AN ABSTRACT OF THE DISSERTATION OF

Deborah L. Clark for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on April 17, 1996. Title: Post-dispersal Seed Fates in a Western Oregon Native Prairie.

Redacted for privacy

Abstract approved: _____

Mark V. Wilson

Knowledge of post-dispersal seed fates and other regeneration characteristics is crucial for predicting abundances and distributions of populations and, ultimately, community species composition and diversity. Seed fate studies, however, are rare primarily due to the difficulty of determining seed fates and causes of mortality.

This thesis investigated post-dispersal seed fates for four species common to western Oregon native prairies: *Bromus carinatus* Hook and Arn. var. *carinatus*, *Cynosurus echinatus* L., *Daucus carota* L., and *Prunella vulgaris* var. *lanceolata* (Barton) Fern. The general approach was to sow seeds of these species into experimentally manipulated field plots for each of two years, and to recover these seeds from the soil one year later to determine their fates (persistance, death, or establishment as seedlings). The effect of mowing on seedling establishment was also addressed. Additional studies focused on the effects of a single mortality factor, fungal disease, on seed and seedling deaths.

The fate of most seeds was death (44%-80%). Few seeds established as seedlings (4%-17%), and mowing did not significantly increase seedling establishment. Only *Daucus carota* formed a persistent seed bank.

Fungal disease generally caused less than 10% mortality. Pot studies corroborated these field results. Other investigators have suggested higher levels of disease in natural vegetation.

Vertebrate predation significantly reduced seed numbers for only *Bromus carinatus* (21%). The largest cause of death for all species for both years was the combined group of other mortality causes (invertebrate predation, interference, and abiotic factors) (52%-73%). The components of this combined group, however, differed among species. The most likely components for *Bromus carinatus* and *Cynosurus echinatus* were interference (competition plus allelopathy) and abiotic factors, although invertebrate predation cannot be ruled out for *Bromus carinatus*. Seedling death due to abiotic factors was most likely the largest component for *Daucus carota*. The most probable components for *Prunella vulgaris* were invertebrate predation and abiotic factors.

Implications of these findings for population patterns and for restoration of native prairies are discussed.

Post-dispersal Seed Fates in a Western Oregon Native Prairie

by

Deborah L. Clark

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented April 17, 1996
Commencement June 1996

Doctor of Philosophy dissertation of Deborah L. Clark presented April 17, 1996

APPR Redacted for privacy

Major Professor, representing Botany and Plant Pathology

Redacted for privacy

Chair of Department of Botany and Plant Pathology

Redacted for privacy

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for privacy

Deborah L. Clark, Author

ACKNOWLEDGMENT

I thank the members of my doctoral committee, Mark V. Wilson, Kenneth B. Johnson, Patricia S. Muir, Donald B. Zobel, and Jeffrey C. Miller, for their encouragement and invaluable advice, particularly during the writing stage. I extend special gratitude to my major professor, Mark Wilson, who always made time for my many research questions, who always had creative suggestions for my many research problems, and who gave me exceptional training as a scientist. I also thank him for providing numerous opportunities to expand my research experiences outside my own dissertation research.

Lisa Lantz kindly allowed the use of her unpublished data collected at our joint study site. I am also grateful for her companionship during long days in the field. I am also indebted to Cynthia Haley for her able assistance with field and laboratory work. Elaine R. Ingham generously conducted the fungal biomass analyses. I thank Kathy Merrifield for graciously sharing the soil sieving facilities.

The Ecology and Forest Pathology lab group was a source of constant support and encouragement throughout my graduate program. I thank each and every one of these people for their unfailing good humor and friendship.

I gratefully acknowledge financial support from a P.F. Yerex Graduate Fellowship, The Nature Conservancy, the Mazamas, and a Sigma Xi Grant-in-Aid of Research. I thank Benton County Parks and Recreation for permission to use the Open Space Park as my study site.

Finally, this project would not have been possible without my husband, Jim Clark. I sincerely thank him for his support.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (CONTINUED)

LIST OF TABLES (CONTINUED)

LIST OF TABLES IN APPENDICES

POST-DISPERSAL SEED FATES IN A WESTERN OREGON NATIVE PRAIRIE

CHAPTER 1

INTRODUCTION

RATIONALE

Events that occur during the regeneration stage of plants' life cycles are crucial in determining the distribution and abundance of plant populations (Grubb 1977, Harper 1977, Grime 1979, Werner 1979, Gross and Werner 1982, Smith 1983). Community composition and diversity are also determined by differences among species in their regeneration characteristics and safe-site requirements (Grubb 1977, Masuda and Washitani 1990, Grime and Hillier 1992). Furthermore, these differences in regeneration patterns affect successional patterns by determining the probability of a species' seedlings colonizing in communities of different successional stages (Gross and Werner 1982, Olff et al. 1994).

The survival of seeds is essential for successful regeneration. Measuring the progressive mortality of seeds caused by different factors allows development of techniques that reduce or increase these losses, consequently controlling the abundance of seedlings (e.g., Lawrence and Rediske 1962). This knowledge of regeneration characteristics, then, is a key to successful control of exotic weeds, management of natural resources, and the restoration and conservation of native species and habitats.

Even though studies on virtually every aspect of seed biology demonstrate the importance of species regeneration from seeds, few studies follow the fate of individual

seeds. Following seed fates is difficult, because most seeds are small and inconspicuous. The biotic and abiotic factors that influence seed death, seed persistence, and seedling establishment are exceptionally difficult to analyze in natural populations. Thus, no balance sheet exits that permits accounting for all the seeds that a plant produces (Chambers and MacMahon 1994).

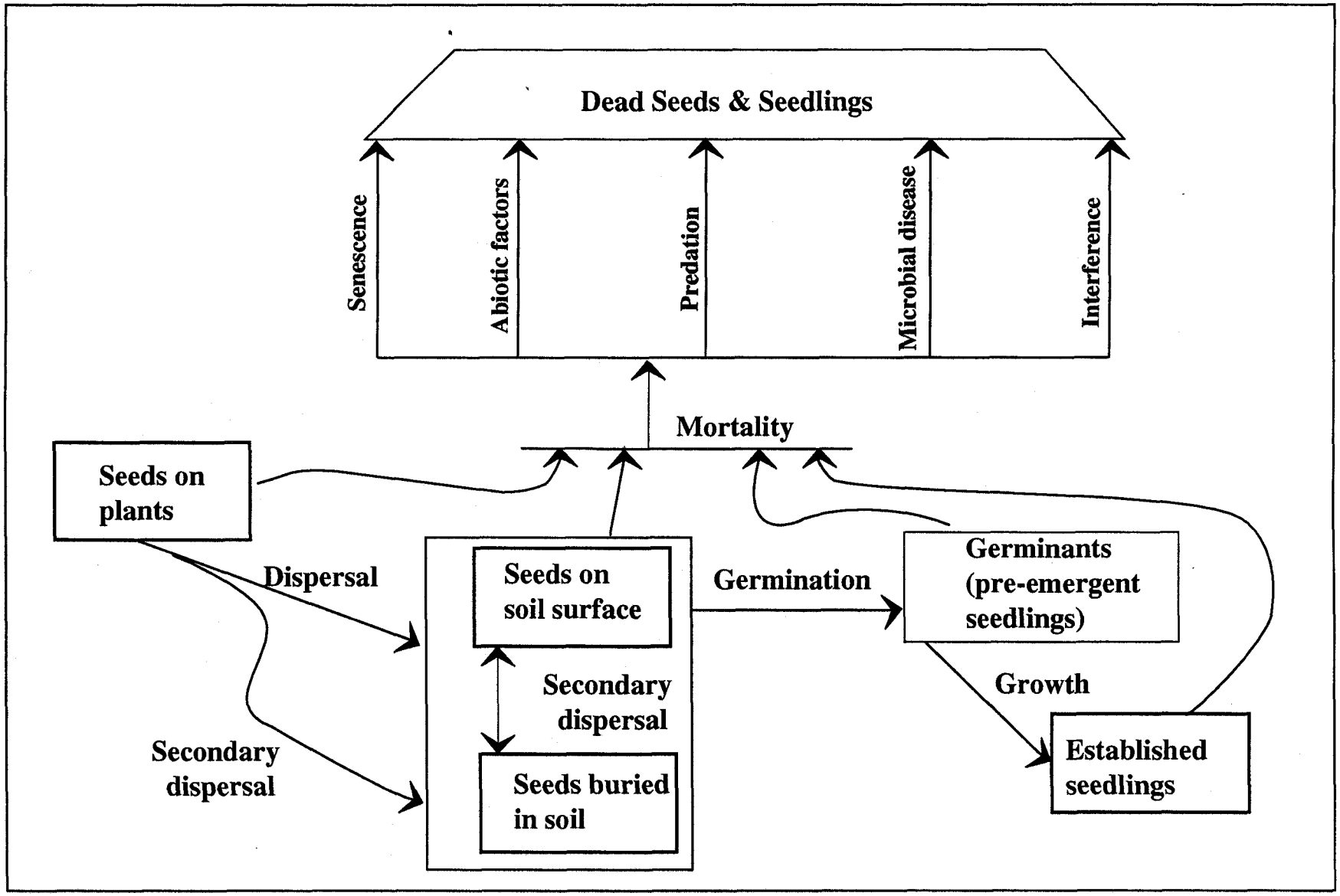
Model

The model in Figure 1.1 outlines the potential pathways that seeds follow after dispersal. Seeds generally arrive on the soil surface, where they remain or move deeper into the soil by burial. Persistent seeds are either dormant or quiescent until conditions are appropriate for germination. Germination can occur immediately after dispersal, or after persistence in the soil. Death can occur at several points along the pathway: immediately after dispersal, during persistence in the soil, or during germination and seedling growth. Seeds generally die from senescence, predation, and microbial disease, while seedlings die from microbial disease, herbivory, interference from neighboring plants, and abiotic factors.

Mortality

It is extremely difficult to determine the causes of death for seeds and germinants (pre-emergent seedlings), especially for small seeds under field conditions. Germination of the seed at a depth from which it fails to emerge is the usual explanation for the large

Figure 1.1. Model describing fates of seeds and the processes controlling the magnitudes of these fates.


Dead Seeds & Seedlings
Senescence
Abiotic factors
Predation
Microbial disease
Interference
Mortality
Seeds on plants
Dispersal
Seeds on soil surface
Germination
Germinants (pre-emergent seedlings)
Secondary dispersal
Growth
Secondary dispersal
Seeds buried in soil
Established seedlings

number of seeds that fail to emerge in most seed bank investigations (Schafer and Chilcote 1970, Cook 1980, Washitani 1985). But, because dead seeds and germinants decay in the soil, death caused by senescence, pathogenic attack, and fatal germination are indistinguishable. Causes of seedling mortality are also difficult to determine. When a young seedling emerges, it may die in a few hours, leaving no detectable trace. Another seedling may immediately emerge in the same place. Even with a dead plant's remains, it is often impossible to assign a cause of death.

As environmental conditions vary unpredictably from year to year, so undoubtedly does the relative importance of mortality factors (e.g., Mack and Pyke 1984). Although a mortality factor causes minor losses at a particular time or place, it may cause major losses at another time and place, depending on changes in the environment. Moreover, variability in magnitude and causes of mortality can prevent competitive exclusion of species, promoting increased community diversity (Crawley and Pacala 1990). On the other hand, consistent mortality from a single factor can potentially decrease population size (Louda 1989, Anderson 1989, Harper 1990), control population spatial distribution (Tadros 1957, van Leeuwen 1989, Louda 1989, Augspurger 1990, Reader and Beisner 1991) and influence community species composition (Borchert and Jain 1978). Furthermore, mortality patterns influence the evolution of a species by determining the relative reproductive success of individual plants. Note that this evolutionary effect can occur even if specific mortality factors have no impact on plant population size (Crawley 1992, Anderson 1989).

Seed fate studies

The handful of studies that have attempted to quantify the post-dispersal fate of seeds in natural vegetation vary in their degree of completeness (Table 1.1). Generally, seed persistence and seedling survival are measured, but rarely are mortality factors determined, with the exception of predation (Table 1.1). This lack of seed fate studies hampers our ability to generalize about seed and seedling mortality patterns. Knowledge of these and other regeneration characteristics is essential to predict population distribution and abundance patterns and ultimately plant community species composition and diversity.

OBJECTIVES

The second chapter of this dissertation reviews the scientific literature on the post-dispersal fates of seeds and the processes controlling these fates, emphasizing studies from grasslands. Specifically, Chapter 2 describes the previously known patterns for each mortality factor (senescence, predation, microbial disease, interference, abiotic environmental factors) and discusses the mechanisms by which seeds and seedlings resist these mortality factors.

The objective of Chapter 3 was to determine, using an experimental approach, the magnitudes of post-dispersal seed fates and the processes controlling these fates for four species common in western Oregon native prairies. The general approach was to sow seeds of these four species into experimentally manipulated field plots for each of two years, and to recover these seeds from the soil one year later to determine their fates.

Table 1.1. Summary of measured post-dispersal seed fates and mortality factors.

Study	Survival		Mortality factors					
	seed	seedling	combined	predation	senescence	abiotic	disease	interference
Lawrence and Rediske 1962 (conifer forest)	x	x		x		x	x	
Sarukhan 1974 (grassland)	x	x	x					
van Baalen 1982 (forest clearings)	x	x	x					
Pavone and Reader 1982 (abandoned pasture)	x	x	x					
Smith 1983 (deciduous forest understory)	x	x		x				
Holthuijuzen et al. 1987 (pastures)	x	x		x				
Alvarez-Bullya and Martinez-Ramos 1990 (tropical forest)	x	x		x				
Kalisz 1991 (forest understory)	x	x		x				
Hughes and Westoby 1992 (sclerophyll vegetation)		x		x				
Horvitz and Schemske 1994 (tropical forest)	x	x		x				
Vander Wall 1994 (semi-desert shrubland)	x	x		x				
Chambers 1995 (alpine herbfields)	x	x	x					

The study species were two grasses, *Bromus carinatus* Hook and Arn. var. *carinatus* H. and A. (native perennial bunchgrass) and *Cynosurus echinatus* L., (non-native annual) and two dicots, *Prunella vulgaris* var. *lanceolata* (Barton) Fern.(native perennial), and *Daucus carota* L., (non-native biennial).

Field experiments were conducted in an upland prairie, dominated by the native bunchgrasses *Festuca idahoensis* var. *roemeri*, *Bromus carinatus* var. *carinatus* and *Elymus glaucus*. The site is one of the few remnants of a vast prairie and oak-savanna ecosystem that covered much of the Willamette Valley until the immigration of settlers into Oregon beginning about the 1840's (Boag 1992). Diaries and letters of these settlers report that extensive portions of Willamette Valley grasslands were burned annually by the indigenous Calapooia people to allow for easier travel, hunting and gathering of food plants (Boag 1992). These fires prevented tree and shrub establishment, thus maintaining the grasslands.

Today these prairies and oak-savannas are considered among the rarest of western Oregon's ecosystems, because of their destruction by agricultural, grazing and urban activities. The remaining parcels are threatened by invasion of both woody species and non-native weeds. Managers of protected remnants want to increase or maintain native species in these prairies, while discouraging non-native invaders. Although the prairies were historically maintained by burning, fire is not always a feasible management tool today because of environmental regulations and proximity of prairies sites to urban areas. Thus, alternatives, such as mowing, have been considered. Mowing can be effective in reducing the abundance of woody plants, but the effect on seed regeneration of native and non-native species in Valley prairies is generally unknown (but see Wilson and Clark

1995). Chapter 5 investigates the effects of mowing on seed regeneration of the four study species. The general approach was to sow seeds of the four species into mowed and unmowed field plots and to compare regeneration from seeds between the treated and untreated plots one year later.

Of all the factors causing seed and seedling death in natural vegetation, microbial disease is the least known. Given the substantial magnitude of seedling death caused by fungi in agricultural systems (Sewell 1981, Harman and Stasz 1986), fungal disease is likely to be a significant cause of the tremendous seedling mortality rate in natural ecosystems (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989, Thompson and Baster 1992, Reader 1993). But the effect of fungi on seeds and seedlings in natural systems is rarely documented in natural vegetation (Burdon 1987, Kranz 1990), due to the inherent difficulties in ascribing seed and seedling losses to one of several possible mortality factors. In particular, quantifying disease effects are especially difficult, e.g., distinguishing between saprophytic decay of senescent seeds and pathogenic attack on seeds and seedlings. One potentially practical method for determining the magnitude of death caused by fungi is to exclude fungi experimentally with fungicides (Burdon 1987, Paul et al. 1989, Harper 1990). Chapter 4 investigates the magnitude of seed and seedling death caused by fungal disease for the study species. The approach used to address this question included both field and pot experiments in which soil-borne pathogens characteristically associated with seed and seedling deaths were chemically excluded.

Chapter 6 summarizes of the results of the three major research topics, (1) the post-dispersal seed fates, (2) the effects of fungal disease on seeds and seedlings in natural vegetation, and (3) the effects of mowing on survival of sowed seeds.

Several ancillary studies, described in the appendices, were necessary to complete the main research investigations. Appendix 1 describes an experiment conducted to determine whether the cages used in Chapters 3 and 5 had effects on seed survival other than exclusion of vertebrate predators. Test results that determined possible adverse fungicide effects on seed germination are reported in Appendix 2. Appendix 3 describes preliminary tests conducted to determine the efficacy of techniques to retrieve seeds from soil after sowing in field plots. Finally, the germination percentages of seeds stored in the laboratory over a period of 2-3 years are reported in Appendix 5.

CHAPTER 2
LITERATURE REVIEW OF POST-DISPERSAL SEED FATES IN GRASSLANDS

INTRODUCTION

The regeneration stage of a plants' life cycle is crucial in determining the distribution and abundance of plant populations (Grubb 1977, Harper 1977, Grime 1979, Werner 1979, Gross and Werner 1982, Smith 1983). Community composition and diversity are also influenced by differences among species in their regeneration characteristics and safe-site requirements (Grubb 1977, Masuda and Washitani 1990, Grime and Hillier 1992). Furthermore, these differences in regeneration patterns can affect successional patterns by determining the probability of a species' seedlings colonizing in communities of different successional stages (Gross and Werner 1982, Olff et al. 1994).

The survival of seeds is essential for successful regeneration. Measurements that quantify the progressive mortality of seeds caused by different factors may allow development of techniques that reduce or increase these losses, consequently controlling the abundance of seedlings (e.g., Lawrence and Rediske 1962). Knowledge of regeneration characteristics, then, is a key to successful exotic weed control, management of natural resources, and the restoration and conservation of native species and habitats.

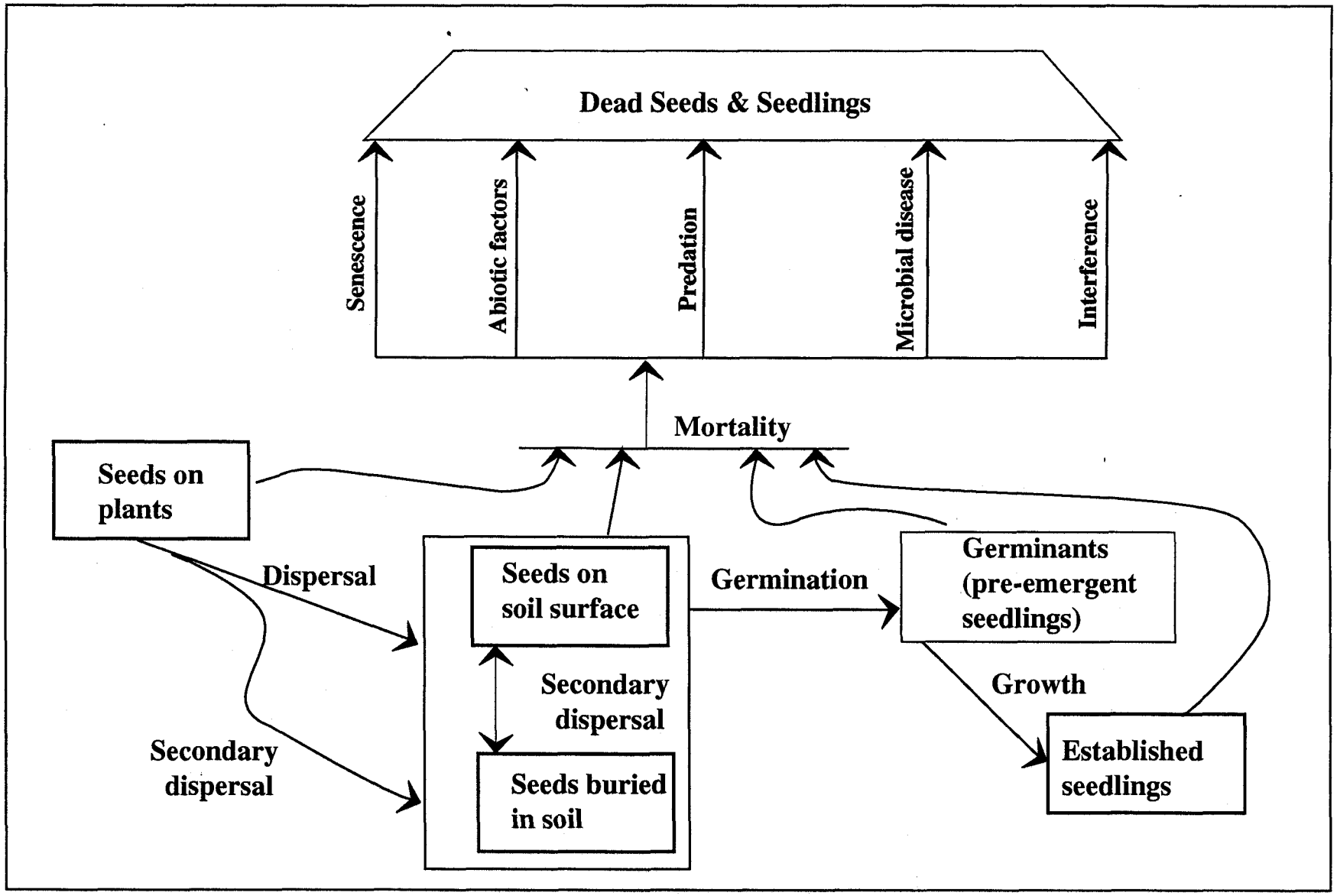
Even though studies on virtually every aspect of seed biology demonstrate the importance of species regeneration from seed, few studies follow the fate of individual seeds. Following seed fates is difficult, because most seeds are small and inconspicuous. The biotic and abiotic factors that influence seed death, seed persistence and seedling

establishment are exceptionally difficult to analyze in natural populations. Thus, no balance sheet exists that permits accounting for all the seeds that a plant produces (Chambers and MacMahon 1994).

The model in Figure 2.1 outlines the potential pathways that seeds follow after dispersal. Seeds generally arrive on the soil surface, where they remain or move deeper into the soil by burial. Persistent seeds are either dormant or quiescent until conditions are appropriate for germination. Germination can occur immediately after dispersal or after persistence in the soil seed bank. Death can occur at several points along the pathway: immediately after dispersal or after persistence in the soil, or during germination and growth. Seeds die from senescence, predation, and microbial disease, while seedlings die from microbial disease, herbivory, interference from neighboring plants, and abiotic factors.

The scope of this review is limited to post-dispersal fates of seeds and the processes influencing these fates, although pre-dispersal mortality and dispersal patterns are important in determining regeneration patterns and consequently, population and community characteristics (Louda 1989, Chambers and MacMahan 1994). The review is divided into three sections, each of which discusses one seed fate, (1) death, (2) germination and growth as seedlings, and (3) persistence as seeds. Mortality patterns and the processes controlling seed and seedling death are presented first, because mortality factors are integrated with and subsequently determine the patterns of the other two seed fates. The ecological patterns associated with each mortality factor are described and then the mechanisms by which seeds and seedlings resist death by that factor are

Figure 2.1. Model describing fates of seeds and the processes controlling the magnitudes of these fates.


Dead Seeds & Seedlings
Senescence
Abiotic factors
Predation
Microbial disease
Interference
Mortality
Seeds on plants
Dispersal
Seeds on soil surface
Germination
Germinants (pre-emergent seedlings)
Secondary dispersal
Growth
Secondary dispersal
Seeds buried in soil
Established seedlings

discussed. Post-dispersal seed fates in grasslands are emphasized, although examples from other ecosystems are used when grassland examples are not available.

SEED FATES

Mortality

Patterns of mortality

Mortality rates are probably highest during the seed and seedling phases of a plant's life (e.g., Sharitz and McCormick 1973, Watkinson 1978, Cook 1979, Hickman 1979, Cavers 1983). Generally, small percentages of the seeds produced by plants emerge and establish as seedlings (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989, Thompson and Baster 1992, Reader 1993).

Demographic studies generally focus on the number of survivors at each stage in the life cycle, with little attention paid to the causes of death (e.g., Burdon et al. 1983, Lee and Hamrick 1983, Dolan and Sharitz 1984, Klemow and Raynal 1985, Kachi and Hirose 1985). In particular, studies rarely distinguish between the causes of seed and seedling death or quantify the relative magnitude of these causes for a particular species. It is doubtful whether a whole schedule of mortality has ever been determined for any plant species in natural vegetation (Harper 1990).

It is extremely difficult to determine the causes of death for seeds and germinants (pre-emergent seedlings), especially for small seeds under field conditions. Germination of the seed at a depth from which it fails to emerge is the usual explanation for the large number of seeds that fail to emerge in many seed bank investigations (Schafer and Chilcote 1970, Cook 1980, Murdoch and Roberts, 1982, Washitani 1985). But, because

dead seeds and germinants decay in soil, death caused by senescence, microbial disease, and fatal germination are indistinguishable.

Causes of seedling mortality are also difficult to determine. When a young seedling emerges, it may die within a few hours, leaving no detectable trace. Another seedling may immediately emerge in the same place (Harper 1990). Even with dead plant remains, it is often impossible to assign a cause of death. Thus, only with extremely tedious, almost continuous observation, such as with a video camera (Fenner 1987), could the true magnitude and causes of seedling mortality be measured. Nevertheless, a few studies have attempted to determine causes of death by (1) monitoring at frequent intervals and making deductions from available evidence, (2) establishing correlations between mortality and relevant factors, such as seedling density, distance from parent, and density of surrounding vegetation, and (3) experimentally manipulating mortality factors by altering the environment (Fenner 1987).

As environmental conditions vary unpredictably from year to year, so undoubtedly does the relative importance of mortality factors for a single species (e.g., Mack and Pyke 1984). Although a mortality factor causes minor losses at a particular time or place, it may cause major losses at another time and place, depending on changes in environment. Moreover, variability in magnitude and causes of mortality can prevent competitive exclusion of plant species, promoting increased species diversity of the community (Crawley and Pacala 1990). On the other hand, consistent mortality from a single factor can potentially decrease population size (Louda 1989, Anderson 1989, Harper 1990), control the population spatial distribution, (Tadros 1957, Janzen 1970, van Leeuwen 1981, Louda 1989, Augspurger 1990) and influence community species

composition (Borchert and Jain 1978). Furthermore, mortality patterns influence the evolution of a species by determining the relative reproductive success of individual plants. Note that this evolutionary effect can occur even when specific mortality factors have no impact on plant population size (Crawley 1992, Anderson 1989).

Senescence

Patterns of longevity

Many studies have attempted to determine the maximum life span of seeds using (1) inferences made from associated materials near the seeds and from the time of last known disturbance, (2) plant materials from herbarium sheets, and (3) experimental data (reviews by Turner 1933, Harrington 1972, Priestly 1986). Seed age has been inferred by estimating the age of the materials found associated with the seeds and of buildings above the excavation site (Odum 1974). Age of buried seeds has also been inferred from the time of last known disturbance (Brenchly 1918, Livingston and Allessio 1968). Estimates of the longest lived seeds range from 150 to several thousand years.

The best documented claims for longevity are those based on known seed collection data and experiments. The ages of long-lived seeds taken from herbarium sheets range from 158 years to more than 200 (Ramsbottom 1942, Turner 1933). Members of the Fabaceae family commonly have the longest lived seeds. Other estimates are based on experiments in which seeds were buried in the soil and samples removed periodically over many years (Beal 1905, Darlington 1922, Turner 1933, Toole and Brown 1946, Youngman 1951, Darlington and Steinbauer 1961, Kivilaan and

Bandurski 1973). Seeds of several species survived 80-100 years under these conditions (Darlington and Steinbauer 1922, 1961, Kivilaan and Bandurski 1981).

Mechanisms

Senescence is death caused by physiological processes. Prior to death, as the seed ages, metabolic processes gradually decline with increasing inactivation of enzymes, including those that control germination (Mayer and Poljakoff-Mayber 1989). The mechanism of seed aging is not fully understood, although several theories (reviews by Roberts 1972, Priestly 1986) suggest various factors are responsible for the generalized degradation of membranes, polymers, and other systems within seeds (Harman and Stasz 1986, Mayer and Poljakoff-Mayber 1989). Genetics control the maximum length of time that seeds can remain viable (Mayer and Poljakoff-Mayber 1989). However, environmental factors ultimately determine the physiological life span of any given seed, i.e., whether the seed will remain viable for the full period allowed by its genome or whether it will lose its viability at some earlier stage. Generally, conditions that greatly reduce metabolic activity, such as low temperatures and high carbon dioxide concentrations, allow the longest seed viability (Mayer and Poljakoff-Mayber 1989). Specifically, species generally fall into three groups based on the relationship between environmental conditions and seed longevity. For seeds of most crops and many non-cultivated species, seed longevity increases with decrease in seed storage moisture content and temperature in a quantifiable and predictable way (Roberts and Ellis 1982). In contrast, recalcitrant seeds (e.g., seeds of many large seeded woody perennials) do not survive desiccation (Roberts and Ellis 1982, Murdoch and Ellis 1992). The third

category shows intermediate seed storage behavior for a limited number of species in which low temperatures and further desiccation injure dry seeds (Murdoch and Ellis 1992).

Animal predation

Patterns of animal predation

The literature on post-dispersal predation and its consequences for plant populations and communities is voluminous, with several thorough reviews (Janzen 1971, Price and Jenkins 1986, Louda 1989, Sallabanks and Courtney 1992, Crawley 1992). Documentation is extensive, showing tremendous losses of potential off-spring to seed predators with 100% losses frequently recorded (Janzen 1971, Reichman 1979, Crawley 1983, 1992, Hendrix 1988). The magnitude of post-dispersal predation for a species varies considerably and is generally unpredictable (Louda 1989, Hulme 1994). The probability of predation varies with habitat, microhabitat, plant species, seed burial, seed density, seed crop size, predator density, year, season, and the availability of alternative food for generalist seed predators (Price and Heinz 1984, Thompson 1987, Louda 1989, Willson and Whelan 1990, Reader and Beisner 1991, Crawley 1992, Hulme 1994). Thus, the search for simple patterns to describe the causes and consequences of variation in seed predation rates has been relatively unsuccessful (Crawley 1992), strongly suggesting that from a plant's "point of view", there are few if any predictable sites for escaping seed predators (Whelan et al. 1991).

Agents

Animals consuming seeds range from rodents, bats and birds to ants, ground beetles, slugs, snails, and earthworms (Crawley 1983, 1992). In contrast to pre-dispersal predators, post-dispersal seed predators tend to be larger, more mobile, generalist herbivores such as rodents and birds (Thompson 1987, Crawley 1992.). Nevertheless, larger insects like ants, lygaeid bugs and carabid beetles can be significant post-dispersal seed predators (Crawley 1992), particularly in deserts and nutrient poor communities (Reichman 1979, Brown et al.1986). In grasslands, rodents are principal post-dispersal seed predators (Hulme 1994) with the effects of ants and, particularly, other invertebrates less well known (Thompson 1987).

Mechanisms resisting animal predation

Predation on large seeds, especially those with thin seed coats, are generally severe, much greater than those on small or hard coated seeds (Janzen 1971, Abramsky 1983, Louda 1989), possibly due to differences in ease of handling by rodents or seed nutrient content (Thompson 1985). Also, small compact seeds are more easily buried in the soil than larger, more elongated seeds, thus reducing seed predation rates (Thompson et al. 1993). But rodents, in contrast to ants and beetles, which are strictly surface foragers, can find and dig up the larger size buried seeds (Abramsky 1979, Reichman 1979, Crawley 1992, Hulme 1994).

Many studies stress the importance of chemical defenses in protecting seeds from disease, predation and herbivory (Ferenczy 1956, Srivastava and Mishear 1971, Halloin 1983, Baker 1989, Waterman 1992). For example, a highly significant correlation exists

between seed persistence of British species in the soil and concentration of ortho-dihydroxyphenol in the seeds (Hendry et al. 1994). Relatively short-lived seeds have lower concentrations of ortho-dihydroxyphenols while longer-lived seeds (more than five years persistence in seed bank) have higher ortho-dihydroxyphenol concentrations (Hendry et al. 1994). Phenolic compounds resist bacterial and fungal attack in *in vitro* studies, and possibly deter predation and herbivory by insects (Feeny 1970), molluscs (Mott 1987), mammals (Tahvanainen et al. 1985). Of the 21 species with the lowest ortho-dihydroxyphenol concentration, 14 were grasses that generally do not form persistent seed banks (Hendry et al. 1994). Furthermore, the higher the nutritional (protein) value of the seed, the greater the concentration of the defense compound, ortho-dihydroxyphenol (Hendry et al. 1994). Thus, these correlations suggest that presence of ortho-dihydroxyphenol deters seed predation, promoting seed persistence in the soil.

Microbial disease

Patterns of microbial disease

In contrast to the multitude of predation studies, ecological studies that quantify seed and seedling mortality by microbial pathogens in natural vegetation are rare (Burdon 1987, Kremer 1993). This lack of documentation results from difficulties of quantifying disease effects, e.g., distinguishing between saprophytic decay of senescent seeds and pathogenic attack of seeds and seedlings. But given the substantial magnitude of seedling death caused by microbial pathogens in agricultural systems (Sewell 1981, Harman and Stasz 1986), pathogens are likely to be a significant cause of the high

seedling mortality rates in natural ecosystems (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1984, Thompson and Baster 1992, Reader 1993).

Microbial decay of seeds and germinants is assumed to cause significant unexplained losses in many seed bank studies (e.g., Roberts and Feast 1972). Direct evidence for death by microbes comes from buried seed studies that quantify the survival of seeds in the soil seed bank or describe seed dormancy patterns (Zorner et al. 1984, Washitani 1985, Granstrom 1987, Pons 1989, 1991, Bridgemohan et al. 1991, Pierce and Cowling 1991, Crist and Friese 1993). Generally, seeds enclosed in bags were buried in the soil, retrieved at regular intervals, and the number of germinants, and viable and dead seeds quantified. Frequently, the percentage of decayed seeds was high, but the investigations did not determine whether decay was saprophytic or pathogenic.

The rare studies investigating causes of seedling mortality by fungi in natural vegetation describe significant mortality by fungi (Lawrence and Rediske 1962, Mack and Pyke 1984, Augspurger 1990). Fungi accounted for the greater part of seed loss during the pre-germination period of Douglas-fir seeds and were also the principle cause of Douglas-fir seedling death (Lawrence and Rediske 1962). Fungal disease (damping-off) ranked from high to very low in importance relative to other mortality factors in a series of studies involving tropical tree seedlings (Augspurger 1990). A significant number of *Bromus tectorum* seedlings died from an infestation of a smut, *Ustilago bullata*, over a three year period in a semi-arid grassland (Mack and Pyke 1984).

Agents

Although seeds are common vectors of bacteria and viruses (Neegarrd 1977), rarely do studies report death of dispersed seeds or seedlings caused by bacterial or viral diseases in agricultural systems. In contrast, many studies investigating seed-bacteria interactions report antagonistic reactions by seedborne bacteria against fungi, potentially protecting the seed from fungal disease (Liu and Vaughan 1965, Kremer et al. 1984, Bruehl 1987).

Pathogens of the genera, *Pythium, Phytophthora, Rhizoctonia, and Fusarium*, are known to be major causes of seed and seedling death in agricultural systems, and are among the most ubiquitous of soilborne fungi (Sewell 1981, Harman and Stasz 1986, Bruehl 1987, Kranz 1990). Yet the effect of these pathogens on seeds and seedlings in natural systems is rarely documented, due to the inherent difficulties in determining cause of death (Burdon 1987, Burdon and Shattock 1980).

Water soluble exudates from seeds and plant roots are the major sources of nutrients for many of these soil-inhabiting fungi are (Harman and Stasz 1986). These exudates stimulate germination of fungal spores and mycelial growth, promoting infection (Sewell 1981, Harman and Staz 1986, Burdon 1987). When physiological aging, weathering, or mechanical damage reduce the integrity of the seed coat, the amount of exudate may increase, increasing the susceptibility of the seed to microbial attack (Halloin 1983).

Because dormant seeds have little physiological activity, the amount of exudate is small, potentially rendering dormant seeds virtually "invisible" to many soilborne pathogens, and thus, minimizing mortality (Burdon and Shattock 1980, Sewell 1981).

But the cell membrane integrity gradually declines as dormant seeds age, resulting in nutrient loss into the surrounding environment, which simulates the fungal and bacterial growth, increasing the risk of pathogen attack (Burdon and Shattock 1980, Burdon 1987). Thus, microorganisms may preferentially colonize and decay seeds that are inferior in quality or are already dying physiologically, accelerating loss of older potentially non-viable seeds rather than killing viable seeds (Burdon 1987). No studies clearly distinguish between decay caused by microbial pathogens and that occurring after seeds die (Harman and Stasz 1986, Kremer 1993).

Seeds also produce exudate during germination. Exudation rates immediately increase at imbibition, releasing soluble organic and inorganic substances, including a variety of sugars and amino acids (Lynch 1978, Burdon and Shattock 1980, Anderson and Baker 1983, Harman 1983, Burdon 1987). Numerous examples of fungal stimulation by these exudates during imbibition are available (Sewell 1981, Harman and Staz 1986, Burdon 1987). Thus, germinants appear to have greater risk of pathogen attack caused by the stimulation of fungal spore germination and mycelial growth compared to young dormant seeds.

Mechanisms resisting microbial disease

The most important component in seed resistance of many crop species to pathogens is a structural defense, the seed coat (Halloin 1983). Resistance in non-crop species may occur in a similar manner, but little quantitative information exists (Kremer 1993). Hard-seed coats consist of a continuous layer of densely packed palisade layers that are mechanical barriers, similar to plant epidermal cells, preventing fungal

penetration (Halloin 1983, Kremer et al. 1984). Once these hard coats are damaged, fungi readily infect the seeds (Harman 1983, Halloin 1983).

Other resistance mechanisms include chemical defenses such toxic compounds in the seed and seed coat (Rice 1984). Seeds of legumes commonly contain alkaloids, which are extremely fungistatic (Baker 1989). Broad-spectrum toxins to microbes include phenolic compounds (Kremer 1993). Both seeds and vegetative parts of plant produce phytoalexins, another chemical resistance mechanism, in response to fungal infection (Keen 1975, Harman 1975).

In addition to structural and chemical defenses, associations with microflora inhibit seed decay by fungi. Microflora, such as fluorescent *Pseudomonas* spp., *Enterobacter cloacae*, *Bacillus subtilis* and *Streptomyces* spp., easily colonize seeds and are antagonistic to various seed rotting fungi (Kremer et al. 1984, Bruehl 1987). Several mechanisms explain how seedborne microorganisms protect against disease. If the seedborne microflora are present in great numbers and can respond quickly to exudates, they consume the exudates before these exudates can overcome fungistasis of pathogenic propagules in the soil (Bruehl 1987). The seed microflora can rapidly produce antibiotics, inhibiting some pathogens (Bruehl 1987). Slightly pathogenic microflora can elicit an incompatible host response, giving the seed temporary resistance to more virulent pathogens (Bruehl 1987). Antagonistic microflora also can compete with the pathogenic fungi for essential growth factors or for infection sites (Bruehl 1987).

Interference

Patterns

Interference is used to refer to the overall adverse influence of one plant on another, thus encompassing both competition, which involves the reduction of resources needed by a neighboring plant, and allelopathy, which refers to the biochemical interactions between organisms caused by the addition of a toxic chemical compound to the environment by one of the organisms (Rice 1984).

Generally, few seeds successfully germinate and grow in dense unmowed grasslands. Most seedlings need small patches of bare soil or short turf, but even here seedling survival can be restricted to species with relatively large seeds (Fenner 1978, Gross and Werner 1982, Gross 1984, Winn 1985, McConnaughay and Bazzaz 1987, Peart 1989, Silvertown and Tremlett 1989, Reader 1991, Thompson and Baster 1992, Wilson and Gerry 1995). The small patches allow seedlings to avoid death apparently caused by competition for space, light, nutrients, and/or water.

Competitive outcomes between seedlings and neighboring plants may be influenced by the formation of mycorrhizae (Janos 1980). The failure to form mycorrhizae, or even a delay in formation, may put a seedling at a competitive disadvantage, particularly in nutrient poor soils (Janos 1980). The magnitude of death due to lack of mycorrhizal formation for grassland species is unknown.

Although competition is a major factor in determining spatial distribution of plants, Rice (1984) suggests that allelopathy probably also plays a role in most, if not all, vegetation spatial distributions. Numerous studies have implicated allelopathy as a major cause of seedling mortality in grasslands (Rice 1984).

Mechanisms resisting death by interference

Species with small seeds (<1 mg) are often unable to establish in competition with perennial vegetation (Gross and Werner 1982, Reader 1993, Gross 1984, Winn 1985). In contrast, Thompson and Baster (1992) and Fenner (1978), report that seed weight has little effect on seedling establishment success.

Plants can resist competition between seedlings and mature neighboring plants by delaying seed germination until conditions are appropriate for promoting maximum seedling survival. One mechanism, canopy-induced seed dormancy, prevents seed germination as long as the seed is shaded by surrounding plants. The light filtering through a leaf canopy is richer in wavelengths from the far-red end of the spectrum (700-800) and poorer in those from the red end (620-680) compared to full daylight (Pons 1992). The plant photoreceptor phytochrome detects the low red/far-red ratios caused by shading from neighboring plants and induces seed dormancy, preventing germination under highly competitive conditions (Pons 1992). Only when gaps occur in vegetation is seed dormancy broken by the high red/far-red ratios resulting from unmodified daylight (Pons 1992). In laboratory studies, leaf filtered light induced seed dormancy in 17 of 27 grassland species (Silvertown 1980), but few studies have confirmed the potential effects of grassland canopy inhibition of germination under field conditions (Pons 1992, but see Deregibus et al. 1994).

Alternative mechanism by which seeds "detect" gaps in vegetation are related to fire. For some species, there is a lack of seed germination until a fire occurs. Heat from the fire, smoke, or chemicals leached from the charate stimulate seed germination at a

time when competition from surrounding vegetation is decreased (Keeley et al. 1985, Bell et al. 1987, Dixon et al. 1995).

Abiotic environmental factors

Patterns

Abiotic factors that directly kill seeds include crushing, burning (Peart 1984), desiccation (Murdoch and Ellis 1992), and extreme temperatures (Murdoch and Ellis 1992). Additional abiotic factors that kill seedlings include drought (Mack and Pyke 1984, Soriano and Sala 1986), frost heaving (Mack and Pyke 1984), nutrient shortages (Grime and Curtis 1976, Chambers et al. 1990), seed burial too deep for emergence (Cook 1980), mechanical inhibition by litter (Bergelson 1990), and poor radicle contact with the soil (Peart 1984). It is unlikely that abiotic processes cause many seed deaths compared to rate of seedling deaths, because of the greater vulnerability of seedlings to these abiotic processes. Fenner (1987) suggests that abiotic factors may cause proportionally more seedling deaths in harsh habitats compared to more mesic habitats, where biotic factors such as grazing and competition may kill more seedlings (e.g., Sharitz and McCormick 1973).

Mechanisms resisting death by abiotic factors

Many of the abiotic factors causing seedling death can be ameliorated by dispersal patterns and by germination requirements or timing. For example, most of the northern British species tested failed to germinate at low temperatures, indicating that the high temperatures required for germination is caused by natural selection restricting

germination to the short but relatively favorable summer (Grime et al. 1981). A high proportion of the species generally found in southern Britain germinated, however, at the lower temperatures and generally had wider temperature ranges for germination compared to the northern species (Grime et al. 1981).

Seed dormancy status often cycles with the seasons, low dormancy correlating with optimal conditions for germination and seedling establishment (Angevine and Chabot 1980, Karssen 1982, Baskin and Baskin 1985, 1989, Fenner 1987, Masuda and Wahsitani 1990, Olff et al. 1994). For example, in habitats with high temperatures and low moisture during the growing season, species show a drought-avoiding germination syndrome (Angevine and Chabot 1980). Dispersed seeds are initially dormant with a low temperature requirement for germination. Later in the summer, seeds are no longer dormant, but temperature requirements for germination generally remain lower than that of the surrounding environment. Sometimes, a germination requirement for extra moisture may also develop. As temperatures decrease and the optimal germination temperature requirement increases during late summer, germination occurs and seedlings grow through the cooler, moister autumn and winter months, with flowering of annuals in early spring (Angevine and Chabot 1980).

Appendages (e.g. awns) on diaspores increase survival during germination by anchoring the diaspore to the soil (Peart 1984), decreasing the probability of dehydration by the emerging radicle (Harper et al. 1955). For seeds with no anchoring devices, soil contact increased with burial (Peart 1984). But because burial can be slow or infrequent, a mechanism, such as dormancy, that delays germination until effective burial occurs is necessary (Peart 1984). Most awned grasses in this study (Peart 1984) germinated

rapidly when supplied with moisture and suitable temperature, in contrast to most unawned species, which exhibited dormancy (Peart 1984). Likewise, rapid germination of many British species is positively correlated with the presence of a pappus, conical seed shape (Asteraceae), hygroscopic awns (Poaceae), and antrose hairs or teeth (Asteraceae and Poaceae) (Grime et al. 1981).

Germination and growth

Patterns and mechanisms

Generally, small percentages of the seeds produced by plants emerge and establish as seedlings (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989, Thompson and Baster 1992, Reader 1993). The mechanisms by which seedlings resist mortality factors, such as predation, microbial disease, abiotic factors, and interference, have been previously discussed in the mortality section. This section focuses on the description of the biological processes (1) germination, which initiates the development of seeds into seedlings, and (2) dormancy, which inhibits germination.

Germination

Germination is the process in which a dry quiescent seed, in response to water uptake, increases metabolic activity and initiates the formation of a seedling from the embryo (Mayer and Poljakoff-Mayber 1989). Exactly when germination ends and growth begins is difficult to define. Commonly, the end of germination is identified by emergence of the radicle from the seed coat (Come and Thevenot 1982, Mayer and Poljakoff-Mayber 1989). Radicle elongation characterizes the transition from a

reversible physiological state to an irreversible state, because, generally, once the radicle pierces the seed coat dehydration will kill the plant. Consequently, this evidence of irreversibility distinguishes growth from germination. Thus, the time at which imbibed seeds cannot be dried again without damage is defined as the stage at which germination ends and the embryo becomes a germinant or seedling (Come and Thevenot 1982, Mayer and Poljakoff-Mayber 1989).

A seed becomes a germinant once germination ends, but the point at which it ceases to be a seedling is much less clear. Generally, it is unsatisfactory to say a young plant is no longer a seedling when it becomes independent of its seed's nutrient reserves because the reserves of different nutrients are depleted at different rates (Fenner 1987). This definition is also impractical for field ecologists, because it is not possible to determine in the field whether the young plants are still mobilizing internal reserves. Morphological characteristics are not particularly helpful because of the diversity of seedling morphology (Fenner 1987). Thus, most investigators define seedling in the context of their own study.

Dormancy

For germination to begin, the following requirements must be met: adequate supply of water, appropriate temperatures, appropriate composition of atmospheric gases, and light (for certain species) (Come and Thevenot 1982, Mayer and Poljakoff-Mayber 1989). If these requirements are not met, germination does not occur and the seed is considered to be quiescent. Seed dormancy, as distinct from quiescence, is caused by some block to germination within the imbibed seed.

Physiological blocks cause embryo dormancy and prevent seed germination, even if the seed's covering structures have been removed (Come and Thevenot 1982). The mechanisms of these physiological blocks are not fully understood (Bewley and Black 1982), but are related to embryo immaturity, special requirements for temperature and light, and the presence of substances inhibiting germination (Come and Thevenot 1982, Baskin and Baskin 1989, Mayer and Poljakoff-Mayber 1989, Murdoch and Ellis 1992). Mechanical blocks, such as a hard seed coat, may prevent a non-dormant embryo from germinating (Come and Thevenot 1982), by preventing passage of water and gases necessary for germination or mechanically constraining the embryo (Mayer and Poljakoff-Mayber 1989, Kremer 1993). This type of dormancy is sometimes defined as inhibition of germination (Come and Thevenot 1982).

Primary or innate dormancy is the term used to describe a dormant seed at the time of dispersal (Harper 1977, Come and Thevenot 1982, Baskin and Baskin 1985, 1989). After-ripening is the process by which some seeds loses primary or innate dormancy (Karseen 1982, Baskin and Baskin 1989, Mayer and Poljakoff-Mayber 1989, Murdoch and Ellis 1992). Whenever an embryo that has lost primary dormancy is placed under conditions preventing germination, certain factors can cause some embryos to lose the capacity to germinate and again become dormant (Karseen 1982, Baskin and Baskin 1989). This new state is called secondary dormancy or induced dormancy (Harper 1977, Come and Thevenot 1982, Baskin and Baskin 1985).

As seeds after-ripen, they pass through a series of states known as conditional dormancy (Baskin and Baskin 1989) before finally becoming nondormant. Initially, as seeds move from dormancy to non-dormancy, seeds can germinate over only a very

narrow range of environmental conditions. After the after-ripening processes are completed, seeds are nondormant and can germinate over the widest range of environmental conditions possible for the species. Then seeds enter secondary dormancy and the conditions over which they can germinate narrow until the seeds can no longer germinate under any conditions. Thus, seeds exhibit a continuum of changes as they pass from dormancy to nondormancy and from nondormancy to dormancy (Karssen 1982, Baskin and Baskin 1985, 1989).

Persistence

Patterns of seed persistence

Grassland seed banks are relatively large (287 - 31,344 seeds per m^2) compared to seed banks of other ecosystems (Rice 1989). They contain a larger proportion of annuals than perennials, generally more forbs than grasses, with many leguminous, hard-seeded species (Rice 1989, Baker 1989). Weedy or fugitive species that colonize disturbances or gaps in vegetation are among the largest component of grassland seed banks (Louda 1989, Rice 1989).

Bakker (1989, cited by Thompson 1987) distinguishes between short-term persistent seed banks, which are relatively short-lived (1-5 years) and can maintain populations after poor seed production, and long-term persistent seed banks (> 5 years), which can contribute to the population regeneration after their extinction from the aboveground vegetation.

Mechanisms by which seeds persist in the soil

To persist in the soil, seeds must first have a genetic makeup for longevity, must avoid death by tolerating abiotic factors in the soil, resisting disease and predation, and finally must delay germination. These processes by which seeds avoid death and delay germination have been previously discussed in this chapter. The following generalizations emerge from these discussions.

Most species that possess persistent seed banks have seeds < 1 mg in mass, while those species with larger seeds, especially those with thin seed coats, possess only transient seed banks (persistence in the soil less than one year) (Thompson and Grime 1979, Louda 1989, Thompson 1987). Seed or fruit weight and the extent to which the seed or fruit shape differs from sphericity are correlated with persistence in the soil seed bank of British herbaceous species (Thompson et al. 1993). Thompson et al. 1993 showed that compact spherical seeds or fruits that weigh less than 3 mg generally form long-term persistent seeds banks. Other studies show that grass seeds that do not maintain persistent seed banks are large and attenuated, often with awns and retrose hairs (Thompson 1987).

The mechanism underlying the above relationship between morphological characteristics and seed persistence is likely ease of burial. Smaller, rounder seeds have a greater probability of burial into the soil than large attenuated seeds (Peart 1984, Thompson et al. 1993). Burial, by preventing light exposure, can induce secondary dormancy in seeds (Pons 1991), inhibiting germination (Pons 1991) and promoting seed persistence. Burial also reduces the risk from fire (Peart 1984) and predation (Reichman

1979, Crawley 1992, Hulme 1994), potentially increasing persistence in the soil seed bank.

Decreased mortality is not sufficient for long term persistence, however, if seeds lack dormancy mechanisms and can germinate rapidly under a wide range of conditions. Comparisons made between species with persistent and transient seed banks show that species with persistent seed banks have seeds that show 50% or less germination immediately after dispersal and require mechanisms such as scarification, chilling, or exposure to light to break dormancy, in contrast to species with transient seed banks, whose seeds show more than 50% germination after dispersal and require no dormancy breaking mechanisms (Grime 1989).

SEED FATE STUDIES

Many "seed fate" studies focus on quantifying seed survival in soil seed banks or describe seed dormancy patterns of buried seeds (Zorrner et al. 1984, Washitani 1985, Granstrom 1987, van Esso and Ghersa 1989, Pons 1989, 1991, Bridgemohan et al. 1991, Pierce and Cowling 1991, Crist and Friese 1993). Generally, seeds enclosed in bags were buried in the soil (protected from animal predators), retrieved at regular intervals, and seed status quantified. The number of viable, non-viable and germinated seeds were counted, but the methodology did not allow for distinction between germinants that would have successfully established as seedlings and those that would not have established (but, see Pierce and Cowling 1991). Moreover, many seeds and germinants decayed, but the investigations did not determine if the decay was saprophytic or pathogenic decay, and thus, the extent of microbial disease was unknown.

An investigation by Lawrence and Rediske (1962) is rare in measuring seed fates and multiple mortality factors in a single study. Few Douglas-fir (*Pseudotsuga menziesii*) seeds (<25%) survived as seeds or seedlings with most death occurring before the start of germination (Lawrence and Rediske 1962). The mortality factors measured included death by fungi, insects, rodents and birds. Fungi accounted for the greater part of seed loss during the pre-germination period and were also the principle cause of seedling mortality (Lawrence and Rediske 1962). The importance of fungi and ground insects in causing mortality of Douglas-fir seeds was unrecognized until these quantitative studies were done, thus, emphasizing the importance of quantifying each process influencing seed fate pathways.

The success of Lawrence and Rediske (1962) in quantifying seed fates was attributable mainly to the technique by which they recovered the sowed seeds. Sowed seeds were tagged with Sc^{46} and later located with a scintillator. Radio-tagging was effective in accounting for more than 95 percent of seed in controlled test plots (Lawrence and Rediske 1962). Moreover, in contrast to seeds of most species, the causes of death for Douglas-fir seeds could easily be determined by the appearance and the condition of the recovered seed or seed hull.

The handful of studies that have attempted to quantify the post-dispersal fate of seeds in natural vegetation vary in their degree of completeness (Table 2.1). Generally, seed persistence and seedling survival are measured, but rarely are mortality factors determined with the exception of predation (Table 2.1). This lack of seed fate studies hampers our ability to make generalizations about seed and seedling mortality patterns. Knowledge of these and other regeneration characteristics are essential for prediction of

population distribution and abundance patterns and ultimately plant community species composition and diversity.

Table 2.1. Summary of measured post-dispersal seed fates and mortality factors.

	Survival		Mortality factors					
Study	seed	seedling	combined	predation	senescence	abiotic	disease	interference
Lawrence and Rediske 1962 (conifer forest)	x	x		x	x	x		
Sarukhan 1974 (grassland)	x	x	x					
van Baalen 1982 (forest clearings)	x	x	x					
Pavone and Reader 1982 (abandoned pasture)	x	x	x					
Smith 1983 (deciduous forest understory)	x	x		x				
Holthuijuzen et al. 1986 (pastures)	x	x		x				
Alvarez-Bullya and Martinez-Ramos 1990 (tropical forest)	x	x		x				
Kalisz 1991 (forest understory)	x	x		x				
Hughes and Westoby 1992 (sclerophyll vegetation)		x		x				
Horvitz and Schemske 1994 (tropical forest)	x	x		x				
Vander Wall 1994 (semi-desert shrubland)	x	x		x				
Chambers 1995 (alpine herbfields)	x	x	x					

CHAPTER 3
POST-DISPERSAL SEED FATES IN A WESTERN OREGON NATIVE PRAIRIE

INTRODUCTION

Rationale

Events that occur during the regeneration stage of plants' life cycles are crucial in determining the distribution and abundance of plant populations (Grubb 1977, Harper 1977, Grime 1979, Werner 1979, Gross and Werner 1982, Smith 1983). Community composition and diversity are also influenced by differences among species in their regeneration characteristics and safe-site requirements (Grubb 1977, Masuda and Washitani 1990, Grime 1992). Furthermore, these differences in regeneration patterns affect successional patterns by determining the probability of a species seedlings colonizing in communities of different successional stages (Gross and Werner 1982, Olff et al. 1994).

The survival of seeds is essential for successful regeneration. Measurements that quantify the progressive mortality of seeds caused by different factors may allow development of techniques that reduce or increase these losses, consequently controlling the abundance of seedlings (e.g., Lawrence and Rediske 1962). This knowledge of regeneration characteristics, then, is a key to successful exotic weed control, management of natural resources, and the restoration and conservation of native species and habitats.

Even though the multitude of studies on every aspect of seed biology demonstrate the importance of species regeneration from seeds, few studies follow the fate of individual seeds. Following seed fates is difficult, because most seeds are small and

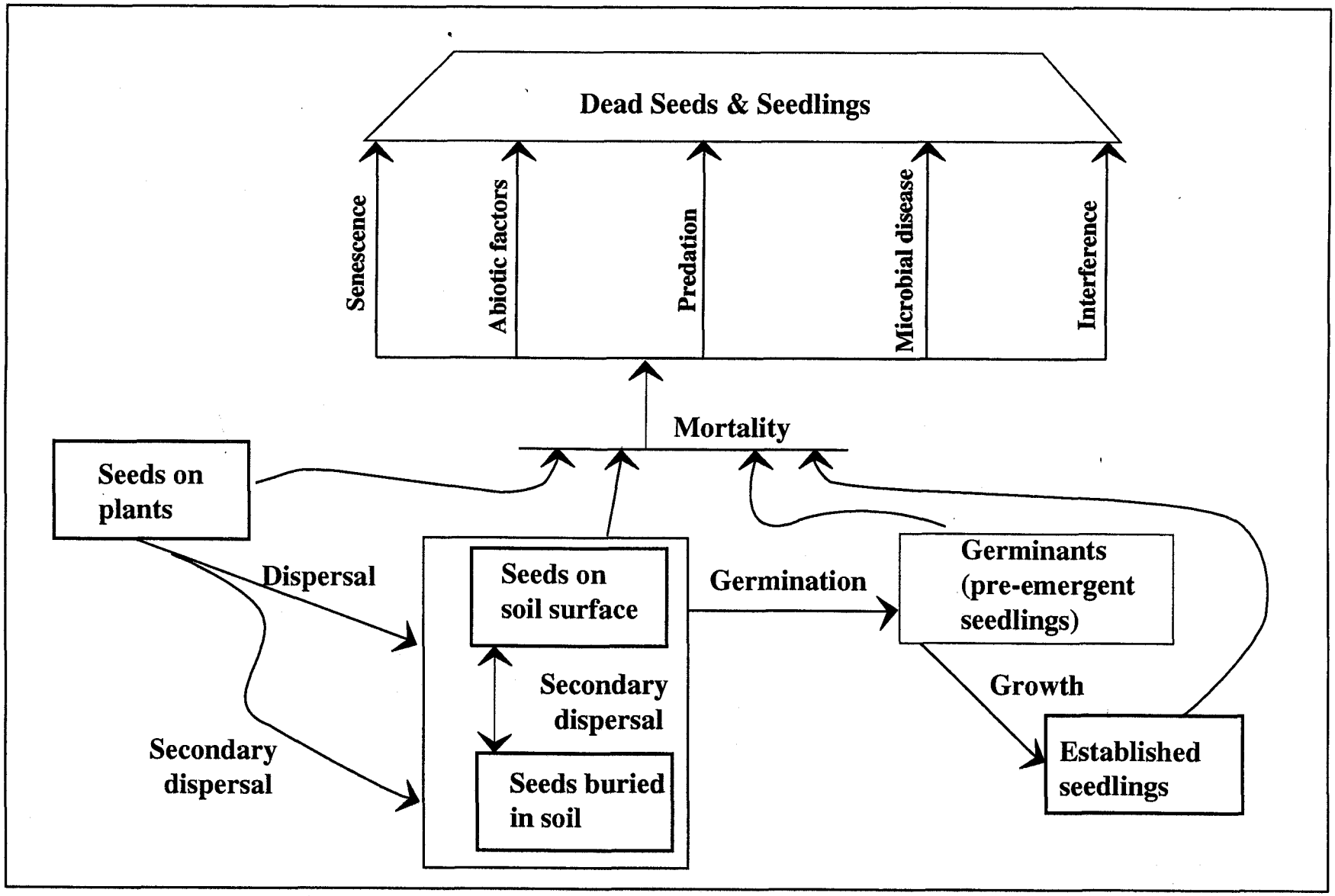
inconspicuous. The biotic and abiotic factors that influence seed death, seed persistence, and seedling establishment are exceptionally difficult to analyze in natural populations. Thus, no balance sheet exists that permits accounting for all the seeds that a plant produces (Chambers and MacMahon 1994).

Model

The model in Figure 3.1 outlines the potential pathways that seeds follow after dispersal. Seeds generally arrive on the soil surface, where they may remain or move deeper into the soil by burial. Persistent seeds are either dormant or quiescent until conditions are appropriate for germination. Germination can occur immediately after dispersal or after persistence in the soil. Death can occur at several points along the pathway: immediately after dispersal, after persistence in the soil, or during germination and growth. Seeds die from senescence, predation, and microbial disease, while seedlings die from microbial disease, herbivory, interference from neighboring plants, and abiotic factors.

It is extremely difficult to determine the causes of death for seeds and germinants (pre-emergent seedlings), especially for small seeds under field conditions. Germination of the seed at a depth from which it fails to emerge is a common explanation for the large number of buried seeds that fail to emerge in most seed bank studies (Cook 1980, Murdoch 1983, Washitani 1985). But, because both dead seeds and germinants decay in the soil, death caused by senescence, pathogenic attack, and fatal germination are indistinguishable. Causes of seedling mortality are also difficult to determine. When a

Figure 3.1. Model describing fates of seeds and the processes controlling the magnitudes of these fates.


Dead Seeds & Seedlings
Senescence
Abiotic factors
Predation
Microbial disease
Interference
Mortality
Seeds on plants
Dispersal
Seeds on soil surface
Germination
Germinants (pre-emergent seedlings)
Secondary dispersal
Growth
Secondary dispersal
Seeds buried in soil
Established seedlings

young seedling emerges, it may die in a few hours, leaving no detectable trace. Another seedling may immediately emerge in the same place. Even with dead plant's remains, it is often impossible to assign a cause of death.

As environmental conditions vary unpredictably from year to year, so undoubtedly does the relative importance of mortality factors (e.g., Mack and Pyke 1984). Although a mortality factor causes minor losses at a particular time or place, it may cause major losses at another time and place, depending on changes in environmental conditions. Moreover, variability in magnitude and causes of mortality can prevent competitive exclusion of plant species, promoting increased species diversity of the community (Crawley and Pacala 1990). On the other hand, consistent mortality from a single factor can potentially decrease population size (Louda 1989, Anderson 1989, Harper 1990), control the population spatial distribution (Tadros 1957, van Leeuwen 1989, Louda 1989, Augspurger 1990, Reader and Beisner 1991) and influence community species composition (Borchert and Jain 1978). Furthermore, mortality patterns influence the evolution of a species by determining the relative reproductive success of individual plants. Note that this evolutionary effect can occur even when specific mortality factors have no impact on plant population size (Crawley 1992, Anderson 1989).

Goal and objectives

The goal of this study was to determine, using an experimental approach, the magnitudes of post-dispersal seed fates and the processes controlling these fates for four species of western Oregon native prairies. The general approach was to sow seeds of

four prairie species into experimentally manipulated field plots in each of two years, and to determine their fates one year later. The specific objectives were:

1. To estimate the numbers of senescent and dormant seeds by testing viability of seeds retrieved from soil samples one year after sowing into field subplots.
2. To estimate the number of seedlings establishing by directly counting seedlings in the field subplots one year after sowing seeds into field plots.
3. To estimate mortality from vertebrate predation by comparing the magnitude of dead seeds and seedlings between caged and uncaged field subplots.
4. To estimate mortality caused by fungal disease by comparing the magnitude of dead seeds and seedlings between fungicide treated and untreated subplots.
5. To estimate the number of seeds moving outside field subplots, by calculating the number of seed surrogates that could not be recovered one year after sowing.

METHODS AND ANALYSIS

Study site

The study site is an upland prairie with silty clay loam (Dixonville soil series) dominated by native bunchgrasses, *Festuca idahoensis* var. *roemeri*, *Bromus carinatus*, and *Elymus glaucus*. This site, part of Open Space Park managed by Benton County Parks, is located approximately 8 kilometers northwest of Corvallis, Oregon, in the foothills of the Coast Range (T11S, R6W, Sec 25, W.M.). The elevation is approximately 300 m, with a 30-50% slope with a westward aspect. The site is one of the few remnants of a vast prairie and oak-savanna ecosystem that covered much of the

Willamette Valley until after the 1840s (Boag 1992). The entire Willamette Valley has a fairly homogeneous climate characterized by mild, wet winters, moderate and dry summers, and cool nights. Measurements made in Corvallis (Owenby and Ezell 1992) show the average annual precipitation is 108 cm, average maximum January temperature is 7.5°C, and maximum average July temperature 26.8° C. The average precipitation during the first year of this study (June 1991-May 1992) was 6.14 cm and for the second year (June 1992-May 1993), 8.84 cm (George Taylor, OR State Climatologist). The average maximum temperature for January 1992 was 9.7°C and for January 1993, 5.3°C. The average maximum temperature for July 1992 was 27.8°C and for July 1993, 23.2°C.

Study species

The four study species, *Bromus carinatus* Hook and Arn. var. *carinatus, Cynosurus echinatus* L., *Daucus carota* L., and *Prunella vulgaris* var. *lanceolata* (Barton) Fern. are dominant at the study site (Table 3.1). The criteria for species selection were that the study species (1) represent a variety of life histories, (2) be common and abundant in western Oregon prairies, and (3) produce sufficient seeds for the research. Nomenclature follows Hitchcock and Cronquist (1973).

The Eurasian *Prunella vulgaris* var. *vulgaris* has the middle cauline leaves about half as wide as long, with broadly rounded base. It grows in the Northwest occasionally in disturbed sites, where it is often dwarfed and prostrate (Hitchcock and Cronquist 1973). The native American *Prunella vulgaris* var. *lanceolata* is ascending or erect, with middle cauline leaves about a third as wide as long and more tapering toward the base (Hitchcock and Cronquist 1973). The variety that is common in undisturbed habitats in

Oregon is generally considered to be the native one (H. Chambers 1995, personal communication).

Table 3.1. Description of the four study species.

Species	Family	Native to Oregon?	Life-span	Seed description
Bromus carinatus var. *carinatus*	Poaceae	yes	perennial	awned
Cynosurus echinatus	Poaceae	no	annual	awned
Prunella vulgaris var. *lanceolata*	Labiatae	yes	perennial	hard-seeded
Daucus carota	Apiaceae	no	biennial	barbed

Throughout this study, the study species will be referred to as *Bromus carinatus, Cynosurus echinatus, Daucus carota* and *Prunella vulgaris.* Although the term seed is used in this study, it refers to the diaspore, i.e., the seed and any associated structures.

Experimental design

Year one, 1991-1992

In mid-July, 1991, twenty 2.2 m × 2.2 m blocks were randomly located at the study site. Ten of these blocks were mowed as part of a different study (Chapter 5). The ten unmowed blocks were used in this present study to estimate seed fates. Within each block, five plots (25 cm × 35 cm) were randomly placed. The following treatments (described in detail below) were randomly applied to one plot of the five plots within each block: 1) cage and fungicide, 2) cage and no fungicide, 3) no cage and fungicide, 4) no cage and no fungicide, and 5) sham cage and no fungicide.

Four subplots, each 5 cm in diameter, were located and permanently marked with nails within each plot. Twenty-five seeds of one species were sowed into a subplot immediately following seed collection at the study site. Seedling data were not collected on all the fungicide treated plots due to time constraints, resulting in unequal samples sizes for seedling counts: $n = 3$ for fungicide treated plots and $n = 10$ for the untreated plots.

Year two, 1992-1993

In late July, 1992, twenty 2.2 m × 2.2. m blocks were randomly placed at the study site. With each block, two plots (25 cm × 35 cm) were randomly placed. One plot was caged as described below and the other plot remained uncaged. Fungicide treatments were not applied the second year. Four subplots, each 5 cm in diameter, were located and permanently marked with nails within each plot. Twenty seeds of one species were sowed into a subplot immediately following seed collection at the study site.

Treatments

Caging treatment

To exclude vertebrate predators, cages (approximately 35 cm × 25 cm × 30 cm high) were made of galvanized metal mesh (1.25 cm) with the bottom edge sunk into the ground about 2.5 - 4 cm. To exclude invertebrate predators, "Tanglefoot" ™, a sticky substance manufactured to trap insects, was applied to the bottom edge of the exclosure up to a height of 2.5 cm and to the soil surface adjacent to the outside edge of the

exclosures. However, insects had access to inside of the cages because of overhanging vegetation, particularly grasses. Thus "Tanglefoot" ™ may have only deterred insects without excluding them. For this reason, "Tanglefoot" ™ was not applied second year.

To test for cage effects other than exclusion of vertebrate predators, sham cages were constructed with one side open, allowing entry of vertebrate predators. Survival of seeds and seedlings did not differ significantly between the sham cage treatment and the unmanipulated treatment (Appendix 1).

Fungicide treatment

The two fungicides used for the fungicide treatment were metalaxyl, N-(2, 6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester and, Captan, N-tricholoro-methylthio-4-cyclohexene-1,2, dicarboximide. Both fungicides are commonly used to prevent pre- and post-emergence damping-off of seedlings caused by fungi (USEPA 1975, Jeffs 1986, Schwinn and Urech 1986, Koepsell and Pscheidt 1995). Captan was sprayed on the soil surface every two weeks and metalaxyl every 30 days, using the manufacturer's recommended rates, beginning in August 1991 and ending May 28, 1992. Seed germination under laboratory conditions did not differ significantly between fungicide treated seeds and untreated seeds for all four study species (Appendix 2).

Data collection

Seedlings

In mid-June of 1992, approximately one year after seeds were sowed in the subplots, the number of seedlings for each of the study species was counted in each subplot. These seedlings numbers were adjusted by subtracting background counts. Background counts for each species were made from the number of established seedlings in subplots not sowed with that study species. Background counts were repeated for the second year study.

Seed retrieval

The soil from the entire microplot (5 cm diameter) was removed with a bulb digger to a depth of 5 cm in mid-to late June and stored at 4° C until it could be sieved. After the soil was sieved with water using sieves to match seed dimensions, the residue was dried at 30° C for two to three days and then stored in plastic bags at room temperature for the next few months until the samples could be examined under a magnifying glass to identify the remaining seeds. Preliminary tests (Appendix 3) showed that this retrieval method was almost 100% effective for *Bromus carinatus* and *Cynosurus echinatus* and almost 90% effective for *Prunella vulgaris*. Because of the background presence of *Daucus carota* seeds in field collected soil samples, more *Daucus carota* seeds were recovered than had been sowed.

Seed viability

The viability of seeds retrieved from the soil samples was determined by germinating the seeds at alternating temperatures of 30° C (day) and 20° C (night) with 14 hours of incandescent and fluorescent light. (Preliminary tests were conducted to determine the appropriate conditions promoting germination for each of the study species.) Seeds that produced at least 1 mm of radicle exposed beyond the seed coat were considered viable. Ungerminated seeds were tested for viability using the tetrazolium viability test (Moore 1985).

The number of dormant and senescent seeds retrieved from the soil was adjusted by subtracting background counts of dormant and senescent seeds. Background seeds counts for *Bromus carinatus*, *Cynosurus echinatus* and *Prunella vulgaris* were made from the samples in which *Daucus carota* seeds were sowed. Background seed counts for *Daucus carota* were made from the samples in which *Prunella vulgaris* seeds were sowed.

Secondary movement

To determine secondary movement of sowed seeds out of the subplots, beads were used as seed surrogates (Table 3.2a,b) and sowed within the caged subplots in the second year. The magnitude of sowed seeds moving outside the subplots was estimated from the number of seed surrogates that could not be recovered one year after sowing. Originally, the intention was to use dead seeds and mark them with paint. However,

Table 3.2a. Weights and dimensions of seed and associated structures for four species of western Oregon native prairies. Sample size for seed dimensions was 10 seeds per species. Sample sizes for the seed weights varied from 3 to 12 replicates each containing 10 air dried seeds. Data are means with standard deviation indicated by *sd*.

Species	length (mm)									
Seeds	with awn	sd	without awn	sd	width (mm)	sd	depth (mm)	sd	mass (mg)	sd
Bromus carinatus	23.2	1.9	12.6	0.4	1.4	0.1	1.2	0.1	8.1	0.0
Cynosurus echinatus	17.3	1.4	4.8	0.2	1.0	0.0	0.7	0.1	1.8	0.0
Daucus carota			2.7	0.3	1.5	0.5	0.5	0.1	0.9	0.0
Prunella vulgaris			1.9	0.1	1.7	0.1	0.8	0.1	1.2	0.0

Table 3.2b. Weights and dimensions of seed surrogates for four species of western Oregon native prairies. Sample size for surrogate dimensions was 10 surrogates. Sample sizes for surrogate weights varied from 3 to 5 replicates. Each replicate contained 5 surrogates for *Bromus carinatus* and 10 surrogates for the other three species. Data are means with standard deviation indicated by *sd*.

Surrogates	length (mm)	sd	diameter of cylinder (mm)	sd	mass (mg)	sd
Bromus carinatus	11.0	0.2	2.0	0.1	83.2	3.5
Cynosurus echinatus	4.4	0.3	2.0	0.1	29.6	0.2
Daucus carota	1.8	0.1	1.2	0.1	6.0	0.2
Prunella vulgaris	1.8	0.1	1.0	0.1	5.4	0.3

numerous paints and other substances were tried, but all leached out or flaked off during the sieving process or would have faded after the seeds had been in the soil for a year.

Data analysis

Model components and magnitudes

For each species, a quantitative model was constructed showing the magnitude of each fate or fate process one year after sowing seeds in the experimental field plots.

1. Loss to secondary dispersal

   Loss to secondary dispersal (*SD*) was calculated as

   $$SD_i = \alpha S_i$$

   where i is 1 for year one and 2 for year two, α is the proportional loss of surrogate seeds in year 2, and S is the number of seeds sowed.

2. Dormant seeds

   The number of dormant seeds (*DO*) was measured directly as the number of viable seeds recovered from the soil in the untreated subplots one year after sowing the seeds.

3. Seedlings

   The number of seedlings (*SL*) was measured directly as the number of seedlings in the untreated subplots one year after sowing seeds.

4. Survival

   Survival (*SU*) was calculated as

   $$SU_i = DO_i + SL_i$$

where i is 1 for year one and 2 for year two, DO is the number of dormant seeds and SL is the number of seedlings.

5. Mortality

Mortality (M) was calculated as

$$M_i = S_i - (DO_i + SL_i + Sd_i)$$

where i is 1 for year one and 2 for year two, S is the number of seeds sowed, DO is the number of dormant seeds recovered from the untreated subplots, SL is the number of seedlings in the untreated subplots, and SD is the loss to secondary dispersal.

6. Loss to senescence

The loss of senescent seeds (SE) was measured directly as the number of non-viable seeds recovered from the soil in the untreated subplots one year after sowing.

7. Loss to vertebrate predation

Loss to vertebrate predation (P) was calculated as

$$P_i = UCD_i - Cd_i$$

where i is 1 for year one and 2 is for year two, CD is the total mortality in the caged subplots and UCD is total mortality in the uncaged subplots.

8. Loss to fungal disease

Loss to fungal disease (F) for year one was calculated as

$$F = NFD - FD$$

where FD is the total mortality in the fungicide treated subplots; and NFD is the total mortality in the non-fungicided subplots.

9. Loss to "other mortality processes"

Loss to "other mortality processes" in year one (O_1) was calculated as

$$O_1 = M_1 - (SE_1 + DO_1 + P_1 + F_1)$$

where M is the total mortality; SE is the number of senescent seeds, DO is the number of dormant seeds, P is loss to predation, and F is loss to fungal disease.

Loss to "other mortality processes" in year two (O_2) was calculated

$$O_2 = M_2 - (SE_2 + DO_2 + P_2)$$

where M is the number of dead seeds and seedlings, SE is the number of senescent seeds, DO is the number of dormant seeds and P is loss to predation.

The number of dormant seeds, senescent seeds and seedlings was adjusted by subtracting the number of background counts. Negative magnitudes were thus possible. Because of different samples sizes used to measure the different seed fate components, the mortality magnitude does not always equal the sum of the reported mortality factors and the survival magnitude does not always equal the sum of the reported means for dormant seeds and seedlings.

Comparisons of fate processes for each species

For year one, magnitudes of six fate processes (disease, vertebrate predation, senescence, "other mortality processes", germination and growth, and persistence) were compared using a one-way randomized block analysis of variance (ANOVA) (Sokal and Rohlf 1981). For year two, magnitudes of six fate processes (vertebrate predation, senescence, "other mortality processes", germination and growth, persistence, and movement) were compared using a one-way randomized block ANOVA (Sokal and Rohfl 1981).

The General Linear Model (GLM) procedure of the SAS Institute statistical software, version 6.08 and type III sum of squares were used throughout all analyses. Inspection of normality plots of residuals showed that the ANOVA assumption of normality was met. Inspection of plots of residuals against predicted values showed that the ANOVA assumption for constant variance was met. When appropriate, differences between means were tested using Fisher's protected least significant difference. For ANOVAs with non-significant treatment effects, analyses were conducted to determine the power of the statistical test to detect a pre-determined effect size between the experimental treatments (Cohen 1969, Peterman 1990, see Appendix 4 for details).

Comparisons of survival between years for each species

One-way analysis of variance (Sokal and Rohlf 1981) was performed comparing survival between year one and year two for each species, using the General Linear Model (GLM) procedure of the SAS Institute statistical software, version 6.08 as described above.

Comparisons of fates among species for each year

Kendall's coefficient of concordance, a statistical test (Conover 1980), was used to compare the ranking of magnitudes of fate processes among species for year one and again for year two. Using this test, an overall measure of agreement of the ranking between species was calculated.

RESULTS

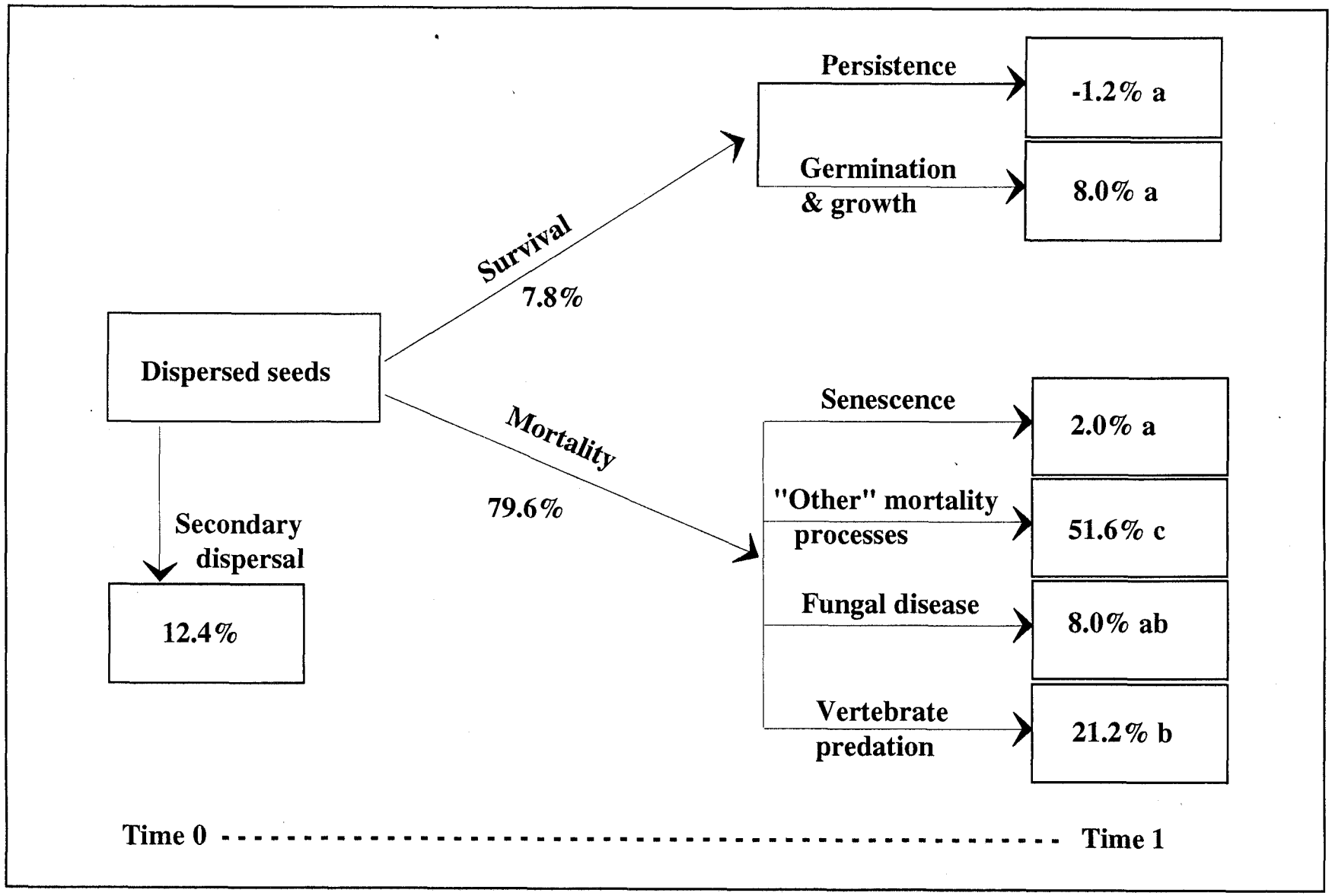
Patterns of seed fates and processes within and between years for each species

Bromus carinatus

Year one (1991-1992)

Many more seeds/seedlings died (79.6%) than survived either as seedlings (8.0%) or as dormant seeds (-1.2%) (Figure 3.2a). (Negative magnitudes were possible when data were adjusted for background counts.) The category, "other mortality processes", (51.6%) were significantly larger than any other causes of death (Figure 3.2a, Tables 3.3 and 3.4). Vertebrate predation caused the second greatest loss of seeds and seedlings (21.2%). The magnitudes of the remaining mortality categories, fungal disease (8.0%) and recovered senescent seeds (2.0%), were small and statistically indistinguishable (Figure 3.2a, Tables 3.3 and 3.4).

Figures 3.2a and 3.2b. Magnitude of post-dispersal seed fates for *Bromus carinatus* var. *carinatus*, a native bunchgrass of western Oregon prairies. Figure 3.2a is for 1991-1992 and Figure 3.2b is for 1992-1993. Magnitudes are expressed as a percentage of the total number of experimentally sowed seeds. Negative magnitudes were possible because data were adjusted for background counts. Magnitudes do not always sum to100% because of differences in sample sizes and methods of calculations. Details on calculations for each fate process are found in the text. Sample sizes and 95% confidence intervals for mean number of seeds in each category are found in Table 3.3. Results of one-way ANOVA comparing fate process magnitudes are found in Table 3.4. Separation of means tests (Fisher's protected LSD, $\alpha = 0.05$) were performed for significant results. The results of this separation of means test are presented in this figure. The magnitude of categories with the same letters are not significantly different from each other.


Persistence
-1.2% a
Germination & growth
8.0% a
Survival
7.8%
Dispersed seeds
Mortality
79.6%
Senescence
2.0% a
"Other" mortality processes
51.6% c
Fungal disease
8.0% ab
Vertebrate predation
21.2% b
Secondary dispersal
12.4%
Time 0
Time 1

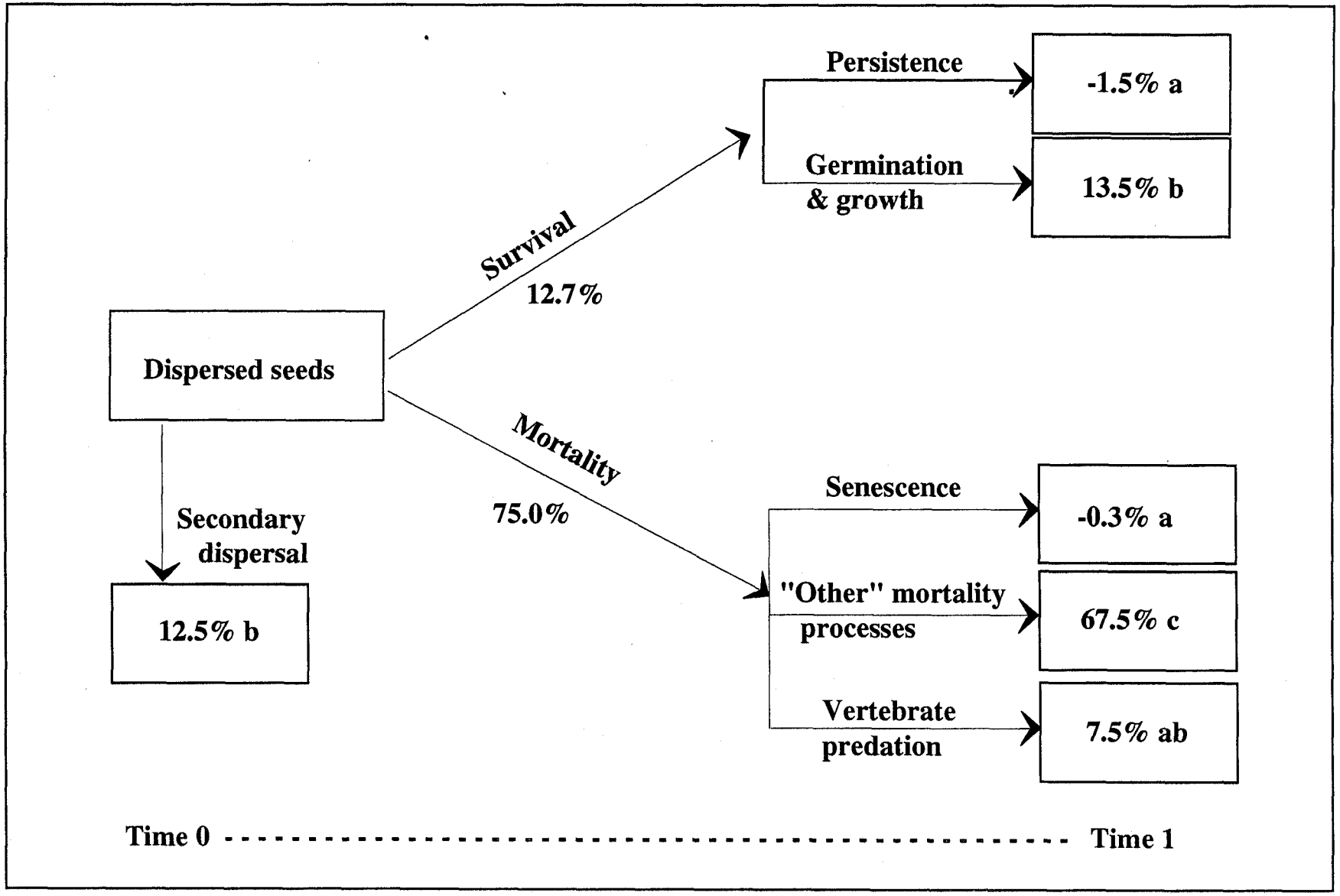

Persistence
-1.5% a
Germination & growth
13.5% b
Survival
12.7%
Dispersed seeds
Mortality
75.0%
Secondary dispersal
12.5% b
Senescence
-0.3% a
"Other" mortality processes
67.5% c
Vertebrate predation
7.5% ab
Time 0
Time 1

Table 3.3. Magnitude of six seed fate processes in 1991-1992 (year 1) for each of four species in western Oregon native prairies. The magnitude is the mean number out of 25 experimentally sowed seeds. "Other mortality processes" are those processes, other than vertebrate predation, fungal disease and senescence, contributing to seed and seedling death. The sample size is indicated by *n*.

Processes	*n*	Magnitude	95% Confidence Interval
a) *Bromus carinatus*			
"other mortality processes"	2	12.9	8.4, 17.3
vertebrate predation	9	5.3	3.2, 7.4
germination and growth	10	2.0	0.0, 4.0
fungal disease	3	2.0	-1.7, 5.6
senescence	9	0.5	-1.6, 2.6
persistence	9	-0.3	-2.4, 1.8
b) *Cynosurus echinatus*			
"other mortality processes"	3	8.8	4.2, 13.3
germination and growth	10	4.3	1.8, 6.8
fungal disease	3	2.4	-2.2, 6.9
vertebrate predation	9	1.6	-1.0, 4.3
persistence	9	0.1	-2.5, 2.7
senescence	9	0.0	-2.6, 2.6
c) *Daucus carota*			
"other mortality processes"	2	9.4	-0.4, 19.2
persistence	8	3.5	-1.4, 8.3
germination and growth	10	2.7	-1.6, 7.1
fungal disease	3	0.3	-7.6, 8.3
senescence	8	0.3	-4.6, 5.1
vertebrate predation	8	-0.5	-5.4, 4.3
d) *Prunella vulgaris*			
"other mortality processes"	3	8.1	1.7, 14.5
fungal disease	3	5.6	-0.8, 12.0
germination and growth	10	4.3	0.8, 7.8
senescence	10	0.5	-3.0, 4.0
persistence	10	0.4	-3.1, 3.9
vertebrate predation	10	-1.7	-5.2, 1.8

Table 3.4. ANOVA comparing the magnitudes of six seed fates in year 1 (1991-1992) for four species in western Oregon prairies. Magnitudes are the number of seeds surviving as seeds or seedlings out of 25 seeds sowed into field plots. Means and sample sizes are presented in Table 3.3. Results of separation of means test (Fisher's protected LSD, $\alpha = 0.05$) are found in Figures 3.2a, 3.3a, 3.4a, and 3.5a.

Source	df	Sum of squares	*F*	*P*
a) *Bromus carinatus*				
block	9	34.3		
fates	5	363.9	7.80	<0.01
error	27	251.9		
b) *Cynosurus echinatus*				
block	9	33.9		
fates	5	203.3	2.76	0.04
error	28	412.7		
c) *Daucus carota*				
block	9	325.0		
fates	5	176.2	0.79	0.57
error	24	1075.2		
d) *Prunella vulgaris*				
block	9	21.1		
fates	5	323.3	2.19	0.08
error	31	915.9		

Year two (1992-1993)

Most seeds/seedlings died (75.0%). Significantly more seeds survived as seedlings (13.5%) than as dormant seeds (-1.5%) (Figure 3.2b, Tables 3.5 and 3.6). "Other mortality processes", which in year two included fungal disease, was significantly larger (67.5%) than any other cause of death (Figure 3.2b, Tables 3.5 and 3.6). The remaining mortality categories, vertebrate predation and recovered senescent seeds, were each less than 10% (Figure 3.2b).

Table 3.5. Magnitude (number of seeds out of 20 experimentally sowed seeds) of six fate processes in 1992-1993 (year 2) for each of four species in western Oregon native prairies. Movement is estimated by number of surrogate seeds moving outside the experimental field plots. "Other processes" are those processes, excluding vertebrate predation and senescence, contributing to death. The sample size is indicated by *n*.

Processes	*n*	Magnitude	95% Confidence Interval
a) *Bromus carinatus*			
"other mortality processes"	19	13.5	11.9, 15.0
germination and growth	20	2.7	1.2, 4.2
movement	20	2.5	1.0, 4.0
vertebrate predation	19	1.5	-0.0, 3.1
senescence	19	-0.1	-1.6, 1.5
persistence	19	-0.3	-1.9, 1.2
b) *Cynosurus echinatus*			
"other mortality processes"	19	14.4	12.5, 16.3
movement	20	4.2	2.3, 6.0
germination and growth	20	2.2	0.3, 4.1
senescence	19	0.0	-1.9, 1.9
persistence	19	0.0	-1.9, 1.9
vertebrate predation	19	-0.9	-2.8, 1.0
c) *Daucus carota*			
"other mortality processes"	19	11.1	7.8, 14.4
movement	20	5.4	2.2, 8.6
germination and growth	20	2.1	-1.1, 5.4
vertebrate predation	19	2.1	-1.2, 5.4
senescence	19	-0.2	-3.5, 3.2
persistence	19	-0.3	-3.6, 3.0
d) *Prunella vulgaris*			
"other mortality processes"	19	14.6	13.5, 15.7
movement	20	5.4	4.3, 6.4
germination and growth	20	0.8	-0.3, 1.8
senescence	19	0.4	-0.7, 1.5
persistence	19	0.3	-0.8, 1.4
vertebrate predation	19	-1.2	-2.3, -0.1

Table 3.6. ANOVA comparing the magnitudes of six seed fates in year 2 (1992-1993) for each of four species in western Oregon native prairies. Magnitudes are the number of seeds surviving as seeds or seedlings out of 20 seeds sowed into field plots. Means and sample sizes are presented in Table 3.5. Results of separation of means (Fisher's protected LSD, $\alpha = 0.05$) are found in Figures 3.2b, 3.3b, 3.4b, and 3.5b.

Source	df	Sum of squares	*F*	*P*
a) *Bromus carinatus*				
block	19	57.1		
fates	5	2506.6	43.92	<0.01
error	91	1038.7		
b) *Cynosurus echinatus*				
block	19	60.5		
fates	5	3125.8	35.10	<0.01
error	91	1620.5		
c) *Daucus carota*				
block	19	166.5		
fate	5	1775.1	6.76	<0.01
error	91	4781.5		
d) *Prunella vulgaris*				
block	19	173.4		
fates	5	3361.6	116.90	<0.01
error	91	523.4		

Comparison between year one and two

Mortality and survival patterns were similar for both years, with most seeds/seedlings dying (Figures 3.2a and 3.2b). Between year survival magnitudes were similar with no statistical differences ($P = 0.42$) (Table 3.7). There was 64% power detect a 10% difference between years. Virtually no seeds survived as dormant seeds either year (Figures 3.2a and 3.2b, Tables 3.3 and 3.5). "Other mortality processes" were the major cause of mortality for both years, even though fungal disease was not included in this

category for the first year (Figures 3.2a and 3.2b). Of the remaining mortality categories for both years, only first year vertebrate predation (21.2%) was greater than 10%.

Table 3.7. Number of seeds and seedlings surviving out of 25 experimentally sowed seeds in 1991-1992 and out of 20 experimentally sowed seeds in 1992-1993 for each of four species in western Oregon native prairies. The sample size for year one was 10 and was 20 for year two. *P* is the probability of falsely rejecting the null hypothesis of no difference of survival between years.

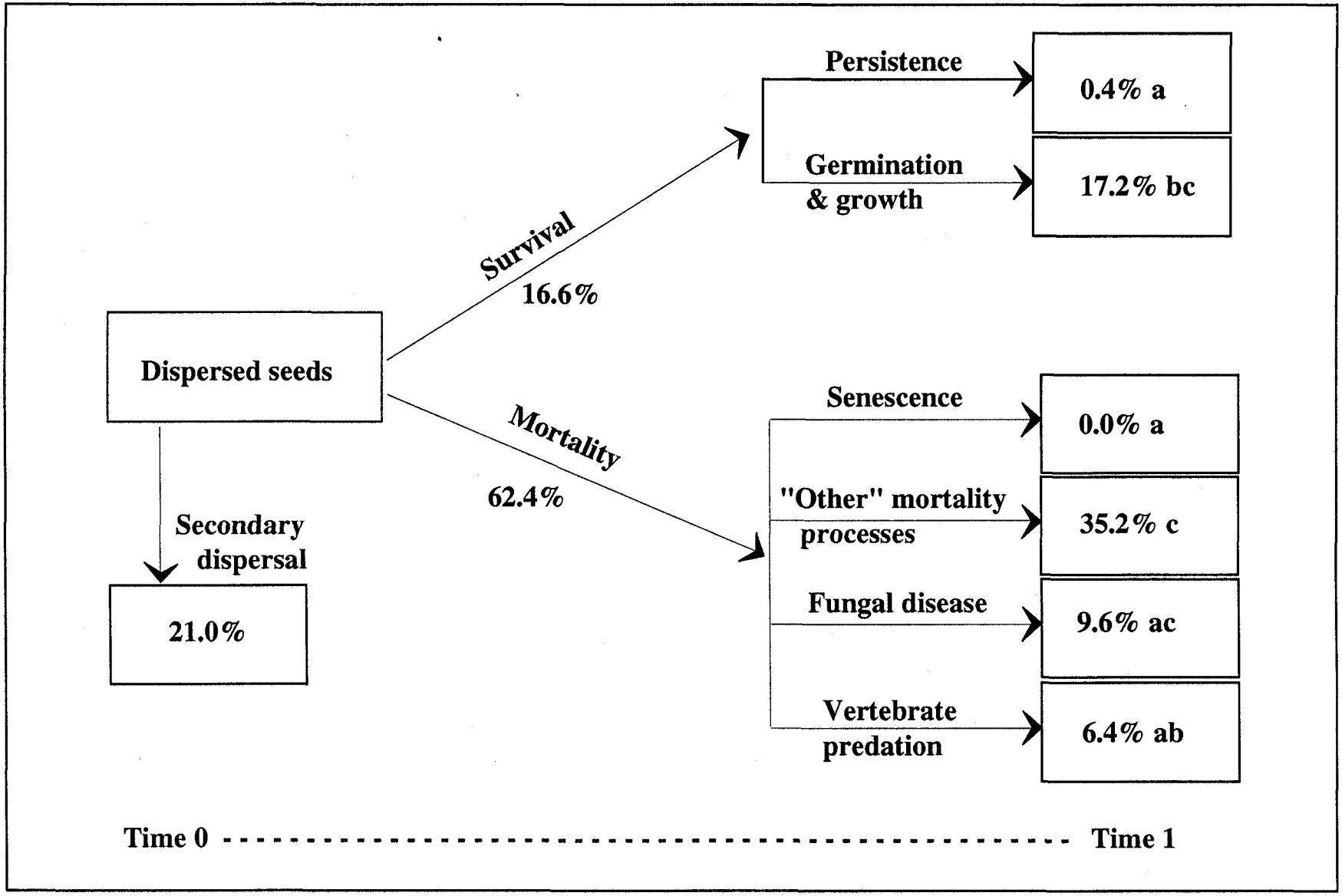
	1991-1992		1992-1993		
Species	mean (%)	95% Confidence Interval	mean (%)	95% Confidence Interval	*P*
Bromus carinatus	7.8	-2.5, 18.0	12.7	5.7, 19.8	0.42
Cynosurus echinatus	16.6	4.1, 29.1	11.6	3.0, 20.2	0.50
Daucus carota	28.6	4.5, 52.7	7.9	-7.8, 23.5	0.15
Prunella vulgaris	18.7	11.7, 25.6	4.5	-0.5, 9.5	<0.01

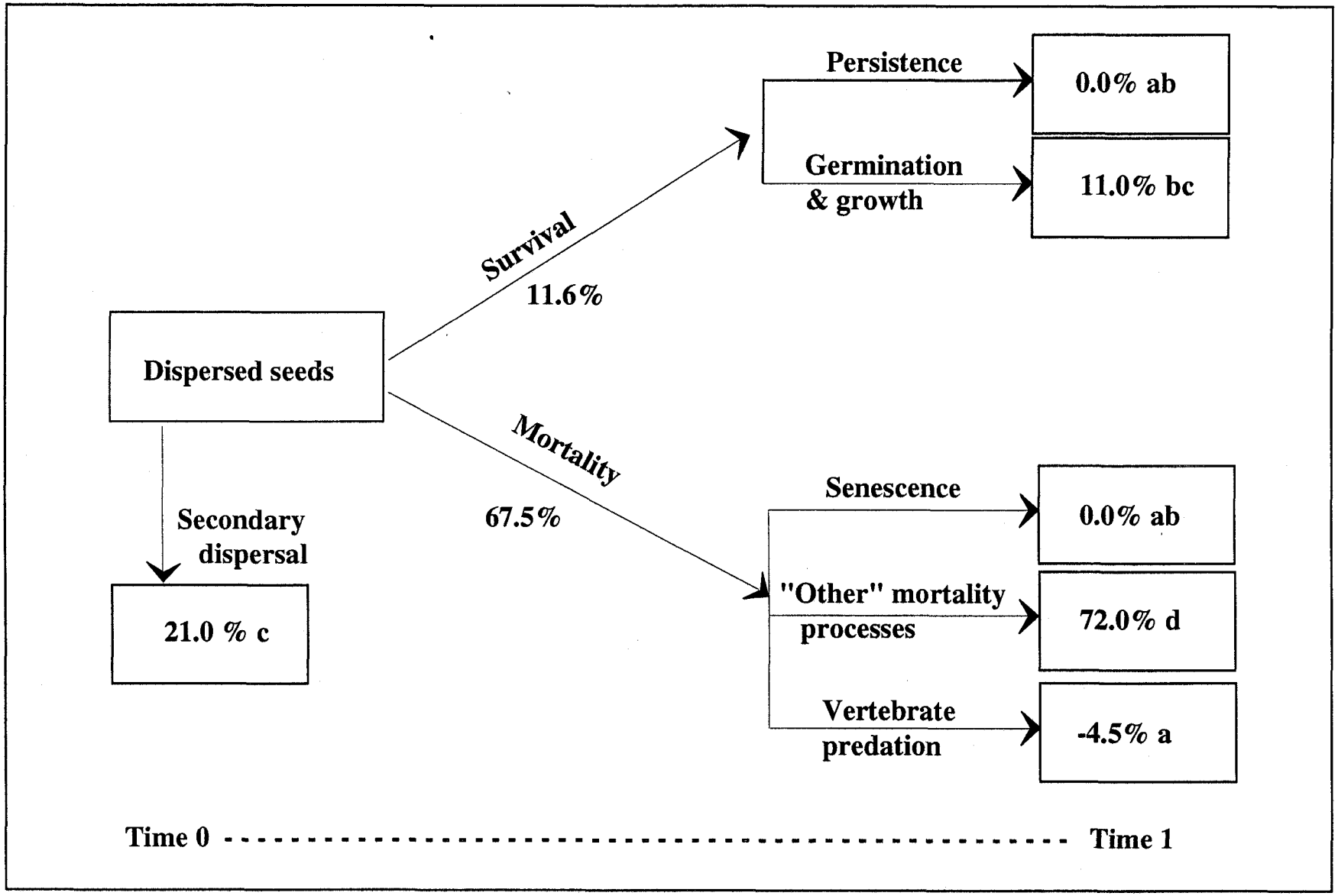
Cynosurus echinatus

Year one (1991-1992)

Many more seeds died (62.4%) than survived, with essentially all the seeds surviving as seedlings (17.2%), rather than as dormant seeds (0.4%) (Figure 3.3a). The largest cause of death was "other " mortality processes (35.2%) (Figure 3.3a, Tables 3.3 and 3.4). The magnitudes of fungal disease, vertebrate predation, and recovered senescent seeds were each less than 10% and not statistically different from each other (Figure 3.3a, Tables 3.3 and 3.4).

Figures 3.3a and 3.3b. Magnitude of post-dispersal seed fates for *Cynosurus echinatus*, a non-native grass of western Oregon prairies. figure 3.3a is for 1991-1992 and Figure 3.3b is for 1992-1993. Magnitudes are expressed as a percentage of the total number of experimentally sowed seeds. Negative magnitudes were possible because data were adjusted for background counts. Magnitudes do not always sum to 100% because of differences in sample sizes and methods of calculations. Details on calculations for each fate process are found in the text. Sample sizes and 95% confidence intervals for mean number f seeds in each category are found in Table 3.3. Results of one-way ANOVA comparing fate process magnitudes are found in Table 3.4. Separation of means tests (Fisher's protected LSD, $\alpha = 0.05$) were performed for significant results. The results of this separation of means test are presented in this figure. The magnitude of categories with the same letters are not significantly different from each other.


Persistence
0.4% a
Germination & growth
17.2% bc
Survival
16.6%
Dispersed seeds
Mortality
62.4%
Senescence
0.0% a
"Other" mortality processes
35.2% c
Fungal disease
9.6% ac
Vertebrate predation
6.4% ab
Secondary dispersal
21.0%
Time 0
Time 1


Persistence
0.0% ab
Germination & growth
11.0% bc
Survival
11.6%
Dispersed seeds
Mortality
67.5%
Secondary dispersal
21.0 % c
Senescence
0.0% ab
"Other" mortality processes
72.0% d
Vertebrate predation
-4.5% a
Time 0
Time 1

Year two (1992-1993)

Mortality (67.5%) was much greater than survival (11.6%) (Figure 3.3b). Survival as seedlings (11.0%) was greater than survival as dormant seeds (0%), although there was no statistical difference between survival categories (Figure 3.3b, Tables 3.5 and 3.6). "Other mortality processes" were the only cause of death (72.0%) (Figure 3.3b, Tables 3.5 and 3.6). Recovered senescent seeds (0.0%) and losses from vertebrate predation (-4.5%) were negligible (Figure 3.3b, Tables 3.5 and 3.6).

Comparison between year one and year two

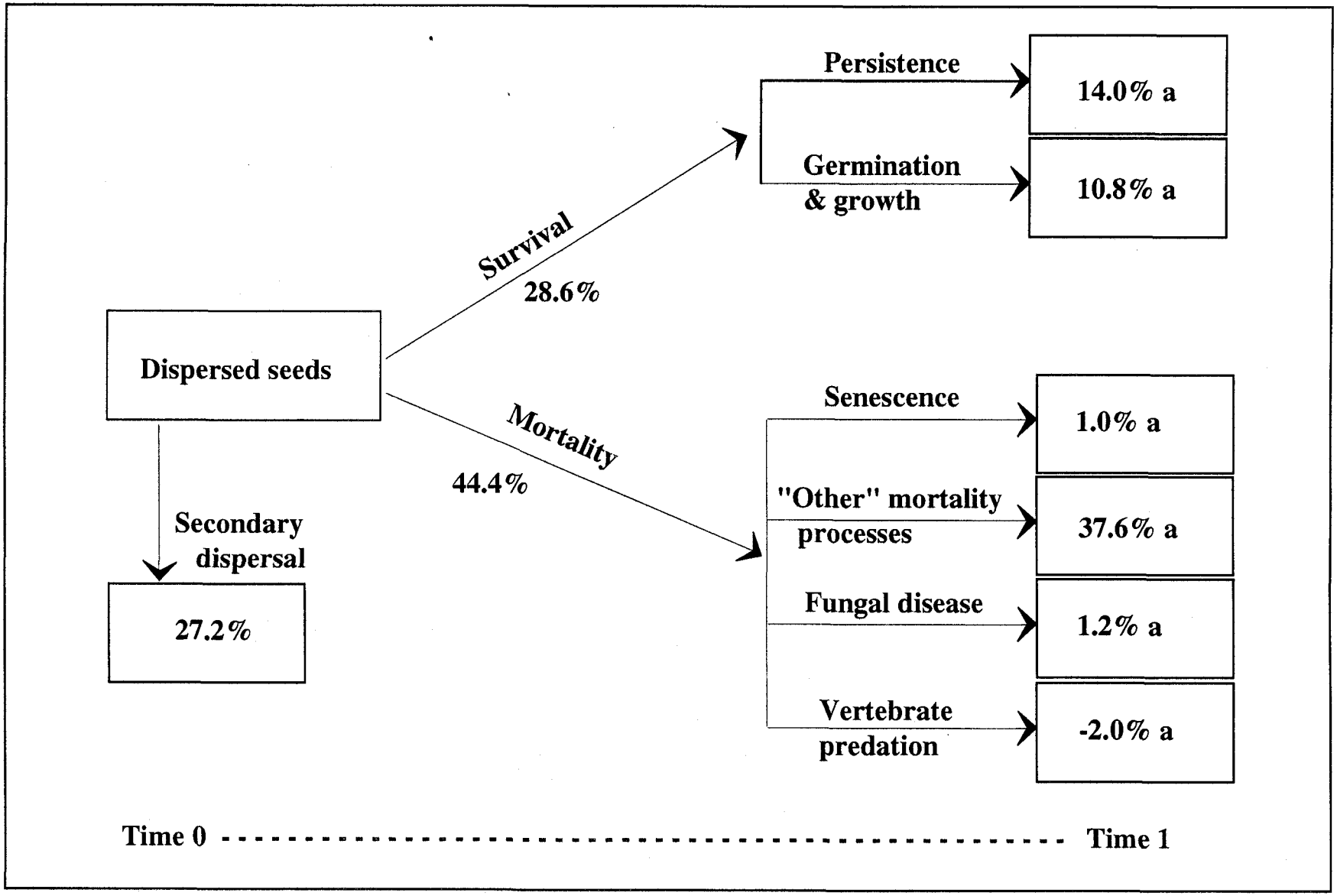
The patterns of mortality and survival were very similar for both years, with few of seeds/seedlings surviving (Figures 3.3a and 3.3b). The survival rates were similar between year one and year two, with no statistical differences ($P = 0.5$) (Table 3.7). There was 94% power to detect a 10% or larger difference between years. The patterns of mortality causes were similar for both years with "other" mortality causes the largest category for both years (Figures 3.3a and 3.3b, Tables 3.3 and 3.5).

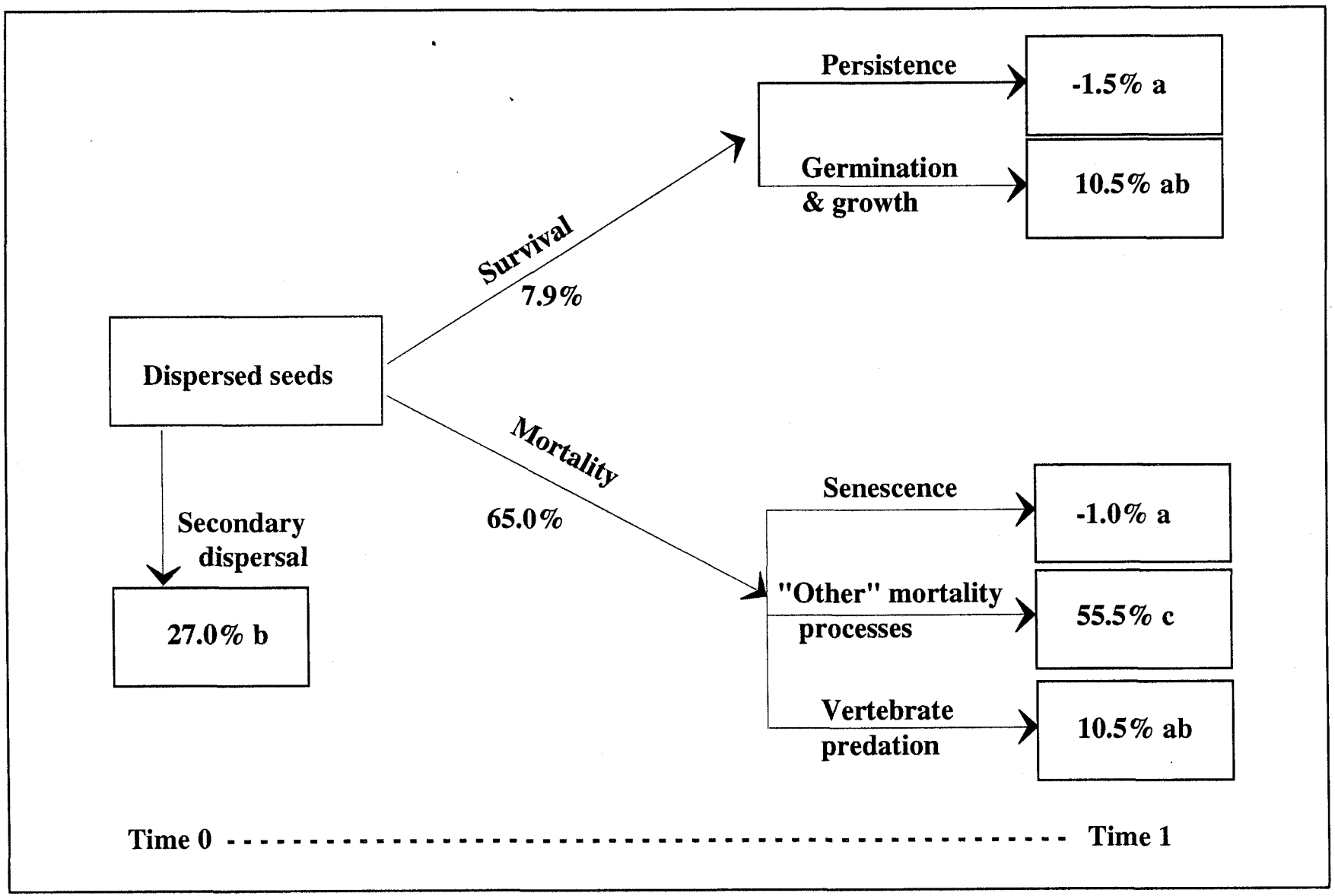
Daucus carota

Year one (1991-1992)

Although mortality was the largest seed fate (44.4%), the survival rate was relatively high (28.6%) with (14%) surviving as dormant seeds (Figure 3.4a). Magnitudes of seed fates did not differ significantly (Table 3.4). Senescence (1.0%), fungal disease (1.2%) and vertebrate predation/herbivory (-2%) contributed little to seed/seedling death,

Figures 3.4a and 3.4b. Magnitude of post-dispersal seed fates for *Daucus carota*, a non-native forb of western Oregon prairies. Figure 3.4a is for 1991-1992 and Figure 3.4b is for 1992-1993. Magnitudes are expressed as a percentage of the total number of experimentally sowed seeds. Negative magnitudes were possible because data were adjusted for background counts. Magnitudes do not always sum to100% because of differences in sample sizes and methods of calculations. Details on calculations for each fate process are found in the text. Sample sizes and 95% confidence intervals for mean number of seeds in each category are found in Table 3.3. Results of one-way ANOVA comparing fate process magnitudes are found in Table 3.4. Separation of means tests (Fisher's protected LSD, $\alpha = 0.05$) were performed for significant results. The results of this separation of means test are presented in this figure. The magnitude of categories with the same letters are not significantly different from each other.


Persistence
14.0% a
Germination
& growth
10.8% a
Survival
28.6%
Dispersed seeds
Mortality
44.4%
Senescence
1.0% a
"Other" mortality
processes
37.6% a
Fungal disease
1.2% a
Vertebrate
predation
-2.0% a
Secondary
dispersal
27.2%
Time 0
Time 1


Persistence
-1.5% a
Germination & growth
10.5% ab
Survival
7.9%
Dispersed seeds
Mortality
65.0%
Secondary dispersal
27.0% b
Senescence
-1.0% a
"Other" mortality processes
55.5% c
Vertebrate predation
10.5% ab
Time 0
Time 1

and "other mortality processes" (37.6%) caused the most deaths (Figure 3.4a, Tables 3.3 and 3.4). There were no statistical differences between the mortality causes given the extremely wide confidence interval for "other mortality processes" (Figure 3.4a, Tables 3.3 and 3.4).

Year two (1992-1993)

The fate of most seeds was death (65.0%), with few seeds surviving either as dormant seeds (-1.5%) or as seedlings (10.5%) (Figure 3.4b, Tables 3.5 and 3.6). "Other mortality processes" were significantly larger (55.5%) than the other causes of mortality (Figure 3.4b and Table 3.5). The remaining causes of death, vertebrate predation and senescence, were each less than 10.5% (Figure 3.4b, Tables 3.5 and 3.6).

Comparison between year one and year two

A substantial number (14.0%) of seeds persisted as dormant seeds the first year; virtually no seeds persisted the second year (Figures 3.4a and 3.4b). For both years "other mortality processes" caused the greatest magnitude of death even though "other mortality processes" don't include fungal disease in year one (Figures 3.4a and 3.4b, Tables 3.3 and 3.5). The remaining causes of death had small magnitudes, each less than 10.5% for both years (Figures 3.4a and 3.4b, Tables 3.5 and 3.7).

Prunella vulgaris

Year one (1991-1992)

Many more seeds/seedlings died (54.4%) than survived, with essentially all the seeds surviving as seedlings (17.2%) rather than as dormant seeds (1.6%) (Figure 3.5a). "Other mortality processes" (32.4%) are the major causes of mortality and fungal disease also at 22.4% (Figure 3.5a), although because of the very wide confidence interval for fungal disease, fungal disease and "other mortality processes" are not statistically distinguishable (Tables 3.3 and 3.4). The magnitudes of recovered senescent seeds and seeds lost to vertebrate predation were each 2% or less (Figure 3.5a, Tables 3.5 and 3.6).

Year two (1992-1993)

Many more seeds/seedlings died (68.5%) than survived either as seedlings (4.0%) or as dormant seeds (1.5%) (Figure 3.5b). The largest proportion (73.0%) died due to "other mortality processes" (Figure 3.5b, Tables 3.5 and 3.6). Two percent or less were lost to senescence (2.0 %) and vertebrate predation (-6.0%) (Figure 3.5b, Tables 3.5 and 3.6).

Comparison between year one and year two

For both years, mortality was much greater than survival rates (Figures 3.5a and 3.5b). Survival was significantly greater ($P < 0.01$) in year one than in year two (Table 3.7) mostly because of decreased germination and growth in year two. The patterns for

both years suggest that "other mortality processes" are the major causes for mortality (Figures 3.5a and 3.5b, Tables 3.3 and 3.5), although the magnitude of fungal disease was 22.4%.

Patterns of seed fates and processes among species

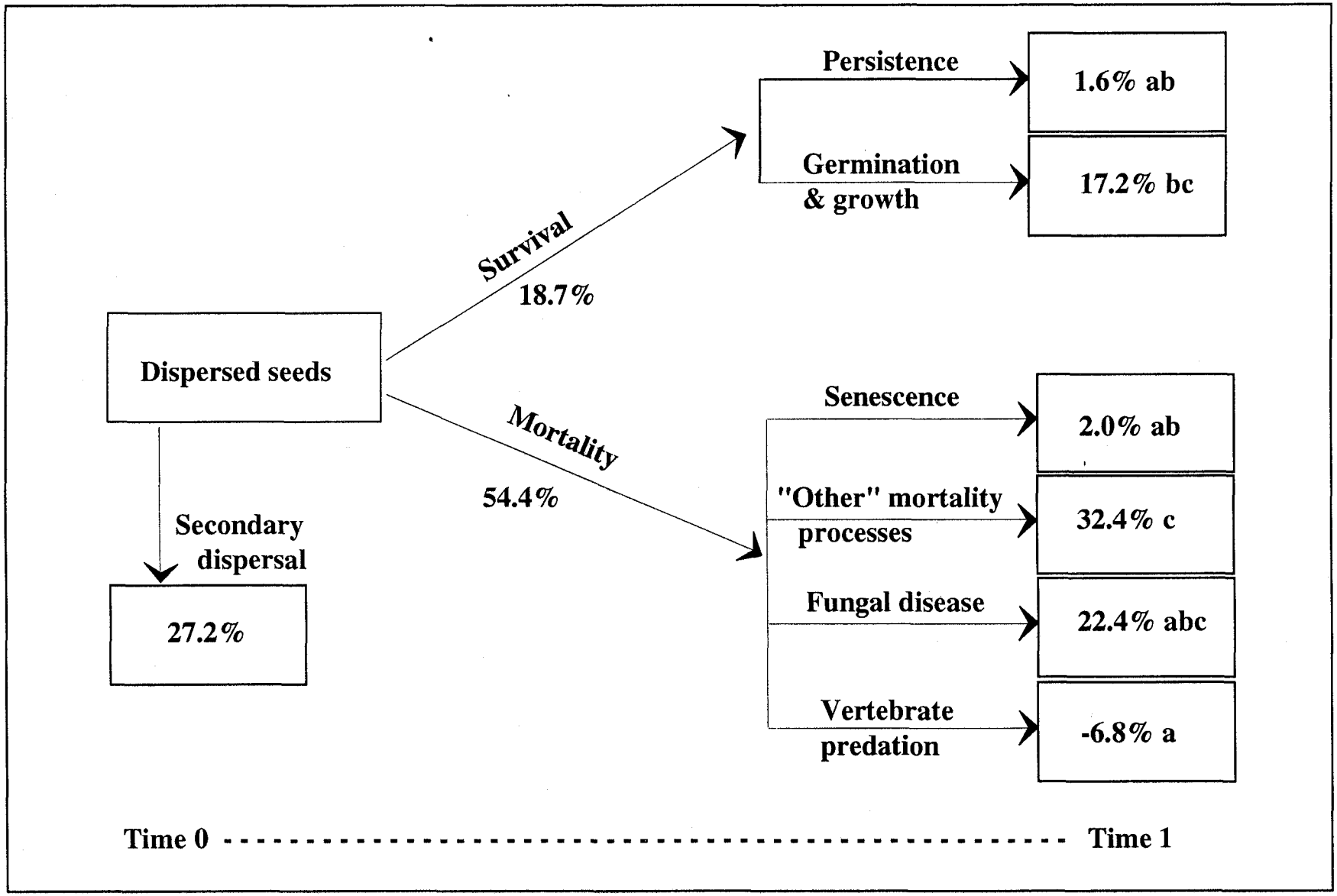
Mortality was high for all four species for both years (Figures 3.2-3.5). Survival was relatively low, with most seeds surviving as seedlings (Figures 3.2-3.5). A major exception to this pattern was *Daucus carota*, in which 14.0% of seeds persisted as dormant seeds the first year (Figure 3.4a).

"Other mortality processes" contributed the largest percentage of dead seeds for all four species for both years, even though the magnitude of fungal disease was not included in "other mortality processes" for year one (Figures 3.2-3.5). Loss of seeds to vertebrate predation was less than 11% for all species for both years with the exception of *Bromus carinatus* in year one, in which the proportion of seeds lost was 21.2% (Figures 3.2-3.5). Fungal disease occurred at magnitudes less than 10% for all species with the

exception of *Prunella vulgaris* in year one (22.4%) (Figures 3.2-3.5). For all species for both years, few senescent seeds (no more than 2%) were recovered (Figures 3.2-3.5).

Ranks of fate magnitudes differed significantly among species for both year one ($P < 0.01$) and year two ($P < 0.01$) (Kendall's test of correspondence, Table 3.8), supporting the description of differences in mortality causes.

Figures 3.5a and 3.5b. Magnitude of post-dispersal seed fates for *Prunella vulgaris* var. *lanceolata*, a native forb of western Oregon prairies. Figure 3.5a is for 1991-1992 and Figure 3.5b is for 1992-1993. Magnitudes are expressed as a percentage of the total number of experimentally sowed seeds. Negative magnitudes were possible because data were adjusted for background counts. Magnitudes do not always sum to100% because of differences in sample sizes and methods of calculations. Details on calculations for each fate process are found in the text. Sample sizes and 95% confidence intervals for mean number of seeds in each category are found in Table 3.3. Results of one-way ANOVA comparing fate process magnitudes are found in Table 3.4. Separation of means tests (Fisher's protected LSD,$\alpha = 0.05$) were performed for significant results. The results of this separation of means test are presented in this figure. The magnitude of categories with the same letters are not significantly different from each other.


Persistence
1.6% ab
Germination
& growth
17.2% bc
Survival
18.7%
Dispersed seeds
Mortality
54.4%
Senescence
2.0% ab
"Other" mortality
processes
32.4% c
Fungal disease
22.4% abc
Vertebrate
predation
-6.8% a
Secondary
dispersal
27.2%
Time 0
Time 1

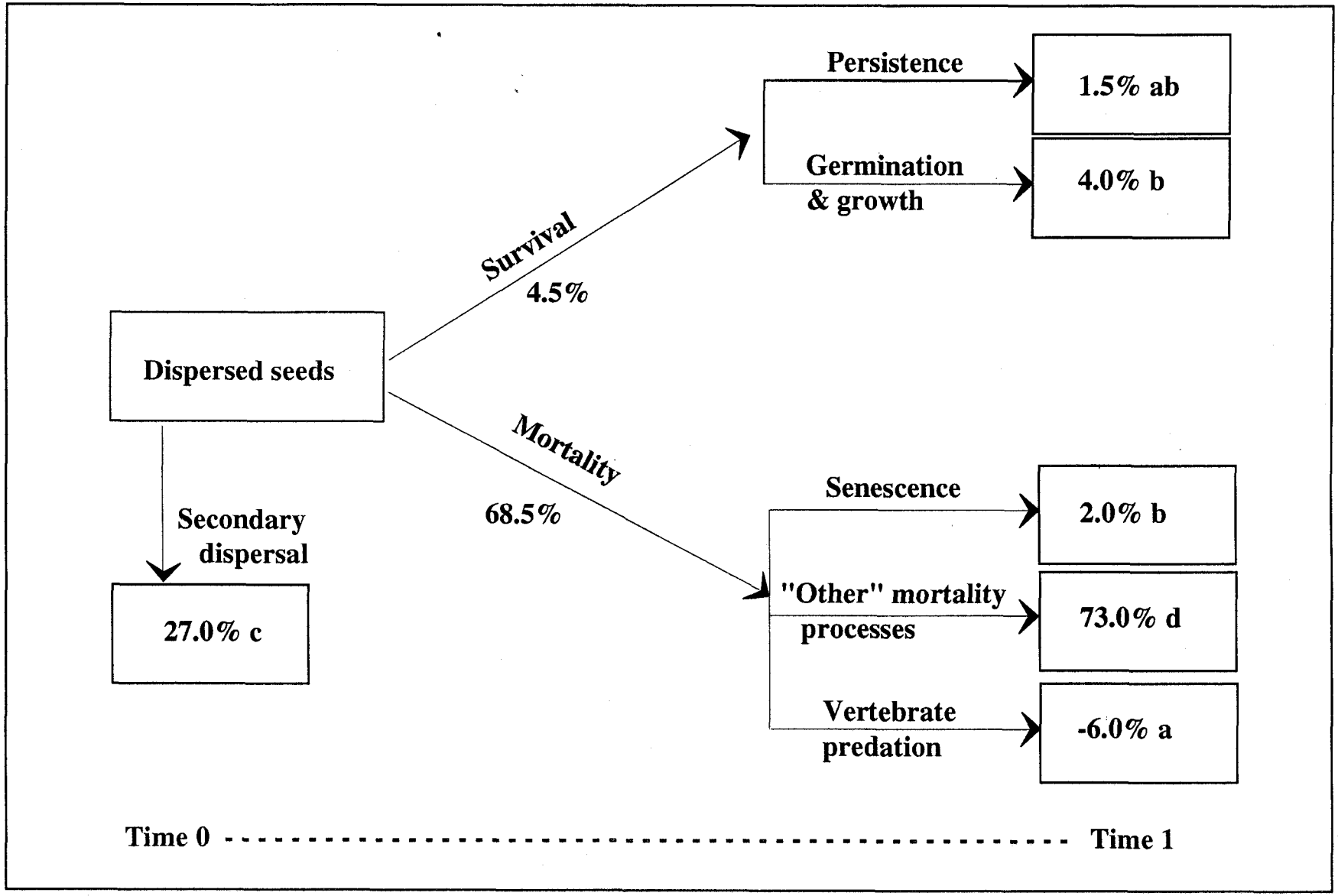

Persistence
1.5% ab
Germination & growth
4.0% b
Survival
4.5%
Dispersed seeds
Mortality
68.5%
Secondary dispersal
27.0% c
Senescence
2.0% b
"Other" mortality processes
73.0% d
Vertebrate predation
-6.0% a
Time 0
Time 1

Table 3.8. Results of the Kendall's coefficient of correspondence comparing ranking of six seed fate processes among four grasslands species, *Bromus carinatus, Cynosurus echinatus, Daucus carota* and *Prunella vulgaris* for 1991-1992 and 1992-1993. Kendall's coefficient of correspondence, *W*, is an overall measure of agreement among the rankings of the fates among the species. Values can range from 0 to 1. The sample statistic, χ^2 is computed from the sum of ranks for each of the fates. *P* is the probability that species differ in the ranking of their fates by chance. The means for the 1991-1992 seed fate processes are presented in Table 3.3. The means for 1992-1993 are presented in Table 3.5.

Year	df	W	χ^2	*P*
1991-1992	6	0.71	17.143	<0.01
1992-1993	5	0.87	17.429	<0.01

DISCUSSION

Patterns of seed fates

Germination and growth

The variability in data of this study was very large (as with most ecological field studies), making it difficult to estimate precise magnitudes of seed fates and processes. In spite of this difficulty, distinctive and consistent patterns emerge. Within a species, seed/seedling survival and mortality patterns were similar between years, in spite of the expectation that patterns would greatly differ between years because of variation in environmental factors (Sharitz and McCormick 1973, Burdon et al. 1983, Mack and Pyke 1989). Furthermore, for all study species most seeds died, with relatively few seeds surviving as seedlings. Causes of mortality differed among species.

Generally, small percentages of seeds produced by grassland species emerge and establish as seedlings (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989,

Thompson and Baster 1992, Reader 1993). Reported percentages of seedling establishment for *Daucus carota* are similar to the percentages in this study (approximately 11%), given the precision of the present estimate. Only 1% of the *Daucus carota* seeds sowed into unmanaged grasslands emerged with about half of the emergents surviving ten months later (Thompson and Baster 1992). Seedling emergence of *Daucus carota* was about 11% of seeds sowed in newly disturbed agricultural fields, 2% in five year old fields and 13% in 15 year old fields (Gross and Werner 1982). First year emergence was 18% of sowed seeds in recently fallowed agricultural fields (Holt 1972). Reported seedling emergence rates for *Prunella vulgaris*[1] range from 0.7 % to 30%, depending on the seed size and the habitat (Winn 1985). This range is somewhat wider than the establishment percentages (4%-17%) for this present study. No studies report seedling establishment rates for *Cynosurus echinatus* and *Bromus carinatus*.

Persistence

Comparison with other studies

Virtually no seeds persisted for three species (*Bromus carinatus, Cynosurus echinatus, Prunella vulgaris*), in contrast to the seeds of *Daucus carota,* some of which persisted in the soil for at least a year. Other studies also report a persistent seed bank for *Daucus carota*, with seed longevity varying between two and at least five years (Gross and Werner 1982, Thompson et al. 1993). Investigations of persistent seed banks for *Prunella vulgaris*[1] describe conflicting results. Roberts (1986) describes a persistent

[1] The variety of *Prunella vulgaris* was not reported in Winn (1985), nor in the other studies cited in this thesis that report results of *Prunella vulgaris* research.

seed bank in which a portion of the seeds persist for 5 years, but Winn (1985) says that *Prunella vulgaris* seeds do not survive more than one winter in the soil, which is consistent with the pattern in this present study. A single study reports the presence of *Cynosurus echinatus* seeds in the soil (Cocks 1994), but because the soils samples were collected in Australia in January, the presence of *Cynosurus echinatus* probably reflects a transient seed bank rather than a persistent seed bank. No studies report seed banks for *Bromus carinatus*.

Patterns of seed persistence for the study species are also similar to community level patterns of many European and North American grassland seed banks, where the seeds of most of the species that are frequent in the vegetation appear to be absent from the seed bank (Roberts 1981, Schenkeveld and Verkaar 1984, Rice 1989). None of the perennial species (*Bromus carinatus* and *Prunella vulgaris*) or grass species (*Bromus carinatus* and *Cynosurus echinatus*) in this study formed a persistent seed bank, which is consistent with other grassland seed banks, which often contain a larger proportion of annuals than perennials (Rice 1989), and are contain more forbs than grasses (Roberts 1981, Rice 1989, Baker 1989). Although weedy or fugitive species that colonize disturbances or gaps in vegetation are among the largest component of grassland seed bank species (Louda 1989, Rice 1989), in this study only one (*Daucus carota*) of three such species (*Cynosurus echinatus, Prunella vulgaris and Daucus carota*) formed a persistent seed bank.

Mechanisms promoting seed persistence

The morphological characteristics of *Daucus carota* seeds match the seed morphological patterns of British herbaceous species that maintain long-term persistent seed banks. Seed or fruit weight and the extent to which the seed or fruit shape differs from sphericity is correlated with persistence in the soil seed bank (Thompson et al. 1993). Compact (spherical) seeds or fruits that weigh less than 3 mg, such as those of *Daucus carota* (Table 3.2a), generally form long-term persistent seed banks (Thompson et al. 1993). Yet, *Prunella vulgaris*, which has small compact seeds similar to *Daucus carota* diaspores (Table 3.2), did not form a persistent seed bank in the present study. Moreover, other studies show that seeds of grass species that maintain persistent seed banks are generally small and compact (Thompson 1987, Thompson and Grime 1979), while seeds of grasses lacking persistent seed banks are large and attenuated, often with awns and retrose hairs, similar to the seed characteristics of both *Bromus carinatus* and *Cynosurus echinatus*, which did not form persistent seed banks in this study .

The mechanism underlying the above relationship between seed morphological characteristics and persistence in the seed bank is likely ease of burial. Smaller, more spherical seeds appear to have a greater probability of burial into the soil than larger, attenuated seeds (Peart 1984, Thompson et al. 1993). Burial, by blocking light, can induce secondary dormancy in seeds (Pons 1991), thus inhibiting germination (Pons 1991) and promoting seed persistence. Burial also reduces the risk from fire (Peart 1984) and predation (Reichman 1979, Crawley 1992, Hulme 1994), potentially increasing persistence in the soil seed bank. In the present study more of the smaller and rounder surrogate seeds (*Daucus carota* and *Prunella vulgaris* surrogates) were lost from the

subplots than with the larger more elongated surrogates (*Bromus carinatus* and *Cynosurus echinatus*), thus supporting the hypothesis that small size and compactness increase seed movement. Although the seed characteristics of both *Daucus carota* and *Prunella vulgaris* seeds seem to promote seed burial, wet *Prunella vulgaris* seeds produce a mucilaginous substance that causes the seeds to adhere to soil particles and litter, potentially hindering seed burial and thus, exposing seeds to processes that limit persistence in the soil.

If seeds are to persist in the soil until conditions are favorable for increased seedling survival, germination must be delayed. Seeds of *Daucus carota* readily germinated in laboratory tests conducted over several months at 30° C (day) and 20° C (night) with 14 hours of light (D. Clark, unpublished data). Other investigations show that seed germination of *Daucus carota* decreases substantially without light (Gross 1984, Pons 1991). Furthermore, *Daucus carota* seeds show a seasonal pattern in some grasslands in availability of non-dormant seeds, with secondary dormancy highest in the summer months and lowest in January through April (Pons 1991). Both these dormancy mechanisms allow *Daucus carota* seeds to avoid germination, potentially promoting seed persistence.

Seeds of the other three study species, which lack persistent seed banks, also germinated readily in laboratory tests conducted over several months at 30° C (day) and 20° C (night) with 14 hours of light (D. Clark, unpublished data). *Prunella vulgaris*[1] also germinated readily in other investigations (Winn 1985). Grasses with awned seeds often germinate immediately when supplied with moisture and a suitable temperature, in contrast to many unawned grass species, which exhibit seed dormancy (Peart 1984).

Furthermore, seeds of many grasses found in habitats characterized by summer drought (such as the Willamette Valley) are capable of germination under a wide range of temperatures, although timing of germination is determined by amount of moisture (Thompson and Grime 1979). Seeds of perennial grass species grown as crops in the Willamette Valley germinate as soon as the soil stays wet long enough (at least 48 hours) for the seed to imbibe and germinate (M. Azevedo 1995, personal communication). Germination of these species continues through the winter until cold soil temperatures limit germination, but germination begins again when soil temperatures warm (M. Azevedo 1995, personal communication). *Cynosurus echinatus* germinates readily under field conditions during fall and early winter months in the Willamette Valley (M. Maret 1995, unpublished data). Limited observations suggest that *Bromus carinatus* also germinates during this time (M. Maret, unpublished data). Except during the summer, therefore, seeds of these grass species make little or no contribution to the seed bank (Thompson and Grime 1979). Taken together, these studies indicate that lack of dormancy and non-restrictive germination requirements are mechanisms reducing long-term seed persistence for *Bromus carinatus*, *Cynosurus echinatus*, and *Prunella vulgaris*.

Mortality

Delaying germination is not sufficient for long-term seed persistence. Seeds must also avoid death. In the following section, the general discussion of mortality factors that caused the very large number of seed and seedling deaths includes those various mortality factors contributing to lack of seed persistence.

Senescence

The magnitude of recovered senescent seeds was consistently small (never more than 2%) for all species for both years. The germination percentages of seeds stored at room temperature for two years after seed collection were more than 70% for *Bromus carinatus* and *Prunella vulgaris* and almost 99% for *Cynosurus echinatus* and *Daucus carota* (Appendix 5). After three years of storage at room temperatures, only seeds of *Bromus carinatus* had less than 85% germination (Appendix 5). These species, thus, have the genetic capability to remain viable several years, suggesting that perhaps senescence was a minor factor contributing to seed deaths in this study. Actual field longevity will depend on environmental conditions experienced by the seeds.

Fungal disease

Loss from disease by common soil fungi in this study was less than ten percent with the possible exception of *Prunella vulgaris*. Other investigations of these four species also report that seedling death caused by fungal disease is less than ten percent (Chapter 4). However, in contrast to these results, the rare studies investigating seed and seedling mortality by fungi in natural vegetation describe significant mortality by fungi (Lawrence and Rediske 1962, Mack and Pyke 1984, Augspurger 1990, but see Lonsdale 1993). Fungi accounted for the greater part of seed loss during the pre-germination period of Douglas-fir seeds and were also the principal cause of Douglas-fir seedling death (Lawrence and Rediske 1962). The magnitude of loss caused by fungal disease (damping-off) ranked from high to very low, relative to other mortality factors

contributing to deaths of tropical tree seedlings (Augspurger 1990). A significant number of *Bromus tectorum* seedlings died from an infestation of a smut *Ustilago bullata* over a three year period in a semi-arid grassland (Mack and Pyke 1984).

Vertebrate predation

Grassland plants can lose large numbers of dispersed seeds to predators, principally vertebrates (Louda 1989, Crawley 1992). Vertebrate predation, in this study, appeared to be an important mortality factor for only one species, *Bromus carinatus*. These results are consistent with a concurrent study conducted at the same study site in which predation caused significant seed losses for *Bromus carinatus*, but caused no significant seed losses for *Cynosurus echinatus* or *Daucus carota* (Appendix 6).

Vertebrate predation of large seeds, especially those with thin seed coats such as *Bromus carinatus,* is generally much greater than predation of smaller seeds, such as *Daucus carota* and *Prunella vulgaris*, possibly due to ease of handling and nutrient content (Thompson 1987). Furthermore, as discussed earlier, small compact seeds such as *Daucus carota* are more easily buried in the soil compared to large awned seeds such as *Bromus carinatus*, thus decreasing susceptibility to predators (Thompson et al.1993).

Presence of toxic compounds in the seeds may have contributed to differences in vertebrate predation rates among the four study species. *Prunella vulgaris*[1] contains high levels of ortho-dihydroxyphenol, a defense compound deterring predation (Hendry et al. 1994). In this same study (Hendry et al. 1994), which compared seed concentrations of ortho-dihydroxphenol with longevity of seeds in the soil seed bank, the

species with the lowest levels of ortho-dihydroxyphenol were grasses (Hendry et al. 1994). Janzen (1971) also commented on the general lack of toxicity of grass seeds.

"Other mortality processes"

The magnitude of "other mortality processes" was generally greater than any measured mortality factor for all four species. Potential processes in this fate category include invertebrate predation, abiotic processes, interference from neighboring plants and bacterial or viral diseases (Table 3.9). Because the magnitude of this category was calculated as the difference between the number of seeds sowed and the number of seeds accounted for in measured fate categories, "other mortality processes" also include any senescent seeds that disappeared due to saprophytic decay. The following section discusses which of these processes were likely components of "other mortality processes" for each of the study species (Table 3.9).

Other microbial diseases

Although seeds are common vectors of bacteria and viruses (Neegaard 1977), rarely do studies report death of dispersed seeds or seedlings caused by bacterial or viral diseases in agricultural systems. In contrast, many studies investigating seed-bacteria interactions report antagonistic reactions by seed-borne bacteria against fungi, potentially protecting the seed from fungal disease (Liu and Vaughan 11965, Kremer et al. 1984, Bruehl 1987). Based on these patterns, it is unlikely bacterial or viral diseases played a large role in mortality of seeds or seedlings in this study (Table 3.9).

Table 3.9. Possible components of "other mortality processes" for four species in western Oregon native prairies. A "+" indicates that the mortality factor is a likely component of "other mortality processes", a "-" indicates that the mortality factor is not likely a component, and "?" indicates evidence is not available to determine whether the mortality factor is a component or not.

Species	"other mortality processes"			
	bacterial/viral diseases	invertebrate predation	abiotic factors	interference
Bromus carinatus	-	?	+	+
Cynosurus echinatus	-	-	+	+
Daucus carota	-	-	+	-
Prunella vulgaris	-	+	+	-

Invertebrate predation

Rodents are often the major predators of dispersed seeds in grasslands (Louda 1989), with negligible losses attributable to invertebrates (Hulme 1994, but see Reader 1991, 1993). To determine whether invertebrate predation was a component of "other mortality processes", the present study in which predation by *only* vertebrates was measured was compared with a concurrent study with the same species at the same study site in which the *combined* predation from both vertebrates and invertebrates was measured (Appendix 6). In the present study significant losses from vertebrate predation occurred only for *Bromus carinatus*. In the concurrent study, significant combined predation occurred for *Bromus carinatus* and *Prunella vulgaris*, but not for *Cynosurus echinatus* and *Daucus carota* (Appendix 6). Thus, comparison of these two studies suggests that invertebrate predation caused insignificant losses for *Daucus carota* and

Cynosurus echinatus (Table 3.9). Given virtually no vertebrate predation for *Prunella vulgaris* in this study and significant seed predation in the concurrent study, invertebrate predation is likely to be a component in "other mortality processes" for *Prunella vulgaris* (Table 10). Predation of *Bromus carinatus* occurred in both studies, and thus, the loss can not be categorized as to vertebrate or invertebrate predation (Table 3.9).

Interference

Interference is used to refer to the overall adverse influence of one plant on another, thus, encompassing both competition, which involves the reduction of resources needed by a neighboring plant, and allelopathy, which refers to the biochemical interactions between organism caused by the addition of a chemical compound to the environment by one of the interacting organisms (Rice 1989).

Generally, few seeds successfully germinate and grow in dense unmowed grasslands. Most seedlings need small patches of bare soil or short turf to avoid death possibly caused by competition for space, light, nutrients or water (Fenner 1978, Gross and Werner 1982, Gross 1984, Winn 1985, McConnaughay and Bazzaz 1987, Peart 1989, Silvertown and Tremlett 1989, Reader 1991, Thompson and Baster 1992, Wilson and Gerry 1995). An alternative explanation for seedling deaths in closed vegetation is allelopathy. Numerous studies have implicated allelopathy as a major cause of seedling mortality in grasslands (review by Rice 1984), but the potential effect in Willamette Valley grasslands is unknown.

Seedling establishment of *Daucus carota* can occur in closed vegetation with few gaps (Gross and Werner 1982), but decreased abundance of surrounding vegetation

enhances emergence and establishment (Holt 1972, Gross and Werner 1982, Silvertown and Tremlett 1989, but see Reader 1993). These patterns contrast with a study conducted concurrently with the present study at the same site (L. Lantz, unpublished data). In this concurrent study, seedling mortality was measured one year after seeds of the four study species were sowed into untreated field plots and into plots in which the aboveground and belowground vegetation had been removed. Seedling mortality of *Daucus carota* and *Prunella vulgaris* did not differ significantly between field plots treated with and without vegetation removal. Seedling mortality significantly decreased in plots with vegetation removal, however, for the two species with the largest seeds, *Bromus carinatus* and *Cynosurus echinatus* (L. Lantz, unpublished data). Sowing seeds of these two species into vegetation that was only mowed (no vegetation removal) did not significantly increase seedling survival of either grass species (Chapter 5), suggesting that competition for light was not a factor. These studies suggest that reduction of space or belowground resources by mature plants is in all likelihood an important component of "other mortality processes" in the present study for two species, *Bromus carinatus* and *Cynosurus echinatus* (Table 3.9).

Abiotic processes

Abiotic factors that directly kill seeds include crushing, burning (Peart 1984), desiccation (Murdoch and Ellis 1992), and extreme temperatures. Additional abiotic factors that kill seedlings include drought (Mack and Pyke 1984, Soriano and Sala 1986), nutrient shortages (Chambers et al. 1990), burial too deep for emergence (Schafer and Chilcote 1970), mechanical inhibition by litter (Bergelson 1990) and poor radicle contact with the soil (Peart 1984). It is likely that abiotic processes cause few seed deaths

compared to the rate of seedling deaths, because of the greater vulnerability of seedlings to these abiotic processes.

Abiotic processes that cause seedling death may be particularly important when seeds readily germinate and lack dormancy mechanisms, such as the seeds of the study species (with the exception of *Daucus carota).* Highly variable environmental conditions or unusual weather conditions can miscue germination so that timing of germination is unsuitable for seedling survival. Seeds of perennial grass crops in the Willamette Valley readily germinate soon after the fall rains (M. Azevedo 1995, personal communication). Seedlings can survive freezing temperatures during the winter, but do not survive repeated thawing and freezing, because frost heaving rips the roots from the soil (M. Azevedo 1995, personal communication). Abiotic factors that cause mortality can not be ruled out for any of the species in this study and are likely to contribute a significant number of seedling deaths (Table 3.9).

Summary of "other mortality processes"

Most deaths caused by "other mortality processes" for *Bromus carinatus* and *Cynosurus echinatus* likely occurred as seedlings either by interference or abiotic processes, although invertebrate predation can not be ruled out for *Bromus carinatus* (Table 3.9). Seedling death due to abiotic processes is most likely the largest component of "other mortality processes" for *Daucus carota* (Table 3.9). For *Prunella vulgaris*, a combination of seed death by invertebrate predators and seedling death by abiotic processes are most likely the largest components of the "other" processes (Table 3.9).

Summary of seed fate patterns

Three fates await seeds after dispersal. Seeds can either persist as seeds, germinate and grow as seedlings, or die. Most of the sowed seeds in this study died, with few seeds germinating and surviving as seedlings for any of the four species for either year. Virtually no seeds persisted for three of the study species, whereas seeds of *Daucus carota* formed a small persistent seed bank for at least one year.

A summary of the factors causing mortality are presented in Table 3.10. "Other mortality processes" were generally the largest cause of death for all four species for both years, even though the "other mortality processes" did not include loss from fungal disease in year one as in year two. The likely components of "other mortality processes" (invertebrate predation, interference or abiotic processes) varied as follows between species. Most deaths caused by "other mortality processes" for *Bromus carinatus* and *Cynosurus echinatus* probably occurred during the seedling stage with a combination of interference and abiotic processes as the most likely causes. Abiotic processes that killed seedlings were likely the principle cause of death for *Daucus carota*. The most probable components of "other mortality processes" for *Prunella vulgaris* were seed death by invertebrate predation and seedling death by abiotic processes.

Table 3.10. Summary of mortality factors for four species common to western Oregon native prairies in 1991-1993. A "+" indicates the mortality factor caused death. A "-" indicates that the mortality factor did not cause death. A "?" means evidence is not available to detemine whether or not the mortality factor caused death.

Species	Mortality factors					
	senescence	disease	vertebrate predation	invertebrate predation	abiotic factors	interference
Bromus carinatus	?	-	+	?	+	+
Cynosurus echinatus	?	-	-	-	+	+
Daucus carota	?	-	-	-	+	-
Prunella vulgaris	?	-	-	+	+	-

Disease by common soil fungi caused less than ten percent mortality, with the exception of *Prunella vulgaris*, in contrast to expectations of much higher magnitudes. Vertebrate predation substantially reduced the survival of only one species, *Bromus carinatus*. Because of the inherent difficulties in measuring seeds and germinants that disappear from microbial decay, the magnitude of seed senescence is unknown. The potential magnitude, however, of seed senescence after one year is possibly minor based on laboratory experiments for all four species.

These patterns of mortality help explain the lack of seed persistence for three of the study species. Seed persistence results from delaying germination and avoiding death. The lack of a persistent seed bank for *Cynosurus echinatus* is not likely caused by seed death, although bacterial and viral diseases cannot ruled out, suggesting that lack of dormancy and non-restrictive germination requirements are the probable mechanisms for lack of seed persistence. Lack of dormancy or non-restrictive germination requirements

and seed death caused by predation are responsible for lack of long-term seed persistence for *Bromus carinatus* and *Prunella vulgaris*.

Implications

From the patterns of the three seed fates (survival as seedling, persistence as seed, or death) observed in this study, predictions can be made about population and community patterns of the four study species. High seed and seedling mortality rates can potentially limit the population sizes of all four study species, particularly because these species have limited or no ability to increase population density by vegetative regeneration, with the possible exception of *Prunella vulgaris*. Predation pressure, if consistent, can limit the population distribution of both *Bromus carinatus* and *Prunella vulgaris*. Because belowground competition is likely a cause of mortality for both *Cynosurus echinatus* and *Bromus carinatus*, their abundance should be greater in areas in which gaps occur more often compared to areas where gaps occur less often, assuming that compensatory mortality does not occur.

Because the results of this study suggest that the annual *Cynosurus echinatus* forms no persistent seed bank, elimination of seed production will quickly extirpate a population of *Cynosurus echinatus* unless off-site seed dispersal occurs. In contrast, a population of *Daucus carota* can persist as seeds in the soil even with no seed production over a period of years. Although the perennial species *Bromus carinatus* and *Prunella vulgaris* lack persistent seed banks, populations can also persist at a site without seed production due to longevity of adults.

Conservation of Native Prairies

To conserve native prairies, managers seek to increase the abundance of native species and reduce the abundance of weedy non-native species. Two approaches can achieve these goals, (1) control of propagule availability and (2) control of mortality factors. The results from this study suggest the following management options.

Native species

Sowing extra seeds of the two native species should increase their population sizes, assuming that safe-sites are not limited. Even if long-distance dispersal occurs, an outside source of seeds is necessary to reintroduce these species on sites where these species are absent, because seeds of the native species did not persist in the soil .

Applying pesticides that prevent vertebrate seed predation for *Bromus carinatus* and invertebrate seed predation for *Prunella vulgaris* before sowing will increase seedling establishment rates, assuming no compensatory mortality by other factors. Sowing seeds in natural or artificial gaps in the vegetation will increase seedling establishment of *Bromus carinatus*.

Non-native species

Controlling propagule availability is more likely to control abundance of the non-native species than is controlling mortality factors. Because the annual *Cynosurus echinatus* lacks a persistent seed bank, removal of the on-site seed source by mowing before seed maturation should eventually eliminate the population. In contrast, removal of seeds before production may not eliminate a population of *Daucus carota* because it

can regenerate from propagules in its persistent seed bank. If, however, the seed bank receives no new seed inputs, it may be virtually eliminated after 5-6 years (Roberts 1986). Disturbances that create gaps in the vegetation decrease seedling death from competition for *Cynosurus echinatus*. Thus, eliminating this type of disturbance will decrease abundance of this species.

CHAPTER 4
EFFECTS OF FUNGAL DISEASE ON SEEDS AND SEEDLINGS IN A WESTERN OREGON NATIVE PRAIRIE

INTRODUCTION

Microbial disease is a generally unstudied but potentially important influence on plant population dynamics and plant community diversity in natural ecosystems (reviews by Burdon and Shattock 1980, Dinoor and Eshed 1984, Burdon 1987, Kranz 1990). Many examples demonstrate the role pathogens have in determining population sizes and distributions, and, thus, community composition (Pratt and Heather 1973, Dinoor and Eshed 1984, Weste 1987, Supkoff et al. 1988, von Broembsen 1989, Kranz 1990, Carlsson et al. 1990, Dobson and Crawley 1994). By controlling the distribution of plant populations, pathogens can enforce absence or rarity of species (Sewell 1981, Harper 1990). Thus, disease in natural vegetation may be unapparent with only rare signs of damage, but nevertheless may be a significant factor regulating population and community dynamics.

Given the substantial magnitude of seedling death caused by microbial pathogens in agricultural systems (Sewell 1981, Harman and Stasz 1986), pathogens are likely to be a significant cause of the tremendous seedling mortality in natural ecosystems (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989, Thompson and Baster 1992, Reader 1993). The pathogens known to be major causes of seed and seedling death (*Pythium, Rhizoctonia, Phytophthora and Fusarium*) are among the most ubiquitous of soil-borne fungi (Sewell 1981, Harman and Stasz 1986, Bruehl 1987). Yet the effect of these fungi on seeds and seedlings in natural systems has rarely been documented (Burdon 1987,

Kranz 1990) due to the inherent difficulties in ascribing seed and seedling losses to one of several possible mortality factors. One potential method for determining the magnitude of death caused by fungi is to exclude them experimentally with fungicides (Burdon 1987, Paul et al.1989, Harper 1990). Although investigators have used chemical exclusion in agricultural experiments (Kreitlow et al. 1950, Michail and Carr 1966, Clements et al. 1982, Dowling and Linscott 1983, Jansen and Ison 1995), few have chemically excluded pathogenic fungi, particularly those causing seedling death, in natural vegetation (Lonsdale 1993)

The objective of this study was to determine the magnitude of seed and seedling death caused by fungal disease for four common plant species in western Oregon native prairies. The approach used to address this question included both field and pot experiments which chemically excluded soil-borne pathogens characteristically associated with seed and seedling deaths were.

METHODS AND ANALYSIS

Study species

The four study species, *Bromus carinatus* Hook and Arn. var. *carinatus*, *Cynosurus echinatus* L., *Daucus carota* L., and *Prunella vulgaris* var. *lanceolata* (Barton) Fern. are dominant at the study site (Table 4.1). The criteria for species selection were that the study species (1) represent a variety of life histories, (2) be common and abundant in western Oregon prairies, and (3) produce sufficient seeds for the research. Nomenclature follows Hitchcock and Cronquist (1973).

Table 4.1. Description of the study species.

Species	Family	Native to Oregon?	Life-span	Seed description
Bromus carinatus var. *carinatus*	Poaceae	yes	perennial	awned
Cynosurus echinatus	Poaceae	no	annual	awned
Prunella vulgaris var. *lanceolata*	Labiatae	yes	perennial	hard-seeded
Daucus carota	Apiaceae	no	biennial	barbed

The Eurasian *Prunella vulgaris* var. *vulgaris* has the middle cauline leaves about half as wide as long, with a broadly rounded base. It grows occasionally in disturbed sites in the Northwest, where it is often dwarfed and prostrate (Hitchcock and Cronquist 1973). The native American *Prunella vulgaris* var. *lanceolata* is ascending or erect, with middle cauline leaves about a third as wide as long and more tapering toward the base (Hitchcock and Cronquist 1973). The variety that is commonly found in undisturbed habitats in Oregon is generally considered to be the native variety (H. Chambers 1995, personal communication).

Throughout this study, the study species will be referred to as *Bromus carinatus, Cynosurus echinatus, Daucus carota* and *Prunella vulgaris.* Although the term seed is used, it is refers to the diaspore, i.e., the seed and any associated structures.

Field experiment

The field experiment was part of a larger study (Chapter 3), which quantified the post-dispersal seed fates of four species in a western Oregon native prairie.

Study site

The study site is an upland prairie, with silty clay loam (Dixonville soil series) dominated by the native bunchgrasses, *Festuca idahoensis* var. *roemeri*, *Bromus carinatus*, and *Elymus glaucus*. This site, part of the Open Space Park managed by Benton County Parks, is located approximately 8 kilometers northwest of Corvallis, Oregon, in the foothills of the Coast Range (T11S, R6W, Sec 25, W.M.). The elevation is approximately 300 m with a 30-50% slope, and a westerly aspect. The site is one of the few remnants of a vast prairie and oak-savanna ecosystem that covered much of the Willamette Valley until after the 1840s (Boag 1992). The entire Willamette Valley has a fairly homogeneous climate with mild, wet winters, moderate and dry summers, and cool nights. Corvallis (Owenby and Ezell 1992) had an average annual precipitation of 108 cm, an average maximum January temperature of 7.5° C, and maximum average July temperature of 26.8° C. The average precipitation during the first year of this study (June 1991-May 1992) was 6.14 cm and for the second year (June 1992-May 1993), 8.84 cm (George Taylor, OR State Climatologist). The average maximum temperature for January 1992 was 9.7°C and for January 1993, 5.3°C. The average maximum temperature for July 1992 was 27.8°C and for July 1993, 23.2°C.

Experimental design and treatments

In early July, 1991, twenty 2.2 m × 2.2 m blocks were randomly located at the study site. Ten of these blocks were mowed as part of the larger study (Chapter 3) and excluded in this present study. Within each block, two randomly placed plots (25 cm × 35 cm) were either treated with fungicides or left untreated. Three other randomly

placed plots in each block received one of three caging treatments as part of the larger study (Chapter 3). Four subplots, each 5 cm in diameter, were located and permanently marked with nails within each plot. Twenty-five seeds of one of the four species were sowed into a subplot immediately following seed collection at the study site. Data were not collected on all the fungicide treated plots, resulting in unequal sample sizes, $n = 4$ for fungicide treated plots and $n = 20$ for untreated plots for each species.

A mixture of two fungicides was used for the fungicide treatment: metalaxyl, N-(2, 6-dimethylphenyl) - N-(methoxyacetyl) alanine methyl ester, and Captan, N-tricholoromethylthio-4-cyclohexene -1,2, dicarboximide. Both fungicides are commonly used to prevent pre- and post-emergence damping-off of seedlings caused by fungi (USEPA 1975, Jeffs 1986, Schwinn and Urech 1986, Koepsell and Pscheidt 1995). Metalaxyl protects seeds and seedlings from disease caused by Oomycetes, e.g.,*Pythium* and *Phytophthora* (Schwinn and Urech 1986). The broad spectrum fungicide Captan was added to protect against seedling diseases caused by other fungi, such as *Rhizoctonia* (Agnihotri 1971, Wainwright and Pugh 1975, USEPA 1975, Jeffs 1986). Captan was sprayed on the soil surface every two weeks and metalaxyl sprayed every 30 days, using the manufacturer's recommended rates, beginning August 1, 1991 and ending May 28, 1992.

Data collection

In mid-June 1992, approximately one year after seeds were sowed, the number of seedlings for each of the study species was counted in each subplot. Background counts for each species were made from the number of seedlings established in subplots not sowed with the study species.

Analysis

The data were analyzed using all components of the larger study (for methods, see Chapter 3). For each species, seedling numbers was analyzed for treatment effects using split-plot analysis of variance (ANOVA) (Steele and Torrie 1960). Mowing was the whole plot factor, with blocks nested within the mowing treatment, and treatment was the subplot factor (with the subplot being a factorial design of caging and fungicide). The General Linear Model (GLM) procedure of the SAS Institute statistical software, version 6.08, and type III sum of squares were used for the analysis. Inspection of normality plots of the residuals showed that the ANOVA assumption of normality was met. Inspection of plots of residuals against predicted values showed that the ANOVA assumption of constant variance was met. The statistical power of this experiment was determined for non-significant results (Cohen 1969, Appendix 4).

Fungicide effectiveness and effects on germination

Additional experiments were conducted prior to and concurrently with the field experiment to determine if the fungicides used in the field experiments affected on seed germination and whether the fungicides were effective in controlling soil pathogenic fungi.

Fungal biomass experiment

To determine the effectiveness of the fungicide treatment in reducing the soil fungal population, fungal biomass was compared between soil samples collected from fungicide treated plots and untreated plots. Six paired plots (each 1 m × 0.5 m) were randomly placed at the study site. One plot of the pair was sprayed on various dates with a mixture of the two fungicides (Captan and metalaxyl), the concentration of which varied between sample dates (described below). The other plot of the pair was left untreated. For the later comparisons, a third plot was added that was sprayed with only water. On six different dates, a composite of three soil samples, totaling approximately 10 grams (wet soil), was collected from the soil surface of each of the plots. The fungal biomass in each sample was measured by the Soil Microbial Biomass Service, Oregon State University, using the fluorscein diacetate method (Ingham and Klein 1984, Lodge and Ingham 1991).

The sampling schedule and fungicide concentrations were as follows:

1. March 5, 1992: Soil samples were collected from six paired plots, half of which received no fungicide treatment and half of which had been sprayed 14 days earlier with Captan and metalaxyl, using the same concentrations as in the field experiment.

2. April 2, 1992: A second set of soil samples was collected from the same six paired plots, 15 days after a second spraying of Captan and metalaxyl, using the same concentrations as in the experiment on seed and seedling fates.

3. April 6, 1992: A third set of soil samples was collected from the same six paired plots, but this time samples were collected 4 days after spraying with Captan and 19 days after spraying with metalaxyl, using the same concentrations as used in the field experiment.

4. April 28, 1992: A fourth set of soil samples was collected from the same six paired plots, six days after the treated plots were sprayed with an increased dosage of fungicide: Captan was increased to five times the field rate and metalaxyl was increased to two times the field rate.

5. May 11, 1992: Five new blocks were randomly located at the study site. Within each block, three plots (each 1 m × 0.5 m) were treated with either a) fungicides, b) water, or c) left untreated. The water treatment was added because the field soil was beginning to dry. Soil samples were collected from the new set of field plots five days after using a fungicide treatment three times the field rate of Captan and two times the field rate of metalaxyl.

6. May 26, 1992: Soil samples were collected from the previous five plots 15 days after application of Captan and metalaxyl. The fungicide dosage of Captan was increased to ten times the field rate, and dosage of metalaxyl was increased to two times the field rate.

For the March and April data, comparisons were made between the soil biomass of the fungicide treated and untreated plots, using a paired t-test. For the May data,

comparisons were made between the soil biomass of the fungicide treated, water treated and non-fungicide treated plots, using a Friedman two-way analysis.

Seed germination

Twenty seeds of each species were sowed in each of ten Petri dishes lined with filter paper. The fungicides Captan and metalaxyl were applied to the seeds in five of the Petri dishes at the same concentrations used in the field experiment. Seeds in the other five Petri dishes were left untreated. The Petri dishes were randomly placed in a germinator at 30° C day/20° C night with 14 hours of light and watered with distilled water as needed. Germination was monitored and recorded weekly. A seed was considered germinated when the radicle emerged from the seed coat. Monitoring continued until no new germination occurred for 10 days.

For each species, the germination percentages were statistically compared between the fungicide treated seeds and non-fungicide treated seeds, using a one-way analysis of variance (Sokal and Rohlf 1981) performed by the statistical software, Statgraphics, version 5.0. Inspection of normality plots of the residuals showed that the ANOVA assumption of normality was met. Inspection of plots of residuals against predicted values showed that the ANOVA assumption of constant variance was met.

Pot experiments 1 and 2

In the second year, 1993, the question of fungal disease in natural vegetation was addressed using a more controlled approach, where seeds were sowed in pots of field soil rather than at the field site.

Pot experiment 1

Methods

In November, 1992, twenty paired samples of field soil with intact vegetation were randomly collected from the study site of the field experiment and placed in pots (approximately 20 cm diameter and 20 cm deep). One-half side of each pot was sowed with twenty seeds of each of the four study species, *Bromus carinatus*, *Cynosurus echinatus*, *Daucus carota,* and *Prunella vulgaris* (80 seeds/pot). The seeds in one of the paired pots (chosen at random) were coated with an organo-mercury fungicide Ceresan, which controls pre-emergent damping off by fungi. The seeds in the other pot of the pair were left untreated. The half side of the pot in which no seeds were sowed was monitored for background seedling establishment of the study species.

The pots were buried in the ground outside University greenhouses so that the soil level of the pots was even with the soil surface level. To simulate field conditions, the pots received water from only rain or snow and were unprotected from outdoor temperatures. Seeding numbers were counted in early February, 1993, and again in late April.

Analysis

The number of seedlings in each the treated and untreated replicate was adjusted for the background counts by subtracting the number of seedlings found in the adjacent control. After adjustment for the background counts, the number of seedlings from the pots with fungicide treated seeds was compared to the number of seedlings from the pots with non-fungicide treated seeds, using a paired t-test (Sokal and Rohlf 1981) for the February data. This analysis was repeated for the April data. Analyses were performed using the statistical package, Statgraphics, version 5.0. Inspection of the variable histogram showed that the assumption of normality was met. The power of the statistical test was determined for non-significant results (Appendix 4).

Fungicide effects on germination

Methods

Prior to the pot experiments a laboratory test was conducted to determine effects of the fungicide used (Ceresan) on germination percentages. Twenty seeds of each species were sowed in each of 20 Petri dishes lined with filter paper. The seeds in ten of the Petri dishes were treated with the fungicide Ceresan at the same concentration used with the pot experiment. The seeds in the remaining dishes were left untreated. The Petri dishes were put in a germinator at 30° C day and 20° C night with 14 hours of light and watered with distilled water when needed. Germination was monitored and recorded weekly. A seed was considered germinated when the radicle emerged. Monitoring continued until no new germination occurred for 10 days.

Analysis

The germination rates were statistically compared between the fungicide treated seeds and non-fungicide treated seeds for each of the four species using a one-way analysis of variance (Sokal and Rohlf 1981) with the statistical software, Statgraphics, version 5.0. Inspection of normality plots of residuals showed that the ANOVA assumption of normality was met. Inspection of residuals against predicted values showed that the ANOVA assumption for constant variance was met.

Pot disease experiment two

The pot disease experiment was repeated in the spring to account for any seasonal differences in optimal conditions for germination and fungal activity.

Methods

Existing pots and soil from the first pot disease experiment were used. The side in which the background counts had been counted for the first pot experiment was divided in half and one of these halves was randomly selected in which to sow the new set of seeds; that is, one-quarter of the entire pot was used as the experimental unit for the new study, pot experiment 2. In early March, 1993, twenty seeds of each species were sowed in each pot (80 seeds/pot). Seeds in one of the pots of the pair (the same pot that received the fungicide treated seeds in the first pot experiment) were treated with the fungicide Ceresan at the same concentration used in the first pot experiment. The seeds in the other pot of the pair were untreated. The remaining quarter of the pot was used to

monitor background counts of seedlings for this second experiment. Final data collection was in late April, 1993.

Analysis

After adjustment for the background counts, the seedling number from the pots with fungicide treated seeds was compared to the seedling number from the pots with non-fungicide treated seeds, using a paired t-test analysis (Sokal and Rohlf 1981). The analysis was performed using the statistical software, Statgraphics, version 5.0. Inspection of the variable histogram showed that the assumption of normality was met. The power of the statistical test was determined for non-significant results (Appendix 4.)

RESULTS AND DISCUSSION

Field experiment

Seedling establishment increased with fungicide treatment for *Cynosurus echinatus, Daucus carota, and Prunella vulgaris* (Table 4.2). The increase was statistically significant, however, for only *Daucus carota* ($P = 0.05$), with no significant interactions (Table 4.3). Thus, the results for *Daucus carota* support the hypothesis that soil-borne pathogenic fungi cause significant seed/seedling mortality in the absence of fungicide

Three possibilities can explain the non-significant results for three species: (1) lack of statistical power to detect differences in the number of seedlings between the treated and untreated plots, (2) ineffective fungicides, or (3) no fungal disease of these

seeds/seedlings. Before concluding that fungal disease is not significantly contributing to seed and seedling mortality, alternatives one and two need to be examined.

Statistical power

Before concluding the treatments had no effects when statistical tests show non-significant results, it is important to calculate the statistical power of the experiment to detect a pre-determined effect difference (effect size) between treatments (Appendix 4).

Table 4.2. Number seedlings establishing out of 25 sowed seeds treated with and without fungicides Captan and metalaxyl in a western Oregon native prairie in 1991-1992 for each of four species. Standard deviation is indicated by sd. The sample size was four for the fungicide treatments and 20 for the non-fungicide treatments when mowed and unmowed blocks were combined.

Species	fungicide		no fungicide	
	mean	sd	mean	sd
Bromus carinatus	4.6	3.8	5.0	4.2
Cynosurus echinatus	9.5	4.6	6.0	4.4
Daucus carota	6.4	5.6	4.5	4.5
Prunella vulgaris	6.3	7.7	4.5	4.0

For this research the objective was to detect at least a 10% seed loss by fungal decay of the original number of sowed seeds. A 10% reduction of the original 25 seeds that were sowed in field experiment is a 2.5 seedling difference between treated and untreated plots.

Table 4.3. Split-plot analysis of variance comparing effects of fungicides Captan and metalaxyl on seedling establishment in 1991-1992 for four species in a western Oregon native prairie. Mowing (a treatment from a larger study) was the whole plot factor with blocks nested within the mowing treatment. The subplot factor was fungicide treatment and caging treatment, which was part of a different study. Means are presented in Table 4.2.

Source	df	sum of squares	F	P
a) *Bromus carinatus*				
mow	1	35.2	2.02	0.17
error	18	313.5		
cage	1	25.9	2.07	0.16
fungicide	1	18.4	1.47	0.24
mow × cage	1	30.9	2.47	0.13
mow × fungicide	1	0.8	0.07	0.80
cage × fungicide	1	0.3	0.02	0.88
error	21	262.6		
b) *Cynosurus echinatus*				
mow	1	0.8	0.04	0.84
error	18	348.7		
cage	1	20.0	1.10	0.31
fungicide	1	7.2	0.39	0.54
mow × cage	1	0.1	0.00	0.95
mow × fungicide	1	17.3	0.95	0.34
cage × fungicide	1	23.8	1.30	0.27
error	23			
c) *Daucus carota*				
mow	1	18.7	0.75	0.40
error	18	447.3		
cage	1	45.8	2.29	0.14
fungicide	1	86.9	4.36	0.05
mow × cage	1	9.1	0.45	0.51
mow × fungicide	1	1.8	0.09	0.77
cage × fungicide	1	5.0	0.25	0.62
error	23	458.6		
d) *Prunella vulgaris*				
mow	1	0.2	0.01	0.91
error	18	332.5		
cage	1	36.9	1.47	0.24
fungicide	1	0.1	0.00	0.96
mow × cage	1	26.9	1.07	0.31
mow × fungicide	1	13.0	0.52	0.48
cage × fungicide	1	57.2	2.27	0.15
error	23	1060.7		

Table 4.4. Power analysis of four experiments that compared seedling establishment between fungicide treatments for four species in a western Oregon native prairie. See the text for descriptions and results of the four experiments: (1) field experiment, (2) pot experiment one, February data, (3) pot experiment one, April data, and (4) pot experiment two. The second and third columns list the power (%) of each experiment to detect an effect size of a 1.5 seedling difference and a 2 seedling difference between fungicide treated treatments. A 2 seedling difference is 10% of the original number of sowed seeds (20) for the pot experiments and is 8% for the field experiment where 25 seeds were sowed. The last column lists the effect size for each experiment that can be detected with 78% power.

Experiments	Power (%) to detect difference of		Effect size
	1.5 seedlings	2 seedlings	at 78% power
a) *Bromus carinatus*			
1. field experiment	63	85	1.8
2. pot exp. one, Feb	52	72	2.2
3. pot exp. one, Apr	83	97	1.4
4. pot exp. two	57	78	2.0
b) *Cynosurus echinatus*			
1. field experiment	>93	>98	1.5
2. pot exp. one, Feb	>98	>99	1.0
3. pot exp. one, Apr	98	>99	0.9
4. pot exp. two	37	55	2.7
c: *Daucus carota*			
1. field experiment	>98	>98	1.1
2. pot exp. one, Feb	>99	>99	0.8
3. pot exp. one, Apr	>99	>99	0.5
4. pot exp. two	>99	>00	0.3
d) *Prunella vulgaris*			
1. field experiment	>98	>98	1.5
2. pot exp. one, Feb	78	98	1.5
3. pot exp. one, Apr	>99	>99	0.4
4. pot exp. two	42	61	2.5

Power analysis for *Cynosurus echinatus*, *Daucus carota* and *Prunella vulgaris* showed at least 93% power to detect a 1.5 or greater seedling difference (6% of the original number of sowed seeds) between treated and untreated field plots (Table 4.4). For *Bromus carinatus*, power was 85% to detect a 2 seedling difference (8% of the original population). In contrast, power to detect 1.5 seedling difference for *Bromus carinatus* was only 58% (Table 4.4).

For three species, *Cynosurus echinatus*, *Daucus carota* and *Prunella vulgaris*, this experiment had sufficient power (at least 78%) to detect potential seedling mortality caused by fungi of 6% or greater of the original number of seeds sowed (25 seeds). For *Bromus carinatus* there was approximately 80% power to detect a mortality rate of 7.2%. Thus, insufficient statistical power does not explain the absence of significant fungicide treatment effects in the field experiment.

Fungicide effectiveness and effects on germination

A second explanation for non-significant results is that the fungicide treatment was less than 100% effective. The fungicides used in this study, Captan and metalaxyl, have good records of effectively protecting seeds and seedlings of crop species from fungal disease (USEPA 1975, Jeffs 1986, Schwinn and Urech 1986, Koepsell and Pscheidt 1995). Furthermore, that seedling establishment of *Daucus carota* increased significantly with the fungicide treatment (Tables 4.2 and 4.3) argues for adequate fungicide effectiveness.

In the fungal biomass experiment, fungal biomass decreased in the fungicide treated plots only on three sample dates (April 6, May 11 and 21), but the decrease was

not statistically significant (April 6, $P = 0.30$; May 11, $P = 0.45$; May 21, $P = 0.25$) (Table 4.5). In contrast, fungal biomass was higher in the treated plots on the first sampling date and as well on the April 28 date (Table 4.5).

Power analyses on the non-significant results was not conducted due to the difficulties in estimating the biologically significant effect size, that is, what is the

Table 4.5. Comparison of soil fungal biomass (micrograms/gram dry weight of soil) between soil samples collected from field plots treated with fungicides Captan and metalaxyl and untreated plots. Soil samples were collected on various dates in 1992 in a western Oregon native prairie. For the March 5 through April 28 sample dates, $n = 6$; for May 11 and 21, $n = 5$. For the March and April samples, comparisons were made between treatments using a non-parametric procedure, the Wilcoxon signed rank test. For the last two sampling dates, comparisons were made between three treatments (fungicide, no fungicide, and water) using a Friedman two-way analysis, a non-parametric procedure. For the Wilcoxon test, P is the probability of falsely rejecting the null hypothesis of no decrease in fungal biomass with the fungicide treatment. For the Friedman test, P is the probability of falsely rejecting the null hypothesis of no differences between fungicide treatments. Standard deviations are indicated by *sd*.

	fungicide		no fungicide		water		
Date	mean	sd	mean	sd	mean	sd	*P*
March 5	45.22	13.14	24.25	10.97			0.48
April 2	63.71	11.52	58.28	28.50			0.30
April 6	55.76	30.52	89.06	56.34			0.30
April 28	60.94	19.58	32.74	11.38			0.48
May 11	21.56	8.34	30.20	17.36	27.02	13.68	0.45
May 21	22.48	14.16	31.60	18.18	40.54	12.39	0.25

minimum decrease in fungal biomass necessary to cause a decrease in seedling mortality? First, it is unknown what proportion of the total fungal biomass is composed of the potential soil-borne pathogens. Thus, a five percent decrease in total fungal biomass might reflect 100% reduction in these pathogens if they comprised five percent of total

fungal biomass. Yet if these pathogenic fungi comprised 40% of the population, a five percent reduction of the total fungal biomass is not as severe.

Table 4.6. Comparison of germination under laboratory conditions between seeds treated with fungicides Captan and metalaxyl and untreated seeds for four species found in western Oregon native prairies. The sample size was five Petri dishes with each replicate containing twenty seeds. Standard deviation is indicated by *sd*. *P* is the probability of differences occurring between the fungicide treatments just by chance.

Species	fungicide		no fungicide		
	mean %	sd	mean %	sd	*P*
Bromus carinatus	83	15.3	86	8.9	0.72
Cynosurus echinatus	98	2.7	98	2.8	0.98
Daucus carota	67	7.6	80	117	0.07
Prunella vulgaris	75	12.3	75	16.6	1.00

A significant decrease in the fungal biomass of the fungicide treated plots would have supported our hypothesis that the fungicides, Captan and metalaxyl, reduced the abundance of the target fungal species. Nevertheless, non-significant results do not necessarily invalidate this hypothesis. Non-target fungi often increase after the reduction of target fungal populations by fungicides, possibly caused by competitive release (Agnihotri 1971). These reports may also explain our initial results on the first sampling date of greater fungal biomass in the treated plots than in the untreated plots (Table 4.5). Thus, the results of this fungal biomass test are ambiguous in that they do not clearly show whether the fungicides metalaxyl and Captan reduced the target population of soil-borne fungi that characteristically cause pre- and post emergence damping off of seedlings.

In the laboratory seed germination experiment, germination was similar between the Captan/metalaxyl treated seeds and the untreated seeds (Table 4.6) with no significant differences (Table 4.5) for all species: *Bromus carinatus* ($P = 0.72$), *Cynosurus echinatus* ($P = 0.98$), *Daucus carota* ($P = 0.07$) and *Prunella vulgaris* ($P = 1.0$), supporting the null hypothesis of no fungicide effects on germination. Analysis shows the following power to detect a 15% difference in germination between treated and untreated seeds: *Bromus carinatus* 39%, *Cynosurus echinatus* >91%, *Daucus carota* 61%, and *Prunella vulgaris* 29%.

Pot experiments 1 and 2

To address the possibility of less than 100% effective fungicide activity, a different fungicide, Ceresan (an organomercury fungicide), was used the next field season. In contrast to the field experiment where the fungicides were sprayed on the soil surface, Ceresan was applied directly to the seeds. Because of the potentially long-lasting toxicity of this fungicide in the soil (Jeffs 1986), the experiment was conducted under more controlled conditions using pots of field soil rather than sowing the seeds directly at the field site.

Seedling establishment for the February pot experiment one was low (< 2 seeds out of 20, Table 4.7), which is often typical of grassland species (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989, Thompson and Baster 1992, Reader 1993). Seedling establishment was similar between fungicide treatments for all four species with no statistical differences: *Bromus carinatus* ($P = 0.95$), *Cynosurus echinatus* ($P = 0.69$), *Daucus carota* ($P = 0.25$), *Prunella. vulgaris* ($P = 0.55$) (Table 4.7).

Data were collected a second time in April for this first pot experiment, the rationale being that fungi may have been killing seeds in the fall even though environmental conditions limited seed germination. Thus, as seed germination continued through the spring, the expectation was that the fungicide treatments would have increased seedling establishment compared to the non-fungicide treatments.

Table 4.7. Comparison of mean numbers of seedlings establishing between pots sowed with seeds treated with fungicide Cerasan and pots sowed with untreated seeds from November 1992 until February 1993 for four species of a western Oregon native prairie. The sample size was twenty paired pots, each of which was sowed with twenty seeds of each of the four species. Seedling numbers are adjusted for background counts. Standard deviations is indicated by *sd*. *P* is the probability of falsely rejecting the null hypothesis of no difference in mean seedling establishment between fungicide treatments (paired t-test).

Species	fungicide		no fungicide		*P*
	mean %	sd	mean %	sd	
Bromus carinatus	1.58	2.97	1.63	2.17	0.95
Cynosurus echinatus	0.42	1.71	0.58	0.84	0.69
Daucus carota	0.21	0.92	0.58	1.39	0.25
Prunella vulgaris	1.26	2.49	0.89	1.20	0.55

Alternatively, the results for the first pot experiment suggested that the conditions during late fall and early winter may limit both seed germination and pathogenic fungi activity. Therefore, the pot experiment was repeated a second time the following March. Seedling establishment was similar between fungicide treatments for the April pot experiment one (Table 4.8) with no significant differences for all four species: *Bromus carinatus* ($P = 0.18$), *Cynosurus echinatus* ($P = 0.20$), *Daucus carota* ($P = 1.00$), and *Prunella vulgaris* ($P = 0.77$). This pattern was repeated for the second pot experiment in

which seedling establishment was similar between fungicide treatments (Table 4.9) with no significant differences for all four species: *Bromus carinatus* ($P = 0.10$), *Cynosurus echinatus* ($P = 0.15$), *Daucus carota* ($P = 1.0$), and *Prunella vulgaris* ($P = 0.26$). Although these non-significant results suggest that fungal disease is not occurring in these pot experiments, statistical power and fungicide effectiveness need to be examined before making this conclusion.

Table 4.8. Comparison of mean number of seeds establishing between pots with seeds treated with and without fungicide Cerasan from November 1992 through April 1993 for four species of a western Oregon native prairie. The sample size was twenty paired pots, each of which was sowed with twenty seeds of each of the four species. Seedling numbers are adjusted for background counts, which allows the negative establishment for *Cynosurus echinatus*. Standard deviation is indicated by *sd*. *P* is the probability of falsely rejecting the null hypothesis of no difference in mean seedling establishment between fungicide treatments (paired t-test).

Species	fungicide		no fungicide		
	mean	sd	mean	sd	*P*
Bromus carinatus	0.79	1.36	1.58	2.22	0.18
Cynosurus echinatus	-0.21	1.65	0.26	0.65	0.20
Daucus carota	0.05	0.71	0.05	0.23	1.00
Prunella vulgaris	0.05	0.23	0.11	0.74	0.77

Statistical power

Analysis show fairly strong statistical power (about 80%) for all three pot experiments to detect at least a two seedling increase in treated seeds, which is a 10% of the original 20 sowed seeds (Table 4.4). Power was virtually 100% to detect 1.5 to 2

Table 4.9. Comparison of number of seeds establishing between pots sowed with seeds treated with and without fungicide Cerasan from March 1993 through April 1993 for four species of a western Oregon native prairie. The sample size was twenty paired pots, each of which was sowed with twenty seeds of each of the four species. Seedling numbersare adjusted for background counts. Standard deviations are indicated by *sd*. *P* is the probability of falsely rejecting the null hypothesis of no difference in mean seedling establishment between fungicide treatments (paired t-test).

Species	fungicide		no fungicide		*P*
	mean	sd	mean	sd	
Bromus carinatus	12.80	3.20	11.40	3.19	0.10
Cynosurus echinatus	5.89	4.39	5.72	3.66	0.15
Daucus carota	0.16	0.37	0.16	0.50	1.00
Prunella vulgaris	0.89	1.85	2.11	4.18	0.26

seedlings increase for *Daucus carota*. In contrast, the power for *Bromus carinatus* ranged from 50 to 95% to detect 1.5 to 2 seedling increase (Table 4.4).

The within-species results were not as consistent for *Cynosurus echinatus* and *Prunella vulgaris* as for *Daucus carota* and *Bromus carinatus*, with power higher for the first pot experiment compared to the second pot experiment (Table 4.4). Even so, for both *Cynosurus echinatus* and *Prunella vulgaris*, power was approximately 80% in the second pot experiment to detect at least a 2.7 seedling difference, which is 13.5% of the original number of seeds sowed (Table 4.4).

In conclusion, this experimental design generally allowed sufficient power (at least 80%) to detect seedling mortality caused by fungi 10% or greater of the original number of seeds sowed (20 seeds). The power was less than 80% to detect a seedling mortality rate 5% or less. Seedling establishment was small in all the pot experiments, often less than the 1.5 to 2 seedling effect size (Tables 4.7, 4.8 and 4.9). These results suggest that lack of germination or other causes of mortality are obscuring any mortality caused by fungi.

Fungicide effectiveness and effects on germination

A second explanation for non-significant results is that the fungicide treatment was less than 100% effective. The organomercury fungicide used in this study, Ceresan, is an eradicant and protectant with a broad spectrum of toxicity to fungi causing disease of crop species (Jeffs 1986, MacSwan and Koepsell 1987, Thomson 1988).

Organomercury fungicides do not generally decrease seed germination (Jeffs 1986). Germination was similar between fungicide treatments in the laboratory seed germination experiment of this study (Table 4.10), with no significant differences for all four species: *Bromus carinatus* ($P = 0.65$), *Cynosurus echinatus* ($P = 0.20$), *Daucus carota* ($P = 0.55$) and *Prunella vulgaris* ($P = 0.10$), thus supporting the hypothesis of no fungicide effects on germination. Analysis shows the following power to detect a 15% difference in germination between treated and untreated seeds: *Bromus carinatus,* 91%; *Cynosurus echinatus,* >93%; *Daucus carota* >93%; *Prunella vulgaris* 68%.

General discussion

In summary, seedling establishment for the study species was generally similar between fungicided treated and untreated seeds for both the pot and field experiments. The results were similar despite conducting the experiments in different years, in field experiments vs. pot experiments, with different fungicides, and with different modes of

Table 4.10. Comparison of germination percentages under laboratory conditions between seeds treated with and without fungicide Cerasan for four grassland species in a western Oregon native prairie. The sample size was ten with each replicate containing twenty seeds. Standard deviation is indicated by *sd*. *P* is the probability of falsely rejecting the null hypothesis of no difference in means between treatments.

Species	fungicide		no fungicide		*P*
	mean	sd	mean	sd	
Bromus carinatus	87.22	10.64	85.00	9.72	0.65
Cynosurus echinatus	97.50	3.54	95.00	4.71	0.20
Daucus carota	92.50	6.35	90.50	7.98	0.55
Prunella vulgaris	82.50	12.75	72.03	13.90	0.10

fungicide application (to soil vs. on seeds). The one exception occurred in the field experiment, where seedling establishment of *Daucus carota* was significantly higher in the fungicide treated plots than in the untreated plots. This result, however, was not repeated with the pot experiments conducted the following year. The generally non-significant results coupled with the fairly high statistical power of these experiments and the past records of effective performance by the experimental fungicides suggest that common soil-borne fungal pathogens did not cause seed/seedling mortality at magnitudes of 10% or greater of the original number of sowed seeds.

Although the agricultural literature on seedling death caused by fungal disease is extensive (Sewell 1981, Harman and Stasz 1986), studies that quantify seed or seedling death by fungi in natural vegetation are rare. Microbial pathogens have been implicated as the cause of unexplained losses in many buried seeds studies, but whether the loss was due to microbial decay of senescent seeds or pathogen attack of living seeds and germinants is unknown (Roberts 1986, Roberts and Neilson 1981, Crist and Friese 1993). The rare studies investigating seed and seedling mortality by fungi in natural vegetation describe significant mortality by fungi, in contrast to the results this present study (Lawrence and Rediske 1962, Mack and Pyke 1984, Augspurger 1990). Fungi accounted for the greater part of seed loss during the pre-germination period of Douglas-fir seeds and were also the principle cause of Douglas-fir seedling death (Lawrence and Rediske 1962). The magnitude of loss caused by fungal disease (damping-off) ranked from high to very low relative to other mortality factors contributing to deaths of tropical tree seedlings (Augspurger 1990). A significant number of *Bromus tectorum* seedlings died from an infection of a smut *Ustilago bullata* over a three year period in a semi-arid grassland (Mack and Pyke 1984).

Using chemical exclusion to determine the effects of fungal disease on seed/seedling mortality has not generally been used in natural systems, though some attempts have been made in agricultural research. Studies involving seedling establishment in agricultural pastures (Kreitlow et al. 1950, Michail and Carr 1966, Clements et al. 1982, Dowling and Linscott 1983, Janson and Ison 1995) report mixed results, but generally demonstrate that fungicides have often had remarkably little effect

in increasing seedling establishment. The statistical power of detecting loss due to fungal disease and the effectiveness of the fungicides used was not investigated in these studies.

To avoid potential problems in data interpretation, several issues need to be considered when conducting chemical exclusion experiments in natural vegetation (Paul et al. 1989). First, the fungicide needs to be effective. Because fungicides are often selective for particular fungal groups, combinations of fungicides may be necessary to exclude the fungi of interest. For example, in this study, metalaxyl protects seedlings only from disease caused by Oomycetes, e.g., *Pythium* and *Phytophthora* (Schwinn and Urech 1986); thus, a broad spectrum fungicide, Captan, was added to protect against seedling diseases caused by other fungi such as *Rhizoctonia* (Agnihotri 1971, Wainwright and Pugh 1975, USEPA 1975, Jeffs 1986). Under field conditions, the effectiveness of the fungicide may vary depending on such factors as soil physical and chemical characteristics, soil moisture, and soil microbial content.

The fungicide needs to persist long enough to answer the specific research question. Repeated applications of metalaxyl and Captan were required in the field experiment of this study because of the short effective time, particularly of Captan (Agnihotri 1971, Wainwright and Pugh 1975, USEPA 1975, Kuthubutheen and Pugh 1979). The pot experiments of this study were terminated shortly after seedling emergence, at which time the organomercury fungicide has limited effects on fungal disease (Jeffs 1986, P. Koepsell 1995, personal communication).

Toxicity to non-target organisms and direct effects on plant species present in the treated vegetation are also important issues. Paul et al. (1989), one of the first studies to address this issue, investigated the effects of several commonly used fungicides on the

growth of 19 wild plant species. The results showed that metalaxyl generally had no adverse effects on any of species tested (Paul et al. 1989). Captan is generally non-toxic to crop species (USEPA 1975).

Another difficulty in measuring the magnitude of fungal disease of seeds and seedlings in natural vegetation is the considerable amount of variability common in ecological field experiments. Disease of seeds and seedlings in natural systems is likely to be markedly patchy due to the strong interaction between fungal activity and abiotic environmental conditions (Burdon 1987, Augspurger 1990). Field studies need to be designed carefully with sufficient statistical power to detect biologically significant levels of fungal disease. From this study, estimates of the variability to be expected in this plant community are now available and will be essential in estimating of sample size and design of future studies.

The results of this study are contrary to what one might expect, given the high seedling mortality caused by fungi in agricultural systems (Sewell 1981). For fungal disease to occur, three components of the disease triangle are necessary: a susceptible host population, a virulent pathogen population and abiotic conditions that promote growth of the fungus relative to the host. Any or all these components may have been missing in this study system.

Even when all three components of the disease triangle are present, many studies in natural vegetation show that disease occurs at insignificant levels (Dinoor and Eshed 1984, Burdon and Shattock 1986, Kranz 1990). One hypothesis is that in natural communities, a variety of homeostatic mechanisms exist in that keep genetic, ecological and pathological factors in dynamic equilibrium, preventing significant disease levels

(Dinoor and Esched 1984). When the relative magnitude of any one of these factors changes, disease levels increase, sometimes resulting in epidemics (Dinoor and Esched 1984, Burdon and Shattock 1986). For example, *Fusarium* spp. cause considerable seedling mortality in plant nurseries, but appear to cause little or no disease in undisturbed natural vegetation (Schisler and Linderman 1984). One explanation suggests that soils of forests and grasslands may have greater diversity of organic matter in the form of litter compared to that of agricultural fields or plant nurseries, potentially increasing the diversity of the saprophytic fungi in the natural vegetation compared to agricultural systems (E.M. Hansen 1995, personal communication). These saprophytic fungi may compete against and reduce the abundance of pathogenic fungi, such as *Fusarium* spp., thus limiting seedling disease. Moreover, in agricultural systems there is probably greater abundance of living tissue (the host for *Fusarium*) than in natural vegetation due to dense planting of the crop and supplemental fertilizer and water, further encouraging the abundance of *Fusarium* and disease.

Summary

Although disease in natural systems has been generally ignored by plant ecologists, considerable evidence indicates that disease, even when unapparent with only rare signs of damage, is an important process influencing plant population dynamics and plant community diversity. This study is one of the first to use chemical exclusion of fungi to determine the effects of fungal disease on seed and seedling mortality in natural vegetation.

This research showed that disease caused by common soil-borne fungi is not contributing to 10% or greater seedling mortality rates for four species commonly found in western Oregon native prairies. The results of the three experiments were similar despite conducting the experiments in different years, in field experiments vs. pot experiments, with different fungicides, and with different fungicide applications (to soil vs. seeds).

CHAPTER 5
EFFECTS OF MOWING ON SEEDLING ESTABLISHMENT IN A WESTERN OREGON NATIVE PRAIRIE

INTRODUCTION

Rationale

The prairies and oak-savannas of the Willamette Valley of Oregon are considered among the rarest of western Oregon' ecosystems. Prairies, dominated by bunchgrasses, were historically maintained by fires set by the indigenous Calapooia people to increase abundance of food plants and for ease of hunting (Boag 1992). The frequent fires prevented the establishment of woody species.

Today, managers of protected prairie remnants want to increase or maintain populations of native species while discouraging invasions of weedy non-native species. However, because of environmental regulations and proximity of remnant prairies to urban areas, fire is not always a feasible management tool and alternatives have been considered. Mowing can be effective in reducing the abundance of woody plants, but the effect on seed regeneration of native and non-native species in Valley prairies is generally unknown (but see Wilson and Clark 1995).

Litter or a leaf canopy from surrounding mature plants reduces the amount of light available to seedlings. The reduced amount of light may also decrease soil surface temperatures and increase soil moisture compared to soil with an open canopy. A leaf canopy also changes light quality by increasing the far-red/red wavelength ratio. This change in light quality can induce seed dormancy, preventing germination under conditions where young seedlings would be exposed to severe competition from mature

plants (Pons 1992). Only when gaps occur in vegetation is seed dormancy broken by the relative high red/far-red ratios of unmodified daylight (Pons 1992). These predictions are supported by laboratory studies that report leaf-filtered light induced seed dormancy in 17 of 27 grassland species (Silvertown 1980). Mowing, thus, has the potential effects of increasing amount of light for emerging seedlings and enhancing germination by breaking canopy-induced seed dormancy (Silvertown 1980, Pons 1992, Deregibus et al. 1994).

Objective

The objective of this study was to determine the effects of mowing on seedling establishment of four species common in western Oregon native prairies. The general approach was to sow seeds of four prairie species into mowed and unmowed field plots and to compare seedling establishment one year later between the treated and untreated plots.

METHODS

Study site

The study site is an upland prairie with silty clay loam (Dixonville soil series) dominated by the native bunchgrasses *Festuca idahoensis* var. *roemeri*, *Bromus carinatus* var. *carinatus*, and *Elymus glaucus*. This site, part of the Open Space Park managed by Benton County Parks, is located approximately 8 kilometers northwest of Corvallis, Oregon, in the foothills of the Coast Range (T11S, R6W, Sec 25, W.M.). The elevation is approximately 300 m, with a 30-50% slope facing west. The site is one of

the few remnants of a vast prairie and oak-savanna ecosystem that covered much of the Willamette Valley until about the1840s (Boag 1992). The entire Willamette Valley has a fairly homogeneous climate with mild, wet winters, moderate and dry summers, and cool nights. Measurements made in Corvallis (Owenby and Ezell 1992) show the average annual precipitation is 108 cm, average maximum January temperature is 7.5° C, and maximum average July temperature is 26.8° C. The average precipitation during the first year of this study (June 1991-May 1992) was 6.14 cm and for the second year (June 1992-May 1993), 8.84 cm (George Taylor, OR State Climatologist). The average maximum temperature for January 1992 was 9.7°C and for January 1993, 5.3°C. The average maximum temperature for July 1992 was 27.8°C and for July 1993, 23.2°C.

Study species

The four study species, *Bromus carinatus* Hook and Arn. var. *carinatus, Cynosurus echinatus* L., *Daucus carota* L., and *Prunella vulgaris* var. *lanceolata* (Barton) Fern. (Table 5.1) are dominant at the study site. The criteria for species selection were that the study species (1) represent a variety of life histories, (2) be common and abundant in western Oregon prairies, and (3) produce sufficient seeds for the research. Nomenclature follows Hitchcock and Cronquist (1973).

The Eurasian *Prunella vulgaris* var. *vulgaris* has the middle cauline leaves about half as wide as long, with a broadly rounded base. It grows in the Northwest occasionally in disturbed sites, where it is often dwarfed and prostrate (Hitchcock and Cronquist 1973). The native American *Prunella vulgaris* var. *lanceolata* is ascending or erect, with middle cauline leaves about a third as wide as long and more tapering toward

the base (Hitchcock and Cronquist 1973). The variety that is common in undisturbed habitats in Oregon is generally considered to be native (H. Chambers 1995, personal communication).

Table 5.1. Description of the four study species.

Species	Family	Native to Oregon?	Life-span	Seed
Bromus carinatus var. *carinatus*	Poaceae	yes	perennial	awned
Cynosurus echinatus	Poaceae	no	annual	awned
Prunella vulgaris var. *lanceolata*	Labiatae	yes	perennial	hard-seeded
Daucus carota	apiaceae	no	biennial	barbed

Throughout this study the four species will be referred to as *Bromus carinatus, Cynosurus echinatus, Daucus carota* and *Prunella vulgaris.* Although the term "seed" is used in this study, it is refers to the diaspore, i.e., the seed and any associated structures.

Experimental design

In mid-July 1991, twenty 2.2 m x 2.2 m blocks were randomly located at the study site. To determine the influence of vegetation canopy removal on seedling establishment, ten blocks were randomly selected and the vegetation mowed to a height of about 5 cm in early July with a gasoline powered "weed-eater". Litter from the mowing was left in place. Within each of the 20 blocks, five plots (25 cm x 35 cm) were

randomly placed. One plot of the five was left untreated and used in this study. The other four plots received various treatments as part of a different study (Chapter 3).

Four subplots, each 5 cm in diameter, were located and permanently marked with nails within each plot. Twenty-five seeds of one of the four study species were sowed into each subplot immediately following seed collection at the study site.

Data collection

In mid-June 1992, approximately one year after seeds were sowed in the subplots, the number of seedlings for each of the study species was counted in each subplot. Background counts for each species were made from the number of seedlings in subplots in which the study species were not sowed.

Statistical analysis

The data were analyzed using all components of the larger study (for methods, see Chapter 3). Seedling establishment of each species was analyzed for treatment effects using split-plot analysis of variance (ANOVA) (Steele and Torrie 1960). Mowing was the whole plot factor with blocks nested within the mowing treatment and treatment was the subplot factor (these treatments, caging and fungicide were part of a larger study, Chapter 3). The General Linear Model (GLM) procedure of the SAS Institute, version 6.08, and type III sum of squares were used for the analysis. Inspection of the normality plots of residuals showed that the ANOVA assumption of normality was met. Inspection of plots of residuals against predicted values showed that the ANOVA assumption for constant variance was met.

For ANOVAs with non-significant treatment effects, analyses were conducted to determine the power of the statistical test to detect a 2 seedling difference, which is 8% of sowed seeds, between the experimental treatments (Appendix 4, Cohen 1969).

RESULTS AND DISCUSSION

Seedling establishment was generally low in the unmowed plots (Table 5.2), which typical of grassland species (Peart 1984, McConnaughay and Bazzaz 1987, Peart 1989, Thompson and Baster 1992, Reader 1993). Establishment rates were somewhat higher in the mowed treatments compared to the unmowed treatments, particularly for *Bromus carinatus* (Table 5.2). The means, however, were not statistically different between treatments for all four species: *Bromus carinatus,* $P = 0.17$; *Cynosurus echinatus*, $P = 0.84$; *Daucus carota* $P = 0.40$; *Prunella vulgaris* $P = 0.91$ (Table 5.3).

The lack of mowing effects is in contrast to the expectation from laboratory studies (Silvertown 1980) in which simulated leaf canopy induced seed dormancy in 27% of *Daucus carota* seeds and 78% of seeds of *Prunella* vulgaris. The expectation was that removal of leaf canopy by mowing would increase the germination rate and potentially, the seedling establishment rate. However, as pointed out by Pons (1992) and Deregibus et al. (1994), germination responses to phytochrome status are greatly affected by levels of other factors, such as temperature, nitrogen, photoperiod and irradiance, which under laboratory conditions do not necessarily correspond to levels under field conditions.

Table 5.2. Number seedlings establishing out of 25 experimentally sowed seeds in mowed and unmowed plots in a western Oregon native prairie in 1991-1992 for each of four species. Ten blocks were mowed out of 20 blocks. Standard deviation is indicated by *sd*.

Species	no mow		mow	
	mean	sd	mean	sd
Bromus carinatus	87.22	10.64	85.00	9.72
Cynosurus echinatus	97.50	3.54	95.00	4.71
Daucus carota	92.50	6.35	90.50	7.98
Prunella vulgaris	82.50	12.75	72.03	13.90

Another mechanism, in addition to disturbance, by which seedlings of these study species escape light competition is by the timing of germination. Seeds of *Cynosurus echinatus* and *Daucus carota* readily germinate under field conditions during fall and early winter months when mature plants have died back (M. Maret, unpublished data), thus escaping the more intense shading found later in the spring and early summer.

The results from this study suggest that mowing will not promote the establishment of the two non-native species, *Cynosurus echinatus* and *Daucus carota*, but neither will mowing promote the establishment of the native species, *Bromus carinatus* and *Prunella vulgaris*. However, to make any generalizations about mowing on seed regeneration, comprehensive studies involving numerous grassland species and sites are required. To make the best management decision involving mowing, other related studies are needed to determine long-term effects of mowing, such as competitive interactions and removal of propagule sources.

Table 5.3. Spilt-plot analysis of variance comparing mowed and unmowed plots on seedling establishment (out of 25 sowed seeds) in 1991-1992 for four species in western Oregon native prairie. Mowing was the whole plot factor with blocks nested within the mowing treatment. The subplot factor was caging and fungicide treatments, which are part of a larger study (Chapters 3 and 4). Means are presented in Table 5.2.

Source	df	sum of squares	F	*P*
a) *Bromus carinatus*				
mow	1	35.2	2.02	0.17
error	18	313.5		
cage	1	25.9	2.07	0.16
fungicide	1	18.4	1.47	0.24
mow × cage	1	30.9	2.47	0.13
mow × fungicide	1	0.8	0.07	0.80
cage × fungicide	1	0.3	0.02	0.88
error	21	262.6		
b) *Cynosurus echinatus*				
mow	1	0.8	0.04	0.84
error	18	348.7		
cage	1	20.0	1.10	0.31
fungicide	1	7.2	0.39	0.54
mow × cage	1	0.1	0.00	0.95
mow × fungicide	1	17.3	0.95	0.34
cage × fungicide	1	23.8	1.3	0.27
error	23	420.0		
c) *Daucus carota*				
mow	1	18.7	0.75	0.40
error	18	447.3		
cage	1	45.8	2.29	0.14
fungicide	1	86.9	4.36	0.05
mow × cage	1	9.0	0.45	0.51
mow × fungicide	1	1.7	0.09	0.77
cage × fungicide	1	4.9	0.25	0.62
error	23	458.6		
d) *Prunella vulgaris*				
mow	1	0.2	0.01	0.91
error	18	304.1		
cage	1	85.7	3.41	0.08
fungicide	1	2.5	0.01	0.75
mow × cage	1	13.5	1.54	0.47
mow × fungicide	1	13.0	0.52	0.47
cage × fungicide	1	57.2	2.27	0.14
error	23	579.3		

CHAPTER 6
CONCLUSIONS

Only a handful of studies have attempted to describe the post-dispersal fates of seeds. Understanding seed fate patterns along with other regeneration characteristics are essential for development of hypotheses on the role of regeneration strategies in determining size and distribution of plant populations, and ultimately species composition and diversity of communities.

The primary reason for the paucity of seed fate studies is the extreme difficulty in determining seed fates and mortality causes. Particularly difficult is distinguishing between seeds that die due to failed germination, saprophytic decay and pathogenic decay. Improvements in techniques for locating or retrieving seeds after experimental sowing is the key to increasing seed fate research. Stringent requirements limit use of the successful technique, labeling seeds with a radioactive tag (Lawrence and Rediske 1962, Vander Wall 1994). Marking seeds with dye or paint can be successful in the short-term for only some species. Some have suggested use of video cameras such as used in animal studies (Fenner 1987). Retrieval of seeds by sieving and microscopic examination is much too labor intensive and time-consuming to be used in extensively.

This thesis investigated post-dispersal seed fates and the processes controlling these fates for four species common in western Oregon native prairies: *Bromus carinatus*, *Cynosurus echinatus*, *Daucus carota*, and *Prunella vulgaris* (Chapter 3). The effect of mowing on one particular seed fate, seedling establishment, was also addressed (Chapter 5). Additional studies focused on the effect of a single mortality factor, fungal disease, on seed and seedling death (Chapter 4).

Three fates await seeds after dispersal (Figure 6.1). Seeds can either persist as seeds, germinate and grow as seedlings, or die. Most seeds of the study species died (80%-44%), with relatively few seeds germinating and surviving as seedlings for both years of the study (Chapter 3). Virtually no seeds persisted for three of the study species, whereas seeds of *Daucus carota* (14%) formed a small persistent seed bank for year one.

"Other mortality processes" were generally the largest cause of death for all four species for two years (73%-32%, Chapter 3). The likely components of "other mortality processes" (invertebrate predation, interference or abiotic processes) differed among species (Table 6.1).

Most deaths caused by "other mortality processes" for *Bromus carinatus* and *Cynosurus echinatus* probably occurred as seedlings, either by interference or abiotic factors, although invertebrate predation can not be ruled out for *Bromus carinatus* (Table 6.1). Seedling death due to abiotic factors is most likely the largest component of "other mortality processes" for *Daucus carota*. The most probable components for *Prunella vulgaris* were seed death by invertebrate predation and seedling death by abiotic factors (Table 6.1).

Disease by common soil fungi in the field experiment caused less than ten percent mortality of seeds and seedlings, with the exception of *Prunella vulgaris* (Chapter 3). Vertebrate predation substantially reduced the seed numbers of only one species, *Bromus carinatus* (21.2%). Because of the inherent difficulties in measuring seeds and germinants

Figure 6.1. Model describing fates of seeds and the processes controlling the magnitudes of these fates.

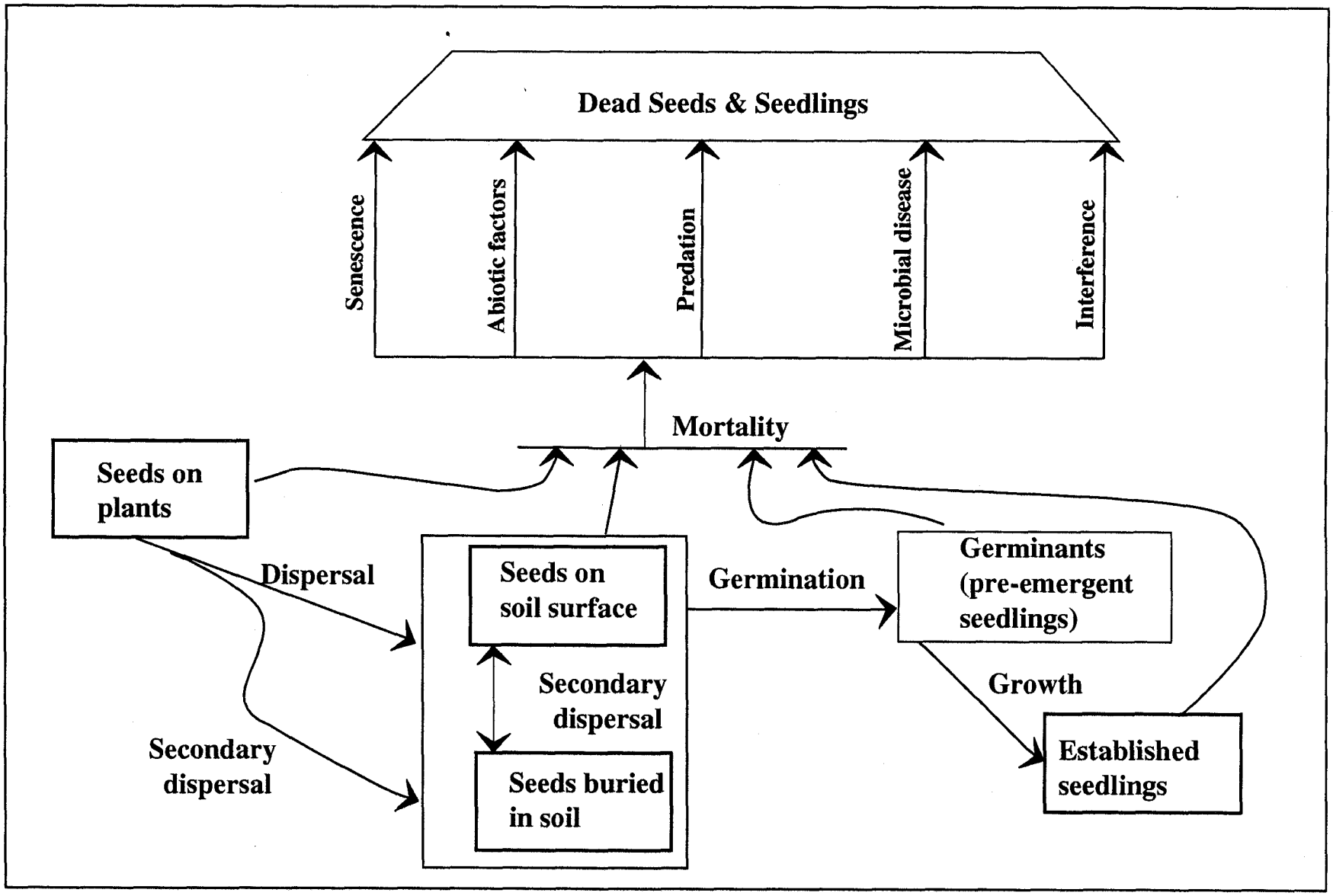

Dead Seeds & Seedlings
Senescence
Abiotic factors
Predation
Microbial disease
Interference
Mortality
Seeds on plants
Dispersal
Seeds on soil surface
Germination
Germinants (pre-emergent seedlings)
Secondary dispersal
Growth
Secondary dispersal
Seeds buried in soil
Established seedlings

Table 6.1. Possible components of "other mortality processes" for four species found in western Oregon native prairies. A "+" indicates that the mortality factor is a likely component of "other" mortality processes, a "_" indicates that the mortality factor is not likely a component, and "?" indicates evidence is not available to determine whether the mortality factor is a component or not.

	"Other" mortality processes			
Species	bacterial/viral diseases	invertebrate predatio	abiotic factors	interference
Bromus carinatus	-	?	+	+
Cynosurus echinatus	-	-	+	+
Daucus carota	-	-	+	-
Prunella vulgaris	-	+	+	-

that disappear due to microbial decay, the magnitude of senescence is unknown. The potential magnitude, however, of seed senescence after one year is possibly minor, based on laboratory experiments for all four species.

These patterns of mortality help explain the lack of seed persistence for three of the study species. Seed persistence depends on avoidance of both germination and death. The lack of a persistent seed bank for *Cynosurus echinatus* is probably not caused by seed death, suggesting that lack of dormancy and non-restrictive germination requirements are the probable mechanisms. Lack of dormancy/non-restrictive germination requirements, and seed death caused by predation are conceivably responsible for the lack of long-term seed persistence for *Bromus carinatus* and *Prunella vulgaris*.

Although the expectation was that the regeneration responses to disturbances would differ among the four species, the increases in seedling establishment in the mowed plots compared to the unmowed plots were not significant for all four species

(Chapter 5). The results suggest that mowing will not promote the establishment of the two non-native species, *Cynosurus echinatus* and *Daucus carota*, but neither will mowing promote the establishment of the native species, *Bromus carinatus* and *Prunella vulgaris*.

These seed fate results have implications for managers who wish to conserve native prairies of the Willamette Valley. Sowing extra seeds of the two native species should increase their populations sizes, assuming that safe-sites are not limited. Even if long-distance dispersal occurs, an outside source of seeds is necessary to reintroduce these species on sites where these species are absent, because seeds of the two native species did not persist in the soil. Applying pesticides that prevent vertebrate predation for *Bromus carinatus* and invertebrate seed predation for *Prunella vulgaris* before sowing will increase seedling establishment rates, assuming no compensatory mortality by other factors. Sowing seeds in natural or artificial gaps in the vegetation will increase seedling establishment of *Bromus carinatus*. Because fungal disease caused few deaths, application of fungicides to seeds is unnecessary.

Controlling propagule availability will likely control abundance of the non-native species better than controlling mortality factors. Because the annual *Cynosurus echinatus* appears to lack a persistent seed bank, removal of the on-site seed source by mowing before seed maturation should eventually eliminate the population. In contrast, seed removal before maturation may not eliminate a population of *Daucus carota,* because it can regenerate from propagules in its persistent seed bank. If, however, the seed bank receives no new seed inputs, the seed bank may be virtually eliminated after 5-6 years.

The additional studies in Chapter 4 investigating the mortality factor of fungal disease on seed and seedling death corroborate the results on fungal disease described in Chapter 3. This research (Chapter 4) generally showed that disease caused by common soil-borne fungi is not contributing to seedling mortality at magnitudes of 10% or greater of the original seed population for all study species. The results of the three experiments described in Chapter 4 were similar despite conducting the experiments in different years, under uncontrolled conditions (field conditions) vs. controlled conditions (pot experiments), with different fungicides, and with different modes of fungicide applications (to soil vs. on seed).

This pattern of small magnitude of death caused by fungal disease is contrary to what one might expect given the considerable magnitude of seedling mortality caused by fungi in agricultural systems (Sewell 1981). For fungal disease to occur, three components of the disease triangle are necessary: (1) a susceptible host population, (2) a virulent pathogen population, and (3) abiotic conditions that promote growth of the fungus relative to the host. Any or all these components may have been missing in this study.

Nevertheless, even when all three components of the disease triangle are present, many studies in natural vegetation show that disease occurs at insignificant levels (Dinoor and Eshed 1984, Burdon and Shattock 1986, Kranz 1990). One hypothesis is that a variety of mechanisms exist in natural communities keeping populations of pathogens in check or balance with host populations, thus, preventing significant levels of disease (Dinoor and Esched 1984). When the relative magnitude of any one of these

mechanisms changes, disease levels increase, sometimes resulting in epidemics (Dinoor and Esched 1984, Burdon and Shattock 1986).

Future research that addresses the question of why soil-borne fungi did not contribute substantially to seedling mortality in this thesis needs to investigate whether all the components necessary for disease to occur are present. First, studies need to determine if common soil-borne pathogens (e.g., *Pythium* spp., *Phytophthora* spp., *Fusarium* spp.) are present at the study site and if so, at what inoculum levels. Next, greenhouse or laboratory studies should determine whether these fungi can infect and cause disease in the study species. Both absence of fungal pathogens or inability to cause disease in the study species can explain lack of disease in the present study. However, if fungal pathogens are present and can cause disease in the study species, more investigations are required to determine the environmental conditions necessary to promote disease.

Although disease in natural systems has been generally ignored by plant ecologists, considerable evidence indicates that disease, even when unapparent , is a significant process influencing plant population dynamics and plant community diversity (Chapter 2). This research is one of the first to use chemical exclusion of fungi to determine the effects of fungal disease on seed/seedling mortality in natural vegetation.

One final note: Statistical power is an integral part of scientific hypothesis-testing, yet it has been largely ignored in the ecological literature (Peterman 1989, Fairweather 1991). Statistical power is the probability of correctly detecting an effect, that is, the probability of rejecting the null hypothesis of no effect when in fact there really is an effect (Cohen 1969). Statistical power should be calculated and reported

when a statistical test fails to reject the null hypothesis of no treatment effects in order to judge whether there really is no effect or whether the test was too weak to examine the null hypothesis properly. The experiments used in this research generally had high power, lending credence to the non-significant results.

BIBLIOGRAPHY

Abramsky, Z. 1983. Experiments on seed predation by rodents and ants in the Israeli desert. Oecologia 57:328-332.

Agnihotri, V.P. 1971. Persistence of Captan and its effects on microflora, respiration, and nitrification of a forest nursery soil. Canadian Journal of Microbiology 17:377-383.

Alvarez-Buylla, E.R. and Martinez-Ramos, M. 1990. Seed bank versus seed rain in the regeneration of a tropical pioneer tree. Oecologia 84:314-325.

Anderson, A.N. 1989. How important is seed predation to recruitment in stable populations of long-lived perennials? Oecologia 81:310-315.

Anderson, J.D. and Baker, J.E. 1983. Deterioration of seeds during aging. Phytopathology 73:321-325.

Angevine, M.W. and Chabot, B.F. 1980. Seed germination syndromes in higher plants. In: Solbrig, O.T., Jain, S., Johnson, G.B., and Raven, P.H. (eds.). Topics in Plant Population Biology. Columbia University Press, New York.

Augspurger, C. K. 1990. Spatial patterns of damping-off disease during seedling recruitment in tropical forests. In: Burdon, J.J. and Leather, S.R. (eds). Pests, Pathogens and Plant Communities. Blackwell Scientific Publications, Oxford.

Baker, H.G. 1989. Some Aspects of the Natural History of Seed Banks. In: Leck, M.A., Parker, V.T., and Simpson, R.L. (eds.). Ecology of Soil Seed Banks. Academic Press, Inc.

Bakker, J.P. 1989. Nature Management by Grazing and Cutting. Kluwer, Dordrecht.

Baskin, J.M. and Baskin, C.C. 1985. The annual dormancy cycle in buried weed seeds: a continuum. Bioscience 35:492-498.

Baskin, J.M. and Baskin, C.C. 1989. Physiology of dormancy and germination in relation to seed bank ecology. In: Leck, M.A., Parker, V.T., and Simpson, R.L. (eds.). Ecology of Soil Seed Banks. Academic Press, San Diego, CA

Beal, W.J. 1905. The vitality of seeds. Bot. Gaz. (Chicago) 40:140-143.

Bell, D.T.S., Vlahos, S. and Watson, L.E. 1987. Stimulation of seed germination of understorey species of the Northern Jarrah Forest of western Australia. Australian Journal of Botany 35:593-599.

Bewley, J.D. and Black, M. 1982. Physiology and Biochemistry of Seeds in Relation to Germination, Vol.2. Springer-Verlag, New York.

Boag, P.G. 1992. Environment and Experience: Settlement Culture in Nineteenth-Century Oregon. University of California Press, Berkeley and Los Angeles, CA.

Borchert, M.I. and Jain, S.K. 1978. The effect of rodent seed predation on four species of California annual grasses. Oecologia 33:101-113.

Brenchley, W.E. 1918. Buried weed seeds. Journal of Agricultural Science 9:1-31.

Bridgemohan, P., Brathwaite, R.A.I., and McDavid, C.R. 1991. Seed survival and patterns of seedling emergence studies of *Rottboellia cochinchinensis* (Lour.) W.D. Clayton in cultivated soils. Weed Research 31:265-272.

Brown, J.H., Davidson, D.W., Munger, J.C., and Inouye, R.S. 1986. Experimental community ecology: the desert granivore system. In: Diamond, J. and Case, T.J. (eds). Community Ecology, Harper and Row, New York, pp. 41-61.

Bruehl, G.W. 1987. Soilborne Plant Pathogens. MacMillam Publishing Co., New York.

Burdon, J.J. 1987. Diseases and Plant Population Biology. Cambridge University Press, Cambridge.

Burdon, J.J. and Shattock, R.C. 1980. Disease in Plant Communities. Applied Biology 5:145-219.

Burdon, J.J., Marshall, D.R., Brown, A.H.D. 1983. Demographic and genetic changes in populations of *Echium plantagineum*. Journal of Ecology 71:667-679.

Carlsson, U., Elmqvist, T., Wennstrom, A., and Ericson, L. 1990. Infection by pathogens and population age of host plants. Journal of Ecology 78:1094-1105.

Cavers, P.B. 1983. Seed demography. Canadian Journal of Botany 61:3578-3590.

Chambers, J.C. 1995. Distrubance, life history strategies, and seed fates in alpine herbfield communities. American Journal of Botany 82:421-433.

Chambers, J.C., MacMahon, J.A., and Brown, R.W. 1990. Alpine seedling establishment: the influence of disturbance type. Ecology 71:1323-1341.

Chambers, J.C. and MacMahon, J.A. 1994. A day in the life of a seed: Movements and fates of seeds and their implications for natural and managed systems. Annual Review of Ecology and Systematics 25:263-292.

Clements, R.O., French, N., Guile, C.T., Golightly, W.H., Lewis, S., and Savage, M.J. 1982. The effect of pesticides on establishment of grass swards in England and Wales. Ann. Appl. Biol. 101:305-313.

Cocks, P.S. 1994. Colonization of a South Australian Grassland by Invading Mediterranea Annual and Perennial Pasture Species. Australian Journal Agricultural Research 45:1063-1076.

Cohen, J. 1969. Statistical Power Analysis for the Behavioral Sciences. Academic Press, New York and London.

Come, D. and Thevenot, C. 1982. Environmental control of embryo dormancy and germination. In: Khan, A.A. (ed.). The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier Biomedical Press.

Conover, W.J. 1980. Practical non-parametric statistics, 2nd edition. Wiley, New York.

Cook, R.E. 1979. Patterns of juvenile mortality and recruitment in plants. In: Solbrig, O.T., Jain, S., Johnson, G.B., and Raven, P.H. (eds.). Topics in Plant Population Biology. Columbia University Press, New York.

Cook, R.E. 1980. The biology of seeds in the soil. In: Solbrig, O.T. (ed.). Demography and Evolution in Plant Populations. University of California, Berkeley.

Crawley, M.J. 1983. Herbivory; The Dynamics of Animal-Plant Interactions. Blackwell Scientific Publications, Oxford.

Crawley, M.J. 1992. Seed predators and plant population dynamics. In: Fenner, M. (ed.). Seeds: The Ecology of Regeneration in Plant communities. pp.157-192. Wallingford, UK, CAB.

Crawley, M.J. and Pacala, S. 1990. Herbivores, plant parasites and plant diversity. In: Toft, C. (ed.). Parasitism: Conflict or Coexistence. Oxford University Press, Oxford.

Crist, T.O. and Friese, C.F. 1993. The impact of fungi on soil seeds: implications for plant and granivores in a semiarid shrub-steppe. Ecology 74:2231-2239.

Darlington, H.T. 1922. Dr. W.J. Beal's seed viability experiment. American Journal of Botany 9:266-269.

Darlington, H.T. and Steinbaur, G.P. 1961. The eighty year period for Dr. W.J. Beal's seed viability experiment. American Journal of Botany 48:321-325.

Deregibus, V.A., Casal, J.J., Jacobo, E.J., Gibson, D., Kauffman, M.,AND Rodriguez, A.M. 1994. Evidence that heavy grazing may promote germination of *Lolium multiflorum* seeds via phytochrome-mediatedd perception of high red/far-red ratios. Functional Ecology 8:536-542.

Dinoor, A. and Eshed, N. 1984. The role and importance of pathogens in natural communities. Annual Review Phytopathology 22:443-466.

Dixon, K.W., Roche, S., and Pate, J.S. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. Oecologia 101:185-192.

Dobson, A. and Crawley, M. 1994. Pathogens and the structure of plant communities. Trends in Ecology and Evolution 9:393-398.

Dolan, R.W. and Sharitz, R.R. 1984. Population dynamics of *Ludwigia leptocarpa* (Onagraceae) and some factors affecting size hierachies in a natural population. Journal of Ecology 72:1031-1041.

Dowling, P.M. and Linscott, D.L. 1983. Use of pesticides to determine relative importance of pest and disease factors limiting establishment of sod-seeded lucerne. Grass and Forage Science 38:179-185.

Fairweather, P.G. 1991. Statistical Power and Design Requirements for Environmental Monitoring. Australian Journal of Marine and Freshwater Research 42.555-567.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581.

Fenner, M. 1978. A comparison of the abilities of colonizers and closed-turf species to establish from seed in artificial swards. Journal of Ecology 66:953-963.

Fenner, M. 1987. Seedlings. New Phytologist 106:35-47.

Ferenczy, L. 1956. Occurrence of antibacterial compounds in seeds and fruits. Acta Biologica Acadamiae Scientiarum Hungaricae (Budapest) 6:317-323.

Granstrom, A. 1987. Seed viability of fourteen species during five years of storage in a forest soil. Journal of Ecology 75:321-331.

Grime, J.P. 1979. Plant Strategies and Vegetation Processes. Wiley, Chichester.

Grime, J.P. 1989. Seed Banks in Ecological Perspective. In: Leck, M.A., Parker, V.T., and Simpson, R.L. (eds.). Ecology of Soil Seed Banks. Academic Press Inc., San Diego.

Grime, J.P. and Hillier, S.H. 1992. The contribution of seedling regeneration to the structure and dynamics of plant communities and larger units of landscape. In: Fenner, M. (ed.). Seeds, The Ecology of Regeneration in Plant Communities. CB International, Wallingford.

Grime, J.P. and Curtis, A.V. 1976. The interaction of drought and mineral nutirent stress in calcareous grassland. Journal of Ecology. 64:975-988.

Grime, J.P., Mason, G., Curtis, A.V., Rodman, J.B., S.R. Mowforth, M.A.G., Neal, A.M., and Shaw, S. 1981. A comparative study of germination characteristics in a local flora. Journal of Ecology 69:1017-1059.

Gross, K.L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. Journal of Ecology 72:369-387.

Gross, K.L. and Werner, P.A. 1982. Colonizing abilities of "biennial" plant species in relation to ground cover: implications for their distributions in a successional sere. Ecology 63: 921-931.

Grubb, P.J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological Review 52:104-145.

Halloin, J.M. 1983. Deterioration resistance mechanisms in seeds. Phytopathology 73:335-339.

Harman, G.E. 1983. Mechanisms of seed infection and pathogenesis. Phytopathology 73:326-329.

Harman, G.E. and Stasz, T.E. 1986. Influence of seed quality on soil microbes and seed rots. In: Physiological-Pathological Interactions Affecting Seed Deterioration. Crop Science Society of America. Special Publication No.12. CSSA, Madison, Wisconsin.

Harper, J.L. 1977. Population Biology of Plants. Academic Press, London.

Harper, J.L. 1990. Pests, pathogens and plant communities: An introduction. In: Burdon, J.J. and Leather, S.R. (eds.). Pests, Pathogens and Plant Communities. Blackwell Scientific Publications, Oxford.

Harper, J.C., Landragen, P.A., and Ludwig, J.W. 1955. The influence of environmental seed and seedling mortality, Vol II. The pathogenic potential of the soil. New Phytologist 54:199-131.

Harrington, J.F. 1972. Seed storage and longevity. In: Kozlowski, T.T. (ed.). Seed Biology, Vol. III. Academic Press, New York.

Hendrix, S.D. 1988. Herbivory and its impact on plant reproduction. In: Lovett Doust, J. and Lovett Doust, L. (eds.). Plant Reproductive Ecology. Oxford University Press, New York.

Hendry, G.A.F., Thompson, K., Moss, C.J., Edwards, E., and Thorpe, P.C. 1994. Seed persistence: A correlation between seed longevity in the soil and *ortho*-dihydroxyphenol concentration. Functional Ecology 8:658-664.

Hickman, J.E. 1979. The basic biology of plant numbers. In: Solbrig,O.T., Jain, S., Johnson, G.B., and Raven, P. H. (eds.). Topics in Plant Population Biology. Columbia University Press, New York.

Hitchcock, C.L. and Cronquist, A. 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle.

Holt, B.R. 1972. Effect of arrival time on recruitment, mortality, and reproduction in successional plant populations. Ecology 53:668-673.

Holthuijzen, A.M.A., Sharik, T.L., and Fraser, J.D. 1987. Dispersal of eastern red cedar (*Juniperus virginiana*) into pastures: an overview. Canadian Journal of Botany 65:1092-1095.

Horvitz, C.C. and Schemske, D.W. 1994. Effects of dispersers, gaps, and predators on dormancy and seedling emergence in a tropical herb. Ecology 75:1949-1958.

Hughes, L. and Westoby, M. 1992. Fate of seeds adapted for dispersal by ants in Australian sclerophyll vegetation. Ecology 1992:1285-1299.

Hulme, P.E. 1994. Post-dispersal seed predation in grassland: its magnitude and sources of variation. Journal of Ecology 82:645-652.

Ingham, E.R. and Klein, D.A. 1984. Soil fungi: relationships between hyphal activity and staining with fluorescein diacetate. Soil Biol. Biochemistry 16:273-278.

Janos, D.P. 1980. Mycorrhizae influence tropical succession. Biotropica 12:56-64.

Jansen, P.I. and Ison, R.L. 1995. Factors contributing to the loss of seed from the seed-bank of *Trifolium balansae* and *Trifolium resupinatum* over summer. Australian Journal of Ecology 20:248-256.

Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forest. American Naturalist 104:501-528.

Janzen, D.H. 1971. Seed predation by animals. Annual Review of Ecology and Systematics. 2:465-492.

Jeffs, K.A. (ed). 1986. Seed Treatment. British Crop Protection Council Publications, Surrey, England.

Kachi, N. and Hirose, T. 1985. Population dynamics of *Oenothera glazioviana* in a sand-dune system with special reference to the adaptive significance of size-dependent reproduction. Journal of Ecology 73:887-901.

Kalisz, S. 1991. Experimental determination of seed bank age structure in the winter annual *Collinsia verna*. Ecology 72:575-585.

Karssen, C.M. 1982. Seasonal patterns of dormancy in weed seeds. In: Khan, A.A. (ed.). The physiology and biochemistry of seed development, dormancy and germination. Elseview Biomedical Press. Amsterdam.

Keeley, J.E., Morton, B.A., Pedrosa, A., and Trotter, P. 1985. Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. Journal of Ecology 73:445-458.

Keen, N.T. 1975. The isolation of phytoalexins from germinating seeds of *Cicer arientinum, Vigna sinensis, Arachis hypogaea* and other plants. Phytopathology 64:91-92.

Kivilaan, A. and Bandurski, R.S. 1973. The ninety year period for Dr. W.J. Beal's seed viability experiment. American Journal of Botany 60:140-145.

Kivilaan, A. and Bandursik, R.S. 1981. The one hundred year period for Dr. W.J. Beal's seed viability experiment. American Journal of Botany 68:1290-1292.

Klemow, K.M. and Raynal, D.J. 1985. Demography of two faculative biennial plant species in an unproductive habitat. Journal of Ecology 73:147-167.

Koepsell, P.A. and Pscheidt, J.W. (eds). 1995. Pacific Northwest Plant Disease Handbook, Oregon State University, Corvallis.

Kranz, J. 1990. Fungal diseases in multispecies plant communities. New Phytologist 116:338-405.

Kreitlow, R.J., Garber, R.J., and Robinson, R.R. 1950. Investigations on seed treatment of alfalfa, red clover, and sudan grass for control of damping-off. Phytopathology 40:883-898.

Kremer, R.J. 1993. Managment of weed seed banks with microorganisms. Ecological Applications 3:42-52.

Kremer, R.J., Hughes Jr., L.B., and Aldrich, R.J. 1984. Examination of microorganims and deterioration resistance mechanisms associated with velvetleaf seed. Agronomy Journal 76:745-749.

Kuthubutheen, A.J. and Pugh, G.J.F. 1979. The effects of fungicides on soil fungal populations. Soil Biol. Biochemistry 11:297-303.

Lawrence, W.H. and Rediske, J.H. 1962. Fate of sown Douglas-fir seed. Forest Science 8:210-218.

Lee, J.M. and Hamrick, J.L. 1983. Demography of two natural populations of musk thistle (*Carduus mutans*). Journal of Ecology 71:923-936.

Liu, S. and Vaughan, E.K. 1965. Control of *Pythium* infection in table beet seedlings by antagonistic microorganisms. Phytopahtology 55:986-989.

Livingston, R.B. and Allessio, M.L. 1968. Buried viable seed in successional field and forest stands, Harvard Forest, Massachusetts. Bulletin of the Torrey Botanical Club 95:58-69.

Lodge, D.J. and Ingham, E.R. 1991. A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. Agriculture, Ecosystems and Environment 34:131-144.

Lonsdale, W.M. 1993. Losses from the seed bank of *Mimosa pigra*: soil micro-organisms vs. temperature fluctuations. Journal of Applied Ecology 30:654-660.

Louda, S.M. 1989. Predation in the dynamics of seed regeneration. In: Leck, M.A., Parker, R.L., and Simpson, R.L. (eds.). Ecology of Soil Seed Banks. Academic, New York.

Lynch, J.M. 1978. Microbial interactions around imbibed seeds. Annals of Applied Biology 89:165-167.

Mack, R.N. and Pyke, D.A. 1984. The demography of *Bromus tectorum*: the role of microclimate, grazing and disease. Journal of Ecology 72:731-748.

MacSwan, I.C. and Koepsell, P.A. (eds.). 1987. Pacific Northwest Plant Disease Handbook, Oregon State University, Corvallis.

Masuda, M. and Washitani, I. 1990. A comparative ecology of the seasonal schedules for 'reproduction by seeds' in a moist tall grassland community. Functional Ecology 4:169-182.

Mayer, A.M. and Poljakoff-Mayber, A. 1989. The Germination of Seeds. Pergamon Press, Oxford.

McConnaughay, K.D.M. and Bazzaz, F.A. 1987. The relationship between gap size and performance of several colonizing annuals. Ecology 68:411-416.

Michail, S.H. and Carr, A.J.H. 1966. Effect of seed treatment on establishment of grass seedlings. Plant Pathology15:60-64.

Moore, R.P. (ed). 1985. Handbook on Tetrazolium Testing. The International Seed Testing Association, Zurich, Switzerland.

Mott, K.E. (ed.). 1987. Plant Molluscicides. John Wiley, Chichester.

Murdoch, A.J. 1983. Environmental control of germination and emergence in *Avena fatua*. Aspects of Applied Biology 4:63-69.

Murdoch, A.J. and Ellis, R.H. 1992. Longevity, Viability and Dormancy. In: Fenner, M. (ed.). Seeds, The Ecology of Regeneration in Plant Communities. CAB International,Wallingford.

Murdoch, A.J.and Roberts, E.H. 1982. Biological and financial criteria of long-term control strategies for annual weeds. Proceedings 1982 British Crop Protection Conference. Weeds 741-748.

Neegaard, P. 1977. Seed pathology, Vol. 1. John Wiley and Sons, New York.

Odum, S. 1974. Seeds in ruderal soils, their longevity and contribution to the flora of disturbed ground in Denmark. Proceedings of the 12th British Weed Control Conference 1131-1144.

Olff, H., Pegtel, D.M., Van Groenendael, J.M. and Bakker, J.P. 1994. Germination strategies during grassland succession. Journal of Ecology 82:69-77.

Owenby, J.R. and Ezell, D.S. 1992. Monthly Station Normals of Temperature, Precipitation, and Heating and Cooling Degree Days 1961-1990, Oregon. Climatography of the United States No. 81. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Climatic Data Center. Asheville, North Carolina.

Paul, N.D., Ayres, P.G., and Wyness, L.E. 1989. On the use of fungicides for experimentation in natural vegetation. Functional Ecology 3:759-769.

Pavone, L.V. and Reader, R.J. 1982. The dynamics of seed bank size and seed state of *Medicago lupulina*. Journal of Ecology 70:537-547.

Peart, M.H. 1984. The effects of morphology, orientation and position of grass diaspores on seedling survival. Journal of Ecology 72:437-453.

Peart, D.R. 1989. Species interactions in a successional grassland, Vol. III. Effects of canopy gaps, gopher mounds and grazing on colonization. Journal of Ecology 77:267-289.

Peterman, R.M. 1989. Application of Statistical Power Analysis to the Oregon Coho Salmon (*Oncorhynchus kisutch*) Problem. Can. J. Fish. Aquat. Sci. 46:1183-1187.

Peterman, R.M. 1990. Statistical power can improve fisheries research and management. Can. J. Fish. Aquat. Sci. 47:2-15.

Pierce, S.M. and Cowling, R.M. 1991. Dynamics of soil-stored seed banks of six shrubs in fire-prone dune fynbos. Journal of Ecology 79:731-747.

Pons, T.L. 1989. Dormancy, germination and mortality of seeds in heathland and inland sand dunes. Acta Bot. Neerl. 38:327-335.

Pons, T.L. 1991. Dormancy, germination and mortality of seeds in a chalk-grassland flora. Journal of Ecology 79:765-780.

Pons, T.L. 1992. Seed responses to light. In: Fenner, M. (ed.). Seeds: The Ecology of Regeneration in Plant Communities. AB International Wallingford.

Pratt, B.H. and Heather, W.A. 1973. The origin and distribution of *Phytophthora cinnamomi* rands in Australian native plant communities and the significance of it association with particular plant species. Australian Journal of Biological Science 26:559-573.

Price, M.V. and Heinz, K.M. 1984. Effects of body size, seed density, soil characterisitics on rates of seed harvest by heteromyid rodents. Oecologia 61:420-425.

Price, M.V. and Jenkins, S.H. 1986. Rodents as seed consumers and dispersers. In: Murray, D.R. (ed.). Seed Dispersal. Academic, Sydney.

Priestly, D.A. 1986. Seed Aging: Implications for Seed Storage and Persistence in the Soil. Cornell University Press.

Ramsbotton, J. 1942. Recent work on germination. Nature (London) 149:658-659.

Reader, R.J. 1991. Control of seedling emergence by ground cover: A potential mechanism involving seed predation. Canadian Journal of Botany 69:2084-2087.

Reader, R.J. 1993. Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. Journal of Ecology 81:169-175.

Reader, R.J. and Beisner, B.E. 1991. Species-dependent effects of seed predation and ground cover on seedling emergence of old-field forbs. American Midland Naturalist 126:279-286.

Reichman, O.J. 1979. Desert granivore foraging and its impact on seed densities and distributions. Ecology 60:1085-1092.

Rice, E.L. 1984. Allelopathy. Academic Press, Inc. Orlando.

Rice, K.J. 1989. Impacts of Seed Banks on Grassland Community Structure and Population Dynamics. In: Leck, M.A., Parker, V.T. and Simpson, R.L. (eds). Ecology of Soil Seed Banks. Academic Press, San Diego, CA.

Roberts, E.H. 1972. Cytological, genetical, and metabolic changes assoicated with loss of viability. In: Roberts, E.H. (ed.). Viability of seeds. Chapman and Hall, London.

Roberts, E.H. and Ellis, R.H. 1982. Physiological, ultrastructural and metabolic aspects of seed viability. In: Khan, A.A. (ed). The Physiology and Biochemistry of Seed Develpment, Dormany and Germination. Elsevier/North Holland Biomedical Press, Amsterdam. pp. 465-485.

Roberts, H.A. 1981. Seed banks in Soil. Advances in Applied Biology 6:1-55.

Roberts, H.A. 1986. Seed persistence in soil and seasonal emergence in plant species from different habitats. Journal of Applied Ecology 23:639-656.

Roberts, H.A. and Feast, P.M. 1972. Fate of seeds of some annual weeds in different depths of cultivated and undistrubed soil. Weed Research 12:316-324.

Roberts, H.A. and Neilson, J.E. 1981. Changes in the soil seed bank of four long-term crop/herbicide experiments. Journal of Applied Ecology 18:661-668.

Sallabanks, R. and Courtney, S.P. 1992. Frugivory, seed predation, and insect vertebrate interactions. Annu. Rev. Entomol. 37:377-400.

Sarukhan, J. 1974. Studies on plant demography: *Ranunculus repens* L., *R. bulbosus* L., R. *acris* L, Part II. Reproductive strategies and seed population dynamics

Schafer, D.E. and Chilcote, D.O. 1970. Factors influencing persistence and depletion in buried seed populations, Part II. The effects of soil temperature and moisture. Crop Science 10:342-345.

Schenkeveld, A.J. and Verkaar, H.J. 1984. The ecology of short-lived forbs in chalk grasslands: Distribution of germinative seeds and its significance for seedling emergence. Journal of Biogeography 11:251-260.

Schisler, D.A. and Linderman, R.G. 1984. Evidence for the involvement of the soil microbiota in the exclusion of *Fusarium* from coniferous forest soils. Canadian Journal of Micorbiology 30:142-150.

Schwinn, F.J. and Urech, P.A. 1986. Progress in the Chemical Control of Diseases Caused by Oomycetes. ACS Symposium, Ser, American Chemical Society, Washington, DC.: The Society (Fungicide Chemistry), pp. 89-106.

Sewell , G.W.F. 1981. Soil-borne pathogens in natural vegetation and weeds of clutivation. In: Thresh, J.M. (ed.). Pests, Pathogens and Vegetation. Pitman Advance Publication Program, Boston, pp. 175-190.

Sharitz, R.R. and McCormick, J.F. 1973. Population dynamics of two competing annual species. Ecology 54:723-740.

Silvertown, J. 1980. Leaf-canopy-induced seed dormancy in a grassland flora. New Phytologist 85:109-118.

Silvertown, J. and Tremlett, M. 1989. Interactive effects of disturbance and shade upon colonization of grassland: an experiment with *Anthriscus sylvestris* (L.) Hoffm., *Conium maculatum* L., *Daucus carota* L. and *Heracleum sphondylium* L. Functional Ecology 3:229-235.

Smith, B.H. 1983. Demography of Floerkea proserpinacoides, a forest-floor annual, Part III. Dynamics of seed and populations. Journal of Ecology 71: 413-425.

Sokal, R.R. and Rohlf, F.J. 1981. Biometry. W. H. Freeman and Company.

Soriano, A. and Sala, O.E. 1986. Emergence and survival of *Bromus setifolius* seedlings in different microsites of a Patagonian arid steppe. Israel Journal of Botany 35:91-100.

Srivastava, V.P. and Mishra, R.M. 1971. Fungal inhibitory agents in seed coats. Phytopathologie Mediterranea 10:127-128.

Steele, R.G.D. and Torrie, J.H. 1960. Principles and Procedures in Statistics. McGraw-Hill, New York.

Supkoff, D.M., Joley, D.B., and Marois, J.J. 1988. Effect of introduced biological control organisms on the density of *Chondrilla juncea* in California. Journal of Applied Ecology 25:1089-1095.

Tadros, T.M. 1957. Evidence of the presence of an edapho-biotic factor in the problem of serpentine tolerance. Ecology 38:14-23.

Tahvanainen, J., Julkunen-Titto, T., and Lavola, A. 1985. Phenolic compounds of willow bark as deterrents against feeding by mountain hare. Oecologia 65:319-323.

Thompson, J.N. 1985. Postdispersal seed predation in *Lomatium* spp. (Umbelliferae): variation among individuals and species. Ecology 66:608-1616.

Thompson, K. 1987. Seeds and seed banks. New Phytologist (suppl.) 106: 23-24.

Thompson, K. and Baster, K. 1992. Establishment from seed of selected Umbelliferae in unmangeged grassland. Functional Ecology 6:346-352.

Thompson, K. and Grime, J.P. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67:893-921.

Thompson, K., Band, S.R. and Hodgson, J.G. 1993. Seed size and shape predict persistence in soil. Functional Ecology 7:236-241.

Thomson, W.T. 1988. Agricultural Chemicals. Book IV, Fungicides. Thomson Publications, Fresno, CA.

Toole, E.H. and Brown, E. 1946. Final results of the Duvel buried seed experiment. J. Agric. Res. 72:201-210.

Turner, J.H. 1933. The viability of seeds. Bull. Misc. Inf., R. Bot. Gard., pp. 257-268.

United States Environmental Protection Agency. 1975. Initial Scientific and Minieconomic Review of Captan (Substitute Chemical Program), Office of Pesticide Programs, Washington, D.C. EPA-540/1-75-012.

van Baalen, J. 1982. Germination ecology and seed population dynamics of *Digitalis purpurea*. Oecologia 53:61-67.

van Esso, M.L. and Ghersa, C.M. 1989. Dynamics of *Sorghum halpense* seeds in the soil of an uncultivated field. Canadian Journal of Botany 67:940-944.

van Leeuwan, B.H. 1981. Influence of micro-organisms on the germination of the monocarpic *Cirsium vulgare* in relation to disturbance. Oecologia 48:112-115.

Vander Wall, S.B. 1994. Seed fate pathways of antelope bitterbrush: dispersal by seed-caching yellow pine chipmunks. Ecology 75:1911-1926.

von Broembsen, S. L. 1989. Invasions of natural ecosystems by plant pathogens. In: Drake, J. A. et al. (eds). Biological Invasions: a Global Perspective. John Wiley & Sons Ltd.

Wainwright, M. and Pugh, G.J.F. 1975. Effect of fungicides on the numbers of micro-organisms and frequency of cellulolytic fungi in soils. Plant and Soil 45:561-572.

Washitani, I. 1985. Field fate of *Amaranthus patulus* seeds subjected to leaf-canopy inhibition of germination. Oecologia 66:338-342.

Waterman, P.G. 1992. Roles for secondary metabolites in plants. In: Chadwick, D.J. and Whelan, J. (eds). Secondary Metabolites: Their Function and Evolution, Ciba Foundation Symposium 171. John Wiley and Sons, Chichester.

Watkinson, A.R. 1978. The demography of a sand dune annual: *Vulpia fasciculata,* Part II. The dynamics of seed populations. Journal of Ecology 66:35-44.

Werner, P.A. 1979. Competition and coexistence of similar species. In: Solbrig, O.T., Jain, S., Johnson, G.B., and Raven, P. (eds). Topics in plant population biology. Columbia University Press, New York.

Weste, G. 1987. The biology of *Phytophthora cinnamomi* in Australasian forests. Annual Review of Phytopathology 25:207-229.

Whelan, C.J., Willson, M.F., Tuma, C.A., and Souza-Pinto, I. 1991. Spatial and temporal patterns of postdispersal seed predation. Canadian Journal of Botany 69:428-436.

Willson, M.F. and Whelan, C.J. 1990. Variation in post-dispersal survival of vertebrate-dispersed seeds: effects of density, habitat, location, season and species. Oikos 57:191-198.

Wilson, M.W. and Clark, D.L. 1995. Research on Habitat Manipulation and Management of Fender's Blue Butterful Areas. Final Report, Oregon Field Office, The Nature Conservancy, Oregon Natural Heritage Program.

Wilson, S.D. and Gerry, A.K. 1995. Strategies for mixed-grass prairie restoration: Herbicide, tilling, and nitrogen manipulation. Restoration Ecology 3: 290-298.

Winn, A.A. 1985. Effects of seed size and microsite on seedling emergence of *Prunella vulgaris* in four habitats. Journal of Ecology 73:831-840.

Youngman, B.J. 1951. Germination of old seeds. Kew Bull. pp. 432-426.

Zorner, P.S., Zimdahl, R.L., and Schweizer, E.E. 1984. Sources of viable seed loss in buried dormant and non-dormant populatins of wild oat (*Avena fatua* L.) seed in Colorado. Weed Research 24:143-150.

APPENDICES

APPENDIX 1
EFFECTS OF SHAM CAGE

OBJECTIVE

Chapter 3 describes a field experiment which the goal was to quantify the post-dispersal seed fates of four species in a western Oregon native prairie. One objective was to determine the magnitude of mortality caused by vertebrate predation by comparing the survival of seeds and seedlings between caged and uncaged plots. This appendix describes an experiment conducted to determine if the cages had any effects other than exclusion of vertebrate predators on seed survival of the four study species, *Bromus carinatus* var. *carinatus*, *Cynosurus echinatus*, *Daucus carota* and *Prunella vulgaris* var. *lanceolata*.

METHODS AND ANALYSIS

The study site, experimental design, details of caging treatments, and data collection are described in Chapter 3. Survival (sum of dormant seeds and seedlings) was compared between the plots with the sham cage and the uncaged plots, using a split-plot analysis of variance (ANOVA) for each species (Steele and Torrie 1960). Mowing (which was part of another study, Chapter 5) was the whole plot factor. The subplot factor consisted of the five treatments: (1) closed cage, (2) no cage, (3) sham cage, (4) fungicide and (5) no fungicide. Because the sample sizes were unbalanced, the General Linear Model (GLM) procedure of the SAS Institute statistical software, version 6.08 and type III sum of squares were used for all analyses. Inspections of normality plots of the residuals showed that the ANOVA assumption of normality was met. Inspection of

residual plots against predicted scores showed that the ANOVA assumption of constant variance was met. When appropriate, differences between means were tested using Fisher's protected least significant difference. For ANOVAs with non-significant treatment effects, analyses were conducted to determine the power of the statistical test to detect a pre-determined effect size between experimental treatments (Cohen 1969, Peterman 1990, see Appendix 4 for details).

RESULTS AND DISCUSSION

Seed and seedling survival did not significantly differ between the sham cage treatment and the uncaged treatment ($P > 0.5$) (Appendix Tables 1.1 and 1.2). The significant treatment effect for *Bromus carinatus* (Appendix Table 1.2) was due to differences between caged and uncaged treatments.

Power analysis shows the experiment had the following power to detect an 8% loss caused by predation of the original 25 seeds sowed: *Bromus carinatus*, 76%; *Cynosurus echinatus*, 76%; *Daucus carota*, 76%; and *Prunella vulgaris*, 80%. Therefore, the results support the hypothesis that the caging treatment had no effects other than prevention of vertebrate predators on survival of the sowed seeds of the four study species.

Appendix Table 1.1. Number of seeds and seedlings surviving out of 25 experimentally sowed seeds in plots with and without a sham cage in 1991-1992 for each of four species found in western Oregon native prairies. The sample size is 20, when mowed and unmowed plots are combined. Standard deviation is indicated by *sd*.

Species	No cage		Sham cage	
	mean	sd	mean	sd
Bromus carinatus	3.1	4.0	2.9	3.5
Cynosurus echinatus	6.3	5.0	6.0	3.9
Daucus carota	5.3	7.8	5.0	4.9
Prunella vulgaris	5.4	3.6	6.2	4.4

Appendix Table 1.2. Split-plot analysis of variance comparing mowing and five fungicide and caging treatments on survival of seeds and seedlings for four species in a western Oregon native prairie in 1991-1992. Mowing (a treatment from a another study, chapter 5) is the whole plot factor. The subplot factors are (1) closed cage, (2) no cage, (3) sham cage, (4) fungi-cide application, and (5) no fungicide application. Means of sham cage and no cage plots are presented in Appendix table 1.1.

Source	df	Sum of squares	*F*	*P*
a) *Bromus carinatus*				
mow	1	75.4	3.62	0.07
error	18	374.7		
treatment	4	168.5	2.98	0.03
mow × treatment	4	87.2	1.54	0.21
error	32	452.3		
b) *Cynosurus echinatus*				
mow	1	78.9	2.77	0.11
error	18	513.5		
treatment	4	95.8	1.59	0.20
mow × treatment	3	11.4	0.25	0.86
error	34	513.8		
c) *Daucus carota*				
mow	1	3.3	0.15	0.70
error	18	391.7		
treatment	4	171.4	0.86	0.50
mow × treatment	4	250.5	1.26	0.30
error	33	1641.0		
d) *Prunella vulgaris*				
mow	1	0.0	0.0	0.97
error	18	478.3		
treatment	4	76.9	0.94	0.45
mow × treatment	4	53.3	0.65	0.63
error	36	738.0		

APPENDIX 2
EFFECTS OF THE FUNGICIDES CAPTAN AND METALAXYL ON SEED GERMINATION

OBJECTIVE

Chapter 3 describes a field experiment which quantified post-dispersal seed fates of four species in a western Oregon native prairie. One objective was to determine the magnitude of mortality caused by fungal disease by comparing the survival of seeds and seedlings between fungicide treated plots and untreated plots (for details, see Chapter 3). This appendix describes tests conducted to determine whether adverse effects of the fungicide treatment had adverse effects on seed germination of the four study species: *Bromus carinatus* var. *carinatus*, *Cynosurus echinatus, Daucus carota*, and *Prunella vulgaris* var. *lanceolata.*

METHODS AND ANALYSIS

Twenty seeds of each species were sowed in each of ten Petri dishes lined with filter paper. The seeds in five of the Petri dishes were treated with the fungicides Captan and metalaxyl at the same concentrations (fungicide per unit of soil) used in the field experiment (Chapter 3). Seeds in the other five Petri dishes were untreated. The Petri dishes were randomly placed in a germinator at 30° C during the day and 20° C at night, with 14 hours of light, and watered with distilled water as needed. Germination was recorded weekly. A seed was considered germinated when the radicle emerged from the seed coat. Monitoring continued until no new germination occurred for 10 days.

For each species, the germination percentages were statistically compared between the fungicide treated seeds and untreated seeds, using a one-way analysis of variance (Sokal and Rohlf 1981) performed by the statistical software package Statgraphics, version 5.0. Inspections of normality plots of the residuals showed that the ANOVA assumption of normality was met. Inspection of plots of residuals against predicted scores showed that the ANOVA assumption of constant variance was met.

RESULTS AND DISCUSSION

Germination did not differ significantly between treated and untreated seeds for all four species: *Bromus carinatus* ($P = 0.72$), *Cynosurus echinatus* ($P = 0.98$), *Daucus carota* ($P = 0.07$) and *Prunella vulgaris* ($P = 1.0$) (Appendix tables 2.1). Analysis showed the following power to detect a 15 percentage point change in germination between treated and untreated seeds: *Bromus carinatus,* 39%; *Cynosurus echinatus,* >61%; *Daucus carota,* 61%; and *Prunella vulgaris*, 29%. The power for this statistical test was limited by the sample size of five Petri dishes.

Appendix Table 2.1. Comparison of germination under laboratory conditions between seeds treated with fungicides Captan and metalaxyl and untreated seeds for four species in western Oregon native prairies. The sample size was five Petri dishes with each replicate con-taining 20 seeds. Standard deviation is indicated by *sd*. *P* is the probability of falsely rejecting the null hypothesis of no differences in means between treatments.

Species	fungicide		no fungicide		
	mean %	sd	mean %	sd	*P*
Bromus carinatus	83	15.3	86	8.9	0.72
Cynosurus echinatus	98	2.7	98	2.8	0.98
Daucus carota	67	7.6	80	11.7	0.07
Prunella vulgaris	75	12.3	75	16.6	1.00

APPENDIX 3
EFFICACY OF SEED RETRIEVAL TECHNIQUES

OBJECTIVE

Chapter 3 describes a field experiment which the goal quantified post-dispersal seed fates of four species in a western Oregon prairie. The general approach included sowing seeds of four prairie species into experimentally manipulated field plots for each of two years, and recovering these seeds from the soil one year later to determine their fates. This appendix describes preliminary tests to determine the efficacy of these retrieval techniques for the following four species: *Bromus carinatus* var. *carinatus*, *Cynosurus echinatus, Daucus carota*, and *Prunella vulgaris* var. *lanceolata*.

METHODS

Ten to twenty seeds of the study species were sowed into 5 cm diameter plots located in undisturbed vegetation at the study site (Chapter 3). The soil containing the seeds was then removed from the plot to a depth of 5 cm with a bulb digger. These soil samples were sieved using water and sieves matched to the size of the seed sowed in a particular sample. The remaining residues were dried at 30° for 2-3 days and then examined for any remaining seeds, using a dissecting microscope or magnifying glass. These extraction methods were identical to those used in the research described in Chapter 3.

RESULTS AND DISCUSSION

Virtually 100% of the larger seeds of *Bromus carinatus* and *Cynosurus echinatus* were recovered (Appendix table 3.1). For *Prunella vulgaris*, the percentage was 88%. Wet *Prunella vulgaris* produce a mucilaginous substance to which dirt particles adhere, covering the seeds. At first the seeds were difficult to detect due to the dirt covering, but with more experience they were easily recognizable. Because of the background presence of *Daucus carota* seeds in the field collected soil samples, more *Daucus carota* seeds sowed were recovered than had been sowed. However, given the effectiveness of the retrieval techniques for the other three species, the decision was made to use these methods to retrieve the sowed seeds from the soil for all four species.

Appendix Table 3.1. Seeds recovered from soil samples (as a percentage of number sowed) for four species of western Oregon native prairies. The sample size is indicated by *n*. Standard deviation is indicated by *sd*.

Species	n	seeds per sample	mean %	sd
Bromus carinatus	4	10	97.5	5.0
Cynosurus echinatus	3	10	100.0	0.0
Daucus carota	4	10	140.0	39.2
Prunella vulgaris	3	20	88.3	10.4

APPENDIX 4
ANALYSIS OF STATISTICAL POWER

INTRODUCTION

Before concluding there are no treatment effects when statistical tests show non-significant results, it is important examine the-statistical power of the experiment to detect a pre-determined effect difference (effect size) between treatments. Power is equal to 1-β, where beta is the probability of committing a Type II error, that of failing to reject the null hypothesis (e.g., there is no difference in seedling establishment between the fungicide treated plots and the untreated plots) when in fact the null hypothesis is false (there is in reality a difference in seedling establishment between treatments). The factors that determine the strength of power are (1) alpha (the probability of committing a Type I error), (2) the sample size, and (3) the true difference between the means under the null hypothesis and the alternate hypothesis. Often, alpha is set at 0.05 and power at 0.80. At these values the relative seriousness of Type II error to Type I error is 0.2/0.05 or 4 to 1. Thus, the mistaken rejection of the null hypothesis is considered four times as serious as mistaken failure to reject. In this thesis, power analyses were performed for all tests with non-significant differences between treatments. The following sections discuss the power analyses for specific statistical tests conducted in this thesis research.

ONE-WAY ANALYSIS OF VARIANCE, t-TESTS AND PAIRED t-TESTS

Statistical power for one-way ANOVAs and t-tests was determined from the power tables of Cohen (1969), which require alpha (the probability of committing a Type I error), the sample size (n), and the standardized effect size (d). The standardized effect

size (*d*) was calculated by first determining the smallest difference or effect between treatments that was considered to be biologically significant. This effect size differed depending on the particular research question and is described in the pertinent chapter. The effect size was then standardized by dividing it by the common standard deviation of the means of the treated and untreated variables.

Statistical power of paired t-tests were determined from the tables of Cohen (1969) as described above for one-way ANOVA and t-tests, with one difference. The standardized effect size calculated above was adjusted by multiplying by the square root of two. This adjustment was necessary because the tables (Cohen 1969) are based on the number of samples in each of two means. With the paired t-test, there is only one set of means.

FACTORIAL ANALYSIS OF VARIANCE

Statistical power for the factorial ANOVA used in the field experiment described in Chapter 4 was determined from the power tables of Cohen (1969), which require alpha (the probability of committing a Type I error), the sample size (*n*), and the standardized effect size (*f*).

There are several ways to compute the standardized effect size (*f*). The method used in this study was based on the relationship between *f* and the effect size, *d*, (computed for t-tests.) If $k = 2$ (where k is the number of levels for a treatment), then $f =$ (1/2)d. The average *d* was calculated from the three pot experiments (described in chapter four) and divided by 2 to compute *f*. This effect size (*d*) from the pot experiments was based on detecting a 2-seedling increase in seedling establishment

between pots with fungicide treated seeds and untreated seeds. Using d from the pot experiments was based on the assumption that the variability in the field experiment would be similar to the average variability in the pot experiments. Because the sample sizes for the fungicide treatment and the nonfungicide treatment were not equal, the sample size (n) was calculated as follows: $n = N/k$, where N is the total number of replicates and k is the number of treatment levels.

In addition to calculating power to detect a 2-seedling increase in seedling establishment between fungicide-treated and untreated plots, the effect size (f) at 78% power was also determined from the power tables of Cohen (1969).

APPENDIX 5
GERMINATION PERCENTAGES OF SEEDS STORED FOR TWO AND THREE YEARS AFTER COLLECTION

OBJECTIVE

The objective of this study was to determine the germination percentages of seeds stored two and three years after collection of four grassland species found in western Oregon native prairies: *Bromus carinatus* var. *carinatus*, *Cynosurus echinatus, Daucus carota*, and *Prunella vulgaris* var. *lanceolata*,

METHODS

The seeds of the four study species were stored in paper bags at laboratory room temperature immediately after field collection for either two or three years. Seeds (28-32) of each species were sowed on moistened filter paper in each of three Petri dishes (only two Petri dishes were used for 1992 collected seeds of *Prunella vulgaris*). The Petri dishes were put in a germinator at 30° C during the day with 14 hours of light and 20° C during the night and watered with distilled water as needed. (These germination conditions were determined preliminary germination tests). Germination was recorded weekly. A seed was considered germinated when the radicle emerged beyond the seed coat. Monitoring continued until no new germination occurred for 10 days.

RESULTS AND DISCUSSION

With the exception of *Bromus carinatus*, germination percentages were similar between seeds stored for two years and those stored for three years after collection

(Appendix Table 5.1). *Bromus carinatus* showed substantial reduction in seed germination after the second year of storage (Appendix Table 5.1). Therefore, the results suggest that the seeds of these four species have the genetic ability to remain viable for at least two years with little senescence.

Appendix Table 5.1. Germination of seeds stored at room temperature in paper bags for approximately two and three years after field collection for each of four species found in western Oregon native prairies. The sample size is indicated by *n*. Each replicate contained 28 and 32 seeds. Standard deviation is indicated by *sd*.

Species	Years stored	*n*	Germination (%)	sd
Bromus carinatus	two	3	73.3	5.8
	three	3	46.7	3.3
Cynosurus echinatus	two	3	98.9	1.9
	three	3	97.8	1.9
Daucus carota	two	3	98.9	1.9
	three	3	86.0	6.4
Prunella vulgaris	two	2	85.7	5.1
	three	3	92.2	3.9

APPENDIX 6
PREDATION

INTRODUCTION

Unpublished data collected by Lisa Lantz (Department of Botany and Plant Pathology, Oregon State University) for her thesis project were re-analyzed to determine whether the *combined* predation from vertebrates and invertebrates caused seed losses for four grassland species found in western Oregon native prairies: *Bromus carinatus* var. *carinatus*, *Cynosurus echinatus*, *Daucus carota*, and *Prunella vulgaris* var. *lanceolata*.

METHODS AND ANALYSIS

Lantz used the same study species and experimental plots as were used for year two (1992-1993) of the research described in Chapter 3. Two plots (25 cm x 35 cm) were randomly located within each of 20 randomly located blocks (2.2 m x 2.2 m). One of the plots within each block was caged (description in chapter 3) to prevent vertebrate predators and the other plot was uncaged. To prevent invertebrate predation, Lantz sowed 5 seeds of the four study species in early summer 1992 into dishes consisting of a Mason jar ring with a cloth bottom. Dishes in the caged plots were elevated approximately 5 cm from the soil surface by a pedestal, which was covered by "Tanglefoot" ™, a product designed to prevent access by crawling insects. Overhanging vegetation was removed from the caged plots to prevent insects from "dropping in". In the uncaged plots, she sowed 5 seeds of each the four study species into dishes sitting on

the ground with no application of "Tanglefoot"™. Dishes were monitored regularly for the next year and the number of missing seeds recorded and replaced.

The number of missing seeds was compared between the treated and the untreated plots by a paired t-test (Sokal and Rohlf 1981), using the statistical software Statgraphics, version 5.0. Inspections of histograms of the variable showed assumptions of normality were met.

RESULTS AND DISCUSSION

The number of missing seeds within the treated plots and the untreated plots was significantly different for two species, *Bromus carinatus* ($P < 0.01$) and *Prunella vulgaris* ($P = 0.01$), rejecting the null hypothesis of no seed predation by either vertebrate or invertebrate predators (Appendix table 6.1). The number of missing seeds in the treated plots was not significantly different from the number of missing seeds in the untreated plots for the other two species, *Cynosurus echinatus* ($P = 0.10$) and *Daucus carota* ($P = 0.37$), supporting the null hypothesis of no seed predation (Appendix table 6.1).

The results suggest that seed mortality was caused by predation by either vertebrate or invertebrate predators for both the native species, *Bromus carinatus* and *Prunella vulgaris*. In contrast, the results suggests that no seed predation occurred for the two non-native species, *Cynosurus echinatus* and *Daucus carota*.

Appendix Table 6.1. Results of a paired t-test comparing the mean number of seeds missing from uncaged plots compared to caged plots for four species in 1992-1993 in Open Space Park, a western Oregon native prairie. The mean difference between uncaged and caged is presente with standard deviation indicated by *sd*.

Species	*n*	mean difference	sd	*P*
Bromus carinatus	20	6.1	3.9	<0.01
Cynosurus echinatus	20	2.0	5.2	0.10
Daucus carota	20	-0.9	4.1	0.37
Prunella vulgaris	20	2.7	4.2	0.01