After dispersal, seeds can germinate and establish as seedlings, persist as seeds, or die. Knowledge of these three seed fates is crucial for understanding the abundance and distribution of plant populations and ultimately, community composition and diversity. Few studies, however, have simultaneously measured these fates, while also examining the factors causing mortality. The goal of this research was to simultaneously quantify the three seed fates and factors causing death (predation and fungal disease) for four species found in prairies in western Oregon, USA. The most common seed fate for the four study species was death (44–80%). Fungal disease, which has seldom been quantified in natural ecosystems, generally caused less than 10% mortality for each of the four species. Vertebrate predation substantially reduced seed numbers only for Bromus carinatus (21%). Of the unmeasured mortality factors, indirect evidence showed invertebrate predation was a cause of death for seeds of only one species, Prunella vulgaris. In addition, competitive pressures caused seedling death for only the two grass species, Bromus carinatus and Cynosurus echinatus. Survival as established seedlings was generally much more common than survival as persistent seed, with the exception of Daucus carota, in which 14% of the sown seeds persisted the first year.

Key words: fungal disease; mortality factors; prairie; seed fates; seed predation; seedling establishment.

Conditions that occur during the regeneration stage of a plant’s life cycle are crucial in determining the distribution and abundance of plant populations (Grubb, 1977; Harper, 1977; Werner, 1979; Gross and Werner, 1982). Community composition and diversity are also influenced by differences among species in their regeneration characteristics and site requirements (Grubb, 1977; Masuda and Washitani, 1990; Grime and Hillier, 1992). Furthermore, these differences in regeneration patterns influence 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).

Although numerous studies focus on every aspect of seed biology and demonstrate the importance of species regeneration from seed, only a handful of studies follow multiple seed fates simultaneously. These studies are from forest systems (Lawrence and Rediske, 1962; Smith, 1983; Alvarez-Buylla and Martinez-Ramos, 1990; Horvitz and Schemes, 1994), shrub communities (Hughes and Westoby, 1992; Vander Wall, 1994), alpine communities (Chambers, 1995), disturbed habitats (Sarukhan, 1974; Holthuijzen et al., 1987; Watkinson et al., 2000), and often focus on a single species (Pavone and Reader, 1982; van Baalen, 1982; Kalisz, 1991).

One of three fates awaits seeds after dispersal. Seeds land on the soil surface, where they may remain or move deeper into the soil by burial. Germination can occur immediately after dispersal or after persistence in the soil. Persistent seeds are either dormant or quiescent until conditions are appropriate for germination. Death can occur at any point along the pathway: immediately after dispersal, after persistence in the soil, or during germination and growth. Factors that cause death include interference from neighboring plants, microbial disease, predation, senescence, and abiotic factors.

Determining seed fates under field conditions is difficult by visual observation because seeds are inconspicuous and show few signs of causes of death. For example, because both dead seeds and germinants decay in the soil, death caused by pathogens, seed senescence, and failure of germinants to emerge from deep burial can be indistinguishable. As a result, experimental studies that manipulate mortality agents like fungal pathogens are necessary to elucidate various seed fates.

The goal of this research was to determine simultaneously multiple seed fates (death, persistence, and seedling establishment) and factors causing death for four species found in western Oregon (USA) native prairies over two years. The general approach was to sow seeds of these species into experimentally manipulated field plots in each of two years, and then measure seedling establishment rates and number of remaining seeds recovered from the soil. The specific objectives were to simultaneously:

1. estimate the numbers of nonviable and viable seeds by testing viability of seeds retrieved from the soil 1 yr after sowing into field plots in two different years;
2. estimate the number of established seedlings by directly counting seedlings in the field plots 1 yr after sowing in two different years;
3. estimate mortality caused by vertebrate predation by comparing the abundance of dead seeds and seedlings between caged and uncaged field plots in two different years;
4. estimate mortality caused by fungal disease by comparing the abundance of dead seeds and seedlings between fungicide-treated and untreated plots;
5. estimate the number of seeds moving outside field plots by calculating the number of seed surrogates no longer in the plots 1 yr after sowing.

MATERIALS AND METHODS

Study site and species—The study site, one of the few remnants of a vast prairie and oak–savanna ecosystem that covered much of the Willamette Val-
until the 1840s (Boag, 1992), is located approximately 8 km northwest of Corvallis, Oregon, USA in the foothills of the Coast Range (T11S, R6W, Section 25, Willamette meridian). The native upland prairie is dominated by native bunchgrasses, Festuca roemerii (Pavlick) E. B. Alexeev, Bromus carinatus Hook and Arn. var. carinatus and Elymus glaucus Buckl. The entire Willamette Valley has a fairly homogeneous climate characterized by mild, wet winters and moderate, dry summers with cool nights.

The four study species, Bromus carinatus var. carinatus (native perennial grass), Cynosurus echinatus L. (nonnative annual grass), Daucus carota L. (nonnative biennial dicot), and Prunella vulgaris var. lanceolata (Barton) Fern. (native perennial dicot) are all dominant at the study site. The native species are potential candidates for restoration projects and the nonnative species are weedy species that invade native prairies. The seeds of the grass species are the largest (8.1 mg for B. carinatus and 1.8 mg for C. echinatus, dry mass) and are awned, while the seeds of the forbs are the smallest (0.9 mg for P. vulgaris and 1.2 mg for D. carota, dry mass). Although the term seed is used in this study, it refers to the diaspore, i.e., the seed and any associated structures.

**Experimental design and treatments**—In mid-July 1991, the first year of the study, 10 2.2 × 2.2 m blocks were randomly located at the study site. Five treatments (described in detail later) were randomly applied to the five plots (25 × 25 cm) 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 robust and filled seeds of one species were sowed into a subplot within 2 wk of seed collection at the study site. The treatment plots contained standing vegetation at the time of treatment and sowing. Seedling data were collected in three fungicide-treated plots and in 10 of the untreated plots.

In late July 1992, the second year of the study, 20 new 2.2 × 2.2 m blocks were randomly placed at the study site. Within each block, one plot (25 × 35 cm) was caged as described later and another plot remained uncaged. Fungicide treatments were not applied the second year. Instead, fungal disease was investigated in a separate experiment with controlled pot studies (D. L. Clark, unpublished manuscript). Four subplots, each 5 cm in diameter, were located and permanently marked with nails within each plot. Twenty robust and filled seeds, one species per subplot, were sowed within 2 wk of seed collection at the study site.

To exclude vertebrate predators, cages (approximately 35 × 25 × 30 cm high) were made of galvanized metal mesh (1.3 cm) with the bottom edge sunk into the ground about 2.5–4 cm. 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 no-cage treatment (Clark, 1996).

We selected a combination of fungicidal compounds, both systemic and protectant, to ensure coverage of all potential soil fungal pathogen groups: metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetil) alanine methyl ester; Drexel Chemical, Memphis, Tennessee, USA] and Captan (N-trichloro-methylcarbamate; Drexel Chemical). Both fungicides and Captan were sprayed on the soil surface every 2 wk and is effective against seed-rotting fungi (US EPA, 1975; Wainwright and Pugh, 1975; Jeffs, 1986). Captain was sprayed on the soil surface every 2 wk and metalaxyl every 30 d, using the manufacturer’s recommended rates, beginning August 1991 and ending 28 May 1992. Seed germination under soil-less laboratory conditions did not differ significantly between fungicide-treated seeds and untreated seeds for all four study species (Clark, 1996).

To determine secondary dispersal of sowed seeds out of the subplots, seed surrogates made of beads similar in shape and size for each of the four species (Clark, 1996) were sowed within the caged subplots in the second year only. The amount of sowed seeds moving outside the subplots was estimated by subtracting the number of beads recovered from the plot from the number sowed. The amount for year 1 was estimated by multiplying the proportion lost in year 2 times the number sowed in year 1. Because the surrogates were smooth and might travel better than seeds due to secondary movement from water, the results represent the high ends for dispersal outside the plots.

**Data collection**—In mid-June of 1992 and again in 1993, approximately 1 yr after seeds were sowed in the subplots, the number of seedlings for each of the study species was counted in each subplot. These seedling numbers were adjusted by subtracting the number of established seedlings in subplots not sowed with that study species.

The soil from each subplot was removed with a bulb digger to a depth of 5 cm in mid- to late June and stored at 4°C until soil could be sieved to eliminate particles larger than seeds. The residue from sieving was dried at 30°C for 2–3 d and then stored in plastic bags at room temperature until the samples could be examined under magnification to identify the remaining seeds. It is unlikely that storage at 4°C (similar to field conditions during the winter) and the drying temperature negatively affected seed viability. Preliminary tests showed that this method was effective for seed retrieval (Clark, 1996).

Viability of seeds retrieved from soil samples was determined by germinating the seeds in Petri dishes lined with filter paper placed in a germination chamber at alternating temperatures of 30°C (day) and 20°C (night) with 14 h of incandescent and fluorescent light. Preliminary tests showed that these conditions promoted germination for each of the study species. Seeds with at least 1 mm of exposed radicle were considered viable. Ungerminated seeds were further tested for viability using the tetrazolium viability test (Moore, 1985). The number of seeds retrieved from the soil was adjusted by subtracting background counts of seeds from subplots not sowed with that study species.

**Data analysis**—The number of persistent seeds (D) was measured directly as the number of viable seeds recovered from the soil in the untreated subplots 1 yr after sowing the seeds. The number of seedlings (L) was measured directly as the number of seedlings in the untreated subplots 1 yr after sowing seeds. Survival (U) was calculated as U = D + L. Loss to secondary dispersal outside the plots (V) was calculated as V = aS, where a is the proportional loss of surrogate seeds measured in year 2 and S is the number of seeds sowed. Mortality (M) was calculated as the difference between the number of seeds sown and the sum of remaining seeds, seedlings, and dispersal, M = S − (D + L + V). The number of nonviable seeds (N) was measured directly as the number of nonviable seeds recovered from the soil in the untreated subplots 1 yr after sowing. Loss to vertebrate predation (P) was calculated as P = M1 − M2, where M1 is total mortality in the uncaged subplots and M2 is the total mortality in the caged subplots. Loss to fungal disease (F) for year 1 was calculated as F = M2u − M2, where M2u is the total mortality in the nonfungicided subplots and M2 is the total mortality in the fungicide treated subplots. Loss to unmeasured mortality factors in year 1 (O1) was calculated by subtraction as O1 = M1 − (N1 + P1 + F1). Loss to unmeasured mortality factors in year 2 (O2) was calculated as O2 = M2 − (N2 + P2).

Because the number of recovered seeds and seedlings for each sample was adjusted by subtracting the number of background counts, mean magnitudes and lower bounds on confidence intervals could have negative values. Even though negative values for seed fates are not biologically meaningful, they are an inevitable outcome of the sampling design and do not interfere with the statistical analysis. Comparisons of the types of survival and causes of death were made for each species using a one-way randomized block analysis of variance (ANOVA) (Sokal and Rohlf, 1981). Assumptions of homogeneity of variances and normality for ANOVA were met. This analysis was repeated for year 2 except loss from secondary dispersal outside experimental plots was included, and mortality by disease was excluded. When appropriate, dif-
Er In 1991–1992 and 1992–1993 for four plant species of western Oregon native prairies. Categories are expressed as a percentage of the total number of experimentally sowed seeds (with 95% confidence intervals). Differences between means were tested using Fisher’s protected least significant difference ($\alpha = 0.05$). Means sharing letters within a single year were statistically indistinguishable. Details on calculations for each fate or mortality factor are found in the text.

**RESULTS**

The type of survival and causes of death differed significantly within years for *Bromus carinatus* in year 1 ($F_{5,27} = 7.80, P < 0.01$) and year 2 ($F_{5,91} = 43.92, P < 0.01$), for *Cynosurus echinatus* in year 1 ($F_{5,28} = 2.76, P = 0.04$) and year 2 ($F_{5,91} = 35.10, P < 0.01$), for *Daucus carota* in year 2 only ($F_{5,91} = 6.76, P < 0.01$), and for *Prunella vulgaris* in year 1 ($F_{5,31} = 2.19, P = 0.08$) and year 2 ($F_{5,91} = 116.90, P < 0.01$).

Death was the primary fate for *Bromus carinatus* (79.6% and 75.0%, Fig. 1). Few seeds established as seedlings (8.0% and 13.5%) and virtually no seeds persisted either year (2.1% and 1.5%, Fig. 1). Most mortality for both years was caused by factors not directly tested (51.6% and 67.5%), even though fungal disease was not included in this category for the first year (Fig. 1). Of the remaining mortality categories for both years, only first-year vertebrate predation (21.2%) was greater than 10%. Loss from secondary dispersal outside the plots accounted for approximately 12% of the sowed seeds (Fig. 1).

Few seeds of *Cynosurus echinatus* survived (16.6% in year 1 and 11.6% in year 2, Fig. 1), with nearly all as seedlings, rather than as persistent seeds. The patterns of mortality were similar for both years, with unmeasured mortality factors the largest category for both years (35.2% and 72.0%, Fig. 1). Loss of seeds to vertebrate predation for both years and fungal disease was less than 10% (Fig. 1). Loss of seeds from secondary dispersal outside the plots was 21% (Fig. 1).

Although mortality was the largest fate category for *Daucus carota* for both years (44.4% and 65.0%), the survival rate was relatively high the first year (28.6%) with most of these surviving as persistent seeds. In contrast, virtually no seeds persisted the second year (−1.5%, Fig. 1). For both years,
factors not tested directly caused the greatest magnitude of death (37.6% and 55.5%), although the magnitude was not significantly different from the other mortality factors in year 1 (Fig. 1). Loss of seeds to vertebrate predation and fungal disease was small, each less than 10.5% for both years (Fig. 1). Loss of seeds to secondary dispersal outside the plots was approximately 27% (Fig. 1).

For both years, mortality of Prunella vulgaris (54.4% and 68.5%) was much greater than survival (18.7% and 4.5%, Fig. 1). Survival as established seedlings was much higher (17.2%) in year 1 than in year 2 (4.0%). The patterns for both years suggest that factors not tested directly are the major causes for mortality (32.4% and 73.0%, Fig. 1), although the magnitude of fungal disease was substantial (22.4%) in year 1. The large amount of variation in the data for fungal disease caused fungal disease and unmeasured mortality factors to be statistically indistinguishable. Loss of seeds to secondary dispersal outside the plots was about 27% (Fig. 1).

**DISCUSSION**

**Causes of mortality**—The experimental manipulation of three mortality factors in this study allowed consideration of the causes of death, which was the most common fate. The number of recovered nonviable seeds suggests losses due to senescence are very low (<2%). It is possible, however, that senescent seeds may have decayed and disappeared between the time of sowing and retrieval from the soil.

The handful of investigations done in natural systems often show considerable mortality of seeds and seedlings from fungal disease (Lawrence and Rediske, 1962; Mack and Pyke, 1984; Augspurger, 1990; Leishman et al., 2000) but sometimes demonstrates low mortality from disease (Schisler and Linderman, 1984; Lonsdale, 1993). The extensive literature on seedling death in agricultural systems shows that mortality by fungi can be substantial (Sewell, 1981; Harman and Stasz, 1986). Death by common soil fungi in the present study was less than 10%, with the exception of Prunella vulgaris. This lack of significant fungal disease may have been caused by lack of fungal pathogens in the soil, resistant plant hosts, or lack of appropriate environmental conditions for disease to occur.

Grassland plants often lose large numbers of dispersed seeds to vertebrate predators (Louda, 1989; Crawley, 1992). In this study and in Lantz (1997) (a concurrent study at the same site), however, vertebrate predation was an important mortality factor for only one species, Bromus carinatus. Vertebrate predation of large seeds is generally much greater than predation of smaller seeds, possibly from visibility, ease of handling, and nutrient content (Thompson, 1987). In addition, compact seeds are more easily buried in the soil, thus decreasing susceptibility to predators (Thompson et al., 1993). These factors are consistent with the low vertebrate predation rates found here for Cynosurus echinatus, Daucus carota, and Prunella vulgaris, whose seeds are all small (<2 mg). Prunella vulgaris seeds also contain high levels of ortho-dihydroxyphenol, a defense compound deterring predation (Hendry et al., 1994), which may also explain the low levels of predation for P. vulgaris seeds.

Likely causes of seed death in the unmeasured mortality factors category are nonfungal disease, invertebrate predation, interference, and abiotic constraints. Although these factors were not manipulated experimentally in this study, indirect evidence suggests which factors might be major causes of death. Seeds are common vectors of bacteria and viruses (Ner-gaard, 1977), yet rarely do studies report death of seeds or seedlings by bacterial or viral diseases. In fact, 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, 1965; Kremer et al., 1984; Bruehl, 1987). Thus, bacterial disease is unlikely to have contributed much to mortality from unmeasured factors.

In general, seed losses due to predation in grasslands are attributed to vertebrate predators rather than invertebrate predators (Louda, 1989; Hulme, 1994; but see Reader, 1991, 1993). Invertebrate predation of the four study species can be indirectly determined by comparing the results of the present study in which predation by only vertebrates was measured with the results of a concurrent study at the same site in the combined predation from both vertebrates and invertebrates was measured (Lantz, 1997). In the concurrent study, cages were used to prevent vertebrate predation, and invertebrate predation of seeds was prevented by sowing seeds into dishes elevated from the soil surface by a pedestal, which was covered by Tanglefoot, a product designed to prevent access by crawling insects. Significant predation (vertebrate and invertebrate combined) occurred for only B. carinatus and P. vulgaris (P ≤ 0.01) (Lantz, 1997). Vertebrate predation in the present study occurred for only B. carinatus but not P. vulgaris. Thus, invertebrate predation was likely to have been an important contribution to the mortality of P. vulgaris from unmeasured factors.

There is widespread evidence for competition limiting the survival of germinants in dense undisturbed grasslands, such as this native prairie. Most seedlings have higher survival in patches of bare soil or short turf, locations relatively free of competition for space, light, nutrients, and water (Fenner, 1978; McConnaughay and Bazzaz, 1987; Thompson and Baster, 1992; Wilson and Gerry, 1995) or allelopathy (Rice, 1984). In the concurrent study (Lantz, 1997), seedling mortality of B. carinatus and C. echinatus decreased significantly in plots with vegetation removal (P ≤ 0.03). Thus, interference from other species, especially competition for belowground resources, may be an important contribution to the mortality of grasses from unmeasured factors.

Abiotic constraints are unlikely to cause death at the seed stage, but are likely important at the more vulnerable seedling stage. Seeds that lack dormancy mechanisms and that readily germinate, like those of B. carinatus, C. echinatus, and P. vulgaris, are particularly vulnerable to adverse abiotic conditions, especially in highly variable weather conditions typical of western Oregon. Abiotic factors cannot be ruled out as an important contribution to mortality in these species.

**Patterns of survival**—Grassland species generally produce few seedlings that successfully establish (Pearl, 1984, 1989; McConnaughay and Bazzaz, 1987; Thompson and Baster, 1992; Reader, 1993). Seedling establishment rates in this study for D. carota and P. vulgaris were similar to those reported elsewhere for these species (Holt, 1972; Gross and Werner, 1982; Thompson and Baster, 1992). No studies report seedling establishment rates for the grasses C. echinatus and B. carinatus.

Daucus carota was the only species in this study with significant seed persistence. Other studies also report a persistent
seed bank for *Daucus carota*, with seed longevity between 2 and 5 yr (Gross and Werner, 1982; Thompson et al., 1993). The morphological characteristics of *Daucus carota* seeds match the seed morphological patterns of British herbaceous species that maintain long-term persistent seed banks (Thompson et al., 1993). Grass species lacking persistent seed banks have seeds that are large and attenuated, often with awns and retrorse hairs (Thompson and Grime, 1979; Thompson, 1987), similar to the seed characteristics of both grasses *B. carinatus* and *C. echiatus*, which did not form persistent seed banks in this study. *Prunella vulgaris*, which has small compact seeds, did not form a persistent seed bank in the present study and was the exception to these patterns. Wet seeds of *P. vulgaris* produce a mucilaginous substance that causes the seeds to adhere to soil particles and litter, potentially hindering seed burial and thus exposing seeds to lethal processes like desiccation and invertebrate predation that limit persistence in the soil. Seed persistence is also promoted by dormancy. *Daucus carota* is the only study species reported to have dormant seeds (Gross, 1984; Pons, 1991), and grasses with awned seeds, like *B. carinatus* and *C. echiatus*, typically are not dormant (Pearl, 1984). Furthermore, many grasses found in habitats characterized by summer drought (such as the Willamette Valley) have nonrestrictive germination requirements, germinating under a wide range of temperatures when moisture is adequate (Thompson and Grime, 1979; Maret, 1996).

**Implications for conservation**—The results of this study are useful for conserving native Willamette Valley upland prairies, among the rarest of Oregon’s ecosystems (ONHP, 1983; Noss et al., 1995). Sowing seeds of the two native species, *B. carinatus* and *P. vulgaris*, is one way to increase their abundance at restoration sites, but low seed persistence (Fig. 1) means few seeds are available from a soil seed bank. Because establishment rates are low for these species (<20%, Fig. 1), sowing rates will need to be high.

The results from this study suggest controlling propagule availability is more likely to be effective in controlling abundance of the nonnative species than is controlling mortality factors of seeds and seedlings. Because seeds of the annual *C. echiatus* do not persist in the soil (Fig. 1), removal of the on-site seed source by mowing or fire before seed maturation should eventually eliminate the population, unless immigration occurs. In contrast, short-term management to reduce its seed production will not eliminate populations of *D. carota* because its seed can persist in the soil (Fig. 1).

**LITERATURE CITED**


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