.00	0.00	0.00	0.00	0.00	0.78	0.35	0.99	0.78	0.36	1.00	0.22	0.00	0.64	0.01	0.03	100	2	1	10
			Low	Hybrid Canola	2.41	-1.15	4.76	11.15	0.32	116.69	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.05	1.00	0.68	0.05	1.00	0.32	0.00	0.95	0.11	0.01	100	2	1	16
				OP Canola	2.25	-0.15	4.44	9.45	0.86	84.38	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.45	1.00	0.84	0.47	1.00	0.16	0.00	0.53	0.08	0.02	100	2	1	23
	Medium	High		Hybrid Canola	-1.01	-1.21	-0.45	0.36	0.30	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.45	0.13	0.00	0.45	0.87	0.55	1.00	100	100	100	5	4	10
				OP Canola	-0.84	-1.23	0.17	0.43	0.29	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.18	-0.03	0.59	0.20	0.00	0.60	0.80	0.40	1.00	100	74.89	100	5	4	45
			Low	Hybrid Canola	-1.04	-1.21	-0.45	0.35	0.30	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.44	0.10	0.00	0.44	0.90	0.56	1.00	100	100	100	4	4	10
				OP Canola	-1.17	-1.22	-1.08	0.31	0.30	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.03	-0.01	0.11	0.05	0.00	0.16	0.95	0.84	1.00	100	100	100	4	4	4
	Low	High		Hybrid Canola	-1.23	-1.26	-1.21	0.29	0.28	0.30	0.00	0.00	0.00	0.00	0.00	0.00	-0.02	-0.06	0.00	0.01	0.00	0.03	0.99	0.97	1.00	100	100	100	4	4	4
				OP Canola	-1.24	-1.28	-1.20	0.29	0.28	0.30	0.00	0.00	0.00	0.00	0.00	0.00	-0.04	-0.08	0.00	0.02	0.00	0.07	0.98	0.93	1.00	100	100	100	4	4	4
			Low	Hybrid Canola	-1.22	-1.27	-1.18	0.29	0.28	0.31	0.00	0.00	0.00	0.00	0.00	0.00	-0.02	-0.07	0.02	0.01	0.00	0.05	0.99	0.94	1.00	100	100	100	4	4	4
				OP Canola	-1.19	-1.27	-1.10	0.30	0.28	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.01	-0.06	0.10	0.05	0.00	0.17	0.95	0.83	1.00	100	100	100	4	4	4
Saskatchewan	High	High		Hybrid Canola	-0.96	-1.08	-0.80	0.38	0.34	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.12	0.32	0.22	0.12	0.32	0.78	0.67	0.88	100	100	100	5	4	6
				OP Canola	-0.79	-1.10	-0.43	0.45	0.33	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.10	0.53	0.32	0.10	0.54	0.68	0.46	0.90	100	100	100	6	4	11
			Low	Hybrid Canola	-0.83	-1.03	-0.65	0.44	0.36	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.15	0.42	0.30	0.15	0.42	0.70	0.58	0.85	100	100	100	6	4	7
				OP Canola	-0.46	-0.88	0.01	0.63	0.41	1.01	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.26	0.69	0.49	0.27	0.70	0.51	0.30	0.73	100	97.28	100	10	5	106
	Medium	High		Hybrid Canola	-1.21	-1.21	-1.20	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
			Low	Hybrid Canola	-1.21	-1.21	-1.20	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
	Low	High		Hybrid Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
			Low	Hybrid Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
Ontario	High	High		Hybrid Canola	3.41	2.18	4.19	30.21	8.84	65.72	0.15	-0.23	0.61	0.27	0.00	0.72	0.71	0.27	0.99	0.99	0.98	1.00	0.01	0.00	0.02	0	0	1.30	1	1	2
				OP Canola	4.53	3.41	5.82	92.45	30.35	335.33	0.05	-0.12	0.27	0.14	0.00	0.37	0.85	0.63	1.00	1.00	0.99	1.00	0.00	0.00	0.01	0	0	0	1	1	1
			Low	Hybrid Canola	4.44	3.39	5.26	84.37	29.77	192.22	0.14	-0.23	0.63	0.27	0.00	0.73	0.72	0.26	1.00	1.00	0.99	1.00	0.00	0.00	0.01	0	0	0	1	1	1
				OP Canola	5.46	4.25	6.75	235.55	70.37	856.78	0.06	-0.12	0.27	0.15	0.00	0.37	0.85	0.63	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0	0	0	1	1	1
	Medium	High		Hybrid Canola	-1.42	-1.49	-1.35	0.24	0.22	0.26	-0.22	-0.29	-0.14	0.00	0.00	0.00	-0.02	-0.03	-0.01	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	3	3	3
				OP Canola	-1.44	-1.60	-1.31	0.24	0.20	0.27	-0.19	-0.38	-0.07	0.00	0.00	0.00	-0.07	-0.11	-0.03	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	3	3	4
			Low	Hybrid Canola	-1.42	-1.49	-1.35	0.24	0.22	0.26	-0.22	-0.29	-0.14	0.00	0.00	0.00	-0.02	-0.03	-0.01	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	3	3	3
				OP Canola	-1.44	-1.60	-1.31	0.24	0.20	0.27	-0.19	-0.38	-0.07	0.00	0.00	0.00	-0.07	-0.11	-0.03	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	3	3	4
	Low	High		Hybrid Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
			Low	Hybrid Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.21	-1.21	-1.21	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
Québec	High	High		Hybrid Canola	-1.28	-1.60	-0.94	0.28	0.20	0.39	-0.62	-0.66	-0.59	0.00	0.00	0.00	0.30	0.08	0.51	0.30	0.08	0.51	0.70	0.48	0.92	100	100	100	4	3	5
				OP Canola	0.66	-0.08	1.29	1.94	0.93	3.62	0.07	0.01	0.17	0.11	0.04	0.21	0.71	0.51	0.83	0.82	0.61	0.92	0.18	0.08	0.39	0	8.15	100	7	4	71
			Low	Hybrid Canola	-1.06	-1.54	-0.62	0.35	0.21	0.54	-0.62	-0.66	-0.59	0.00	0.00	0.00	0.42	0.13	0.65	0.42	0.13	0.65	0.58	0.35	0.87	100	100	100	4	3	7
				OP Canola	1.22	0.35	2.24	3.38	1.41	9.37	0.08	0.02	0.18	0.12	0.05	0.22	0.76	0.60	0.86	0.89	0.75	0.96	0.11	0.04	0.25	0.00	1.75	52.10	4	2	13
	Medium	High		Hybrid Canola	-1.22	-1.23	-1.22	0.30	0.29	0.30	-0.02	-0.02	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.22	-1.22	-1.21	0.30	0.29	0.30	-0.01	-0.01	-0.01	0.00	0.00	0.01	0.00	-0.01	0.00	0.00	0.00	0.01	1.00	0.99	1.00	100	100	100	4	4	4
			Low	Hybrid Canola	-1.22	-1.22	-1.21	0.30	0.29	0.30	-0.02	-0.02	-0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	1.00	0.99	1.00	100	100	100	4	4	4
				OP Canola	-1.22	-1.22	-1.22	0.30	0.29	0.30	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
	Low	High		Hybrid Canola	-1.22	-1.22	-1.21	0.30	0.30	0.30	-0.01	-0.01	0.00	0.00	0.00	0.00	-0.01	-0.01	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.23	-1.24	-1.22	0.29	0.29	0.29	0.00	0.00	0.00	0.00	0.00	0.00	-0.03	-0.04	-0.02	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
			Low	Hybrid Canola	-1.22	-1.22	-1.21	0.30	0.30	0.30	-0.01	-0.01	0.00	0.00	0.00	0.00	-0.01	-0.01	0.00	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4
				OP Canola	-1.23	-1.24	-1.22	0.29	0.29	0.29	0.00	0.00	0.00	0.00	0.00	0.00	-0.03	-0.04	-0.02	0.00	0.00	0.00	1.00	1.00	1.00	100	100	100	4	4	4

When σ_W survival is zero a value of 0.001 is used instead.

Mean estimates are calculated using 1 run of T=10,000 iterations.

Confidence intervals are calculated using a bootstrapped short-term forecast of T=3 iterations using 10,000 bootstrap replicates.

Table 4-11. Bootstrapped differences and ratios (hybrid – OP; hybrid / OP) for canola demonstrating the benefit of using equivalence hypothesis tests over null hypothesis tests in precautionary principle scenarios.

										Null Hypothesis Test	Equivalence test				
										H_0 is rejected (-), or H_0 is not rejected (+)	H_1 is not rejected (+) or H_1 is rejected (-)				
										H_0: $\lambda_1 - \lambda_2 = 0$	H_1: $\lambda_1 / \lambda_2 \leq 1/(1+x)$ or $\lambda_1 / \lambda_2 \geq (1+x)$				
										H_A: $\lambda_1 - \lambda_2 \neq 0$	H_2: $1/(1+x) < \lambda_1 / \lambda_2 < (1+x)$				
			Differences ($\lambda_{Hybrid} - \lambda_{OP}$)			Ratios ($\lambda_{Hybrid} / \lambda_{OP}$)				95% CI	90% CI, x varies from 1% - 50%				
Site	Disturbance	Density	estimate	L.C.I	U.C.I.	estimate	L.C.I	U.C.I.	Quadrant		1%	5%	10%	20%	50%
Alberta	High	High	-1.02	-15.51	18.65	0.89	0.56	1.52	4	+	-	-	-	-	-
		Low	1.70	-20.51	76.64	1.18	0.33	5.28	4	+	-	-	-	-	-
	Medium	High	-0.07	-0.78	0.22	0.84	0.42	1.50	3	+	-	-	-	-	-
		Low	0.04	-0.04	0.34	1.14	0.91	1.85	1	+	-	-	-	-	-
	Low	High	0.00	-0.01	0.02	1.01	0.98	1.05	1	+	-	-	+	+	+
		Low	-0.01	-0.03	0.01	0.97	0.91	1.02	1	+	-	-	+	+	+
Saskatchewan	High	High	-0.07	-0.30	0.08	0.85	0.58	1.19	1	+	-	-	-	-	-
		Low	-0.20	-0.62	0.03	0.69	0.43	1.01	3	+	-	-	-	-	-
	Medium	High	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
		Low	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
	Low	High	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
		Low	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
Ontario	High	High	-62.24	-287.30	-14.27	0.33	0.17	0.64	4	-	-	-	-	-	-
		Low	-151.18	-751.10	-12.86	0.36	0.15	0.81	4	-	-	-	-	-	-
	Medium	High	0.00	-0.03	0.05	1.02	0.89	1.21	1	+	-	-	-	-	+
		Low	0.00	-0.03	0.05	1.02	0.89	1.21	1	+	-	-	-	-	+
	Low	High	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
		Low	0.00	0.00	0.00	1.00	1.00	1.00	1	+	+	+	+	+	+
Québec	High	High	-1.66	-3.35	-0.67	0.14	0.09	0.27	3	-	-	-	-	-	-
		Low	-3.03	-8.86	-1.05	0.10	0.05	0.23	3	-	-	-	-	-	-
	Medium	High	0.00	0.00	0.00	1.00	0.99	1.00	1	+	+	+	+	+	+
		Low	0.00	0.00	0.00	1.00	1.00	1.01	1	+	+	+	+	+	+
	Low	High	0.00	0.00	0.01	1.01	1.00	1.02	1	-	-	+	+	+	+
		Low	0.00	0.00	0.01	1.01	1.00	1.02	1	-	-	+	+	+	+

For the null hypothesis test, a failure to reject the null hypothesis is a failure to prove that there is a difference.

For the equivalence test, a fauluer to reject the primary hypothesis is a failure to prove that the populations are equal at the level of 'x'.

The interpretation of '+' and '-' is not exactly equal between the null hypothesis test and the equivalence test.

When σ_W survival is zero a value of 0.001 is used instead.

The equivalence test uses a 90% CI because it represents two one tailed tests.

A 1-2α confidence interval tests at the α level for an equivalence test.

The Classical hypothesis test uses a 95% CI to test at the level of α.

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Chapter 5 General discussion and conclusions

5.1 Summary of Results

Population matrix modeling could be useful for assessing and comparing the fitness of GM and comparator crops. However, field experiments that collect demographic information for GM crops need to be more robust, and 'substantial equivalence' needs to have a much stricter definition to be useful. Experiments that span many years (>10?) and focus on acquiring precise data for important LLP would provide much more robust stochastic estimates due to increased precision as well as an increased chance to sample low-occurrence, high-productivity years for medium and low disturbance regimes.

Objective 1 results

We believe that we have successfully accomplished our goal to develop field-based methods to collect demographic data from GM crops and suggest modifications to increase precision in important LLP estimates. The assessment endpoint scenarios that we discussed should result in easier comparisons, and can be used in conjunction with equivalence testing. Finally, we demonstrated the differences between null and equivalence hypothesis testing, and discussed why those differences are important in the context of an EBRA.

Objective 2 results

For the comparison of the weedy/invasive propensity of hybrid canola and OP canola, our stochastic analysis suggests that hybrid canola is no more weedy than OP canola and

may be less weedy in certain environments. Our elasticity analysis suggests that GM traits that increase seed survival in the seedbank should be avoided, but traits that increase seedling survival and fecundity may not be a problem in medium and low disturbance regimes. Larger datasets that comprise more years would increase the robustness of our analysis.

5.2 Future Research

Reasonable values for substantial equivalence and acceptable extinction thresholds should be determined by proponents of GM crops and regulators. Accepted values for $\boldsymbol{T}$ to be used in short-term forecasts should also be discussed.

Due to climate change, it's entirely possible that datasets may actually 'expire'. Long term analyses that study the predictive ability of datasets that span across different years should be initiated.

More complex models that allow for mating between crops and non-annual weedy relatives could be investigated. Additionally, the fitness differences between hybrid offspring of crops that have multiple GM traits which segregate independently could be investigated using LTRE analyses.

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