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Insect pests and pathogens compromise the persistence of two endemic and rare *Braya* (Brassicaceae)

By

© Susan E. Squires

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ABSTRACT

Rare and threatened plant species face a variety of threats to their persistence including habitat degradation, non-native herbivores, and pathogens. In this study we explored the effects of a non-native, agricultural pest and three pathogens on two rare vascular plants restricted to a unique ecosystem. Agro-ecosystems support many non-native insects, but their potential to find and impact rare, native plants is largely unknown. *Plutella xylostella* L. (diamondback moth) is a global agricultural pest of the Brassicaceae family, including the endangered *Braya longii* (Fernald) (Long's braya) and threatened *B. fernaldii* (Abbe) (Fernald's braya) that are endemic to the limestone barrens of Newfoundland, Canada. The immigration of *P. xylostella* from southern overwintering sites to this unique ecosystem was monitored with pheromone traps between 2003 and 2005. At the same time individually tagged *Braya* were monitored for the presence and impact of *P. xylostella* and three pathogens. Since habitat loss and deterioration is still the most important threat to the persistence of endangered species, the frequency of each pest was compared between *Braya* populations growing on anthropogenically disturbed and undisturbed habitat.

Between 2003 and 2005, 30% of *B. longii* and 16% of *B. fernaldii* were infested by *P. xylostella,* 8.6% of the total *B. longii* population died from root rot (*Fusarium* sp.), 18% of *B. longii* on anthropogenically disturbed sites were infected with an unidentified pathogen causing their flowering stalks to rot, and 27% of *B. fernaldii* in northern sites were infected with an unidentified pathogen causing flowering stalk and leaf deformities.

Impacted plants contributed between 9% and 75% less seeds to annual seed production than healthy, flowering plants and had a statistically higher probability of mortality. The majority (66%-100%) of pathogen infections occurred on anthropogenically disturbed habitat.

Stage based transition matrices created from these data and summarized into deterministic projections predict *Braya* populations will decline over the next 10 years. *P. xylostella* may negatively impact the persistence of other rare Brassicaceae worldwide because they can infest rare plants growing in native vegetation, especially when the vegetation is sparse, and they, as do the pathogens, preferentially damage flowering plants. Modelling suggests that the management of pathogens in anthropogenically disturbed populations will most improve the population growth rate, where as the management of *P. xylostella* in undisturbed populations will most improve the population growth rate. Presently, insufficient attention is directed to the impacts of both native and non-native agricultural pests on rare host plants; hence, there is a need for both the conservation and agricultural communities to cooperate in mitigating their impacts on native biodiversity.

For my husband, Liam,

thank-you for

your love,

your prayers,

your sacrifices and help,

your constant patience,

and your infectious humour.

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbol / Abbreviation	Meaning
ANOVA	Analysis of variance
cm	Centimetre
°C	Degrees Celsius
df	Degrees of freedom
F	F-value or F-ratio
km	Kilometre
λ	Lambda
L	Litre
m	Metre
m^2	Squared metre
ml	Millilitre
mm	Millimetre
N	Sample size
N_o	Population size at time 0
N_t	Population size at time t
P or p	Probability
R^2	Correlation coefficient
SE	Standard error
T or t	Time
χ^2	Chi-square
$\pm$	Plus or minus
%	Percentage
=	Equal
<	Less than
$\leq$	Less than and equal to
>	Greater than
$\geq$	Greater than and equal to
i.e.	Which means
e.g.	For example

CHAPTER ONE

GENERAL INTRODUCTION

1.1. RARE PLANT CONSERVATION ISSUES

1.1.1. Rare plant concerns – Habitat loss and degradation

Like all organisms, plants struggle to exist in changing environments that result in dynamic interactions with their habitat, herbivores, and pathogens. Anthropogenic disturbance, especially habitat loss, degradation, and fragmentation remain the greatest threats to ecosystem health and biodiversity, including the survival of rare plant species (Saunders et al., 1991; Sumina, 1994; Brooks et al., 2002; Venter et al., 2006), followed closely by the threats of invasive insect and plant species, and climate change (Thomson, 2005; Westoby and Burgman, 2006). Historically the conversion of natural habitat into agro-ecosystems has been the principal cause of habitat loss (Tilman, 1999), followed by loses due to forestry, urban sprawl, and mining. Barley, maize, rice, and wheat alone occupy a total of 588 million hectares or approximately 40% of the Earth's 1,470 million hectares of cropland (Tilman, 1999). These agro-ecosystems support native and non-native insect and pathogen species, often in abundance.

Plant populations living on anthropogenically degraded habitats are typically exposed to changes in species diversity (Sumina, 1994; Forbes et al,. 2001), higher numbers of invasive species (MacDougall and Turkington, 2005), and altered physical conditions, such as higher soil temperatures (Chambers et al., 1990), resulting in changes in their survival, reproduction, and growth rates (Chambers, 1995; Forbis et al., 2004).

Although natural disturbances, such as fire, often cause demographic changes and alter physical conditions, individual plant species and plant communities have co-evolved with, and are adapted to these natural disturbances (Quintana-Ascencio et al., 2003). The successful recovery of many rare plant species now require that biotic threats, such as new insect herbivores and pathogens, are managed within the context of an already fragmented, lost, and/or deteriorated habitat.

1.1.2. Rare plant concerns – Pathogens

Many microorganisms, such as bacteria, fungi, and viruses, cause diseases in plants. The impact of a particular pathogen is strongly influenced by environmental conditions (Agrios, 2005), and plant pathogens can reach epidemic levels when these conditions within an ecosystem are anthropogenically disturbed (Augspurger, 1990). For example, root rot caused by *Fusarium* species increases when the plant is exposed to intermittent drought or excessive water and soil compaction (Agrios, 2005). The negative effect of pathogens on plant reproduction and/or survival suggests that pathogens can have a detrimental impact on plant population size (Alexander and Burdon, 1984; Alexander and Antonovics, 1988; Colling and Matthies, 2004) even to the point of increasing their risk of extinction (Barrett et al., 2008). However, the impact of pathogens on the persistence of rare plants is still not commonly studied. The most comprehensive analyses of the impacts of pathogens on plant population biology was undertaken by Burdon in 1987; however, many of the examples used were agriculturally based as there were few studies in natural ecosystems.

1.1.3. Rare plant concerns – Non-native herbivores

All plants in every ecosystem are at risk of being eaten by an herbivore, thus the importance of herbivory in influencing plant population sizes, life-history strategies, and evolution are fundamental ecological questions. Herbivory is usually considered to be detrimental to the fitness of the host plant (Marquis, 1984; Crawley, 1985; Belsky, 1986; Gurevitch et al., 2002; Strauss and Zangerl, 2002; for an alternative view see: Harris, 1973; McNaughton, 1983). Herbivores can limit the distribution and density of plants, including endangered and threatened species, within natural ecosystems (Louda, 1983; Crawley, 1985, 1989, 2005; Doak, 1992; Louda and Potvin, 1995; Louda and Rodman, 1996; Bevill et al., 1999; Gurevitch et al., 2002; Strauss and Zangerl, 2002; Labandeira, 2002; and Fröborg and Eriksson, 2003). Defoliation can impede growth rate, forcing plants to remain small and non-reproductive (Marquis, 1984). The herbivory of reproductive structures can result in the partial or complete destruction of buds, flowers, and fruit and this has consequences on plant demography by decreasing the regeneration and colonization ability of a plant population, as well as changing its spatial distribution (Crawley, 1989; Strauss, 1997; Adler et al., 2001; Strauss and Zangerl, 2002).

Plutella xylostella (L) (Lepidoptera: Plutellidae) (diamondback moth) is native to the Mediterranean, where before the introduction of insecticides in the 1940's, it was not a problematic agricultural pest (Talekar and Shelton, 1993). *P. xylostella* was the first insect documented to become resistant to insecticides, such as dichlorodiphenyltrichloroethane (DDT) (Ankersmit, 1953; Johnson, 1953). The widespread use of broad-spectrum insecticides caused a decline in the abundance of the

natural predators of *P. xylostella* and an increase in its population size. Since these initial population increases, *P. xylostella* has migrated or been introduced through the transport of plants and seeds to Europe, North America, and Asia making it the most universally dispersed of all Lepidoptera and one of the most problematic agricultural pests in the world (Talekar and Shelton, 1993). The host plants of *P. xylostella* are all members of the family Brassicaceae (mustard), such as the crop plants in the *Brassica oleracea* (L.) complex (e.g.; cabbage, broccoli, collards, and cauliflower), *Brassica napus* (canola), *B. rapa* (field mustard) (Talekar and Shelton, 1993), and in some rare cases non-Brassicaceae host plants (Löhr and Gathu, 2002). *P. xylostella* control can be costly, for example, in 1995, 1.25 million hectares of infested canola in western Canada were sprayed with insecticide to control *P. xylostella,* costing producers between $45 and $52 million dollars (CAN) (Dosdall et al., 2001). A similar infestation occurred in western Canada in 2001 resulting in the need to spray 1.8 million hectares of canola with insecticide (Dosdall et al., 2001).

The low winter temperatures in northern countries, such as Canada, Russia, and northern Asia, are not conducive to the overwintering of *P. xylostella* (Butts and McEwen, 1981; Smith and Sears, 1982; Talekar and Shelton, 1993; Dosdall et al., 2001; Capinera, 2008). Although *P. xylostella* are poor flyers, travelling distances of less than 200 m (Mo et al., 2003) and heights of two metres from the ground (Capinera, 2008), the adults are transported on high-altitude wind currents from southern agro-ecosystems for thousands of kilometres per day for several days to disperse to northern countries (Harcourt, 1986; Talekar and Shelton, 1993; Hopkinson and Soroka, 2010). Each year, *P.*

xylostella invade Canada from the United States (Smith and Sears, 1982; Braun et al., 2004; Hopkinson and Soroka, 2010). There are four to six generations of *P. xylostella* annually in Canadian agro-ecosystems, but in warmer countries, such as the United States, where there is continuous breeding; there are seven to 15 generations of *P. xylostella* annually (Capinera, 2008).

P. xylostella is a specialist of the Brassicaceae and uses glucosinolates, chemicals unique to the Brassicaceae, and visual factors as cues to locate their host plant (Louda and Mole, 1991; Aliabadi and Whitman, 2001; Alan and Renwick, 2002; Couty et al., 2006). Sinigrin, sinalbin, and glucocheirolin are feeding stimulants and the allyl isothiocyantes, and glucosinolate metabolites are oviposition stimulants (Talekar and Shelton, 1993). While glucosinolates are the key olfactory cue for host plant recognition by *P. xylostella*, large plant size (Karban, 1997), high plant density (Root, 1973; Risch et al., 1983; Yamamura and Yano, 1999), sparse vegetated "backgrounds" (Smith, 1969; Risch et al., 1983; Yamamura and Yano, 1999), and the presence of reproductive structures (Karban, 1997) are also positive visual cues for host plant recognition.

P. xylostella females lay 11-188 eggs during the ovipostion period (Talekar and Shelton, 1993). The incubation period can last approximately one week but is highly affected by temperature (Talekar and Shelton, 1993; Liu et al., 2002). *P. xylostella* larvae go through four larval stages where during the first instar larvae mine the spongy mesophyll tissue in the leaves and the older instar larvae feed on the remainder of the leaf tissue, flowers, fruit, and seeds (Talekar and Shelton, 1993). Heavy rain is a major source of mortality for *P. xylostella* because the larvae are easily displaced from leaves by

raindrops (Kobori and Amano, 2003). The fourth-instar larva constructs an open-network cocoon on leaves, stems, or nearby material, such as rocks, where it will remain for four to 15 days as it develops into an adult moth (Talekar and Shelton, 1993). *P. xylostella* adults are slender, grayish brown, 8.5 mm long, and when their wings are folded they display three light coloured, diamond-shaped spots (Capinera, 2008).

In agro-ecosystems in the United States, management of *P. xylostella* occurs when their population density is above 0.3 larvae per plant (Capinera, 2008). Insecticides are still commonly used worldwide to manage *P. xylostella* outbreaks because insecticides are inexpensive (Capinera, 2008). However, *P. xylostella* has become resistant to all previous insecticides, including DDT (Ankersmit, 1953; Johnson, 1953; Talekar and Shelton, 1993) and it is expected that this will happen with any current and new insecticides. The use of alternative control methods, such as trap cropping (Åsman, 2002; Shelton and Nault, 2004; Badenes-Perez et al., 2006), sprinkler irrigation that mimics rainfall (Harcourt, 1986; Waklsaka et al., 1991; Kobori and Amano, 2003), mass trapping to disrupt reproductive success (Silverstein, 1981), and biological control (Capinera, 2008) have been extensively studied for *P. xylostella* and are being used more commonly in integrated pest management programs.

1.2. *BRAYA LONGII* AND *BRAYA FERNALDII*

Of the 127 vascular plants on the Canadian species at risk list, 18 are endemic to Canada (Species at Risk, 2002). Three of these species, *Braya longii* (Fernald) (Long's

braya), *Braya fernaldii* (Abbe) (Fernald's braya), and *Salix jejuna* (Fernald) (Barren's willow), are endemic to limestone barrens of the Great Northern Peninsula of Newfoundland and ranked as globally (G1), nationally (N1) and provincially (S1) critically imperilled (Hermanutz et al., 2002). Harvard botanists, Bayard Long and M. L. Fernald, first discovered *B. longii* in 1924 and *B. fernaldii* in 1925 (Meades, 1996a, b). In 1997, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recommended *B. longii* and *B. fernaldii* to be designated as endangered and threatened, respectively under the federal *Species at Risk Act* (Species at Risk Act, 2002). In 2001, *B. longii* and *B. fernaldii* were designated as endangered and threatened, respectively under the Newfoundland and Labrador provincial *Endangered Species Act* (Endangered Species Act, 2001).

1.2.1. Distribution and habitat

The limestone barrens of the island of Newfoundland are limited to a fragmented, narrow strip of land on the west coast of the Great Northern Peninsula that are part of the Strait of Belle Isle ecoregion (Hermanutz et al., 2002). While this ecoregion is a hotspot for plant diversity, containing 114 of Newfoundland's 271 rare plant species (Bouchard et al., 1991), it represents only 1.7% (1,820 km^2) of the land area of Newfoundland. Within this area, *B. longii* is found in five populations within a range of 6 km between Yankee Point and Shoal Cove, and in one isolated population, Anchor Point, 14 km to the south, and *B. fernaldii* is found in 16 populations within a range of 190 km from the Port au Choix National Historic Site to the Burnt Cape Ecological Reserve (Figure 1.1;

Hermanutz et al., 2002; Hermanutz et al., 2009). All populations are 13 to 15 m above sea level and a maximum of 1.5 km from the ocean (Greene, 2002).

Braya grow in limestone habitat that has been fragmented and disturbed by both natural and anthropogenic processes. Naturally, the arctic-like weather of the Great Northern Peninsula has fragmented each population by creating a landscape with scattered patches of suitable plant habitat. In accordance with the intermediate disturbance hypothesis (Grime, 1973; Greene, 2002), *Braya* species exploit gaps in the vegetation produced by small-scale disturbances, such as frost action and wind and soil erosion, to survive (Noel, 2000; Greene, 2002). Frost action on the limestone barrens has lead to the formation of patterned substrate, such as sorted circles and sorted stripes, a phenomenon that is common in polar, subarctic, and alpine regions (Washburn, 1956; Mann, 2003). Anthropogenically disturbed habitats have undergone larger scale disturbance to the substrate and vegetation and contain homogenous gravel substrates with no patterned or sorted substrate and low plant species diversity (Greene, 2002; Rafuse, 2005). Both *Braya* species can grow in both sorted and unsorted substrates (Greene, 2002; Tilley, 2003).

1.2.2. Population size, trends, and conservation concerns

Historically, the limestone barrens have suffered from intense habitat fragmentation and destruction as a result of quarrying activity, road construction, and development (Janes, 1999; Hermanutz et al., 2002; Tilley et al., 2005). The result of these disturbances has been the degradation of large areas of habitat in all *B. longii* populations

and half of the *B. fernaldii* populations (Hermanutz et al., 2002). The 1998 to 2000 census revealed that 75% of the global *B. longii* population (7,235 individuals) and 57% of the global *B. fernaldii* population (3,434 individuals) were growing on anthropogenically disturbed substrate. During the 2008 census, 5,549 flowering *B. longii* and 3,282 flowering *B. fernaldii* were counted, of which the vast majority, 91% and 90% respectively, were found on anthropogenically disturbed substrate (Hermanutz et al., 2009).

Anthropogenic mortality of *Braya* increased substantially from 1968 to 1990 when quarries removed limestone for road material (Janes, 1999), the construction of a highway bisected some *Braya* populations and community development destroyed *Braya* habitat and populations (Hermanutz et al., 2002). In some areas of the limestone barrens the use of limestone gravel to level areas of land, support power lines or build roads has created patches of anthropogenically disturbed habitat that both *Braya* species are capable of invading. These degraded patches account for 31% of habitat within *Braya* populations (Hermanutz et al., 2009). It has been observed worldwide that anthropogenically disturbed habitats have an increased likelihood of exploitation by insect herbivory and pathogens than natural habitats (Ouborg and Biere, 2003).

As in all ecosystems, climate change is and will continue to play a significant role in the long-term survival of *Braya* without global intervention. Recent data show that changing climatic regimes on the Great Northern Peninsula may lead to an overall increase of approximately 4°C in the mean annual air temperature by the 2080's (Slater,

2005), a phenomenon that may lead to both direct plant mortality and indirect plant mortality due to increasing pest and pathogen pressure or competition.

1.2.3. Biology of *Braya longii* and *Braya fernaldii*

Both *Braya* species are taxonomically closely related and have similar life histories and ecology (Hermanutz et al., 2002). *B. longii* and *B. fernaldii* are both small (1–10 cm and 1–7 cm tall, respectively), herbaceous perennials with linear-spatulate leaves and white, four-petalled flowers, arranged in a raceme (Hermanutz et al., 2002). *B. longii* differs from *B. fernaldii* in having larger petals, smaller sepals, and pubescent fruit (Meades, 1996a, b; Parsons, 2002). Both *Braya* have scapose racemes of small white flowers (Harris, 1985) and flower from the middle to the end of June, start producing fruit by mid July, and have mature fruits by mid August (Parsons and Hermanutz, 2006). *Braya* have contractile taproots for secure anchorage in frost-heaved substrates and die back to the crown during winter.

Braya growing on anthropogenically disturbed sites are larger, have higher reproductive output, grow in densities at least 10 times those found in undisturbed sites (Hermanutz et al., 2002) and flower earlier (Donato, 2005). For these reasons anthropogenically disturbed sites have commonly been considered to be 'optimal' habitats for *Braya* (Hermanutz et al., 2002), but this misconception is rooted in the productivity of these populations rather than their long-term viability. *B. longii* and *B. fernaldii* are self-pollinating (Parsons, 2002) and produce, on average, 2.2 and 1.3 flowering stalks per plant, respectively, on undisturbed substrate and 5.6 and 4.3

flowering stalks per plant, respectively, on anthropogenically disturbed substrate (Hermanutz et al., 2009). Each fruit produces between 9.0 and 16.6 seeds resulting in hundreds of seeds being produced per year per plant (Hermanutz et al., 2009). *B. longii* seeds weigh approximately 2.5 times more than *B. fernaldii* seeds (Hermanutz et al., 2002). Similar species have a long-lived seed bank, however the size and distribution of either *Braya* species seed bank is unknown (Hermanutz et al., 2002; Tilley, 2003).

1.3. THESIS OUTLINE

Low species diversity, high levels of habitat disturbance, degradation, and fragmentation, abundance of a food source, and insecticide use in anthropogenically modified ecosystems, such as agro-ecosystems, has created population explosions of some insect herbivores and pathogens (Capinera, 2008). Agro-ecosystems and their associated pests are often in close proximity to natural ecosystems. Some agricultural pests disperse into nearby native ecosystems and have detrimental effects on the plants seed production, especially those plants already suffering from anthropogenic disturbance (McKone et al., 2001). The severity of this problem increases when the agricultural pest's host plant is already a rare or endangered species (Oostermeijer, 2003). Few studies have documented the effect of agricultural insects and pathogens, on the distribution and density of rare plants (McKone et al., 2001) and thus the consideration of this factor in conservation and management strategies.

In effectively manage the rare *B. longii* and *B. fernaldii*, it is vital to understand the impact of the agricultural pest *P. xylostella* on plant growth, reproduction, and survival. To determine this, the infestation rate, survival, and reproductive success of *P. xylostella* on *Braya* was monitored for three years and the amount of larval feeding on leaf biomass and reproductive output recorded. These analyses are reported in Chapter two, "*Agricultural insect pest compromises survival of two endemic Braya (Brassicaceae)*" and published in *Biological Conservation,* volume 142 (Squires et al., 2009).

In Chapter three, "G*lobal agricultural pest can find endangered plants within native habitats*", I expand on our understanding of an agricultural pest's ability to find rare host plants by analyzing whether visual cues known to be used by insects in natural and agricultural ecosystems influence the level of infestation of *P. xylostella* on *Braya* in their native ecosystem. I test the influence of host plant population size, host plant size, presence of reproductive structures on host plants, host plant abundance and density, non-host plant abundance, and the presence of cultivated Brassicaceae on *P. xylostella* infestation frequency and abundance. While for *P. xylostella* glucosinolates are a vital chemical attractant for host plant recognition and oviposition (Talekar and Shelton, 1993), odours such as those released by glucosinolates do not lead insects directly to a host plant; visual cues also play an important role (Bernays and Chapman, 1994). Couty et al. (2006) found that when a host plant was hidden (i.e., absence of visual cues) the number of *P. xylostella* landing on non-host plants significantly increased. For rare host-plants, it maybe more important to understand visual cues than chemical cues as visual

cues maybe able to be manipulated through restoration efforts (i.e.; lower plant density) to facilitate management of the pest and conservation of the rare host plant.

As habitat loss and fragmentation are still the most important threat to the persistence of endangered species, it was important to compare the impacts of *P. xylostella*, and other biotic threats on anthropogenically and naturally disturbed substrate. In Chapter four, "*Are rare plant populations on disturbed habitats less valuable for conservation?*" I compare at the presence of insect and pathogenic threats and their subsequent impact on *Braya* seed production and survival in populations growing on anthropogenically disturbed and undisturbed habitat. Comparing the infestation rate and impacts of pests in each habitat type will enable scientists and managers to determine the value of anthropogenic sites in the management of these rare species.

The threat of an agricultural pest to a rare plant, or the value of suboptimal habitat (anthropogenically disturbed) on the survival of a rare plant has never before been incorporated into models of the extinction probability of rare species, such as the commonly used population viability analysis. In Chapter five, "*Persistence of rare plants threatened by pests depends on habitat disturbance*", I outline the demography of *B. longii* and *B. fernaldii* and illustrate how their vital rates change under anthropogenically disturbed and insect and pathogenic pressure. Without knowing how these plant species respond to these threats, it will be impossible to adequately and accurately predict changes in plant population sizes as ecosystems continue to suffer from increasing anthropogenic pressure.

In Chapter six, I summarize the significance and implications of the results from Chapters two, three, four, and five for *B. longii* and *B. fernaldii* and other rare vascular plants. I focus on the influence of human disturbance, in the form of anthropogenically disturbed habitat and agricultural pests, on rare plant ecology. Results from these studies have implications in the management of *B. longii* and *B. fernaldii*, and other rare flora of the limestone barrens. This research furthers our knowledge about the effects of human disturbance on the survival of long-lived rare plant species and key issues in restoration of habitats and species, a necessity to understand as ecosystems all over the world suffer from habitat loss and degradation.

1.4. CO-AUTHORSHIP STATEMENT

This research was conducted independently but under the supervision of Drs. Luise Hermanutz and Peggy Dixon and with the support of Dr. Murray Colbo. I was responsible for the design and execution of field and laboratory studies, but was assisted with data collection by Eddie Donato and Casidhe Dyke in 2003, Natalie Bonnell, Joni Driscoll, Ginette Rafuse, and Sarah Critch in 2004, and Julie Robinson and Natalie Bonnell in 2005 and 2006. Insect pheromone traps were collected by Shirley Alyward (Parks Canada) in 2004 and 2005 and Port au Choix, Cook's Harbour, and Flower's Cove Green Team members from 2003 to 2005. I trained all assistants, who then conducted their work under my supervision following protocols which I designed. I entered and analysed all data and interpreted the results.

I wrote the original drafts of the manuscripts that constitute the chapters of this thesis. Chapters two through five are co-authored by Drs. Hermanutz and Dixon. Both Drs. Hermanutz and Dixon provided financial support, shared in the design of the project, and provided comments on draft manuscripts. The manuscripts were revised based on their comments, as well as comments from Dr. Colbo and colleagues, all of whom are listed in the acknowledgments.

Publications, both published and anticipated, arising from this thesis are:

Chapter 2 Squires, S.E., Hermanutz, L., and Dixon, P.L. 2009. Agricultural insect pest compromises survival of two endemic *Braya* (Brassicaceae). Biological Conservation 142: 203-211.

Chapter 3 Squires, S. E., Dixon, P.L., and Hermanutz, L. 2009. Global agricultural pest can find endangered plants within native habitat.

Chapter 4 Squires, S.E., Hermanutz, L. and Dixon, P.L. 2009. Are rare plant populations on disturbed habitats less valuable for conservation?

Chapter 5 Squires, S.E., Hermanutz, L. and Dixon, P.L. 2009. Persistence of rare plants threatened by pests depends on habitat disturbance.

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Figure 1.1. Map of the Great Northern Peninsula, Newfoundland, Canada showing the approximate location of *Braya* populations and their level of disturbance (adapted from Hermanutz et al., 2002). At Shoal Cove and at Anchor Point East there is one *Braya longii* and one *B. fernaldii* population. Study sites are located in populations written in italics.

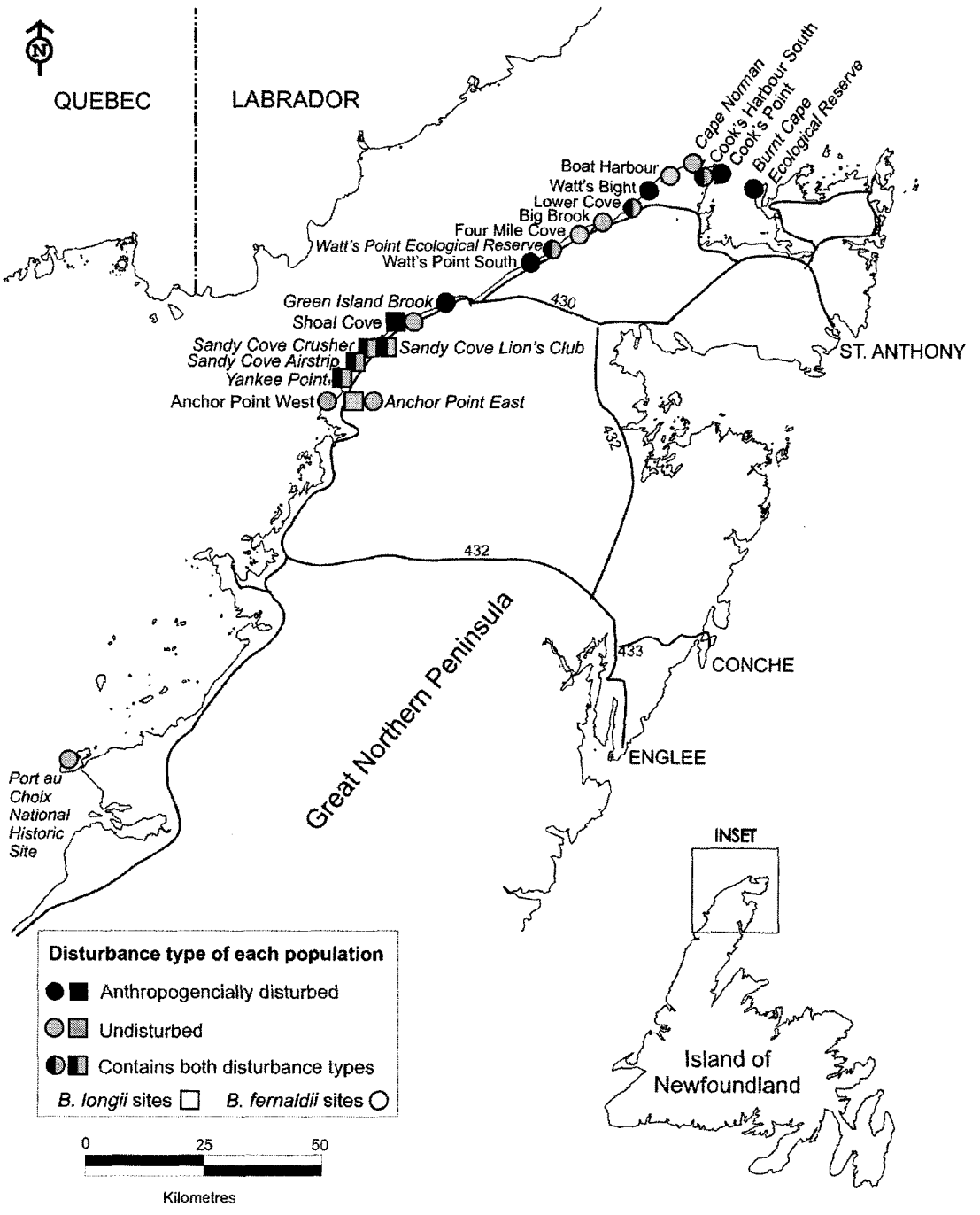
QUEBEC
LABRADOR
Cape Norman
Cook's Harbour South
Cook's Point
Burnt Cape Ecological Reserve
Boat Harbour
Watt's Bight
Lower Cove
Big Brook
Four Mile Cove
Watt's Point Ecological Reserve
Watt's Point South
Green Island Brook
Shoal Cove
Sandy Cove Crusher
Sandy Cove Airstrip
Yankee Point
Sandy Cove Lion's Club
Anchor Point West
Anchor Point East
430
ST. ANTHONY
432
432
433
CONCHE
ENGLEE
Great Northern Peninsula
Port au Choix National Historic Site
INSET
Island of Newfoundland
Disturbance type of each population
Anthropogencially disturbed
Undisturbed
Contains both disturbance types
B. longii sites
B. fernaldii sites
0
25
50
Kilometres

Figure 1.1.

CHAPTER TWO

AGRICULTURAL INSECT PEST COMPROMISES SURVIVAL OF TWO ENDEMIC *BRAYA* (BRASSICACEAE)

2.1. ABSTRACT

Agro-ecosystems support many native and non-native insects, often in abundant quantities, but the potential of these insects to invade and degrade natural ecosystems is largely unknown. *Plutella xylostella* L. (diamondback moth) is a global agricultural pest that is not native to North America. It feeds on members of the Brassicaceae family, including the endangered *Braya longii* (Fernald) (Long's braya) and threatened *B. fernaldii* (Abbe) (Fernald's braya) which are endemic to the limestone barrens of Newfoundland, Canada. The immigration of *P. xylostella* from overwintering sites in the United States to this rare natural ecosystem was monitored with pheromone traps between 2003 and 2005. After their mass immigration in early summer, females lay eggs on an average of 30% of the *B. longii* and 16% of the *B. fernaldii* population. Larval feeding reduces the mean seed output of *B. longii* infested plants by 60%, from 10.8 to 4.3 seeds per fruit, and damages 26% of their leaves. There are residual and long-term effects of this herbivory, as many dead *Braya* had higher numbers of eggs, and subsequent leaf and fruit damage one to three years before they died. High summer air temperatures and low precipitation allowed this pest to become multivoltine, resulting in additive damage to *Braya* individuals. Presently, insufficient attention is directed to the impacts of agricultural pests on native ecosystems and rare host plants; hence, there is a

need for both the conservation and agricultural communities to cooperate in mitigating the impacts of these pests on native biodiversity.

2.2. INTRODUCTION

Agro-ecosystems support an enormous diversity and biomass of insect species. Unlike the many studies on the impacts of the agricultural weeds and modified crops on natural systems (Kling, 1996; Sagoff, 2005), very few studies have investigated ecosystem and community level impacts of agricultural insect species within natural environments, especially on rare ecosystems and species (McKone et al., 2001). McKone et al. (2001) determined that *Diabrotica* species (corn rootworm beetles) have the ability to disperse from corn into prairie remnants where they feed extensively on the pollen of native *Helianthus* species (sunflowers) reducing seed set up to 15%. They concluded that these beetles are likely one of a number of agricultural pests that negatively affect plants in prairie remnants.

Plutella xylostella (L.) (Lepidoptera: Plutellidae) (diamondback moth) is a global pest of the Brassicaceae, including agriculturally important crops such as broccoli and cabbage (Talekar and Shelton, 1993). *P. xylostella* is cosmopolitan where its host plants occur, either as a permanent pest that survives year round or as an annual migrant during the growing season where it is unable to overwinter due to low winter temperature (i.e., Canada) (Butts and McEwen, 1981; Smith and Sears, 1982; Dosdall et al., 2001), making it one of the most problematic agricultural pests worldwide (Talekar and Shelton, 1993).

The success of *P. xylostella* is due to a variety of attributes including resistance to pesticides (Shelton et al., 1996), long-distance dispersal via high altitude wind currents (Harcourt, 1986; Talekar and Shelton, 1993; Hopkinson and Soroka, 2010), and recent changes in climatic limits that allow *P. xylostella* to survive further north (Shelton et al., 1996).

P. xylostella can survive on wild Brassicaceae species (Talekar and Shelton, 1993; Badenes-Perez et al., 2005) and in some cases non-Brassicaceae host plants (Löhr and Gathu, 2002). Typical agricultural host plants such as cabbage occur only in small patches on the Great Northern Peninsula of Newfoundland, Canada; thus when *P. xylostella* arrives by wind dispersal, they survive on rare, native, and non-agricultural Brassicaceae species (Hermanutz et al., 2002; Tilley et al., 2005). *P. xylostella* larvae were first seen on two rare and endemic Brassicaceae species, *Braya longii* (Fernald) (Long's braya) and *Braya fernaldii* (Abbe) (Fernald's braya) in 1995 and were identified as a potential threat in the *Braya* Recovery Plan (Hermanutz et al., 2002). *B. longii* and *B. fernaldii* are both small (1–10 cm and 1–7 cm tall, respectively), herbaceous perennials with linear-spatulate leaves and white, four-petalled flowers, arranged in a raceme (Hermanutz et al., 2002). *B. fernaldii* has pubescent fruits and smaller petals and leaves than *B. longii* (Parsons and Hermanutz, 2006). Parsons and Hermanutz (2006) reported that 44% of *B. longii* and 57% of *B. fernaldii* buds were completely eaten by *P. xylostella*. The Committee on the Status of Endangered Wildlife in Canada has designated *B. longii* as "endangered" and *B. fernaldii* as "threatened" (Species at Risk Act, 2002); both species are endemic to the globally rare limestone barrens on the

northern tip of the Great Northern Peninsula and are ranked globally (G1), nationally (N1) and provincially (S1) as critically imperiled.

The objectives of this study were to evaluate the impact of this non-native agricultural pest on the long-term persistence of *B. longii* and *B. fernaldii* by investigating: (1) *P. xylostella* population dynamics (infestation rate and survival) on *B. longii* and *B. fernaldii*; (2) the number of generations of *P. xylostella* that can occur on *Braya* under varying climatic regimes; (3) the impact of *P. xylostella* larval feeding on leaf biomass and reproductive output; and (4) the cumulative impact of *P. xylostella* feeding on mortality of both *Braya* species. A 3-year study from 2003 to 2005 was initiated to address these objectives. This study attempts to improve our understanding of the effects of agricultural pests on natural ecosystem biodiversity and function by determing their ability to survive on and impact a rare or endangered plant species within its native ecosystem.

2.3. MATERIALS AND METHODS

2.3.1. Study populations

The limestone barrens are characterized by a cool, wet, and windy climatic regime that supports tundra-like vegetation (Banfield and Jacobs, 1998). *B. longii* and *B. fernaldii* exploit gaps in the vegetation produced by small-scale disturbances, such as frost action and wind and soil erosion, to survive (Noel, 2000). The population dynamics and effect of *P. xylostella* on *Braya* was studied on six *B. longii* (Figure 1.1; Sandy Cove

Crusher anthropogenic and natural, Sandy Cove Airstrip anthropogenic and natural, Sandy Cove Lion's Club anthropogenic, and Yankee Point anthropogenic) and four *B. fernaldii* populations (Figure 1.1; Port au Choix Natural Historic Site, Anchor Point, Cape Norman, and Burnt Cape Ecological Reserve). The populations spanned the entire ranges of both *Braya* species (190 km distribution of *B. fernaldii*; 25 km of *B. longii*), including populations inside and outside protected areas (Hermanutz et al., 2002).

2.3.2. Population dynamics of *P. xylostella*

The arrival of *P. xylostella* moths onto *Braya* habitat was assessed using 17 Phero Tech delta pheromone traps, which were set up throughout the 10 populations the first week of June in 2003, 2004, and 2005. The *P. xylostella* pheromone lure used in traps is highly specific (Chisholm et al., 1983) and widely used in integrated pest management programs to monitor the presence of *P. xylostella* moths (Baker et al., 1982; Walker et al., 2003). Based on the area from which traps attract male moths, two traps were placed in all populations except very small ones (i.e., <30 m width or length), where one trap was used. Traps were set in the same location each year at a height of 0.3 m above the ground (Baker et al., 1982; Kmec and Weiss, 1997) and a minimum distance of 30 m apart. Traps were checked weekly until August for the number of new moths. Pheromone lures were stored in a freezer to minimize degradation until they were placed in the traps and new pheromone lures were used each year. To maximize trapping efficiency, the sticky inserts were changed whenever the number of insects reached 50 or there was a significant amount of debris present on the insert.

To determine the frequency and timing of occurrence of the immature stages of *P. xylostella* on *Braya* the number of *P. xylostella* eggs, larvae, and pupae were counted on approximately 100 individually tagged plants from all ages, reproductive stages, and sizes in each of the six *B. longii* (2003, n = 525; 2004, n = 506; 2005, n = 542) and four *B. fernaldii* (2003, n = 346; 2004, n = 372; 2005, n = 401) populations two to three times per month from June to August in each year of the study. At the end of August, the percentage of leaves and fruit damaged by *P. xylostella* larval feeding was recorded using a ratio scale (0% = no damage, 12.5% = ≤25% damage, 37.5% = 26-50% damage, 62.5% = 51-75% damage, and 87.5% ≥ 76% damage; Said and Itulya, 2003). In each year of the study and in 2006 the survival of tagged plants was assessed. To determine if *P. xylostella* feeding increases the mortality of infested plants, the maximum number of eggs recorded during the survey period, leaf damage, and fruit damage was compared among plants that survived and plants that died in the year of the plant's death as well as one, two, and three years prior to the plant's death.

Visual inspection of plants can underestimate larval infestation as early instar larvae reside within reproductive structures (Parsons, 2002). To determine the level of larval infestation and damage, five plants with a high density of eggs on their leaves were tagged each year at three *B. longii* populations. From each of these plants, two buds, flowers, and/or fruit were randomly collected two to four times per month from June to August every year. To minimize any negative impact on seed productivity, sample sizes were purposely kept small and samples taken from only those *B. longii* populations where *P. xylostella* infestation has historically been the highest (Parsons and Hermanutz,

unpublished data). Immediately upon collection, samples were stored in 75% ethanol. The reproductive structures were dissected within three months of collection and the numbers of larvae, larval entry/exit holes, and undamaged seeds present in each fruit were counted.

2.3.3. *P. xylostella* survival

Air temperature and precipitation data were collected hourly from a weather monitoring network which was established in 2003 and 2004 (Donato, 2005) because the development rate and survival of *P. xylostella* is controlled by temperature (Liu et al., 2002) and larvae are extremely susceptible to drowning (Harcourt, 1986; Kobori and Amano, 2003). Harcourt (1957) determined that *P. xylostella* took 283 degree days above a threshold of 7.3°C to develop from egg to adult. Average daily temperature increases of a few degrees can accumulate sufficient degree days to allow multiple generations of *P. xylostella* to develop (Liu et al., 2002). Air temperatures from six dual temperature HOBO Pro/External temperature loggers and nine HOBO XT loggers, and air temperature and precipitation from three weather stations were analysed.

2.3.4. Statistical analysis

Analyses of variance (ANOVAs) were completed using MINITAB (version 13 for Windows) with alpha set at 0.05, provided the assumptions of normality and homogeneity outlined by the general linear model were met. If the assumptions were not met or the response variable was binomial, logistic regressions were completed with SAS

(version 9.1 for Windows) using GENMOD, as outlined by the generalized linear model. Analyses were completed to determine whether there were significant differences in oviposition, in numbers of moths trapped, and in leaf and fruit damage between *Braya* species, among populations, and among years. Further analyses were completed for each species to determine whether *P. xylostella* infestation was a result of prior infestation. For fruit collected from *B. longii* individuals, the amount of seed production per fruit was compared among years, among populations, and between fruit with and without larvae or evidence of larval feeding. Analyses were also completed to determine whether there were significantly more eggs, leaf damage, and fruit damage on dead plants than on plants that survived in the year of their death or one to three years prior to their death.

2.4. RESULTS

2.4.1. Population dynamics of *P. xylostella*

Population dynamics of *P. xylostella* adults varied among years and from univoltine to multivoltine. In 2003 and 2004 *P. xylostella* first arrived on *Braya* sites at the end of June, with mass immigration occurring the first week of July, when 75% of the total number of moths was caught (Figure 2.1). In contrast to 2003, in August 2004 a second group of *P. xylostella* was caught in the pheromone traps in *B. longii* populations (Figure 2.1a). Arrival of *P. xylostella* was earlier in 2005 (Figure 2.1); and densities peaked the last week of June, second week of July, and first week of August (Figure 2.1).

In all three years of the study, traps caught significantly more *P. xylostella* (4.4X) at *B. longii* populations than at *B. fernaldii* populations (Figure 2.1) ($F_{1,15}$=15.13, p<0.001).

In each year, *P. xylostella* eggs were laid within two weeks of the first captures of moths. In 2003 and 2004, eggs were first seen and reached a maximum density on *B. longii* and *B. fernaldii* during the first week of July (Figure 2.1). In 2005, eggs were first seen a month earlier than in 2003 and 2004 (first week of June; Figure 2.1). The percentage of *B. longii* and *B. fernaldii* infested with *P. xylostella* eggs varied significantly among years, with infestation lowest in 2004 and highest in 2005 (χ^2=25.81, df=2, p<0.0001, binomial distribution; Figure 2.2). *B. longii* populations had more eggs and significantly more plants infested than *B. fernaldii* populations (χ^2=33.03, df=1, p<0.0001, binomial distribution; Figure 2.2).

In August 2004 a second group of *P. xylostella* eggs were found on individuals of both *Braya* species, coinciding with the second group of *P. xylostella* caught in pheromone traps (Figure 2.1). There was *P. xylostella* eggs on *B. longii* and *B. fernaldii* throughout the growing season in 2005, resulting in individual plants being infested with two or three sets of *P. xylostella* eggs (Figure 2.2).

Infestation in any one year increased the probability of future infestation in both species. Infested *B. longii* and *B. fernaldii* individuals had a 35% probability while non-infested individuals had a 19% probability of being infested the following year ($F_{1,1780}$=49.97, p<0.0001), and a 46% probability versus a 24% probability of being infested two years later ($F_{1,1560}$=22.28, p<0.0001).

First instar larvae mine the spongy mesophyll tissue of the *B. longii* and *B. fernaldii* leaves, whereas later instar larvae forage on the external portions of the leaves, buds, flowers, and fruit. These later instar larvae are visible in the field approximately two weeks after egg deposition. Dissections of *B. longii* reproductive structures (buds, n=81; flowers, n=234; and fruit, n=443) showed that *P. xylostella* larvae infested less than 15% of buds and flowers in all years, but an average of 48% of fruit (range of 23% to 67%) over the three year period (Figure 2.3). *P. xylostella* larvae were the only insect found to infest *B. longii* reproductive structures. Pupae were not usually found on *B. longii* and *B. fernaldii*, with the exception of a few observed in August and September in each year of the study. A small number of pupae were found on other material on the site, such as rocks.

2.4.2. Multiple generations of *P. xylostella*

The average monthly air temperature was higher in July 2004 than July 2003 (Table 2.1). This increase in temperature coincided with a second group of *P. xylostella* caught in the pheromone traps. In 2004, *P. xylostella* were first trapped on June 13th and a second peak of *P. xylostella* were caught in pheromone traps on August 1st (Figure 2.1), during which time there were 279 cumulative degree days on *B. longii* sites and 253 cumulative degree days on *B. fernaldii* sites above the developmental baseline of 7.3°C (Harcourt, 1957).

The average air temperature between June and August increased again from 2004 to 2005 in *Braya* populations, with the exception of August, which was cooler in 2005

than 2004 in *B. fernaldii* populations (Table 2.1). Again this increase in temperature coincided with multiple groups of *P. xylostella* adults, eggs, and larvae throughout the growing season. In 2005, eggs were present on the plants during the first week of pheromone trapping, suggesting that the moths most likely arrived before trapping was initiated. Although the exact date the moths arrived in 2005 is not certain, egg development could not have begun until May 27th because there was no day before this with a temperature above 7.3°C. *P. xylostella* were caught every week of trapping but reached a peak on July 11th (Figure 2.1). In *B. longii* populations there were 272 cumulative degree days above 7.3°C between May 27th and the July 11th peak and 244 cumulative degree days between the July 11th and the August 8th peaks. Although *P. xylostella* was present during this time in *B. fernaldii* populations and its phenology was similar to that seen in *B. longii* populations, climate data indicate that the cumulative degree days above 7.3°C were not sufficient (130 and 170) to allow for multiple generations of *P. xylostella*.

Both the weather stations on the *B. fernaldii* populations in Port au Choix and the *B. longii* populations at the Sandy Cove Airstrip natural recorded higher amounts of rainfall in 2003 than 2004. Rain was especially heavy during the third week of July in 2003. At this time, the larvae were beginning to feed on the external portions of the leaves (Figure 2.1) when they are easily displaced by rain. The cumulative rainfall amounts from May to August at the Sandy Cove Airstrip weather station were 182 mm in 2003 and 173 mm in 2004. These cumulative rainfall amounts are approximately 50%

lower than that of the 30-year normal (1971-2000) (401 mm) for summer precipitation produced from the nearby Flower's Cove climate station (Environment Canada, 2005).

2.4.3. Damage caused by *P. xylostella* larval feeding

Leaf and fruit damage: *B. longii* individuals had an average leaf damage that was 9% higher ($F_{1, 623}$=14.78, $p<0.001$) and average fruit damage that was 20% higher ($F_{1,486}$=39.43, $p<0.001$) than *B. fernaldii* individuals (Figure 2.4). The highest level of fruit damage occurred in 2005 in both species (Figure 2.4), when *P. xylostella* adult and egg numbers were also highest (Figure 2.1). In that year, 45% of fruit on single flowering and 50% of fruit on multiple flowering *B. longii* individuals were damaged. *P. xylostella* larvae were the only insect ever observed feeding on *B. longii* or *B. fernaldii* leaves or fruit.

Of the 443 fruit sampled from *B. longii* individuals between 2003 and 2005, 48% had evidence of *P. xylostella* larval feeding (Figure 2.3). The number of seeds per fruit decreased by 60% or from 10.8 ± 0.22 seeds per fruit in undamaged fruit to 4.3 ± 0.31 seeds per fruit in fruit with a larva ($F_{1,436}$=5.43, $p<0.020$) or evidence of previous larval damage ($F_{1.436}$=235.93, $p<0.0001$). Fruit are small and were only ever infested by one *P. xylostella* larvae. As a result changes in seed set per fruit due to *P. xylostella* larval infestation will be similar among all fruit with larval damage and can be generalized across populations regardless of differences between population infestation rates of *P. xylostella* eggs. By using the average number of seeds per damaged and undamaged fruit, total number of fruit per plant, and proportion of fruit damaged per plant we determined

that the average seed production per infested *B. longii* over the study period was 295 ± 27 seeds per plant ((seed production/plant = (proportion of damaged fruit x 4.3 seeds) + (proportion of undamaged fruit x 10.8 seeds)). Since the expected seed production per plant in the absence of *P. xylostella* feeding (seed production /plant = number of fruit x 10.8 seeds) is estimated to be 412 ± 36 seeds, *P. xylostella* caused the seed production of *B. longii* to decrease by 29%.

Multiple generations of *P. xylostella* resulted in an increase in leaf and fruit damage. In 2004, *B. longii* and *B. fernaldii* individuals infested with *P. xylostella* eggs in both June and July, and in July only, had the same average July leaf (5%, 6%) and fruit (0%) damage; however, individuals with eggs in both months had a higher average August leaf (23%; $F_{1,101}$=1.48, p=0.226) and fruit (19%; $F_{1,101}$=1.19, p=0.277) damage than individuals with eggs in July only (17%, 16%). Although the increase was not statistically significant, the same pattern was seen on *B. longii* and *B. fernaldii* in 2005. In 2005, four sampled *B. longii* and two sampled *B. fernaldii* individuals were infested with three sets of *P. xylostella* eggs, one for each generation (Figure 2.2). These individuals had a higher average July leaf (19%) and fruit damage (35%) compared to those individuals with only one set (6%; 11%) and two sets (6%; 17%) of eggs. Statistical analysis was not completed because the number of plants infested with three sets of eggs was extremely small. The fruit damage in 2005 was higher than any other year and, for the first time, higher than the leaf damage (Figure 2.4).

Plant mortality rates: Intense larval feeding on infested plants not only decreased leaf biomass and seed productivity, but it also increased mortality. This is not evident in the year the plant dies (year t); in that year the number of *P. xylostella* eggs (χ^2=9.53, df=1, p=0.002, binomial distribution), leaf damage (χ^2=396.66, df=4, p<0.0001, binomial distribution), and fruit damage (χ^2=24.31, df=4, p<0.0001, binomial distribution) caused by larval feeding is significantly higher on plants that survived than plants that died. The pattern of survival and mortality was statistically similar for both *Braya* species (χ^2=0.03, df=1, p=8738, binomial distribution). However, *B. longii* and *B. fernaldii* that died had higher leaf damage (χ^2=14.86, df=4, p=0.005, binomial distribution) and fruit damage (χ^2=14.31, df=4, p=0.0064, binomial distribution) the years prior to their death (t-1, t-2, and t-3) than plants that survived (Figure 2.5). Individuals did not have higher densities of eggs (χ^2=0.42, df=4, p=0.5158, binomial distribution) the years prior to their death (t-1, t-2, and t-3) than plants that survived (Figure 2.5). The specific year of previous infestation was not significant (χ^2=1.61, df=2, p=0.4464, binomial distribution), meaning that results were the same one, two, and three years prior to death.

2.5. DISCUSSION

The conservation of threatened and endangered plant species requires constant effort in the face of mounting threats, such as habitat loss, invasive species, anthropogenic disturbance, and climate change (Noel, 2000; Thomson, 2005; Westoby and Burgman, 2006). This study suggests that agricultural pests should be added as a

potent threat. Documenting the ability of an agricultural pest to survive and reproduce on rare and endangered plant species suggests we must expand our understanding of the effect of agro-ecosystems on natural ecosystems. Our research illustrates that an agricultural insect pest, *P. xylostella,* can have negative effects on the survival and reproductive output of *B. longii* and *B. fernaldii,* two globally rare plant species. Although it is known that *P. xylostella* can survive on weeds in the Brassicaceae family (Talekar and Shelton, 1993), it has not previously been documented that *P. xylostella* can survive on rare Brassicaceae.

Three years of trapping data indicate that *P. xylostella* usually arrive at *Braya* sites in early July. Similarly across Canada, pheromone trapping indicates that these moths arrive in spring or early summer (Smith and Sears, 1982; Hopkinson and Soroka, 2010). Research into the wind trajectories that carry these insects north suggests that the first capture of moths in pheromone traps in Canada is linked to the influx of migrant moths from their overwintering sites (Smith and Sears, 1982; Hopkinson and Soroka, 2010). While it is not known from where the Newfoundland immigrants originated in the United States, *P. xylostella* immigrants in Saskatchewan and Manitoba are likely from southern Texas (Braun et al., 2004; Hopkinson and Soroka, 2010). Our research indicates that the damage to *B. longii* and *B. fernaldii* depends on the number of *P. xylostella* that arrive from overwintering sites, the timing of their arrival, and the success of their invasion. These three factors are dependent on the size of the migrant population, their success travelling north, and the climatic conditions of the area where they arrive (Smith and Sears, 1982; Hopkinson and Soroka, 2010).

In 2005, *P. xylostella* appeared in *Braya* habitats at least four weeks earlier than previously recorded. This had serious consequences on *B. longii* and *B. fernaldii* as it extended the developmental time of *P. xylostella*, allowing them to survive for three generations. Research in Svalbard, Norway, where *P. xylostella* are also immigrants, suggests that climate change may cause the air masses that transport them north to occur earlier and more frequently (Coulson et al., 2002). Climate change may also be responsible for the recent ability of various Lepidoptera, including *P. xylostella,* to travel on wind currents to islands in the sub-Antarctic and southern South America where they feed on native species (Convey, 2005). The impact of *P. xylostella* on these native species is unknown. While climate change will affect wind currents and thereby the dispersal of these and other widely distributed insects, it may also affect both the source populations and the ecosystems to which these insects immigrate (Kiritani, 2006).

Liu et al. (2002) reported that the survival rate of *P. xylostella* from egg to adult was the highest (80%) at 14°C and dropped dramatically at 10°C (19.2%). Higher air temperatures (12.4°C in June to 16.6°C in August) and an earlier arrival of *P. xylostella* in the summer of 2005 led to a shorter development time for eggs and larvae and a longer developmental period, which facilitated three *P. xylostella* generations, compared to two generations in 2004, and a single generation in 2003. The air temperature was consistently higher in *B. longii* populations than in *B. fernaldii* populations, as was the infestation rate and damage of *P. xylostella*. The 30-year normal for 1971 to 2000 (7.9°C in June to 13.1°C in August in Flower's Cove) (Environment Canada, 2005) and previous climatic studies (Donato, 2005) suggest that the historical summer climatic regime on the

limestone barrens was not ideal for *P. xylostella* survival because of low temperatures and high precipitation. However, recent data show that changing climatic regimes on the Great Northern Peninsula may lead to an overall increase of approximately 4°C in the mean annual air temperature by the 2080's (Slater, 2005).

Reduced rainfall in 2004 compared to 2003 may also have contributed to the success of *P. xylostella* in 2004. Heavy rain is a major source of mortality for *P. xylostella* because the larvae are easily displaced from leaves by raindrops (Kobori and Amano, 2003). This effect is so pronounced that some sectors of the agricultural industry use an irrigation system that mimics rainfall to increase the mortality rate of *P. xylostella* larvae (Waklsaka et al., 1991). Continued lower-than-normal summer rainfall will lead to a marked reduction in mortality of *P. xylostella* eggs and larvae. This is expected to have a detrimental effect on *B. longii* and *B. fernaldii*, through higher levels of leaf and fruit damage and possibly higher mortality rates. Potential climatic changes in temperature and rainfall may further increase the ability of *P. xylostella* to reproduce and survive for multiple generations within a growing season (Shelton et al., 1996). Multiple generations of *P. xylostella* pose a threat to seed production, and therefore, possibly the population viability of *B. longii* and *B. fernaldii*.

Unlike damage to agricultural crops, damage to *B. longii* and *B. fernaldii* is a threat to their long-term persistence. Death of adult plants is linked to previous leaf and fruit damage, presumably because *P. xylostella* feeding weakens the plant so that death is more probable in subsequent years. Death is not linked to previous densities of *P. xylostella* eggs, most probably because on some plants eggs do not hatch, larvae do not

survive to feed, or larvae move to another plant, resulting in no damage to the originally infested plant. The reason that *P. xylostella* infestation and damage is not high in the year the plant dies is that previously-damaged plants die early in the growing season prior to the current year's moth infestation and damage. *P. xylostella* larvae also consistently feed on *Braya* reproductive structures, deceasing seed production. The loss of seeds is a threat to the long-lived seed banks of *B. longii* and *B. fernaldii* have (Hermanutz et al., 2002) as large plants contribute disproportionately to the seed bank and suffer a higher mortality rate. Examination of the fruit of *B. longii* revealed that the fruit usually are infested with larvae and this infestation substantially decreases the seed set. Multiple generations pose an increased threat to seed production as later generations of larvae are present during seed development and therefore increase fruit damage and seed loss. The effect of *P. xylostella* on *B. longii* and *B. fernaldii* should be incorporated into future predictions of their population size and extinction probability, such as those made with population viability analysis. Such an analysis would help determine whether the impact of *P. xylostella* infestation on *B. longii* and *B. fernaldii* survival and seed production needs to be reduced using methods such as mass trapping of moths using pheromone baited traps (Silverstein, 1981).

B. longii and *B. fernaldii* are not the only rare Brassicaceae species colonised and damaged by *P. xylostella* in Newfoundland. The provincial government of Newfoundland and Labrador has designated *Neotorularia humilis* ((C.A. Mey) Hedge and J. Léonard) (low northern rockcress) (Brassicaceae) as endangered because it is globally uncommon (G4) and provincially rare (S1) (Tilley et al., 2005). In 2005 *P.*

xylostella eggs were observed on a few *N. humilis* individuals and subsequently *P. xylostella* was identified as a potential threat in the Low Northern Rockcress Recovery Strategy (Tilley et al., 2005). This infestation provides further evidence that rare Brassicaceae species worldwide may be threatened by *P. xylostella.* There are 56 rare Brassicaceae species in Canada (Canadian Endangered Species Conservation Council, 2006) and since *P. xylostella* is now able to disperse to every continent (Talekar and Shelton, 1993; Convey, 2005), any Brassicaceae species could be at risk. Agricultural pests, both native and non-native, are supported in enormous quantities on agricultural host plants; however few studies have investigated the ability and success of these abundant insects to negatively affect host plants in natural ecosystems. In cases such as *B. longii* where the host plant is a species at risk of extinction, infestation by an agricultural pest is not a matter of loss of yield or money, but a loss of species diversity; hence, there is an urgent need for the conservation and agricultural communities to cooperate in mitigating the impacts of these pests on native biodiversity.

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Table 2.1. A comparison of the average monthly air temperature (°C) in *Braya longii* and *B. fernaldii* populations over the study period.

Species	Month /Year	2003 (± SE)	2004 (± SE)	2005 (± SE)
B. longii	June	9.94 ± 0.45	6.72 ± 0.21	12.40 ± 0.13
	July	9.36 ± 0.20	13.19 ± 0.23	15.67 ± 0.11
	August	No data	16.56 ± 0.15	16.65 ± 0.10
B. fernaldii	June	8.53 ± 0.23	6.34 ± 0.21	8.13 ± 0.18
	July	8.18 ± 0.19	12.37 ± 0.22	12.14 ± 0.17
	August	No data	15.92 ± 0.18	14.01 ± 0.20

Figure 2.1. Population trends of *Plutella xylostella* on A) *Braya longii* and B) *B. fernaldii* populations in each study year. Adult numbers were determined from pheromone trap captures and eggs and larvae were counted on individually tagged plants. Standard error bars are not shown because standard error was always less than 0.1.

Figure 2.2. The average percentage (± SE) of tagged A) *Braya longii* and B) *B. fernaldii* individuals infested with eggs during each *Plutella xylostella* generation in each year. There was only one *P. xylostella* generation in 2003 and only two *P. xylostella* generations in 2004.

Figure 2.3. The percentage (± SE) of all sampled *Braya longii* reproductive structures infested with, or damaged by, *Plutella xylostella* larvae in 2003, 2004, and 2005.

Figure 2.4. A comparison of the average percentage (±SE) of leaves and fruit damaged by *Plutella xylostella* feeding on infested A) *Braya longii* and B) *B. fernaldii* individuals in each year of the study. (Damage scale: 0% = no damage, 12.5% ≤25% damage, 37.5% = 26%-50% damage, 62.5% = 51%-75% damage, and 87.5% ≥76% damage).

Figure 2.5. The average number (± SE) of *Plutella xylostella* eggs, larval caused leaf damage, and larval caused fruit damage on A) *Braya longii* and B) *B. fernaldii* in a given year (year t), one year prior (year t-1), two years prior (year t-2), and three years prior (year t-3) to the survival or death of an individual.

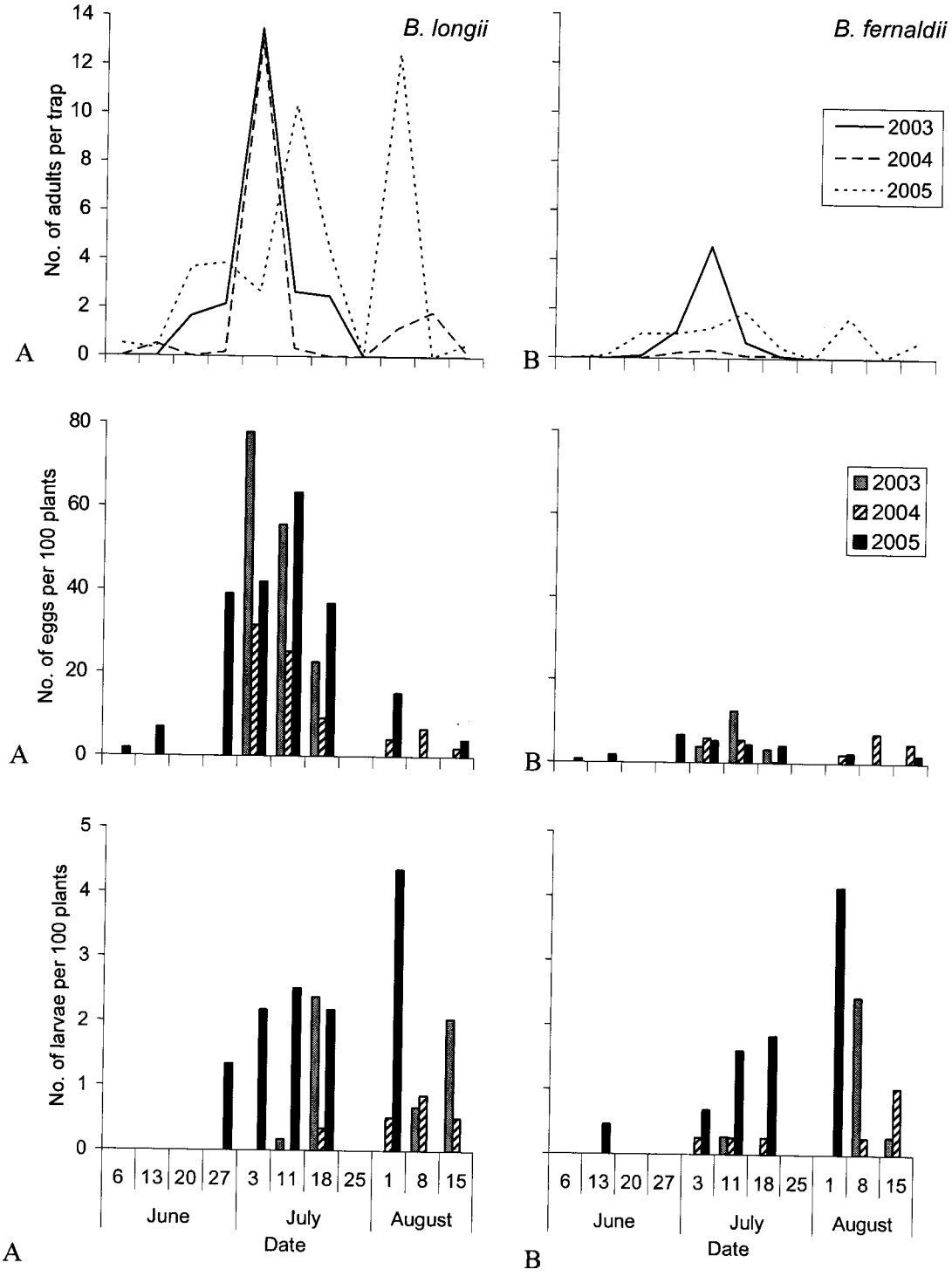
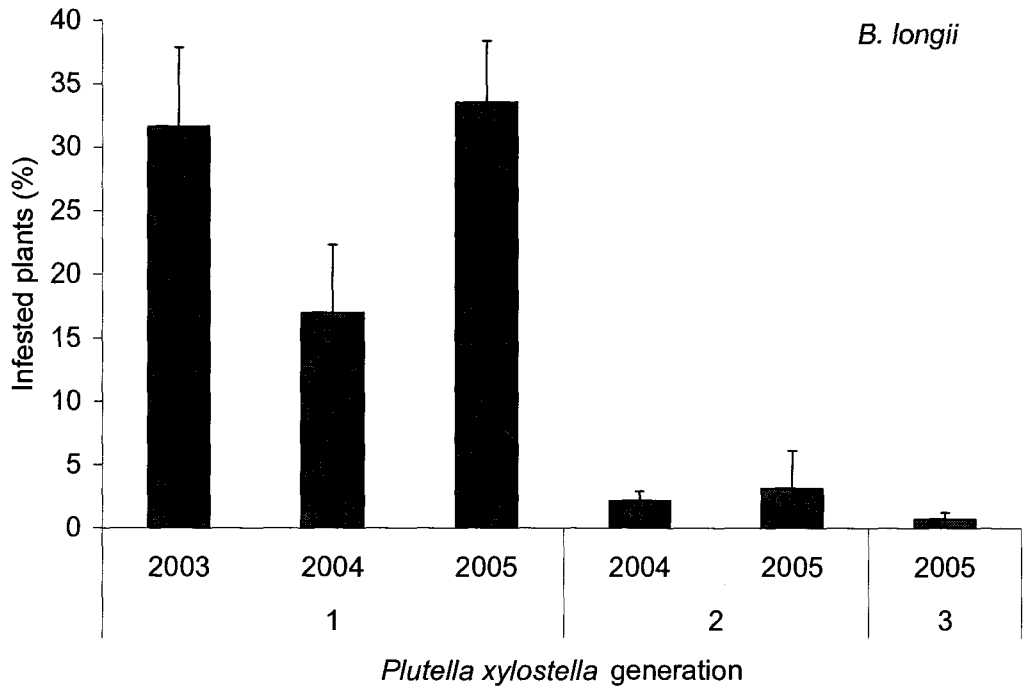
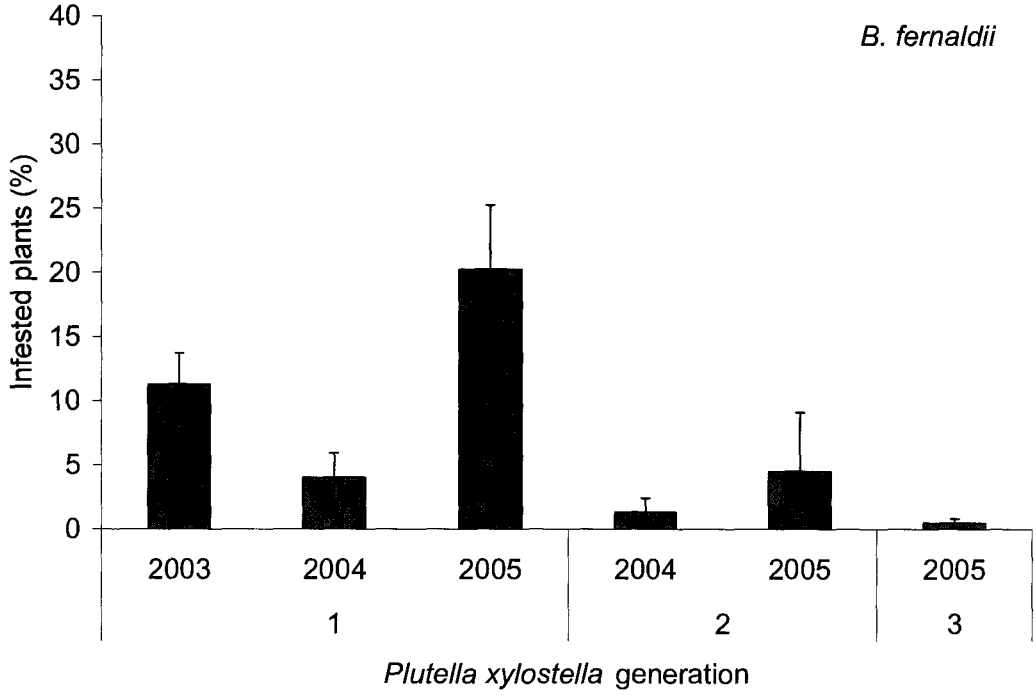

B. longii
B. fernaldii
No. of adults per trap
2003
2004
2005
A
B
No. of eggs per 100 plants
No. of larvae per 100 plants
6 13 20 27 3 11 18 25 1 8 15
June
July
August
Date

Figure 2.1.


B. longii
Infested plants (%)
40
35
30
25
20
15
10
5
0
2003
2004
2005
2004
2005
2005
1
2
3
Plutella xylostella generation

A


B. fernaldii
Infested plants (%)
40
35
30
25
20
15
10
5
0
2003
2004
2005
2004
2005
2005
1
2
3
Plutella xylostella generation

B

Figure 2.2.

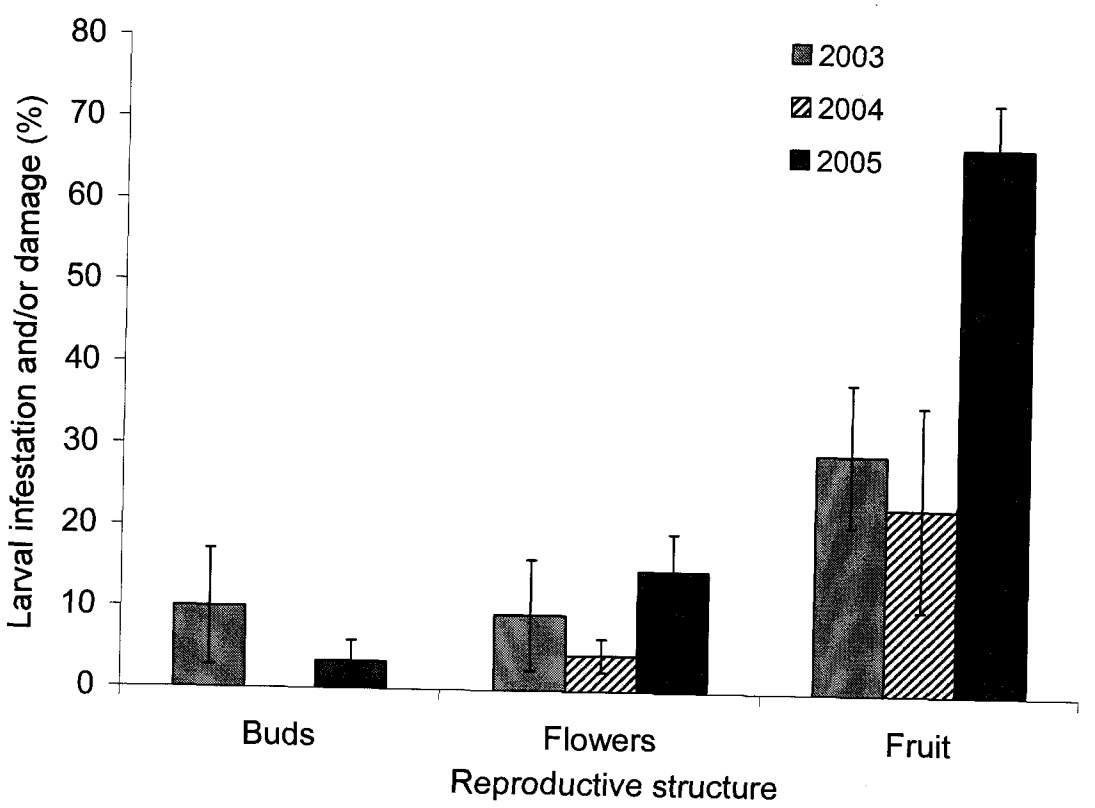
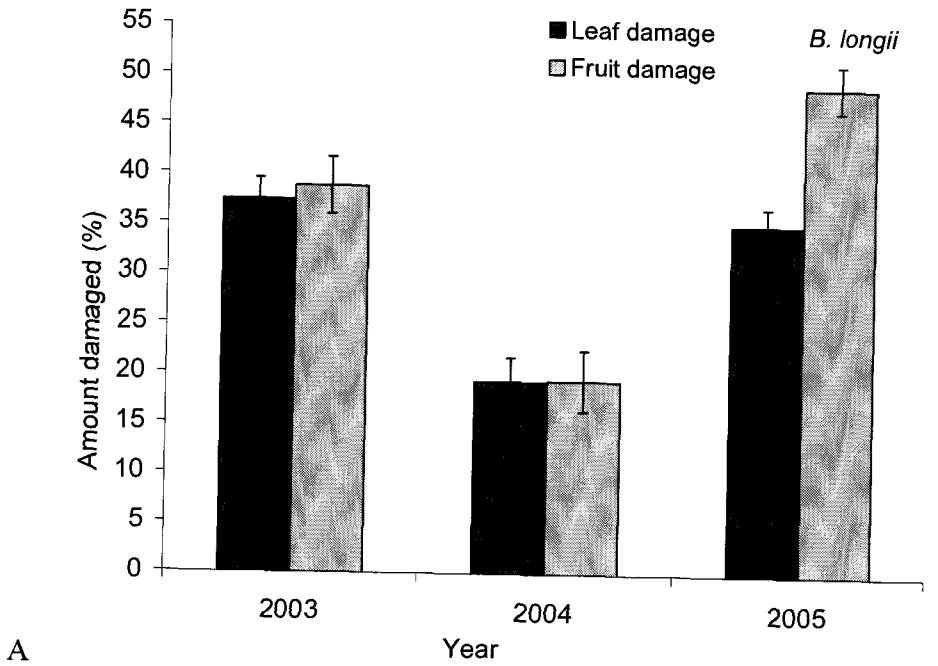
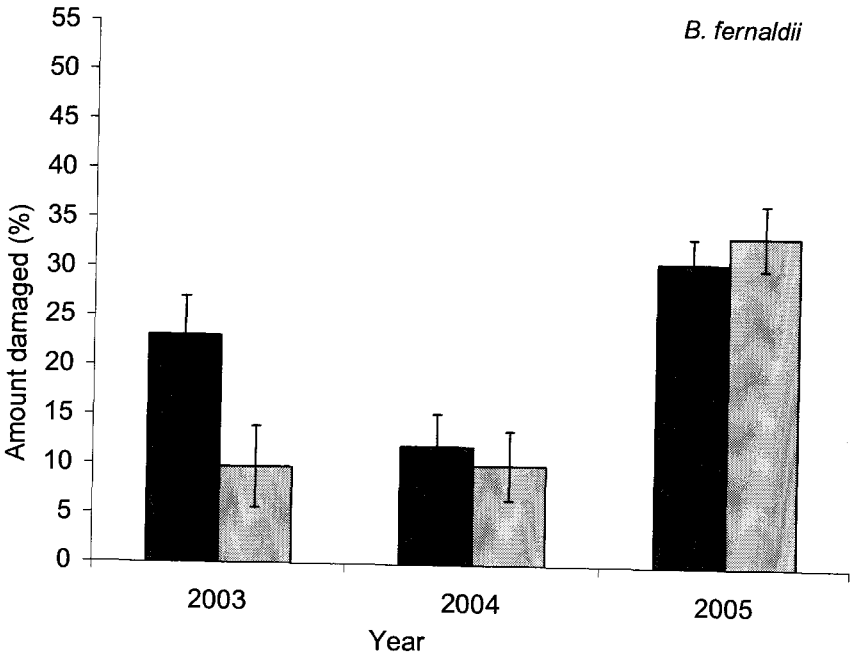

80
70
60
50
40
30
20
10
0
Larval infestation and/or damage (%)
2003
2004
2005
Buds
Flowers
Fruit
Reproductive structure

Figure 2.3.


Leaf damage
Fruit damage
B. longii
Amount damaged (%)
55
50
45
40
35
30
25
20
15
10
5
0
2003
2004
2005
Year

A


B. fernaldii
Amount damaged (%)
55
50
45
40
35
30
25
20
15
10
5
0
2003
2004
2005
Year

B

Figure 2.4.

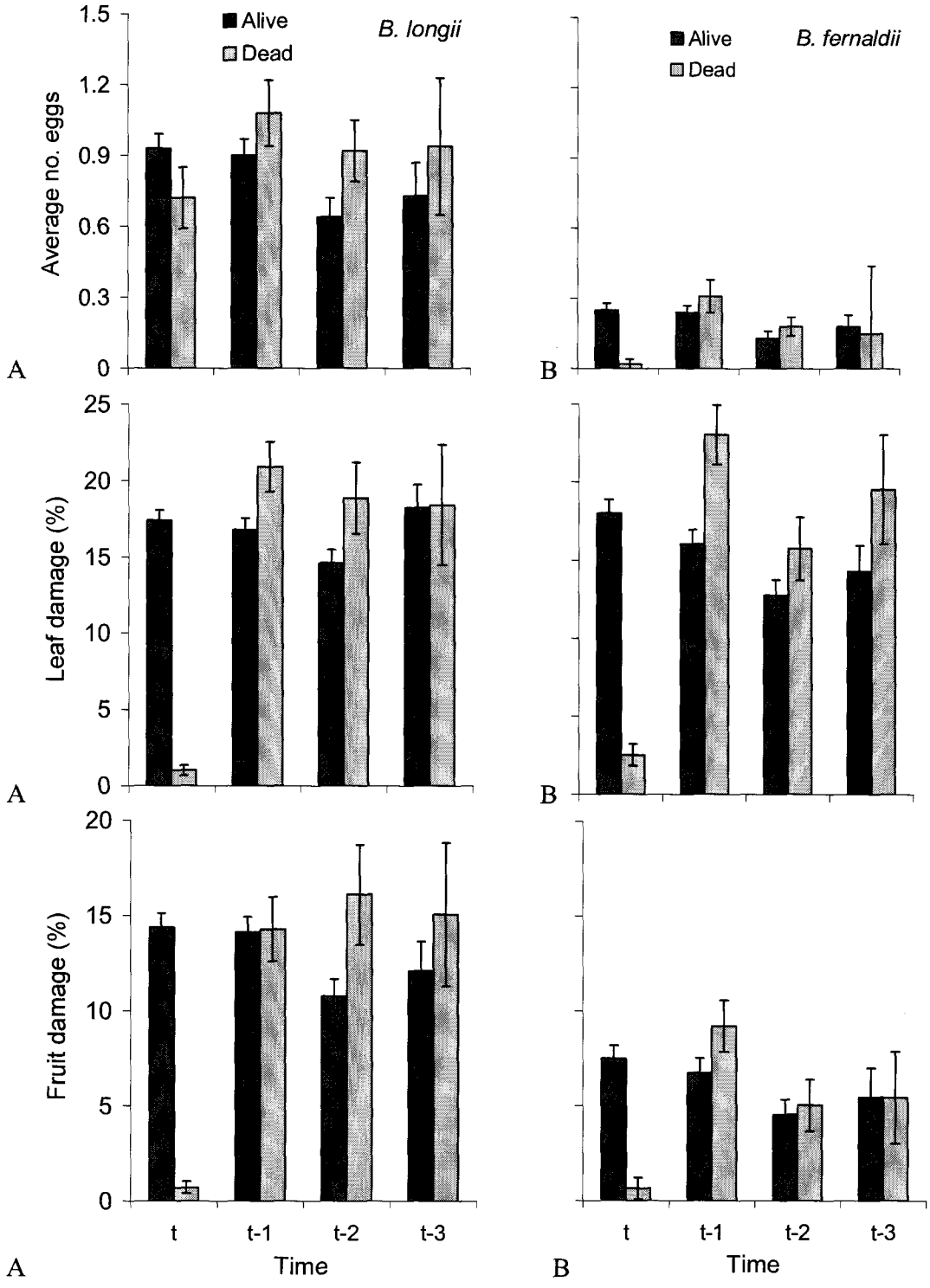

Alive
Dead
B. longii
B. fernaldii
Average no. eggs
Leaf damage (%)
Fruit damage (%)
A
B
Time
t
t-1
t-2
t-3

Figure 2.5.

CHAPTER THREE

GLOBAL AGRICULTURAL PEST CAN FIND ENDANGERED PLANTS WITHIN NATIVE HABITATS

3.1. ABSTRACT

Agricultural pests can harm rare plants growing outside the agro-ecosystem. In contrast to agro-ecosystems, these non-agricultural hosts grow at low densities in relatively species-rich ecosystems. *Plutella xylostella* (diamondback moth) (L.) is a highly problematic agricultural pest on Brassicaceae that can locate rare plants in their native ecosystems. This insect is highly dispersive and infests the rare Brassicaceae, *Braya longii* (Fernald) (Long's braya) and *B. fernaldii* (Abbe) (Fernald's braya). *B. longii* and *B. fernaldii* have been designated as endangered and threatened in Canada, respectively and are endemic to the limestone barrens on the island of Newfoundland. We evaluated the factors affecting infestation rates by monitoring infested and non-infested *Braya* within the entire ranges of both *Braya* species and by placing experimental cabbage transplants on *B. longii* sites. *P. xylostella* infested 30% of *B. longii* and 16% of *B. fernaldii* annually between 2003 and 2005. Flowering individuals, regardless of size, had a 2.8-fold higher probability of *P. xylostella* infestation than non-flowering individuals. Infestation was positively associated with a higher percent cover of *Braya* and higher density of flowering *Braya*, supporting the resource concentration hypothesis, and was not affected by the presence of a common, agricultural host crop (cabbage). In contrast, the presence of native, non-host vegetation was not associated with a decrease in

P. xylostella infestation on *Braya*. *P. xylostella* may negatively impact the persistence of other rare members of this family worldwide, especially in ecosystems with little native vegetation cover, such as alpine tundra and deserts, because it can find and infest plants regardless of the presence of native vegetation and damage flowering rather than non-flowering plants.

3.2. INTRODUCTION

Although agricultural insect pests can have catastrophic impacts on ecosystems, little is known about their consequently higher population densities impact on host species growing in natural ecosystems (Squires et al., 2009). In natural ecosystems, insect herbivory decreases when the host plant is small (Price,1991; Karban, 1997), does not have flowers (Karban, 1997), is in a small population (Colling and Matthies, 2004), or at a low density (Stanforth et al., 1997). Research in agro-ecosystems has attempted to determine the visual cues for host plant selection as a component of pest management. It has been suggested that highly vegetated "backgrounds" (Smith, 1969; Finch and Kienegger, 1997; Finch and Collier, 2000), sparse host plant density (Root, 1973; Risch et al., 1983; Yamamura and Yano, 1999), and the presence of preferred host species (Åsman, 2002; Shelton and Nault, 2004; Badenes-Perez et al., 2006) all decrease the ability of insects to find a host because the plant is less apparent.

Recently, agricultural insect pests have been recorded to negatively effect plants in natural ecosystems (McKone et al., 2001; Squires et al., 2009); however, the relative importance of the visual cues used by agricultural pests to locate non-crop host plants in natural ecosystems has not been addressed (Couty et al., 2006). To mitigate the impact of agricultural pests in natural ecosystems we must understand their ability to locate these host plants within natural vegetation communities. Communities which do not exhibit the characteristics typical of agro-ecosystem monocultures in which they thrive, including high host densities, low species diversity, large host size, and low host reproductive output. For rare host-plants, it maybe more important to understand visual cues than chemical cues as visual cues maybe able to be manipulated through restoration efforts (i.e.; lower plant density) to facilitate management of the pest and conservation of the rare host plant.

Eggs of *Plutella xylostella* (L.) (diamondback moth), a serious pest of cultivated Brassicaceae, were first observed on the endangered, *Braya longii* (Fernald) (Long's braya; Brassicaceae) and the threatened, *B. fernaldii* (Abbe) (Fernald's braya; Brassicaceae) in 1995 (Hermanutz et al., 2002). These two *Braya* species are endemic to the limestone barrens of the Great Northern Peninsula of Newfoundland (Canada). Due to very limited agricultural production on the Northern Peninsula, typical host plants such as *Brassica oleracea* var. *capitata* (L.) (cabbage), occur only in small patches associated with home gardens. Thus when *P. xylostella* arrive annually by wind dispersal from the south because they are unable to overwinter due to low winter temperatures, they oviposit and develop on rare, native, and non-agricultural Brassicaceae (Squires et al., 2009).

The two congeneric *Braya* species have similar but not identical morphologies and life histories (Parsons and Hermanutz, 2006). Mature *B. longii* individuals are taller than *B. fernaldii* (1-10 cm and 1-7 cm respectively), have longer petals than *B. fernaldii* (4-5 mm and 2-4 mm respectively; Parsons and Hermanutz, 2006), and seed weights approximately 2.5 times heavier than *B. fernaldii* (Hermanutz et al., 2002). Both species are endemic to this small area of Newfoundland, and therefore, the presence of *P. xylostella* poses a very serious threat to their survival and reproductive output (Squires et al., 2009). Host plant recognition cues recorded from natural and agricultural ecosystems will be important in determining how *P. xylostella* locates these very rare plants in their natural ecosystem and will be crucial in developing management strategies that will minimise the impact of *P. xylostella* on *Braya*.

In agro-ecosystems *P. xylostella* locates its host plants through a combination of olfactory visual cues (Bernays and Chapman, 1994; Badenes-Perez et al., 2004). For *P. xylostella* and other Brassicaceae specialists, glucosinolates are the main chemical attractant for host plant recognition and oviposition (Talekar and Shelton, 1993). *P. xylostella* is capable of pre-alighting recognition behaviour, such that it can recognise a preferred host plant based on olfactory cues while in flight (Bukovinszky et al., 2005). However, odours such as those released by glucosinolates alone do not lead insects directly to a host plant; visual cues also play an important role (Bernays and Chapman, 1994). Couty et al. (2006) found that when a host plant was hidden (i.e., absence of visual cues) the number of *P. xylostella* landing on non-host plants significantly increased. Given the highly dispersive ability of *P. xylostella* (Harcourt, 1986; Talekar and Shelton,

1993), its considerable reproductive potential (Sarfraz et al., 2006), and its ability to survive on both common (Talekar and Shelton, 1993) and rare Brassicaceae (Squires et al., 2009), it is probable that *P. xylostella* is negatively affecting natural ecosystems globally, but its impact has not been assessed.

The objectives of this study were to determine whether the following visual cues influence the level of infestation of *P. xylostella* on *B. longii* and *B. fernaldii* in their native ecosystem: (1) host plant population size, (2) host plant size, (3) presence of reproductive structures on host plants, (4) host plant abundance and density, (5) apparency as a function of non-host plant abundance, and (6) presence of cultivated Brassicaceae. These visual cues are known to attract insect herbivores in natural ecosystems and *P. xylostella* in agro-ecosystems. Globally it is not known how many rare Brassicaceae are or have been hosts for *P. xylostella.* By determining the visual cues *P. xylostella* uses in recognizing *Braya* species as hosts, conservation biologists can evaluate the extent to which other rare members of the Brassicaceae and other ecosystems with similar characteristics might be at risk from *P. xylostella* infestation and damage.

3.3. MATERIALS AND METHODS

3.3.1. Study populations

The limestone barrens of Newfoundland are characterized by a cool, wet, and windy climatic regime that supports tundra-like vegetation (Banfield and Jacobs, 1998). In undisturbed habitat, *Braya* exploit gaps in the slow-growing vegetation produced by

small-scale disturbances, such as frost action and wind and soil erosion (Noel, 2000). Anthropogenically disturbed habitat (generated by quarrying and road building) contains only homogenous gravel with no patterned ground, and has lower plant species diversity than undisturbed habitat (Greene, 2002; Rafuse, 2005), making it more ecologically similar to conventional agricultural systems than undisturbed habitat.

The cues that *P. xylostella* use to find *Braya* were studied over three growing seasons in six *B. longii* (Figure 1.1; Sandy Cove Crusher anthropogenic and natural, Sandy Cove Airstrip anthropogenic and natural, Sandy Cove Lion's Club anthropogenic, and Yankee Point anthropogenic) and four *B. fernaldii* study populations (Figure 1.1; Port au Choix Natural Historic Site, Anchor Point, Cape Norman, and Burnt Cape Ecological Reserve) between 2003 and 2005. These populations are geographically separated, span the entire ranges of both *Braya* species (190 km distribution of *B. fernaldii*; 25 km of *B. longii*), and contain areas of undisturbed native and/or anthropogenically disturbed habitat. Approximately 100 plants from all ages, reproductive stages (flowering and non-flowering), and sizes in each of the six *B. longii* (2003, n = 525; 2004, n = 506; 2005, n = 542) and four *B. fernaldii* (2003, n = 346; 2004, n = 372; 2005, n = 401) populations were individually tagged using a nail, flagging tape, and a uniquely numbered aluminum tag and was monitored weekly from June to August in each year for the presence of *P. xylostella.*

3.3.2. *Braya* population size

To determine whether host plant population size influences the level of *P. xylostella* infestation on *Braya*, the percentage of plants infested with *P. xylostella* eggs, recorded from individually tagged *Braya,* was compared to the total number of flowering plants in each population and the total population size. The total number of flowering plants in each *Braya* population was counted between 1998 and 2000, and again in 2008 (Hermanutz et al., 2002, Hermanutz et al., 2009). Due to the small size of non-flowering *Braya*, only flowering plants can reliably be seen and counted in a general survey. The total population size (flowering and non-flowering plants) was estimated from permanent plots assessed in 2008, in which the number of plants in each reproductive stage was recorded. The proportion of flowering plants in the permanent plots was compared with the total number of flowering plants per population and then multiplied by the number of non-flowering plants in the permanent plots to generate an estimate of the total number of non-flowering plants in each population. This resultant number added to the original 2008 population count of flowering plants is an estimate of the total population size.

3.3.3. *Braya* size and reproductive output

To determine if the size or reproductive output of *Braya* affects the ability of *P. xylostella* to infest *Braya*, the presence of *P. xylostella* eggs on the individually tagged plants were compared among size and reproductive stages. To obtain measurements of size and reproductive output, the basal diameter, longest leaf, and longest fruiting stalk were measured and the numbers of stalks and numbers of flowers and fruits per stalk

were counted at the end of the growing season in August on each individually tagged plant. The basal diameter of the plant or width at the caudex and the length of the longest leaf were measured with digital callipers to the nearest hundredth of a millimetre. The number of flowers and fruit were counted on one randomly chosen flowering stalk per individual. The number of flowers was counted by summing the number of fruit and the number of unfertilized flowers, which was obvious due to the presence of empty pedicles.

3.3.4. *Braya* and non-host abundance and density

To determine if *Braya* abundance, *Braya* density, or natural, non-host plant vegetation cover affects *P. xylostella* infestation rates among populations, digital pictures were taken of each individually tagged plant and its surrounding vegetation in 2004. A 30 cm x 30 cm plot was positioned over each tagged *Braya*, such that the plant was in the centre and a picture was taken 1m above the ground parallel to the surface (Figure 3.1). The pictures were analysed by placing a digital grid (24 x 24), with each block measuring 0.6 cm, over the picture and recording the presence or absence of non-host plant vegetation and *Braya* at each cross-hair to determine percent cover of each category. There were a total of 529 cross-hairs per grid. In addition, the numbers of *Braya* in each reproductive stage (single and multiple rosettes of leaves and single and multiple flowering stalks) were counted to determine *Braya* density.

3.3.5. Presence of cultivated Brassicaceae in local vegetable gardens

To determine if the presence of a cultivated Brassicaceae, which are known to attract *P. xylostella* in agro-ecosystems, influence the level of *P. xylostella* infestation on *Braya*, vegetable gardens containing cabbage between *Braya* populations were monitored in 2003, 2004, and 2005. The types of crops growing in each garden, the date cabbage were transplanted, and the use of insecticides was recorded. The distance between each garden and the nearest *Braya* population was determined using a global positioning system (GPS). The arrival of *P. xylostella* moths onto *Braya* habitat was compared to their arrival in the gardens using 19 Phero Tech delta pheromone traps, which were set up the first week of June in each year, throughout the 10 *Braya* study populations and two gardens growing cabbage. Once a month the number of *P. xylostella* eggs, larvae, and pupae present on individually tagged *Braya* in their natural habitat and 10 cabbage transplants in each garden were counted.

The *P. xylostella* pheromone lure used in traps is highly specific (Chisholm et al., 1983) and widely used in integrated pest management programs to monitor the presence of these moths (Baker et al., 1982; Walker et al., 2003). Based on the area from which traps theoretically attract male moths, two traps were placed in all populations except very small ones (i.e., <30 m width or length), such as the gardens, where one trap was used. Traps were set in the same location each year at a height of 0.3 m above the ground (Baker et al.,1982; Kmec and Weiss, 1997) and a minimum distance of 30 m apart. Traps were checked weekly from June until August for the number of new moths. Pheromone lures were stored in a freezer to minimize degradation until they were placed in the traps

and new pheromone lures were used each year. To maximize trap efficiency, the sticky inserts were changed whenever the number of insects trapped reached 50 or there was a significant amount of debris present on the insert.

3.3.6. Presence of cultivated Brassicaceae (cabbage) in *Braya* populations

To evaluate whether *P. xylostella* might prefer cultivated Brassicaceae to *Braya*, eight cabbage transplants were placed in each of four *B. longii* populations in 2005. The transplants (six to eight leaves each) were purchased locally from the same source local communities used for their gardens and were grown from seed in the spring of 2005. The transplants were placed in 15 cm plastic pots with commercial topsoil (pro-mix), and the pots were buried in the ground so that the top of each pot was level with the substrate surface. Limestone rocks were placed over the edge of the pots to mimic the natural limestone substrate and to keep the pots in position. Transplants in pots were placed at equal distance throughout the distribution of *Braya* in each population on June 7 and the number of *P. xylostella* adults, eggs, larvae, and pupae were counted monthly.

3.3.7. Statistical analysis

Linear regressions were completed to determine if an increase in the percentage of plants infested with *P. xylostella* per population coincided with an increase in the size of *Braya* populations, as recorded from the 1998 to 2000 and 2008 surveys of flowering plants, and a 2008 estimate of the total population size.

To determine whether there were differences in any of the growth or reproductive measurements (i.e., basal diameter, longest leaf, longest flowering stalk, number of flowering stalks or numbers of flowers per stalk) among years, disturbance regimes, and between plants infested and not infested with *P. xylostella* eggs, analyses of variance (ANOVAs) were completed using MINITAB (version 13 for Windows) with alpha set at 0.05. ANOVAs were used to determine if the number of adult moths trapped in pheromone traps differed significantly between *Braya* species and local vegetable gardens, and among years. The assumptions of normality and homogeneity outlined by the general linear model were tested to ensure analyses with this model were appropriate. Interaction terms were included in all models.

If the assumptions were not met or the response variable was binomial, logistic regressions were completed with SAS (version 9.1 for Windows) using GENMOD, as outlined by the generalized linear model. Logistic regressions were used to test the effects of the percent cover of *Braya,* the total density of *Braya,* the density of multiple, flowering *Braya*, and reproductive stage on the presence and absence of *P. xylostella* eggs on *Braya*. Similar analyses were also completed to determine whether there were significant differences in the presence and absence of *P. xylostella* eggs on *Braya* (binomial response variable) between species and disturbance regimes (anthropogenically disturbed or undisturbed), among populations and reproductive stages, and the percent cover of natural vegetation. Logistic regressions were also used to determine whether or not there were significant differences in the presence or absence of *P. xylostella* larvae

between *Braya* and cabbage in gardens and among years and between *Braya* and cabbage transplants placed in *Braya* populations. Interaction terms were included in all models.

3.4. RESULTS

3.4.1. *Braya* population size

Infestation levels of *P. xylostella* on both *Braya* species did not increase with an increase in the *Braya* population size (both flowering and total number of individuals; Figure 3.2). The total number of *Braya* (non-flowering and flowering plants) ranged from 393 to 136,133 *Braya* per population. While the five most heavily infested study populations had an average population size that was larger than the five least infested study populations (28,371 ± 26,942 and 1,704 ± 807 respectively), the increase in *P. xylostella* infestation was not related to an increase in *Braya* population size (R^2adjusted = 0.0%, $F_{1,8}$=0.650, p=0.443). Of the five populations growing on anthropogenically disturbed habitat, four were among the five most infested populations and three were among the five largest populations. The total number of flowering plants in each *Braya* study population ranged from 150 to 2,400 during the first census (Figure 3.2a; Hermanutz et al., 2002) and 54 to 3,324 during the second census (Figure 3.2b; Hermanutz et al., 2009). While the five most heavily infested study populations had an average flowering population size of 3.1 fold (1998-2000 census) and 4.3 fold (2008 census) larger than the five least infested study populations (Figure 3.2a,b), the increase in *P. xylostella* infestation was not related to an increase in the flowering *Braya*

population size (1998-2000 census: R^2adjusted = 6.6%, $F_{1,8}$=1.64, p=0.237 and 2008 census: R^2adjusted = 0.0%, $F_{1,8}$=0.996 p=0.348).

3.4.2. *Braya* size and reproductive output

Individuals infested with *P. xylostella* eggs were significantly larger (basal diameter, longer leaves and flowering stalks), and more reproductive (more flowering stalks and flowers per stalk) than individuals with no eggs (Table 3.1). The presence of flowers was more important than the size of the plants in determining *P. xylostella* infestation rate. Flowering plants, including small individuals with only one rosette of leaves, were infested 2.8 times more often than non-flowering plants of all sizes (Figure 3.3). Flowering plants with multiple rosettes of leaves were infested with *P. xylostella* eggs more often than any other plant type (χ^2=257.27, df=3, p<0.0001, Poisson distribution; Figure 3.3).

Braya growing on anthropogenically disturbed habitat are the largest and produce the most flowering stalks of all individuals. *B. longii* and *B. fernaldii* growing on anthropogenically disturbed habitat had significantly longer leaves ($F_{1,1566}$=2.25, p=0.133 and $F_{1,1114}$=10.44, p=0.001 respectively), and flowering stalks ($F_{1,852}$=28.98, p<0.0001 and $F_{1,578}$=22.23, p<0.0001 respectively) and significantly more flowering stalks ($F_{1,12.52}$=12.82, p<0.0001 and $F_{1,577}$=2.02, p=0.94 respectively) and flowers per stalk ($F_{1,853}$=32.09, p<0.0001 and $F_{1,577}$=14.65, p<0.0001 respectively) than *Braya* growing on undisturbed habitat. *B. longii* and *B. fernaldii* growing on anthropogenically disturbed

habitat have a similar basal diameter to plants growing in undisturbed habitat ($F_{1,1568}$=1.91, p=0.168 and $F_{1,1114}$=0.86, p=0.383 respectively).

3.4.3. *Braya* and non-host vegetation cover and density

The percent cover of natural vegetation did not differ significantly between *Braya* infested or not infested with *P. xylostella*; however, infestation rates were positively associated with a higher percent cover of *Braya* and higher density of flowering *Braya.* For example, tagged *Braya* infested with *P. xylostella* eggs had, on average, 2.6 ± 0.4% of the sample plots (30 cm x 30 cm plot) covered with *Braya*, which is a significantly higher percent cover than the average percent cover (1.5 ± 0.3%) of *Braya* immediately surrounding tagged *Braya* not infested with *P. xylostella* eggs in 2004 (χ^2=21.07, df=1, p<0.0001, binomial distribution) (Figure 3.4a,b). The majority of infestations occur on *Braya* growing on anthropogenically disturbed habitat (Squires, Chapter 4), which had a significantly higher percent cover of *Braya* in the sample plots (3.33 ± 0.53%) than *Braya* growing on undisturbed habitat (1.85 ± 0.26%) (χ^2=4.77, df=1, p=0.0290, binomial distribution). The percent cover of natural vegetation around individually tagged *Braya* was similar whether *Braya* were infested with *P. xylostella* eggs (20.3 ± 2.1%) or not (20.7 ± 1.6%) (χ^2=1.09, df=1, p=0.2957, binomial distribution) (Figure 3.4c,d).

The density of *Braya* in the sample plot of a tagged *Braya* infested (2.23 ± 0.16) and not infested (2.01 ± 0.09) with *P. xylostella* eggs was not statistically different (χ^2=2.01, df=1, p=0.1564, binomial distribution), because infested and not infested *Braya* were surrounded by similar densities of single flowering, single non-flowering, and

multiple non-flowering plants. However, infested *Braya* were surrounded by significantly higher densities of multiple flowering plants (0.75 ± 0.07) than *Braya* that were not infested (0.53 ± 0.07) (χ^2=18.05, df=7, p=0.0117, binomial distribution). *Braya* growing on anthropogenically disturbed habitat had a significantly higher density of *Braya* in the sample plots (3.08 ± 0.14) than *Braya* growing on undisturbed habitat (1.27 ± 0.07) (χ^2=4.23, df=1, p=0.0398, binomial distribution) and a significantly higher density of multiple flowering plants in the sample plots (0.85 ± 0.06) than *Braya* growing on undisturbed habitat (0.32 ± 0.03) (χ^2=4.14, df=1, p=0.0418, binomial distribution).

3.4.4. Presence of cultivated Brassicaceae in local vegetable gardens

The presence of cabbage gardens did not affect the probability that *Braya* would be infested by *P. xylostella* because in each year of the study *P. xylostella* were caught in pheromone traps in *Braya* populations at least three weeks earlier than in pheromone traps in the gardens (Figure 3.5). Changes in the numbers of adults caught per trap followed similar patterns of highs and lows between *Braya* populations and the gardens (Figure 3.5). For most sampling dates, similar or higher numbers of *P. xylostella* were caught per pheromone trap in *Braya* populations than in gardens ($F_{1,48}$=6.79, p = 0.012).

In 2003, there were five gardens growing cabbage within the distribution of *B. longii* and they were on average 0.96 km away from any *B. longii* population. At the same time, there were four gardens growing cabbage within the distribution of *B. fernaldii* and they were on average 6.31 km from a *B. fernaldii* population. In 2004, four of these gardens were not replanted and one new garden was added to the study. In 2005

the same six gardens were monitored. Residents planted an average of 41 cabbage transplants per garden between May 25th and June 20th of 2003, 2004, and 2005. All but one garden contained a combination of *Brassica rapa* variety *rapa* (turnip), *Allium* species (onion), *Solanum tuberosum* (potato), *Beta vulgaris* (beet), and *Daucus carota* subspecies *sativus* (carrot). One resident used intercropping with *Allium* species to minimize insect herbivory on cabbage and another resident (2003 only) used a granular insecticide to control *Delia* species (root maggots).

The cabbage gardens supported significantly higher numbers of *P. xylostella* larvae and pupae per plant than *Braya*. In early August, the number of *P. xylostella* larvae per cabbage ranged from a high of 0.3 larvae per plant (24 larvae per 90 plants) in 2003 to a low of 0.05 larvae per plant (3 larvae per 60 plants) in 2004. At the same time, the number of *P. xylostella* larvae ranged from less than 0.05 larvae per *B. longii* in 2004 to 0.08 larvae per *B. longii* in 2005 and was always lower than 0.05 larvae per *B. fernaldii* (χ^2=24.63, df=1, p<0.0001, binomial distribution). With the exception of two pupae observed on *Braya* in August and September of 2003, pupae were not seen on *Braya*, but were found in cabbage gardens in each year of the study. On average there were 0.3 *P. xylostella* pupae per plant (24 pupae per 90 plants) in 2003 and 0.07 *P. xylostella* pupae per plant (4 pupae per 60 plants) in 2004 and 2005.

3.4.5. Presence of cultivated Brassicaceae (cabbage) in *Braya* populations

The presence of young cabbage transplants in *Braya* populations did not decrease the infestation probability of *P. xylostella* on *Braya*. Cabbage transplants placed in *B.*

longii populations showed no evidence of being infested by *P. xylostella* eggs or larvae on June 12th or July 3rd of 2005, while 45% of *B. longii* in those same populations in 2005 were infested with *P. xylostella* eggs. Evidence of *P. xylostella* infestation was observed in two of the four populations and of all 32 cabbage transplants only 28% were infested with *P. xylostella*. This was not statistically higher than the 12% larval infestation recorded on cabbage in the gardens in 2005 and the 15% larval infestation recorded in those same *B. longii* populations in 2005 (χ^2=4.02, df=2, p=0.1340, binomial distribution).

3.5. DISCUSSION

The plant characteristics that *P. xylostella* use as visual cues to find agricultural host plants are well known (Talekar and Shelton, 1993; Badenes-Perez et al., 2005a,b; 2006); however, whether or not the moth uses those same visual cues to find rare host plants within native plant communities has not been studied (Couty et al., 2006). Our research illustrates that the presence of flowers and size of *Braya* plants are more important than other visual cues known to attract *P. xylostella* in agro-ecosystems, such as sparse non-host vegetation and the presence of a typical host species. *P. xylostella* infestation increased in areas of high *Braya* density and percent cover, supporting similar studies (Root, 1973; Stanforth et al., 1997), but was not affected by the presence of native plant vegetation. Future studies need to consider these results within the context of olfactory cues *P. xylostella* use in host recognition and plant chemical defenses; however,

for the conservation of non-agricultural host-plants, it maybe more important to understand visual cues which can be manipulated *in situ* to facilitate recovery efforts.

B. longii and *B. fernaldii* infested by *P. xylostella* were most often the largest of all *Braya* individuals and more often flowering than non-flowering. Since the number of flowering stalks increases with increases in plant size, *P. xylostella* seem to select the largest, most reproductive plants. Small flowering plants had eggs more frequently than large non-flowering plants, indicating that presence of flowering structures is more important than plant size in the probability of infestation. In contrast, agricultural crops infested by *P. xylostella,* such as cabbage, have highly reduced flowers or are harvested before flowering occurs, (such as with *Brassica oleracea* var. *botrytis* (broccoli); Dixon, 2006), suggesting flowering is not a factor in the selection process of herbivores for many agricultural host plants. Since agricultural crops are bred to be genetically uniform, their size tends to be more similar than non-agricultural plant size (Dixon, 2006), suggesting plant size may be less important in the selection process of herbivores in agro-ecosystems than in the selection of wild host plants with a variety of sizes (Price, 1991).

Individual *Braya* plants infested with *P. xylostella* eggs had a higher percent cover of surrounding *Braya* plants and density of multiple flowering plants in the immediate surrounding area (30 cm x 30 cm plot) than plants that were not infested, but *Braya* population size did not affect *P. xylostella* infestation rate. This is consistent with known *P. xylostella* behaviour, where adult moths were recorded in higher densities in large patches of host plants than in small patches of host plants (Bukovinszky et al., 2005) and supports the resource concentration hypothesis (Root, 1973). *P. xylostella*

infestation was not affected by the presence of native plant vegetation because the native vegetation on the limestone barrens is naturally too sparse to disrupt the host-finding behaviour of *P. xylostella*.. Moths were found to thoroughly search one area for host plants, such that only plants in the immediate vicinity were infested because *P. xylostella* move little after landing (Bernays and Chapman, 1994). Flowering plants also contain higher concentrations of glucosinolates, the chemicals known to attract *P. xylostella* (Smallegange et al., 2007). This indicates that populations where the percent cover of *Braya* and density of multiple flowering plants is highest, such as those growing on anthropogenically disturbed habitat, will continue to support the highest infestation levels of *P. xylostella*. These populations therefore attract *P. xylostella,* and management options, such as reducing *Braya* densities while increasing their distribution within populations in an effort to mimic those populations found in natural conditions should be investigated (Squires, Chapter 4; Squires, Chapter 5).

The percent cover of natural vegetation was not different in the area surrounding infested and not infested *Braya*. While the presence of 'background' vegetation, such as clover, is known to decrease the infestation rate of *P. xylostella* in agro-ecosystems, it seems only effective when clover accounts for greater than 50% of the ground cover (Finch and Kienegger, 1997) or when the abundance of vegetation is such that the leaves of host and non-host vegetation overlap or intermingle (Couty et al., 2006). Only one *Braya* population had a percent cover of natural vegetation of greater than 50% suggesting that natural vegetation on the limestone barrens is too sparse to disrupt the host-finding behaviour of *P. xylostella*. These results do not support the common

observation that a lower plant apparency is a result of higher amounts of background vegetation (Smith, 1969), a trend that may be similar in ecosystems with relatively little vegetation cover, such as limestone outcrops, polar habitats, alpine tundra, and deserts.

Talekar and Shelton (1993) reported that in the absence of favoured cultivated hosts *P. xylostella* will maintain itself on Brassicaceae weeds, but our research indicates that rare Brassicaceae may be at risk even when favoured cultivated hosts are available. Cabbage is a typical host plant of *P. xylostella,* but a survey of all local vegetable gardens showed that the cabbage did not attract *P. xylostella* to the area. *P. xylostella* infested the cabbage in the gardens after they infested *Braya*, indicating that local vegetable gardens are not a threat and need not be removed as a management action. Further research using cabbage as a trap crop in areas where *Braya* were present showed that cabbage transplants had fewer *P. xylostella* larvae than *B. longii*. These results suggest that the possibility that cabbage can be used as a management tool to lure *P. xylostella* away from *Braya* is limited, but there may be merit in testing other cultivated Brassicaceae.

The reason for *P. xylostella*'s preference of *Braya* over cabbage in field trials is not clear; however, it may be due to one or a combination of chemical cues and leaf morphology that provides the cabbage with some resistant to *P. xylostella*. *Braya* may not have evolved the same profile of chemical glucosinolate deterrents produced by other members of the Brassicaceae family (Louda and Mole, 1991; Aliabadi and Whitman, 2001). Future studies need to determine the concentrations and combination of glucosinolates in both *Braya* species and compare those to typical agricultural host crops for which the profiles are known. Also, unlike the cabbage variety used, both *Braya*

species have a glossy leaf that is dark green in colour, two of the most important morphological characters found to increase host plant selection of agricultural crops by *P. xylostella* (Dixon, 2006; Sarfraz et al., 2006). A lack of leaf pubescence is also an important morphological character in host plant selection. *B. fernaldii* has pubescent leaves (Hermanutz et al., 2002), a deterrent to *P. xylostella* oviposition (Sarfraz et al., 2006; Løe et al., 2007), where as *B. longii* has glabrous leaves (Hermanutz et al., 2002). This may contribute to the lower infestation rate of *B. fernaldii* than *B. longii.*

Other jurisdictions have recorded *P. xylostella* infestation on native vegetation but infestation dynamics, host identity, and host impacts remain unknown (Convey, 2005). Rare Brassicaceae worldwide should be monitored for infestation by *P. xylostella* and other hyper abundant agricultural pests, especially those in areas where insects immigrate annually and typical host crops are not available to support the population, and in habitats with low vegetation cover. *B. longii* and *B. fernaldii* are two of five rare Brassicaceae known to be infested and damaged by *P. xylostella* on the island of Newfoundland (Canada). *Neotorularia humilis* ((C.A. Mey) Hedge and J. Léonard) (low northern rockcress) is endangered and was infested in 2005 with *P. xylostella* eggs (Tilley et al., 2005), *Arabis alpina* subspecies *alpine* (L.) (alpine rockcress) was infested in 2008 with both *P. xylostella* eggs and larvae, and *Draba incana* L. (hoary whitlowgrass) was infested in 2004 with *P. xylostella* larvae. Furthermore, eggs of another agricultural pest of Brassicaceae, *Pieris rapae* (L.) (cabbage white butterfly) have been recorded on large, flowering *B. longii* in sparse amounts (Squires, unpublished data).

P. xylostella cannot overwinter in Canada (Butts and McEwen, 1981) and therefore are adapted to the southern agro-ecosystems from which they originate. However, *P. xylostella* have retained their ability to find non-cultivated, wild host crops such as *Braya* species and appear to use the presence of flowers, a large plant size, and a high density of *Braya* in the immediate area as important visual cues for host selection. *P. xylostella* infestation is not affected by the *Braya* population size, the presence of a cultivated agricultural crop, or the amount of native background vegetation. While *P. xylostella*'s interaction with *Braya* does not affect their evolution (due to the inability to overwinter), this insect is acting as an agent of natural selection for the evolution of *Braya*. *P. xylostella* negatively impact the survival and reproductive output of *Braya* as they infest and damage large flowering rather than non-flowering plants (Squires et al., 2009). The impacts of *P. xylostella* and other agricultural pests should be studied on wild Brassicaceae globally to determine the severity and distribution of the negative impacts of agricultural pests on natural ecosystems, and their potential long-term selective impacts.

3.6. LITERATURE CITED

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Table 3.1. A comparison of the average size and reproductive ability (± SE) of tagged *Braya longii* and *B. fernaldii* individuals, with and without *Plutella xylostella* eggs.

Plant characteristic /	*B. longii*			*B. fernaldii*		
P. xylostella eggs	Yes	No	Significance	Yes	No	Significance
Basal diameter (mm)	18.0 ± 0.7	8.6 ± 0.2	$F_{1,1568}$=299.63, p<0.0001	18.3 ± 0.9	9.1 ± 0.3	$F_{1,1114}$=204.82, p<0.0001
Longest leaf (mm)	15.1 ± 0.3	10.9 ± 0.2	$F_{1,1568}$=161.42, p<0.0001	13.0 ± 0.4	9.1 ± 0.1	$F_{1,1114}$=141.57, p<0.0001
Longest stalk	51.2 ± 1.3	39.4 ± 0.9	$F_{1,852}$=40.38, p<0.0001	31.5 ± 1.3	19.5 ± 0.6	$F_{1,578}$=84.36, p<0.0001
No. of flowering stalks	4.4 ± 0.3	1.8 ± 0.1	$F_{1,852}$=71.55, p<0.0001	3.8 ± 0.3	1.9 ± 0.1	$F_{1,578}$=69.90, p<0.0001
No. of flowers/stalk	8.0 ± 0.2	6.5 ± 0.1	$F_{1,852}$=31.42, p<0.0001	8.4 ± 0.3	6.2 ± 0.2	$F_{1,578}$=43.50, p<0.0001

Figure 3.1. Images of 30 cm x 30 cm plots in a A) anthropogenically disturbed and B) undisturbed habitat used to determine the effect of *Braya* and natural vegetation abundance and *Braya* density on *Plutella xylostella* host finding ability.

Figure 3.2. A comparison of the percentage of plants infested by *Plutella xylostella* and the A) 1998-2000 and B) 2008 flowering plant population size of each *Braya* population. The data points for populations on anthropogenically disturbed habitat are circled.

Figure 3.3. The percentage of each *Braya longii* and *B. fernaldii* plant type infested with *P. xylostella* eggs over the study period (2003-2005). Plant types are SN- single rosette and non-flowering, SF- single rosette and flowering, MN- multiple rosettes and non-flowering and, MF- multiple rosettes and flowering.

Figure 3.4. The percent cover (± SE) of A) *Braya* and C) natural vegetation surrounding *Braya longii* infested and not infested with *Plutella xylostella* eggs in 2004 on undisturbed (N) and anthropogenically disturbed (A) populations (parts B and D refer to *B. fernaldii*). The study populations are ordered on the x-axis from lowest (left) to highest (right) percentage of plants infested.

Figure 3.5. The average number of *Plutella xylostella* adults per pheromone trap per week in A) 2003, B) 2004, and C) 2005 over the growing season in *Braya* populations and in cabbage gardens. (Note- no data were collected the week of July 25th in any year).

A

B

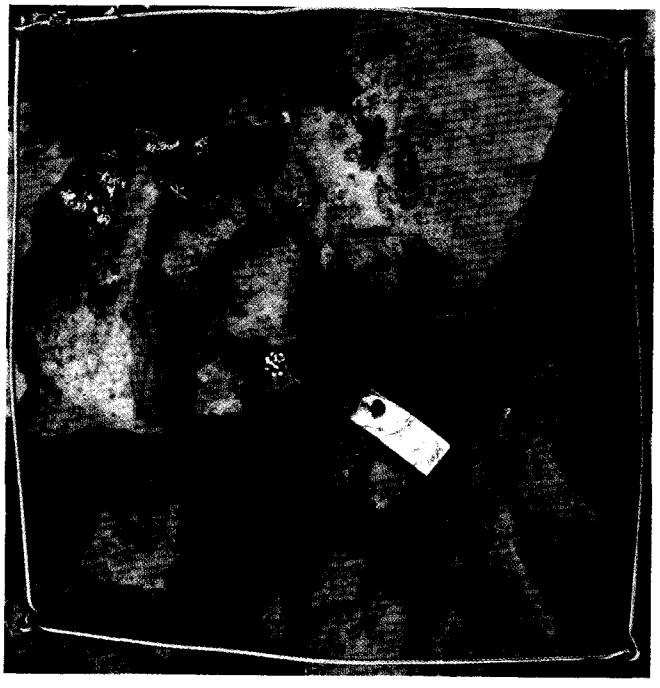
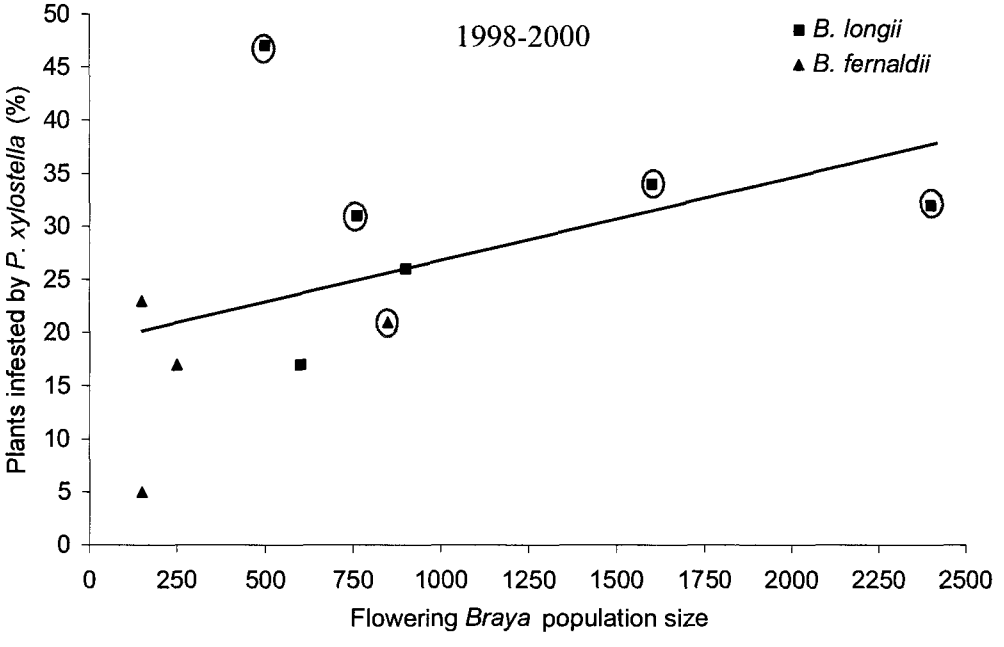
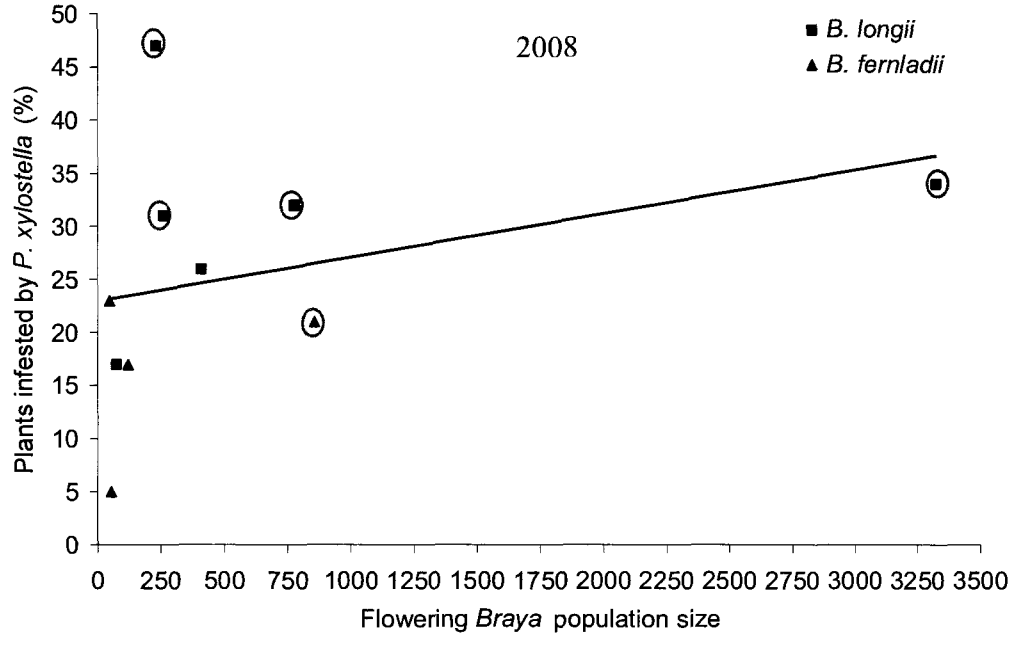


Figure 3.1.


1998-2000
B. longii
B. fernaldii
Plants infested by P. xylostella (%)
0
5
10
15
20
25
30
35
40
45
50
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
Flowering Braya population size

A


2008
B. longii
B. fernladii
Plants infested by P. xylostella (%)
0
5
10
15
20
25
30
35
40
45
50
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
3000
3250
3500
Flowering Braya population size

B

Figure 3.2.

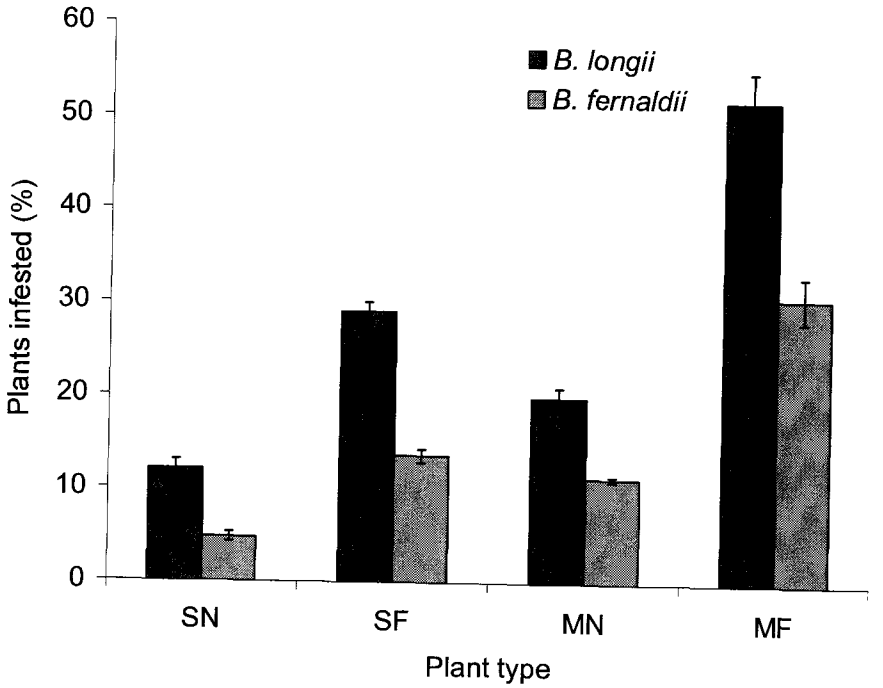

60
50
40
30
20
10
0
Plants infested (%)
B. longii
B. fernaldii
SN
SF
MN
MF
Plant type

Figure 3.3.

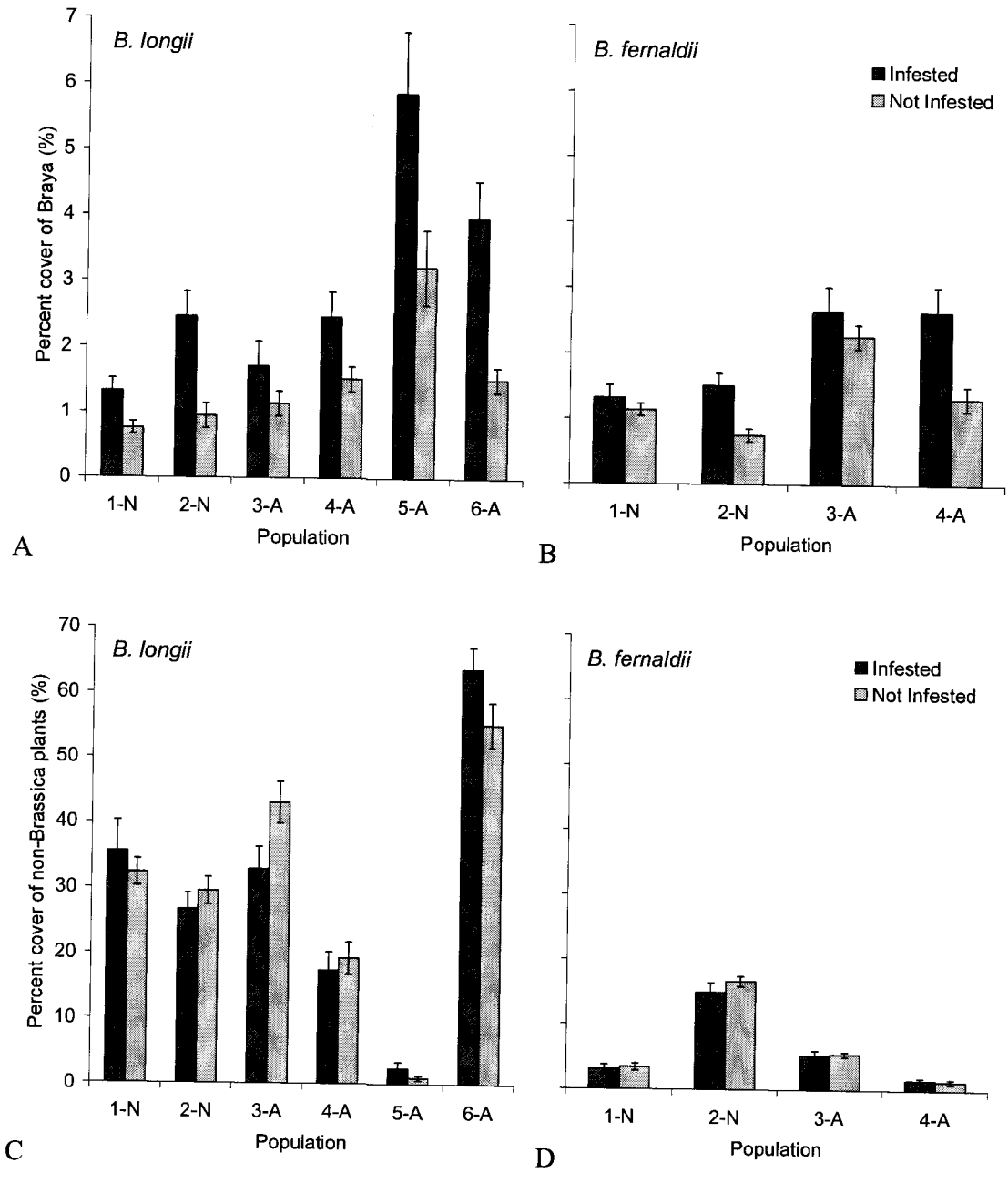

B. longii
Percent cover of Braya (%)
0
1
2
3
4
5
6
7
1-N
2-N
3-A
4-A
5-A
6-A
Population
A
B. fernaldii
Infested
Not Infested
1-N
2-N
3-A
4-A
Population
B
B. longii
Percent cover of non-Brassica plants (%)
0
10
20
30
40
50
60
70
1-N
2-N
3-A
4-A
5-A
6-A
Population
C
B. fernaldii
Infested
Not Infested
1-N
2-N
3-A
4-A
Population
D

Figure 3.4.

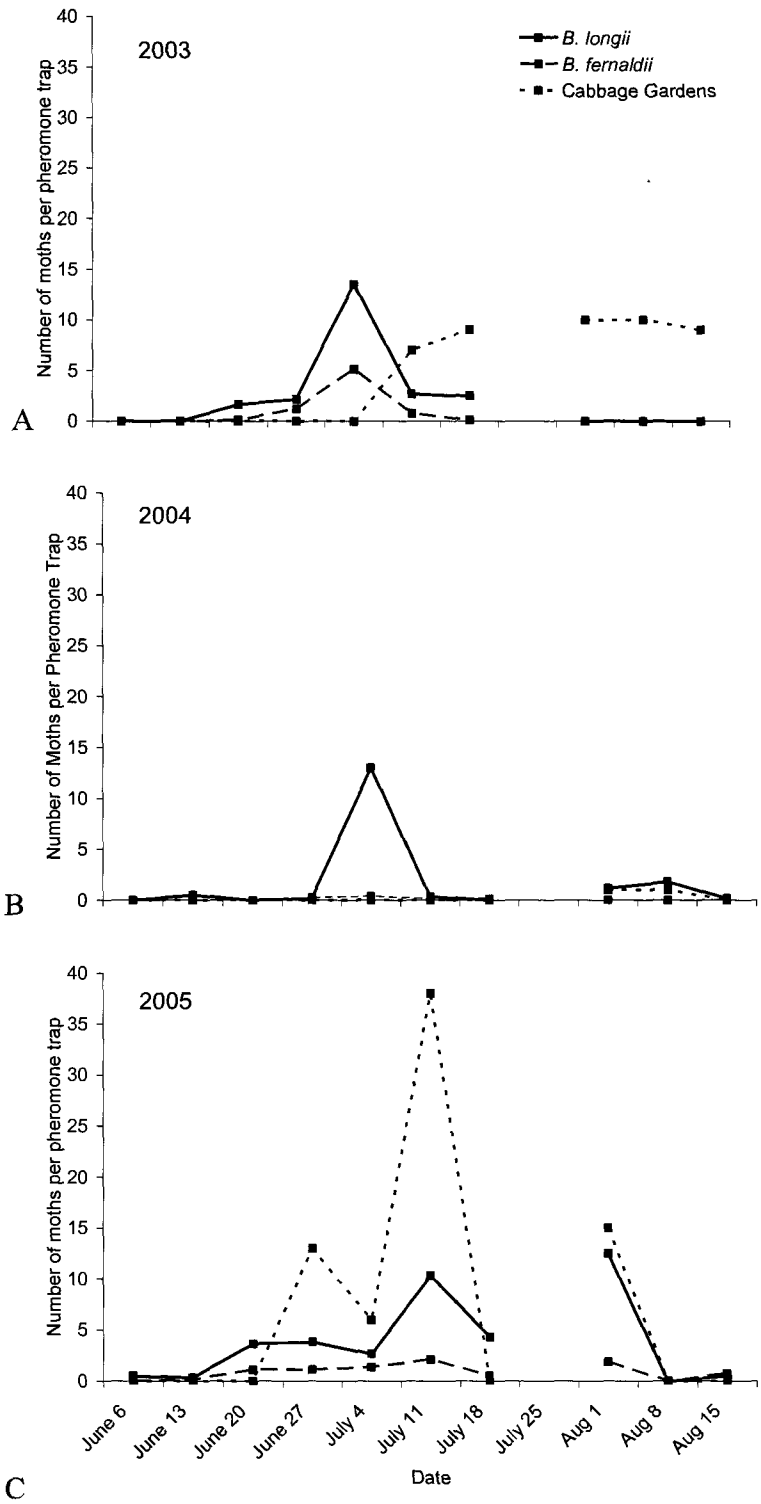

2003
B. longii
B. fernaldii
Cabbage Gardens
Number of moths per pheromone trap
A
2004
Number of Moths per Pheromone Trap
B
2005
Number of moths per pheromone trap
June 6
June 13
June 20
June 27
July 4
July 11
July 18
July 25
Aug 1
Aug 8
Aug 15
Date
C

Figure 3.5.

CHAPTER FOUR

ARE RARE PLANT POPULATIONS ON DISTURBED HABITATS LESS VALUABLE FOR CONSERVATION?

4.1. ABSTRACT

Habitat loss and fragmentation is the most important threat to the persistence of endangered species, requiring conservation biologists to consider the use of anthropogenically disturbed habitats in species recovery and management plans. I surveyed individually tagged *Braya longii* (Fernald) (endangered) and *B. fernaldii* (Abbe) (threatened) for the presence of insect and pathogenic threats and their subsequent impact on seed production and survival to compare the health of *Braya* populations growing on anthropogenically disturbed and undisturbed habitat. Both *Braya* species are endemic to the limestone barrens of Newfoundland, Canada.

Between 2003 and 2005, 30% of surveyed *B. longii* and 16% of surveyed *B. fernaldii* were infested and damaged by *Plutella xylostella* (L.), 8.6% of the *B. longii* population died from root rot, 18% of *B. longii* on anthropogenically disturbed sites were infected with an unidentified pathogen causing their flowering stalks to rot, and 27% of *B. fernaldii* in northern sites were infected with an unidentified pathogen causing had flowering stalk and leaf deformities. A large majority (66%-100%) of the pathogen infections occurred on anthropogenically disturbed sites. The presence of each pest, except for the pathogen causing *Braya* flowering stalks to rot, was linked with a statistically significant increase in mortality. Plants infested with *P. xylostella* or infected by the pathogen causing flowering stalks to rot contributed between 9% and 31% less

seeds to annual seed production than healthy, flowering plants. Due to their large size, plants that died because of infection by the pathogens causing deformities and root rot would have contributed between 31% and 75% more seeds to annual seed production than healthy, flowering plants that survived. Presently, anthropogenic sites are considered important reservoirs for *Braya* seeds in the *Braya* Recovery Plan and have received legal protection. However, their ability to act as pest reservoirs and their lack of within population genetic diversity brings into question the conservation value of populations on degraded habitat. Our research suggests that endangered plant populations surviving on heavily disturbed or degraded habitats need to be screened for potential negative impacts to the remaining populations and that some habitats may need to be restored prior as part of recovery efforts.

4.2. INTRODUCTION

Despite the increasing prominence of threats such as invasive species, habitat loss and fragmentation remain the largest obstacle to rare species recovery (Kerr and Deguise, 2004), especially that of rare vascular plants (Venter et al., 2006). Often, habitat loss is complete, but more often the habitat is heavily disturbed and degraded rather than destroyed. Some organisms, especially plants that exploit natural disturbances and have pre-existing seed banks (Forbes and Jefferies, 1999), may survive in these suboptimal habitats because they are released from natural competition for space and resources (Winker et al., 1995). In such situations, degraded or disturbed habitat must be considered in recovery and management plans.

Before degraded or disturbed habitats are protected and/or restored, recovery teams must consider that plant populations living on degraded habitats are typically exposed to changes in species diversity (Sumina, 1994; Forbes et al., 2001), higher numbers of invasive species (MacDougall and Turkington, 2005), and altered physical conditions, such as higher soil temperatures (Chambers et al., 1990), resulting in changes in their survival, reproduction, and growth rates (Chambers, 1995; Noel, 2000; Forbis et al., 2004). Although natural disturbances, such as fire, often cause demographic changes and alter physical conditions, individual plant species and plant communities have co-evolved with and are adapted to these disturbances (Quintana-Ascencio et al., 2003). While rare plant species may be able to survive in degraded habitats, the appropriateness of maintaining populations on such habitats without restoration has not been assessed to determine whether these threats will put populations within natural habitats at risk, currently and into the future.

The limestone barrens of the Great Northern Peninsula of Newfoundland, Canada support a diverse and rare flora, harbouring 114 of Newfoundland's 271 rare plant species (Bouchard et al., 1991). Brassicaceae species, *Braya longii* (Fernald) (Long's braya) and *B. fernaldii* (Abbe) (Fernald's braya) are endemic to the limestone barrens and are designated as endangered and threatened, respectively (Species at Risk Act, 2002). Similar to rare habitats worldwide, the limestone barrens and their flora have been threatened by habitat loss and degradation. The threat increased substantially from 1968 to 1990 when quarries removed limestone (Janes, 1999) for the construction of a highway that bisected some *Braya* populations and community development destroyed *Braya* habitat and populations (Hermanutz et al., 2002). In some areas of the limestone barrens

utility operators and municipalities have used limestone gravel to level areas of land, support power lines or build roads has created patches of anthropogenically disturbed habitat on which both *Braya* species are capable of surviving. It is possible that in some situations *Braya* species colonised these new disturbed areas from nearby undisturbed areas; however, it is more likely that *Braya* species grew from seeds present in the limestone gravel that was moved in the disturbance, as seeds have a very localised dispersal distance (Tilley, 2003). These disturbed patches account for 31% of habitat within *Braya* populations (Hermanutz et al., 2009). Currently, approximately 90% of the global populations of both *B. longii* and *B. fernaldii* are growing on these anthropogenically disturbed populations (Hermanutz et al., 2009); therefore evaluation of the role of these disturbed populations in long-term survival and persistence of *Braya* is imperative to future management strategies.

B. longii and *B. fernaldii* are both small (1–10 cm and 1–7 cm tall, respectively), herbaceous perennials with linear-spatulate leaves and white, four-petalled flowers, arranged in a raceme (Hermanutz et al., 2002). *Braya* growing on anthropogenically disturbed populations are larger, have higher reproductive output, grow in densities at least 10 times those found in undisturbed populations (Hermanutz et al., 2002) and flower earlier (Donato, 2005). For these reasons anthropogenically disturbed populations have commonly been considered by some to be 'optimal' habitats for *Braya*. This misconception is rooted in the productivity of these populations rather than their long-term viability. While it may be true that anthropogenically disturbed habitats harbour the majority of the *B. longii* and *B. fernaldii* population, they also harbour the entire suite of insect and disease threats to these rare species (Hermanutz et al., 2002); however we do

not know the impacts of these pests and pathogens on the long-viability of the populations.

The first pathogen documented to infect *Braya* was collected from *B. fernaldii* at Boat Harbour, Newfoundland and Labrador in 1925 (Figure 4.1; Fernald, 1950). This unidentified pathogen was thought to be a virus or bacterium, and results in leaf and flowering stalk deformities and leaf pubescence that cause a loss in reproductive output (Hermanutz et al., 2002). The same suite of symptoms was recorded on *B. fernaldii* individuals in the Watt's Point Ecological Reserve (Northern Peninsula, Newfoundland, Canada) in 1995 (Meades, 1996). In addition to this pathogen, *Braya* populations are hosts to two insect pests. In 1995, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (diamondback moth) larvae were found on *B. longii* and *B. fernaldii* (Meades, 1996). *P. xylostella* is a global agricultural pest on members of the Brassicaceae family and is not native to North America (Talekar and Shelton, 1993). In the late 1990's, *Delia* species (root maggots) were detected in the caudex of *B. longii* that were wilted on anthropogenically disturbed populations (Hermanutz et al., 2002). In 2002, research concluded that this insect was *Delia platura* (Meigen) (Diptera: Anthomyiidae) (seed corn maggot) (Hermanutz and Parsons, 2003).

Presently, anthropogenic populations are considered important reservoirs for *B. longii* and *B. fernaldii* seeds in the *Braya* Recovery Plan (Hermanutz et al., 2002) and have received protection under the provincial *Endangered Species Act* of Newfoundland and Labrador (Endangered Species Act, 2001). The objectives of this study were to improve our understanding of the value of anthropogenically disturbed populations to the preservation of these endemic species by determining the: (1) extent to which

anthropogenically disturbed habitat are included in North American plant species recovery efforts; (2) infestation rate and cumulative impact (i.e., damage and mortality) on *B. longii* and *B. fernaldii* as a result of each pest on both anthropogenic and natural populations; (3) presence and impact of any other pests; and (4) threat of the pests to nearby undisturbed or natural populations. Comparing the infestation rate and impacts of pests in each habitat type will enable scientists and managers to determine the value of anthropogenic populations and habitats in the management of these rare species.

4.3. MATERIAL AND METHODS

4.3.1. Anthropogenically disturbed habitat in North America

To determine the extent to which anthropogenically disturbed habitats are included in North American plant species recovery efforts, a survey of Canadian and United States recovery documents was completed. All available recovery documents for flowering plants listed as endangered were downloaded from the Canadian Species at Risk Public Registry (2009) and the United States Fish and Wildlife Service (2009) and assessed to determine if the plant species grew on only natural habitat, only on anthropogenically disturbed habitat, or both habitat types.

4.3.2. Study populations

The limestone barrens are characterized by a cool, wet and windy climatic regime that supports tundra-like vegetation (Banfield and Jacobs, 1998). *B. longii* is presently distributed into six and *B. fernaldii* into 16 geographically separate populations within the

limestone barrens (Hermanutz et al., 2009) (Figure 1.1). Of these 22 populations, nine are growing in undisturbed habitat, six are growing in anthropogenically disturbed habitat and seven contain areas of both undisturbed and anthropogenically disturbed habitat (Figure 4.1). Undisturbed habitats contain exposed bedrock and patterned substrate created by frost action (Greene, 2002), and have high plant species diversity (Hermanutz et al., 2002). Anthropogenically disturbed habitats have obvious disturbance to the substrate and vegetation and therefore contain homogenous gravel substrates with no patterned ground and low plant species diversity (Greene, 2002; Rafuse, 2005).

Nine *B. longii* and eight *B. fernaldii* study sites were established in 2003 to compare the infestation rate, damage, and mortality of *Braya* caused by pests in anthropogenically disturbed and undisturbed habitat (Figure 1.1). Four of the five *B. longii* populations contain both anthropogenically disturbed and undisturbed habitat, therefore study sites were established in each disturbance type of each population. No *B. fernaldii* population contains areas of both disturbance types therefore only one study site was established in each of the eight populations. The study sites spanned the entire ranges of both *Braya* species (190 km distribution of *B. fernaldii*; 25 km of *B. longii*), including populations inside and outside protected areas (Figure 1.1). Between 30 and 100 individually tagged plants from all ages, reproductive stages, and sizes on each of the nine *B. longii* sites (2003, n = 678; 2004, n = 674; 2005, n = 670) and eight *B. fernaldii* sites (2003, n = 488; 2004, n = 544; 2005, n = 573) were assessed two to three times per month from June to August from 2003 to 2005 for any symptoms of, and subsequent damage by, insect infestation and once a month for any symptoms of pathogen infection. The number of flowering stalks was recorded from each tagged plant in August to assess

the extent of damage caused by disease or insects on reproductive structures and to determine if pest presence was correlated with a particular reproductive stage. The survival of each individually tagged plant was also assessed each year and in 2006 to determine if infection or infestation increases the probability a plant will die the following year.

4.3.3. Monitoring unidentified pathogen

Tagged plants observed with leaf and/or flowering stalk deformities and an increased degree of pubescence, were considered infected with the unidentified pathogen first noted in 1925. From each study site where the described symptoms were observed on at least one plant, a single flowering stalk was collected from all flowering tagged plants, which had more than one flowering stalk. This included plants that had and did not have symptoms of pathogen infection. The seeds from these flowering stalks were grown as part of an *ex situ* conservation program at the Memorial University Botanical Garden (Driscoll, 2006) to determine if plants exhibited similar symptoms of infection.

4.3.4. *Plutella xylostella* infestation

To determine the level of infestation and subsequent damage in anthropogenically disturbed populations compared with undisturbed populations, the presence and density of *P. xylostella* eggs, as well as the damage caused by *P. xylostella* larval feeding on *B. longii* and *B. fernaldii* were recorded on each individually tagged plant two to three times per month from June to August in each year of the study. At the same time, the percentage of leaves and fruit damaged by *P. xylostella* larval feeding was recorded using

a ratio scale (0% = no damage, 12.5% = ≤25% damage, 37.5% = 26-50% damage, 62.5% = 51%-75% damage, and 87.5% ≥ 76% damage) (Squires et al., 2009).

4.3.5. Monitoring *D. platura* infestation

Infestation by *D. platura*, as indicated by wilting (Hermanutz and Parsons, 2003), was monitored in the same populations and on the same individually tagged plants as above. The roots of each wilted plant were not removed and inspected as this would have required an extensive amount of habitat disturbance, which is illegal under the *Species at Risk Act* (2002) and the *Endangered Species Act* (2001). General surveys of populations were conducted once a month from June to August. *Braya* observed in these surveys to be infested with *D. platura* according to the criteria above, were included in the mortality count and the number of flowering stalks measured.

Since *D. platura* is usually a secondary feeder on material that is already dying, five freshly wilted plants (one to two days after the onset of wilting) were collected and analysed (Dr. George White, retired Phytopathologist, Agriculture and Agri-Food Canada, Ottawa) to determine the original source of mortality. Eight *B. longii* individuals growing at Memorial University Botanical Garden from seed collected from healthy plants growing on anthropogenically disturbed sites were inoculated with the most common isolate collected from wilted plants. The roots of each plant were scraped with a sterile, metal needle to produce an opening and then a 0.5 ml fungus: 5 ml distilled, sterile water mixture was poured into the wound. The inoculated plants, as well as two control plants, which were also scraped, were watered regularly and checked every three days for one month for signs of wilting. These plants were younger (one or two years old)

than the wild plants that died (> five years); however inoculating *B. longii* in the wild was not possible because it is against provincial and federal legislation to harm endangered or threatened species (Endangered Species Act, 2001; Species at Risk Act, 2002).

4.3.6. Monitoring *Braya* populations for any other pests

All individually tagged plants in each *B. longii* and *B. fernaldii* populations were monitored for the presence of and damaged caused by previously unrecorded pests at the same time they were checked for the presence of *P. xylostella*, *D. platura*, and the unidentified pathogen. Identifying any unidentified pests was not a priority, as it is vital to first understand the pests' impact on both the viability of *Braya* individuals and populations to determine if further studies are warranted.

4.3.7. Statistical analysis

Analyses of variance (ANOVAs) were completed using MINITAB (version 13 for Windows) with alpha set at 0.05, provided the assumptions of normality and homogeneity outlined by the general linear model were met. If the assumptions were not met or the response variable was binomial (i.e., dead/alive or presence/absence), logistic regressions were completed with SAS (version 9.1 for Windows) using GENMOD, as outlined by the generalized linear model. Analyses were completed to determine whether there were significant differences in the numbers of plants infested among years (2003-2005), study sites, plant size, presence of flowers and disturbance regimes (anthropogenically disturbed or undisturbed). Further analyses were completed to determine if *Braya* mortality in year t+1 was a result of pathogen infection in year t, or

another factor such as populations, plant size, presence of flowers or disturbance regime. Analyses were also completed to determine whether there was significantly more *P. xylostella* leaf and fruit damage on dead plants than on plants that survived one to three years prior to their death in both disturbance regimes.

4.4. RESULTS

4.4.1. Anthropogenically disturbed habitat in North America

A review of the 24 Canadian (Species at Risk Public Registry, 2009) and 281 of the 573 United States recovery strategies posted online for endangered, flowering plants (U.S. Fish and Wildlife Service, 2009) revealed that currently 33% of Canadian listed plants and 17% of USA listed plants grow at least partially on anthropogenically disturbed habitat.

4.4.2. Unidentified viral or bacterial pathogen

No *B. longii* population or southern *B. fernaldii* population (Port au Choix National Historic Site and Anchor Point) (Figure 1.1) monitored between 2003 and 2005 had individuals which showed symptoms of leaf and flowering stalk deformities and/or an increase in the degree of pubescence. Three of the five northern *B. fernaldii* populations (Burnt Cape Ecological Reserve, Cape Norman, and Watt's Point Ecological Reserve; Figure 1.1) had individuals which exhibited symptoms. Of the tagged *B. fernaldii* at these three sites, an average of 27% were infected each year with the pathogen (Figure 4.1a – Unidentified 1). Of the infected plants, the majority (90%) were

growing within anthropogenically disturbed habitat at the Burnt Cape and the Watt's Point Ecological Reserves (χ^2=26.95, df=1, p< 0.0001, binomial distribution) (Figure 4.1b).

Of the individuals that exhibited the described symptoms during the study, 92% showed these symptoms by the end of July in each year. After symptoms first appeared in June they gradually increased in severity throughout the summer. *B. fernaldii* with these symptoms had a significantly higher mortality rate than those plants without symptoms; 35% of infected plants died the year following infection compared to only 18% of plants showing no symptoms (χ^2=8.55, df=1, p=0.0035, binomial distribution) on the same sites (Figure 4.2 - Unidentified 1). *B. fernaldii* infected with this pathogen had, on average, slightly more flowering stalks (Table 4.1 - Unidentified 1; χ^2=3.45, df=1, p=0.0634, binomial distribution) compared with plants growing on the same sites that did not show any symptoms. By using the average number of stalks per infected and uninfected plant, the average number of fruit per stalk and the average number of seeds per fruit (Squires and Hermanutz, unpublished data), we determined that the average seed production per uninfected *B. fernaldii* on anthropogenically disturbed sites over the study period was 56 ± 13 seeds per plant compared to 81 ± 12 seeds per plant for infected *B. fernaldii* growing on the same sites. The infected plants would have contributed a higher proportion of seed (31% more) to the annual seed production than the plants that were not infected but they did not because the infected flowering stalks rarely or never produced seeds.

Over the study period, a single flowering stalk was collected from 93 plants on sites with plants that showed symptoms of infection. No *B. fernaldii* seedling or adult (1+ years) grown in the *ex situ* program at the Memorial University Botanical Garden

developed the described symptoms of this pathogen, including those grown from seed of infected plants.

4.4.3. *Plutella xylostella* infestation

The majority of *P. xylostella* infestations occurred on individuals growing on anthropogenically disturbed habitat (Figure 4.3). On average over the study period, 36% of *B. longii* and 19% of *B. fernaldii* individuals growing on anthropogenically disturbed habitat were infested, compared with 21% of *B. longii* and 14% of *B. fernaldii* growing on undisturbed habitat (χ^2=7.10, df=1, p=0.0077, binomial distribution).

The difference between the infestation rate on anthropogenically and undisturbed habitat remains constant or increases as the number of generations of *P. xylostella* within a growing season increase. In 2004 and 2005, climatic conditions were warmer and drier allowing more generations of *P. xylostella* to survive and reproduce than in 2003 (Squires et al., 2009). During the second *P. xylostella* generations in 2004 and 2005, the percentage of tagged *B. longii* and *B. fernaldii* infested was on average, two and three times higher, respectively, on anthropogenically disturbed habitat than on undisturbed habitat (Figure 4.3). In 2005, a small percentage of tagged *Braya* were infested with a third *P. xylostella* generation, all of which, with the exception of 0.3% of *B. fernaldii* growing on undisturbed habitat, were growing on anthropogenically disturbed habitat (Figure 4.3).

The average number of eggs per infested *Braya* was similar on anthropogenically disturbed (2.7 ± 0.1 (*B. longii*); 1.5 ± 0.1 (*B. fernaldii*)) and undisturbed (2.4 ± 0.2 (*B. longii*); 1.6 ± 0.2 (*B. fernaldii*)) habitat. Consequently, infested plants growing on

anthropogenically disturbed and undisturbed habitat had similar amounts of leaf and fruit damage (Figure 4.4). The only exception was that *B. longii* growing on anthropogenically disturbed had significantly more fruit damage than those growing on undisturbed habitat ($F_{1,345}$=5.03, p=0.026; Figure 4.4a). This damage caused the seed set of *B. longii* to decline by 29% (Squires et al., 2009) and was linked to the mortality of both *Braya* species. *B. longii* and *B. fernaldii* growing on both disturbance types that died had 1.3 times higher leaf damage (χ^2=14.86, df=4, p=0.005, binomial distribution) and 1.2 times higher fruit damage (χ^2=14.31, df=4, p=0.0064, binomial distribution) in the years prior to their death than plants that survived.

4.4.4. *D. platura* and *Fusarium* root fungus

Freshly wilted plants (one to two days after the onset of wilting) collected in 2003 were not infested with *D. platura.* It was determined from these newly wilted plants that the roots first decayed as a result of infection by a root fungus of the genus *Fusarium* (Dr. George White, personal communication) and were subsequently infested by *D. platura.* Dr. White isolated *Fusarium equisetum* and *F. avenaceum* (most common) from the roots of dead *B. longii* individuals. None of the eight *ex situ B. longii* that had been inoculated with a *F. avenaceum* isolate or the control plants exhibited signs of wilting. While it is still not known which or even if, either of these fungi causes *B. longii* to die, the roots of every sampled, dead *B. longii* that wilted showed symptoms of severe root decomposition typical of common *Fusarium* infections (Fravel et al., 2003).

A total of 599 *B. longii* individuals, including an average of 26% of the tagged *B. longii* on anthropogenically disturbed sites each year, that died between 2003 and 2005

were wilted with severely decaying roots; symptoms of *Fusarium* infection (Figure 4.1a). No dead plants or wilting symptoms were seen in the *B. fernaldii* populations or in any undisturbed *B. longii* populations during the study period (Figure 4.1b). Mortality due to root decomposition was highest in 2003 (313 individuals) and declined in 2004 (233 individuals) and 2005 (53 individuals). Mortality due to root decomposition was lowest (12%) in June and highest (73%) in August. Based on population counts reported in the *Braya* Recovery Plan (Hermanutz et al., 2002), this mortality is equivalent to a loss of 8.6% of the *B. longii* population over a three year period.

Direct mortality due to this pathogen is not the only problem for *Braya*, indirect losses due to a decline in annual seed production influence long-term population persistence. Since *B. longii* individuals that died had, on average, significantly more flowering stalks (Table 4.1; χ^2=10.43, df=1, p=0.0012, negative binomial distribution) than non-symptomatic plants growing on anthropogenically disturbed habitat, the dead plants would have contributed a higher proportion of seed to the annual seed production than the plants that survived (i.e., those not infected). By using the average number of stalks per infected and uninfected plant, the average number of fruit per stalk, and the average number of seeds per fruit (Squires and Hermanutz, unpublished data), we determined that the average seed production per uninfected *B. longii* on anthropogenically disturbed habitat over the study period was 148 ± 29 seeds per plant compared to 579 ± 46 seeds per plant for infected *B. longii* growing on the same habitat. Infected (i.e., dead) plants would have contributed 75% more seeds to annual seed production than plants that survived (i.e., those not infected).

4.4.5. Previously unrecorded pathogen

In 2003, a series of previously unobserved symptoms were observed on 16 tagged *B. longii* growing on anthropogenically disturbed habitat; flowering stalks of *B. longii* changed colour and subsequently became mouldy. The symptoms always began at a single fruit that changed colour from green to pink and then to white. Symptoms then spread up and down the flowering stalk until the entire stalk was white. The flowering stalk then became covered in a blue mould and wilted, which prevented seeds within the fruits from dispersing.

Similar to the *Fusarium* infection and root decomposition, in 2003 only *B. longii* individuals growing on anthropogenically disturbed habitat showed these symptoms. By 2004 the number of study sites with affected plants increased from three anthropogenically disturbed *B. longii* sites to all four anthropogenically disturbed and both undisturbed *B. longii* sites and one anthropogenically disturbed (Burnt Cape Ecological Reserve) and one undisturbed (Anchor Point) *B. fernaldii* site. These same sites were infected in 2005. On average, 18% of tagged *B. longii* (Figure 4.1 - Unidentified 2) and 2% of *B. fernaldii* (Figure 4.1) were infected each year. While the symptoms were observed on *B. fernaldii* in 2004, the majority (86%) of infested plants over the study period were *B. longii* (χ^2=98.57, df=1, p<0.0001, binomial distribution). Although the pathogen apparently dispersed to undisturbed habitat in 2004 and 2005, the majority of all *B. longii* (66%) and *B. fernaldii* (67%) with the described symptoms were growing on anthropogenically disturbed sites (χ^2=6.52, df=1, p=0.0107, binomial distribution) (Figure 4.1b).

On average 72% of the flowering stalks (and their seed) on infected *Braya* died, while none died on healthy plants. Infected and uninfected *B. fernaldii* had similar numbers of flowering stalks (Table 4.1 - Unidentified 2; χ^2=0.89, df=1, p=0.3466, binomial distribution). Theoretically, this means infected and uninfected plants could contribute similar amounts of seed to the annual seed production. By using the average number of stalks per uninfected plant and the average number of healthy stalks remaining per infected plant, the average number of fruit per stalk, and the average number of seeds per fruit (Squires and Hermanutz, unpublished data), we determined that the average seed production per uninfected *B. fernaldii* over the study period was 53 ± 22 seeds per plant compared to 48 ± 25 seeds per plant for an infected *B. fernaldii* (9% decline) growing on the same sites. Infected *B. longii* had statistically higher numbers of flowering stalks (Table 4.1 - Unidentified 2; χ^2=17.79, df=1, p=0.0001, binomial distribution) than non-symptomatic plants and would have contributed a higher proportion of seed to the annual seed production than the plants that were not infected. We determined that the average seed production per uninfected *B. longii* over the study period was 161 ± 31 seeds per plant compared to 112 ± 31 seeds per plant for infected *B. longii* growing on the same sites. Infected plants would have contributed 31% more seeds to annual seed production than plants that were not infected.

Although reproductive output was clearly compromised by this pathogen, the probability of death was also statistically higher for infected *B. longii* during the length of this study. Of infected *B. longii* individuals, 23% died the following year, whereas 15% of flowering individuals with no symptoms and in the same populations died the following year (χ^2=2.05, df=1, p=0.1526, binomial distribution) (Figure 4.2 –

Unidentified 2). In contrast, infection did not compromise the survival of *B. fernaldii* individuals. Of infected *B. fernaldii* individuals, 8% died the following year, whereas 16% of flowering individuals with no symptoms in the same populations died the following year (χ^2=2.80, df=1, p=0.0945, binomial distribution) (Figure 4.2).

4.4.6. Cumulative probability of pathogen infection

Plants exhibiting symptoms of one pathogen were unlikely to exhibit symptoms of another pathogen in the same year. Only thirteen *B. longii* that wilted and died as a result of root rot, possibly by a *Fusarium* species, also showed symptoms of flowering stalks rotting (unidentified pathogen 2). Only one *B. fernaldii* that showed symptoms of leaf and flowering stalk deformities and/or an increase in the degree of pubescence (unidentified pathogen 1) also showed symptoms of unidentified pathogen 2. Since the probability of exhibiting symptoms of one pathogen is typically independent of exhibiting symptoms of another pathogen, the probability of exhibiting symptoms of each pathogen can be summed to determine the minimum probability of being infected by any one of the pathogens.

Flowering *B. longii* growing on anthropogenically disturbed habitat have the highest probability (0.49) of being infected by a pathogen (Table 4.2). Both flowering and non-flowering *B. fernaldii* growing on anthropogenically disturbed habitat have almost equivalent probabilities of being infested by a pathogen (0.31 and 0.29 respectively) (Table 4.2). On undisturbed habitat, both flowering and non-flowering plants of both *Braya* species have relatively low probabilities (0.0 – 0.14) of being infected by a pathogen (Table 4.2).

4.5. DISCUSSION

Habitat loss, degradation, and fragmentation remain the greatest threats to ecosystem health and rare species survival (Saunders et al., 1991; Sumina, 1994; Brooks et al., 2002; Venter et al., 2006). Conservation biologists protect populations on degraded or fragmented habitat simply because undisturbed habitat exists only in small fragments or not at all. While the protection of populations on anthropogenically disturbed habitat can be important and currently exist in between 17% and 33% of North American recovery documents, it should not be assumed that it is the best conservation approach. Our research indicates that *B. longii* and *B. fernaldii* populations that are resident in anthropogenically disturbed habitat should be restored because these habitats may threaten the health and viability of natural populations by acting as reservoirs from which pests can develop and possibly colonise natural populations.

B. longii growing on anthropogenically disturbed populations suffer higher levels of *P. xylostella* infestation, and infection by *Fusarium* species and an unidentified pathogen causing flowering stalks to rot, than undisturbed populations. High levels of mortality and a high to complete loss of reproductive output are associated with each of these threats. The overall health of *B. fernaldii* populations are better because they are less affected by *P. xylostella* and the unidentified pathogen causing flowering stalks to rot, and so far have not been infected by *Fusarium*. Mortality of *B. fernaldii*, especially on anthropogenically disturbed populations appears to be mainly a result of the unidentified pathogen causing leaf and flowering stalk deformities and an increase in the degree of pubescence. The populations currently infected are closest in proximity to Boat Harbour, the population where the pathogen was first reported in 1925 (Fernald, 1950).

Although *B. longii* and *B. fernaldii* have been studied since the early 1980s (Hermanutz et al., 2002) and monitored continuously since 1998 (Hermanutz et al., 2009), three of the four pests were first seen between 1995 and 2003. Since the majority of these threats are relatively new, it is possible that the number of pests affecting *Braya* populations may continue to increase. The fact that the majority of these infestations and infections occur on anthropogenically disturbed habitat and the unidentified pathogen causing flowering stalks to rot moved from anthropogenic to undisturbed populations, indicates that populations on anthropogenically disturbed habitat may be acting as "foci" for pests. The long-term stability of populations growing on undisturbed habitat cannot be guaranteed without the removal or reduction of the risk of these pests.

The reason anthropogenically disturbed *Braya* populations are affected more severely and frequently by pests than undisturbed sites is likely a combination of the host-finding and dispersal abilities of the pests themselves, as well as *Braya* health on anthropogenically disturbed habitat. *P. xylostella* uses a suite of visual and chemical cues to locate its host plants including host plant size and the presence of flowers (Bernays, 1994; Badenes-Perez et al., 2004). *B. longii* and *B. fernaldii* growing on anthropogenically disturbed habitat have basal diameters that are 1.7 and 1.5 times larger, respectively, and they produce 2.5 and 3.3 times more flowering stalks than those growing on undisturbed habitat (Hermanutz et al., 2009), making plants on anthropogenically disturbed habitats easier to locate and nutritionally superior. The *B. longii* and *B. fernaldii* populations most highly infected with pathogens are also the sites with the lowest plant species diversity and the highest *Braya* density (Hermanutz et al., 2002; Rafuse, 2005; Hermanutz et al., 2009), creating a situation that can promote the

spread of many pathogens (Anderson et al., 1986; Gilbert and Hubbell, 1996). This is compounded by the presence of activity by off-road vehicles (Hermanutz et al., 2002) that have the potential to carry pathogens within and between populations.

A lack of genetic differentiation between sites can make the larger population more susceptible to infection (Parker, 1985; Brown et al., 1991) and may be another reason why *Braya* populations growing on anthropogenically disturbed habitat are infected by numerous pathogens where as those on undisturbed habitat are not. Parsons and Hermanutz (2006) determined that there was a lack of genetic differentiation among anthropogenically disturbed *B. longii* populations and only a moderate amount among anthropogenically disturbed *B. fernaldii* populations. They suggested that this was a result of gravel, and therefore seeds, having been moved among sites during the original disturbances caused by limestone quarrying and road building. In contrast, undisturbed populations of each species are highly genetically differentiated from one another.

The potential interaction between insects and diseases must also be considered in determining the value of anthropogenically disturbed habitat. Given that *P. xylostella* and the pathogens primarily affect the same demographic group of *Braya* populations (flowering individuals), interactions at one or several levels seems probable, but need to be quantified. Research on plant-insect herbivore-pathogen interactions suggests that insect herbivores may act as vectors of disease (Strauss and Zangerl, 2002) or by stressing their host plant and thereby allowing for pathogen infections (Hatcher, 1995). A review by Hatcher (1995) on the relationship between above-ground insect herbivores and below-ground plant pathogenic fungi noted that interactions between these trophic groups usually affect a common host plant.

Over the three year study the number of generations of *P. xylostella* and the number of plants infested increased in years of warmer and drier summer weather (Squires et al., 2009). Concurrently, there was a consistent decline in the number of *B. longii* infected by *Fusarium* species from 2003 to 2005 on each site and many more populations, including those on undisturbed habitat, showed symptoms of the pathogen causing leaf and flowering stalk deformities and increased pubescence. The number of *B. fernaldii* infected with this unidentified pathogen did not fluctuate over time or in different climatic regimes. Because these diseases may respond differently to weather patterns, it is difficult to predict what will happen in the coming decades when the mean annual air temperature of the limestone barrens habitat is predicted to warm by as much as 4°C by the 2080's (Slater, 2005), but it is likely that without intervention these threats will continue.

Management of the pathogens will be improved when further studies determine the species responsible and their methods of infection and dispersal. With that knowledge the usefulness of mitigation techniques, such as removing infected plant material from the sites can be appropriately assessed. *B. longii* and *B. fernaldii* seed are only able to disperse approximately 50 cm, as they are small, round seeds with no adaptation for long distance dispersal by wind or animals (Tilley, 2003). The dispersal capability of *P. xylostella* and many pathogens is much higher (Talekar and Shelton, 1993; Hatcher, 1995) and may have resulted in their spread to *Braya* on undisturbed habitats. There are potential management strategies, such as mass trapping, for *P. xylostella*; however their success in mitigating infestation and subsequent damage in recent studies on *Braya* was limited (Hermanutz et al., 2006). Mass trapping worked well on sites less than 10 m^2, the

control site had 3.7 times as many eggs per plant as the experimental site (with traps), but results were inconclusive on sites greater than $10m^2$ (Hermanutz et al., 2006). It will be important to determine the ability of researchers and the general public walking between populations to act as dispersal agents for any of the pathogens.

Restoration of anthropogenically disturbed habitat should be considered as a management option to deal with both *P. xylostella* and diseases. Although the act of restoring habitat is complicated and must be carefully planned (Miller and Hobbs, 2007) it may include reducing the density of *Braya* on anthropogenically disturbed habitat (Hermanutz et al., 2002; Colling and Matthies, 2004; Hermanutz et al., 2009) and increasing the species diversity to mimic that of undisturbed habitat (Sumina, 1994; Noel, 2000). The restoration of anthropogenically disturbed *Braya* populations may have an evolutionary benefit; insects and pathogens that select host plants on the basis of their health and size may permanently change the genetic make-up of *B. longii* and *B. fernaldii* populations. A wide array of research on host plant genetic heterogeneity suggests that the conservation of plants in fragmented landscapes must consider the negative impact of genetic erosion due to selective pressures (Saunders et al., 1991; Gilbert and Hubbell, 1996; Stockwell et al., 2003; Heal et al., 2004).

It is unlikely that the high levels of pest incidence and subsequent mortality of *B. longii* and *B. fernaldii* observed on anthropogenically disturbed habitat will cease without intervention. While anthropogenically disturbed populations have historically been valued for their larger population size, their ability to harbour insects and disease may actually negate their usefulness to the point that they may be detrimental to conservation efforts unless they can be restored. In conclusion, the conservation value of

anthropogenically disturbed habitat should be individually evaluated within the management planning framework to ensure these fragments are in fact contributing to the long-term persistence of rare plants, and not having negative impacts on their future. This will become more important as continued habitat loss and degradation force managers and biologists to further understand plant ecology on differing levels of habitat integrity. Conservation biologists need to analyse differences in threat frequency and plant demography on undisturbed versus anthropogenically disturbed habitats and state these in recovery documents to ensure appropriate recovery actions are taken. They also need to ensure that habitat protection is a priority within recovery efforts. Our research suggests that degraded habitats need to be screened for potential negative impacts on endangered plant populations and that some sites may need to be restored to promote recovery efforts.

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Table 4.1. A comparison of the number of flowering stalks (± SE) on individually tagged *Braya longii* and *B. fernaldii* infected and not infected with a pathogen.

Pathogen	Species	Infected	Not Infected
Fusarium	*B. longii*	13.3 ± 0.8	3.6 ± 0.2
Unidentified 1	*B. fernaldii*	3.5 ± 0.5	2.7 ± 0.2
Unidentified 2	*B. longii*	5.3 ± 0.5	2.4 ± 0.1
Unidentified 2	*B. fernaldii*	3.1 ± 0.8	2.3 ± 0.1

Table 4.2. The probability of a flowering and non-flowering *Braya* individual in each disturbance type (A -Anthropogenically disturbed or U- Undisturbed) exhibiting symptoms of a pathogen infection.

Species	Reproductive stage	Disturbance	Probability of infection			
			Fusarium	Unidentified 1	Unidentified 2	Any pathogen
B. longii	Flowering	A	0.26	0.00	0.23	0.49
		U	0.00	0.00	0.14	0.14
	Non-flowering	A	0.00	0.00	0.00	0.00
		U	0.00	0.00	0.00	0.00
B. fernaldii	Flowering	A	0.00	0.27	0.02	0.29
		U	0.00	0.03	0.03	0.06
	Non-flowering	A	0.00	0.31	0.00	0.31
		U	0.00	0.03	0.00	0.03

Figure 4.1. The A) average annual percentage (±SE) of tagged *Braya longii* and *B. fernaldii* infected over the study period with one of three pathogens and the B) average annual percentage (±SE) of those pathogen infections which occurred on anthropogenically disturbed sites.

Figure 4.2. A comparison of the average annual mortality rate (±SE) of tagged *Braya longii* and *B. fernaldii* the year following an infection by one of the three pathogenic threats.

Figure 4.3. The average percentage (±SE) of tagged A) *Braya longii* and B) *B. fernaldii* infested with eggs during each *Plutella xylostella* generation in each year of the study on undisturbed and anthropogenically disturbed sites. There was only one generation of *P. xylostella* in 2003 and only two generations of *P. xylostella* in 2004.

Figure 4.4. A comparison of the average annual leaf and fruit damage (±SE) caused by *Plutella xylostella* larval feeding on infested A) *Braya longii* and B) *B. fernaldii* growing on each disturbance type. (Damage scale: 0% = no damage, 12.5% ≤25% damage, 37.5% = 26%-50% damage, 62.5% = 51%-75% damage, and 87.5% ≥76% damage).

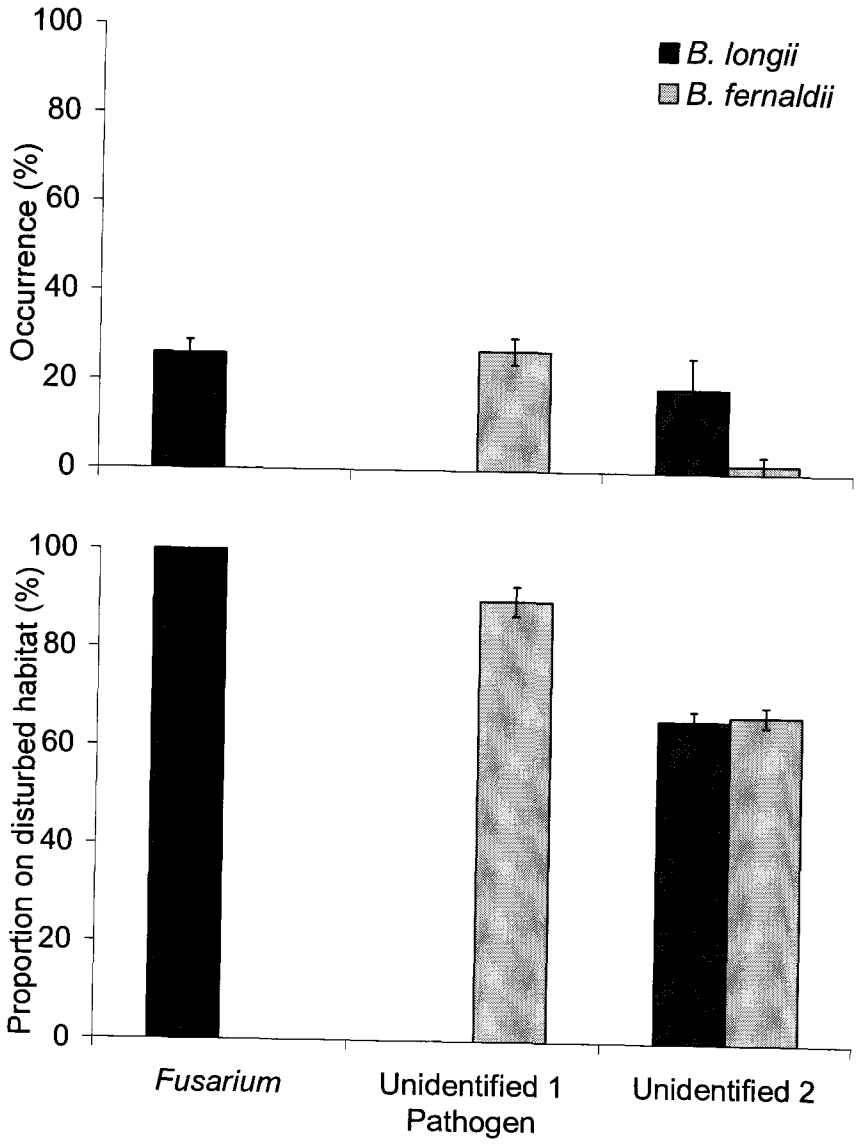
100
80
60
40
20
0
Occurrence (%)
B. longii
B. fernaldii
A
100
80
60
40
20
0
Proportion on disturbed habitat (%)
Fusarium
Unidentified 1
Pathogen
Unidentified 2
B

Figure 4.1.

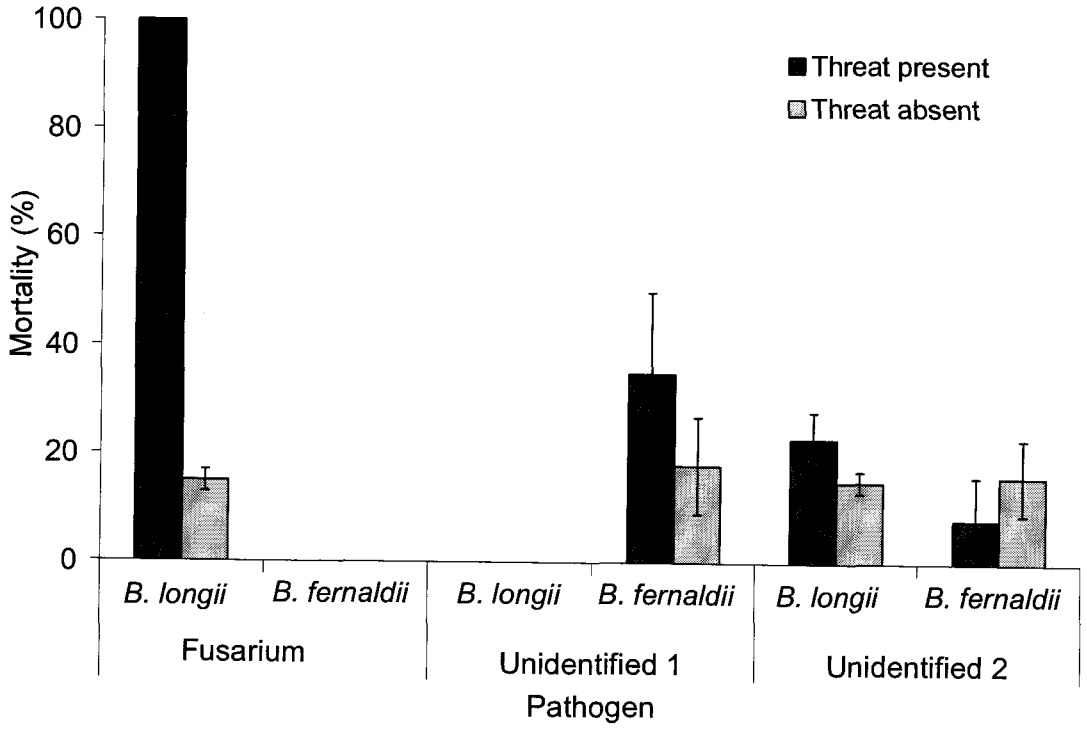
100
80
60
40
20
0
Mortality (%)
Threat present
Threat absent
B. longii
B. fernaldii
B. longii
B. fernaldii
B. longii
B. fernaldii
Fusarium
Unidentified 1
Unidentified 2
Pathogen

Figure 4.2.

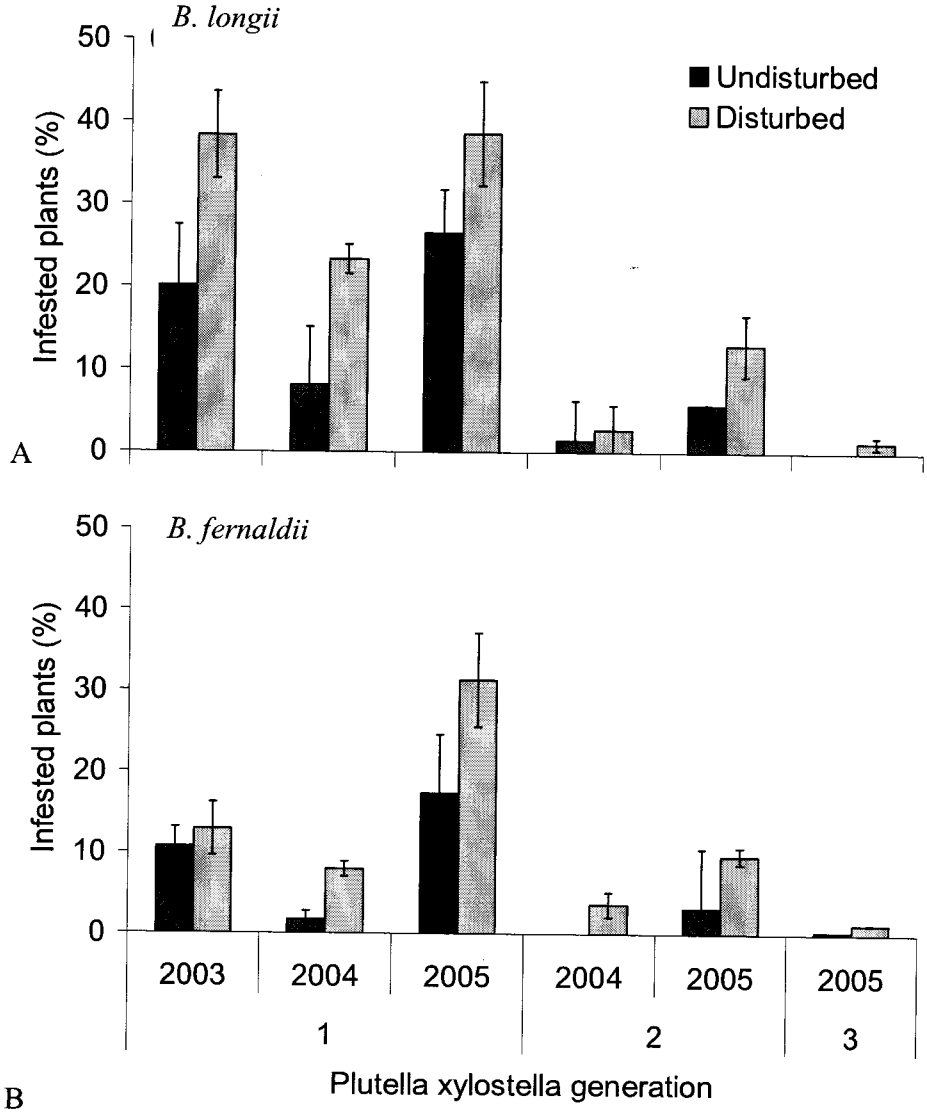
A
B. longii
Undisturbed
Disturbed
Infested plants (%)
50
40
30
20
10
0
B
B. fernaldii
Infested plants (%)
50
40
30
20
10
0
2003
2004
2005
2004
2005
2005
1
2
3
Plutella xylostella generation

Figure 4.3.

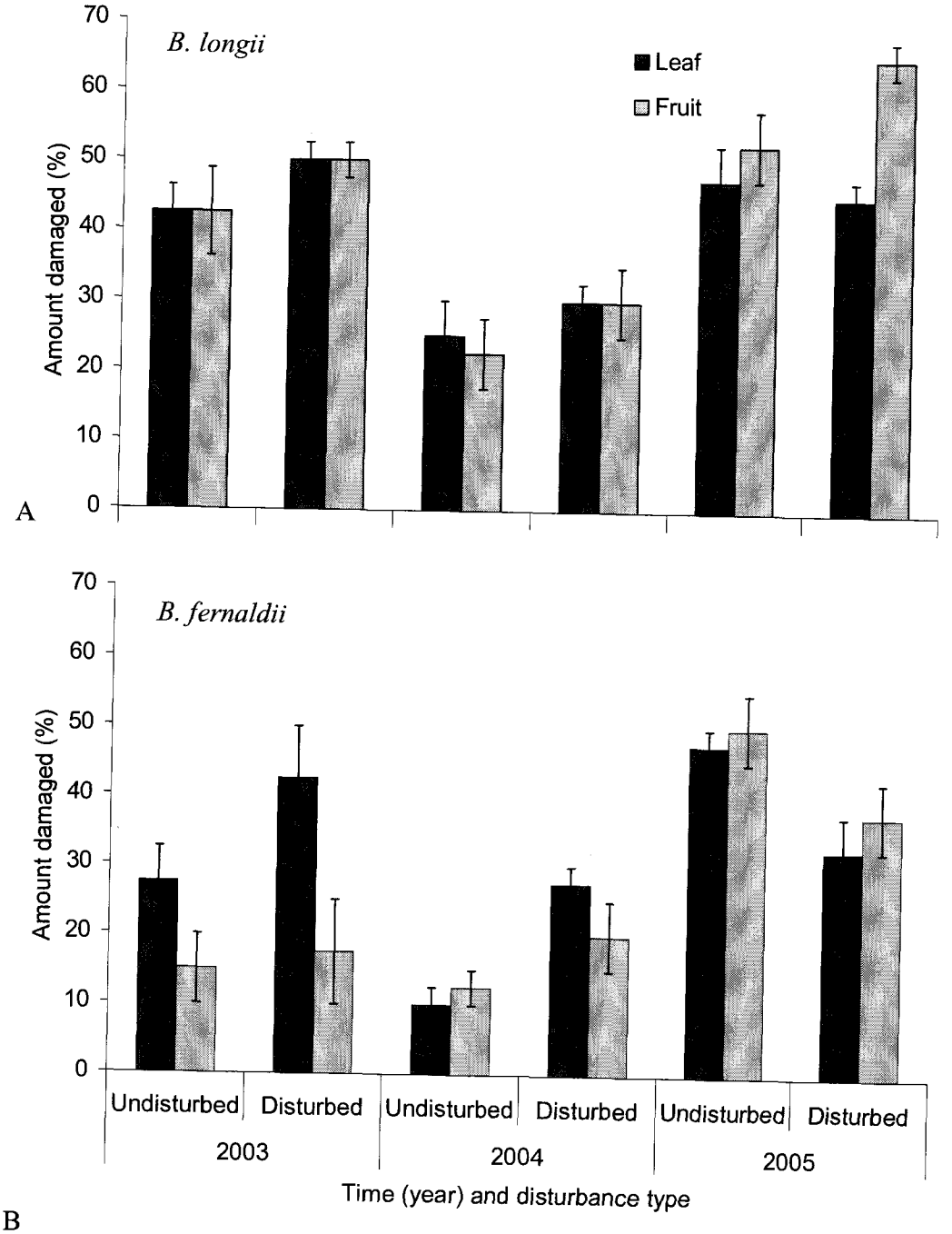
A
B. longii
Leaf
Fruit
Amount damaged (%)
70
60
50
40
30
20
10
0
B. fernaldii
Undisturbed
Disturbed
2003
2004
2005
Time (year) and disturbance type

Figure 4.4.

CHAPTER FIVE

PERSISTANCE OF RARE PLANTS THREATENDED BY PESTS DEPENDS ON HABITAT DISTURBANCE

5.1. ABSTRACT

Populations of *Braya longii* (Long's braya, endangered) and *B. fernaldii* (Fernald's braya, threatened) declined between 1998 and 2008. These rare Brassicaceae are endemic to the limestone barrens on the northern tip of the Great Northern Peninsula of Newfoundland. Demographic data was recorded annually from 1998 to 2006 in five *B. longii* and eight *B. fernaldii* populations, including populations in undisturbed and anthropogenically disturbed substrate. Stage based transition matrices created from these data and summarized into deterministic projections suggest future declines over the next 10 years for each *Braya* species on both disturbance types, except *B. longii* populations on anthropogenically disturbed substrate. The population viability of *Braya* populations on undisturbed substrate is vulnerable to increased mortality of large, flowering plants, where as *Braya* populations on anthropogenically disturbed substrate are vulnerable to declines in seedling survival and seed production. Currently *Braya* reproduction and survival is threatened by *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (diamondback moth) infestation and infection by three pathogens. Between 2003 and 2006 the impacts from each of these threats on the demography of *Braya* was determined. Management scenarios were explored using the baseline, deterministic models and adjusting the current survival rates to reflect the survival rates of plants unaffected by the pests. The

removal of any one threat improved the population viability of *Braya;* however, populations on anthropogenically disturbed substrates were projected to be most improved by the removal of pathogenic threats where as populations on undisturbed substrates were projected to be most improved by the removal of *P. xylostella.* Intervention, including the prevention of mortality due to pests and the restoration and introduction of populations into undisturbed, unoccupied habitat will help improve long-term population viability.

5.2. INTRODUCTION

The International Union for Conservation of Nature (IUCN) and the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) promote the use of demographic data in quantitative analysis such as population projections, to assess the impact of threats, success of management strategies, and persistence of populations (IUCN, 2008; COSEWIC, 2009). Analyzing demographic and census data to predict the possible fate of plant populations began in the 1970's and has developed into an array of population viability analyses (Harper and White, 1974; Bierzychudek, 1999). Structured populations, those populations in which individuals do not all equally contribute to the growth of the population, are most commonly assessed using population projection or transition matrices (Morris and Doak, 2002). The early uses of population transition matrices in plant ecology were to assess the probability of a population reaching a threshold, such as extinction, either under the current conditions or potential management

scenarios (Menges, 1990), and to determine the impact of specific threats that may compromise viability, such as herbivores (Doak, 1992; Erhlén, 1995). Since then, population viability analysis has been used to assess the impact of fire suppression (Quintana-Ascencio et al., 2003), climate change (Maschinski et al., 2006), and trampling (Maschinski et al., 1997) on plant persistence. These models have not been used to compare the extinction probability of rare plant populations in natural habitats to those growing in anthropogenically disturbed habitats and whether these degraded habitats should be included in restoration efforts (Squires, Chapter 4). They have also never been used to determine the effects pathogens or an agricultural insect herbivore on vital rates (Squires, Chapter 3), and hence population viability. Population viability analysis is thought to be most useful in situations where there are complex interactions a requirement to quantitatively evaluate various management scenarios (Brigham and Schwartz, 2003).

All plant species from all ecosystems are at risk of being negatively affected by herbivores or pathogens, thus the importance of these biotic threats in influencing plant population sizes, life-history strategies, and evolution are fundamental ecological questions. The negative impacts of herbivores on plants have been extensively studied and while much is known about their impact on the destruction of host plant biomass and reproductive output (Gurevitch et al., 2002; Labandeira, 2002; Strauss and Zangerl, 2002), the extent to which herbivores limit the distribution and density of plants, and thus their demographic vital rates, is less well understood (Louda, 1982; Louda and Rodman, 1996). The negative effect of pathogens on plant reproduction and/or survival suggests

that pathogens can have a detrimental impact on plant population size (Alexander and Burdon, 1984; Alexander and Antonovics, 1988; Burdon, 1993; Colling and Matthies, 2004) even to the point of increasing the risk of extinction (Barrett et al., 2008). However, their impact on rare plants is still not commonly studied. The most comprehensive analyses of the impacts of pathogens on plant population biology were by Burdon (1987); however the majority of case studies evaluated were agricultural based due to the general lack of research on the impacts of pathogens in natural ecosystems.

The endangered, *Braya longii* (Fernald) (Long's braya; Brassicaceae) and the threatened, *B. fernaldii* (Abbe) (Fernald's braya; Brassicaceae) are endemic to the limestone barrens on the northern tip of the Great Northern Peninsula of Newfoundland (Canada). Both these limestone barrens endemics are affected by *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (diamondback moth), a global agricultural pest of Brassicaceae, and three pathogens. *P. xylostella* eggs were first observed on *Braya* in 1995 (Hermanutz et al., 2002) and have since become a very serious threat to their reproductive output and survival (Squires et al., 2009). Between 2003 and 2005 plants infected with pathogens contributed 9% to 75% fewer seeds to annual seed production than healthy, flowering plants and have an increased probability of mortality (Squires, Chapter 4). A large majority (66%-100%) of the pathogen infections occurred in populations growing on anthropogenically disturbed substrate (Squires, Chapter 4). These degraded patches account for 31% of habitat within *Braya* populations (Hermanutz et al., 2009). Currently, approximately 90% of the global populations of both *Braya* species (91% of *B. longii* populations and 90% of *B. fernaldii* populations) are growing on these

anthropogenically disturbed sites (Hermanutz et al., 2009); therefore evaluation of the long-term viability of populations on these disturbed sites is imperative to the development of a long-term management strategy.

The objectives of this study were to determine the: (1) demography of *B. longii* and *B. fernaldii* in populations on anthropogenically disturbed habitat and undisturbed habitat; (2) extinction probabilities of *Braya,* in each habitat type; and (3) extent to which the persistence of *Braya* is improved with the removal of insect and pathogenic threats. Without answering these questions it will be impossible to determine the appropriate management options for each species to ensure their long-term persistence.

5.3. MATERIALS AND METHODS

5.3.1. Study populations

The limestone barrens are characterized by a cool, wet and windy climatic regime that supports tundra-like vegetation (Banfield and Jacobs, 1998). *B. longii* is presently distributed into six and *B. fernaldii* into 16 geographically separate populations within the limestone barrens (Hermanutz et al., 2009). Of these 22 populations, nine are growing in undisturbed substrate, six are growing in anthropogenically disturbed substrate and seven contain areas of both undisturbed and anthropogenically disturbed substrate. Undisturbed habitats contain exposed bedrock and patterned substrate created by frost action (Greene, 2002), and has higher plant species diversity (Hermanutz et al., 2002). Anthropogenically disturbed habitats on the limestone barrens have obvious disturbance to the substrate and

vegetation and contain homogenous gravel substrates with no patterned ground and lower plant species diversity (Greene, 2002; Rafuse, 2005).

Demographic, threat, and population size data were recorded from five *B. longii* and eight *B. fernaldii* populations. Of the five *B. longii* populations, four include data collected from both undisturbed and anthropogenically disturbed substrate and one contains only anthropogenically disturbed substrate (i.e., nine study populations). Five of the *B. fernaldii* populations contain only undisturbed substrate and the remaining three contain only anthropogenically disturbed substrate.

5.3.2. Study species

B. longii and *B. fernaldii* are both small (1-10 cm and 1-7 cm tall respectively) perennials that are taxonomically closely related and have similar life histories and ecology (Hermanutz et al., 2002; Parsons and Hermanutz, 2006). *B. longii* and *B. fernaldii* have scapose racemes of small white flowers (Harris, 1985) and flower from the middle to the end of June, start producing fruit by mid-late July, and have mature fruits by mid to late August (Parsons and Hermanutz, 2006). *Braya* species produce between 9.0 and 16.6 seeds per fruit (Hermanutz et al., 2009); however *B. longii* seeds weigh approximately 2.5 times more than *B. fernaldii* seeds (Hermanutz et al., 2002). Soil seed bank, seed rain, and natural recruitment analyses concluded that the *Braya* seed bank is spatially and temporally variable and that *Braya* seed are only able to disperse approximately 50 cm (Tilley, 2003), suggesting that the distribution of both *Braya*

species within a site and between sites is limited by dispersal and density-dependent seedling mortality.

Both *Braya* species are infested by *P. xylostella*. *P. xylostella* typically infests agriculturally important crops such as broccoli and cabbage (Talekar and Shelton, 1993). *P. xylostella* is an annual migrant to Canada during the growing season where it is unable to overwinter due to low winter temperature (Butts and McEwen, 1981; Smith and Sears, 1982; Dosdall et al., 2001). *P. xylostella* can survive on wild Brassicaceae species (Talekar and Shelton, 1993) and in some cases non-Brassicaceae host plants (Löhr and Gathu, 2002). On the Great Northern Peninsula of Newfoundland, Canada agricultural Brassicaceae, such as cabbage occur only in small patches and when *P. xylostella* arrives by wind dispersal, they survive and oviposit on rare, native, and non-agricultural Brassicaceae species (Hermanutz et al., 2002; Squires et al., 2009).

Three pathogens have become a serious issue in *B. longii* and *B. fernaldii* recovery. *B. fernaldii* are infected by only one of these pathogens (unidentified pathogen 1) in any significant amount. This pathogen was first documented infecting *B. fernaldii* at Boat Harbour, Newfoundland and Labrador in 1925 (Fernald, 1950) and it causes leaf and flowering stalk deformities and increased leaf pubescence. The same suite of symptoms was recorded on *B. fernaldii* individuals in the Watt's Point Ecological Reserve, Newfoundland and Labrador in 1995 (Meades, 1996). *B. longii* are infected by two different pathogens. In the late 1990's *Fusarium* infection was found in *B. longii* growing on anthropogenically disturbed habitat and caused their roots to rot. In 2003 an unidentified pathogen (unidentified pathogen 2) was found in *B. longii* growing on

anthropogenically disturbed habitat which killed flowering stalks. The same symptoms were found in 2004 on *B. longii* growing on undisturbed habitat and a minimal number of *B. fernaldii* growing in both disturbance types.

5.3.3. Collection of demographic data

The data necessary to derive survival, reproductive, and life cycle stages transition probabilities of *Braya* were recorded from 30 individually tagged plants in each of nine *B. longii* and eight *B. fernaldii* study populations in August of each year from 1998 to 2006 (*B. longii* – n= approximately 270 plants per year; *B. fernaldii* – n= approximately 240 plants per year). Individually tagged plants were located throughout the entire ranges of both *Braya* species (190 km distribution of *B. fernaldii*; 25 km of *B. longii*), including populations inside and outside protected areas (Figure 1.1). Five of the nine *B. longii* populations and three of the eight *B. fernaldii* populations contain anthropogenically disturbed habitat. The tagged individuals represented adult plants from all reproductive stages (vegetative growth or flowering) and sizes (single or multiple rosettes of leaves) in each *Braya* population.

The total plant reproductive output of each plant was determined by counting the number of flowering stalks per plant, and multiplying that by the number of fruit per stalk and the average number of seeds per fruit. The total number of flowering stalks per plant and the number of fruit per stalk were counted in August on each individually tagged plant. The average number of seeds per fruit was determined for each species, in each

disturbance type, at each site, by collecting one flowering stalk per year from plants with multiple flowering stalks and counting the number of seeds in two of the fruit.

Seedlings are too small and crowded together to be individually tagged as described above, therefore seedling survival rates were determined by monitoring seedling emergence and survival from permanent seed addition plots from 2002 to 2006 (Tilley, 2003). Seeds (n= 15 to 25) were added to 50 cm x 50 cm plots (*B. longii* - n=18; *B. fernaldii* - n=22) in four populations of undisturbed substrate, after they were cold stratified for two to three months to ensure that they had completed their winter dormancy process. The seed coats of all seeds were then scarified with fine sandpaper to mimic the natural action of seed movement through the soil profile due to frost heave. The seeds used for this experiment were harvested in 2001 and planted in 2002. Each seed was laid on the soil and gently patted into the soil. Plots were observed every August to determine seedling survival each year.

The probability of a seed becoming a seedling could not be accurately determined from the seed addition plots as these seeds were stratified, scarified, and placed on the soil so that density dependence was not a factor. Therefore the number of seedlings recorded in the permanent monitoring plots (see population census) was divided by the total number of flowering plants to determine the probability of a plant producing (or seed becoming) a seedling for each species and each disturbance type. Although this estimate is likely an underestimate as it incorporates mortality associated with the seed stage already included in the model it is considered to be a better estimate of the seed to seedling stage because it incorporates the high natural mortality rate of seedlings early

after their emergence due to density dependence factors. The majority of seeds disperse a very short distance (< 50 cm) from a parent plant (Tilley, 2003) therefore the permanent plots likely include all seedlings.

A seed bank experiment was initiated in 2004 to determine the longevity of *Braya* seeds in soil. Seeds were sealed in mesh bags and buried in 19 L pails filled with sand and placed outside at Memorial University Botanical Garden. To determine the longevity of seeds within the seed bank and the proportion of seeds which germinate, seeds were exhumed and tested for germination in the spring of each year for three years (2005, 2006 and 2007). Seeds were rinsed with deionized water to remove excess sand, soaked for 30 minutes in diluted dish soap (2 L water, 2 ml dish soap), rinsed three times in deionized water, soaked for 10 minutes in a 1% bleach solution, and rinsed three more times in deionized water to decrease the probability of mould developing on the seeds before they could germinate. Seeds were then scarified with sand paper, placed in a Petri dish on damp filter paper, and placed in a growth chamber set with a daytime temperature of 16°C (16 hours) and a night time temperature of 10°C (8 hours) and germination was checked daily for 21 days.

5.3.4. Population census

Between 1998 and 2000 and again in 2008 the numbers of flowering *Braya* were counted (Hermanutz et al., 2009) and recorded separately in populations containing areas of both undisturbed and anthropogenically disturbed habitat. In June 2008, one to five permanent monitoring plots (2 m x 1 m) were established within each of the nine *B.*

longii and eight *B. fernaldii* study populations that are monitored for demographic data. In August of 2008, the same time demographic data were recorded on individually tagged *Braya* and the number of each plant stage (seedling, vegetative growth, single rosette, flowering, and multiple rosette, flowering) with the exception of the seed stage was recorded from each monitoring plot to determine the average composition of *B. longii* and *B. fernaldii* populations on undisturbed and anthropogenically disturbed substrate (Figure 5.1).

The total number of flowering plants in *B. longii* and *B. fernaldii* study populations on undisturbed and anthropogenically disturbed substrate recorded in the 2008 census was divided by the average proportion of flowering plants in the permanent monitoring plots within those same populations to determine the estimated total *Braya* population size (i.e., all lifecycle stages except seeds). The proportion of plants in each lifecycle stage was multiplied by the estimated total *Braya* population size to determine the total number of plants in each lifecycle stage within a population. The number of seeds produced by each *Braya* population was determined by multiplying the number of single, flowering plants by the average number of seeds produced per single, flowering plant and adding it to the number of multiple, flowering plants multiplied by the average number of seeds produced per multiple, flowering plant. These estimates were used as the 2008 population vectors for the population viability analysis.

5.3.5. Data analyses and modelling

A population viability analysis is based on matrices that link the probability of survival and reproduction to the probability of moving from one lifecycle stage to another. These vital rates were determined for each *Braya* population and the data were pooled to create four baseline population viability models which reflect the species specific differences, as well as the variation due to disturbance type (1) *B. longii* undisturbed sites (n = 708 plants); 2) *B. longii* anthropogenically disturbed sites (n = 1052 plants); 3) *B. fernaldii* undisturbed sites (n = 998 plants); and 4) *B. fernaldii* anthropogenically disturbed sites (n = 678 plants).

Each baseline population viability model summarizes demographic data into stage-based projection matrices (Lefkovitch, 1965) in the form $n_{t+1} = An_t$, where A is a matrix of transition probabilities outlining the contribution of each stage to all other stages and n_t and n_{t+1} are population vectors at time t and time t+1. Transition probabilities for growth were determined by dividing the total number of *Braya* individuals that moved from lifecycle stage x in census t to lifecycle stage y in census t+1, by the total number of individuals that started in lifecycle stage x and survived to census t+1. The reproductive contribution of each life cycle stage (first row of matrix A) was determined by multiplying the survival probability of each lifecycle stage by the average number of seeds that stage produces in a year. All seeds are assumed to remain within a population because *Braya* seed was found to disperse, on average, approximately 50 cm (Tilley, 2003).

These matrices can be multiplied by a population vector containing the number of individuals in each size class at present (t) to predict the population size the following year (t+1). Repeated iterations of the model result in a projection of the population growth (lambda) and extinction rate. These projections were determined using a computer program written in MATLAB (version 7.7.0471 (R2008b), MathWorks Inc.), which included an elasticity analysis of the matrix projection models to identify the vital rate that had the largest proportional impact on the population growth rate (Morris and Doak, 2002).

5.3.6. Management scenarios

To determine the most destructive biotic threat to the population viability of declining *Braya* populations, the presence of *P. xylostella* and pathogens (Squires, Chapter 4), as well as their influence on *Braya* reproduction and survival was recorded on each individually tagged plant two to three times per month from 2003 to 2006 (Squires et al., 2009). The survival rate of adult *Braya* the year after (year t+1) the individual was either infested and damaged by *P. xylostella* or infected by a pathogen was compared with the survival rate of adult *Braya* in year t+1 that were neither infested by *P. xylostella* nor infected by a pathogen. The difference between these survival rates was determined for all adult *Braya* life cycle stages for both *B. longii* and *B. fernaldii* in each disturbance regime. The survival rates for all adult *Braya* life cycle stages within each of the base models was increased by this percentage to assess the population growth rate and extinction probability of *Braya* when these threats were removed. Neither pest has ever

been found on *Braya* seedlings, nor was there evidence of damage to seedlings by these pests (Squires et al., 2009; Squires, Chapter 4).

5.3.7. Statistical analysis

To determine whether there were 1) differences in the amount of seed bank mortality between species and disturbance regimes, and among years and 2) whether there were differences in seed germination between species and among years, analyses of variance (ANOVAs) were completed using MINITAB (version 13 for Windows) with alpha set at 0.05. The assumptions of normality and homogeneity outlined by the general linear model were tested to ensure analyses with this model were appropriate. Interaction terms were included in all models.

5.4. RESULTS

5.4.1. Demography of *Braya*

The lifecycles of *B. longii* and *B. fernaldii* are sufficiently similar to enable representation by a single lifecycle diagram, which divides their lifecycle in seven stages: seeds, four seedling stages (year one to four), and three adult stages (Figure 5.1). *Braya* seedlings are classified as having two cotyledons and/or fewer than four true leaves, where as *Braya* adults are reproductive and/or have greater than four true leaves. Seed burial experiments indicated that seeds can remain viable in the seed bank for a minimum of three years. On average, survival of seeds over three years within the seed bank was 47

± 5%, ranging from 15% to 81%, and did not differ significantly between species ($F_{1,11}$=3.14, p=0.218), among years ($F_{1,11}$=0.23, p=0.678), or between disturbance types ($F_{1,11}$=0.10, p=0.906). In year one, the average survival of seed for each species in each disturbance type ranged from 42% to 51%. These averages were included in the demographic and management models.

The probability of a seed becoming a seedling was recorded from permanent monitoring plots and ranged from 0.01 to 0.07 seedlings per flowering *Braya*. The survival probabilities for each seedling stage after emergence used in the population viability analysis was those recorded from the *in situ* seed addition plots. Of those seedlings which emerged, 69% of *B. longii* and 67% of *B. fernaldii* survived until August of 2003. Between 2004 and 2006, survival of those seedlings ranged from 54% to 77% on *B. longii* sites and 67% to 83% on *B. fernaldii* sites but did not differ significantly between species ($F_{1,7}$=0.74, p=0.454) or among years ($F_{1,7}$=0.29, p=0.829). After four years of growth, seedlings were considered large enough (i.e., greater than four true leaves) to be included in the vegetative growth stage. Less than 0.01% of seeds planted in 2002 emerged after 2003.

B. longii has a higher survival and reproductive rate for every adult lifecycle stage than *B. fernaldii*, with both species having the lowest probability of surviving within the multiple flowering stage (Table 5.1). For both species, the probability of survival is higher for adult stages if the population is growing on undisturbed compared to anthropogenically disturbed substrate (Table 5.1). The reproductive output (number of seeds per plant) of a multiple rosette, flowering *B. longii* and *B. fernaldii* on undisturbed

substrate is on average seven times and five times higher, respectively, than a single rosette, flowering *Braya* (Table 5.1). This difference increases to 18 times higher for a *B. longii,* and 17 times higher for a *B. fernaldii* if the plant is growing on anthropogenically disturbed substrate.

Although the actual probability of moving between or staying within a lifecycle stage differs between *Braya* species, the overall pattern for both *Braya* species is similar. Individuals have the highest probability of remaining within the vegetative growth and multiple flowering stages than growing or regressing to another stage (Figure 5.2). Both *B. longii* and *B. fernaldii* on anthropogenically disturbed substrate move more quickly through their lifecycle than *Braya* growing on undisturbed substrate because they have a higher probability of moving out of the vegetative growth stage into either the single or multiple flowering stages and moving out of the single flowering stage to the multiple flowering stage (Figure 5.2). By moving quickly to the multiple flowering stage, *Braya* growing on anthropogenically disturbed substrate have a higher seed output per individual per year, but a shorter lifespan.

5.4.2. Population census

The number of adult, reproductive *Braya* counted in the 2008 census declined by 81% and 49% in *B. fernaldii* undisturbed and anthropogenically disturbed populations respectively and declined by 70% in *B. longii* undisturbed populations from the previous 1998 to 2000 census (Figure 5.3; Appendix A). The number of *B. longii* decreased slightly (5%) in anthropogenically disturbed populations (Figure 5.3; Appendix A).

Based on the proportions of adult reproductive *Braya* in the permanent plots compared to the 2008 census, the total size of each *Braya* population could be determined (Figure 5.4). In 2008, the total size of *Braya* populations (all stages except seeds) range from 1,942 plants in undisturbed *B. fernaldii* populations to 124,224 plants in anthropogenically disturbed *B. longii* populations (Table 5.2). The number of seeds produced yearly in each *Braya* population by adult, reproductive *Braya,* as estimated from the 2008 census and the average number of seeds produced per flowering individual, ranged from 51,264 seeds in undisturbed *B. fernaldii* populations to 7,781,266 seeds in anthropogenically disturbed *B. longii* populations (Table 5.2).

5.4.3. Extinction probability of *Braya* in different disturbance regimes

Between the two censuses (1998 to 2000 and 2008) the number of adult, reproductive *B. longii* within the study populations on undisturbed substrate declined by 70% (ranging from declines of 54% to 93% among populations; Figure 5.3; Appendix A) and in anthropogenically disturbed populations declined by 5% (ranging from increases of 1,489% to declines of 68% among populations; Figure 5.3; Appendix A). Similarly, the number of adult, reproductive *B. fernaldii* within the study populations on undisturbed substrate declined by 81% (ranging from declines of 52% to 100% among populations; Figure 5.3; Appendix A) and in anthropogenically disturbed populations declined by 49% (ranging from increases of 1% to declines of 99% among populations; Figure 5.3; Appendix A). The changes in population size that are expected based on the projections (Table 5.3; Table 5.4; Figure 5.5) are more than these actual declines

observed in the total *B. fernaldii* population but less than the actual declines observed in the total *B. longii* population. For both species the changes in population size seen in the projections fall within the possible outcomes suggested by the among population variation recorded between the two census.

Under the current demographic model, and as supported by the actual declines seen between 1998 and 2008, without intervention *B. fernaldii* could go extinct within the next 80 years and *B. longii* (on undisturbed substrate only) could go extinct within the next 150 years. The vital rates that have the largest proportional impact on the population growth rate differ between populations growing on undisturbed and anthropogenically disturbed substrate (Table 5.5; Table 5.6). *Braya* populations growing on undisturbed substrate are highly affected by the survival and transition of plants within the adult lifecycle stages, especially those that remain within the vegetative growth stage, move from the vegetative growth to the single flowering stage, and move to and remain in the multiple flowering stage (Table 5.5a; Table 5.6a). For *B. longii* this improvement was large enough to stabilize the population growth rate. In contrast, *Braya* populations growing on anthropogenically disturbed substrate are most affected by the survival and transition of plants within the seed and seedling lifecycle stages, especially the number of seeds produced by a multiple flowering individual and the ability of plants to move from the seed to seedling stages (Table 5.5b; Table 5.6b); these plants are tending towards a more short-lived perennial life history compared with those plants exposed to a natural disturbance regime.

5.4.4. Management scenarios

Both *P. xylostella* and the pathogens had a negative affect the population viability of *B. longii* and *B. fernaldii* in both disturbance regimes. The removal of both *P. xylostella* and the pathogens caused the largest improvement in the growth rate of all *Braya* populations, except for the growth rate of *B. longii* on anthropogenically disturbed sites; these populations were most improved by the removal of the mortality associated with *Fusarium* infection (Figure 5.5). For *B. longii* and *B. fernaldii* populations growing on undisturbed sites, the removal of *P. xylostella* damage caused a larger improvement in the population growth rate (*B. longii*- 8.0%, *B. fernaldii*- 10.0%) than the removal of a pathogen (*B. longii*- 1.8%, *B. fernaldii*- 0.3%) (Figure 5.5). In contrast, the removal of a pathogen caused a larger improvement in the population growth rate of *B. longii* and *B. fernaldii* populations growing on anthropogenically disturbed sites (*B. longii*- 1.8%, *B. fernaldii*- 10.7%) than the removal of *P. xylostella* damage (*B. longii*- 1.1%, *B. fernaldii*- 7.4%) (Figure 5.5).

5.5. DISCUSSION

The numbers of *B. longii* and *B. fernaldii* have declined in almost all populations in the past ten years due to the negative sustained damage associated with both non-native pests and pathogens. Without intervention, deterministic projection matrices predict future declines of 60% and 90% in most populations. Intervention, including the

prevention of pest and pathogen induced mortality will improve population viability. Given the high degree of degraded habitats throughout the ranges of most rare plants, our study suggests that any demographic projections need to incorporate disturbance regimes and how these affect the population and species persistence across their ranges, and be incorporated into management strategies.

Although the actual probabilities of moving between or staying within a lifecycle stage differs between each species, the overall pattern for both *Braya* species is identical; this supports the strategy of managing the species similarly, as outlined in the *Braya* Recovery Plan (Hermanutz et al., 2002). However, the demography of *B. longii* and *B. fernaldii* is different for populations growing on anthropogenically disturbed compared with those growing on undisturbed substrates, suggesting that management of these plants on different disturbance regimes must address these habitats with different strategies. Both *B. longii* and *B. fernaldii* populations on undisturbed substrate are characterized by higher adult survival rates and a high probability of plants reverting to the vegetative growth stage than *Braya* populations on anthropogenically disturbed substrates.

In similar analyses evaluating the impact of herbivory on perennial plants, a decrease in adult or established plant survival due to herbivory was found to be an important factor in the viability of the population (Louda and Potvin, 1995; Fletcher et al., 2001; Ehrlén, 2003). *Braya* populations on undisturbed substrate are particularly vulnerable to the mortality of large, flowering plants because they provide the largest input to total seed production. Herbivory by *P. xylostella* has an impact on the survival

and reproductive output of large, flowering plants more often than other plant types (Squires, Chapter 3), which is why the hypothetical removal of this threat improved the viability of these populations over a ten year period more than the removal of pathogens.

Braya populations on anthropogenically disturbed substrate are particularly vulnerable to declines in seedling survival and seed production and therefore tend towards a more short-lived perennial life history than *Braya* populations on undisturbed substrate. This is a potentially significant evolutionary difference and an important aspect to consider in planning restoration programs. Previous studies confirm that seedling recruitment in plant populations increases with increasing seed production (Louda, 1982, Eriksson and Ehrlén, 1992; Louda and Potvin, 1995; Ehrlén and Eriksson, 2000). Currently, the most significant loss to seed production and therefore annual inputs to the seed bank in populations on anthropogenically disturbed substrate are flowering stalk mortality and adult mortality due to pathogen infection; therefore their hypothetical removal greatly improves the long-term viability of *Braya* populations. The probability of a seed surviving to the seedling stage is low in all *Braya* populations, suggesting that seed mortality is high *in situ*. This in turn may limit the number of viable seeds within the seed bank and, therefore, annual inputs via seed production are likely important in replenishing the seed bank and subsequent recruitment of seedlings into local populations.

The removal of either *P. xylostella* or the pathogens will be difficult. There are potential management strategies, such as mass trapping for *P. xylostella*, however their success in mitigating infestation and subsequent damage in recent studies on *Braya* was

limited (Hermanutz et al., 2006). Precautionary measures, such as limited vehicle traffic and between site visits, should be put in place to prevent the spread of pathogens among sites by humans through off-road vehicles and walking until the identification and dispersal mechanism of these pathogens is determined. Management of the pathogens will be improved when further studies determine the species responsible and their methods of infection and dispersal. Future restoration of populations must consider how to safely introduce seed to ensure populations that are created or maintained limit pathogen survival and spread (i.e., clean seed prior to planting to prevent spread of pathogens and plant seed as scattered, low density patches, on undisturbed habitat).

If mitigation of pests is not possible or unsuccessful then the density and distribution of *Braya* within each population on undisturbed and anthropogenically disturbed substrate should be restored to natural levels recorded in populations on undisturbed substrate. The introduction of *Braya* into undisturbed, unoccupied habitat should also be considered. Restoration of populations, species historical distribution, and habitat has been suggested and used previously as a tool to prevent extinction (Maunder, 1992; Maschinski and Duquesnel, 2006; Miller and Hobbs, 2007; Menges, 2008). Recent work on the restoration of *Braya* indicates that seeds planted within suitable but unoccupied habitat can successfully germinate and survive (Tilley, 2003; Pelley, unpublished data). The seeds needed for restoration and the maintenance of genetic diversity of all *Braya* populations should continue to be preserved with living and seed bank *ex situ* populations to ensure their maintenance regardless of population decline.

The most pronounced weakness in previous studies using population viability analysis has been the lack of robust demographic data. Most datasets include low sample sizes and short timeframes of data collection (e.g., over only four years) (Menges, 2000; Reed et al., 2002), as well as a lack of information on the critical stage of seed bank and seed to seedling transitions (Menges and Quintana-Ascencio, 2004). In the literature it is not rare to encounter transition probabilities estimated from one or a few individuals (Münzbergová and Ehrlén, 2005) or seed bank size and survival values based on the limited data (Ehrlén, 1995; Menges and Quintana-Ascencio, 2004; Miller et al., 2009). In the case of *B. longii* and *B. fernaldii,* the availability of a longer term data set with hundreds of individuals since 1998 on adult vital rates, allowed for a more rigorous evaluation of risk and extinction. While these models are based on limited years of seed bank and seedling survival data, and therefore could be improved upon further study of these life cycle stages, the models are based on similar years of *in situ* and *ex situ* seed bank analysis as other population viability analysis (Quintana-Ascencio et al., 2003; Miller et al., 2009). Seed bank analyses are often missing from population viability analysis (Menges, 2000).

As continued years of monitoring improve the sample size of demographic data at individual sites, site specific population viability analysis should be completed to develop site specific management plans because all sites are not affected by threats, and therefore in decline to the same degree (Appendix A). While the total population of *B. longii* on anthropogenically disturbed habitat is projected to increase, in the last ten years the *B. longii* population size has decreased, and quite severely, in three of the five populations.

Site specific population projections will help us to better understand what vital rates and/or population compositions are controlling these growth rates.

Population viability analyses are an effective tool to understand the impact of threats and the success of subsequent management plans (Maschinski et al., 1997) and are most useful when comparing relative, and not absolute, changes in a population growth rate and extinction probability over short time periods (Beissinger and Westphal, 1998; Menges, 2000) as done in this study. The speed of the decline facing *B. longii* and *B. fernaldii* populations requires immediate action to mitigate biotic threats and should focus on removing threats facing undisturbed populations. The removal of any one threat improved the population viability of *Braya;* however, *Braya* populations on anthropogenically disturbed substrates were projected to be most improved by the removal of pathogenic threats where as *Braya* populations on undisturbed substrates were projected to be most improved by the removal of *P. xylostella.* Intervention, including the reduction or prevention of mortality caused by pests and the restoration and introduction of populations into undisturbed, unoccupied habitat will help improve the long-term population viability.

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Table 5.1. The average annual reproductive output and probability of survival for *Braya longii* and *B. fernaldii* in each lifecycle stage in each disturbance type.

Disturbance	Lifecycle Stage	*B. longii*		*B. fernaldii*	
		Survival	Reproduction	Survival	Reproduction
Undisturbed	Seed	0.46	0.002	0.51	0.013
	Seedling 1	0.69	0	0.67	0
	Seedling 2	0.77	0	0.67	0
	Seedling 3	0.69	0	0.83	0
	Seedling 4	0.54	0	0.79	0
	Vegetative growth	0.85	0	0.82	0
	Single flowering	0.90	61.70	0.82	63.10
	Multiple flowering	0.86	432.30	0.78	332.10
Anthropogenic	Seed	0.49	0.002	0.42	0.013
	Seedling 1	0.69	0	0.67	0
	Seedling 2	0.77	0	0.67	0
	Seedling 3	0.69	0	0.83	0
	Seedling 4	0.54	0	0.79	0
	Vegetative growth	0.75	0	0.64	0
	Single flowering	0.81	113.60	0.69	155.40
	Multiple flowering	0.69	2,017.00	0.52	2,679.20

Table 5.2. The population vectors for *Braya longii* and *B. fernaldii* on undisturbed and anthropogenically disturbed substrate. Vectors are based on the ratios between the total number of flowering plants recorded from the permanent monitoring plots and those recorded from the 2008 census. Seed values are estimated from multiplying the average number of seeds produced per single and multiple flowering individuals by the number of single and multiple flowering *Braya* recorded in the 2008 census.

Lifecycle Stage	*B. longii*		*B. fernaldii*	
	Undisturbed	Anthropogenic	Undisturbed	Anthropogenic
Seed	129,677	7,781,266	51,264	1,089,625
Seedling 1	167	14,200	44	377
Seedling 2	167	14,200	44	377
Seedling 3	167	14,200	44	377
Seedling 4	167	14,200	44	377
Vegetative growth	2,167	64,375	1 534	3,426
Single flowering	233	1,262	97	465
Multiple flowering	267	3,787	136	406
Total population size	133,011	7,907,490	53,206	1,095,431

Table 5.3. The stage based, transition matrices for A) *Braya longii* undisturbed and B) *B. longii* anthropogenically disturbed outlining the probabilities that an individual at stage x, time t will move to stage y, time t+1 and the stage fecundity. Stages: S- seed, SL- seedling, VG- vegetative growth, SF- single rosette, flowering plant, and MF- multiple rosettes, flowering plant.

A

Stage	S	SL	SL	SL 3	SL 4	VG	SF	MF
S	0.001	-	-	-	-	-	55.53	371.78
SL 1	0.01	-	-	-	-	-	-	-
SL 2	-	0.69	-	-	-	-	-	-
SL 3	-	-	0.77	-	-	-	-	-
SL 4	-	-	-	0.69	-	-	-	-
VG	-	-	-	-	0.54	0.51	0.45	0.11
SF	-	-	-	-	-	0.15	0.17	0.14
MF	-	-	-	-	-	0.05	0.18	0.50

B

Stage	S	SL	SL	SL 3	SL 4	VG	SF	MF
S	0.001	-	-	-	-	-	92.02	1391.73
SL 1	0.03	-	-	-	-	-	-	-
SL 2	-	0.69	-	-	-	-	-	-
SL 3	-	-	0.77	-	-	-	-	-
SL 4	-	-	-	0.69	-	-	-	-
VG	-	-	-	-	0.54	0.32	0.24	0.06
SF	-	-	-	-	-	0.14	0.15	0.04
MF	-	-	-	-	-	0.10	0.27	0.38

Table 5.4. The stage based, transition matrices for A) *Braya fernaldii* undisturbed and B) *B. fernaldii* anthropogenically disturbed outlining the probabilities that an individual at stage x, time t will move to stage y, time t+1 and the stage fecundity. Stages: S- seed, SL- seedling, VG- vegetative growth, SF- single rosette, flowering plant, and MF- multiple rosettes, flowering plant.

A

Stage	S	SL	SL	SL 3	SL 4	VG	SF	MF
S	0.001	-	-	-	-	-	51.74	259.04
SL 1	0.005	-	-	-	-	-	-	-
SL 2	-	0.67	-	-	-	-	-	-
SL 3	-	-	0.67	-	-	-	-	-
SL 4	-	-	-	0.83	-	-	-	-
VG	-	-	-	-	0.79	0.53	0.39	0.14
SF	-	-	-	-	-	0.11	0.18	0.08
MF	-	-	-	-	-	0.03	0.11	0.39

B

Stage	S	SL	SL	SL 3	SL 4	VG	SF	MF
S	0.001	-	-	-	-	-	107.10	1393.18
SL 1	0.004	-	-	-	-	-	-	-
SL 2	-	0.67	-	-	-	-	-	-
SL 3	-	-	0.67	-	-	-	-	-
SL 4	-	-	-	0.83	-	-	-	-
VG	-	-	-	-	0.79	0.22	0.24	0.05
SF	-	-	-	-	-	0.11	0.10	0.04
MF	-	-	-	-	-	0.07	0.13	0.18

Table 5.5. The elasticity matrices for A) *Braya longii* undisturbed and B) *B. longii* anthropogenically disturbed. Values in bold represent the vital rates that had the largest proportional impact on the population growth rate. Stages: S- seed, SL- seedling, VG- vegetative growth, SF- single rosette, flowering plant, and MF- multiple rosettes, flowering plant.

A

Stage	S	SL 1	SL 2	SL 3	SL 4	VG	SF	MF
S	0.0001	0	0	0	0	0	0.0117	0.0715
SL 1	0.0832	0	0	0	0	0	0	0
SL 2	0	0.0832	0	0	0	0	0	0
SL 3	0	0	0.0832	0	0	0	0	0
SL 4	0	0	0	0.0832	0	0	0	0
VG	0	0	0	0	0.0880	**0.1509**	0.0320	0.0072
SF	0	0	0	0	0	0.0722	0.0196	0.0148
MF	0	0	0	0	0	0.0502	0.0434	**0.1105**

B

Stage	S	SL 1	SL 2	SL 3	SL 4	VG	SF	MF
S	0.0001	0	0	0	0	0	0.0061	**0.1135**
SL 1	**0.1196**	0	0	0	0	0	0	0
SL 2	0	**0.1196**	0	0	0	0	0	0
SL 3	0	0	**0.1196**	0	0	0	0	0
SL 4	0	0	0	**0.1196**	0	0	0	0
VG	0	0	0	0	**0.1196**	0.0488	0.0054	0.0017
SF	0	0	0	0	0	0.0426	0.0067	0.0022
MF	0	0	0	0	0	0.0840	0.0334	0.0579

Table 5.6. The elasticity matrices for A) *Braya fernaldii* undisturbed and B) *B. fernaldii* anthropogenically disturbed. Values in bold represent the vital rates that had the largest proportional impact on the population growth rate. Stages: S- seed, SL- seedling, VG- vegetative growth, SF- single rosette, flowering plant, and MF- multiple rosettes, flowering plant.

A

Stage	S	SL 1	SL 2	SL 3	SL 4	VG	SF	MF
S	0.0100	0	0	0	0	0	0.0189	0.0607
SL 1	0.0796	0	0	0	0	0	0	0
SL 2	0	0.0796	0	0	0	0	0	0
SL 3	0	0	0.0796	0	0	0	0	0
SL 4	0	0	0	0.0796	0	0	0	0
VG	0	0	0	0	0.0796	**0.2300**	0.0324	0.0075
SF	0	0	0	0	0	**0.0755**	0.0237	0.0068
MF	0	0	0	0	0	0.0440	0.0309	0.0704

B

Stage	S	SL 1	SL 2	SL 3	SL 4	VG	SF	MF
S	0.0015	0	0	0	0	0	0.0100	**0.1138**
SL 1	**0.1238**	0	0	0	0	0	0	0
SL 2	0	**0.1238**	0	0	0	0	0	0
SL 3	0	0	**0.1238**	0	0	0	0	0
SL 4	0	0	0	**0.1238**	0	0	0	0
VG	0	0	0	0	**0.1238**	0.0470	0.0080	0.0015
SF	0	0	0	0	0	0.0421	0.0059	0.0021
MF	0	0	0	0	0	0.0911	0.0263	0.0319

Figure 5.1. Generalized lifecycle diagram of *Braya longii* and *B. fernaldii*. The coefficients P_{ij} correspond to the probability that an individual in stage j at time t will transition to the stage i at time t+1. Stages: S- seed, SL- seedling, VG- vegetative growth, SF- single rosette, flowering plant, and MF- multiple rosettes, flowering plant.

Figure 5.2. Probabilities that A) *Braya longii* and B) *B. fernaldii*, on undisturbed or anthropogenically disturbed habitat, in an adult lifecycle stage during census (t) will transition to another or remain in the same lifecycle stage or died in census (t+1). Stages: VG- vegetative growth, SF- single flowering, MF- multiple flowering, and D- death.

Figure 5.3. A comparison of the number of flowering *Braya longii* and *B. fernaldii* counted on undisturbed and anthropogenically disturbed substrate in the 1998-2000 census and the 2008 census.

Figure 5.4. The percentage of plants in each lifecycle stage in permanent plots surveyed on *Braya longii* A) undisturbed and B) anthropogenically disturbed substrate and *B. fernaldii* C) undisturbed and D) anthropogenically disturbed substrate. Stages: SL- seedling, VG- vegetative growth, SF- single rosette, flowering plant, and MF- multiple rosettes, flowering plant.

Figure 5.5. Deterministic growth rates (λ) and projected A) *Braya longii* undisturbed, B) *B. longii* anthropogenically disturbed, C) *B. fernaldii* undisturbed, D) *B. fernaldii*

anthropogenically disturbed population sizes for current and management models over a 10 year period.

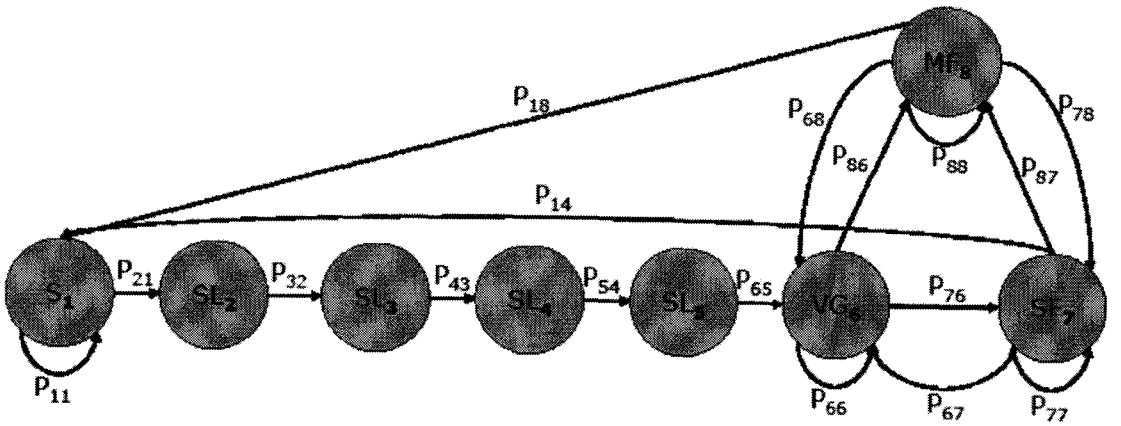
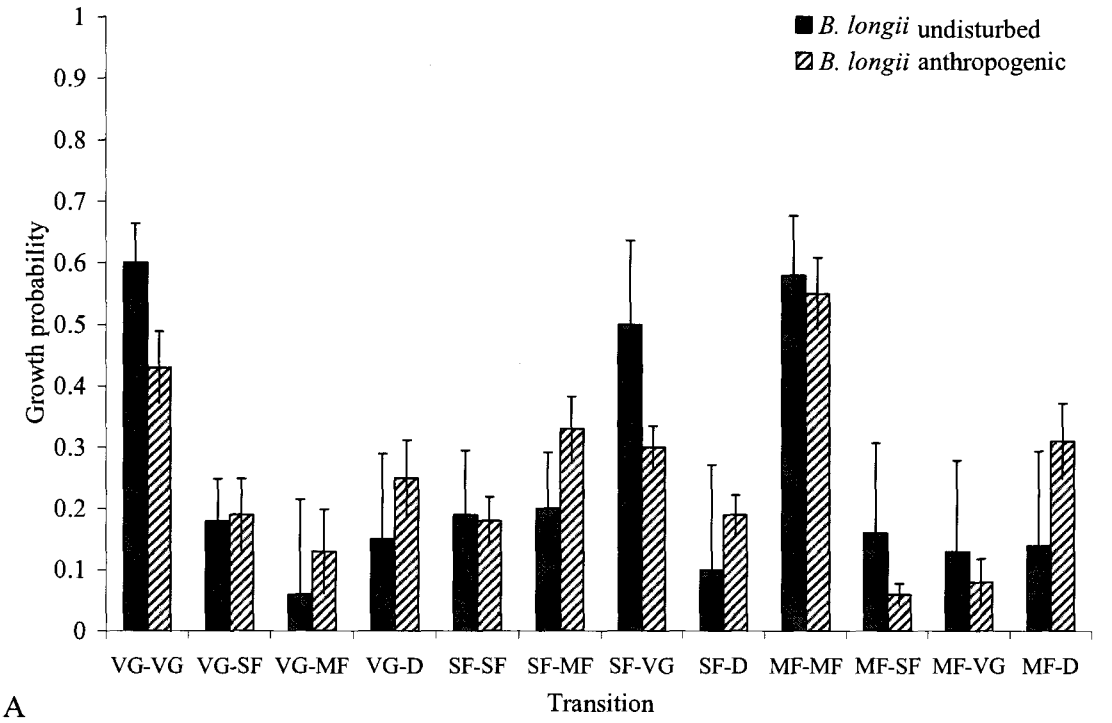
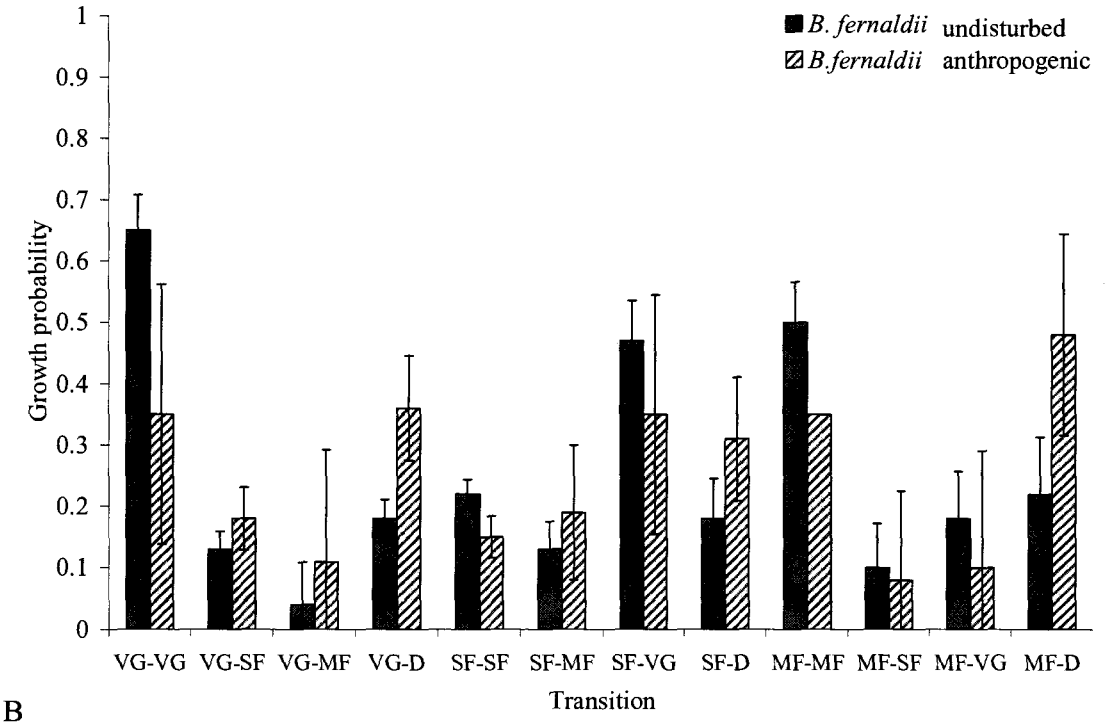

p_{18}
p_{14}
p_{68}
p_{86}
p_{88}
p_{87}
p_{78}
p_{21}
p_{32}
p_{43}
p_{54}
p_{65}
p_{76}
p_{11}
p_{66}
p_{67}
p_{77}
S_1
SL_2
SL_3
SL_4
SL_5
VG_6
SF_7
MF_8

Figure 5.1.


B. longii undisturbed
B. longii anthropogenic
Growth probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
VG-VG VG-SF VG-MF VG-D SF-SF SF-MF SF-VG SF-D MF-MF MF-SF MF-VG MF-D
Transition

A


B. fernaldii undisturbed
B.fernaldii anthropogenic
Growth probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
VG-VG VG-SF VG-MF VG-D SF-SF SF-MF SF-VG SF-D MF-MF MF-SF MF-VG MF-D
Transition

B

Figure 5.2.

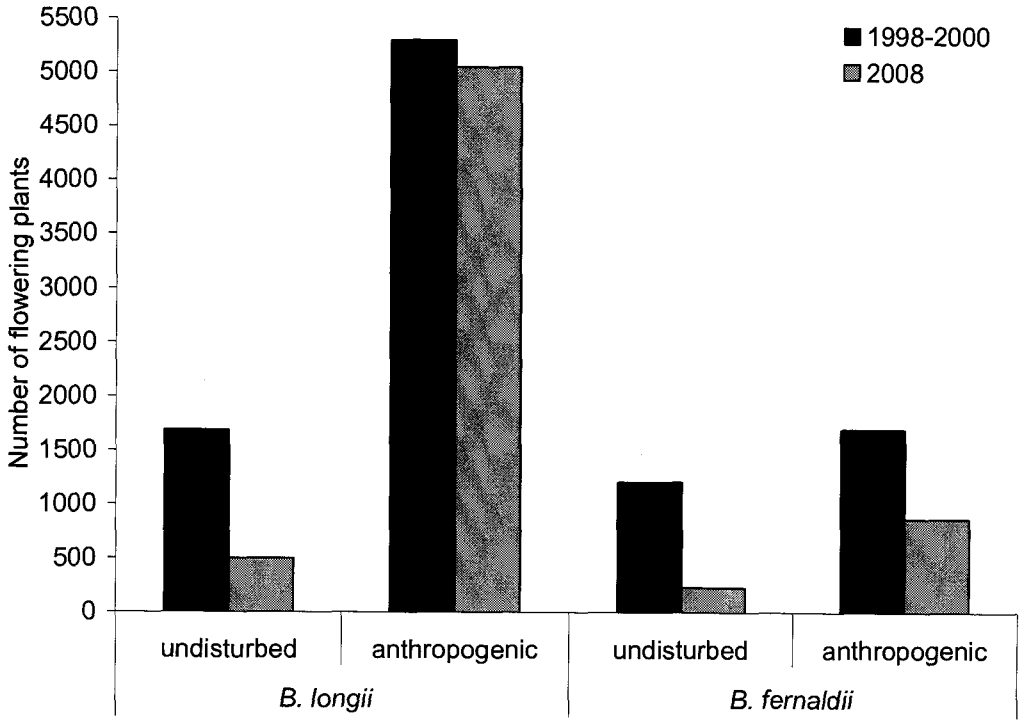

5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Number of flowering plants
1998-2000
2008
undisturbed
anthropogenic
undisturbed
anthropogenic
B. longii
B. fernaldii

Figure 5.3.

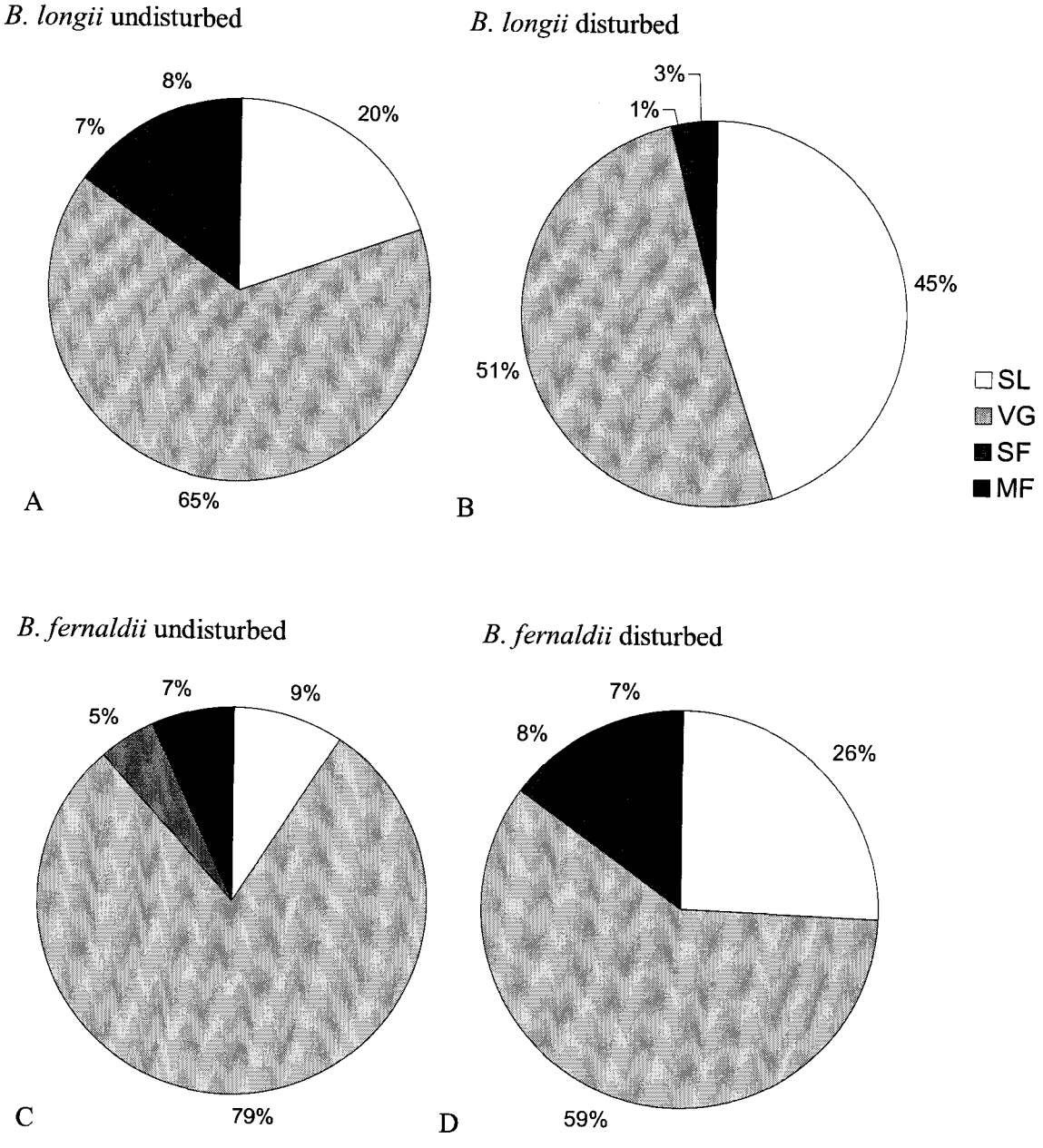

B. longii undisturbed
8%
7%
20%
65%
A
B. longii disturbed
3%
1%
45%
51%
B
SL
VG
SF
MF
B. fernaldii undisturbed
7%
9%
5%
79%
C
B. fernaldii disturbed
7%
8%
26%
59%
D

Figure 5.4.

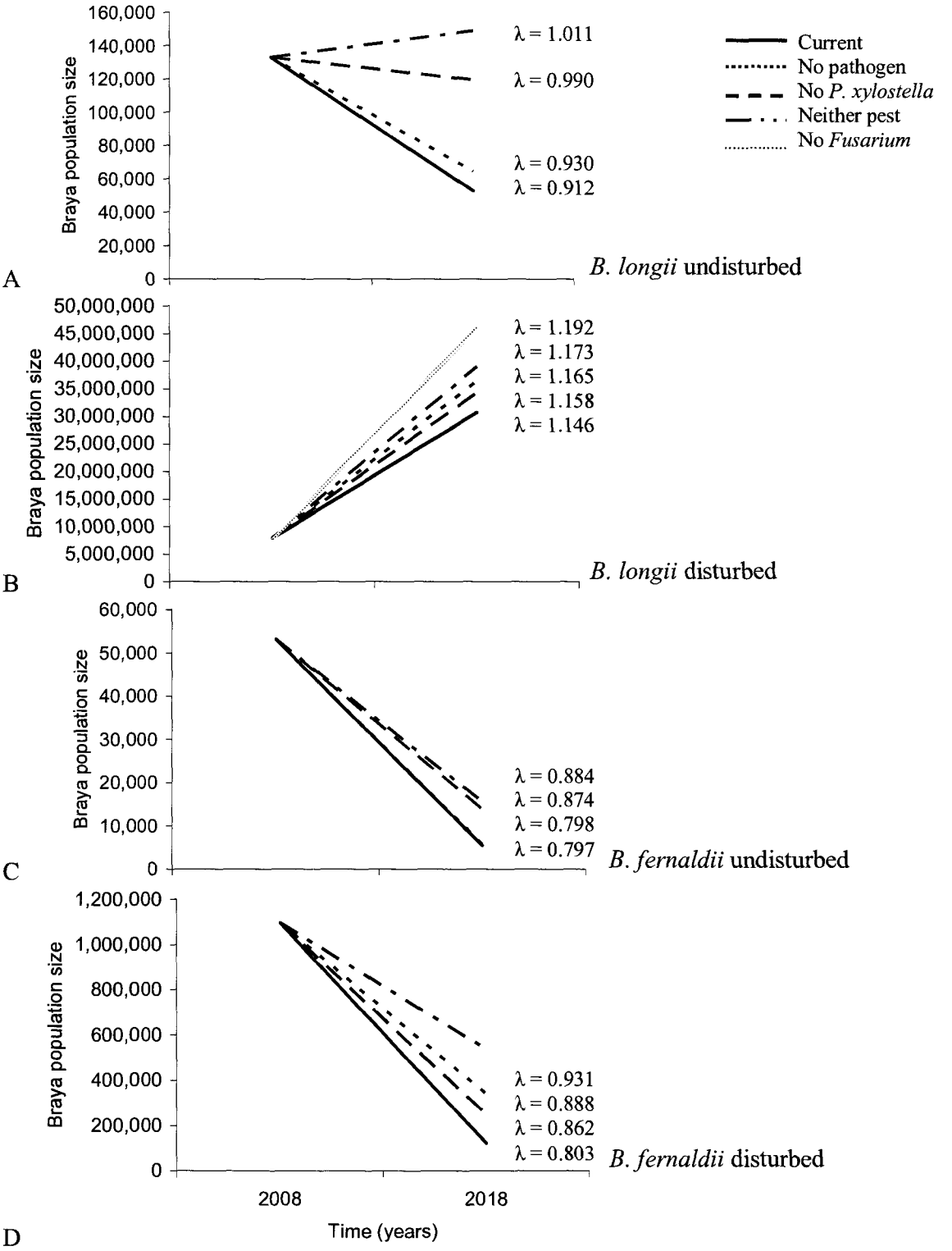

Braya population size
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
λ = 1.011
λ = 0.990
λ = 0.930
λ = 0.912
Current
No pathogen
No P. xylostella
Neither pest
No Fusarium
A
B. longii undisturbed
Braya population size
50,000,000
45,000,000
40,000,000
35,000,000
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
λ = 1.192
λ = 1.173
λ = 1.165
λ = 1.158
λ = 1.146
B
B. longii disturbed
Braya population size
60,000
50,000
40,000
30,000
20,000
10,000
0
λ = 0.884
λ = 0.874
λ = 0.798
λ = 0.797
C
B. fernaldii undisturbed
Braya population size
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
λ = 0.931
λ = 0.888
λ = 0.862
λ = 0.803
B. fernaldii disturbed
2008
2018
Time (years)
D

Figure 5.5

CHAPTER SIX

SUMMARY AND CONCLUSION

As the world continues to produce more food in response to our increasing population, it is critical that agricultural practices be modified to minimize environmental impacts, such as those of agricultural pests on our already threatened natural ecosystems. This study has shown that agricultural pests, especially *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (diamondback moth) are not just surviving on weeds in disturbed areas but can and are feeding extensively and reproducing on *Braya,* non-agricultural rare plants in natural ecosystems.

In Chapter two, I determined the infestation rate, survival, reproductive success and impact of *P. xylostella* on the endangered, *Braya longii* (Fernald) (Long's braya; Brassicaceae) and the threatened, *B. fernaldii* (Abbe) (Fernald's braya; Brassicaceae), which are endemic to the limestone barrens of Newfoundland, Canada. I found that after their mass immigration in early summer, female *P. xylostella* laid eggs on an average of 30% of the *B. longii* and 16% of the *B. fernaldii* population. Larval feeding reduced the mean seed output of infested *B. longii* by 60%, from 10.8 to 4.3 seeds per fruit, and damaged 26% of their leaves. There are residual and long-term effects of this herbivory, as many dead *Braya* had higher numbers of eggs, and subsequent leaf and fruit damage one to three years before they died. High summer air temperatures and low precipitation

allowed this pest to become multivoltine, resulting in additive damage to *Braya* individuals.

In order to mitigate the impacts on natural ecosystems, specifically the limestone barrens, it was important to determine how *P. xylostella* find *Braya* in their natural ecosystems, which typically consist of smaller, less reproductive plants at lower densities, and occur in communities with a higher species diversity compared to agricultural crops. In Chapter three, I evaluated the factors affecting infestation rates by monitoring infested and non-infested *Braya* within the entire ranges of both *Braya* species and placing experimental cabbage transplants on *B. longii* sites. Flowering individuals, regardless of size, had a 2.8-fold higher probability of *P. xylostella* infestation than non-flowering individuals. Infestation was positively associated with a higher percent cover of *Braya* and higher density of flowering *Braya*, supporting the resource concentration hypothesis, and was not affected by the presence of a common, agricultural host plant (cabbage). In contrast, the presence of native, non-host vegetation did not decrease *P. xylostella* infestation on *Braya*. Our research suggests that *P. xylostella* may also negatively impact the persistence of other rare members of this family worldwide, especially in ecosystems with little native vegetation cover, such as alpine tundra and deserts, because the insect can find and infest plants regardless of the presence of native vegetation and they damage flowering rather than non-flowering plants.

Habitat loss and fragmentation is still the most important threat to the persistence of endangered species, requiring conservation biologists, such as the Limestone Barrens Species at Risk Recovery Team, to consider the use of anthropogenically disturbed

habitats in species recovery and management plans. As a result, it was vital to compare the impact of *P. xylostella* and any other pest on *Braya* in both anthropogenically disturbed and undisturbed habitat. In Chapter four, I surveyed individually tagged *B. longii* and *B. fernaldii* for the presence of insect and pathogenic threats and their subsequent impact on seed production and survival to compare the health of *Braya* populations growing on anthropogenically disturbed and undisturbed habitat. Between 2003 and 2005, 8.6% of the *B. longii* population died from root rot, 18% of *B. longii* on anthropogenically disturbed sites were infected with an unidentified pathogen causing their flowering stalks to rot, 27% of *B. fernaldii* in northern sites had flowering stalk and leaf deformities, and 30% of surveyed *B. longii* and 16% of surveyed *B. fernaldii* were infested and damaged by *P. xylostella*. A large majority (66%-100%) of the pathogen infections occurred on anthropogenically disturbed habitat and during the study period one pathogen spread from anthropogenically disturbed populations to undisturbed populations. The presence of each pest, except for the pathogen causing *Braya* flowering stalks to rot, was linked with a statistically significant increase in mortality. Plants infested with *P. xylostella* or infected by the pathogen causing flowering stalks to rot contributed between 9% and 31% less seeds to annual seed production than healthy, flowering plants. Due to their large size, plants that died because of infection by the pathogens causing deformities and root rot would have contributed between 31% and 75% more seeds to annual seed production than healthy, flowering plants that survived. Presently, anthropogenically disturbed habitats are considered an important reservoir for *Braya* seeds in the *Braya* Recovery Plan and have received legal protection. However,

their ability to act as pest reservoirs and their lack of within population genetic diversity brings into question the conservation value of populations on degraded habitat. Our research suggests that degraded habitats need to be screened for potential negative impacts on endangered plant populations and that some sites may need to be restored to improve recovery efforts of all populations.

To determine the longer-term impacts of both *P. xylostella* and the pathogenic threats on both *Braya* species all data, as well as nine years of demographic data (1998-2006) were incorporated in Chapter five into a population viability analysis. Stage based transition matrices were created and summarized into deterministic projections. With the exception of *B. longii* populations on anthropogenically disturbed substrate, these models suggest future declines over the next 10 years for each *Braya* species on both disturbance types. The demography of *Braya* on anthropogenically disturbed and undisturbed habitat is different as *Braya* populations on undisturbed substrate is vulnerable to the mortality of large, flowering plants, where as *Braya* populations on anthropogenically disturbed substrate are vulnerable to declines in seedling survival and seed production. Management options were explored using the baseline, deterministic models and adjusting the current survival rates to reflect the survival rates of plants unaffected by the pests. The removal of any one threat improved the population viability of *Braya;* however, populations on anthropogenically disturbed substrates were projected to be most improved by the removal of pathogenic threats where as populations on undisturbed substrates were projected to be most improved by the removal of *P. xylostella.* Intervention, including the prevention of pest and pathogen mortality and the restoration

and introduction of *B. longii* and *B. fernaldii* populations into undisturbed, unoccupied habitat will improve their long- term population viability and ensure their persistence in perpetuity.

Our research clearly illustrates that we have the ability to dramatically influence the ecology and persistence of rare plant species, such as the endangered and threatened *B. longii* and *B. fernaldii,* through the indirect impacts of agriculture and habitat disturbance. Trends suggest that both agricultural and habitat disturbance will increase with expansions in the global human population and therefore likely play an ever larger role in plant ecology. Conservation biologists and managers will need to study rare plant ecology and communities will need to develop land use plans within the context of these threats to ensure appropriate management decisions are made and threats to biodiversity are removed or minimized.

APPENDIX A

2002 AND 2008 *BRAYA* POPULATION CENSUS

Adapted from:

Hermanutz, L., Squires, S., and Pelley, D. 2009. 2008 Limestone barrens research report. Wildlife Division, Department of Environment and Conservation, Government of Newfoundland and Labrador, Corner Brook, Canada.

Table 1. A comparison of the total number of flowering *Braya longii* counted in both undisturbed (N) and anthropogenically disturbed (D) habitat in 2002 and 2008.

Population	Disturbance	2002 Count	2008 Count
Anchor Point East	N	50	Not counted
Yankee Point	N	10	2
	D	1 600	3 224
Sandy Cove Airstrip	N	900	411
	D	2 400	778
Sandy Cove Lion's Club	N	180	12
	D	760	261
Sandy Cove Crusher	N	800	75
	D	500	230
Shoal Cove	D	35	556
Total		7 235	5 549

Table 2. A comparison of the total number of flowering *Braya fernaldii* counted in both undisturbed (N) and anthropogenically disturbed (D) habitat in 2002 and 2008.

Population	Disturbance	2002 Count	2008 Count
Port au Choix	N	150	54
Anchor Point east	N	250	121
Anchor point west (St. Barbe)	N	650	12
Shoal Cove	N	50	1
Green Island Brook		Not counted	2 056
Watt's Point South	D	800	12
Watt's Point	N	75	5
	D	50	2
Four Mile Cove	N	40	Not counted
Big Brook	N	3	3
Lower Cove	N	200	21
	D	100	Not counted
Watt's Bight	D	20	62
Boat Harbour	N	20	Not counted
Cape Norman	N	150	46
Cook's Point	D	25	17
Cook's Harbour south	N	0	14
	D	1	0
Burnt Cape	D	850	857
Total		3 434	3 283