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**Alien *Brassica*: Variation in Performance Among and Within Species
and Locations**

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at
Lincoln University
by
Ross Meffin

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Abstract of a thesis submitted in partial fulfilment of the
requirements for the Degree of Doctor of Philosophy.

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Our ability to consistently predict which taxa will become invasive and where, and understanding of the drivers of this variation is limited. A better understanding of how variation in traits within species and biotic and abiotic conditions within regions influence patterns of invasion is needed to improve our understanding of the processes underlying invasions and our ability to manage alien species.

The taxonomic unit used to assess alien invasiveness is usually the species. There is considerable intraspecific variation in plant traits, but the extent to which these differences can affect invasiveness is unknown. I tested the hypothesis that most of the variation in plant performance among taxa would reside at the species level (relative to subspecies and varieties).

I quantified variation in performance among taxa of alien *Brassica* with data from a seed sowing experiment comprising 24 taxonomically stratified varieties (six subspecies, three species) used to parameterise hierarchical models.

Around 30 times more variation resided among varieties within species than among species. Differences in the viability rates of the sown seeds explained some of the differences in performance. My results show that intraspecific taxa of alien species can vary significantly in performance and suggest novel genotypes may pose a different invasion risk to that currently established for the species. Weed risk assessment protocols could be modified to include varietal traits such as seed viability in assessments.

The availability of seeds and bare ground both influence whether plant populations establish, but the degree to which they influence persistence is less well known. The taxonomic level at which most variation in alien performance resides may shift under different regimes of seed and site availability.

I quantified the effects of relaxing seed and site limitation on the performance of a taxonomically stratified selection of 12 varieties of alien *Brassica*, and looked for shifts in the taxonomic level at which most variation in performance resided. Seeds were sown at two densities (400 seeds/m² and 4000 seeds/m²), and with and without pre-sowing soil disturbance.

Both a higher sowing density and pre-sowing disturbance increased *Brassica* emergence, but only disturbance increased survivorship, and neither resulted in significantly higher population growth rates. Most variation in performance among taxonomic levels resided at the varietal level regardless of seed density or disturbance. While high seed density and disturbance can increase seedling recruitment, unless aliens can pass subsequent ecological filters persistence is unlikely.

Alien plant populations often become extinct due to demographic and environmental stochasticity, unsuitable traits, and Allee effects. Such aliens may appear to be naturalised if new populations are founded by dispersed or immigrant propagules. Differentiating between transient and persistent populations is crucial to our understanding and management of invasions.

I recorded feral alien *Brassica* populations in three annual surveys, along with measures of seed sources, site and management characteristics that may influence the probability of presence and survival. I identified correlates of population presence and survival, and quantified the probability of population survival from year to year.

Brassica would likely not persist in the landscape beyond ten years without anthropogenic seed input. Only around 40% of populations survived two or more years after foundation, but new populations focused around transportation routes and seed companies compensated for extinctions. Stochasticity and trait-site mismatch were causes of population extinction. Adventive aliens may be controlled by managing propagule sources; accounting for population extinctions is important to accurately assess distributions from cumulative presence data.

Further research is required to identify which traits drive variation in plant performance and invasiveness within species. In addition we need to be able to identify taxa which are most likely to contain substantial intraspecific variation in these traits. Applying the precautionary principle would suggest that the same restrictions should apply to the importation of new plant varieties, subspecies and genotypes as are applied to the importation of new plant species.

Keywords: alien plants, *Brassica napus*, *Brassica rapa*, intraspecific variation, invasion, partitioning variation, plant traits, population extinction, population persistence, repeatability, roadside vegetation, seed addition, seed limitation, seed mass, seed viability, site limitation, stochasticity, subspecies, survival, variety.

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Chapter 1

Introduction

1.1 Overview

1.1.1 Background

It is widely recognised that invasive species cause serious ecological and economic damage (2000, Pimentel et al. 2001, Crooks 2002, Vila et al. 2011, Pysek et al. 2012). This has driven a large research effort to understand the processes underlying invasion and identify potential invaders with the goal of mitigating these impacts (Pheloung et al. 1999, Mack et al. 2000, Champion and Clayton 2001, Byers et al. 2002, Leung et al. 2002, Hulme 2006, Cousens 2008, Hayes and Barry 2008, Gasso et al. 2010, Kumschick and Richardson 2013). This research has sought to understand why some alien taxa become invasive while others do not (Mack 1996, Pheloung et al. 1999, Kolar and Lodge 2001, Hayes and Barry 2008), and why some sites are invaded while others are not (Hastings et al. 2005, Pysek and Hulme 2005, Theoharides and Dukes 2007, Simberloff 2009). While much progress has been made, we still lack the ability to consistently predict which taxa will become invasive and where (Pysek et al. 1995, Mack et al. 2000, Byers et al. 2002, Hulme 2012).

The default approach in invasion ecology has been to treat species as the unit of observation, with the implicit assumption that most variation in traits related to invasiveness resides at the level of species, and that differences within species are comparatively small and negligible (e.g. Stohlgren et al. 1999, Pimentel et al. 2000, Sakai et al. 2001, Grotkopp et al. 2002, Strayer et al. 2006, Kumschick and Richardson 2013). This is despite longstanding recognition that variation within species may be an important driver of variation in invasiveness (McNeill 1976, Williamson 1992), and a small but growing body of studies that suggest there may be considerable variation in important ecological characteristics and invasiveness within alien species (Saltonstall 2002, Kinter and Mack 2004, Vellend et al. 2010, Merrill et al. 2012, Hierro et al. 2013). An understanding of how intraspecific variation can affect invasion outcomes may improve our understanding of the processes underlying invasions and our ability to predict the risks posed by alien species.

1.1.2 Objectives

In this thesis I investigate variation in the performance of a genus of alien plants among taxa and sites. This thesis was motivated by the question: Do imports of new plant species necessarily pose a greater risk than new varieties or genotypes of alien species already in a recipient region? I used

Brassica as a model system to answer this question because it has a well-defined and diverse hierarchical taxonomy (Tsunoda et al. 1980, Gupta 2009, Ramchiary et al. 2011, USDA 2013) and several species are widely naturalised worldwide and in New Zealand (Pessel et al. 2001, Randall 2002, Heenan et al. 2004b, Gulden et al. 2008, Peltzer et al. 2008, Mizuguti et al. 2011). I reframed the motivating question as a testable hypothesis for the genus *Brassica*: most variation in the performance of alien *Brassica* resides among species rather than within species. My core question was thus:

A) Does most variation in the performance of alien *Brassica* reside among species, or is there similar or greater variation in performance within species?

Before tackling the central question of my thesis I aimed to gain a better understanding of my chosen model system by asking:

B) What are the drivers of naturalisation of *Brassica*, i.e. biotic and abiotic correlates of population presence and persistence, and how do these vary among taxa?

C) What is frequency and annual rate of persistence of populations of alien *Brassica* taxa in the study system, and how do these vary among taxa?

Building on results obtained in objectives A, B and C, along with a knowledge the factors associated with *Brassica* naturalisation from the literature I asked:

D) Can limited recruitment of *Brassica* be overcome by increasing the availability of safe sites and seeds?

And I tested the robustness of my answer to A in an additional ecologically realistic scenario:

E) Does increasing the availability of safe sites and seeds alter how performance is partitioned among taxonomic levels?

1.1.3 Structure

The thesis consists of five chapters; the three data chapters (chapters two to four) are presented as manuscripts prepared for submission to scientific journals. At the time of submission none of these manuscripts have been submitted for publication.

In chapter one I discuss the choice of *Brassica* as a model system, and provide a concise review of relevant literature (the current species-based approach in invasion ecology; intraspecific variation in plant traits and naturalisation with a focus on *Brassica*; *Brassica* biology, ecology, and naturalisation). Chapter two presents the results of three annual field surveys of feral *Brassica* populations in Canterbury, in which I aim to identify biotic and abiotic correlates of population presence and persistence at landscape and local scales, and quantify population frequency and annual rate of persistence (objectives **B** and **C**).

Chapter three tackles the core question of my thesis (objective **A**); using a seed sowing experiment, I aim to identify how variation in *Brassica* performance is partitioned among taxonomic levels (species, subspecies and varieties) and locations (among and within plots).

In chapter four I manipulate seed sowing density and pre-sowing disturbance in a second seed sowing experiment to investigate if increasing the availability of seeds and safe sites can overcome limitations to *Brassica* establishment or alter the partitioning of performance among taxonomic levels (objectives **D** and **E**).

In chapter five I summarise, synthesise and discuss the main results. Appendices present material which would be presented as supplementary material in published papers, or would be omitted from published papers but is included here for completeness and to preserve data collected and analyses performed as part of the thesis.

1.2 Choice of *Brassica* as Model System

The core question that I sought to answer was: Does most variation in the performance of alien *Brassica* reside among species, or is there similar or greater variation in performance within species? The high economic importance of *Brassica* cultivation in New Zealand makes this a practical test case of the issues involved. Active breeding programs (e.g. Lammerink and Hart 1985, Gowers and Nicol 1989, Ellis and Farrell 1995, Douglas 2005, Carter 2007, Christey et al. 2008, Jong et al. 2010) can be enhanced by the importation of new varieties and genotypes.

The known ability of *Brassica* to naturalise and its well defined and diverse hierarchical taxonomy make the genus an ideal system to test whether most taxonomic variation in naturalisation risk lies at the species level. Within many species of the genus are nested subspecies, and further nested within these are many varieties which have diverse traits selected to suit various uses, climates and markets (USDA 2013). The genus is widespread and has a long history of artificial selection, resulting in considerable variation in morphology, breeding traits, ploidy, phenology and ecological tolerances

(Tsunoda et al. 1980, Gupta 2009, Ramchiary et al. 2011). Several *Brassica* species are known to have naturalised worldwide (Randall 2002); within Canterbury, New Zealand at least four species (*B. rapa*, *B. napus*, *B. oleracea* and *B. nigra*) are known to form naturalised and casual populations along rural roadsides and other disturbed areas (Heenan et al. 2004b, Peltzer et al. 2008).

The choice of *Brassica* as a model system requires that there are some limitations on how broadly the results can be interpreted. The species chosen to test my core hypothesis are closely related, (U 1935), and I deliberately set out to maximise variation within species, which can be unusually large in *Brassica* (Tsunoda et al. 1980, Gupta 2009, Ramchiary et al. 2011). Furthermore the *Brassica* are generally annual and biennial. Populations of annual and biennial taxa are by their nature often transient, forming part of a larger metapopulation, whereas many of the plants considered to be amongst the most problematic invaders are persistent perennials. The results presented here may not be generalizable to other genera, for example those containing less variation within species or perennial taxa.

1.3 Species-based approach in Invasion Ecology

The default approach in invasion ecology has been to treat species as the unit of observation, with the implicit assumption that most variation in traits related to invasiveness or invasion risk resides at the level of species, and that differences within species are comparatively small and negligible (e.g. Stohlgren et al. 1999, Pimentel et al. 2000, Sakai et al. 2001, Grotkopp et al. 2002, Strayer et al. 2006, Kumschick and Richardson 2013). There has rarely been any attempt to justify or explicitly acknowledge this assumption, although many authors have noted the potential importance of intraspecific variation in determining invasion outcomes. Nearly forty years ago McNeill (1976) pointed out that concepts such as species, and even variety and ecotype, could not fully reflect the variation among organisms, and that variation within species may be just as important as differences between species to the study and control of weeds. Assessments of the potential invasiveness of genetically modified organisms have also pointed out that even relatively small genetic differences within a species can markedly alter ecological characteristics, potentially resulting in significantly increased invasiveness (National_Research_Council 1989, Williamson 1992, Williamson and Fitter 1996). These reviews compiled a number of cases which suggest that genetic variation within species resulted in differences in invasion success (see below, 1.4 Intraspecific Variation in Plant Traits and Invasiveness). These case studies, however, were unable to establish a causal link between increased invasiveness and variation in genes or traits within species. Despite these indications that taxa may vary significantly in invasiveness within species, no studies have explicitly set out to quantify how variation in invasiveness is partitioned among taxonomic levels, and few studies have demonstrated

variation in invasiveness within species (see below, 1.4 Intraspecific Variation in Plant Traits and Invasiveness).

The use of a species-based approach in invasion ecology most likely stems from three sources. First, using species as the unit of observation is prevalent throughout the biological sciences; indeed, species is generally viewed as the fundamental unit of our taxonomic system (Mishler and Donoghue 1982, Luckow 1995, Lee 2003). Second, intraspecific taxa are often difficult to identify on the basis of morphology (e.g. Garcia et al. 2002, Curn and Zaludova 2007). Other identification techniques, such as flow cytometry and AFLP, can be expensive and time consuming, and may still be unable to provide sufficient taxonomic resolution (Campbell et al. 2003), although the situation is improving (Agarwal et al. 2008). Data such as herbarium records and plant trait databases are also frequently not available at the level of subspecies, cultivars or varieties. Together these factors make studies of invasion at taxonomic levels below that of species difficult, and without a strong motivating research objective, hard to justify. Third, invasion ecology has a long history dating back to the 19th century when, for example, agriculturists had a keen interest in understanding what allowed weeds to invade cultivated fields. However, the current intense interest in the field is a relatively recent phenomenon, beginning with Elton's (1958) seminal work on the topic, and research has burgeoned over the last 30 years (Richardson and Pysek 2008, Pysek and Hulme 2009). Perceived priorities such as disentangling the influences of alien species traits, biotic and abiotic environmental characteristics, introduction history and pathways, and anthropogenic habitat modification (to name a few of the multiple factors interacting to determine invasion outcomes) have been challenging (Catford et al. 2009, Romanuk et al. 2009, Heger et al. 2013). The sometimes serious impacts of invasive aliens along with the increasing rate at which species are being transported around the globe have lent urgency to the research program. As a result the possibility that intraspecific variation may result in substantial variation in invasiveness within species has been largely overlooked, or put aside to focus on more obvious research priorities.

1.4 Intraspecific Variation in Plant Traits and Invasiveness

Trait variation within species can arise from differential artificial or natural selection. Artificial selection often aims to increase productivity or survival; for example targeting traits such as seed mass, fecundity, tolerance of harsh environmental conditions and pests, growth rate, ploidy and biomass (Williams and Hill 1986, Habekotte 1993, Kumar 1995, Kelly 2006, Marhold et al. 2010, Ramchiary et al. 2011, Meyer et al. 2012). Altering these traits in such ways may also increase the risk of invasion (Sutherland 2004, Thuiller et al. 2005, Thuiller et al. 2006, Hayes and Barry 2008, Sanders 2010, Schlaepfer et al. 2010, van Kleunen et al. 2010). Natural selection acting on genotypes, ecotypes and subspecies from different regions can lead to differences in fitness among conspecifics

in new habitats depending on the match between conditions in the source and recipient region (Saji et al. 2005, Nosil 2007, Sobel et al. 2010, Lekberg et al. 2012).

A recent meta-analysis quantified the proportions of variation residing among and within species for 10 commonly studied plant traits, using a database of 2.88 million trait entries for 69 000 plant species, and 3 million ancillary data which were used to account for trait variation due to phenotypic plasticity (Kattge et al. 2011). This study gives the best picture currently available of how variation in plant traits is distributed among and within species. The results showed that while most variation in the studied traits resides among species, intraspecific variation was substantial and accounted for around 40% of the overall variation in three traits (leaf phosphorus content per dry mass, leaf nitrogen content per area, and light saturated photosynthetic rate per leaf area). Around 10% of the overall variation in seed mass, 18% of the variation in plant height, and 30% of the variation in specific leaf area was within species. Of the traits analysed, that with the least variation residing within species (7%) was leaf longevity.

Intraspecific variation can also be substantial in traits thought to influence invasiveness such as seed mass (Baker 1965, Rejmanek et al. 2005), fecundity (Mason et al. 2008), tolerance of physical stress (Chahal et al. 2013, Gabler and Siemann 2013), growth rate (Grotkopp et al. 2010, Pan et al. 2012), and ploidy (Munzbergova 2006, Halverson et al. 2008). Here I focus on *Brassica* because intraspecific trait variation in the genus is comparatively well-documented and *Brassica* is the model system used in this thesis, but examples from other genera are included where examples from *Brassica* were not available. Seed mass can vary widely within species. Hendrix and Sun (1989) documented variation in seed mass of 3 - 16 fold within Umbellifer species, while Voller et. al (2012) suggested there is typically around 10 fold variation in seed mass within grassland species. Studies which compare the fecundity of genotypes within species while controlling for the confounding effects of environment are not common, but there can clearly be substantial variation. Miri (2007) compared 16 varieties of *B. napus* in a common garden experiment and found mean seed production per plant varied by more than threefold. Crawley et al. (1993) also found a threefold variation in the number of siliques produced by three *B. napus* varieties grown in common gardens. Physical stresses which can affect plant performance include saline soils, frost, and drought. Qasim et al. (2003) subjected *B. napus* varieties to salt stress in a common glasshouse and found a salt tolerant genotype originating from Australia had significantly higher biomass and seed yield than a non-salt tolerant genotype originating from Denmark. *Brassica napus* varieties also differ significantly in their survival and when subjected to frost (Rapacz 1999), and performance when subjected to water stress (Rad and Zandi 2012). Growth rates also vary significantly among genotypes within species. Among nine genotypes

of clover (*Trifolium repens*) originating from Europe, the Middle East, China, New Zealand and Australia there was a 3 fold difference in growth rates when grown in standardised conditions (Hofmann et al. 2000). Rapid cycling *B. rapa* (RCBr) completes its life-cycle far more rapidly than conventional annual and biennial varieties and can produce up to ten generations per year (Williams and Hill 1986, Kelly 2006). Brassicaceae is well known for its large variation in ploidy (Marhold and Lihova 2006); *B. rapa* has diploid, triploid and tetraploid varieties (Elling et al. 2010), while *Cardamine* species can have cytotypes ranging as many as six ploidy levels (Marhold et al. 2010).

Little is known about the magnitude of variation in invasiveness within species. Williamson (1992), and Williamson and Fitter (1996) in reviews of the potential hazards of genetically modified organisms and the characteristics of successful invaders compiled examples of how small genetic differences can alter ecological characteristics and may lead to increased invasiveness. However, these examples were generally speculative, pertained to taxa that were classified as (closely related) distinct species, or did not show a causative link between within species variation in genes or traits and variation in invasiveness. For example, it has been speculated that the observed switch of *Epilobium angustifolium* from a rare British native herb to a wide-spread invasive species early this century may have been due to the introduction of new genes from garden stocks or North American congeners (Salisbury 1961), but evidence to support this interpretation is lacking. Another example illustrates how changes in ploidy can give rise to invasive genotypes, even without any new genes, although the new genotype bears its own species epithet. Common cordgrass, *Spartina anglica*, is an allelotetraploid that arose from hybridisation of *Spartina maritima* and *Spartina alterniflora* (Marchant 1967). Neither of the progenitor taxa are invasive, but *S. anglica* invades waterways and intertidal flats and is considered to be a serious pest in the U.K., Asia, Australia, New Zealand and North America (Hedge and Kriwoken 2000, Grevstad et al. 2003, An et al. 2007, Nehring and Hesse 2008). Similarly, *Impatiens capensis*, introduced from North America is invasive in England, while its allospecies *I. noli-tangere* (Hulten and Fries 1986) is a rare and declining English native, yet trait differences between the two appear to be trivial (Perrins et al. 1992). *Chondrilla juncea* is an obligate apomict alien introduced into Australia from the Mediterranean. As a result of its obligate apomixis there are at least three genetically isolated, distinct races (Burdon et al. 1981). The rust fungus *Puccinia chondrolina* was introduced in 1971 as a control agent, but was found to be effective against only one of the races of *C. juncea* (Burdon et al. 1981), suggesting subspecific genetic differences confer an invasive advantage on the other two races, albeit in the face of anthropogenic control.

More recently studies of the relationship between the magnitude of within species genetic diversity and invasiveness have suggested that genotypes within species may differ in invasiveness. It has been suggested that high genetic diversity may increase invasiveness, but it remains unclear

whether any increase in performance is the result of genetic diversity per se, or a sampling effect where more diverse genetic mixtures of an invader are more likely to include high performance, invasive genotypes (Dlugosch and Parker 2008). Vellend et al. (2010) added mixtures of dandelion (*Taraxacum officinale*) seeds containing from one to five dandelion genotypes to plots of Kentucky bluegrass (*Poa pratensis*) and measured dandelion performance. More diverse mixtures performed better. While this result may be in part due to increased invasiveness due to higher genetic diversity, the identity of genotypes was more important in explaining performance than genetic diversity, and the authors suggested that the better performance of more diverse dandelion mixtures was largely due to a sampling effect and the eventual dominance of highly productive dandelion genotypes (Vellend et al. 2010). Furthermore, in a separate common garden experiment Vellend et al. (2009) found differences of over an order of magnitude in plant size and seed production among six *T. officinale* genotypes, as well as significant differences in the timing of reproduction. While the authors did not test for links between these traits and invasiveness or population performance, such variation among genotypes suggests there is potential for selection among genotypes during invasion (Vellend et al. 2009).

A few studies have shown a link between genetic or trait differences and invader performance. Perhaps the best studied case is that of cheatgrass (*Bromus tectorum*). Cheatgrass was introduced into New Zealand and the USA from Europe over 100 years ago. In New Zealand it has proved to be a benign casual occupant of disturbed areas such as roadsides, while in the USA it has invaded large areas of intact shrub- and grassland, resulting in a range of economic, community and ecosystem impacts (Kinter and Mack 2004). Common greenhouse experiments indicate that differences in performance between the two recipient regions can be explained by the introduction of different genotypes, with plants from North American populations being larger and more vigorous than those from New Zealand populations (Kinter and Mack 2004). Genetic analysis indicates that specialist *B. tectorum* genotypes are allowing further expansion into previously uninvaded habitats in the USA (Merrill et al. 2012). Another example of the introduction of genotypes with traits leading to increased invasiveness was documented by Hierro et al. (2013). They used a reciprocal common garden experiment to show that the seed mass of demes of the invasive ruderal *Centaurea solstitialis* sourced from its introduced range in Argentina was twice that of demes sourced from its native range in Turkey, and that this difference contributed to the invasion success of *C. solstitialis* in Argentina. Even very small within species differences can manifest as dramatically different ecological characteristics resulting in invasion and serious impacts; molecular evidence indicates that alien Euro-Asian (Saltonstall 2002) and Mediterranean (Lambertini et al. 2012) haplotypes of the cosmopolitan species *Phragmites australis* have been introduced into the USA (Guo et al. 2013). These alien haplotypes have higher biomass, growth rate and fecundity than the native lineage

(Belzile et al. 2010, Mozdzer and Zieman 2010), and have invaded large areas where the species was previously absent, displacing the native genotype and other native species (Saltonstall 2002, Guo et al. 2013).

1.5 *Brassica* Biology, Ecology and Naturalisation

The genus *Brassica* (*Brassicaceae*) comprises mostly monocarpic annuals and biennials native to Western Europe, the Mediterranean and temperate Asia. It has a long history of intense artificial selection, and is one of the most diverse genera of cultivated plants. The six commonly cultivated *Brassica* species are interrelated; three diploid species (*B. oleracea*, *B. nigra* and *B. rapa*) appear to have given rise to three amphidiploids (*B. napus*, *B. juncea* and *B. carinata*) via interspecific hybridisation (U 1935). The genus contains considerable variation in morphology, breeding traits, ploidy, phenology and ecological tolerances (Tsunoda et al. 1980, Gupta 2009). Recently the genus has been the subject of considerable genetic modification research, and has been widely utilised in studies of the risks posed by genetically modified crops through gene flow via hybridisation (Bing et al. 1996, Allainguillaume et al. 2006, FitzJohn et al. 2007, Devos et al. 2009).

Brassica are widely grown in New Zealand and other temperate regions globally for crops, seed and forage, and are of considerable economic importance. Fifteen species are recognised as having been imported into New Zealand (MAF 1998) of which 8 are considered naturalised, occurring primarily on road-verges and wasteland (Table 1.1, Webb et al. 1988, Heenan et al. 2002, Heenan et al. 2004a, Heenan et al. 2008); the wild turnip (*B. rapa oleifera*) is notable for being widespread and displaying weedy characteristics (Heenan et al. 2004b). Within these species there are approximately 45 subspecies and varieties, encompassing approximately 200 cultivars and hybrids (Gareth Holmes, personal communication).

Germination in *Brassica* is epigeal, and is primarily regulated by temperature (Gomez-Campo 1999). There appears to be a linear relationship between temperature and the time to germination, up to some optimum temperature; for *B. oleracea* germination typically commences after accumulation of around 1000 degree hours above 4.5 °C, thus at 10 °C germination commences in 9 days and 90% of seeds will have germinated within 13 days (Wagenvoort et al. 1981). There is considerable genetic variation within species in the time taken for seeds to germinate (Diepenbrock 2000). In the vegetative stage *Brassica* are characterised by a rosette in which leaves are formed in spiral succession around a single shoot apex. There is an approximately linear relationship between temperature and the rate of leaf formation between 6 °C, when the rate is zero, and an optimum at around 20 °C (Morrison and McVetty 1991). Conditions for optimum growth in *Brassica* are moist soils high in Nitrogen (Wright et al. 1988, Taylor et al. 1991, Bozkurt et al. 2011), temperatures

around 20 °C (Morrison and McVetty 1991, Jensen et al. 1996), with a minimum of competition from other vegetation (Miller and Schemske 1990, Miller 1995, Diepenbrock 2000) and post-emergence disturbance (Eager et al. 2013). The time until flowering varies widely among and within *Brassica* species, as well as with temperature (Gomez-Campo 1999); there is also evidence that some genotypes are sensitive to photoperiod, requiring short days to flower (Parkinson 1952, Tommey and Evans 1991). Some taxa are nominally biennial although this most likely reflects the usual time taken to complete the juvenile stage and reach the conditions necessary to trigger flowering, rather than a strict requirement to pass through a winter before flowering (Gomez-Campo 1999). Seeds form in siliques; the number of seeds produced depends largely on the strength of competition for resources, plant size, availability of nitrogen and water and shading (Sharma and Ghildiyal 1992, Gammelvind et al. 1996, Gomez-Campo 1999).

Research on *Brassica* naturalisation has focussed on oilseed rape (canola, *Brassica napus*) due to its commercial importance and concerns about gene flow from genetically modified varieties and the large areas cultivated (Pessel et al. 2001, Heenan et al. 2004b, Gulden et al. 2008, Knispel and McLachlan 2010, Mizuguti et al. 2011). Feral populations are common along road verges in areas of production globally (Randall 2002, Heenan et al. 2004b, Saji et al. 2005, Knispel and McLachlan 2010, Squire et al. 2011). Few of these studies have investigated biotic and abiotic site characteristics which influence *Brassica* naturalisation, although they are thought to rely on disturbance to establish in predominantly perennial roadside habitats (Crawley and Brown 1995). Observations suggest weed control practices such as mowing and herbicide application may disturb established vegetation and facilitate *Brassica* establishment (Knispel and McLachlan 2010). Most research has focussed on propagule supply and mechanisms of recruitment. Specifically, whether populations are: 1) transient populations reliant on seed immigration to maintain a presence in the landscape, 2) persistent due to longevity of seeds below ground and extended dormancy resulting in survival through seed banks, or 3) persistent due to reproduction and local recruitment. This is largely due to an interest in whether populations persist and can act as reservoirs for transgenes (Ellstrand et al. 1999, Jenkins 2005, FitzJohn et al. 2007), but also reflects the fact that propagule pressure is likely to have a major influence on naturalisation (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009). Surveys of *Brassica* populations on the Canterbury plains indicated they were more frequent close to towns and seed storage and certification facilities, these locations are suggested to be proxies for propagule pressure (Peltzer et al. 2008). Studies in the UK showed *B. napus* populations to be short lived, unable to recruit amongst established perennial vegetation and reliant on seed inputs spilled from trucks to maintain a continuing presence in the landscape (Crawley et al. 1993, Crawley and Brown 1995, 2004). Other studies have found populations may be more persistent and implicated seed banks as being a major seed source, suggesting seeds may be able to survive below ground for up to

10 years (Hails et al. 1997, Pessel et al. 2001, Pivard et al. 2008b). Evidence for local recruitment is scantier although seed set has been recorded in up to 30 - 48% of feral populations (Elling et al. 2009). Still other studies have found contributions from all these processes, suggesting around half of populations are ephemeral (35% resulting from seed escapes from adjacent fields and 15% from seeds spilt during transport) and half persistent (40% from seed banks and 10% from local recruitment) (Pivard et al. 2008a). Whether immigrant seeds come from adjacent fields or spills during transport is also debated; again evidence supports both (Crawley and Brown 1995, Pivard et al. 2008b). While there is good evidence that trucks do spill seeds during transport (von der Lippe and Kowarik 2007, Bailleul et al. 2012), and that major roads tend to have more populations than minor ones (Pivard et al. 2008a, Knispel and McLachlan 2010), it is unclear whether this road effect is the result of higher propagule pressure of different management and disturbance regimes (although see Crawley and Brown 1995). Furthermore, there may be significant inter-year variation in the relative importance of these processes (Peltzer et al. 2008).

1.6 Summary

In this thesis my primary objective is to answer the question: Does most variation in the performance of alien *Brassica* reside among species, or is there similar or greater variation in performance within species? I use *Brassica* as a model system to test the hypothesis that most of the variation in performance in the genus *Brassica* resides at the species level rather than among subspecies or varieties, and that the taxonomic level of species is therefore the appropriate level to quantify invasion risk. *Brassica* makes an ideal system to test this hypothesis due to its known ability to naturalise, well defined hierarchical taxonomy, widespread cultivation in New Zealand and internationally, and economic importance.

Table 1.1 Summary of *Brassica* species naturalised in New Zealand

Species	Life cycle	Distribution	Habitat	Height (m)	Flowering Time	Petal Colour
<i>fruticulosa</i>	biennial to perennial	Palmerston North, Raumati, Wellington	Waste land, roadsides, cliffs, and sandy places	< 1	Jan - Dec	yellow
<i>juncea</i>	annual	Northland, Canterbury	Roadsides, sandy beaches, waste land	1 - 2	Sep - Feb	yellow
<i>napus</i>	annual or biennial	Throughout NZ	Locally naturalised on coasts, and an occasional casual escape from cultivation on roadsides and in cultivated fields	< 1.5	(Sep)-Oct-Dec-(Apr)	yellow
<i>nigra</i>	annual	Auckland (Waitemata Harbour), Canterbury, Otago	A rare casual weed on waste land and among crops	0.6 - 1 - (2)	Dec - May	yellow
<i>oleracea</i>	biennial to perennial	Throughout NZ	Locally established on coastal cliffs, a casual cultivation escape inland	1 - 2 - (3)	(Jul)-Oct-Nov-(Apr)	pale yellow or white
<i>oxyrrhina</i>	annual	Known from Tokerau Beach, Northland only	Established on sand dunes among lupin scrub	< 0.50	Nov - Jan	cream with purple veins
<i>rapa</i>	annual	Throughout NZ	Common and persistent weed of pastures, roadsides, waste places and gardens	< 1.5	(Jul)-Sep-Feb-(Jun)	yellow
<i>tournefortii</i>	annual	Auckland, Tauranga, Taranaki, Wanganui, Horowhenua, Marlborough, Canterbury	Occasionally established in sandy coastal sites, especially in North; casual in railway yards and near flour mills in South	< 1	(Jul)-Oct-Mar-(Jun)	pale yellow, becoming whitish, veined with yellow or sometimes violet

Chapter 2

Landscape-Level Persistence and Distribution of Casual Alien Feral Crops Linked to Seed Transport

2.1 Abstract

Alien plant populations often become extinct due to demographic and environmental stochasticity, unsuitable traits, and Allee effects. Such aliens may appear to persist and be naturalised if new populations are founded by dispersed or immigrant propagules at a rate on average equal to that of extinction. Differentiating between transient and persistent populations is crucial to our understanding and management of invasions.

I recorded roadside alien *Brassica* populations present on the Canterbury Plains in New Zealand in three annual surveys, along with measures of seed sources, site and management characteristics that may influence the probability of population presence and survival. Using GLMs and proportional-hazard models I assessed which factors were associated with population presence and survival, and the probability of population survival from year to year.

Brassica appears to be adventive and may fail to persist in the landscape much beyond ten years without anthropogenic seed input. Around 60% of populations were extinct two years after foundation, but seed rain focused around transportation routes and seed companies founded new populations, compensating for the rate of extinction. Presence of *Brassica* populations was not related to proximity to *Brassica* fields or other feral populations. Stochasticity and trait-site mismatch were major causes of population extinction. Adventive aliens can be controlled by managing propagule sources, and accounting for population extinctions is important to accurately assess distributions from cumulative presence data.

2.2 Introduction

Understanding the factors that drive variation in the temporal and spatial distribution of introduced plants is central to plant invasion biology (Pysek and Hulme 2005, Jongejans et al. 2008). A key question is often whether populations of alien plants are transient populations or persistent due to successful local recruitment and thus potential sources of further spread (Pessel et al. 2001, Claessen et al. 2005, Pergl et al. 2012). Transient populations can maintain a species' presence in the

landscape and give the appearance of population persistence. This can occur both when continued external propagule inputs (such as accidental anthropogenic seed dispersal) result in sink populations continually appearing and disappearing in the landscape, and when seed emigration from populations prior to local extinction founds new populations in new locations, resulting in a dynamic metapopulation at the landscape scale (Shmida and Ellner 1984, Pulliam 1988, Crawley and Brown 1995, 2004). Removing the propagule supply of aliens which are present in the landscape only as sink populations will prevent further populations being founded and offers the hope of easy control or eradication (e.g. Hayward 2006); species which establish persistent populations and metapopulations are likely to be harder to control. Differentiating among transient, persistent and metapopulations, and identifying the factors which drive these differences is crucial to our understanding of the processes underlying alien invasions and efforts at their management and control.

Plant populations can be founded if propagules are present, and biotic and abiotic conditions are at least a suitable match to plant requirements for germination and recruitment to occur; however these conditions may differ from those which allow the population to persist (D'Antonio et al. 2001). Factors which prevent newly founded plant populations from persisting fall into four broad categories. First, most new alien populations consist of relatively few individuals, making them vulnerable to local extinction due to stochastic variation in demographic rates and environmental conditions (Shaffer 1981, Lande 1993, Mack et al. 2002). In the absence of external seed inputs, chance variations in the vital rates of small populations are likely to eventually result in local extinction if a large proportion of individuals suffer unusually low recruitment, survival or fecundity in a given year (Shaffer 1981, Lande 1993, Stephan and Wissel 1994, Liebhold and Bascompte 2003); a commonly cited figure is that populations of less than 50 individuals are unlikely to avoid this fate (Keiding 1975, Shaffer 1981). Year to year variations in environmental factors which influence the ability of aliens to establish, such as the availability of disturbed sites for germination (Crawley and Brown 1995), herbivory and seed predation (Maron and Vila 2001), climate (MacDougall et al. 2008), or more extreme events such as fires and floods (Lande 1993), can all lead to even quite large populations becoming extinct (Cohen 1979, Leigh 1981, Falk and Holsinger 1992), although larger populations tend to be more persistent due to being buffered against environmental stochasticity both above and below ground (Cohen 1966, Matthies et al. 2004). In particular, disturbance is thought to be an important driver of many invasions allowing aliens to establish in vacant sites and access resources previously monopolised by existing plants (Davis et al. 2000, Mack et al. 2000), newly founded populations may be unable to recruit and become locally extinct without disturbance in years subsequent to their foundation. Metapopulations tend to be buffered against the effects of demographic and environmental stochasticity (Gilpin and Hanski 1991). If several sub-populations are present in the landscape at any one time the likelihood of all of them experiencing a random

drop in demographic rates simultaneously is reduced. The degree to which metapopulation is buffered against fluctuations in the environment depends on the extent to which those fluctuations covary across occupied patches (Stacey and Taper 1992). Second, Allee effects, i.e. reduced survival or reproductive success at low densities, can drive populations below a threshold density where extinction becomes inevitable (Courchamp et al. 1999, Taylor and Hastings 2005). Third, biotic or abiotic conditions may be a poor match for the alien's requirements for growth and reproduction, leading to an inexorable decline in numbers to extinction (Maron and Vila 2001, Levine et al. 2004). Fourth, alien populations may be eradicated by either targeted or non-targeted management (Navie et al. 2010, Baider and Florens 2011).

Feral *Brassica* populations are commonly found along road verges in areas where commercial *Brassica* crops are grown, and in the vicinity of *Brassica* seed transportation routes and hubs (Crawley and Brown 1995, Heenan et al. 2004b, Saji et al. 2005, Knispel and McLachlan 2010, Squire et al. 2011). In particular, populations of oilseed rape (*B. napus*) are widely studied with the aim of elucidating whether they are able to persist at the population or landscape scale without external propagule inputs or are transient population sinks, and which propagule sources, biotic and abiotic variables may drive these differences (Pessel et al. 2001, Crawley and Brown 2004, Gulden et al. 2008, Pivard et al. 2008a, Knispel and McLachlan 2010, Mizuguti et al. 2011). The propagules which found roadside feral *B. napus* populations are thought to originate from trucks spilling seed during transport, and seed spilled from adjacent fields either at the time of sowing or harvest (Crawley and Brown 1995, Garnier and Lecomte 2006, Peltzer et al. 2008, Pivard et al. 2008a, Knispel and McLachlan 2010). Estimates of the proportions of seeds originating from these sources vary, and are likely to differ among systems. Most studies suggest that *B. napus* populations are sinks and reliant on continual seed immigration and disturbance to maintain a presence in the landscape (Crawley and Brown 1995, 2004, Peltzer et al. 2008, Pivard et al. 2008b, Knispel and McLachlan 2010). However, there is evidence that some populations may persist without external seed inputs (Pessel et al. 2001, Pivard et al. 2008a, Squire et al. 2011). Little is known about what proportion of populations persist, and how these populations differ from those which fail to become self-sustaining (Garnier and Lecomte 2006, Pivard et al. 2008a); however it seems likely that frequent disturbance and characteristics which buffer populations from stochasticity such as larger population size or a long lived seed bank are likely to promote persistence (Cohen 1966, Pessel et al. 2001, Claessen et al. 2005).

On the Canterbury Plains in New Zealand populations of four species of alien *Brassica* occur along roadsides, of these *B. rapa* dominates - 62% of populations found in a 2003 survey were *B. rapa* (Heenan et al. 2004b, Peltzer et al. 2008). These species are considered naturalised (Heenan et al.

2004b), but it is unclear whether their continued presence in the landscape is due to continual seed rain founding a series of transient sink populations (i.e. *Brassica* are adventive), the existence of a self-sustaining meta-population, or individual self-sustaining populations. It is also unknown whether the prevalence of *B. rapa* reflects higher propagule pressure or less stringent biotic and abiotic conditions required for the foundation and/or persistence of populations of this species. In three annual field surveys I systematically recorded the occurrence of populations of *Brassica* species, along with measures of propagule pressure, and site characteristics which may influence the likelihood of populations being founded or persisting.

I set out to determine 1) whether populations were transient sink populations or persistent, and the probability of population survival from one year to the next; 2) which factors were associated with population presence and population survival from one year to the next, and which factors may explain why some populations are transient and while others persist: i.e. species identity, biotic and abiotic site characteristics, management practices, and propagule sources (seed spill from trucks, seed spill from adjacent fields, reproduction and seed set, and immigration from other nearby *Brassica* populations resulting in a metapopulation). In line with current knowledge I expected that most populations would be transient and that population presence would be driven by external seed inputs and disturbance, while population persistence would be associated with larger populations in unmanaged sites. The results have implications for understanding the processes underlying invasions by alien species and targeting management.

2.3 Methods

2.3.1 Study Area

The study was conducted in the rural districts of the Canterbury Plains, New Zealand. To include the arable plains while excluding mountainous areas and Banks Peninsula the study area was defined as the portion of the plains with a slope of less than 10°. This was done using ArcView GIS and resulted in a total study area of 9543 km² (Heenan et al. 2004b, Peltzer et al. 2008). The climate is temperate, with a mean annual precipitation of 740.9 mm and mean annual temperature of 12.2 °C (1981 – 2010, Darfield EWS, NIWA). Frosts are frequent in spring and winter, and inland areas may be covered by snowfall in winter. The area is primarily farmland, with dairy and beef cattle, along with sheep accounting for most land use. Much of the area is thus pasture, although *Brassica* crops (notably kale, *B. oleracea acephala*; turnip, *B. rapa rapa*; and swede, *B. napus napobrassica*) are also grown as fodder, particularly over winter. There is an emerging biofuel industry, for which oilseed rape (*B. napus napus*) is the primary source crop. In addition there is a seed multiplication industry which produces *B. rapa*, *B. napus*, *B. oleracea* and *B. juncea* seeds.

The area is covered by a network of roads, ranging from minor dirt and gravel roads to sealed state highways. These roads have a verge, ranging in width from approximately 2 to 5 metres, where the resident vegetation is largely a reflection of the species grown in neighbouring fields; perennials dominate, particularly alien grasses (*Lolium perenne* and *Dactylis glomerata*) and clovers (*Trifolium repens* and *T. pratense*). Naturally occurring populations of introduced *Brassica* (mostly *B. rapa*, with some *B. napus*, *B. juncea* and *B. oleracea*) occur along these road verges, although these are comparatively rare (Heenan et al. 2004b, Peltzer et al. 2008). The vegetation of the road verges is managed to control weed populations (including *Brassica*) to variable extents and this can range from frequent mowing and spraying with herbicides (particularly around drainage ditches and fence-lines) to no management.

2.3.2 Field Surveys

From 2010 to 2012 vehicular surveys of *Brassica* populations along roadsides were conducted in September and October, to coincide with the peak flowering time for *Brassica*. I used the sampling regime established in a 2003 survey of roadside *Brassica* populations in the study area so that my results would be comparable to those of the 2003 survey (Heenan et al. 2004b, Peltzer et al. 2008). I conducted surveys by motor vehicle at as near as possible to a constant speed of 70 km/h; *Brassica* produce prolific yellow flowers, readily distinguishable from other roadside species by their colour and arrangement, allowing them to be easily identified from a passing motor vehicle at this speed. I visited each of fifty 3 × 3 km squares surveyed by Heenan et al. and Peltzer et al. in their 2003 survey (4.7% of the total study area); I travelled the entire length of all roads contained in each square and recorded any *Brassica* populations encountered. From each randomly located square I drove to the next randomly located square by the route with the shortest distance, thus I considered these routes to be randomly located in the landscape; following Heenan et al. and Peltzer et al. (2004b, 2008) I recorded any *Brassica* populations encountered en route between squares. A log was kept of the route taken in 2010 (total length = 2200 km), and the same route was followed in 2011 and 2012. In 2010 morphological identification of *Brassica* was confirmed by flow cytometry where necessary (Heenan et al. 2004b); in 2011 identification was confirmed using AFLP (See Appendix A for methodology). All morphological identifications were confirmed in these analyses so identification in 2012 was based on morphology alone. I conducted the 2010 and 2012 surveys with an assistant. In 2011 I trained two field assistants who subsequently conducted most of the vehicular survey independently; they collected samples of *Brassica* from each population they located for subsequent morphological and AFLP identification.

The following variables were recorded for each *Brassica* population located: species, subspecies (for *B. napus* and *B. rapa*), number of *Brassica* plants and location. For populations of up to 100 individuals, the number of plants was counted; above this the population size was estimated by determining the area occupied by 100 plants and using this to extrapolate the total population size. The geographic location and altitude of each population was recorded using GPS. I expected the probability of a *Brassica* population being present, and the likelihood of it persisting from year to year, to be influenced by propagule pressure, site and management characteristics, and climate, so I collected data to quantify these for each *Brassica* population. Variables associated with propagule pressure were land use in the adjacent field (*Brassica* or other), distance to the nearest location of a company handling bulk quantities of agricultural seed, distance to the nearest *Brassica* population in the previous year, and road type. Seeds which escape fields during sowing may establish populations in the same year in adjacent road verges. Data about land use in the year prior to populations being recorded were unobtainable, so I was unable to account for seeds which may have escaped during harvest and germinate that autumn or the following spring. Traffic is thought to be a major dispersal pathway for *Brassica* seeds (Crawley and Brown 1995), state highways are thus likely to experience higher propagule input than minor or unsealed roads. Roads closer to seed companies are likely to carry more seed transportation trucks, and are expected to be positively associated with *Brassica* presence. In addition, populations which set seed are an additional source of seeds; these may be dispersed and found populations at other sites, although it is thought dispersal of *Brassica* seeds along roadsides is usually limited to a few metres (Garnier et al. 2008). Site characteristics were management (mowed or sprayed with herbicides), occurrence along a water course or in a depression, disturbance and vegetation type. Water courses were mostly artificial drainage and irrigation ditches and culverts, but also included streams; these are moist and often less intensively managed than more accessible road side areas. Depressions were any surface irregularities which prevented mowing of the vegetation down to the same level as the surrounding area. Disturbances (e.g. by vehicles or stock) are thought to facilitate *Brassica* establishment by reducing competition for space and nutrients (Crawley and Brown 2004, Claessen et al. 2005). Vegetation was characterised as consisting of predominantly perennial or annual/biennial species. Climatic variables (mean winter and summer precipitation, plus mean annual, maximum January and minimum July and air temperatures) were obtained from the National Institute of Water and Atmospheric Research (NIWA) 500 × 500 m resolution climate maps (Tait 2007a, b); these covary with altitude. I expected *Brassica* populations to be more frequent in locations with higher precipitation and temperatures.

To be able to contrast characteristics of sites where *Brassica* were present and those where they were absent, in 2010 I recorded the same propagule pressure, site and management characteristics, climatic variables, location and altitude at 10 randomly selected sites where *Brassica* did not occur

within each of the 50 surveyed squares (500 total). These sites were selected by calculating one tenth of the total road length in each plot, and stopping regularly at this interval within the plot. No *Brassica* populations were recorded at these sites in 2011 or 2012. Although some variables may change between years at the specific sites measured (i.e. mowing, herbicide use, disturbance, adjacent land use, distance to the nearest *Brassica* population in the previous year), I assumed that the variables recorded at these sites in 2010 characterised sites where *Brassica* were absent in the landscape, and could be used to characterise sites where *Brassica* were absent in 2011 and 2012.

To be able to determine whether the density of *Brassica* seeds in the soil at a site influenced the likelihood of a *Brassica* population persisting until the following year, from each population present in summer 2010 I collected soil samples in March 2011, at the end of the growing season and after seed set had occurred. From a 2 × 2m plot at the centre of each population I collected 10 randomly located samples, each of 100 ml of soil; these were bulked to make a single sample of 1 L of soil from each population (n = 121). Each bulked sample was spread evenly to a depth of approximately 2 cm over a bed of sterile seed germination mix (60% Peat, 40% sterilised pumice, plus 2kg Osmocote exact mini (16-3.5-9.1), 4kg Dolomite, 1kg Hydrifloin per cubic metre) on a tray within 48 hours of collection (Roberts 1981, Gross 1990). These were kept in a glasshouse and watered sufficiently to keep moist. Every two weeks any germinated seedlings were pricked out and the number of *Brassica* seedlings recorded, until no further germination occurred.

2.3.3 Analysis

Influence of Seed Sources, Management and Local Site Characteristics on the Probability of *Brassica* Population Presence

To test which factors influenced the probability of a *Brassica* population being present at a given location in each year (2010 - 2012), I fitted a model for each year using the function `glm` in R (R Development Core Team 2012) with *Brassica* presence/absence at each site as the binary response variable, which I treated as binomially distributed. To test for differences between *B. rapa* and the other *Brassica* species recorded (*B. napus*, *B. oleracea* and *B. juncea*) I fitted the model to data from just *B. rapa* populations, and to data from all other species combined (other species occurred too infrequently to provide enough data to fit models for each species individually).

I included explanatory variables to assess which seed sources were related to the probability of *Brassica* population presence (distance to the nearest seed company, road type, adjacent land use, distance to the nearest *Brassica* population in the previous year), to assess how management practices were related to the probability of *Brassica* presence (site mowed or sprayed with

herbicides), and to assess how abiotic and biotic site characteristics were related to the probability of *Brassica* presence (disturbance, water course, depression, vegetation type, precipitation and air temperature). Distance to the nearest *Brassica* population in the previous year was omitted from the model for 2010 because no data were available on the locations of *Brassica* populations in 2009. Continuous variables were standardised to make them comparable by subtracting the mean and dividing by two standard deviations (Gelman A 2007). Climatic variables covaried with each other and elevation so I selected one measure each of temperature and precipitation which best explained the probability of a *Brassica* population being present. I fitted simple models with *Brassica* presence/absence as the response variable and each climatic variable as the explanatory variable; models using mean annual minimum temperature and mean summer rainfall had the lowest AIC values so I retained only these climatic variables in my analysis. To fit the models I included all significant variables in a maximal model and used step-wise backwards selection based on AIC values to arrive at the best model.

Survival of *Brassica* Populations

To test which variables influenced the likelihood of *Brassica* populations persisting from year to year I fitted Cox proportional hazard models in R (R Development Core Team 2012) using the function `coxph` from the package `survival`. I used this modelling method because the data was censored; I did not know whether populations recorded in 2010 were newly formed that year, or had survived from 2009 or earlier; similarly, populations recorded in 2012 may persist beyond the study timeframe. The Cox method is a semi-parametric procedure analogous to multiple regression. It is most often used to examine the effects of explanatory variables on the survival times of subjects and is appropriate for censored datasets because it allows for survival beyond the bounds of observations (Cox 1972, Caesar 2003). Here Cox proportional hazard models were used to assess the baseline yearly survival probability of *Brassica* populations, and the relative effects of management, propagule pressure, climate, and abiotic and biotic site variables on the yearly survival probability of *Brassica* populations.

To assess the baseline yearly survival probability of *Brassica* populations I first fitted a model without any explanatory variables; this model gives a measure of the overall yearly survival rate of *Brassica* populations and the associated uncertainty (Cox 1972). I fitted a second model which allowed me to test which management practices, site characteristics, and seed sources influence yearly survival of *Brassica* populations, and whether the probability of survival of *B. rapa* populations differed from that of other species. I fitted this model with all the explanatory variables used when modelling the probability of *Brassica* presence/absence (see above). Additionally, I expected larger populations may have higher yearly survival rates due to being buffered against environmental and demographic

stochasticity (Cohen 1966, Matthies et al. 2004), so for each population I included the mean population size (over the three survey years) as an additional explanatory variable. To test if *B. rapa* populations had a greater probability of persisting than populations of other species I included species (classified as *B. rapa* or other) as an additional explanatory variable. Management, disturbance, vegetation type and adjacent land use data could not be recorded for sites prior to the first detection of a population; for example if a population was first recorded in 2011, these data were not recorded in 2010. To account for this I used the mean of the available observations for these variables. This gives a measure of the probability of each state occurring in any given year; i.e. that a site was mowed, sprayed, disturbed, had annual/biennial vegetation or was adjacent to a *Brassica* crop. I arrived at the best models by including all explanatory variables in a maximal model which I simplified by AIC backwards step-wise selection.

To quantify the baseline yearly survival probability of *B. rapa* populations and that of other *Brassica* species I re-fitted the model without explanatory variables to the data for *B. rapa* populations and the data for all other *Brassica* species combined. To test if different factors were associated with survival of *B. rapa* populations and other *Brassica* species, I re-fitted the model with explanatory variables to the data for *B. rapa* populations and the data for all other *Brassica* species combined. *Brassica napus*, *B. oleracea* and *B. juncea* populations did not occur frequently enough to have sufficient data points to fit a model for each of these species individually.

To test whether populations which had already survived at least one year had a greater probability of surviving to the next year than newly founded populations, I fitted two additional Cox proportional hazard models; one to the data from populations present in 2010 and 2011, and another to data from populations absent in 2010 but present in 2011. These models did not have any explanatory variables and allowed me to estimate the probabilities of established and newly founded *Brassica* populations surviving to the next year, along with the associated uncertainties.

To test whether the density of seeds in the soil at the end of the 2010/2011 growing season affected the probability of population survival until the following year I fitted a binomial GLM using the populations present in 2010 as the dataset. The response variable was the presence or absence of each population in 2011 which I treated as binomially distributed, and the explanatory variable was the number of seeds germinated per litre of soil in soil samples collected in autumn 2011.

2.4 Results

2.4.1 Frequency, Dynamics, Size and Taxonomic Composition of *Brassica* Populations

A total of 401 separate *Brassica* populations were recorded in all surveys (Table 2.1). Populations were widespread but infrequent; over the three survey years there was on average around one population present per 15km of road, or 6.4 individuals per kilometre of road. Most populations did not survive for more than one or two years; the percentage of populations recorded in 2010 that were still present in 2011 was only 43.8%, and by 2012 this was only 15.7% (Table 2.1, see also Fig. 2.1 black bars vs. grey bars).

There was a large turnover of populations from year to year: in the interval from 2010 to 2011 around half of the populations became extinct, but around twice as many new populations were founded; in the interval from 2011 to 2012 around three quarters of the populations became extinct, but a similar number of new populations were founded (Table 2.1, Populations Extinct and Populations Founded in Previous Year). The total number of populations recorded was variable among years (mean \pm s.e., 150 ± 19), and tended to increase over the course of the study. Around 60% of surveyed 3×3 km squares contained no *Brassica* populations in each year; as such these proved to be unsuitable replicate units for a statistical analysis of variation in the number of populations among years.

Most populations were small, comprising fewer than ten individuals, few populations comprised more than 1000 individuals, and the largest was estimated to contain 5000 individuals (Fig. 2.1). Much of the increase in the number of populations from year to year comprised small populations of a few individuals (Fig. 2.1).

The taxonomic composition of populations was remarkably stable among years (Table 2.1), comprising mostly *B. rapa* ($63.2 \pm 2.3\%$), followed by *B. napus* ($24.9 \pm 2.5\%$) with smaller amounts of *B. oleracea* ($3.0 \pm 2.0\%$) and *B. juncea* ($6.1 \pm 1.4\%$). *B. rapa oleifera* was the most common taxon, comprising nearly half of all populations ($47.9 \pm 0.5\%$).

Table 2.1 Summary of *Brassica* populations recorded on the Canterbury Plains field surveys over three years, 2010 - 2012, showing the total number of populations and turnover in each year, and the percentage of populations belonging to each taxon recorded.

	2010	2011	2012	mean \pm s.e.
Populations: Total	121	177	190	150 \pm 19
Total Extinct in Previous Year	-	68	143	105 \pm 21
Total Founded in Previous Year	-	124	156	130 \pm 13
<i>Brassica rapa</i> (%)	64.5	57.6	68.4	63.2 \pm 2.3
<i>oleifera</i> (%)	48.8	47.5	47.4	47.9 \pm 0.5
<i>chinensis</i> (%)	8.3	9.0	19.5	12.3 \pm 3.6
<i>rapa</i> (%)	7.4	1.1	1.6	3.4 \pm 2.0
<i>Brassica napus</i> (%)	24.0	29.9	27.4	24.9 \pm 2.5
<i>napus</i> (%)	21.5	28.8	27.4	25.9 \pm 2.2
<i>napobrassica</i> (%)	2.5	1.1	0	11.2 \pm 0.7
<i>Brassica rapa</i> \times <i>napus</i> (%)	4.1	5.6	0.5	2.8 \pm 1.2
<i>Brassica oleracea</i> (%)	0.8	1.1	1.1	3.0 \pm 2.0
<i>Brassica juncea</i> (%)	6.6	5.6	2.6	86.1 \pm 1.4

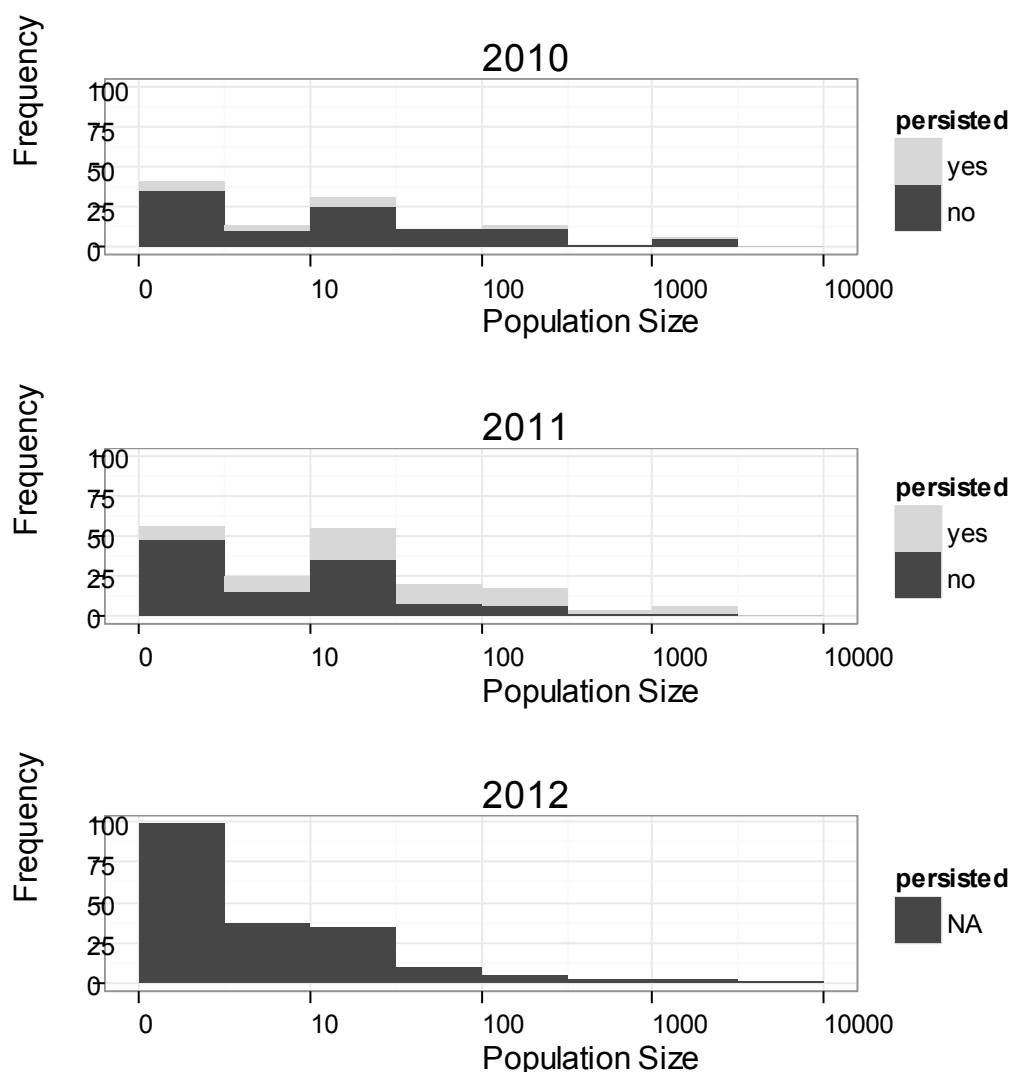


Figure 2.1 Frequency histograms of *Brassica* populations recorded on roadsides on the Canterbury Plains in three annual surveys from 2010 to 2012, classed by the number of *Brassica* individuals in the population (log scale). Black bars indicate populations that were extinct in the following year; grey bars indicate populations that survived until the following year (not shown for 2012, no survey was conducted in 2013).

2.4.2 Influence of Seed Sources on the Probability of *Brassica* Population Presence

There was a clear link between the probability of *Brassica* presence and transportation routes; *Brassica* populations were most likely to occur on state highways, followed by sealed roads, and then unsealed roads (Table 2.2, positive parameter estimate for state highway and sealed road compared to unsealed road). This effect had the biggest magnitude of all variables tested, and was consistent among years. *Brassica* populations were also more likely to be found close to seed companies (Table 2.2, positive distance to seed company parameter estimate). The bulk of populations occurred within 20km of the nearest seed company, with a median distance of 6.2 km in 2010, 7.5 km in 2011 and 6.1

km in 2012. The estimated probability of *Brassica* presence declined sharply as distance to the nearest seed company increased (Fig. 2.2). *Brassica* populations were consistently more frequent close to these seed transportation hubs in all years, although the asymptotic baseline probability of occurrence far from seed companies tended to increase over the three survey years (Fig. 2.2). Neither location adjacent to a cultivated *Brassica* field, nor distance to the nearest feral *Brassica* population in the previous year were significantly linked to the probability presence of feral populations, indicating these are not major seed sources for founding new populations.

Table 2.2 Results of GLMs of the probability of *Brassica* population presence on the Canterbury Plains for each year from 2010 - 2012, showing mean regression estimates \pm standard errors of significant variables. Factor variables are in comparison to the reference class. A dash indicates the variable was not retained in the best model for that year. Significance: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$.

	2010		2011		2012	
Sealed Road	3.01 \pm 0.63	***	2.21 \pm 0.42	***	2.72 \pm 0.46	***
State Highway	3.83 \pm 0.69	***	4.83 \pm 0.67	***	4.63 \pm 0.61	***
Distance Seed Company	1.08 \pm 0.27	***	1.34 \pm 0.38	***	1.06 \pm 0.33	**
Water course	1.06 \pm 0.32	***	0.92 \pm 0.43	*	1.26 \pm 0.38	***
Mowed	-1.53 \pm 0.28	***	-4.40 \pm 0.41	***	-3.27 \pm 0.35	***
Disturbed	0.93 \pm 0.27	***	1.31 \pm 0.34	***	2.04 \pm 0.31	***
Sprayed	-		-		1.50 \pm 0.37	***
Mean Summer Precipitation	-		-		1.20 \pm 0.31	***
Vegetation: annual/perennial	2.13 \pm 0.34	***	-		-	
Distance to Population in Previous Year	-		-		-	
Adjacent Land Use	-		-		-	
Mean Annual Temp	-		-		-	
Depression	-		-		-	

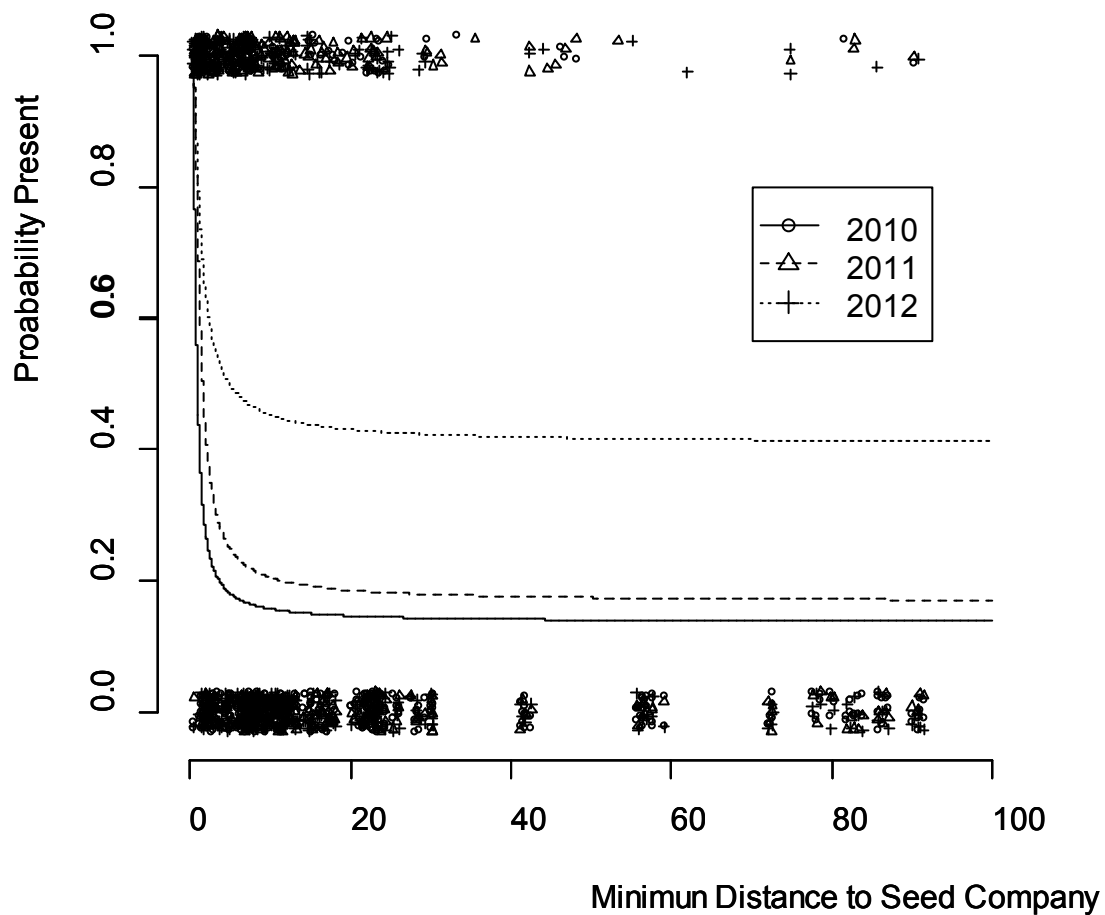


Figure 2.2 The relationships between the probability of *Brassica* population occurrence and the distance to the nearest company handling bulk quantities of agricultural seed. Lines show relationships derived from parameter estimates of GLMs fitted to data from field surveys in 2010 - 2012, symbols indicate survey sites where *Brassica* populations were present (i.e. probability present = 1) and absent (i.e. probability present = 0).

2.4.3 Influence of Management and Local Site Characteristics on the Probability of *Brassica* Population Presence

Water courses, mowing and disturbance had consistently significant relationships with the probability of *Brassica* presence (Table 2.2). *Brassica* populations were consistently more common along watercourses and in disturbed locations than other sites in all years, while management of vegetation by mowing appears to reduce the frequency of *Brassica* populations. Other measured variables were non-significant in explaining the probability of *Brassica* presence in most years (Table 2.2). Populations were more likely to be found in sites with predominantly annual and biennial

vegetation in 2010. Surprisingly, in 2012 *Brassica* populations were more likely to occur at sites managed with herbicides. Sites with higher winter precipitation also more frequently had populations in 2012.

2.4.4 Survival of *Brassica* Populations

The Cox proportional hazards model fitted to data for all *Brassica* species without any explanatory variables was used to estimate the baseline yearly survival probability of *Brassica* populations. Populations had a mean 61.0% chance of surviving one year (95% CI: 55.8% - 66.7%), and a mean 44.0% chance of surviving for two years (95% CI: 35.4% - 49.9%, Fig. 2.3). Populations which had already survived for one year or more appeared to be more persistent in subsequent years than newly founded populations (Fig. 2.4 a and b); the probability of survival from 2011 to 2012 was significantly lower for newly founded populations (18.4%, 95% CI: 12.2% - 22.8%) than for populations which had already survived one year (60%, 95% CI: 49% - 73.5%).

Testing site characteristics for their influence on the probability of *Brassica* population survival showed these differed from those associated with the probability of population presence; larger populations growing on the margins of watercourses or in depressions were more likely to persist (Table 2.3). The risk of populations sited on the banks of watercourses becoming extinct in a given year was reduced by 45.2% compared to other populations. Growing in a depression reduced the annual risk of extinction by 56.3%. Each increase in population size by one individual decreased the annual risk of extinction by 0.3%. While the p-value for the parameter estimate for population size was only marginally significant ($p = 0.053$), AIC values indicated significant support for retaining this term in the final model ($\Delta AIC = 7.7$). Surprisingly, *Brassica* populations at sites controlled with herbicide sprays tended to be more persistent, with 33.1% reduction in annual extinction risk. Disturbance appeared not to affect population persistence.

The probability of survival of *Brassica* populations from one year to the next was not related to variables associated with seed supply (road type, distance to seed company, land use and 2010 seed bank). The number of seeds found in the seed bank in autumn 2010 was non-significant in explaining the probability of population survival from 2010 until 2011 ($p = 0.09$); this suggests there is at best a weak link between persistence and the presence of a long-lived seed bank or successful reproduction and seed set.

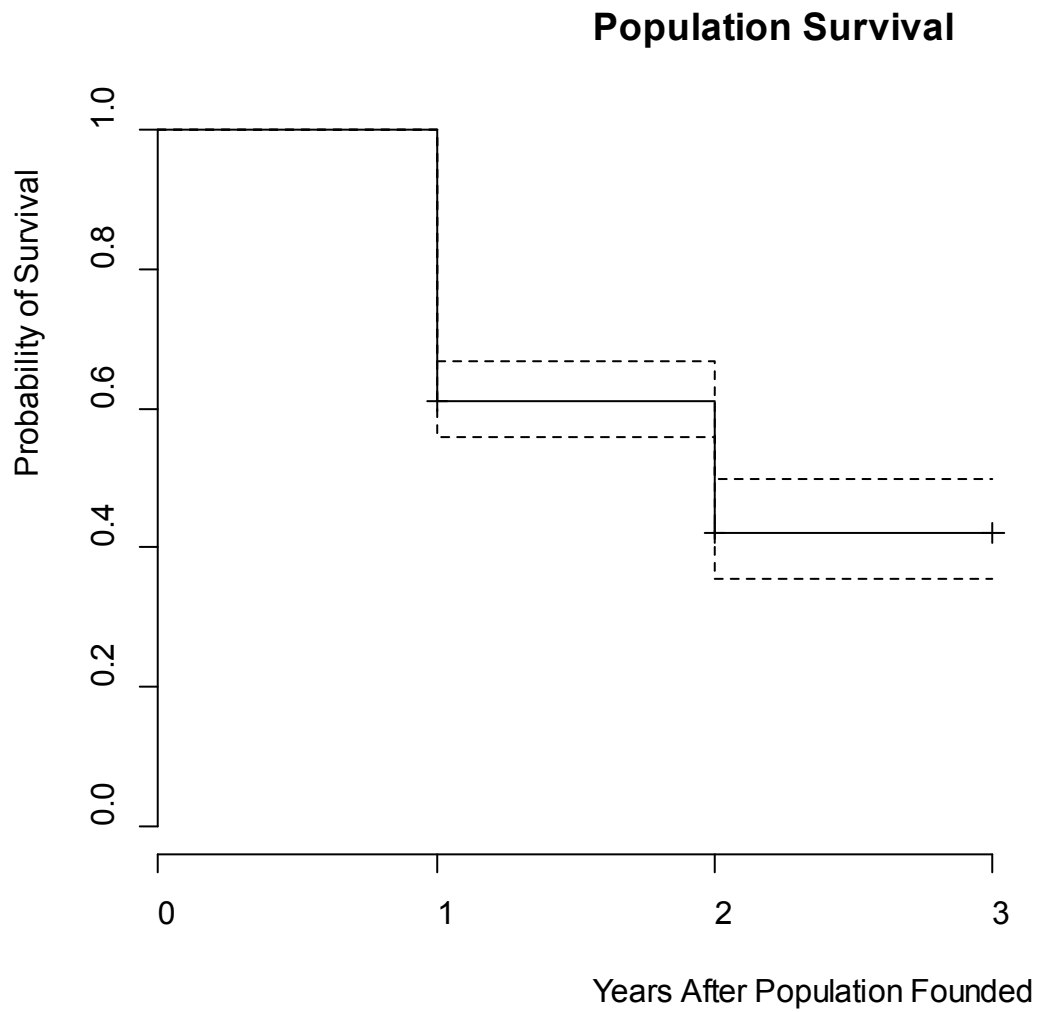


Figure 2.3 Results of a Cox proportional hazards analysis based on 3 years' survey data (2010 - 2012) showing estimated survival probability of *Brassica* populations on the Canterbury Plains the associated 95% confidence interval.

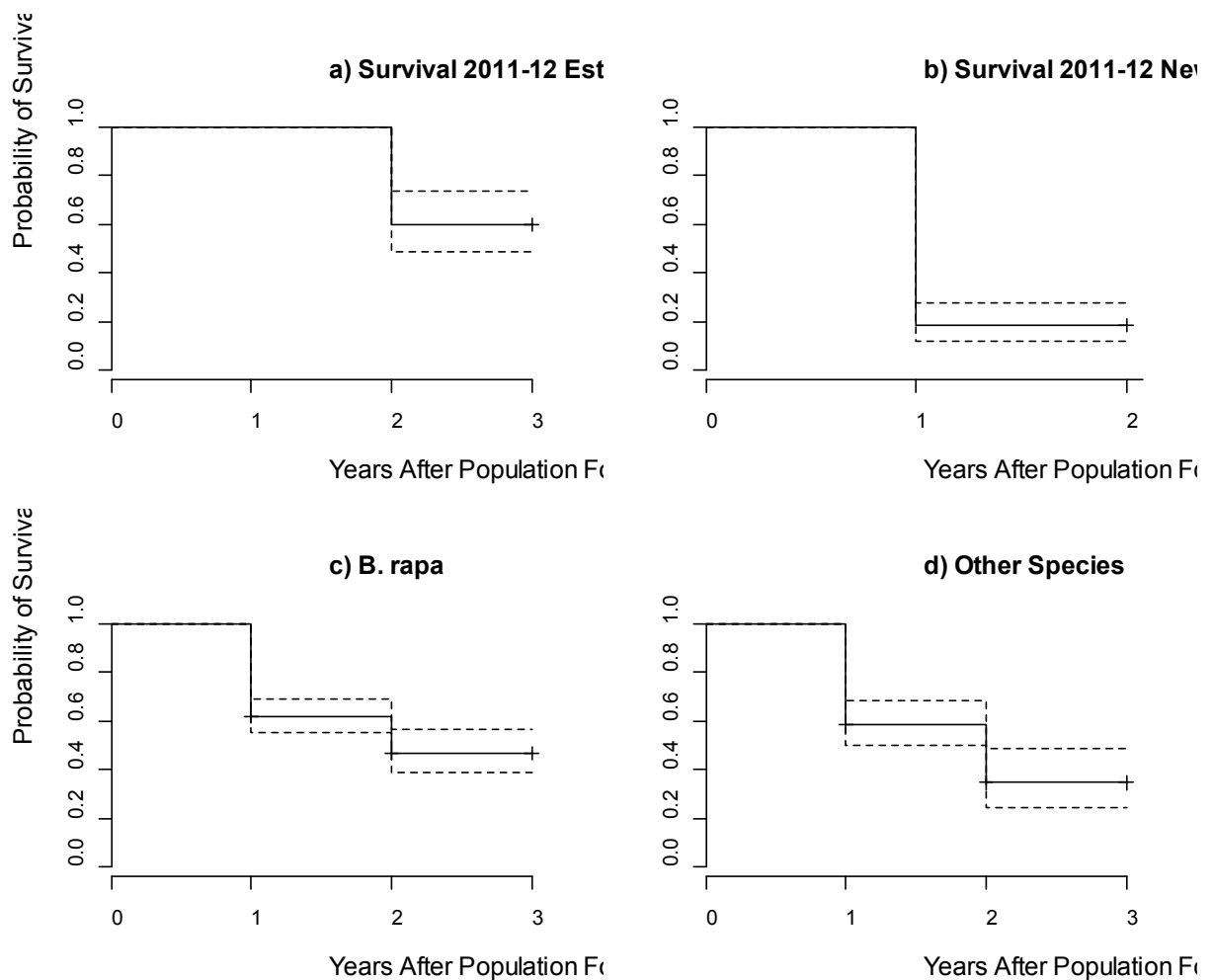


Figure 2.4 Results of Cox proportional hazards analyses showing estimated survival probability of *Brassica* populations on the Canterbury Plains the associated 95% confidence intervals. a) Survival probability of populations from 2011 to 2012 for populations already present in 2010. b) Survival probability of populations from 2011 to 2012 for populations newly founded in 2011. c) Survival probability of *B. rapa* populations based on 3 years' survey data (2010 - 2012).

Table 2.3 Results Cox proportional hazard models of survival of *Brassica* populations on the Canterbury Plains 2010 - 2012, showing mean parameter estimates \pm standard errors, coefficient exponentials, z values and associated p values, fitted to data for all species combined, and separately for *B. rapa* and other species (*B. napus*, *B. oleracea* and *B. juncea*). The coefficient exponentials are interpretable as the proportional effect of that coefficient on the probability of a population becoming locally extinct e.g. holding all other covariates constant, a population sited on a water course has on average 0.548 the risk of becoming extinct each year compared to populations not sited on a water course, an increase in population size of one individual reduces the yearly risk of population extinction by 0.3%.

	Estimate \pm s.e.	e ^{Estimate}	z	Pr(> z)
All species				
Water course	-0.601 \pm 0.207	0.548	-2.898	0.004
Depression	-0.828 \pm 0.296	0.437	-2.799	0.005
Population Size	-0.003 \pm 0.001	0.997	-1.938	0.053
Sprayed	-0.263 \pm 0.101	0.769	-2.595	0.009
<i>B. rapa</i>				
Water course	-0.889 \pm 0.277	0.411	-3.208	0.001
Depression	-1.288 \pm 0.428	0.276	-3.008	0.003
Population Size	-0.002 \pm 0.001	0.998	-1.498	0.134
Sprayed	-0.257 \pm 0.122	0.773	-2.110	0.035
Other Species				
Population Size	-0.021 \pm 0.008	0.980	-2.612	0.009
Sprayed	-0.377 \pm 0.189	0.686	-2.010	0.044

2.4.5 Species Differences

Few differences among species were found in the variables related to the probability of *Brassica* population presence or survival. GLMs assessing variables related to the probability of presence generally retained the same terms and effect directions when fitted to data for *B. rapa* and pooled data for the other three *Brassica* species (Table 2.4). Of the core variables that were significant in explaining the probability of *Brassica* presence in all three survey years (road type, distance to the nearest seed company, presence of a water course, mowing and disturbance), there were very few differences in which terms were retained and their effect directions between *B. rapa* and the other species (Table 2.4).

Species identity (*B. rapa* or other) was non-significant in explaining the probability of *Brassica* populations surviving from year to year, indicating that *B. rapa* populations are not more likely to

survive than populations of other species (Table 2.3, see also Fig. 2.4 c and d). Cox proportional hazard models fitted to data for *B. rapa* retained the same terms and effect directions as when the model was fitted to the data for all species (Table 2.3). While the p-value for the parameter estimate for population size was non-significant for *B. rapa* (0.134), AIC values indicated some support for retaining this term in the final model ($\Delta\text{AIC} = 4.7$). Unlike *B. rapa*, the probability of survival of *B. napus*, *B. oleracea* and *B. juncea* populations (combined data) was not influenced by whether the population was sited along a water course or in a depression.

Table 2.4 Results of GLMs of the probability of *Brassica* population presence on the Canterbury Plains for each year from 2010 - 2012, fitted separately to data for *B. rapa* and other *Brassica* species (*B. napus*, *B. oleracea* and *B. juncea*) combined, showing the effect direction of significant variables ('+' indicates a positive parameter estimate, '-' indicates a negative parameter estimate). An 'NA' indicates the variable was not retained in the best model for that year.

	2010		2011		2012	
	<i>B. rapa</i>	Other Species	<i>B. rapa</i>	Other Species	<i>B. rapa</i>	Other Species
Sealed Road	+	+	+	+	+	+
State Highway	+	+	+	+	+	+
Distance Seed Company	+	+	+	+	+	+
Water course	+	NA	+	+	+	NA
Mowed	-	-	-	-	-	-
Disturbed	+	+	+	+	+	+
Sprayed	NA	NA	NA	NA	+	NA
Mean Summer Precipitation	NA	NA	NA	NA	+	+
Vegetation: annual/perennial	+	+	NA	NA	NA	NA
Distance to Population in Previous Year	NA	NA	NA	NA	NA	NA
Adjacent Land Use	NA	NA	NA	NA	NA	NA
Mean Annual Temp	+	NA	NA	NA	NA	NA
Depression	NA	NA	NA	NA	NA	NA

2.5 Discussion

In this study I set out to determine whether alien *Brassica* maintained their presence on the Canterbury Plains via self-sustaining populations or metapopulations, or were reliant on continual seed immigration to persist, which factors were associated with *Brassica* population presence and survival, and whether these differed among species.

2.5.1 Survival of Populations

Estimates of Populations Survival

Despite the appearance of persistence due to a continuous presence in the landscape, the overwhelming majority of *Brassica* populations on the Canterbury plains are transient populations that are not self-sustaining. The main driver establishing *Brassica* populations is clearly seed spills from trucks during transportation; there was a strong consistent link between *Brassica* presence and both road type and distance to seed company. In the absence of these seed inputs *Brassica* would be unlikely to persist in the landscape in the long term. Just 15% of populations present in 2010 survived until 2012. This may, however, underestimate the probability of persistence because it is likely that some of these populations were present prior to 2010; accounting for this with a survival analysis suggested that around 40% of populations may occupy a site for two years following their foundation. Although there is no continuous record of populations recorded in the original 2003 survey, 5% of populations observed in 2010 occurred at or in close proximity to sites where *Brassica* was present in 2003 (Heenan et al. 2004b, Peltzer et al. 2008). Whether these have persisted throughout this period or have gone extinct only to be recolonized later is not known, but would put a maximum of 5% persistence beyond 10 years. These results are similar to the patterns observed in the UK where roadside sites were occupied by *B. napus* for a mean of 2.1 years and a median of 1.5 years, with a small minority of sites occupied for as long as ten years (Crawley and Brown 1995, 2004). Pessel et al. (2001) and Squire et al. (2011) similarly put maximum persistence times of feral *Brassica* populations at 10 years.

Implications of Low Population Survival

The issue of persistence of alien plant populations is largely neglected in the literature (but see Wade et al. 1997, Pysek et al. 2001, Pergl et al. 2012) despite many invasive aliens displaying large spatial and temporal fluctuations in distribution (Pysek and Hulme 2005) and often being associated with temporally variable anthropogenic habitats and disturbance (e.g. Stapanian et al. 1998, Chytrý et al. 2005, Alexander et al. 2009, Pysek et al. 2010). Understanding population persistence is important from two standpoints. First, where population persistence is low but new populations are continually founded by fresh external propagule inputs, controlling or eliminating the propagule supply should result in the decline and eventual extinction of the invader, offering an effective method of control that avoids many of the difficulties in attempting to control established populations (Hobbs and Humphries 1995). Second, assessments of species distributions in plant atlases and the invasion literature are often based on cumulative survey and herbarium records (Petrik et al. 2010). Failing to account for those populations which have become extinct can result in significant overestimates of a species' distribution. Pergl et al. (2012) checked persistence of historical *Heracleum mantegazzianum*

populations in the Czech republic and found that only around half of grid cells assumed to be occupied actually contained populations of the invader. Studies of the persistence of alien plant populations are few and find highly variable rates of persistence, e.g. Pergl et al. (2012) found 24% of populations recorded over approximately the previous 100 years had persisted, while Pyšek et al. (2001) found up to 90% persistence of three invasive herbs in historical sites. Such variation in population persistence has been attributed to differences in climate, land use, management (Lienert et al. 2002, Pergl et al. 2012) as well as differences among species in life form (Stehlik et al. 2007) and seed longevity (Stocklin and Fischer 1999).

Factors Influencing Population Survival

Here, environmental and demographic stochasticity, Allee effects, and a mismatch between plant requirements and site conditions all appear to contribute to the failure of *Brassica* populations to persist in the long term. Population size appears to be a major determinant of the likelihood of persistence; larger populations had a significantly greater probability of surviving. Theory (Shaffer 1981, Lande 1993), and empirical data (Elam et al. 2007, Firestone and Jasieniuk 2012) support the idea that this is because they are buffered against the negative effects of stochasticity and less susceptible to Allee effects. It is difficult to disentangle these two processes, but species differences do give some insight and suggest that stochasticity is more likely the cause of extinction than Allee effects (see 2.5.4 below).

My results showed that the (post seed set) number of seeds in the soil was at best marginally significant in explaining population survival to the following year, suggesting that successful reproduction or the presence of a long-lived seed bank are not the main constraints on population persistence. This is not to say that seed banks do not play a role, they may still allow plant populations to survive unusually harsh years in which conditions are unsuitable for successful growth and reproduction, and re-occupy a site after an interval of absence (Philippi 1993).

If the availability of seeds does not limit persistence, this indicates that a continuation of suitable conditions for emergence and growth from year to year may instead be a control of population persistence. Drainage and irrigation ditches may provide sites with less stochastic variability in the availability of bare ground, moisture and nutrients (Truscott et al. 2005). *Brassica* are usually grown in moist, fertile, low-competition, undisturbed sites (i.e. fields, Wright et al. 1988, Champolivier and Merrien 1996, Albert et al. 2012); it seems likely that drainage and irrigation ditches provide the best match to these conditions out of the available roadside sites. Many other roadside sites appear to be too temporarily variable and/or a poor match to *Brassica* attributes, preventing populations from successfully emerging or reproducing in successive years. In common with Pivard et al. (2008a), I

found that *Brassica* populations which had already survived one year were more likely to survive an additional year than newly founded populations. These results suggest there is something about these established populations or the sites they occupy that increases the probability of survival. My results show that the number of seeds in the soil does not significantly increase the probability of population survival, suggesting that populations at these sites do not persist due to the presence of a long-lived seed bank. Most likely these are additional sites that have conditions more similar to those in agricultural fields than the majority of roadside sites. Alternatively these sites may be occupied by *Brassica* taxa with attributes that are a better match to roadside conditions; *Brassica* have substantial intraspecific variation in attributes, some of which may facilitate increased performance in comparatively harsh sites. In this study I was unable to identify *Brassica* taxa with sufficient resolution to test this possibility.

The importance of site characteristics such as management regime, and the type and timing of disturbance in determining the probability of population survival is further illustrated by the relationships of the probability of *Brassica* persistence with herbicide application and occurrence in depressions. Sites sprayed with herbicides were favourable for *Brassica* persistence, such sites are often annually sprayed to control vegetation and maintain bare patches around posts, road markers and fences (Heenan et al. 2004b). This annual disturbance appears to give *Brassic*as a competitive advantage over other (predominantly perennial) roadside vegetation, and may enhance local recruitment (Crawley and Brown 2004, Heenan et al. 2004b, Knispel and McLachlan 2010). Populations sited in depressions tended to be more persistent. Because road-verges are usually mown with wide motorised mowers, these areas are usually not cut as short as the surrounding vegetation, *Brassic*as here may survive as intact rosettes, or have a better chance of survival after being cut due to higher residual biomass (Wilson and Clark 2001).

2.5.2 Seed Sources

It appears that accidental dispersal of seeds from trucks transporting seeds from seed companies to farms is the main vector founding new *Brassica* populations. Density of seeds in the soil, distance to the nearest *Brassica* population and proximity to a *Brassica* crop all had no effect on the probability of *Brassica* presence, while major transport routes and roads close to seed companies were more likely to have *Brassica* populations. Previous studies have found similar patterns (Crawley and Brown 1995, Pivard et al. 2008a), but have been unable to rule out the possibility that the higher frequency of *Brassica* populations on major roads may be due to differences in management or disturbance regimes (Pivard et al. 2008a). In this case this possibility can be discounted. I conducted a seed sowing experiment in the same study region in which *Brassica* seeds were added to roadside plots at a fixed rate, thus controlling for propagule pressure (Chapter 3). This showed that *Brassica*

performance did not differ among road types. It seems few roadside populations are founded by seed immigration from neighbouring fields or nearby populations in this system. Seeds spilled during harvest may be an additional propagule source for which I was unable to account; however most *Brassica* crops observed during surveys were fodder rather than seed crops so generally did not set seed.

2.5.3 Presence of Populations

Frequency of Populations

Brassica populations were infrequent compared to similar studies in Europe and Canada. I found 6.4 *Brassica* individuals per kilometre of road, the majority of which were *B. rapa*; on the M25 in the UK Crawley and Brown (2004) reported around 50 plants per kilometre of *B. napus*, and in Canada Knispel and McLaughlan (2010) recorded 5 - 550 *B. napus* plants per kilometre. The difference likely reflects the dominance of *Brassica* seed crops in the UK and Canada, which support large *B. napus* oilseed industries (Crawley and Brown 1995, Knispel and McLachlan 2010). This industry necessarily involves the transportation of larger quantities of harvested seed. By contrast most *Brassica* populations in Canterbury were *B. rapa*, which is grown for fodder rather than seed, and only requires transportation of seed to establish the crop - seeds are not produced, harvested or transported. Canterbury does support an oilseed industry, but this is not as established in New Zealand as in Canada or Europe, a relatively small amount of seed is produced (Hampton et al. 2012); *B. napus* accounted for only around one quarter of the recorded *Brassica* populations.

Dynamics of Population Frequency

The number of *Brassica* populations recorded increased over the course of the surveys. Roadside *Brassica* populations have occurred in the area for decades making it unlikely that this represents an invasion in progress (Peltzer et al. 2008). A lag effect cannot be discounted (Taylor and Hastings 2005), but this seems unlikely given the low rates of population survival and the lack of evidence for a self-sustaining metapopulation. *Brassica* and other ruderal annuals tend to have large inter-annual variation in abundance as a result of variation in climate influencing demographic rates such as germination and fecundity (Franco and Silvertown 1996, Grime 2001); other studies have recorded similar variation in the frequency of *Brassica* populations and attributed this to inter-annual variation around a dynamic equilibrium driven by climatic factors (Crawley and Brown 2004, Peltzer et al. 2008). Such dynamic metapopulations are well documented in annual and biennial species, although generally new populations result from reproduction and dispersal prior to local extinction rather than anthropogenic seed input (Hanski 1998). Following the same sampling protocol as this study, previous work identified 107 populations in 2003, and 142 populations in 2005 (Heenan et al. 2004b, Peltzer et al. 2008). In 2010 I recorded 121 populations, suggesting variation around an equilibrium

rather than an increase in the number of populations. It is also possible that the increase in number of *Brassica* populations over the course of the surveys reflects a measurement bias rather than a true increase, both my study and the 2003 -2005 study recorded an increase in the number of populations over their courses. Although we followed the same route each year, sites where *Brassica* were present in the previous year were checked explicitly and this may have given rise to an increase in sampling effort from year to year. Also we may have become more effective at finding *Brassica* over the course of the study. Much of the increase was in small populations of a few individuals which are relatively hard to detect, lending some support to these possibilities. Alternatively there may have been an increase in the transportation of *Brassica* seeds over the course of the study, and thus an increase in propagule supply; given that most populations are ephemeral results of seed spills during transportation this seems possible. The probability of *Brassica* populations occurring far from seed companies appeared to increase over the course of the survey suggesting transportation of seeds may have increased; however, no data on the total quantities of *Brassica* seed transported were available to test this definitively and this perceived increase may again reflect higher detection probabilities as we became more effective at finding *Brassica* populations.

Factors Influencing Presence of Populations

Brassica populations are most common in un-mown, disturbed sites along the banks of water courses where other annual and biennial species dominate.

The association between disturbance and the establishment of populations of alien plants is well documented, particularly for fast-growing annual taxa (Davis et al. 2000, Mack et al. 2000). Sites in which annuals and biennials dominate experience high annual turnover, and this 'carousel' effect is thought to allow opportunities for aliens to establish (Vandermaarel and Sykes 1993).

Mowing appears to be an effective management tool for limiting the occurrence of annuals such as *Brassica*. However, it should be noted we relied on the presence of flowers to detect populations from a passing vehicle; it seems possible that at least some of the observed effect of mowing may result from removal of flowers and thus reduced detectability of mown populations. The other main vegetation management practice on roadsides is herbicide application. Here spraying with herbicides generally had no effect on the probability of *Brassica* presence, and was positively associated with *Brassica* persistence, and presence in 2012. A similar positive relationship between herbicide use and *B. napus* presence was reported by Knispel et al. (2010). It seems spraying acts to disturb established perennial vegetation and actually provides opportunities for ruderal species to establish and persist.

It is more difficult to establish the mechanisms which support more frequent *Brassica* populations in sites such as drainage and irrigation ditches. These sites are generally impractical to mow, and experience regular disturbances in the form of high flow events and annual dredging, i.e. disturbances are likely timed to allow emergence but not prevent subsequent growth and maturation. These may also be sites with moist, fertile conditions similar to those in which *Brassica* are usually grown (i.e. fields, Wright et al. 1988, Champolivier and Merrien 1996, Albert et al. 2012). In addition water courses may act as secondary dispersal pathways (Soomers et al. 2010), although my results suggest that few populations are founded by seeds dispersing from other nearby populations.

2.5.4 Species Differences

The factors associated with the presence of populations differed little among species; however the factors associated with the survival of *B. rapa* populations showed some different patterns to those associated with the other *Brassica* species. *Brassica rapa* populations were far more likely to survive when located along water courses such as drainage and irrigation ditches, whereas for the combined *B. napus*, *B. oleracea* and *B. juncea* group there was a stronger signal that population size increased the probability of population survival. This indicates that *B. napus*, *B. oleracea* and *B. juncea* may be more sensitive to stochastic and Allee effects, whereas persistence of *B. rapa* populations is more strongly influenced by site characteristics. Allee effects are likely to be particularly important for self-incompatible species (Elam et al. 2007, Levin et al. 2009), which include *B. rapa* and *B. oleracea* but not *B. napus* (Tochigi et al. 2011), which made up the bulk of populations in the ‘other species’ group. It thus seems likely that the strong influence of population size on survival of *B. napus* (along with *B. oleracea* and *B. juncea*) reflects a greater susceptibility to stochastic effects. The results from the seed sowing experiment described in Chapter 4 suggest that *B. rapa* is better able to take advantage of disturbance than the biennials *B. napus* and *B. oleracea* (*B. juncea* is annual but populations were rare). Drainage and irrigation ditches are frequently disturbed by both high flow events and maintenance, particularly in winter and spring. It seems likely that *B. rapa* is able to rapidly grow and reproduce in this regularly disturbed environment, thereby reducing its sensitivity to stochasticity, while *B. napus* and *B. oleracea* are less able to capitalise on this frequent disturbance and remain vulnerable to stochastic effects.

2.5.5 Conclusions

In this system, despite giving the impression of persistence in the landscape, populations of an alien weed are generally short-lived, becoming extinct within a few years due to demographic and environmental stochasticity, inappropriate attributes for successful emergence, survival and

reproduction, and Allee effects. Rather than being naturalised (i.e. persisting in the landscape through successful reproduction) as is generally thought (Webb et al. 1988, Heenan et al. 2004b), in this system *Brassica* would be better considered as adventive (Pysek 1995). Few studies of alien plants consider population persistence explicitly (Pergl et al. 2012); failure to do so may not only result in overestimation of alien abundance and distribution when cumulative records are used, it may also miss opportunities for control or eradication. In this system it seems likely that if seed loss from trucks could be controlled or limited, for example by the more widespread adoption of tight fitting covers, roadside *Brassica* populations would decline significantly and may be all but absent from the landscape in around a decade. Other apparently naturalised alien species may in fact display such adventive population dynamics, in particular cultivated species where large quantities of seed are transported. Anthropogenic propagule dispersal is a major vector of alien plant spread (Hodkinson and Thompson 1997). Even when a species is self-sustaining, controlling additional human mediated propagule inputs could potentially reduce the species' range or abundance, and prove to be a valuable management tool. We need to better document persistence of alien plant populations, develop a better understanding of the controls of population persistence (Rouget and Richardson 2003), and integrate these into efforts at mapping distributions and control.

Chapter 3

Introducing New Varieties of Alien Plants May Pose Novel Biosecurity Risks

3.1 Abstract

The taxonomic unit used to assess alien invasiveness is usually the species. There is considerable intraspecific variation in plant traits, but the extent to which these differences can affect invasiveness is unknown. Alien performance also varies among sites within a region. Quantifying the relative magnitudes of variation in invader performance among and within species and sites is key to efforts to understand invasions and identify invasive taxa.

I quantified performance of alien *Brassica* using a seed sowing experiment comprising 24 taxonomically stratified varieties (six subspecies, three species) plus a naturalised reference variety. Twenty-five seeds of each variety were sown separately into 25 × 25 cm cells of 80 altitudinally stratified plots (25 taxa × 4 replicates = 100 cells/plot). For each plot and taxon I measured covariates likely to influence performance. Using data from this experiment I quantified three measures of performance: number of cotyledons emerged per cell, numbers of plants per cell over time, and rate of population growth.

I partitioned variation in performance among taxa and locations using hierarchical models, and tested which covariates explained some of this variation. I tested the hypothesis that most variation in plant performance among taxa would reside at the species level (relative to subspecies and varieties), i.e. that species was the most appropriate taxonomic unit for assessing the performance and invasiveness of alien plants.

Around 90% of the variation in plant population performance was due to spatial variation among and within plots. Within the taxonomic hierarchy there was around 30 times more variation among varieties within species than there was among species. These patterns were established at emergence and persisted in numbers through time. Differences in the viability rates of the seeds at the time of sowing explained some of the differences in performance.

My results show that subspecific taxa of alien species can vary significantly in performance and suggest novel genotypes may pose a different invasion risk to that currently established for the species. Weed risk assessment protocols could be modified to screen for novel invasive genotypes within species by including variables such as seed viability in assessments.

3.2 Introduction

Much effort has been directed at predicting which alien plant species are likely become invasive (see Mack 1996, Reichard and Hamilton 1997, Pheloung et al. 1999, Mack et al. 2000, Kolar and Lodge 2001, Thuiller et al. 2005, Theoharides and Dukes 2007, Hayes and Barry 2008, Gurevitch et al. 2011). Implicit throughout this research is the assumption that species can be used as the unit of observation for assessing the invasiveness of alien plants (e.g. Rejmanek and Richardson 1996, Pheloung et al. 1999, Champion and Clayton 2001, Grotkopp et al. 2002, Daehler et al. 2004, Pysek et al. 2009, McGregor et al. 2012). The assumption is that most of the variation in invasion success resides at the species level and that variation below this level can be ignored on the grounds that individuals of the same species should have essentially the same probability of invading, given they share similar attributes. However, for over 30 years it has been recognised that variation within species may be important in assessing the risk posed by a given taxa (McNeill 1976, Williamson 1992, Mack 1996).

What delimits a 'species' is often not clear (de Queiroz 2005, Hey 2006), and organisms nominally belonging to the same species can display a wide range of genetic, reproductive, and morphological variability (Hajjar and Hodgkin 2007). Substantial genotypic and trait variation within species can arise from differential artificial or natural selection. Breeding programs artificially select specific traits to produce new varieties and cultivars, such as rapid cycling *Brassica rapa* (RCBr), which can produce up to ten generations per year (Williams and Hill 1986, Kelly 2006). Artificial selection often focuses on traits that increase productivity or survival, many of which may also increase the risk of invasion; for example, seed mass and viability, fecundity, tolerance of harsh environmental conditions and pests, growth rate, ploidy and biomass (Williams and Hill 1986, Habekotte 1993, Kumar 1995, Kelly 2006, Ramchiary et al. 2011, Meyer et al. 2012). Natural selection can produce novel genotypes, ecotypes and subspecies with new traits adapted to local conditions, such as early flowering in *B. rapa* to avoid drought (Franks 2011). Hybridisation within and among species can also give rise to novel genotypes within species, sometimes showing differences such as enhanced fecundity in association with increased invasiveness (Culley and Hardiman 2009, Ellstrand 2009, Ridley and Ellstrand 2009, Schierenbeck and Ellstrand 2009). Recently, genetic modification has become another source of new genes within species, and modified genes may spread beyond the original recipient taxon through hybridisation and introgression. Such genes are often chosen to confer radically new attributes such as resistance to herbicides and insect herbivory (Williamson 1992, Ellstrand et al. 1999, Warwick et al. 2009).

There is also evidence that within species there can be substantial variation in invasion risk. *Bromus tectorum*, native to Europe, is considered benign and casual in New Zealand while in the USA it has

invaded large areas of intact shrub- and grassland (Mack 1981, Kinter and Mack 2004). Common greenhouse experiments indicate that differences in performance between the two recipient regions can be explained by the introduction of different genotypes, with plants from North American populations being larger and more vigorous than those from New Zealand populations (Kinter and Mack 2004). Genetic analysis indicates that specialist *B. tectorum* genotypes are allowing further expansion into previously uninvaded habitats in the USA (Merrill et al. 2012). In a reciprocal common garden experiment, Hierro et al. (2013) found that seed mass of demes of the invasive ruderal *Centaurea solstitialis* sourced from its introduced range in Argentina was twice that of demes sourced from its native range in Turkey, and that this difference contributed to the invasion success of *C. solstitialis* in Argentina. Even very small differences within species can manifest as dramatically different ecological characteristics resulting in invasion; molecular evidence indicates that an alien haplotype of the cosmopolitan species *Phragmites australis* has been introduced into the USA in the last 200 years; this alien haplotype has invaded large areas where the species was previously absent and displaced previously occurring native genotypes, likely altering recipient ecosystem structure and function (Saltonstall 2002).

Seed demographic parameters and traits may play a crucial role in influencing the establishment probability of aliens, and can vary widely within species. Recruitment of seedlings from seeds is not only a prerequisite for establishment, it is considered to be a major bottleneck in the establishment of plant populations (Tilman 1997, Clark et al. 2007, James et al. 2011). The ability of taxa to recruit to seedling stage following the arrival of seeds in a new locality is a major constraint on the ability of taxa to successfully establish in a new location. The relationship between seed mass and establishment is well documented; taxa with higher seed masses are generally less limited by the availability of suitable sites in recruitment to seedling stage (Stanton 1984, Moles and Westoby 2002, Clark et al. 2007, Maron et al. 2012). Seed mass commonly varies by an order of magnitude within species, and a significant component of this variation is thought to be due to genetic differences (Hendrix and Sun 1989, Beaulieu et al. 2007, Voller et al. 2012). Seed viability is also likely to influence the establishment probability of a taxon following the arrival of seeds. Plant species can vary widely in the viability of the seeds they produce; seeds of taxa produced for agriculture can vary in viability within a species, but are generally high (>80%), whereas the viability rates of seeds of wild plants can be much lower (Niedzielski et al. 2009). Thus at a fixed propagule supply rate, the number of viable propagules may vary widely among taxa, influencing the probability of establishment. The rate at which seeds remain viable below ground may also influence establishment probability, and can vary significantly within species (Vivian-Smith and Panetta 2009). Taxa with seeds with very low below ground survival rates are reliant on encountering conditions suitable for germination in the first year, whereas taxa with seeds which can remain viable below ground for extended periods have

more opportunities to encounter a favourable growing season and are in effect buffered against inter-year variability in conditions (Timmins and Owen 2001, Gulden et al. 2003, Claessen et al. 2005).

A climatic match between source and recipient regions is considered to be a fundamental requirement for the establishment and invasion of alien plants (Thuiller et al. 2005, Hayes and Barry 2008). Climate can vary considerably within a region, for example in the lee slopes of mountain ranges where rain shadows and altitudinal gradients can result in large temperature and precipitation differences over relatively short distances (Vinton and Burke 1997). Taxa within a species can vary in traits related to climatic adaptation such as tolerance of frost or water stress, leading to differential adaptation to sites with varying climate within regions (Volis 2009). Thus it might be expected that, within a species, taxa with traits adapted to different climatic conditions (e.g. ecotypes or agricultural varieties bred to be suited to different climates) will vary in their performance among locations within a region (Sexton et al. 2002, Ebeling et al. 2008).

Studies which have quantified the effects of variation among sites on alien plants have found large differences in performance, even at relatively fine spatial scales. In an experimental assessment of the density of safe sites for establishment of the alien *Hieracium lepidulum* in montane forests and grasslands, Miller et al. (In Press) found a more than twofold difference in the density of safe sites among forest plots, and a nearly tenfold difference among grassland plots. Minton and Mack (2010) tested the invasive potential of four alien species in a single field, and found spatial differences in population performance of up to three orders of magnitude. Similar variation was reported between just two sites by Davis et al. (2010) in their experimental assessment of the invasion risk of *Camelina sativa*.

In this study, I use *Brassica* as a model system to test the hypothesis that most of the variation in population performance and invasion risk in the genus *Brassica* would reside at the species level rather than among subspecies or varieties, and that the taxonomic level of species is therefore the appropriate level to quantify invasion risk. *Brassica* makes an ideal system to test this hypothesis due to its known ability to naturalise and well defined hierarchical taxonomy. In addition, the genus has a long history of artificial selection, resulting in considerable variation among varieties in morphology, breeding traits, ploidy, phenology and ecological tolerances (Tsunoda et al. 1980, Gupta 2009, Ramchiary et al. 2011). Specifically, I aimed to 1) partition the variation in population performance among taxonomic levels (species, subspecies, variety) and among plots in order to understand the key drivers of variation in invasion success in *Brassica*; 2) identify covariates related to taxonomy and location which explain variation in *Brassica* performance among taxa and sites; 3) quantify likelihood of establishment of 25 *Brassica* taxa sourced from four climatically different locations using a simple

demographic model parameterised with data obtained by experimentally introducing seeds into plots in an ecologically relevant setting.

3.3 Methods

3.3.1 Taxa

The six commonly cultivated *Brassica* species are interrelated; three diploid species (*B. oleracea*, *B. nigra* and *B. rapa*) appear to have hybridised to give rise to three amphidiploids which have been endowed with species epithets, *B. napus*, *B. juncea* and *B. carinata* (U 1935). These species each contain two or more subspecies, which are further divided into varieties and cultivars (i.e. stable under propagation), selected to suit various uses, climates and markets. For example *B. rapa* contains varieties ranging from root vegetables (turnips, *B. rapa rapa*), to leaf vegetables (e.g. bok choy, *B. rapa chinensis*), oilseed crops (*B. rapa oleifera*), and RCB (*B. rapa*). Seventeen *Brassica* species are known to have naturalised worldwide (Randall 2002, USDA 2013). Within the study region (Canterbury, New Zealand) nine subspecies belonging to six species (*B. rapa chinensis*, *B. rapa glabra*, *B. rapa oleifera*, *B. rapa rapa*, *B. napus napobrassica*, *napus napus*, *B. oleracea acephala*, *B. oleracea gongylodes*, *B. juncea juncea*, *B. nigra*, and *B. tournefortii*), have been recorded as naturalised or casual populations along rural roadsides and other disturbed areas (Heenan et al. 2004b, Peltzer et al. 2008). Of these, the wild turnip (*B. rapa oleifera*) is the most common and can occur as large, persistent populations.

I selected taxa to encompass the potential variation in traits and behaviour among and within species, focussing on the three widely cultivated *Brassica* species considered naturalised in the study area (Heenan et al. 2004b): *B. rapa* (turnip and bok choy/pak choy), *B. oleracea* (cabbage and kale), and *B. napus* (oilseed rape/canola and swede/rutabaga). I selected two subspecies within each species, and then four varieties within each subspecies. In order to widely sample variation in plant traits and behaviour within species I sourced the four varieties within each subspecies from each of four geographic regions with differing climates; North America (Maine), Europe (UK and Germany), Australia and New Zealand (Table 3.1). The aim was to select varieties adapted to conditions in their source region, with the expectation that this would influence their likelihood of establishment in New Zealand (Thuiller et al. 2005, Hayes and Barry 2008). I expected New Zealand varieties to perform best across the study area because they are well adapted to conditions in the study area. Imported varieties should perform worse than New Zealand varieties; Australian varieties should in general be better adapted to warmer, drier conditions and should perform better in plots with these conditions; European and North American varieties are expected to be better suited to colder winters and should perform better in plots with these conditions. Seeds of these varieties were sourced from commercial seed companies. In addition I included seeds collected from the field of a

taxon widely naturalised in the study region and other areas of the world, *B. rapa oleifera* (Randall 2002, Heenan et al. 2004b, Pallett et al. 2004, Gulden et al. 2008), as a reference against which to compare the performance of the commercial varieties. *B. rapa oleifera* seed was collected from a single large, persistent population in the study area. Species and subspecies were recognised following the nomenclature of the GRIN online database (USDA 2013). I did not differentiate between varieties and cultivars; in practice all are likely to be varieties. All of the study taxa are closely related and thus liable to hybridise under open pollination (e.g. Heenan and Dawson 2005). The study thus comprised: 3 species × 2 subspecies × 4 commercial varieties + 1 × naturalised reference variety = 25 taxa, arranged hierarchically in three nested levels (varieties within subspecies, subspecies within species, and species). The species of all varieties were confirmed using amplified fragment length polymorphism (AFLP, see Appendix A). Most subspecies were biennials, with the exception of the annuals *B. rapa chinensis* and *B. rapa oleifera*, and *B. napus napus* which includes both annual and biennial varieties (Table 3.1). However, many biennial *Brassicas* can exhibit a plastic response to stress resulting in flowering and seed set in the first year (bolting).

3.3.2 Location

The study was conducted in the rural districts of the Canterbury Plains, New Zealand, in an area bounded by the Rakaia and Waimakariri Rivers to the North and South, the coast to the East and the foothills of the Southern Alps to the West. The region lies in the lee of the Southern Alps, and precipitation varies more than two-fold among locations within the study area (Tait 2007a, b). The area is primarily grazed farmland, much of it is intensively irrigated for dairy pasture, but sheep and beef cattle are also farmed. It is also the centre of *Brassica* cultivation in New Zealand due to its favourable climate and soils. *Brassica* are grown as arable crops, for animal feed, seed multiplication, and an emerging biofuel industry.

The area is covered by a network of roads, ranging from minor dirt and gravel roads to sealed state highways. These roads have a verge, ranging in width from approximately 2 to 5 metres, where the resident vegetation is largely a reflection of the species found in neighbouring fields; perennials dominate, particularly exotic grasses (*Lolium perenne* and *Dactylis glomerata*) and clovers (*Trifolium repens* and *T. pratense*). Road verges are the main habitat in which feral populations of *Brassica* species occur in the study area (mostly *B. rapa*, with some *B. napus*, *B. juncea* and *B. oleracea*), although these are comparatively rare (Chapter 2, Heenan et al. 2004b, Peltzer et al. 2008). Road verges in the study area are often disturbed by vehicles, farm machinery and stock. Management of the vegetation along these roadsides is primarily the responsibility of the adjoining landholder, and thus varies from frequent mowing and spraying with herbicides (particularly around drainage ditches

and fence-lines), to little or none. In addition the local government carries out occasional herbicide spraying of power-line poles and reflector posts.

Table 3.1 *Brassica* taxa used in the 2011 seed sowing experiment. OSR is oilseed rape. Source zone abbreviations are N. Am (North America) and NZ (New Zealand). Viability is the proportion of viable seeds at the time of sowing as measured using the protocols of the ISTA (Don 2003).

Species	Subspecies	Common Name	Variety	Source Zone	Life Cycle	Viability (mean \pm s.e.)
<i>napus</i>	<i>napus</i>	Canola/OSR	Griffin	N. Am	annual	76.6 \pm 1.5
			Telfer	Australia	annual	98.0 \pm 0.7
			Flash	NZ	biennial	96.0 \pm 0.9
			Tactic	Europe	biennial	92.6 \pm 0.9
	<i>napobrassica</i>	Swede	Purpletop Whiteglobe	N. Am	biennial	92.0 \pm 1.9
			Dominion	Australia	biennial	100.0
			Major Plus	NZ	biennial	99.8 \pm 0.3
			Airlie	Europe	biennial	89.2 \pm 1.3
			Champion	N. Am	biennial	98.2 \pm 0.8
			Sovereign	Australia	biennial	89.7 \pm 1.6
<i>oleracea</i>	<i>acephala</i>	Kale	Gruner	NZ	biennial	72.0 \pm 3.0
			Merlin	Europe	biennial	98.3 \pm 0.7
	<i>capita</i>	Cabbage	Red Express	N. Am	biennial	78.5 \pm 1.9
			Arixos NS	Australia	biennial	81.4 \pm 2.1
			Summercross	NZ	biennial	91.3 \pm 1.6
			Greyhound	Europe	biennial	89.6 \pm 1.4
<i>rapa</i>	<i>chinensis</i>	Pak Choi	Black Summer	N. Am	annual	99.2 \pm 0.4
			Rubens	Australia	annual	97.7 \pm 1.0
			Mei Quing	NZ	annual	96.5 \pm 1.2
			Canton White	Europe	annual	96.7 \pm 0.9
	<i>rapa</i>	Turnip	Mammoth Purpletop	Australia	biennial	96.5 \pm 1.2
			Barkant	NZ	biennial	99.2 \pm 0.4
			Frisia	Europe	biennial	99.5 \pm 0.5
			American Purpletop	N. Am	biennial	95.5 \pm 1.0
	<i>oleifera</i>	Wild Turnip	wild turnip	NZ	annual	44.4 \pm 3.0



Figure 3.1 Layout of a typical plot used in the 2011 seed sowing experiment to quantify *Brassica* performance and naturalisation risk in roadside habitats. Transect tapes run between the plot corners which were permanently marked with metal pegs, tapes were used to position the metal frame shown over cells. Replicate rows run parallel to the road.

3.3.3 Experimental Design

I selected 80 randomly located replicate sites to carry out seed sowing experiments. The sites were chosen first by stratifying the study area by elevation into four bands with elevation ranges: 0-100, 100-200, 200-300 and 300-400 masl; within each elevation band I located 20 sites drawn at random from a regular grid of 3482 points which covered the entire study area (approximately 2000 km²). In many cases a selected point was not located on a roadside, in these cases the site was repositioned at the nearest point on a roadside. I selected *Brassica* varieties from different source climates (see above), so I anticipated that the performance of these varieties would differ depending on climatic variables such as temperature and rainfall, which in the study area covary strongly with elevation. Sites were therefore stratified by elevation to provide a balanced sample representative of the climatic conditions across the study area.

In August 2011, at each of the 80 sites an experimental plot was established that comprised 100 cells arranged in a checkerboard pattern. Each plot had four rows, each with 25 cells (each 25 × 25 cm), with a gap between the two middle rows to allow easy access to all cells (Fig. 3.1). Replicate cells allowed me to quantify within plot variation in *Brassica* performance. The 25 cells of each row were each sown with 25 seeds (400 seeds/m²) from one of the 25 varieties, with each cell randomly assigned a variety. A density of 400 seeds/m² is similar to the seed density estimated to occur when *Brassica* seed is spilled during transport (Bailleul et al. 2012), which is likely to be the main source of

seeds for naturally occurring *Brassica* populations in the study region (Chapter 2, Crawley and Brown 1995). To account for the linear nature of roadside habitats, the four replicate rows were arranged parallel to the road at a distance of 0.5, 0.75, 1.25 and 1.5m from the road edge. Each site and the surrounding area were checked for naturally occurring *Brassica* prior to sowing and over the course of the experiment but none were found. During sowing gaps were mistakenly introduced at unknown locations in two plots making subsequent identification of the taxon in some cells uncertain. These plots were in different elevation bands and were excluded from the analysis.

To estimate survival rates of seeds in the soil, I buried individual mesh bags containing 100 seeds of each variety at a randomly selected subset of 12 sites stratified by elevation (3 sites in each 100m elevation band). Seed bags were buried at the same time as the plots were sown, exhumed one year later and any whole, intact seeds tested for viability by staining with tetrazolium (Lakon 1949). The viability of seeds at the time of sowing may influence taxonomic patterns of germination and subsequent population performance, so this was tested using the ISTA protocol (Don 2003). For each taxon seed mass was calculated by weighing 10 replicated batches of 250 seeds, dividing by each measurement by 250 and taking the mean of the 10 replicates.

In 2012 the experiment was repeated with a subset of 12 of the taxa used in 2011 (two of each subspecies), and a subset of 20 of the plots used in 2011 (5 from each elevation band). In 2012 a balanced, fully factorial design was used; seeds were sown at 400 seeds/m² and 4000 seeds/m² into disturbed and undisturbed cells. For inter-annual comparisons here I consider only data from 2012 pertaining to undisturbed cells sown with 400 seeds/m².

3.3.4 Data Collection

Seeds were added to the plots at the beginning of August 2011. Subsequently a randomly drawn subset of 8 plots, 2 from each of the four elevation bands, was monitored weekly to check for germination. First germination occurred at the end of August 2011, approximately 4 weeks after sowing; all plots were censused twice in September 2011 to capture the initial flush of germination, then monthly until the end of the growing season in February 2012. A final census was carried out in July 2012 to check for any over-wintering individuals.

At each census the number of *Brassica* individuals in each cell was counted. These were recorded as one of five life-stages: cotyledon, seedling (first true pair of leaves), adult (rosette or bolting), flowering, or fruiting. For fruiting individuals, both the number of siliques and the number of seeds per silique for five randomly selected siliques was recorded. Seeds were counted by shining a bright light through the silique to make the seeds visible. The total number of seeds was estimated as the mean number of seeds from the five siliques counted multiplied by the number of siliques.

Different climatic conditions are expected to affect varieties sourced from different climates in different ways: that is I expected source climate and local climate variables (including plot shading) to show interactions in their effects on *Brassica* performance. To measure local climate, data on mean winter and summer precipitation, and annual, maximum January and minimum July air temperature were extracted from the National Institute of Water and Atmospheric Research (NIWA) 500 × 500 m resolution climate maps (Tait 2007a, b). Characteristics of the plot and surrounding site thought to influence *Brassica* performance were recorded prior to sowing and at each census. These covariates were: plot elevation, vegetation height, percent bare ground, adjacent road type (sealed or unsealed), and shading (whether 50% or more of the plot was shaded throughout the day during initial germination in September). Percent bare ground was quantified separately for each of the four replicate rows (classed: 5%, 6 - 20%, 21 - 50% and >50%). Vegetation height was measured at the four corners of each plot; the mean height of the two corners nearest the road was used for the two rows closest to the road, and the mean height of the two corners further from the road was used for the two rows further from the road. Gravel road verges are frequently covered in fine dust from passing traffic, and the verges are frequently used by vehicles passing in opposite directions, both of which may result in higher *Brassica* mortality. Field surveys show gravel roads have fewer *Brassica* populations than sealed ones; this may reflect a difference in habitat or propagule pressure (Chapter 2).

3.3.5 Analysis

All analysis was conducted in R (R Development Core Team 2012).

Measures of *Brassica* Performance

I calculated three measures of *Brassica* performance, rate of population increase (λ), number of cotyledons emerged per cell (G), and total number of individuals per cell through time (L).

Lambda was estimated as the ratio of the total number of individuals (including seeds) per cell after one year to the number of seeds added per cell at the beginning of the experiment (Crawley 1986, Crawley et al. 1993, Norton et al. 2005, Tozer et al. 2008, Minton and Mack 2010). Lambda provides the most reliable measure of establishment potential, however survival of sown seeds to adulthood and reproduction was extremely rare (out of 200,000 seeds sown, just 776 individuals survived to adulthood and only 15 individuals set seed, see Appendix B); this resulted in a highly irregular λ distribution where individuals which set seed were outliers and there were too few observations to partition variation in λ into taxonomic and spatial components (Appendix B).

I chose L as the best measure of *Brassica* performance. L gives a measure of both plant density and the length of time for which individuals persisted; this metric includes individuals which were well

established but died prior to reproduction due random disturbance. Using L also allowed me to use data from all censuses in one model thereby maximising statistical power.

Recruitment of seedlings from seed (emergence) is widely considered to be a major bottleneck in plant establishment that may have a large influence on subsequent population dynamics (Tilman 1997, Clark et al. 2007, James et al. 2011). To test if the observed distribution of variation in performance among taxonomic levels and among/within plots was established at emergence I chose G as an additional measure of *Brassica* performance.

Partitioning *Brassica* Performance among Taxonomic and Spatial Components

To examine how variation in *Brassica* performance was partitioned among taxonomic levels and among and within plots, I fitted mixed-effect models using the function MCMCglmm, which estimates model parameters in a Bayesian framework (Hadfield 2010). I fitted a longitudinal model with L as the response variable (referred to hereafter as model LT), and a model with G as the response variable (referred to hereafter as model GT). My goal was to partition the *total* variation in *Brassica* performance among taxonomic levels and among/within plots so I fitted these models with an intercept only and no fixed effects. Covariates are expected to explain some of the variation in performance and may account for more variation at one taxonomic level than others, e.g. viability rate may account for a large amount of variation in performance at the varietal level, including this covariate as a fixed effect would then reduce the proportion of variation in performance residing at that level.

To account for the non-independence of cells nested within plots, I included plot as a random effect in GT and LT models. To account for the non-independence of repeated measures on cells through time, I included cell and time as additional random effects in the LT model. I treated the total number of seeds germinated in each cell and the total number of individuals in each cell as Poisson distributed. To account for overdispersion in Poisson distributions the MCMCglmm function fits an overdispersion term as an additional random effect.

The GT model thus took the form:

Total number of seeds germinated \sim Poisson(μ)

The mean (μ) was modelled using a log link function as:

$\log(\mu) = b_0 + \text{species/subspecies/variety} + \text{plot} + \text{overdispersion}$

The nested random terms species/subspecies/variety were modelled such that each variety had a coefficient drawn from a normal distribution with mean given by its subspecies and variance

estimated from the data, each subspecies had a coefficient drawn from a normal distribution with mean given by its species and variance estimated from the data, and species coefficients were drawn from a normal distribution with mean 0 and variance estimated from the data:

$$\text{variety} \sim \text{Normal}(\text{subspecies}, \sigma_1^2)$$

$$\text{subspecies} \sim \text{Normal}(\text{species}, \sigma_2^2)$$

$$\text{species} \sim \text{Normal}(0, \sigma_3^2)$$

For plot:

$$\text{plot} \sim \text{Normal}(0, \sigma_4^2)$$

Plus the overdispersion term which is: $\sim \text{Normal}(0, \sigma_5^2)$

The LT model was identical to the GT model except the response variable was the number of *Brassica* individuals counted per cell at each time period, and cell and time were included as additional random effects:

$$\log(\mu) = b_0 + \text{species/subspecies/variety} + \text{plot/cell} + \text{time} + \text{overdispersion}$$

cell was modelled such that each cell had a coefficient drawn from a normal distribution with mean given by its plot.

$$\text{cell} \sim \text{Normal}(\text{plot}, \sigma_6^2)$$

$$\text{time} \sim \text{Normal}(0, \sigma_7^2)$$

All models were run with (uninformative) default priors and 100,000 iterations and a burn-in of 5000 iterations, MCMC samples were thinned to include every tenth sample. Model convergence was checked by running each model three times and overlaying plots of the MCMC chains for each model parameter; for each parameter in each model all three chains converged around a common mean estimate which did not vary during the course of the simulation, indicating all models converged.

I partitioned the variation in G and L among taxonomic levels (species, subspecies, and varieties) and among/within plots. Models were fitted using Bayesian methods which allowed me to directly calculate the proportion of variation in *Brassica* performance, R , explained by each component, and the associated uncertainties, using their posterior distributions (Nakagawa and Schielzeth 2010). For Poisson distributed models this requires accounting for variance of the Poisson distribution and the

associated overdispersion, in addition to the variation associated with the specified random effects themselves (Nakagawa and Schielzeth 2010). Variance of the Poisson distribution (first term below) and overdispersion (second term below) can be estimated as:

$$\log\left(\frac{1}{e^{b_0}} + 1\right) + \sigma_5^2$$

(Nakagawa and Schielzeth 2010). Thus in GT, R_i , the proportion of variation in *Brassica* performance explained by the component with variance σ_i^2 , was calculated as:

$$R_i = \frac{\sigma_i^2}{\sum_{i=1}^5 \sigma_i^2 + \log\left(\frac{1}{e^{b_0}} + 1\right)}$$

Variation in *Brassica* performance due to the Poisson process and associated overdispersion reside at the lowest hierarchical level in the model, for the GT model this is equivalent to the variation in performance due to differences within plots. Thus for the GT model the proportion of variation in *Brassica* performance due to differences within plots was:

$$R = \frac{\sigma_5^2 + \log\left(\frac{1}{e^{b_0}} + 1\right)}{\sum_{i=1}^5 \sigma_i^2 + \log\left(\frac{1}{e^{b_0}} + 1\right)}$$

The total variation in *Brassica* performance due to the taxonomic component (species + subspecies + variety) in G models was calculated as:

$$R = \frac{\sum_{i=1}^3 \sigma_i^2}{\sum_{i=1}^5 \sigma_i^2 + \log\left(\frac{1}{e^{b_0}} + 1\right)}$$

And total variation in *Brassica* performance due to the spatial component (among plot + within plot) in GT model was calculated as:

$$R = \frac{\sum_{i=4}^5 \sigma_i^2 + \log\left(\frac{1}{e^{b_0}} + 1\right)}{\sum_{i=1}^5 \sigma_i^2 + \log\left(\frac{1}{e^{b_0}} + 1\right)}$$

To ensure results from the LT model were comparable to that from the GT model, variation in *Brassica* performance explained by time was excluded when calculating the proportion of variation explained by taxonomic and spatial components. For the LT model, variation due to differences among cells was included in the calculations, i.e. total variation in *Brassica* performance (excluding time) was calculated as:

$$\sum_{i=1}^6 \sigma_i^2 + \log(1/e^{b_0} + 1)$$

Otherwise variation in the LT models was partitioned in the same manner as the GT model.

For each taxonomic and spatial component I then calculated the mean and 95% credible interval for R . Credible intervals are the Bayesian equivalent of confidence intervals, containing 95% of the iterations in the posterior distribution, and were calculated using the function `summary.mcmc` from the R package `coda` (Plummer et al. 2006).

Variation in *Brassica* Performance Accounted for by Covariates

To test which covariates might account for some of the variation in *Brassica* performance, I fitted two additional models, identical to GT and LT with the exception that covariates were included as fixed effects; these models are referred to as GV and LV respectively. Covariates used in V models were: precipitation, mean annual temperature, shade, percent bare ground, vegetation height, seed viability rate, seed mass, source zone (North America, Europe, Australia or New Zealand), and road type. For GV I used winter precipitation and for LV I used summer precipitation, reflecting the timing of the life-stages in each model. Varieties from different source zones are likely to respond differently to precipitation, temperature and shading; to test for this I included the interaction of source zone with these variables as an additional fixed effects in V models. Mean January temperature, mean July temperature and elevation were strongly correlated with annual temperature and not included as covariates in V models, mean annual temperature explained most variation in *Brassica* performance. I did not include annual below ground seed survival rates as a covariate because these covaried with seed viability and seed mass (Pearson's correlation between seed viability and below ground seed survival rate = -0.74, $p = 2 \times 10^{-5}$; Pearson's correlation between seed mass and below ground seed survival rate = -0.39, $p = 0.05$). Table 3.2 summarises the fixed effect variables, including overall cell means and standard errors. Coefficient estimates of the fixed effects were assessed for significance using MCMC p-values, which are calculated as the minimum proportion of MCMC estimates lying to one side of zero. For factor fixed effects, coefficients and their significance were calculated in relation to a reference class of the factor (Table 3.2). The two V models (GV and LV) had the same random effects as GT and LT, but the fixed effect terms were:

$b_0 + b_1(\text{viability rate}) + b_2(\text{source zone}) + b_3(\text{percent bare ground}) + b_4(\text{vegetation height}) + b_5(\text{shade}) + b_6(\text{mean winter precipitation}) + b_7(\text{mean annual temperature}) + b_8(\text{road type}) + b_9(\text{seed mass}) + b_{10}(\text{source zone} \times \text{shade}) + b_{11}(\text{source zone} \times \text{mean winter precipitation}) + b_{12}(\text{source zone} \times \text{mean annual temperature})$

I partitioned variation in G and L among taxonomic and spatial components for the GV and LV models in the same way as for the GT and LT models.

Table 3.2 For the 2011 seed sowing experiment, summary of explanatory variables included in the GV model (total number of seeds germinated per 25 × 25 cm cell) and LV model (total number of *Brassica* individuals per 25 × 25 cm cell in each census), showing variable type (factor or continuous), levels (for factors), and mean, standard error, maximum and minimum values (continuous variables). For factor variables the reference class against which others are compared in G and L models is indicated with a '0'.

Variable	Type	Levels	Mean ± s.e.	Max	Min
Source Zone	Factor	0 (New Zealand) 1 (Australia) 2 (Europe) 3 (North America)	NA	NA	NA
Road	Factor	0 (unsealed) 1 (sealed)	NA	NA	NA
Bare Ground	Factor	0 (0 - 5%) 1 (5 - 20%) 2 (21 - 50%) 3 (51 - 100%)	NA	NA	NA
Shade	Factor	0 (unshaded) 1 (shaded)	NA	NA	NA
Vegetation Height (cm)	Continuous	NA	13.1 ± 5.9	0	99
Mean Summer Precipitation (mm)	Continuous	NA	179.3 ± 12.2	125.2	235.4
Mean Winter Precipitation (mm)	Continuous	NA	214.9 ± 9.8	159.7	273.4
Mean Annual Temperature (°C)	Continuous	NA	11.3 ± 0.1	10.7	11.8
Viability Rate (%)	Continuous	NA	90.8 ± 0.2	44.4	100.0
Seed Mass (mg)	Continuous	NA	3.39 ± 0.2	1.28	5.35

To gauge how much of the total variation in *Brassica* performance was explained by covariates I compared T and V models. I expected covariates to account for some of the variation in *Brassica* performance, and thus the proportion of variation explained by taxonomic and spatial components to shift between T and V models. Covariates related to spatial differences were measured at the plot level (climate, road type, shading, percent bare ground), with the exception of vegetation height, for which two values were calculated for each plot. Covariates related to attributes of the study taxa were measured at the level of variety (climatic source zone of varieties, viability rates in germination tests, seed mass). Thus if covariates related to spatial differences account for more variation in *Brassica* performance than covariates related to attributes of the taxa, I would expect less variation to reside among plots in the V models than the T models; if covariates related to attributes of the taxa account for more variation in *Brassica* performance than covariates related to spatial differences, I would expect less variation to reside among varieties in the V models than the T models.

Partitioning Viability of *Brassica* seeds among Taxonomic Levels

Variation in the viability rates of the sown *Brassica* seeds may exert a large influence on variation in subsequent performance. To quantify how this variation was partitioned among taxonomic levels I followed the modelling protocol described in the section 'Partitioning *Brassica* Performance among Taxonomic and Spatial Components'.

I fitted a model using MCMCglmm and with the proportion of viable seeds in each test replicate (Don 2003) as the response variable. I fitted the model with no fixed effects (intercept only) and the random effects were variety nested within subspecies nested within species. I treated the proportional response variable as binomially distributed.

The model thus took the form:

Proportion of viable seeds \sim Binomial(n,p)

The probability of viability (p) was modelled using a logit function as:

$\text{logit}(p) = b_0 + \text{species/subspecies/variety} + \text{overdispersion}$

The nested random terms species/subspecies/variety were modelled such that each variety had a coefficient drawn from a normal distribution with mean given by its subspecies and variance estimated from the data, each subspecies had a coefficient drawn from a normal distribution with mean given by its species and variance estimated from the data, and species coefficients were drawn from a normal distribution with mean 0 and variance estimated from the data:

variety \sim Normal(subspecies, σ_1^2)

subspecies \sim Normal(species, σ_2^2)

species \sim Normal(0, σ_3^2)

Plus the overdispersion term which is: \sim Normal(0, σ_4^2)

For a binomial distribution, the variance of the binomial distribution is $\pi^2/3$ (Nakagawa and Schielzeth 2010). So R_i , the proportion variation in *Brassica* seed variability explained by the component with variance σ_i , was:

$$R_i = \frac{\sigma_i^2}{\sum_{i=1}^4 \sigma_i^2 + \pi^2/3}$$

For each taxonomic component I then calculated the mean and 95% credible interval for R using the function `summary.mcmc` from the R package `coda` (Plummer et al. 2006).

Comparison between Years

To compare results from 2011 and 2012 seed sowing experiments, I performed the following analyses using only data from plots and taxa that were in both the 2011 and 2012 experiments.

To test whether partitioning variation in *Brassica* performance among taxonomic and spatial components yielded consistent patterns between years, I fitted GT and LT models to the data from the 2012 cohort. This allowed me to compare the relative amounts of variation explained by each taxonomic and spatial component between years. In addition I tested whether *Brassica* taxa showed similar patterns of relative invasiveness in 2011 and 2012, and whether sites were similarly suitable or unsuitable for *Brassica* invasion in 2011 and 2012, using mean λ values for each taxa and site. Mean λ value distributions were non-normally distributed, so I used Spearman's rank correlation to test for significant correlations between years of mean λ values of taxa and sites. This gives an indication of whether *Brassica* performance is consistent among taxa and sites, or varies depending on the year.

3.4 Results

3.4.1 Variation in *Brassica* Performance Among Taxa (T models)

The performance of *Brassica* varieties within a species differed more from each other than the performance of different species, and differences among varieties accounted for the most variation in performance of any taxonomic level, the opposite of what I hypothesised (Fig. 3.2a). That is, at a given location, the relative performance of taxa was primarily determined by differences attributable to their variety rather than their species. These were large differences, with variation among varieties more than 30 times greater than variation among species. Differences among varieties accounted for around 6% of the total variation in numbers of *Brassica* individuals per cell through time (L), whereas differences among species accounted for 0.2% of variation in L (Fig 3.2a, comparing LT for variety and species). This pattern was established at recruitment from seed; around 10% of the total variation in numbers of cotyledons per cell (G) was explained by differences among varieties, whereas differences among species accounted for just 0.3% of variation in G (Fig. 3.2a, comparing GT for species and variety). Differences among subspecies accounted for a small amount of the total variation in G and L (around 1.5% and 0.4% respectively, Fig 3.2a, LT and GT for subspecies).

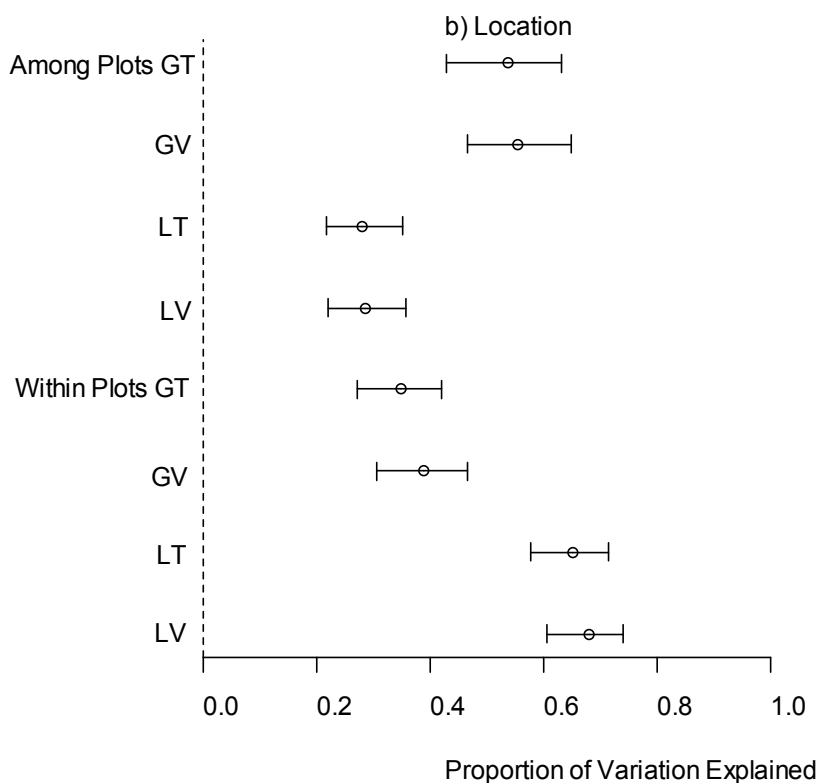
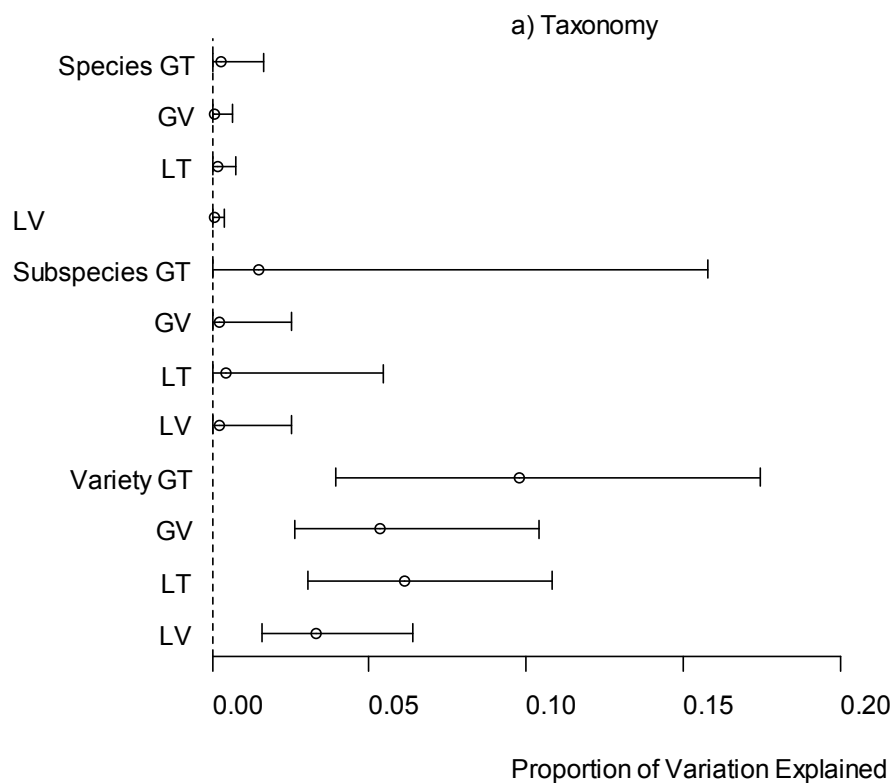


Figure 3.2 For the 2011 seed sowing experiment, the proportion of variation in numbers of *Brassica* seeds germinated per 25 × 25 cm cell (G) and total *Brassica* numbers per cell in each census (L) explained by a) taxonomic (species, subspecies and variety) differences and b) spatial (among and within plot) differences, along with the associated 95% credible intervals. Shown are results from are models fitted with intercept only (T, partitioning total variation) and models fitted with covariates as explanatory variables (V, Fixed effects: viability rate, vegetation height and cover, road type, precipitation, mean annual temperature, plot shading, geographic zone and its interaction with the previous three variables). n=7800 (G), n = 54,600 (L). Variation in *Brassica* performance due to time in L models has been omitted to allow comparison of taxonomic and spatial components between G and L models.

These results were consistent between years (2011 and 2012). Variety accounted for around 10% of variation in number of seeds germinated and 5% of *Brassica* numbers through time in both 2011 and 2012 (comparing Figures 3.2a and 3.3), while species accounted for less than 1% of variation in *Brassica* performance in both years. The ranked mean λ values of varieties were strongly correlated between years ($r = 0.79$, $p = 0.0019$), indicating that relative performance of varieties was reasonably consistent between years.

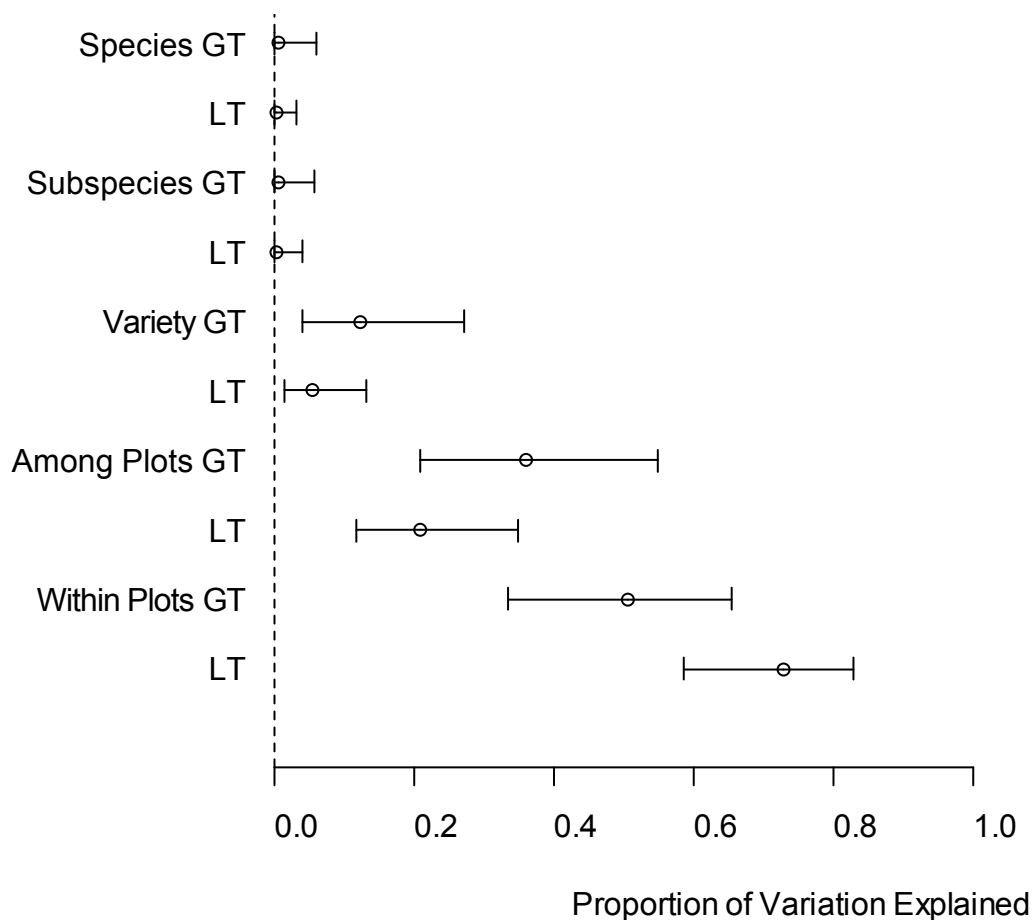


Figure 3.3 For the 2012 seed sowing experiment, the proportion of variation in numbers of *Brassica* seeds germinated per 25 × 25 cm cell (G) and total *Brassica* numbers per cell in each census (L) explained by differences among varieties within subspecies within species, among plots and within plots, along with the associated 95% credible intervals. Shown are results from models fitted with intercept only (T, partitioning total variation). $n=1920$ (G), $n = 17,280$ (L). Variation in *Brassica* performance due to time in L models has been omitted to allow comparison of taxonomic and spatial components between G and L models.

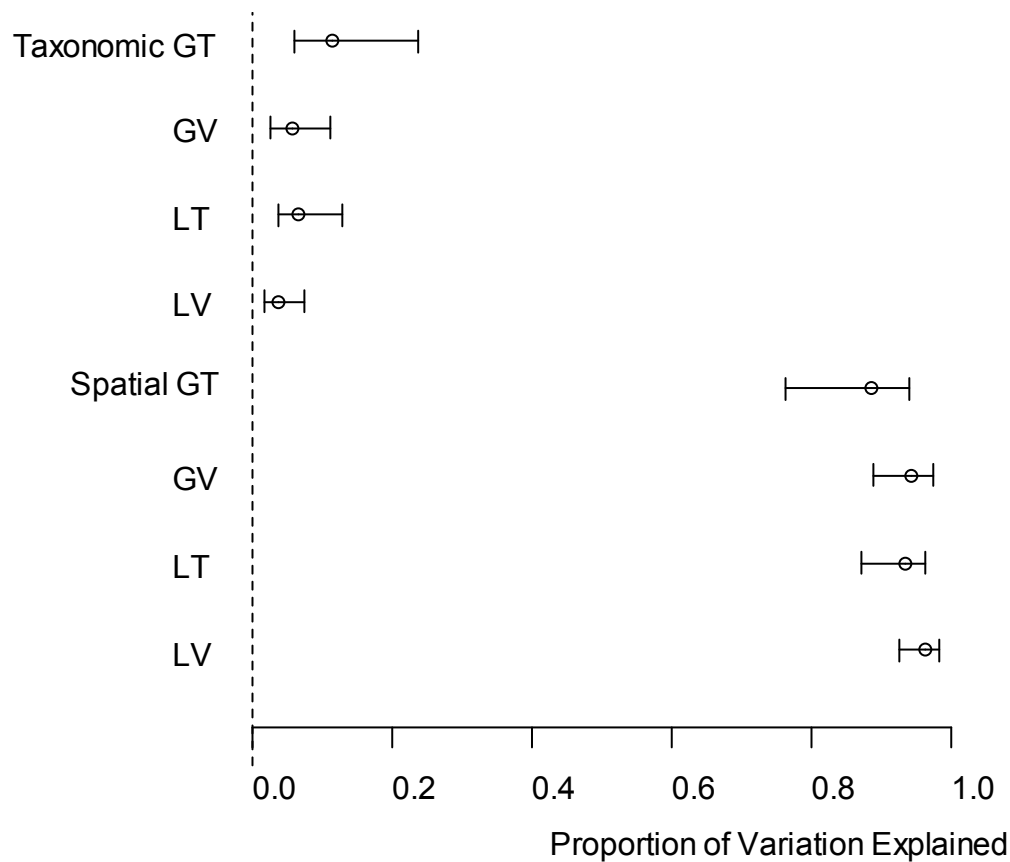


Figure 3.4 For the 2011 seed sowing experiment, the proportion of variation in numbers of *Brassica* seeds germinated per 25×25 cm cell (G) and total *Brassica* numbers per cell in each census (L) explained by taxonomic (species + subspecies + variety) and spatial (among plot + within plot) components along with the associated 95% credible intervals. Shown results from are models fitted with intercept only (T, partitioning total variation) and models fitted with covariates as explanatory variables (V, Fixed effects: viability rate, vegetation height and cover, road type, precipitation, mean annual temperature, plot shading, geographic zone and its interaction with the previous three variables). $n=7800$ (G), $n = 54,600$ (L). Variation in *Brassica* performance due to time in L models has been omitted to allow comparison of taxonomic and spatial components between G and L models.

3.4.2 Variation in *Brassica* Performance Among and Within Plots (T models)

Most variation in *Brassica* performance was due to differences among and within plots (Fig 3.2b). Spatial differences accounted for around 88% of the variation in G and 93% of the variation in L (Fig. 3.2b, GT and LT spatial, among plot + within plot), i.e. spatial differences influenced numbers of *Brassica* per cell through time more than it did numbers of cotyledons per cell. Most of the variation in G was due to differences among plots (around 54%, Fig 3.2b, GT among plot), while most of the variation in L was due to differences within plots (around 65%, Fig 3.2b LT within plot), i.e. variation in numbers of *Brassica* cotyledons per cell was mostly determined by coarse-scale differences among plots, while variation in numbers of *Brassica* per cell through time was mostly determined by fine-scale differences within plots. These patterns were consistent between years; in both years spatial differences accounted for around 90% of variation in G in both years, and over 90% of variation in L (Comparing Figs. 3.2b and 3.3). In contrast to the consistency of taxonomic ranks between years, ranked plot mean λ values were poorly correlated between the 2011 and 2012 cohorts ($\text{cor} = 0.16$, $p = 0.50$), indicating that relative suitability of plots for *Brassica* varied widely between years.

3.4.3 Effects of Covariates related to taxonomy on *Brassica* Performance (V models)

Covariates explained little of the variation in *Brassica* performance; V models differed little from T models qualitatively or quantitatively (Figs. 3.2 and 3.4). Most of the variation in performance accounted for by covariates was in those related to taxonomy, and that resided mostly at the varietal level (Fig. 3.4, less variation in performance accounted for by taxonomy in V compared to T models, Fig. 3.2a, less variation in performance accounted for by variety in V models compared to T models).

The viability rates of seeds at the time of sowing had a significant effect on numbers of cotyledons emerged and numbers of *Brassica* through time (Tables 3.3 and 3.4). Most variation in the viability of *Brassica* seeds resided among varieties, suggesting variation in this demographic parameter drove a significant proportion of the variation in performance among varieties (Table 3.5). However, even after accounting for variation in *Brassica* performance due to viability rates, within the taxonomic hierarchy most variation in performance still resided among varieties (see comparison of L and V models above). The viability rates of seeds of the commercial varieties were high, $92.6 \pm 1.6\%$ (mean \pm s.e., Table 3.1). The wild reference taxon (*B. rapa oleifera*) had markedly lower viability than the commercial varieties, only $44.4 \pm 3.0\%$ (mean \pm s.e.). To exclude the possibility that this seed had been harvested before maturing, the viability rate was confirmed by collecting seed from the same population the following year and retesting its viability ($43.8 \pm 5.2\%$, mean \pm s.e.). There was no significant difference in viability between seed collected in the two years ($t=0.14$, $\text{df} = 11.1$, $p = 0.9$). Seeds with higher mass showed higher recruitment to cotyledon stage, although this was only

marginally significant ($p = 0.078$, Table 3.3), and this effect did not persist in *Brassica* numbers through time (Table 3.4).

Climatic variation among sites overall had little effect on *Brassica* performance, and generally did not have a different effect on varieties sourced from different climatic zones. Varieties sourced from Australia, Europe and North America generally performed as well as each other and local New Zealand varieties. Fewer seeds of varieties sourced from North America emerged in plots with higher winter precipitation and mean annual temperatures compared to varieties sourced from New Zealand (Table 3.3, significant zone \times precipitation and zone \times temperature interactions), but this effect did not persist to influence numbers through time (Table 3.4).

Table 3.3 For the 2011 seed sowing experiment, coefficient estimates and 95% credible intervals for the fixed effects of model GV (total number of seeds germinated per 25 \times 25 cm cell). Significant pMCMC values (< 0.05) indicated in bold. Interaction terms are indicated with a 'x.' Factor variables are in comparison to the reference level for that factor (Bare Ground 0 – 5%, Road: Unsealed Road, Zone: New Zealand, Shade: Unshaded). Random effects: plot, variety, species, subspecies. $n = 7800$. pMCMC values are calculated as the smallest proportion of estimates from the posterior distribution lying to one side of 0.

Parameter	Estimate	95% CI		pMCMC
		Lower	Upper	
Seed Viability Rate	0.370	0.206	0.540	$< 2 \times 10^{-4}$
Seed Mass	0.144	-0.021	0.305	0.078
Vegetation Height	0.001	-0.032	0.034	0.947
Bare Ground: 5 - 20%	-0.147	-0.272	-0.029	0.018
21- 50%	-0.073	-0.348	0.190	0.582
50%	-0.032	-0.367	0.268	0.848
Road: Sealed Road	0.268	-0.324	0.861	0.353
Zone: North America (N. Am)	-0.245	-0.684	0.180	0.264
Australia (Aus)	-0.084	-0.554	0.345	0.700
Europe (Eur)	-0.077	-0.507	0.379	0.716
Precipitation	-0.080	-0.491	0.353	0.688
Precipitation \times N. Am	-0.093	-0.178	-0.001	0.036
\times Aus	0.029	-0.059	0.113	0.510
\times Eur	0.065	-0.021	0.149	0.135
Temperature	0.346	-0.066	0.761	0.104
Temperature \times N. Am	-0.128	-0.226	-0.022	0.016
\times Aus	-0.030	-0.130	0.060	0.571
\times Eur	-0.006	-0.103	0.094	0.907
Shade: Shaded	0.268	-0.349	0.904	0.417
Shaded \times N. Am	-0.065	-0.224	0.081	0.410
\times Aus	0.066	-0.077	0.218	0.383
\times Eur	0.114	-0.032	0.258	0.121

Table 3.4 For the 2011 seed sowing experiment, coefficient estimates and 95% credible intervals for the fixed effects of model LV (total number of *Brassica* individuals per 25 × 25 cm cell in each census). Significant pMCMC values (< 0.05) indicated in bold. Interaction terms are indicated with a '×.' Factor variables are in comparison to the reference level for that factor (Bare Ground 0 – 5%, Road: Unsealed Road, Zone: New Zealand, Shade: Unshaded). Random effects: cell, plot, variety, species, subspecies, time. n = 54600. pMCMC values are calculated as the smallest proportion of estimates from the posterior distribution lying to one side of 0.

Parameter	Estimate	95% CI		pMCMC
		Lower	Upper	
Seed Viability Rate	0.412	0.216	0.631	0.001
Seed Mass	0.155	-0.046	0.366	0.136
Vegetation Height	-0.034	-0.059	-0.009	0.006
Bare Ground: 5 - 20%	0.102	0.057	0.154	$< 2 \times 10^{-4}$
21- 50%	0.129	0.063	0.195	$< 2 \times 10^{-4}$
50%	0.168	0.074	0.251	0.001
Road: Sealed Road	0.045	-0.573	0.688	0.890
Zone: North America (N. Am)	-0.031	-0.216	0.155	0.724
Australia (Aus)	-0.169	-0.348	0.013	0.063
Europe (Eur)	0.039	-0.131	0.219	0.672
Precipitation	-0.307	-0.866	0.348	0.312
Precipitation × N. Am	-0.031	-0.216	0.155	0.724
× Aus	-0.169	-0.348	0.013	0.063
× Eur	0.039	-0.131	0.219	0.672
Temperature	0.041	-0.583	0.647	0.912
Temperature × N. Am	-0.037	-0.231	0.157	0.697
× Aus	-0.128	-0.306	0.067	0.187
× Eur	0.063	-0.117	0.239	0.487
Shade: Shaded	0.272	-0.435	0.952	0.445
Shaded × N. Am	-0.126	-0.326	0.101	0.252
× Aus	0.050	-0.151	0.266	0.638
× Eur	0.138	-0.067	0.338	0.191

Table 3.5 For the seeds of the 25 taxa used in the 2011 seed sowing experiment, the proportion of variation in viability rates at the time of sowing partitioned among taxonomic levels showing mean and 95% credible intervals for each level (variety, subspecies, species) plus the variation explained by the binomial distribution of viable/dead seeds and the associated overdispersion.

	Mean	Lower CI	Upper CI
Variety	0.43	3×10^{-3}	0.62
Subspecies	0.04	3×10^{-19}	0.32
Species	0.02	4×10^{-19}	0.20
Binomial distribution + overdispersion	0.52	3×10^{-3}	0.67

3.4.4 Effects of Covariates related to location on *Brassica* Performance (V models)

Few covariates related to location showed a significant relationship with *Brassica* performance (Tables 3.3 and 3.4). V models showed more variation in *Brassica* performance accounted for by spatial differences compared to T models, indicating that covariates related to taxonomy accounted for more variation in performance than covariates related to location (Fig. 3.4). Numbers of cotyledons emerged were generally lower in cells with intermediate amounts of bare ground (Table 3.3, 6 - 20% and 21 - 50% in comparison to the reference class of 0 - 5%), although this relationship was only significant for the 5 - 20% class. However, *Brassica* numbers through time tended to be higher in cells with more bare ground (Table 3.4, 0 - 5% reference class compared to all others). Plots with taller vegetation tended to have fewer *Brassica* through time (Table 3.4). Road type was non-significant in explaining *Brassica* performance, suggesting that the higher frequency of *Brassica* populations on sealed roads observed in the field is due to higher propagule pressure.

3.5 Discussion

3.5.1 Variation in *Brassica* Performance Among Varieties

In this experiment I tested the hypothesis that most of the variation in population performance of alien *Brassica* would be found among rather than within species. My results suggest that in this system the opposite is true; most of the variation in performance that could be attributed to differences among taxa was due to differences among varieties rather than differences among species or subspecies.

Attempts to understand the mechanisms and processes underlying invasion, and to assess the risk of taxa becoming invasive, have focussed on species as the unit of observation (e.g. Mack 1996, Reichard and Hamilton 1997, Pheloung et al. 1999, Daehler et al. 2004, Theoharides and Dukes 2007). Species is the default unit at which much biological science is conducted, and this approach has led to significant progress and advances in our understanding and management of biological invasions (see Mack 1996, Pheloung 1999, Williamson 1999, Kolar and Lodge 2001, Callaway and Maron 2006, Catford et al. 2009, van Kleunen et al. 2010, Blackburn et al. 2011, Gurevitch et al. 2011, Pysek et al. 2012). Nonetheless, this is not the first indication that alien taxa may vary considerably in their ecological characteristics and invasiveness within species (e.g. Saltonstall 2002, Kinter and Mack 2004, Vellend et al. 2010, Hierro et al. 2013). In light of this it should be recognised that some of our current knowledge regarding the invasiveness of alien species may reflect the average invasiveness of a number of disparate intraspecific taxa, or the invasiveness of one or a few intraspecific taxa that have been the subject of study. Unknown genotypes developed through breeding, hybridisation or

sourced from novel populations may pose an invasion risk different to that at which the species is currently assessed. Importantly, in the area of weed risk assessment (WRA), a species which has been assessed as low risk may actually contain high risk intraspecific taxa. One can envisage a hypothetical situation where the invasive genotype of *B. tectorum* has not yet been introduced to the USA: invasiveness in other regions is given significant weight in determining the outcome of WRAs such as the Australian WRA (Pheloung et al. 1999); based on the evidence of the benign New Zealand genotype, such WRAs may assess the species as low risk, leaving the door open for the invasive genotype to be transported to and establish. Other species may yet prove to play out such a scenario.

We need to assess the degree to which intraspecific differences may influence invasiveness in a wider range of taxa. The species tested here are closely related, and I deliberately set out to maximise variation among varieties; further experiments with a broader range of species are required to test if the within species variation in alien performance found here is common. If it exists only within certain taxa it will be useful to investigate if there are any features which could be used to flag such taxa for further investigation, such as a long history of breeding, multiple uses by humans for the same species, or widely varying morphology.

3.5.2 Seed Parameters

The observed patterns of variation in performance among taxa were established at emergence, suggesting that differences in seed performance were the main drivers of this variation. The relationship between seed mass and establishment potential is well documented (e.g. Stanton 1984, Burke and Grime 1996, Maron et al. 2012), although most evidence only shows a relationship in early life-history stages (Moles and Westoby 2002, Clark et al. 2007). Here there was only a marginally significant effect of seed mass increasing recruitment and this failed to translate to higher numbers through time. It may be that here variation in seed mass among taxa was too small for the effect on establishment to be detected, particularly with other large sources of variation in performance among locations and taxa. Instead, differences in seed viability among varieties drove patterns of emergence which persisted in numbers of individuals through time. This was the only taxonomic attribute that showed a strong and persistent effect on performance. The seed viability rates of the commercial taxa were all within a reasonable range for agricultural seeds, yet this relatively small amount of variation was enough to drive significant differences in performance that persisted through the entire life-cycle of the plants. Given that variation in seed viability resulted in substantial variation in performance, where often cited variables such as seed mass and climate did not, it seems that variable seed viability may result in differing invasiveness for some subspecific taxa of introduced alien species. Even having accounted for the variation in performance due to seed

viabilities, there were still substantial unexplained differences in emergence and subsequent performance among varieties. Further studies investigating the effect of multiple seed traits on emergence and establishment may shed light on the mechanisms driving these differences.

3.5.3 Climatic Match

I expected that much of the difference in performance among varieties would be driven by the differential adaption of varieties sourced from different climatic zones to the climatic conditions in the study area. However little of the observed variation in performance was attributable to interactions between source and recipient climate. New Zealand varieties did not perform better than other varieties, and Australian varieties did not perform better in warmer drier plots, nor did North American and European varieties perform better in cooler plots. Indeed, varieties from North America showed lower emergence rates than New Zealand varieties in cooler and wetter locations, but this effect was absent in numbers through time, suggesting any effect of climate on performance was short lived and minor compared to other factors. This is surprising given that a climatic match between source and recipient region is considered to be one of the main predictors of establishment and invasion potential (Thuiller et al. 2005, Hayes and Barry 2008). It seems that other factors limiting establishment overwhelmed any climatic signal.

3.5.4 Variation in *Brassica* Performance Among Locations

Despite significant variation in performance among varieties, most of the variation in performance was due to differences among and within plots. Of the measured covariates related to spatial differences, only vegetation cover and height were significant in explaining variation in performance; *Brassica* tended to perform better in sites with more bare ground and shorter vegetation. This is in keeping with the documented relationship between open, disturbed, low competition sites and invasibility (Hobbs and Huenneke 1992, Crawley and Brown 1995, Burke and Grime 1996, Crawley and Brown 2004). Having accounted for this there was still a large amount of unexplained variation in performance among locations. Explaining this variation was not a major goal of this study and is difficult without data for other potential covariates such as soil characteristics; collecting these data for all potential covariates in all 80 plots would have been a considerable undertaking. Agricultural *Brassica* varieties such as those used here are generally suited to sites where high nitrogen and moisture levels occur in combination with low competition and infrequent disturbance (i.e. fields, Wright et al. 1988, Champolivier and Merrien 1996, Dixon 2006, Bozkurt et al. 2011). These conditions may rarely occur in combination in roadside habitats; in particular low competition sites are likely to be unfavourable for growth due to low nitrogen or moisture availability and/or frequent disturbance; conversely sites with high nitrogen and moisture availability, and infrequent disturbance are likely to be occupied by well-established perennial grasses and other competitors making

establishment difficult (Burke and Grime 1996). The unexplained variation in performance is thus most likely a reflection of tough environmental conditions for *Brassica* in the study plots. Overlaying the factors limiting *Brassica* establishment creates a mosaic of sites of varying suitability; while some sites were suitable for germination, survival at these sites was low and variable, and sites suitable for reproduction were few and far between. Notably the sites where *Brassica* had the highest λ were not consistent between years, suggesting that the factors limiting establishment at each site varied between years. Furthermore most variation in the numbers of cotyledons emerged was due to differences among plots but most variation in *Brassica* numbers through time was due to differences within plots, suggesting that processes operating at relatively fine scales were responsible for much of the variation in post-emergence mortality. Disturbances by vehicles and stock using the road verges seem likely to result in variable mortality at fine spatial scales and between years, although without measures of disturbance for each cell this remains speculative.

3.5.5 Establishment Risk

Surprisingly, despite sowing seeds of taxa known to naturalise in the study region (Chapter 2, Heenan et al. 2004b, Peltzer et al. 2008) at 80 sites and at densities similar to those expected to occur in the field (Bailleul et al. 2012) performance was generally poor and seed set infrequent. From these results the naturalisation risk of all varieties appears to be low, despite the reference variety *B. rapa oleifera* occurring as large persistent populations (Chapter 2, Heenan et al. 2004b, Peltzer et al. 2008) and, less frequently, populations of *B. napus* and *B. oleracea* also occurring. This suggests 80 plots randomly located on road verges in a study area of approximately 2000 km² were insufficient to sample those sites where the reference taxon performs well enough to form self-sustaining populations. This highlights the difficulties of experimentally assessing naturalisation risk in the face of substantial variation in performance among sites. Indeed most experimental WRAs use far fewer sites to make assessments (e.g. Minton and Mack 2010, Davis et al. 2011). Crawley et al. (1993) reported a similar outcome when they sowed seeds of three varieties of oilseed rape (*B. napus napus*) into 12 locations; without pre-sowing disturbance only a few vigorous individuals set seed and as a result mean λ estimates for these varieties ranged from approximately 0.001 to 0.1. How then do seeds of these taxa reach suitable sites in the field? It is possible that the markedly higher below ground annual survival rate of *B. rapa oleifera* seeds (0.32 versus 0.009 for other varieties) may allow it to persist in the seed bank and thus exploit the additional dimension of time, taking advantage of favourable conditions when they arise, as with some annuals in temporally variable systems (Timmins and Owen 2001, Gulden et al. 2003, Claessen et al. 2005). Due to their low below-ground seed survival rates, however, commercial varieties are largely reliant on seed dispersal via spills from trucks during transport to reach suitable sites (Crawley and Brown 1995, Pessel et al. 2001, von der Lippe and Kowarik 2007, Bailleul et al. 2012). It appears that this vector is effective

enough to reach sites suitable for large flowering individuals to grow, despite the rarity of these sites on roadsides.

The infrequent seed set resulted in highly non-normal lambda distributions with outliers. This prevented me from partitioning taxonomic and spatial components of variation in λ . While numbers of *Brassica* emerged and numbers through time give a good measure of the relative performance of the taxa, λ provides a definitive measure of establishment potential. Further studies which obtained statistically tractable λ distributions may help strengthen conclusions concerning the importance of intraspecific variation in determining invasibility. It is notable that three of the four varieties to achieve $\lambda > 1$ in a cell were *B. rapa chinensis* ('Black Summer', 'Mei Quing', and 'Rubens'). This hints that there may be some taxonomic clustering of naturalisation potential at the subspecific level. Available evidence suggests *Brassica* naturalisation success is most strongly influenced by propagule pressure and disturbance (Chapter 2, Crawley and Brown 1995, 2004, Claessen et al. 2005, Pivard et al. 2008a, Knispel and McLachlan 2010). Future experiments manipulating disturbance and propagule pressure may prove fruitful.

3.5.6 Conclusions

The results presented here add experimental evidence to that of case studies (e.g. Saltonstall 2002, Kinter and Mack 2004, Vellend et al. 2009, Vellend et al. 2010, Merrill et al. 2012) which indicate that alien taxa within species can vary markedly in their ecological characteristics, performance and invasiveness. As yet it is unclear how common such variation within species is, or how often it translates in practice to variation in invasion outcomes. Resolving this will require studies across a broad range of taxa; experimental studies such as this one, as well as reciprocal common garden (e.g. Hierro et al. 2013) and genetic studies (e.g. Saltonstall 2002, Merrill et al. 2012) may all be useful approaches. It may prove useful to refine current WRA protocols to screen for intraspecific variation in traits and demographic parameters linked to invasiveness. The results here suggest that seed demographic parameters and traits (in particular viability, but also others such as seed mass) may be important characteristics influencing variation in invasiveness within short-lived herbaceous species. Such data are simple and inexpensive to measure and could be incorporated into existing WRAs with comparative ease.

Chapter 4

High Seed Density and Disturbance Increase Alien *Brassica* Emergence and Survival but Not Population Growth.

4.1 Abstract

The availability of seeds and bare ground are both thought to strongly influence whether plant species can establish at a given site, although the degree to which they influence population persistence is less well known. Differences in traits among and within species also influence invasion outcomes, and which traits are most important may depend on the strength of seed and site limitation. Thus the taxonomic level at which most variation in alien performance resides may shift under different regimes of seed and site availability.

I set out to quantify the effects of relaxing seed and site limitation on the germination, survivorship and population increase of a taxonomically stratified selection of 12 varieties alien *Brassica*, and look for shifts in the taxonomic level at which most variation in performance resided. Seeds of these varieties were sown into roadside plots at two densities (400 seeds/m² and 4000 seeds/m²) and with and without pre-sowing soil disturbance. The fates of the resulting populations were recorded and used to parameterise models of plant performance. I assessed the influence of sowing rate and pre-sowing disturbance on *Brassica* performance at different life-stages, and partitioned and compared taxonomic components of variation.

Both a higher sowing density and pre-sowing disturbance increased *Brassica* emergence, but only disturbance increased survivorship, and neither resulted in significantly higher population growth rates. Most variation in performance among taxonomic levels variation resided at the varietal level regardless of seed density or disturbance. While high seed density and disturbance can increase seedling recruitment, unless aliens have suitable adaptations to pass subsequent ecological filters, long term establishment is unlikely.

4.2 Introduction

Understanding what determines the distribution of plant populations is central to community assembly theory and the study of plant invasions (e.g. Tilman 1997, Chesson 2000, Levine and Rees 2002). Which plant taxa establish at a given site is often limited by seed availability (Lockwood et al.

2005, Simberloff 2009, McGregor et al. 2012) and the availability of suitable sites for germination, growth, and reproduction (Hobbs and Huenneke 1992, Davis et al. 2000, Myers and Harms 2009).

A population is seed limited if adding more seeds results in an increase in the density of the taxa at the site, i.e. in the absence of seed addition, seeds fail to reach all possible recruitment microsites at saturating densities (Eriksson and Ehrlén 1992, Turnbull et al. 2000, Clark et al. 2007). As seeds are added at higher densities, seed limitation should decrease and asymptotically approach zero as all available microsites become saturated (Poulsen et al. 2007); i.e. the proportion of seeds which emerge as seedlings should tend to zero. Seed limitation is thought to be related to seed mass (Turnbull et al. 2000, Maron et al. 2012); seed addition experiments tend to show that for species with larger seeds recruitment is more seed limited (see reviews by Moles and Westoby 2002, Clark et al. 2007).

Site limitation occurs when enough seeds arrive at a site so that all possible recruitment microsites are saturated, i.e. availability of suitable microsites is the limiting constraint on population size (Clark et al. 1998, Nathan and Muller-Landau 2000). Site limitation is thought to be substantially reduced following soil disturbance and in early successional habitats (Turnbull et al. 2000, Clark et al. 2007). The specific mechanism is unclear, but it is generally assumed that soil disturbance creates bare ground where germination can occur, and reduces competition with established vegetation for resources (Turnbull et al. 2000, Maron et al. 2012). The performance of taxa with ruderal attributes that allow them to rapidly colonise bare ground and utilise resources (i.e. rapid growth and maturation, high fecundity, such as in many successful invaders) is most likely to be constrained by limited availability of bare ground (Grime 2001).

Seed and site limitation often interact such that the effect of disturbance on recruitment depends on the rate of seed supply, with greatest recruitment in disturbed sites at high rates of seed supply (Clark et al. 2007, Britton-Simmons and Abbott 2008, Eschtruth and Battles 2009). In a recent seed sowing experiment (Chapter 3) I added 200,000 *Brassica* seeds to 80 roadside plots. Surprisingly, emergence and subsequent survival rates were low even though plots were located in sites similar to those where large, mature *Brassica* populations are documented in the study area (Chapter 3, Heenan et al. 2004b, Peltzer et al. 2008). Other studies quantifying *Brassica* performance have also documented low rates of population increase (e.g. Crawley et al. 1993). There is a strong link between seed availability and *Brassica* distribution (Chapter 2, Crawley and Brown 1995, Peltzer et al. 2008, Knispel and McLachlan 2010), however *Brassica* distribution is also linked to disturbance (Chapter 2, Crawley and Brown 2004, Knispel and McLachlan 2010). In this study I aimed to test the hypothesis that *Brassica* emergence and subsequent performance are limited by seed and/or

microsite availability, by adding *Brassica* seeds to cells of roadside plots at two densities, with and without disturbance of soil and vegetation.

Sites that are suitable for recruitment of cotyledons or seedlings may not be suitable sites for subsequent survival to adulthood or reproduction. Thus post-emergence mortality will generally lead to an increase in the degree to which a population is site limited through life-history stages (Clark et al. 1998, Turnbull et al. 2000, Muller-Landau et al. 2002, Clark et al. 2007). Firm conclusions about how seed and site limitation interact to determine plant distributions require following the fate of emerged seedlings until the reproductive stage (Myers and Harms 2009). However most studies assess seed and site limitation at the seedling stage, and studies which follow alien populations through to adulthood and reproduction are rare (but see Swope and Parker 2010). Thus the literature may contain a bias overstating the importance of seed limitation and it is unclear whether documented measures of seed limitation are linked to population persistence. In addition to site limitation, density-dependant processes may influence survival of seedlings to adulthood, i.e. at higher densities seedlings may incur higher rates of mortality at later life-stages due to increased intraspecific competition or likelihood of attack by pathogens and herbivores (Burdon and Chilvers 1982, Condit et al. 1994). Studies seeking to quantify the degree to which a species is site limited should be careful to distinguish density-dependent mortality from site limitation.

Limitation of recruitment by the availability of seeds and/or microsites is also likely to vary among species with different traits. Furthermore, plant traits and performance can vary as much or more within species as among them (Chapter 3); we may thus expect that limitation of recruitment by the availability of seeds and/or microsites may also vary within species. For example, seed mass can vary substantially not only among species, but also within species (Hendrix and Sun 1989, Beaulieu et al. 2007, Voller et al. 2012). There is a well-documented relationship between seed mass and the strength of seed limitation (Turnbull et al. 1999, Turnbull et al. 2000, Moles and Westoby 2002, Clark et al. 2007, Maron et al. 2012, Lonnberg and Eriksson 2013), so we might expect the strength of seed limitation to also vary substantially both among and within species. Seed viability can also vary substantially among within species (Chapter 3) due to genetic differences, maternal effects, the age of seeds and the environmental or storage conditions they encounter. In chapter three I identified seed viability as a parameter that drove some of the variation in performance among varieties. At a given site the number of seeds required to saturate all suitable microsites with viable seeds should vary with seed viability. In addition, the degree to which a taxon is site limited is not solely determined by the availability of bare ground; for example climatic match is considered to be an important control of invader establishment, and microsite suitability is likely to vary along environmental gradients such as temperature and precipitation (Thuiller et al. 2005, Hayes and Barry

2008). Species or varieties within species originating from different climates and introduced into a new region will differ in how well they are adapted to the new climate, i.e. they will differ in the degree to which climate limits in which sites they can establish. In Chapter three I found that most variation in *Brassica* performance resided among varieties, with little variation among species. If *Brassica* recruitment is seed and/or site limited, there may be a shift in the taxonomic level at which most variation in performance resides when seed and site limitation are manipulated.

In this study I ask whether the performance of alien *Brassica* is constrained by the availability of seeds, or suitable sites, how these relationships vary with seed attributes and climate, and how they vary among taxa and life-history stages. Specifically: 1) Can low emergence and subsequent poor performance in *Brassica* (e.g. Chapter 3, Crawley et al. 1993) be overcome by manipulating seed and/or microsite availability? 2) Does increasing seed and/or microsite availability change the way in which variation in *Brassica* performance is partitioned among taxonomic levels? 3) Does the relative importance of seed and/or microsite availability change through time, along environmental gradients or vary among taxonomic groups due to variation in parameters such as seed mass and viability?

4.3 Methods

4.3.1 Taxa

I used 12 alien *Brassica* taxa equally drawn from each of three species and six subspecies (two each from *B. rapa rapa*, *B. rapa chinensis*, *B. napus napus*, *B. napus napobrassica*, *B. oleracea acephala* and *B. oleracea capita*), with the aim of quantifying variation in performance at each taxonomic level (species, subspecies and variety). The study thus comprised: 3 species \times 2 subspecies \times 2 varieties = 12 taxa, arranged hierarchically in three nested levels (varieties within subspecies, subspecies within species, and species). I aimed to quantify the maximum potential variation in the genus at each taxonomic level; to maximise the potential variation in performance among varieties I chose the two varieties from each subspecies which had the best and worst performance (population growth rate, λ) in the seed sowing experiment described in chapter three. I defined species and subspecies according to the nomenclature of the GRIN online database (USDA 2013), with subspecies being finer taxonomic subdivisions of species. Varieties were further taxonomic subdivisions within subspecies. I did not differentiate between varieties and cultivars; in practice all are likely to be varieties, the taxa are closely related and thus liable to hybridise under open pollination (e.g. Heenan and Dawson 2005). The species of all varieties sown were confirmed using amplified fragment length polymorphism (AFLP, see Appendix A). The seed mass of each taxon was measured as described in Chapter three. The taxa used included both annual and biennial taxa (Table 4.1), although many biennial *Brassic*as can exhibit a plastic response to stress resulting in flowering as seed set in the first

year (bolting). The taxa used have many traits and behaviours linked to seed limitation (Clark et al. 2007): they have small to mid-sized seeds (mean values 1.28 - 4.52 mg), low annual below ground seed survival (Chapter 3), are aliens, and were absent from my study sites.

Table 4.1 *Brassica* taxa used in the 2011 seed sowing experiment. OSR is Oilseed rape. Viability is the proportion of viable seeds at the time of sowing as measured using the protocols of the ISTA (Don 2003), seed mass in milligrams.

Species	Subspecies	Common Name	Variety	Viability (mean \pm s.e.)	Seed Mass (mean \pm s.e.)
<i>napus</i>	<i>napus</i>	Canola/OSR	Griffin	76.6 \pm 1.5	3.34 \pm 0.01
			Flash	96.0 \pm 0.9	4.52 \pm 0.03
	<i>napobrassica</i>	Swede	Dominion	100.0	3.16 \pm 0.02
			Major Plus	99.8 \pm 0.3	2.87 \pm 0.05
<i>oleracea</i>	<i>acephala</i>	Kale	Sovereign	89.7 \pm 1.6	3.80 \pm 0.10
			Gruner	72.0 \pm 3.0	4.38 \pm 0.07
	<i>capita</i>	Cabbage	Red Express	78.5 \pm 1.9	3.49 \pm 0.04
			Greyhound	89.6 \pm 1.4	4.50 \pm 0.03
<i>rapa</i>	<i>chinensis</i>	Pak Choi	Rubens	97.7 \pm 1.0	2.13 \pm 0.02
			Canton White	96.7 \pm 0.9	2.13 \pm 0.01
	<i>rapa</i>	Turnip	Barkant	99.2 \pm 0.4	2.94 \pm 0.05
			Purpletop Whiteglobe	95.5 \pm 1.0	1.28 \pm 0.06

4.3.2 Location

The experiment was carried out in the same study region as the seed sowing experiment described in Chapter three, using a subset of the locations (see below) where the plots in that experiment were sited. See section 3.3.2 for a description of the study region.

4.3.3 Experimental Design

For my experiment I chose the 20 sites where *Brassica* performed best (had the highest λ) from the 80 randomly located sites used in chapter three. In the seed sowing experiment described in chapter three, recruitment was low and few *Brassica* individuals survived to set seed, as a result I was unable to partition λ , the rate of population increase, among taxonomic levels. In this study I aimed to increase survivorship so that I would obtain enough observations of individuals with seed set to partition λ among taxonomic levels. *Brassica* performance may depend on climatic variables such as temperature and rainfall. These covary with elevation; in the study region this ranges from 0 - 400m, so I chose the five top-performing sites from each of the four 100m elevation intervals described in Chapter three. Climatic variables did not explain any significant variation in *Brassica* performance in the chapter three experiment; however, in this experiment I relaxed constraints on establishment (by

experimentally manipulating seed and site limitation), without these strong barriers to establishment climatic variables may significantly affect *Brassica* performance.

In May 2012 at each site an experimental plot was established that consisted of 96 cells arranged in a 48×2 checkerboard pattern. Sowing rate and disturbance were experimentally manipulated in a randomised fully factorial design with the two rows as replicates within plots. Seeds were added at two rates (25 seeds per cell, 400 seeds/m² and 250 seeds per cell, 4000 seeds/m²). The lower rate is representative of rates that may occur due to seed spill and escapes from neighbouring fields (Bailleul et al. 2012) and allowed me to compare results with my previous experiment (Chapter 3); the higher rate is likely to be greater than generally occurs, but allowed me to test if low recruitment and poor performance could be overcome by increasing seed availability. To test if low recruitment and poor performance could be overcome by increasing the availability of suitable (i.e. disturbed) microsites, I disturbed designated cells by removing all vegetation and breaking up the underlying soil to a depth of 5cm with a mattock prior to sowing; undisturbed cells did not receive any treatment. A fully factorial design allowed me to test for interactions between sowing rate and disturbance. Each row thus consisted of 12 taxa \times 2 sowing rates \times 2 disturbance regimes, with each combination in a randomly determined cell. To minimise unplanned disturbances which may confound the experimental disturbance treatment, I sited plots in the least disturbed part of the road verge, as close as possible to the fence-line. To account for small scale variations in conditions and the linear nature of roadside habitats, the two replicate rows were arranged parallel to the fence-line at a distance of 0.25, 0.5m. Each site and the surrounding area were checked for naturally occurring *Brassica* prior to sowing and over the course of the experiment; however none were found.

4.3.4 Data Collection

Subsequent to sowing all plots were monitored weekly to check for germination. After first germination in the last week of June 2012 all plots were censused monthly until June 2013 (13 censuses). In each census each cell was checked and the number of *Brassica* individuals recorded. These were recorded as one of five life-stages: cotyledon, seedling (first true pair of leaves), adult (rosette or bolting), flowering and fruiting. For fruiting individuals the number of seeds was recorded. Seeds were counted by shining a bright light through the silique to make the seeds visible.

Microsite suitability may vary along temperature and precipitation gradients. To measure local climate, data on mean summer precipitation, and mean annual air temperature were extracted from the National Institute of Water and Atmospheric Research (NIWA) 500 \times 500 m resolution climate maps (Tait 2007a, b). Microsite suitability may also depend on whether plots were shaded during

June (i.e. winter and the time when emergence occurred) due to lower air temperatures and more frequent frosts. Plots were recorded as shaded if they were shaded throughout day during June.

4.3.5 Analysis

All analysis was conducted in R (R Development Core Team 2012).

Measures of *Brassica* Performance

To allow me to test if increasing the availability of seeds and/or available sites increased *Brassica* emergence and subsequent survival I calculated the following measures of *Brassica* performance, rate of population increase (λ), total number of individuals per cell through time (L), and number of cotyledons emerged per cell (G).

Lambda was estimated as the ratio of the total number of individuals per cell after one year (including seeds) to the number of seeds added per cell at the beginning of the experiment (Crawley 1986, Crawley et al. 1993, Norton et al. 2005, Tozer et al. 2008, Minton and Mack 2010). See Appendix B for details of the methods used to calculate λ . To examine whether a higher seed sowing density or pre-sowing disturbance increased the rate of population increase I tested for pairwise significant differences in λ values between experimental treatments (seed sowing rate and pre-sowing disturbance).

I chose L as the best measure of *Brassica* performance. L gives a measure of both plant density and the length of time for which individuals persisted; this metric includes individuals which were well established but died prior to reproduction. Using L also allowed me to use data from all censuses in one model thereby maximising statistical power. L contains repeated measures in each census, making it impossible to compare means of L among seed sowing and disturbance treatments. I therefore calculated a related measure of survival, number of flowering individuals per seed sown in each cell. This metric is bounded between 0 and 1 and is independent of sowing density, 0 indicates no seed limitation (i.e. establishment limitation = 1), 1 indicates absolute seed limitation (i.e. establishment limitation = 0) and 0.5 indicates that seed and establishment limitation equally constrain survival to flowering (Clark et al. 2007).

Recruitment of seedlings from seed (emergence) is widely considered to be a major bottleneck in plant establishment that may have a large influence on subsequent population dynamics (Tilman 1997, Clark et al. 2007, James et al. 2011). To test if sowing rate and pre-sowing disturbance influence emergence and subsequent survival differently, I chose G as an additional measure of *Brassica* performance. The related measure, number of cotyledons emerged per seed sown gives a

measure of seed limitation at emergence. This metric is bounded between 0 and 1 and is independent of sowing density, 0 indicates no seed limitation (i.e. establishment limitation = 1), 1 indicates absolute seed limitation (i.e. establishment limitation = 0) and 0.5 indicates that seed and establishment limitation equally constrain emergence (Clark et al. 2007).

Effect of Sowing Rate and Pre-Sowing Disturbance on the Proportion of Variation in *Brassica* Performance (L) Explained by Taxonomic Levels

To test if increasing seed and/or microsite availability changed the way in which variation in *Brassica* performance was partitioned among taxonomic levels I fitted mixed-effect models using the function MCMCglmm, which estimates model parameters in a Bayesian framework (Hadfield 2010). I fitted four models corresponding to the experimental seed sowing density and disturbance treatments: using the data from undisturbed cells sown with 400 seeds/m², the data from disturbed cells sown with 400 seeds/m², the data from undisturbed cells sown with 4000 seeds/m², and the data from disturbed cells sown with 4000 seeds/m². I fitted longitudinal models with L as the response variable. Lambda provides the most reliable measure of establishment potential; however, survival of sown seeds to adulthood and reproduction was extremely rare. *Brassica* set seed in just 25 out of 1920 cells (1.3%). This resulted in a highly irregular λ distribution where cells in which seed set occurred were outliers and there were too few observations to partition variation in λ into taxonomic and spatial components. My goal was to partition the *total* variation in *Brassica* performance among taxonomic levels and among/within plots so I fitted these models with an intercept only and no fixed effects. I included variety nested within subspecies nested within species as random effects in each model. To account for the non-independence of cells nested within plots and of repeated measures on cells through time, I included cell and time as random effects. I treated the total number of seeds germinated in each cell and the total number of individuals in each cell as Poisson distributed. To account for overdispersion in Poisson distributions the MCMCglmm function fits an overdispersion term as an additional random effect. The models thus took the form:

Total number of individuals per cell at each census \sim Poisson(μ)

The mean (μ) was modelled using a log link function as:

$$\log(\mu) = b_0 + \text{species/subspecies/variety} + \text{plot/cell} + \text{time} + \text{overdispersion}$$

The nested random terms species/subspecies/variety were modelled such that each variety had a coefficient drawn from a normal distribution with mean given by its subspecies and variance

estimated from the data, each subspecies had a coefficient drawn from a normal distribution with mean given by its species and variance estimated from the data, and species coefficients were drawn from a normal distribution with mean 0 and variance estimated from the data:

$$\text{variety} \sim \text{Normal}(\text{subspecies}, \sigma_1^2)$$

$$\text{subspecies} \sim \text{Normal}(\text{species}, \sigma_2^2)$$

$$\text{species} \sim \text{Normal}(0, \sigma_3^2)$$

For plot, cell and time:

$$\text{plot} \sim \text{Normal}(0, \sigma_4^2)$$

$$\text{cell} \sim \text{Normal}(\text{plot}, \sigma_5^2)$$

$$\text{time} \sim \text{Normal}(0, \sigma_6^2)$$

Plus the overdispersion term which is: $\sim \text{Normal}(0, \sigma_7^2)$

All models were run with (uninformative) default priors and 100,000 iterations after a burn-in of 5000 iterations, MCMC samples were thinned to include every tenth sample. Model convergence was checked by running each model three times and overlaying plots of the MCMC chains for each model parameter; for each parameter in each model all three chains converged around a common mean estimate which did not vary during the course of the simulation, indicating all models converged.

For details of how I partitioned variation in L among taxonomic levels using longitudinal Poisson distributed mixed effects models fitted with MCMCglmm, refer to the procedure described in section 3.3.5.

Effects of Sowing Rate and Pre-Sowing Disturbance on *Brassica* Performance (G, L and λ) and Variation with Climate and Seed Parameters

To test whether species' λ values differed in their responses to a higher seed sowing density or pre-sowing disturbance I compared λ values among experimental treatments using data for each species separately. Lambda values had highly non-normal distributions, and could not be transformed to

approximate normal distributions, making standard approaches for comparing means inappropriate. To account for this, I tested for significant differences with the procedure of Herberich, Sikorski et al. (2010) and the packages 'multcomp' and 'sandwich' (Zeileis 2006, Hothorn et al. 2008), which is robust to non-normal distributions and accounts for multiple tests of significance.

To test whether the higher seed sowing density or pre-sowing disturbance overcame seed and/or site limitation to significantly increase number cotyledons emerged per seed sown, or number of flowering individuals per seed sown, I tested for pairwise significant differences in these metrics among experimental treatments using t-tests on log-transformed data. I used a Holm Bonferroni correction to account for multiple tests of significance.

To test how increasing seed and/or microsite availability influenced emergence and survival, and how these relationships changed along environmental gradients and varied with seed parameters I fitted models using the function MCMCglmm (Hadfield 2010) with G and L as the response variables. These models are referred to as GV and LV respectively and took the same form as the models GV and LV models described in Chapter 3. They were fitted with sowing rate, pre-sowing disturbance and covariates of *Brassica* performance (mean annual air temperature, mean summer precipitation, shading, seed viability and seed mass) as fixed effects. I expected there may be interactions between sowing density and covariates of *Brassica* performance, pre-sowing disturbance and covariates of *Brassica* performance, and sowing density and pre-sowing disturbance so I included each of these interaction terms as fixed effects. The fixed effect terms were:

$$b_0 + b_1(\text{sowing rate}) + b_2(\text{pre-sowing disturbance}) + b_3(\text{mean annual temperature}) + b_4(\text{mean summer precipitation}) + b_5(\text{shade}) + b_6(\text{seed viability}) + b_7(\text{seed mass}) + b_8(\text{sowing rate} \times \text{mean annual temperature}) + b_9(\text{sowing rate} \times \text{summer precipitation}) + b_{10}(\text{sowing rate} \times \text{shade}) + b_{11}(\text{sowing rate} \times \text{seed viability}) + b_{12}(\text{sowing rate} \times \text{seed mass}) + b_{13}(\text{pre-sowing disturbance} \times \text{mean annual temperature}) + b_{14}(\text{pre-sowing disturbance} \times \text{summer precipitation}) + b_{15}(\text{pre-sowing disturbance} \times \text{shade}) + b_{16}(\text{pre-sowing disturbance} \times \text{seed viability}) + b_{17}(\text{pre-sowing disturbance} \times \text{seed mass})$$

The GV and LV models were fitted to the entire data set (i.e. data from cells sown with both 25 and 250 seeds, as well as disturbed and undisturbed cells). Table 4.2 summarises the fixed effect variables, including overall cell means and standard errors. Coefficient estimates of the fixed effects were assessed for significance using MCMC p-values, which are calculated as the minimum proportion of MCMC estimates lying to one side of zero. For factor fixed effects, coefficients and their significance were calculated in relation to a reference class of the factor (Table 4.2).

Table 4.2 Summary of explanatory variables included in the GV (total number of seeds germinated per 25 × 25 cm cell) and LV (total number of *Brassica* individuals per 25 × 25 cm cell in each census), showing variable type (factor or continuous), levels (for factors) and mean, standard error, maximum and minimum values (continuous variables). For factor variables the reference class against which others are compared in G and L models is indicated with a '0'.

Variable	Type	Levels	Mean ± s.e.	Min	Max
Sowing Rate	Factor	0 (400 seeds/m ²) 1 (4000 seeds/m ²)	NA	NA	NA
Pre-sowing Disturbance	Factor	0 (undisturbed) 1 (disturbed)	NA	NA	NA
Shade	Factor	0 (unshaded) 1 (shaded)	NA	NA	NA
Mean Summer Precipitation (mm)	Cont.	NA	179.1 ± 0.6	125.5	223.1
Mean Winter Precipitation (mm)	Cont.	NA	216.9 ± 0.5	170.1	259.3
Viability Rate (%)	Cont.	NA	91.0 ± 0.2	72.0	100.0
Seed Mass (mg)	Cont.	NA	3.21 ± 0.08	1.28	4.52

Testing for Density Dependent Mortality

To test if mortality prior to flowering was due to density dependent effects rather than site limitation I fitted models using MCMCglmm with the proportion of seedlings surviving to adulthood and flowering in each cell as the response variable. I fitted the models with the maximum number of seedlings in each cell as a fixed effect (explanatory variable); the random effects were variety nested within subspecies nested within species, and cell, to account for the non-independence of cells nested within plots. I treated the proportional response variable as binomially distributed.

The models thus took the form:

Proportion of seedling surviving to adulthood ~ Binomial(n,p)

Proportion of seedling surviving to flowering ~ Binomial(n,p)

The probability of survivorship (p) was modelled using a logit function as:

$$\text{logit}(p) = b_0 + b_1(\text{maximum number of seedlings}) + \text{species/subspecies/variety} + \text{overdispersion}$$

The nested random terms species/subspecies/variety were modelled such that each variety had a coefficient drawn from a normal distribution with mean given by its subspecies and variance estimated from the data, each subspecies had a coefficient drawn from a normal distribution with mean given by its species and variance estimated from the data, and species coefficients were drawn from a normal distribution with mean 0 and variance estimated from the data:

variety $\sim \text{Normal}(\text{subspecies}, \sigma_1^2)$

subspecies $\sim \text{Normal}(\text{species}, \sigma_2^2)$

species $\sim \text{Normal}(0, \sigma_3^2)$

Plus the overdispersion term which is: $\sim \text{Normal}(0, \sigma_4^2)$

Partitioning of Seed Parameters among Taxonomic Levels

To test how variation in seed parameters which may be related to the strength of seed and site limitation were partitioned among taxonomic levels I followed the modelling protocol described in section 3.3.5.

I fitted a models using MCMCglmm and with the proportion of viable seeds in each test replicate (Don 2003) as the response variable, and with seed mass from each replicate measure as the response variable. I fitted the models with no fixed effects (intercept only) and the random effects were variety nested within subspecies nested within species. I treated the proportion of viable seeds as binomially distributed, and seed mass as normally distributed.

The models thus took the form:

Proportion of viable seeds $\sim \text{Binomial}(n, p)$

The probability of viability (p) was modelled using a logit function as:

$\text{logit}(p) = b_0 + \text{species/subspecies/variety} + \text{overdispersion}$

and

Seed mass $\sim \text{Normal}(\mu, \sigma^2)$

With mean μ and variance σ^2 estimated from the data.

The nested random terms species/subspecies/variety were modelled such that each variety had a coefficient drawn from a normal distribution with mean given by its subspecies and variance estimated from the data, each subspecies had a coefficient drawn from a normal distribution with mean given by its species and variance estimated from the data, and species coefficients were drawn from a normal distribution with mean 0 and variance estimated from the data:

variety $\sim \text{Normal}(\text{subspecies}, \sigma_1^2)$

subspecies $\sim \text{Normal}(\text{species}, \sigma_2^2)$

species $\sim \text{Normal}(0, \sigma_3^2)$

Plus the overdispersion term which is: $\sim \text{Normal}(0, \sigma_4^2)$

For a binomial distribution, the variance of the binomial distribution is $\pi^2/3$ (Nakagawa and Schielzeth 2010). So R_i , the proportion variation in *Brassica* seed variability explained by the component with variance σ_i , was:

$$R_i = \frac{\sigma_i^2}{\sum_{i=1}^4 \sigma_i^2 + \pi^2/3}$$

For a normal distribution R_i , the proportion variation in *Brassica* seed mass explained by the component with variance σ_i , was:

$$R_i = \frac{\sigma_i^2}{\sum_{i=1}^4 \sigma_i^2}$$

For each taxonomic component I then calculated the mean and 95% credible interval for R using the function `summary.mcmc` from the R package `coda` (Plummer et al. 2006).

4.4 Results

4.4.1 Emergence (G)

Emergence rates varied greatly among cells, with few or no cotyledons emerging many cells, but close to 100% emergence in others (Fig. 4.1). The numbers of *Brassica* cotyledons to emerge per cell (G) showed strong positive relationships with both sowing rate and pre-sowing disturbance, indicating that emergence of *Brassica* was constrained by both the availability of seeds and suitable sites (Fig 4.1, initial peak in numbers following emergence, Table 4.3). There was no significant interaction between sowing rate and pre-sowing disturbance (Table 4.3, S.R. 4000 \times Disturbed parameter estimate), i.e. disturbance did not have a different effect on *Brassica* emergence in cells sown with 400 seeds/m² than in cells sown with 4000 seeds/m², suggesting that even at the higher sowing density not all empty microsites were occupied.

In undisturbed cells just over 10% seeds emerged as cotyledons, while in disturbed cells more than twice as many cotyledons emerged per seed sown (around 25%), suggesting that there were still significant barriers to successful emergence even when vegetation was removed and the soil

disturbed prior to sowing (Table 4.4). There was a small but significant increase in mean number of cotyledons per seed sown in cells sown with 4000 seeds/m² compared to cells sown with 400 seeds/m², in both disturbed and undisturbed cells (Table 4.4). That is, emergence was less constrained by the availability of sites at the higher seed sowing density, suggesting that the higher propagule pressure helped overcome barriers to establishment.

Emergence generally did not vary significantly along climatic gradients or with seed parameters; however, the number of cotyledons emerged was lower in shaded plots, but only in disturbed cells (Table 4.3, Disturbed × Shaded interaction). Seed mass and viability had no effect on emergence.

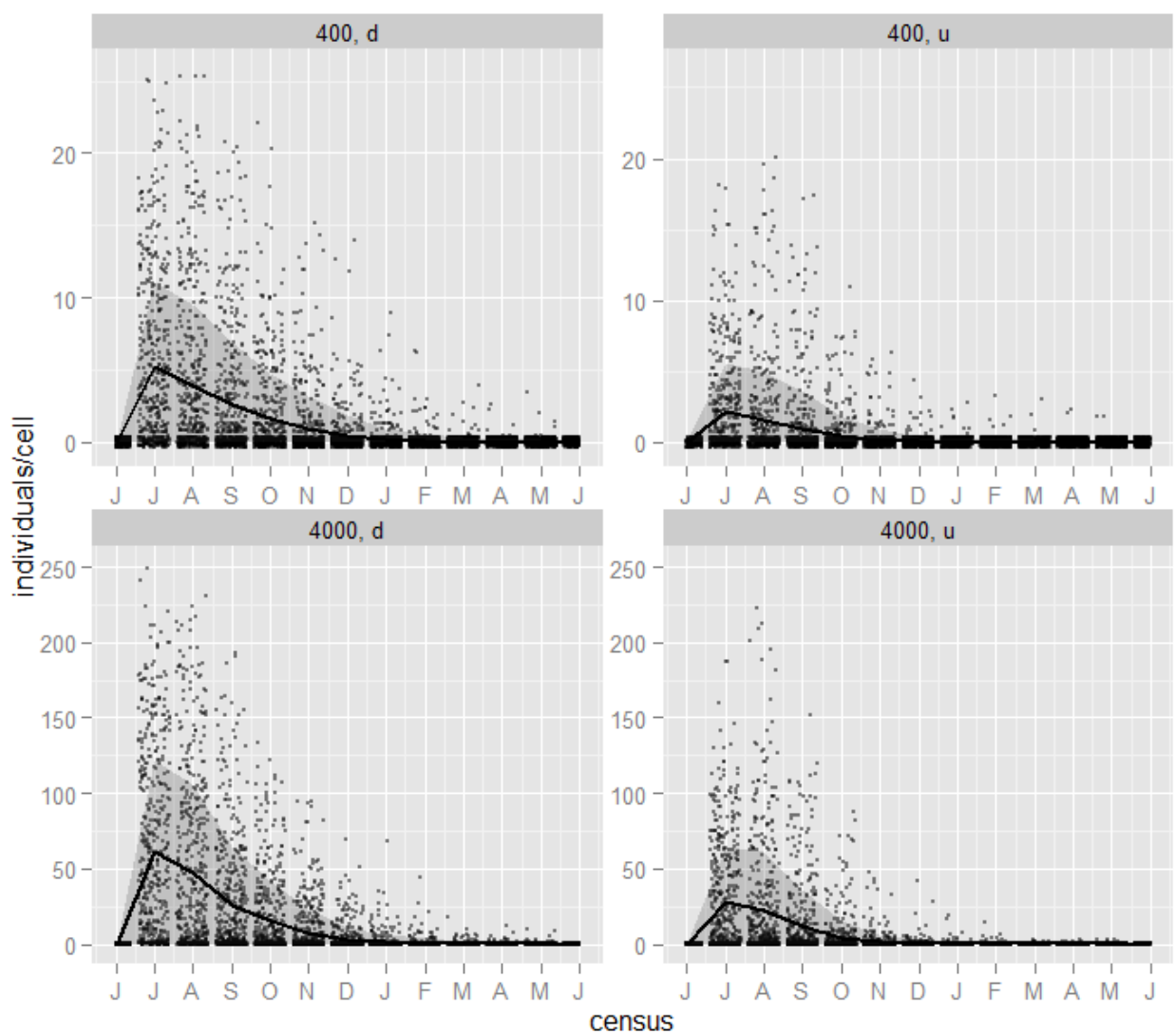


Figure 4.1 Numbers of *Brassica* individuals per 25 × 25 cm cell from each census (grey dots, jittered for clarity) at each census of the 2012 seed sowing experiment. 400 = seeds sown at 400 seeds/m² 4000 = seeds sown at 4000 seeds/m², d = cell vegetation removed and soil disturbed prior to sowing, u = no disturbance treatment. Shown are mean numbers per cell (thick black line) and one standard deviation (grey shaded area).

Table 4.3 Coefficient estimates, 95% credible intervals (CI), and pMCMC values for the fixed effects of the GV model (total number of seeds germinated per 25 × 25 cm cell). Interaction terms are indicated with a '×', significant variables written in bold. Factor variables are in comparison to the reference level for that factor; these are Sowing Rate (S.R): 25 seeds/cell, Disturbance: Undisturbed, Bare Ground: 0 – 5%, Shade: Unshaded. Random effects: plot, variety, species, subspecies. n = 1920.

Parameter (germ)	Estimate	Lower CI	Upper CI	pMCMC
S.R. 250	2.247	2.079	2.424	<2 × 10⁻⁴
Disturbed	1.113	0.934	1.285	<2 × 10⁻⁴
S.R. 250 × Disturbed	0.004	-0.203	0.221	0.965
Shade: Shaded	-0.168	-1.756	1.340	0.830
Precipitation	-0.505	-1.407	0.489	0.282
Temperature	-0.141	-0.989	0.667	0.725
Viability Rate	0.157	-0.300	0.655	0.476
Seed Mass	0.268	-0.217	0.750	0.231
S.R. 250 × Shaded	0.083	-0.190	0.349	0.559
× Precipitation	-0.146	-0.312	0.002	0.071
× Temperature	0.092	-0.043	0.231	0.201
× Viability Rate	0.012	-0.110	0.126	0.851
× Seed Mass	0.057	-0.060	0.179	0.356
Disturbed × Shaded	-0.432	-0.706	-0.162	0.001
× Precipitation	0.119	-0.046	0.261	0.133
× Temperature	-0.024	-0.157	0.121	0.732
× Viability Rate	0.098	-0.021	0.215	0.107
× Seed Mass	-0.008	-0.130	0.109	0.900

Table 4.4 From the 2011 *Brassica* seed sowing experiment, cotyledons per seed sown, flowering individuals per seed sown, and λ values for all taxa combined and each species separately (mean \pm standard error), for cells sown with 400 seeds/m², 4000 seeds/m² and with and without pre-sowing disturbance. Letters in parentheses indicate significant differences in the means; for cotyledons per seed sown and flowering individuals per seed sown means were compared using t-tests on log-transformed data; λ distributions were highly non-normal and heteroscedastic, to account for this means were compared using the methods of Herberich, Sikorski et al. (2010).

	Sown Seed Density (seeds/m ²)	Undisturbed	Disturbed
Cotyledons per Seed Sown (cell mean \pm standard error)	400	0.11 \pm 0.01 (a)	0.24 \pm 0.01 (c)
	4000	0.13 \pm 0.01 (b)	0.27 \pm 0.01 (d)
Flowering Individuals per Seed Sown (cell mean \pm standard error)	400	0.0003 \pm 0.0002 (a)	0.006 \pm 0.001 (b)
	4000	0.0004 \pm 0.0002 (a)	0.005 \pm 0.001 (b)
λ (cell mean \pm standard error)	400	0.024 \pm 0.013 (a)	0.060 \pm 0.029 (a)
	4000	0.012 \pm 0.001 (a)	0.045 \pm 0.021 (a)
λ <i>B. napus</i> (cell mean \pm standard error) <i>napus</i>	400	0.051 \pm 0.038 (a)	0.056 \pm 0.030 (a)
	4000	0.014 \pm 0.001 (a)	0.021 \pm 0.004 (a)
λ <i>B. oleracea</i> (cell mean \pm standard error) <i>napus</i>	400	0.003 \pm 0.001 (a)	0.002 \pm 0.001 (a)
	4000	0.002 \pm 0.001 (a)	0.004 \pm 0.002 (a)
λ <i>B. rapa</i> (cell mean \pm standard error) <i>napus</i>	400	0.019 \pm 0.001 (a)	0.121 \pm 0.080 (a)
	4000	0.020 \pm 0.001 (a)	0.110 \pm 0.061 (a)

Table 4.5 Coefficient estimate, 95% credible intervals (CI), and pMCMC values for the fixed effects of the LV model (total number of *Brassica* individuals per 25 × 25 cm cell in each census). Interaction terms are indicated with a '×', significant variables written in bold. Factor variables are in comparison to the reference level for that factor; these are Sowing Rate (S.R): 25 seeds/cell, Disturbance: Undisturbed, Bare Ground: 0 – 5%, Shade: Unshaded. Random effects: cell, plot, variety, species, subspecies, and time. n = 23040.

Parameter (long)	Estimate	Lower CI	Upper CI	pMCMC
S.R. 250	2.322	2.087	2.563	$<2 \times 10^{-4}$
Disturbed	1.864	1.617	2.098	$<2 \times 10^{-4}$
S.R. 250 × Disturbed	-0.052	-0.339	0.231	0.729
Shade: Shaded	-0.294	-2.229	1.693	0.741
Precipitation	-0.802	-2.268	0.647	0.275
Temperature	-0.690	-1.987	0.575	0.284
Viability Rate	0.149	-0.417	0.764	0.581
Seed Mass	0.389	-0.208	0.983	0.202
S.R. 250 × Shaded	-0.178	-0.587	0.204	0.373
× Precipitation	-0.199	-0.495	0.076	0.162
× Temperature	-0.120	-0.356	0.135	0.339
× Viability Rate	-0.016	-0.177	0.143	0.847
× Seed Mass	-0.004	-0.171	0.163	0.955
Disturbed × Shaded	-1.021	-1.406	-0.624	$<2 \times 10^{-4}$
× Precipitation	-0.262	-0.538	0.034	0.073
× Temperature	-0.415	-0.666	-0.162	0.001
× Viability Rate	0.151	-0.016	0.312	0.066
× Seed Mass	0.029	-0.145	0.187	0.745

4.4.2 Numbers through Time (L)

The numbers of *Brassica* per cell through time (L) showed strong positive relationships with both sowing rate and pre-sowing disturbance, indicating that survivorship was constrained by both the availability of seeds and suitable sites (Fig 4.1, numbers persisting through time, Table 4.5). There was no significant interaction between sowing rate and pre-sowing disturbance (Table 4.5, S.R. 4000 × Disturbed parameter estimate), i.e. disturbance did not have a different effect on *Brassica* survivorship in cells sown with 400 seeds/m² than in cells sown with 4000 seeds/m². If all available microsites were saturated at a density of less than 4000 seeds/m², we would expect that a lower proportion of seeds would emerge in disturbed cells sown with 4000 seeds/m² than sown with 400 seeds/m², thus this result suggests that even at the higher density not all empty microsites were occupied. Pre-sowing disturbance had a greater positive effect on *Brassica* survivorship than germination, while a higher seed density had a smaller positive effect on L than on G (comparing parameter estimates for Disturbed and S.R. 4000 between tables 4.3. and 4.5).

Mean numbers of flowering individuals per seed sown were very low, indicating that survival to flowering was strongly limited by the availability of safe sites (Table 4.4). More individuals survived to flowering following pre-sowing disturbance; the number of flowering individuals per seed sown differed by more than an order of magnitude between disturbed and undisturbed cells (Table 4.4), indicating that pre-sowing disturbance increased the availability of safe sites for flowering more than tenfold. Despite this, even in disturbed cells the availability of safe sites limited survival to flowering far more than seed limitation (relative strengths of site and seed limitation around 0.995 vs. 0.005, Table 4.4). There was no significant difference in the numbers of flowering individuals per seed sown between cells sown with 400 seeds/m² and cells sown with 4000 seeds/m² in either disturbed or undisturbed cells.

There was significant variation in *L* along climatic gradients, but only in disturbed cells. There was a negative relationship between shading and *Brassica* performance in disturbed cells, and this was stronger in the *L* model than the *G* model, indicating shading reduced survivorship as well as emergence (Tables 4.3 and 4.5, Disturbed × Shaded interactions). Numbers through time were also significantly lower in disturbed cells in warmer plots (Table 4.5, Disturbed × Temperature interaction). Higher precipitation had a marginally negative relationship with *L* in disturbed cells. Seed parameters generally had no significant effect on *L*; however there was a marginal positive relationship between seed viability and *L* in disturbed cells (Table 4.5).

4.4.3 Seed Set and Population Increase (λ)

Naturalisation (establishment of a persistent population, which is indicated by $\lambda > 1$) was patchy and localised (Table 4.6). After an initial peak in numbers in July, one month after sowing, mortality was high and few individuals remained by the following January (Fig 4.1). Seed set occurred in just 25 cells (1.3%), in five plots. All varieties set seed except two varieties of *B. oleracea*: ‘Sovereign’ and ‘Red Express’. However fecundity was generally low (14.1 ± 4.2 seeds/flowering individual), excluding one unusually fecund individual (*B. rapa rapa* ‘Purpletop Whiteglobe’: 1972 seeds). As a result of this fecund individual, ‘Purpletop Whiteglobe’ was the only variety to naturalise at the plot scale (Table 4.6). At the cell scale 6 varieties, representing all species except *B. oleracea* had $\lambda > 1$ (Table 4.6), each in a single cell except ‘Purpletop Whiteglobe’ which had $\lambda > 1$ in two cells. Individuals in these seven cells did not appear to pass through a rosette stage, but ‘bolted’, progressing directly from seedling to flowering stages.

Table 4.6 Estimated population growth rates (λ) for each of the 12 *Brassica* varieties grown in the 2012 seed sowing experiment. Shown are λ values for the study region as a whole, and maximum values at the plot and cell scale. $\lambda > 1$ indicates an increasing population, written in bold.

Species	Subspecies	Variety	Estimated λ Values		
			Landscape (mean \pm s.e.)	Best Plot	Best Cell
<i>Napus</i>	<i>napobrassica</i>	Dominion	0.042 \pm 0.011	0.35	1.74
		Major Plus	0.009 \pm 0.006	0.13	0.96
	<i>napus</i>	Flash	0.032 \pm 0.028	0.55	4.40
		Griffin	0.057 \pm 0.038	0.77	6.06
<i>Oleracea</i>	<i>acephala</i>	Gruner	0.005 \pm 0.001	0.02	0.15
		Sovereign	0.003 \pm 0.001	0.03	0.12
	<i>capita</i>	Greyhound	0.001 \pm 0.001	0.03	0.22
		Red Express	0.001 \pm 0.001	0.02	0.08
<i>Rapa</i>	<i>chinensis</i>	Canton White	0.054 \pm 0.017	0.39	2.83
		Rubens	0.010 \pm 0.006	0.18	0.94
	<i>rapa</i>	Barkant	0.055 \pm 0.036	0.74	5.82
		Purpletop Whiteglobe	0.152 \pm 0.092	1.58	12.54

Few data were available to analyse the relationships between λ and the experimental treatments (sowing rate and disturbance). Mean λ was higher in disturbed cells (Table 4.4), suggesting that pre-sowing disturbance increased the availability of safe sites for establishment, and improved *Brassica* performance. However, this difference was non-significant because it was driven by the small number of outlier cells where seed set occurred. Twenty-two out of 25 (88%) of cells in which seeds were produced were those subject to disturbance prior to sowing, and a total of 4764 seeds was produced in disturbed cells compared to a total of 186 seeds produced in undisturbed cells.

Surprisingly, mean λ was higher in cells sown at the lower seed density (400 seeds/m²) than those sown at the higher seed density (4000 seeds/m²). This was true in both disturbed and undisturbed cells (Table 4.4), suggesting that *Brassica* establishment is highly site limited, even with pre-sowing disturbance. However, this difference was non-significant because it was driven by the small number of outlier cells where seed set occurred. Sowing cells with ten times the number of seeds did not result in ten times greater seed output: a total of 4207 seeds was produced in cells sown with 4000 seeds/m² compared to a total of 743 seeds produced in cells sown with 400 seeds/m². Sixteen out of 25 (64%) of cells in which seeds were produced were those sown with 4000 seeds/m².

The effects of pre-sowing disturbance on λ differed among species; notably in disturbed cells species' mean λ values were significantly correlated with the frequency with which naturalised populations were documented in field surveys (Chapter 2, $\text{cor} = 0.98$, $p = 0.09$). *Brassica rapa* mean λ values were more than five times greater in disturbed than undisturbed cells (Table 4.4). This difference was not apparent for *B. napus* or *B. oleracea*, suggesting *B. rapa* was better able to exploit disturbances than *B. napus* or *B. oleracea*. The difference in λ between disturbed and undisturbed cells for *B. rapa* was again driven by a small number of cells and was non-significant when cells sown with 400 and 4000 seeds/m² were tested for differences separately; combining data from the two sowing rate treatments increased statistical power and yielded a significant difference in mean λ between disturbed and undisturbed cell for *B. rapa* ($p = 0.05$). Two cells which had unusually high fecundity were both disturbed cells; in one a single *B. rapa* 'Purpletop Whiteglobe' individual produced 1972 seeds, in the other 19 *B. rapa* 'Barkant' individuals produced 1451 seeds. For both *B. napus* and *B. oleracea* there were no significant differences in mean λ values among experimental treatments (Table 4.4). *Brassica napus* had higher mean λ values in cells sown with 400 seeds/m² than cells sown with 4000 seeds/m², but this difference was driven by the few cells in which *B. napus* set seed and was non-significant. This difference for *B. napus* appeared to drive the higher λ values in cells sown with 400 seeds/m² than cells sown with 4000 seeds/m² for all species combined.

4.4.4 Density Dependent Mortality

There was no evidence of increased mortality of *Brassica* prior to adulthood or flowering in plots which had higher densities of seedlings (Table 4.7), indicating that seedling mortality was due to a lack of suitable sites for growth rather than density dependant processes. The lower mean λ for *B. napus* in cells sown with 4000 seeds/m² than in cells sown with 400 seeds/m² (Table 4.4) suggested that *B. napus* mortality may have been density dependant, however a model of *B. napus* mortality prior to flowering showed mortality was not significantly related to seedling density (results not shown).

Table 4.7 Coefficient estimates, credible intervals (CI) and pMCMC values from the binomial models fitted with the proportion of seedlings dying prior to adulthood and flowering as the response variables and the maximum number of seedlings in each 25 × 25 cm cell as the explanatory variable.

Parameter	Estimate	Lower CI	Upper CI	pMCMC
max. seedlings (mortality prior to adulthood model)	-0.004	-0.011	0.002	0.165
max. seedlings (mortality prior to flowering model)	-0.008	-0.022	0.008	0.335

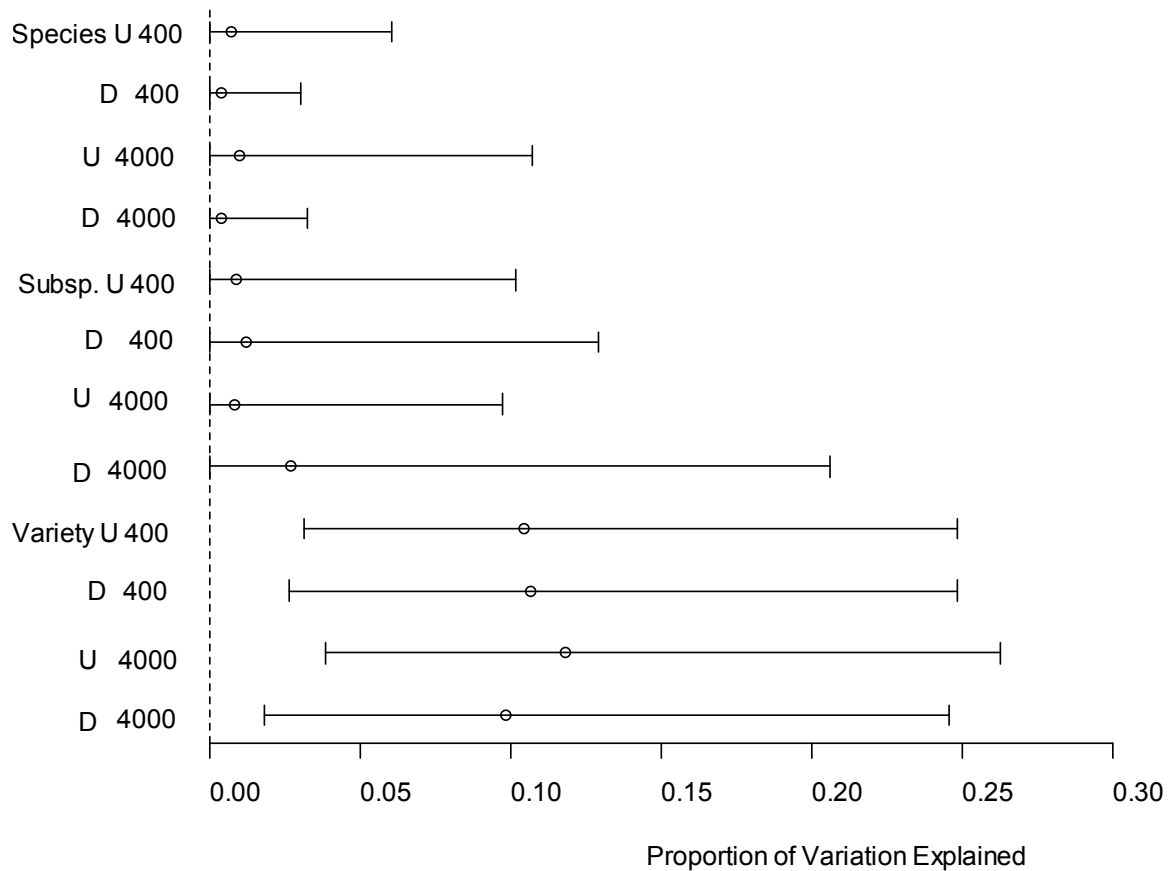


Figure 4.2 Proportion of variation in *Brassica* performance (L, number of individuals per 25 × 25 cm cell at each census) explained by each taxonomic level (species, subspecies, variety) in the 2012 seed sowing experiment. 400 = seeds sown at 400 seeds/m², 4000 = seeds sown at 4000 seeds/m², D = cell vegetation removed and soil disturbed prior to sowing, U = no disturbance treatment. n = 23040.

4.4.5 Partitioning of Performance Among Taxonomic Levels

Neither a higher sowing rate nor pre-sowing disturbance altered the broad of partitioning of *Brassica* performance among taxonomic levels (Fig. 4.2). In all factorial treatment combinations of sowing rate and pre-sowing disturbance species accounted for little of the variation in L (around 1-2% of the total variation in L), while most of the variation in L due to differences among taxa resided at the varietal level (around 10% of the total variation in L). This indicates that even when constraints on establishment and survivorship (seed and site availability) are relaxed, differences among varieties still account for most of the variation in performance due to differences among taxa.

4.4.6 Partitioning of Seed Parameters Among Taxonomic Levels

There were large uncertainties around estimates of how much variation in seed mass and viability resided at each taxonomic level. Most variation in seed mass was among varieties (mean: 0.71, 95% CI: 0.04 - 0.98), with smaller amounts of variation among species (mean 0.20, 95% CI: 2×10^{-16} - 0.95), and subspecies (mean 0.06, 95% CI: 1×10^{-16} - 0.61); the normal distribution accounted for 0.02 of the variation (95% CI: 2×10^{-3} - 0.06). Most variation in seed viability was also among varieties (mean: 0.44, 95% CI: 0.03 - 0.62), with smaller amounts of variation among species (mean 0.02, 95% CI: 4×10^{-19} - 0.22) and subspecies (mean 0.04, 95% CI: 2×10^{-18} - 0.31); the binomial distribution and overdispersion accounted for 0.50 of the variation (95% CI: 2×10^{-3} - 0.64).

4.5 Discussion

In this study I experimentally tested the effects of two seed sowing densities and pre-sowing disturbance on the performance of alien *Brassica*, with the expectations that both a higher seed sowing density and pre-sowing disturbance would increase emergence, survival and the rate of population increase (λ); that pre-sowing disturbance would have more effect on *Brassica* survival and λ than sowing density; and that the taxonomic level at which most variation in *Brassica* performance resides may differ when the constraints of seed and site limitation were relaxed.

4.5.1 Effects of Sowing Density and Pre-Sowing Disturbance on Emergence

It has long been suggested that disturbed sites with bare ground have low resistance to alien establishment (Putwain et al. 1968, Gross and Werner 1982, Jutila and Grace 2002, Price et al. 2011), that high propagule pressure can overcome biotic and abiotic resistance to establishment (Von Holle and Simberloff 2005, Colautti et al. 2006, McGregor et al. 2012), and more recently that the interaction of these two variables can have a large influence on whether invaders can establish (Britton-Simmons and Abbott 2008, Eschtruth and Battles 2009). In this experiment *Brassica* emergence was indeed increased by both the addition of seeds at higher densities and pre-sowing disturbance, indicating that emergence is both seed and site limited. Previous seed addition studies (Chambers 2000, Traveset et al. 2003, Clark et al. 2007), including those using *Brassica* (Crawley et al. 1993), have tended to find that only a small proportion of seeds sown successfully establish as seedlings. Here, most seeds failed to emerge even with pre-sowing disturbance and densities of seeds ten times greater than estimated to occur in the field (Bailleul et al. 2012), and there was no interaction between seed sowing density. *Brassica* emergence was primarily constrained by the availability of safe sites rather than seeds, and the conditions which made sites suitable for emergence were not limited to the availability of bare ground. Indeed, there was no indication of a

saturation of available bare microsites for germination at the higher sowing density; pre-sowing disturbance approximately doubled the emergence rate regardless of seed sowing density. Some factor other than the availability of bare ground limited emergence as much at the low sowing density as at the high sowing density, indicating that the availability of bare microsites was not the main constraint on emergence.

There are two possible explanations for the low emergence rates recorded: seeds were subject to high mortality rates between sowing and emergence, or high rates of seed predation between sowing and germination. Freezing and thawing of the seed bed is thought to be a major contributor to pre-emergence mortality rates of seeds and cotyledons in areas where frosts occur (James et al. 2011), and may have been significant contributor to mortality here. Morning frosts were frequently observed at the study sites during June and July, and appeared to be more severe and persistent in shaded sites. Notably, fewer cotyledons emerged in disturbed, shaded sites; this most likely reflects increased mortality due to frost damage, in undisturbed sites it seems that vegetation may have had a facilitative effect by buffering air and soil temperatures (Brooker et al. 2008). Climatic match is considered to be an important determinant of alien establishment success (Thuiller et al. 2005, Hayes and Barry 2008), but despite considerable variation in climate among experimental sites, climate appears to have had little effect on patterns of emergence apart from mortality due to frost. Alternatively, below ground mortality may have been due to attack by pathogens; when the protective structures of the seed coat are lost, cotyledons become more susceptible to pathogen attack (Harper 1977). Negative effects of pathogens on seedling emergence are thought to be strongest when growth and emergence are slow (Harper et al. 1955, Kirkpatrick and Bazzaz 1979). Here most cotyledons emerged quite rapidly, within one month of sowing, however attack by pathogens may still have been a significant cause of pre-emergence mortality. Seed predation can be a major constraint on alien plant establishment (Ostfeld et al. 1997, Parker et al. 2006), but without data on rates of seed predation at the experimental sites the contribution of seed predation to the low rates of emergence is difficult to assess. When sown at high densities there was an increase in the number of cotyledons emerged per seed sown, indicating that the high seed density overcame some barrier to establishment, one possibility is that seed predators became satiated at the higher seed density (Turnbull et al. 2000). However, even in disturbed cells, where seeds would have been easily visible and exposed to predation by birds and small mammals, emergence rates of up to 100% were recorded, suggesting that any seed predation was highly variable may not have been a major factor. Some seeds were non-viable at the time of sowing and it is likely some remained dormant; however it is unlikely that these two factors alone account for the low emergence rates; seed viability was non-significant in models explaining emergence, and together non-viable and dormant seeds account for only around 10% of seeds sown (mean viability was around 91% and dormancy

over one year around 0.1%). Finally, while censuses were conducted as frequently as possible, some cotyledons may have emerged died prior to the next census without being recorded.

Surprisingly neither seed mass nor seed viability explained any of the variation in *Brassica* emergence or survival, regardless of sowing density or pre-sowing disturbance. In Chapter three, seed viability explained a significant amount of the variation in emergence and subsequent performance. In this chapter I used fewer taxa (with less variation in seed mass and viability) at fewer sites; the most likely explanation for the non-significance of seed mass and seed viability in models of performance is that the taxa used did not vary enough in these parameters, and/or that the smaller number of replicates resulted in insufficient statistical power to detect any effect of seed mass or viability on performance.

4.5.2 Effects of Sowing Density and Pre-Sowing Disturbance on Survivorship and Population Increase

The positive effect of a higher seed sowing density on *Brassica* performance diminished through life-stages, and did not increase the proportion of seeds which reach flowering stage. Indeed, mean λ was lower in cells sown with the higher density of seeds. This is in keeping with the findings of other studies which tend to show that plants are most seed limited at the early stages of their life cycle but become more site limited with time (Clark et al. 2007, McGlone et al. 2011). It is perhaps not surprising that adding seeds often results in a flush of recruitment (seed limitation), followed by mortality at later life-stages due to unfavourable conditions (site limitation). The conditions required for successful emergence are likely to differ from those for survival, which may differ again from those required for flowering and seed set at sufficient rates for a population to be self-sustaining. In effect individuals must pass through a series of ecological filters as they progress through their life cycle; individuals must have passed the filters influencing progress to all previous life-stages before they can progress to the next life-stage. Thus the cumulative site conditions required become more stringent for each life-stage; i.e. plants become successively more site limited as they progress through their life cycle. For example, for annuals in systems such as the one studied here, the processes controlling emergence are, most likely, seed predation and below ground mortality due to frost or pathogen attack. Until the cotyledons emerge above ground and begin to photosynthesise, seedlings rely on energy reserves within the seed itself, and are therefore largely insulated from competition with neighbouring plants for resources prior to emergence (Raven et al. 2005). After emerging above ground seedlings are less buffered from variations in air temperature and the availability of moisture, and are vulnerable to herbivory by mammals, insects and gastropods, in addition to attack by a new suite of pathogens; many may die due desiccation, frost, grazing and infection (Fenner 1987). Finally, flowering and seed set are resource intensive; for a population to

persist, not only must individuals survive to flower, they must have accumulated enough resources to produce enough seeds for the population to be self-sustaining (Campbell and Halama 1993, Biere 1995).

Such filtering concepts have been advanced before (Richardson et al. 2000, Mitchell et al. 2006, Catford et al. 2009, Kempel et al. 2013), and fit well with the results here. Arrival of seeds overcame the first barrier to establishment (i.e. seed limitation), but in the long term the density of seeds had little effect on population trajectories. Competition with surrounding vegetation further constrained establishment and survival. It was only once this filter had been passed (i.e. in disturbed cells) that climate had a significant effect on performance. The low rates of survival and population increase recorded indicate that there were further filters on *Brassica* performance that my experimental design was unable to identify. Indeed, survival and seed set were so low, even with pre-sowing disturbance and seed densities ten times those expected to occur in the field, that reliable estimates of the rates of population increase were difficult to obtain. This was particularly surprising given that populations of all three species used in the experiment are known to occur in the study area, in sites very similar to my experimental sites (Chapter 2). Future studies could fruitfully measure or manipulate post-establishment disturbance, herbivory and the availability of resources such as moisture and nitrogen (Wright et al. 1988, Taylor et al. 1991) to identify the roles these potential filters may play in constraining the establishment and persistence of alien *Brassica* populations. Such studies may also succeed in obtaining more robust data on rates of population increase which would allow λ to be partitioned among taxonomic levels.

4.5.3 Effects of Sowing Density and Pre-Sowing Disturbance on Performance Partitioned Among Taxonomic Levels

There was no shift in the taxonomic level at which most variation in *Brassica* performance resided when seeds were sown at a higher density or into disturbed locations; the result from Chapter three that most variation in *Brassica* performance resides among varieties seems robust under these conditions. This was an important test of the findings of Chapter three because it is likely that many naturalised *Brassica* populations grow in disturbed sites (Chapter 2, Crawley and Brown 2004, Claessen et al. 2005); the possibility existed that when seeds arrived at disturbed sites different traits may have influenced *Brassica* performance than when they arrived at sites that had not been disturbed (as in Chapter 3) and that most variation these traits may have resided among species.

However, λ values gave some indications that much of the variation in important traits for successful reproduction in the field sites may have resided among species. Indeed, while I was unable to

partition variation in λ among taxonomic levels, of the three *Brassica* species used in this experiment, only *B. rapa* population growth rates showed a strong positive response to pre-sowing disturbance. It seems that *B. rapa* possessed traits and behaviours that allowed it to capitalise on the availability of bare ground (rapid maturation and seed set, i.e. bolting) despite the stressful conditions indicated by the low survival and seed set recorded, while bolting was rare in *B. napus* and *B. oleracea*. Notably, with pre-sowing disturbance, the relative population growth rates of the three species closely matched the relative frequencies of populations in the field, suggesting the observed patterns could be generated by differences among species in the ability to capitalise on disturbance, and may not be the result of difference among species in propagule supply. Indeed, a high seed density had little effect on survival to flowering, seed set and λ , suggesting the observed link between the frequency of *Brassica* populations in the field and proxy measures of seed supply (Chapter 2, Crawley and Brown 1995, Pessel et al. 2001, Peltzer et al. 2008) probably reflects wider dispersal and chances of seed reaching disturbed sites rather than the effects of seeds arriving at higher densities (Sax and Brown 2000, Lockwood et al. 2005, Simberloff 2009). It should be noted that while my results indicate that there may be important differences among species' abilities to successfully set seed, there may well have been similar or greater differences in these abilities among varieties within species; however, I was unable to quantify these. My results were able to show that most variation in both emergence and numbers through time resided among varieties; differences among species only become apparent because the only individuals which reproduced were those which showed a plastic response to stress by bolting.

4.5.4 Conclusions

It has been suggested that high propagule pressures may override other constraints on invasion such as species traits or biotic resistance (Von Holle and Simberloff 2005, Colautti et al. 2006, McGregor et al. 2012), but here this was not the case. Seeds arriving at high densities may give rise to transient populations of seedlings (Turnbull et al. 2000), but there was no evidence that a higher seed density increased the likelihood of a self-sustaining population forming. Similarly the sites with bare ground are considered to be highly invasible (Hobbs and Huenneke 1992, Davis et al. 2000), but the availability of bare ground alone may not be sufficient for an invader to establish. After the arrival of seeds in a vacant microsite, even one with a benign climate, there appear to be many subsequent ecological filters which limit survival and ultimately the establishment of self-sustaining populations. We need to develop a deeper understanding of these filters and how they interact with each other and taxa traits.

Seed addition studies seeking to quantify the performance of alien plants which follow populations to reproduction are comparatively rare (Turnbull et al. 2000, Clark et al. 2007). In studies which relax

the constraints of seed and site limitation an initial flush of seedlings may give the impression of invasion, but if we want robust estimates of the likelihood of self-sustaining populations forming, it is important to follow the fate of the introduced plants for at least one full generation (Turnbull et al. 2000). The results here suggest that plastic responses to stress and environmental variation may be important in facilitating some invasions. In particular, in disturbed, high stress environments bolting may be a key trait allowing invaders to gain a foothold. Future studies could explicitly examine the costs and benefits of bolting in low and high stress environments to better elucidate the role of this trait in invasion outcomes, and it could potentially be included in future weed risk assessment protocols along with seed viability and seed mass (Chapter 3).

Chapter 5

General Discussion

5.1 Do new plant species necessarily pose a greater risk than new varieties or genotypes of alien species already in a recipient region?

My results show that there can be far more variation in performance within alien plant species than among them; in the genus *Brassica* most of the variation in performance that could be attributed to differences among taxa was due to differences among varieties rather than differences among species or subspecies. This suggests that new varieties and genotypes of species already present in a region can potentially pose an equivalent or greater biosecurity risk than new species, and raises the possibility that the importation of new varieties and genotypes may present a new and unaccounted for biosecurity risk. *Brassica* represent a practical test case of the issues involved in the importation of plants due to the commercial importance of the genus, which gives rise to an interest in importing new varieties and genotypes to facilitate breeding programs (Lammerink and Hart 1985, Gowers and Nicol 1989, Ellis and Farrell 1995, Douglas 2005, Carter 2007, Christey et al. 2008, Jong et al. 2010).

It should be noted that the species used in my model system are closely related (U 1935), and I deliberately set out to maximise variation within species. Furthermore I specifically chose *Brassica* as a model system to test my hypothesis that most variation in performance attributable to taxonomic differences would reside among species because there is substantial variation in traits within species of the genus (Tsunoda et al. 1980, Gupta 2009, Ramchiary et al. 2011). This made *Brassica* an excellent system to test for the possibility that substantial variation in performance may reside within species, but my results may not be generalizable to other genera, which in general contain far fewer taxa and less variation in traits within species. This study is perhaps best viewed as a proof of concept for which the system most likely to give a positive result was chosen. Having demonstrated that in principle subspecific taxa can differ more than species, it remains to be seen how broadly applicable this principle is. In addition, due to the low survival rates of *Brassica*, even when seeds were sown at high densities into disturbed cells, I was unable to obtain data that would allow me to partition variation in the rate of population increase (λ) among taxonomic levels. However, I was able to show the overwhelming majority of variation in two other measures of performance (number of cotyledons emerged and numbers through time) attributable to taxonomic differences resided among varieties. The generality or otherwise of the results obtained here needs to be established

with further studies on a range of genera, with studies of genera with less diversity within species, and of genera containing taxa with sufficient performance and/or invasiveness to yield λ values that can be partitioned among taxonomic levels. Thus despite the above limitations, my results give a strong indication that in some genera at least, invasion risk can vary significantly within species. Further evidence to support this view come from other studies which suggest alien taxa may vary considerably in their ecological characteristics and invasiveness within species (Saltonstall 2002, Kinter and Mack 2004, Vellend et al. 2010, Merrill et al. 2012, Hierro et al. 2013).

5.2 Does increasing the availability of safe sites and seeds alter how performance is partitioned among taxonomic levels?

The result that most variation in *Brassica* performance that can be attributed to differences among taxa proved robust to manipulations of important drivers of *Brassica* naturalisation, seed availability and disturbance (Crawley and Brown 1995, Pessel et al. 2001, Crawley and Brown 2004, Claessen et al. 2005, Pivard et al. 2008a). With these experimental manipulations I sought to match the conditions in which feral *Brassica* populations are found. This was an important test that my results were valid under ecologically realistic conditions.

5.3 What are the drivers of naturalisation of *Brassica*, i.e. biotic and abiotic correlates of population presence and persistence, and how do these vary among taxa?

Can limited recruitment of *Brassica* in the study system be overcome by increasing the availability of safe sites and seeds?

The two main drivers of the presence of *Brassica* populations identified in this thesis are disturbance and propagule pressure (Chapters 2 and 4). This matches well with the conclusions of other studies of feral *Brassica* (Crawley and Brown 1995, Pessel et al. 2001, Caesar 2003, Crawley and Brown 2004, Claessen et al. 2005, Pivard et al. 2008a). The relationship between open, disturbed, low competition sites and invasibility is well documented, particularly for fast growing annuals and biennials such as *Brassica* (Hobbs and Huenneke 1992, Crawley and Brown 1995, Burke and Grime 1996, Crawley and Brown 2004). There is also a well-known relationship between propagule pressure and invasion success (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009). Management and site biotic and abiotic conditions (such as presence of a water course, mowing, herbicide use, precipitation and dominant vegetation type) appear to act as higher order filters that influenced post-emergence population survival once more fundamental filters such as the availability of seeds and disturbed sites have been passed (Richardson et al. 2000, Mitchell et al. 2006, Catford et al. 2009, Kempel et al. 2013).

While of course the presence of seeds is a prerequisite for the establishment of a population, disturbance appears to play a more crucial role in regulating the foundation and subsequent performance of populations. Even when I experimentally added seeds, thus controlling for propagule pressure, most of the variation in *Brassica* performance was spatial, indicating site characteristics such as disturbance drove the large variation in performance among and within sites (Chapter 3). When I manipulated seed density and the availability of bare ground (Chapter 4) both increased recruitment; however following addition of seeds at high density the increase in plant numbers was short lived, producing a flush of seedlings which failed to persist; by contrast in disturbed cells higher plant numbers were maintained through time and to later life-stages. It seems that higher seed densities have little effect on establishment success for *Brassica* in this system. Indeed, the observed link between the frequency of *Brassica* populations in the field and proxy measures of seed supply (Chapter 2, Crawley and Brown 1995, Pessel et al. 2001, Peltzer et al. 2008) probably reflects wider dispersal of seeds in space and time, resulting in increased chances of seed reaching suitable (i.e. disturbed) sites rather than the effects of seeds arriving at higher densities (Sax and Brown 2000, Lockwood et al. 2005, Simberloff 2009). Disturbance as measured during field surveys was non-significant in explaining the probability of population persistence; however population size was important, likely because larger populations are better buffered against stochastic inter-annual variation in the variables controlling recruitment, survival and reproduction, (Shaffer 1981, Lande 1993, Stephan and Wissel 1994, Mack et al. 2002, Liebhold and Bascompte 2003), such as disturbance (Crawley and Brown 1995, 2004, Claessen et al. 2005).

5.4 What is frequency and annual rate of persistence of populations of alien *Brassica* taxa in the study system, and how do these vary among taxa?

Brassica populations were infrequent in the landscape compared to the results of similar studies in Europe and Canada. This is most likely reflection of the relatively small numbers of seeds transported in Canterbury, where *Brassica* are predominantly fodder crops, compared to regions where *Brassica* seeds are harvested for oil production. The oilseed industry is relatively small in Canterbury at this stage, but if production of oilseeds increases in the future there would likely be a concomitant increase in the frequency of feral *Brassica* populations on the Canterbury plains unless strict measures were in place to limit the accidental escape of seeds during transportation.

Evidence from both field surveys and experiments indicates that *Brassica* populations in the study system are overwhelmingly short lived, and in the absence of continued seed inputs *Brassica* would likely fail to persist in the landscape. Field surveys indicated that most populations do not survive

beyond the first year or two, with a maximum 5% persistence beyond years. Field survey data identified stochastic variability in environmental conditions and demographic rates as the main causes of population extinction (Shaffer 1981, Lande 1993, Mack et al. 2002). In the absence of external seed inputs, chance variations in the vital rates of small populations are likely to eventually result in local extinction if a large proportion of individuals suffer unusually low recruitment, survival or fecundity in a given year (Shaffer 1981, Lande 1993, Stephan and Wissel 1994, Liebhold and Bascompte 2003) and Allee effects (Courchamp et al. 1999, Taylor and Hastings 2005). There was also some evidence from field surveys that *Brassica* were poorly adapted to the bulk of roadside habitat, while drainage and irrigation ditches appeared to provide more amenable conditions. In experiments I sowed seeds of taxa known to naturalise in the study region (Chapter 2, Heenan et al. 2004b, Peltzer et al. 2008), into a large number of replicate sites located in roadside habitat similar to those where feral *Brassica* populations are observed, at seed densities far higher than those expected to occur in the field (Bailleul et al. 2012), and with pre-sowing disturbance of soil and vegetation. Despite this, performance was generally poor and seed set infrequent, leading to rates of population increase generally well below one, indicating populations were rapidly heading towards extinction. This poor performance is further strong evidence of a mismatch between *Brassica* traits and the roadside habitats.

Despite the markedly higher frequency of *B. rapa oleifera* populations than other *Brassica* species in the field, and higher below ground seed survival rate than other *Brassica* species, field surveys showed only minor differences between *B. rapa* and other *Brassica* species in the annual probability of persistence and the factors influencing population survival. However my experiment manipulating the availability of bare ground in chapter four suggests *B. rapa* is better able take advantage of disturbance than the biennials *B. napus* and *B. oleracea*, and its high below ground seed survival rate may allow it to persist below ground in years where conditions are unfavourable (Timmins and Owen 2001, Gulden et al. 2003, Claessen et al. 2005), i.e. years with little or no disturbance.

Brassica are considered naturalised in the study system (Webb et al. 1988, Heenan et al. 2004b), but it seems they would more accurately be termed adventive. The naturalisation risk of all varieties included in my experiments must be considered low. However it should be noted that there was significant variation in the performance of the varieties tested, and it cannot be ruled out that other varieties than those included in my experiments may be able to form self-sustaining populations. In addition in the second seed sowing experiment where I manipulated the availability of seeds and bare ground (Chapter 4) I did not include the reference variety *B. rapa oleifera*. This was due to insufficient collected seed to sow at the high seed density used in this experiment. It is possible that *B. rapa oleifera* may have performed substantially better than the other taxa used when the

constraints of seed and site availability were relaxed. However field survey evidence suggests that the differences between feral populations of *B. rapa* (which are mostly *B. rapa oleifera*) and other *Brassica* taxa are minor. Furthermore, if changes in the handling and transportation of *Brassica* seed led to larger spills, founding larger populations, it is conceivable that these may be large enough to overcome stochastic and Allee effects and survive in the long term.

5.5 Implications and Recommendations for Further Research

5.5.1 Low Population Survival

In common with the system studied here, many invasive aliens display large spatial and temporal fluctuations in distribution (Pysek and Hulme 2005) and are frequently associated with stochastic disturbance and human activities (e.g. Stapanian et al. 1998, Chytry et al. 2005, Alexander et al. 2009, Pysek et al. 2010). Despite this, persistence of alien plant populations is rarely considered (but see Wade et al. 1997, Pysek et al. 2001, Pergl et al. 2012).

Neglecting to quantify and account for the persistence of alien plant populations may result in overestimation of alien abundance and distribution and miss opportunities for control or eradication. Cumulative survey and herbarium records are often used as the basis for assessments of species distributions in plant atlases and the invasion literature (Petrik et al. 2010). Failing to account for those populations which have become extinct can result in significant overestimates of a species' distribution. Further studies are required to determine the rates of persistence of alien plant populations and to verify current assessments of abundance and distribution after accounting for populations which may have failed to persist. These studies are likely to be particularly pertinent for alien plants which are associated with frequent, widespread or high density anthropogenic seed dispersal, in particular species grown as crops, pasture, and ornamentals (Hodkinson and Thompson 1997). As with *Brassica* in this system, such plants may often have short-lived populations and rely on anthropogenic seed rain to maintain their presence in the landscape. In this case, cumulative records would tend to overestimate their abundance and distribution.

Where alien plants are adventive rather than naturalised, controlling or eliminating the propagule supply offers an effective method of control that may avoid many of the difficulties in attempting to control established populations (Hobbs and Humphries 1995). In my study system it seems likely that if seed loss from trucks could be controlled or limited the frequency of roadside *Brassica* populations would significantly decline, and they may be all but absent from the landscape in around a decade. Anthropogenic propagule dispersal is a major pathway for the spread of alien plants (Hodkinson and Thompson 1997) and controlling additional human mediated propagule inputs could potentially

reduce the range or abundance of even fully naturalised species. Further studies which aim to identify adventive alien species that could be managed by better control of propagule sources would likely bring benefits such as reduced costs and greater efficiency of control; species which could be fruitfully investigated include those which are intensively used by humans and produce numerous seeds, such as grasses and seed crops.

5.5.2 Intraspecific Variation

At present most research on biological invasions and efforts to identify potentially harmful aliens through risk assessment protocols uses species as the unit of observation (e.g. Mack 1996, Reichard and Hamilton 1997, Pheloung 1999, Pheloung et al. 1999, Williamson 1999, Kolar and Lodge 2001, Daehler et al. 2004, Callaway and Maron 2006, Theoharides and Dukes 2007, Catford et al. 2009, van Kleunen et al. 2010, Blackburn et al. 2011, Gurevitch et al. 2011, Pysek et al. 2012). My results, in conjunction with a growing body of work that suggests there may be significant intraspecific differences in invasiveness (Saltonstall 2002, Kinter and Mack 2004, Vellend et al. 2010, Merrill et al. 2012, Hierro et al. 2013), raises the possibility that some of our current assessments of invasiveness may in fact be measures of the average invasiveness of a sample of subspecific taxa, or the invasiveness of one or a few subspecific taxa that have been the subject of study. Unknown genotypes developed through breeding, hybridisation or sourced from novel populations may pose an invasion risk different to that at which the species is currently assessed.

At present it is unclear how common large variation in traits and performance within species is, or how often it translates to variation in invasion outcomes. Studies across a broad range of taxa are required; experimental studies which partition performance among taxonomic levels (e.g. Chapters 2 and 3), as well as reciprocal common garden experiments (e.g. Hierro et al. 2013) and genetic studies (e.g. Saltonstall 2002, Merrill et al. 2012) may all be useful approaches. Invasive genera which contain substantial variation within species may be fruitful targets for further research, these include, but are not limited to, *Acacia* (Kriticos et al. 2003, Wardill et al. 2005), *Pinus* (Wheeler and Guries 1982, Sykes 2001), *Phragmites* (Belzile et al. 2010, Lambertini et al. 2012, Guo et al. 2013), *Chrysanthemoides* (Weiss 1984), and *Bromus* (Kinter and Mack 2004, Chambers et al. 2007, Merrill et al. 2012). Future research should aim to identify factors (e.g. a long history of breeding, multiple uses by humans for the same species, widely varying morphology, wide geographic distributions, or a propensity to hybridise or form polyploids) which could be used to identify genera and species that are likely to contain substantial intraspecific variation in traits or invasiveness, and could be targeted for more intensive risk assessment of intraspecific taxa.

My finding that there can be significant variation in the performance of alien plants within species, and that this may result in significant variation in invasiveness, has important implications in the area of weed risk assessment (WRA). A species which has been assessed as low risk may actually contain high risk subspecific taxa. Managers and policymakers should consider the costs and benefits of modifying WRA protocols to screen for intraspecific variation in traits linked to invasiveness. My results suggest that seed parameters (in particular viability, but also others such as seed mass) may be important characteristics influencing variation in invasiveness within short-lived herbaceous species. Such data are simple and inexpensive to measure and could be incorporated into existing WRAs with comparative ease. Despite being widely considered to be a strong control on the naturalisation success of aliens (Pheloung et al. 1999, Thuiller et al. 2005, Hayes and Barry 2008), in this system at least, climatic match between source and recipient region were non-informative in predicting performance. It seems that climatic match may be a higher order ecological filter on establishment than other factors such as bare ground for establishment (Chapters 2 & 4, Miller and Schemske 1990, Miller 1995, Diepenbrock 2000) and post-emergence disturbance (Eager et al. 2013). Climatic match between source and recipient region may not be a consistent predictor of the invasiveness of alien plants due to the overwhelming influence of lower order ecological filters.

Further work is required to identify which parameters drive differences in performance and invasiveness within species; I identified seed performance as a driver of some of the variation in performance among species (Chapter 3), but even having accounted for these there was still much unexplained variation in performance within species. Common garden experiments paired with glasshouse studies to quantify the traits of the taxa used (e.g. Kempel et al. 2013) could be a powerful tool which could be used to identify key traits which drive variation in invasiveness within species, and these traits could then be incorporated into WRA protocols. Substantial intraspecific variation has been documented in parameters linked invasiveness such as seed viability (Chapter 3), seed mass (Baker 1965, Hendrix and Sun 1989, Rejmanek et al. 2005, Voller et al. 2012), fecundity (Miri 2007, Mason et al. 2008), tolerance of physical stress (Rapacz 1999, Qasim et al. 2003, Chahal et al. 2013, Gabler and Siemann 2013), growth rate (Williams and Hill 1986, Hofmann et al. 2000, Grotkopp et al. 2010, Pan et al. 2012), and ploidy (Marhold and Lihova 2006, Munzbergova 2006, Halverson et al. 2008, Elling et al. 2009). Investigating these parameters would be a good start towards identifying which traits drive variation in performance and invasiveness within species. It is important to note, however, that even at the species level research has been unable to identify a suite traits that reliably indicates invasion potential; there is considerable variation among taxa and study systems in the traits linked to invasiveness (Pysek et al. 1995, Mack et al. 2000, Byers et al. 2002, Hulme 2012). Given this, and the difficulties in obtaining reliable identification, distribution and trait data for intraspecific taxa, it is likely to be difficult to identify traits which can be consistently

used to assess differences in invasion risk within species. From a WRA perspective, applying the precautionary principle would suggest that the same restrictions should apply to the importation of new plant varieties, subspecies and genotypes as are applied to the importation of new plant species. In jurisdictions where a “white list” approach is applied, this would mean all new taxa, whether new species or new intraspecific taxa of an existing species, would require risk assessment prior to introduction (Csurhes et al. 2006, Simberloff 2006, Invasive_Species_Council 2009), and this may require a combination of WRA protocols using trait and biogeographic data (e.g. Pheloung et al. 1999) and field trials (e.g. Davis et al. 2011).

Appendix A

Confirming Identification of *Brassica* Taxa Using AFLP

A.1 Methods

Identification of *Brassica* seed used in the seed sowing experiments (Chapters 3 and 4, Table A.1) was carried out using amplified fragment length polymorphism (AFLP) by Gareth Holmes and Gary Houlston, Landcare Research, Lincoln, New Zealand.

A pilot study was undertaken to determine the suitability of the DNA isolation method and the effect of bulk sampling of seedlings on AFLP banding patterns (e.g. Vos et al. 1995). Profiles from two individual seedlings were compared to those resulting from bulking five seedlings (inclusive of the individuals) for lineages of two diploid, and usually self-incompatible species (*B. rapa* and *B. oleracea*) and two tetraploid and self-compatible species (*B. napus* and a naturalised *B. juncea*). Two selective amplification primer pairs were used following the AFLP methods described below.

A.1.1 Sampling and Preparation

For each taxa I grew ten plants in a glasshouse; leaf tissue was sampled from young leaves of five randomly selected plants (total 50-100 mg) and bulked for analysis. This was stored at -80°C until analysis. Bulking of leaves was undertaken to capture some of the genetic variation likely to be present within varieties as is discussed by Warwick et al (2008). The leaf tissue was then freeze-dried before grinding using a Retsch Mixer-Mill 301 with a Qiagen TissueLyser adaptor. Genomic DNA was subsequently isolated using a QIAxtractor robot (Qiagen, Valencia, USA). In some cases, fresh leaf material was frozen in liquid nitrogen and DNA isolated using either an i-Genomic Plant Kit (Intron Biotechnology, Korea) or DNeasy Plant Mini kit (Qiagen, Valencia USA). Representative samples were checked for DNA quantity and quality using a Nanodrop 3000 (ThermoScientific) and by electrophoresis through a 1% agarose gel and staining with ethidium bromide.

A.1.2 AFLP protocol

Screening for amplified fragment length polymorphisms (AFLPs) was undertaken based on the protocol of Vos et al. (1995) with modifications. Adapter and primer sequences are given in Table A.2. For each sample, a combined DNA restriction/ligation was undertaken in a 10 µl reaction containing 1.8 µl DNA isolate (ca. 40-120 ng), 10 U *EcoRI* and 1 U *MseI* (NEB), 0.5 µM *EcoRI* adaptor, 5 µM *MseI* adaptor, 0.1 U T4 DNA ligase and 1X T4 DNA ligase buffer (Fermentas), 50 mM NaCl₂, 0.5 µg BSA (NEB) and 3 µl dH₂O. Mixtures were incubated for 3 hours at 37°C followed by 20 minutes at 65°C, then diluted 5-fold with dH₂O for use as DNA template for the following step.

Pre-amplification was most commonly performed in 25 µl reactions with 3 µl of the diluted adapter-ligated DNA, 0.5 U iTaq DNA polymerase and 1x Mg-free PCR buffer (Intron Biotechnology), 2 mM MgCl₂, 120 µM dNTPs, 0.2 µM EcoRI and 0.2 µM MseI pre-amplification primers, and 15.2 µl of dH₂O. PCR was performed with the following thermal profile: 2 mins at 72°C; 30 cycles of 30 sec at 94°C, 30 sec at 56°C, and 2 mins at 72°C; with a final extension step of 10 mins at 60°C. The concentration of resultant PCR products were assessed by electrophoresis through 1.5% agarose gels stained with ethidium bromide and then normalised by diluting 5 to 10-fold with dH₂O. The diluted pre-amplification products were used as the DNA template for subsequent selective amplification.

We performed a pilot screen of several selective amplification primer combinations to assess the number and resolution of bands for each before proceeding to the next stage. For sample analysis, four selective amplification primer combinations were used, each using EcoRI and MseI selective primers with different tri-nucleotide 3' tails (Table A.2). The EcoRI primers utilised were labelled with fluorochromes (Applied Biosystems, Life Technologies) to enable subsequent product visualisation. PCR amplification was performed in 10 µl reactions containing 1.5 µl of diluted pre-amplification product, 0.25 U iTaq DNA polymerase and 1x Mg-free PCR buffer (Intron Biotechnology), 2 mM MgCl₂, 70 µM dNTPs, 80 ng BSA (NEB), 0.05 µM labelled EcoRI selective primer (Applied Biosystems), 15.1 ng MseI selective primer, and 15.2 µl of dH₂O. PCR was performed using a touchdown protocol as per follows: 2 minutes at 94°C; 13 cycles of 30 sec at 94°C, 30 sec at 65°C (with a -0.7°C reduction each subsequent cycle), 2 minutes at 72°C; 22 cycles of 30 sec at 94°C, 30 sec at 56°C, 2 minutes at 72°C; with a final extension step of 10 minutes at 72°C. PCRs were performed using either GenePro Thermal Cycler (Bioer Technology) or Eppendorf Mastercycler (Eppendorf). Selective amplification products were separated and visualised by EcoGene Ltd. (Auckland, New Zealand) using an AB1 Genescan 3130XL sequencer with ROX500 internal size standard.

A.1.3 Data scoring and analysis

Resultant data files were scored using the program GeneMarker V.1.91 (SoftGenetics LLC, USA). Amplicons with peak intensities of more than 50 relative fluorescence units (rfus) and between 75 to 520 bp were scored as present (1) or absent (0) for each polymorphic locus. The genetic profiles of each sample were then checked locus by locus and extra bands scored down to 35 rfus where the overall peak intensity for the sample was low due to poor amplification. Summary statistics were generated and Principal Components Analysis (PCA) was performed in GenAlex version 6.1 (Peakall and Smouse 2006).

A.2 Results

PCA of the AFLP data (Figure A.1) explained 67.31% of the variation on the first two axis (axis 1, 47.79%, axis two 19.52%). Axes 1 and 2 showed clear divisions among the taxa which matched the species identifications provided by the seed suppliers. These groups were tightly clustered indicating low levels of genetic variation. In conjunction with the supplied identifications from seed companies, this can be interpreted to mean taxa in each of the three groups belong to the same species, and that those are the species identified by suppliers. The rightmost group, corresponding to *Brassica rapa*, was less tightly clustered, indicating wider genetic diversity. Within this group, two sub-groups can be differentiated along axis 2, which correspond to the sub-species identification provided by the seed suppliers (*B. rapa rapa* and *B. rapa chinensis*). The weedy reference variety, *B. rapa oleifera* was located within the *B. rapa rapa* group, indicating it is more genetically similar to this subspecies than *B. rapa chinensis*. The two *B. oleracea* subspecies, *B. oleracea capita* and *B. oleracea acephala* show some separation along Axis 1, but these groups are not clearly defined.

Table A. 1 Table of the 25 taxa used in the seed sowing experiments showing species, subspecies and variety as identified by the suppliers. ID numbers refer to the numbers in Figure A.1.

Species	Subspecies	Variety	ID	Source Zone
<i>napus</i>	<i>napobrassica</i>	major plus	1	New Zealand
<i>napus</i>	<i>napobrassica</i>	airlie	14	Europe
<i>napus</i>	<i>napobrassica</i>	american purpletop	15	North America
<i>napus</i>	<i>napobrassica</i>	dominion	18	Australia
<i>napus</i>	<i>napus</i>	tactic	21	Europe
<i>napus</i>	<i>napus</i>	telfer	22	Australia
<i>napus</i>	<i>napus</i>	flash	23	New Zealand
<i>napus</i>	<i>napus</i>	griffin	24	North America
<i>oleracea</i>	<i>acephala</i>	gruner	3	New Zealand
<i>oleracea</i>	<i>acephala</i>	merlin	12	Europe
<i>oleracea</i>	<i>acephala</i>	champion	17	North America
<i>oleracea</i>	<i>acephala</i>	sovereign	20	Australia
<i>oleracea</i>	<i>capita</i>	arixos	5	Australia
<i>oleracea</i>	<i>capita</i>	summercross	7	New Zealand
<i>oleracea</i>	<i>capita</i>	greyhound	9	Europe
<i>oleracea</i>	<i>capita</i>	red express	11	North America
<i>rapa</i>	<i>chinensis</i>	rubens	4	Australia
<i>rapa</i>	<i>chinensis</i>	mei quing	6	New Zealand
<i>rapa</i>	<i>chinensis</i>	canton white	8	Europe
<i>rapa</i>	<i>chinensis</i>	black summer	10	North America
<i>rapa</i>	<i>oleifera</i>	wild	25	New Zealand
<i>rapa</i>	<i>rapa</i>	barkant	2	New Zealand
<i>rapa</i>	<i>rapa</i>	frisla	13	Europe
<i>rapa</i>	<i>rapa</i>	purpletop white globe	16	North America
<i>rapa</i>	<i>rapa</i>	mammoth purple top	19	Australia

Table A. 2 Adapter and primer sequences used in the AFLP analysis of the 25 *Brassica* taxa used in the seed sowing experiments.

Adapters	Primer
Eco-F	5'-CTC GTA GACTGC GTA CC
Eco-R	5'-AAT TGG TAC GCA GTC TAC
Mse-F	5'-GAC GAT GAG TCC TGA G
Mse-R	5'-TAC TCA GGA CTC AT
Pre-selective amplification	
EcoRI+A	5'-GAC TGC GTA CCA ATT CA
MseI+C	5'-GAT GAG TCC TGA GTA AC
Selective amplification	
EcoRI+ACC (NED)	5'- GAC TGC GTA CCA ATT CACC
MseI+CTG	5'- GAT GAG TCC TGA GTA ACT G
EcoRI+AGG (JOE)	5'- GAC TGC GTA CCA ATT CAG G
MseI+CTA	5'- GAT GAG TCC TGA GTA ACT A
EcoRI+ACT (FAM)	5'-GAC TGC GTA CCA ATT CACT
MseI+CAT	5'-GAT GAG TCC TGA GTA ACA T
EcoRI+AAC (NED)	5'- GAC TGC GTA CCA ATT CAA C
MseI+CAG	5'- GAT GAG TCC TGA GTA ACA G

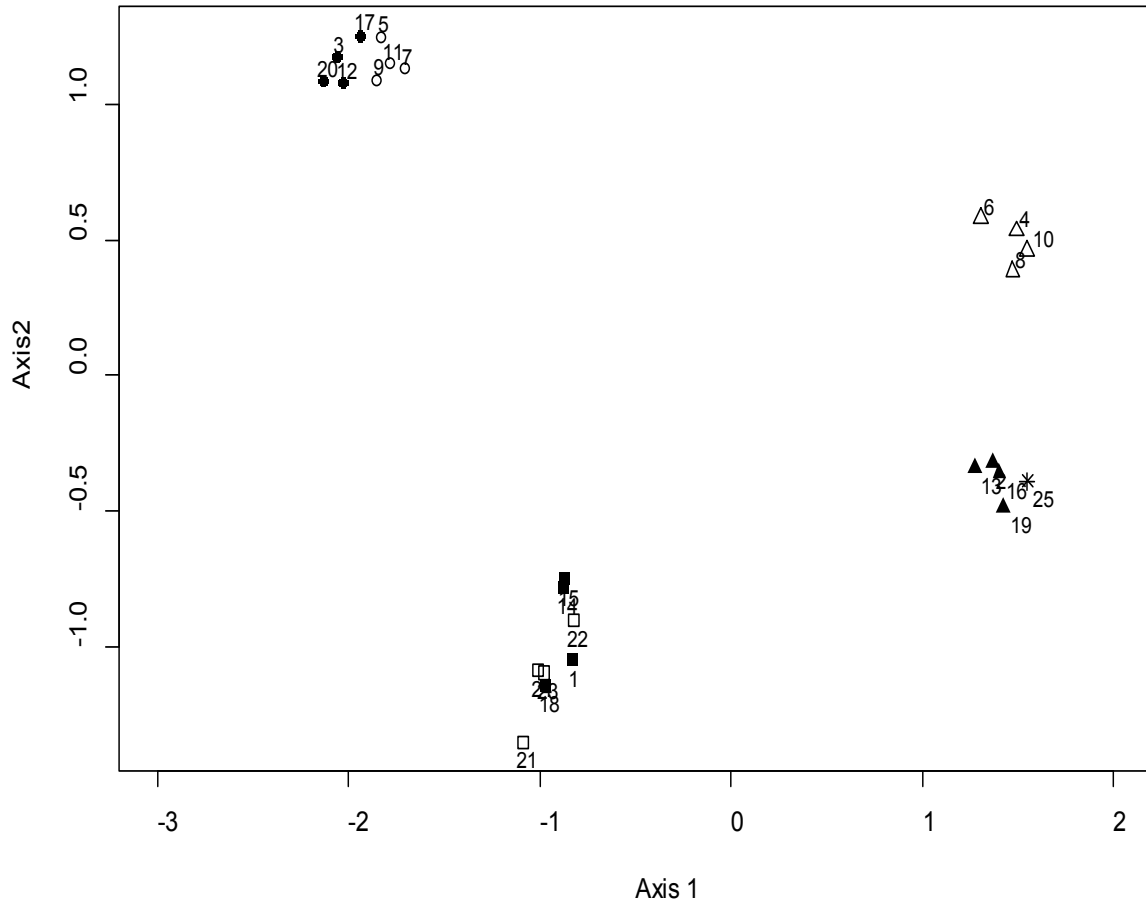


Figure A. 1 Principal Components Analysis plot of AFLP data for the 25 taxa used in the seed sowing experiments. Symbols indicate the identification of the taxa provided by the companies from which the seed was sourced; solid circles = *B. oleracea acephala*, open circles = *B. oleracea capita*, solid squares = *B. napus napobrassica*, open squares = *B. napus napus*, solid triangles = *B. rapa rapa*, open triangles = *B. rapa chinensis*, asterisk = *B. rapa oleifera*. Numbers indicate the ID numbers of the taxa, see Table A.1.

Appendix B

Lambda and *Brassica* Establishment

B.1 Methods

To quantify and compare the likelihoods of establishment for the commercial taxa and *B. rapa oleifera*, I calculated the finite rate of increase, lambda (λ), for each cell. For a given taxa and set of environmental conditions, $\lambda > 1$ (the establishment criterion) indicates the taxa will increase in abundance, while $\lambda < 1$ indicates the taxa will become extinct (Crawley et al. 1993). Calculating λ for each cell allowed me to detect fine-scale spatial differences in likelihood of establishment. In addition, for each taxon I calculated the mean λ for all cells, plus the maximum λ obtained for a single plot and single cell. These latter are the likelihoods of establishment at the most favourable sites and microsites across the study for each taxon.

Brassica are monocarpic annuals and biennials, but in this experiment all plants set seed and died or died without reproducing within one year of sowing; thus all *Brassica* individuals were seeds at the beginning of the experiment (sown seeds) and at the end of the experiment (remaining viable seeds below ground plus seeds produced by mature plants in the intervening year). Lambda was estimated as the ratio of the total number of seeds per cell after one year to the number of seeds added per cell at the beginning of the experiment (Crawley 1986, Crawley et al. 1993, Norton et al. 2005, Tozer et al. 2008, Minton and Mack 2010). Twenty five seeds were sown in each cell, so λ for each cell was estimated as:

$$\lambda = \frac{(25 - g)p_s + N_{SN}}{25}$$

Where g is the number of sown seeds that germinated during the year after sowing, p_s is the proportion of ungerminated seeds remaining viable after one year, N_{SN} is the total number of seeds produced per cell by individuals which germinated and survived to reproduce. The left hand term of the numerator refers to viable seeds that carry over from one year to the next, while right hand term refers to the number of seeds produced by plants that grow and set seed. Here I assume that seed migration rates among cells are zero, all seeds produced enter the seed bank, and all seeds that germinate emerge as cotyledons. The first assumption is justified by the documented low mobility of *Brassica* seeds (Garnier et al. 2008). The second assumption is justified as no germination was recorded after seed set in cells where seeds were produced. The third assumption is justified by the short time (2-4 weeks) between sowing and emergence, and necessitated by the difficulty of quantifying below ground mortality of germinated seeds.

Methodological Approach

Matrix models are a popular tool for demographic modelling, they can be used to calculate λ values and to assess the sensitivity of λ to population vital rates using elasticity analyses (Caswell 1989). However, for this data set matrix models would have been an unnecessarily complex method for calculating λ . Elasticity analyses of population vital rates would have been largely uninformative due to the nature of the data set (see below); furthermore, the main goal of the study was to partition *Brassica* performance among taxonomic levels, not to understand which life-history stages limited *Brassica* establishment.

In this experiment all individuals were seeds both at the start and at the end, one year later. That is, there were no cotyledons, seedlings, rosettes or flowering individuals at the beginning or end of the one year projection interval which would be used in a stage-structured matrix model. As a result the transition probabilities to or from each of these life-stages would be zero. If a stage-based matrix model was constructed the only two non-zero transitions in a Leslie matrix would be seed to seed transitions: i.e. that of a seed persisting as a viable seed below ground and that of a seed germinating, surviving to maturity, and producing more seeds. Sensitivity analysis could examine the sensitivity of λ to these two non-zero transitions (Caswell 1989). However such an analysis would not be very informative; for a population to establish ($\lambda > 1$) would clearly require an increase in the germination-survival-reproduction transition probability.

An alternative to matrix models is to estimate λ as the number of individuals in a population at time $t+1$ divided by the number of individuals at time t (e.g. Crawley 1986, Crawley et al. 1993, Bishop 2002, Norton et al. 2005, Tozer et al. 2008, Garren and Strauss 2009, Minton and Mack 2010, Stanton-Geddes et al. 2012). This is the approach I have used here. In the case of established populations, or transplant studies this may be done using the numbers of seedlings or adult plants (Bishop 2002, Garren and Strauss 2009, Stanton-Geddes et al. 2012). In seed addition studies it is usual to use the ratio of the number of seeds produced after one year to the number of seeds added at the beginning of the study (Norton et al. 2005, Tozer et al. 2008, Minton and Mack 2010). This ignores the carry-over of viable seeds below ground, but this can be accounted for by including an estimate of survival of seeds below ground in the total population estimate (Crawley et al. 1993).

B.2 Results

An initial peak in germination 4 weeks after sowing was followed by a rapid decline in mean numbers of *Brassica* per cell (Fig. B.1). Most individuals did not survive beyond the cotyledon or seedling stage; observation suggests mortality was due to competition (cotyledons and seedlings which failed to emerge from dense vegetation), water stress (desiccated cotyledons and seedlings), herbivory,

mowing and spraying by landowners, and damage from vehicles and stock. No individuals successfully overwintered, and all plants were dead by the July census one year after sowing.

Establishment (as determined by $\lambda > 1$) was rare due to low survivorship and very infrequent seed set; λ was greater than 1 in only 5 cells. *B. rapa oleifera* did not set seed in any cells, and consequently failed to achieve $\lambda > 1$ in any cell. Six commercial varieties set seed in 12 cells in six plots, comprising a total of 15 individuals (Table B.1). These individuals did not pass through a rosette stage, but 'bolted', progressing directly from seedling to flowering stages. Three of the varieties which set seed were sufficiently fecund to achieve $\lambda > 1$ in two plots (Table B.1); two were *B. rapa chinensis* ('Canton White', plot 72; and 'Rubens', plot 40), the other was *B. napus napus* ('Flash', plot 72). An additional *B. rapa chinensis* variety ('Black Summer') achieved $\lambda > 1$ in a single cell in plot 40 (Table A3.2.1).

In those cells where no plants set seed (99.6%) λ values were determined by the estimated numbers of viable seeds remaining below ground after one year and ranged from 0 to 0.03. The 0.4% of cells where seed set occurred had λ values as high as 9.67. Those cells where seed set occurred were clearly of ecological significance, but could not be statistically analysed as they were too few in number and extreme outliers of the data distribution; as a result I was unable to fit a model using MCMCglmm to partition among taxonomic levels and locations (Hadfield 2010), see Chapter 3 for details of model fitting.

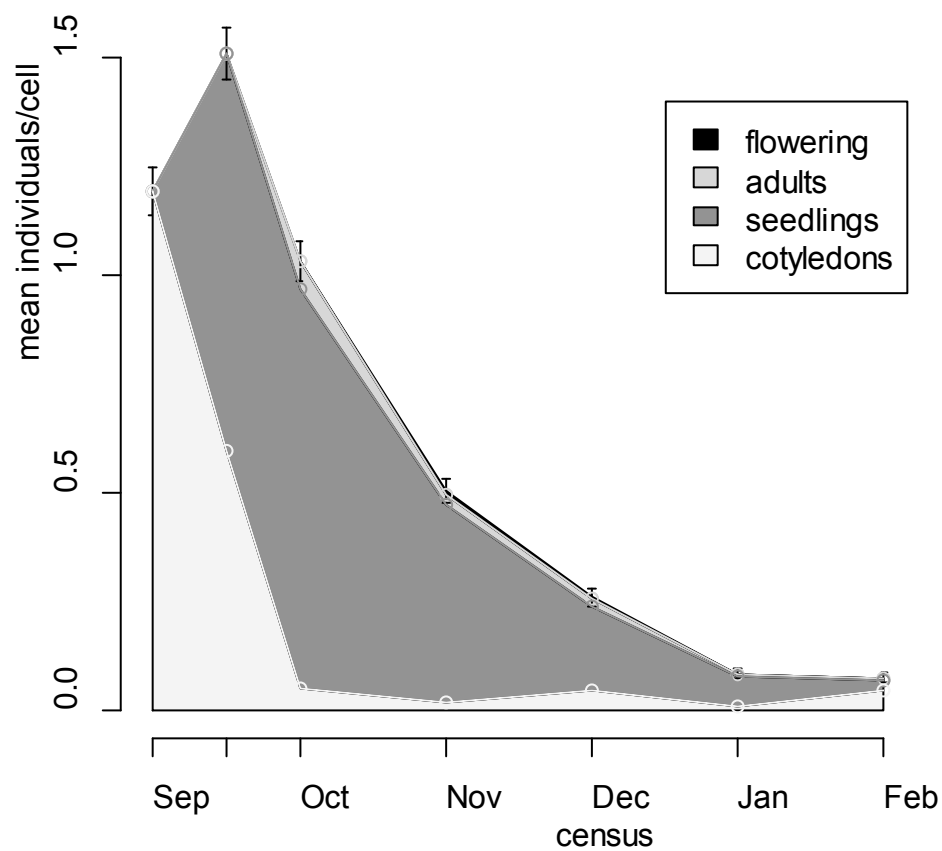


Figure B.1 Mean total *Brassica* numbers per 25 × 25 cm cell at each of seven censuses in the 2011 roadside seed sowing experiment, with 95% confidence intervals for total numbers. Shading represents division by life history stage as indicated.

Table B. 1 Estimated population growth rates (λ) for each of the 25 *Brassica* varieties grown in the 2011 seed sowing experiment. Shown are total seeds produced for each taxon, λ values for the study region as a whole, and maximum values at the plot and cell scale. $\lambda > 1$ indicates an increasing population, written in bold.

species	subspecies	variety	total seeds produced	estimated λ values		
				landscape (mean \pm se)	best plot	best cell
<i>napus</i>	<i>napus</i>	Flash	248	0.021 ± 0.016	1.277	5.099
		Griffin	-	$0.018 \pm 1 \times 10^{-4}$	0.019	0.019
		Tactic	-	$0.010 \pm 1 \times 10^{-4}$	0.011	0.011
		Telfer	-	$0.001 \pm 6 \times 10^{-6}$	0.001	0.001
	<i>napobrassica</i>	Airlie	-	$0.010 \pm 8 \times 10^{-5}$	0.011	0.011
		American Purple Top	-	$0.003 \pm 2 \times 10^{-5}$	0.003	0.003
		Dominion	-	$0.029 \pm 2 \times 10^{-4}$	0.032	0.032
		Major Plus	-	$0.001 \pm 2 \times 10^{-5}$	0.002	0.002
<i>oleracea</i>	<i>acephala</i>	Champion	-	$0.003 \pm 2 \times 10^{-5}$	0.003	0.003
		Gruner	-	$0.005 \pm 4 \times 10^{-5}$	0.005	0.005
		Merlin	-	$0.006 \pm 5 \times 10^{-5}$	0.007	0.007
		Sovereign	-	$0.002 \pm 2 \times 10^{-5}$	0.003	0.003
	<i>capita</i>	Arixos	-	0 ± 0	0.000	0.000
		Greyhound	-	0 ± 0	0.000	0.000
		Red Express	-	$0.001 \pm 4 \times 10^{-6}$	0.001	0.001
		Summercress	-	$0.001 \pm 6 \times 10^{-6}$	0.001	0.001
<i>rapa</i>	<i>chinensis</i>	Black Summer	94	0.013 ± 0.012	0.937	3.745
		Canton White	518	0.082 ± 0.033	3.330	9.670
		Mei Quing	-	0.014 ± 0.002	0.131	0.490
		Rubens	120	0.019 ± 0.015	1.285	4.737
	<i>rapa</i>	Barkant	10	0.020 ± 0.001	0.118	0.417
		Frisia	-	$0.009 \pm 8 \times 10^{-5}$	0.010	0.010
		Mammoth Purple Top	-	$0.013 \pm 8 \times 10^{-5}$	0.014	0.014
		Purple Top White Globe	14	0.021 ± 0.002	0.159	0.578
<i>oleifera</i>	<i>oleifera</i>	Wild	-	0.311 ± 0.001	0.318	0.318

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