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Seed bank dynamics: the role of fungal pathogens and climate change

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Summary

1. One of the climate change scenarios predicted for the UK is warmer winters and additional summer rainfall, which may favour growth and survival of fungal pathogens. We tested several hypotheses on the fate of persistent seeds in the soil and the role of fungal pathogens under this predicted climate change.

2. We buried seed bags containing fungicide-treated and non-fungicide-treated seeds of four species with persistent seed banks (*Convolvulus arvensis* L., *Lotus corniculatus* L., *Medicago lupulina* L. and *Rubus fruticosus* L.) under control and simulated climate change (winter warming plus supplemented summer rain) conditions, and monitored seed survival over 1 to 2 years.

3. Fungicide treatment resulted in a significant increase in the percentage of intact seeds recovered for only two of the four species, *M. lupulina* and *R. fruticosus.* Seeds of *M. lupulina* that were treated with fungicide remained viable in the soil for longer than non-treated seeds. Thus, the effect of fungal pathogens on seed persistence in the soil appears to be species specific.

4. There was no significant effect of the simulated climate (winter warming plus supplemented summer rain) on seed persistence in the soil, for any of the four species. Neither was a significant climate × fungicide treatment interaction found for any of the four species. Thus, it does not appear that the conditions provided in the simulated climate plots favoured the growth and survival of fungal pathogens affecting the soil seed banks of the four species studied here.

5. The use of fungicides in manipulative experiments and the importance of field experiments that simulate predicted climate change are discussed.

Key-words: Dormancy, fungicide, global warming, seed mortality

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Introduction

Seed banks are thought to play a critical role in vegetation dynamics. The presence of a reserve of dormant seeds in the soil can stabilize population dynamics by spreading risk and diminishing large fluctuations in response to short-term environmental perturbations (Harper 1977; Cavers 1983; Venable & Brown 1988). Grime (1989) suggested that soil seed banks may facilitate population recovery following disturbance. Chesson (1986) has shown, with the use of theoretical models, how seed banks can create a 'storage effect' that permits coexistence of species in temporally varying environments. Further, it has been suggested that a long-lived seed bank, by conserving genetic diversity, may buffer populations against changes due to genetic drift, selection or immigration (Levin 1986), and provide a population with more flexibility to cope with change (Baker 1989).

The decline in seed numbers in the soil follows the pattern of an exponential decay curve, with variation amongst species in the constant percentage lost each year (Harper 1977; Cavers 1983). This seed loss from the soil bank may be due to either successful germination and emergence, or mortality from several causes: germinating too deep, ageing and loss of viability, or attack by predators or pathogens. The vast majority of seeds in the soil (up to 90%) suffer mortality due to one of these causes (Cook 1980).

It is highly likely that fungal pathogens are a significant cause of mortality of seeds in the soil (Kirkpatrick

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& Bazzaz 1979; Burdon 1987; Crist & Friese 1993). Burdon (1987) has described several types of fungi that potentially affect seeds. These are: (1) surface-contaminating fungi which may affect seeds, either directly through necrotic action or indirectly through the production of toxic metabolic wastes; (2) internally borne fungi which may increase the metabolic activity of seeds and hence reduce their long-term viability; and (3) soil-borne fungi, which are generally poorly documented. Previous studies have found that seed loss from the seed bank is reduced by fungicide treatment; for example, by 10–16% for the invasive woody shrub *Mimosa pigra* (Lonsdale 1993) and by 39 and 47% for the two pioneer tropical tree species *Cecropia insignis* and *Micronia argentia*, respectively (Dalling, Swaine & Garwood 1998), but only by 0·1–0·2% for two *Trifolium* species in Australian pasture (Jansen & Ison 1995). Hendry *et al*. (1994) found a highly significant correlation between seed persistence in the soil and the *ortho*-dihydroxyphenol concentration of seeds, in a survey of 81 herbaceous species in Britain. They suggested that *ortho*-dihydroxyphenol may act by deferring or decreasing the rate of decomposition by microbes and by defending against herbivory. The correlation between seed persistence in the soil and *ortho*-dihydroxyphenol concentration of seeds suggests that the action of fungal pathogens in the soil may be a strong selective force for species with a soil seed bank. Other studies have also found antimicrobial substances in the seeds of species which are persistent in the soil, for example *Digitalis purpurea* and *Hypericum pulchrum* (Warr, Thompson & Kent 1992), and *Abutilon theophrasti* (Kremer 1986).

The two most important factors influencing fungal pathogen distribution are temperature and moisture, where high levels of precipitation resulting in high humidity and soil moisture, along with warm temperatures, favour pathogen growth and survival (Burdon 1987). Global climate models have predicted that the UK will probably experience a warming that will be most marked overwinter (CCIRG 1991; CCIRG 1996; Houghton *et al*. 1996). Additionally, there is likely to be an increase in anticyclonic behaviour during the summer, which may result in a 20% increase in precipitation (CCIRG 1991; CCIRG 1996; Houghton *et al*. 1996). Thus, it seems a reasonable assumption that warmer mean annual temperatures and higher summer rainfall may result in increased fungal activity, which has important implications for seed bank dynamics. The study reported here tests the following hypotheses:

1. fungal pathogens account for a significant proportion of the mortality of seeds in the soil;
2. supplemented summer rain and winter warming will result in a reduction in the number of viable seeds in the seed bank, and
3. such a reduction in the number of viable seeds in the seed bank can be attributed to enhanced fungal pathogen activity.

We tested these hypotheses using four British herbaceous species which are reported as having persistent soil seed banks. We used a long-term field experiment at Wytham, UK to provide the climate manipulations, and buried seed bags containing fungicide-treated and non-fungicide-treated seeds under control and simulated climate change (warmer winter, supplemented summer rain) conditions. Seed survival was monitored over 2 years.

Materials and methods

STUDY SITE

The study was conducted within a Jurasic corallian limestone grassland at Wytham Woods, Oxfordshire, UK. A long-term climate manipulation field experiment was set up there in 1993/94 to study the effects of predicted climate change on vegetation, invertebrates and plant–animal interactions. The study site was situated within a central 1 ha of a 10-ha ex-arable field, in which cultivation ceased in 1982 (Gibson 1986). The hectare was fenced to exclude deer (the primary wild herbivore) and sheep (used for management of surrounding grassland).

FUNGICIDE TREATMENT

The activity of many systemic fungicides is specific to particular groups of fungi (Paul, Ayres & Wyness 1989), while protectant fungicides have broad-spectrum activity. Hence, it is important to select a combination of fungicidal compounds, both systemic and protectant, to ensure coverage of all potential fungal pathogen groups. The fungicides we used, with details of their common names and activities, are listed in Table 1. The application rate was 2 ml Baytan, 1 g Apron T mixed with 1 ml distilled water, and 1 g Benomyl mixed with distilled water (forming a slurry), per 2000 seeds. Ridomil and Roveral were applied to the soil surface at 6-monthly intervals, at the rate of 0·2 g of active ingredient per m^2.

EXPERIMENTAL DESIGN

Only a brief description of the climate manipulations and overall design is provided here. Further details are given in Cummins *et al*. (1995) and Masters *et al*. (1998). A randomized block design, with five replicates, was used to simulate the effects of warmer winters with either wetter or drier summers. There were six treatments per block: (1) control (ambient climatic conditions); (2) winter warming (WW: 3 °C elevation over ambient applied by heating cables on the soil surface, from 1 November to 30 April the following year); (3) summer drought (SD: complete drought during July and August imposed by mobile non-invasive rain shelters); (4) supplemented summer rainfall (SR: 20% supplementation of the 10-year mean weekly rainfall applied

Table 1. Trade name, common name, activity and fungal groups active against, for the fungicides used in the experiment

Trade name[a]	Common name	Activity	Mastigo-mycotina	Asco-mycotina	Basidio-mycotina	Examples
Baytan	triadimenol	systemic		x	x	
	fuberidazole			x		Fusarium
Apron T	metalaxyl	systemic, protectant	x			Pythium, Phytophthora
	thiabendazole	broad-spectrum				Botrytis, Fusarium,
				x		Rhizoctonia, Septaria
Benomyl	benzimidazole	systemic, protectant		x	x	
Ridomil	metalaxyl	systemic, protectant	x			
	copper oxychloride	protectant	x			Phytophthora
Roveral	iprodione					Botrytis, Sclerotina, Altenarea,
				x		Rhizoctina, Septaria

[a]Fungicides were provided by Ciba Agriculture, Cambridge, UK (Apron T, Ridomil), Du Pont (Benomyl) and Zeneca (Baytan).

as evenly sprayed deionized water); (5) WW + SD, and (6) WW + SR. These manipulations were applied to 3 m × 3 m plots with 2 m walkways between. Treatments began in winter 1993/94 and the experiment is ongoing. In this study, we consider only two of the treatments, control and WW + SR.

Fifty seeds of each of four species were mixed with soil from the study site and placed in nylon mesh bags, measuring approximately 5 cm × 5 cm. Seeds were obtained from commercial seed suppliers and are guaranteed of native origin. The four species used were *Convolvulus arvensis* L., *Lotus corniculatus* L., *Medicago lupulina* L. and *Rubus fruticosus* L. Within each relevant climate plot of the five blocks, we buried eight (*M. lupulina* and *R. fruticosus*) or four (*C. arvensis* and *L. corniculatus*) seed bags, at approximately 5 cm depth and in random locations, half of which contained seeds treated with fungicide. One bag per fungicide treatment per climate, selected randomly, was exhumed at 6-monthly intervals after burial. Thus, the factors fungicide treatment (treated or control), climate (supplemented summer rain plus winter warming or control) and time (four times for *M. lupulina* and *R. fruticosus* and two times for *C. arvensis* and *L. corniculatus*) were completely factorial, with blocks providing the replication. This design resulted in 80 bags (4000 seeds) buried for each of *M. lupulina* and *R. fruticosus* and 40 bags (2000 seeds) buried for each of *C. arvensis* and *L. corniculatus*. Originally, three species were selected for study (*M. lupulina* L., *R. fruticosus* L. and *Trifolium repens* L.) and buried within the blocks; however, as *T. repens* had 100% germination by the end of the first 6 months it was excluded from the study and replaced with *Convolvulus arvensis* L. and *Lotus corniculatus* L. for the second year.

Seed bags of *M. lupulina* and *R. fruticosus* were buried on 10 September 1994, while those of *C. arvensis* and *L. corniculatus* were buried on 8 September 1995. All bags were buried approximately 10 cm in from the edge of the plot and 20 cm apart. Each was marked with a colour-coded plastic tag to enable retrieval. Seed bags were dug up on 15 May 1995 (*M. lupulina* and *R. fruticosus* only), 18 September 1995 (*M. lupulina* and *R. fruticosus* only), 7 May 1996 (all species) and 21 October 1996 (all species).

Exhumed seed bags were examined under a compound microscope and the number of seeds which were intact, non-viable (soft to touch) or germinated (seed with radicle emerged) and the number of empty seed coats were recorded. We did not use standard chemical tests of seed viability (i.e. tetrazolium) as the pink colour of the tetrazolium stain could not be distinguished from the pink dye of the fungicide treatment. However, intact seeds that were firm to touch were assumed to be viable, and were easily distinguished from the soft-to-touch or mushy seeds that were classed as non-viable. Intact empty seed coats were attributed to germination, so that the total number of assumed germinated seeds was calculated as the number of germinated seeds plus the number of empty seed coats.

STATISTICAL ANALYSES

All analyses were performed as GLM analyses within Minitab Version 11·0 (Minitab Inc., State College, PA). Climate, fungicide treatment and time were treated as fixed effects. The response variables examined were percentage of intact seeds and percentage of assumed germinated seeds. Values were square root arcsin transformed for analysis. Analyses for each species were performed separately as species were buried for different periods of time.

Results

Medicago lupulina

There was no effect of climate on the percentage of intact seeds exhumed from the buried bags ($F_{1,58} = 0{\cdot}07$, $P = 0{\cdot}79$). There were strong effects of both fungicide treatment ($F_{1,58} = 169{\cdot}06$, $P < 0{\cdot}001$) and time ($F_{3,58} = 34{\cdot}82$, $P < 0{\cdot}001$) on the percentage of intact seeds remaining, with fungicide treatment having a strong protective effect on the seeds, and the percentage of intact seeds decreasing with time (Fig. 1a). There was a significant fungicide–time interaction

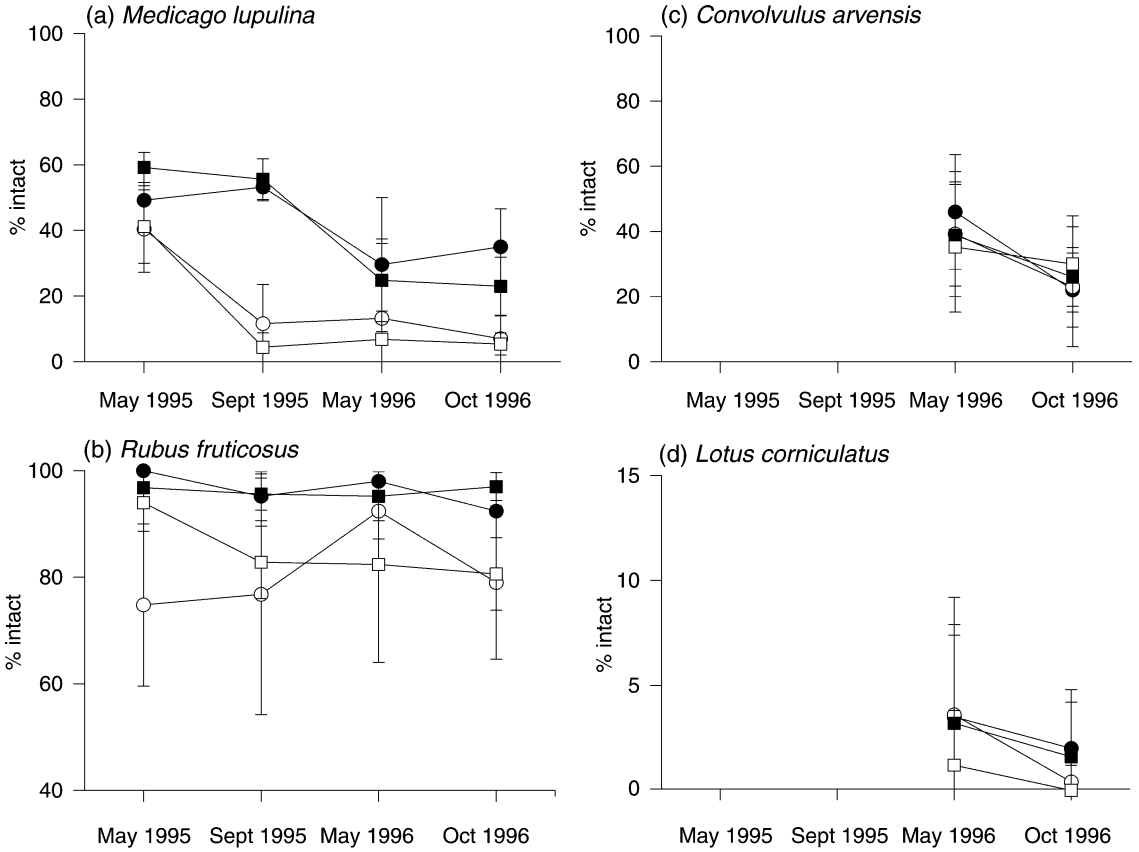

Fig. 1. The percentage of intact (viable) seeds remaining after burial in seed bags for four species: (a) *Medicago lupulina*; (b) *Rubus fruticosus*; (c) *Convolvulus arvensis* and (d) *Lotus corniculatus*. The symbols represent the two climate treatments: □, supplemented summer rainfall/winter warming; ○, control. Solid symbols represent fungicide-treated seeds, while open symbols represent non-fungicide-treated seeds. Note that the *y*-axes for (b) and (d) differ from those for (a) and (c).

($F_{3,58}$ = 12·66, P < 0·001), where fungicide treatment delayed the decline in the number of intact seeds over time (Fig. 1a).

There was a significantly higher percentage of germinated seeds in the control than in the supplemented summer rain/winter warming climate ($F_{1,58}$ = 8·66, P = 0·005). Fungicide treatment ($F_{1,58}$ = 90·49, P < 0·001) and time ($F_{3,58}$ = 7·16, P < 0·001) also significantly affected the percentage of germinated seed, with seeds treated with fungicide having a higher percentage germination and the difference in percentage germination between control and supplemented summer rain/winter warming increasing through time for fungicide-treated seeds (fungicide–time interaction $F_{3,58}$ = 5·35, P = 0·003; Fig. 2a).

Rubus fruticosus

The percentage of intact seeds was always significantly higher for fungicide-treated seeds than for controls ($F_{1,59}$ = 56·09, P < 0·001). There was no direct effect of climate on the percentage of intact seeds ($F_{1,59}$ = 0·20, P = 0·66), although there was a significant climate × fungicide–time interaction ($F_{3,59}$ = 3·90, P = 0·01) (Fig. 1b). The number of germinated seeds was very low, and consequently only time showed any significant effect on percentage germination ($F_{3,59}$ = 3·81, P = 0·01).

Convolvulus arvensis

The only significant factor in the percentage of intact seeds was time ($F_{1,30}$ = 7·43, P = 0·01) where the percentage of intact seeds decreased with time (Fig. 1c). However, both fungicide treatment ($F_{1,30}$ = 10·37, P = 0·003) and time ($F_{1,30}$ = 12·70, P = 0·001) affected the percentage germination of seeds, with higher percentage germination for fungicide-treated seeds and increased germination over time (Fig. 2c).

Lotus corniculatus

The number of intact seeds recovered was extremely low, so that no significant effects of any of the factors or their interactions were found (Fig. 1d). However, climate had a significant effect on the percentage of germinated seeds ($F_{1,31}$ = 5·05, P = 0·03), with a higher percentage germination in control than in supplemented summer rain/winter warming plots (Fig. 1d). There were significant main fungicide ($F_{1,31}$ = 53·55, P < 0·001) and time ($F_{1,31}$ = 4·53, P = 0·04) effects as well as a significant fungicide–time interaction effect ($F_{1,31}$ = 5·45, P = 0·03) on percentage germination. The percentage germination of non-fungicide-treated seeds increased with time while that of fungicide-treated seeds did not (Fig. 2d).

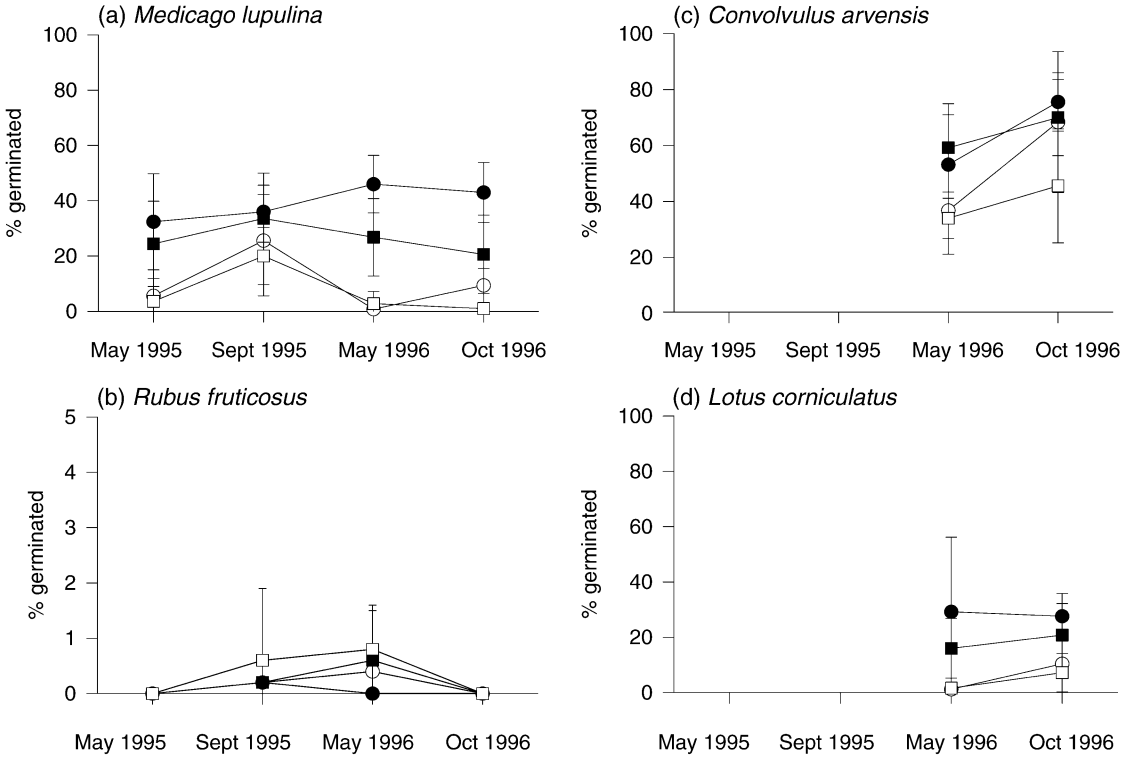

Fig. 2. The percentage of germinated seeds after burial in seed bags for four species: (a) *Medicago lupulina*; (b) *Rubus fruticosus*; (c) *Convolvulus arvensis* and (d) *Lotus corniculatus*. The symbols represent the two climate treatments: □, supplemented summer rainfall/winter warming; ○, control. Solid symbols represent fungicide-treated seeds, while open symbols represent non-fungicide-treated seeds. Note that the *y*-axis for (b) *Rubus fruticosus* is scaled differently to those for (a) (c) and (d).

Discussion

This study tested three hypotheses. The first was that fungal pathogens account for a significant proportion of seed mortality in the soil. We used the number of intact seeds remaining as an inverse measure of mortality. For two of the four species, *M. lupulina* and *R. fruticosus*, treatment of seeds with fungicide resulted in a significant increase in the number of intact seeds found (in *M. lupulina*, 43% intact for fungicide-treated seeds compared to 15% for non-fungicide-treated seeds; in *R. fruticosus*, 96% compared to 83%, respectively). Furthermore, seeds of *M. lupulina* that were treated with fungicide remained viable in the soil for longer than non-treated seeds. For seeds of *C. arvensis* and *L. corniculatus*, fungicide treatment had no significant effect on seed survival. Thus the effect of fungal pathogens on seeds in the soil seed bank appears to be species-specific, rather than general.

The second hypothesis was that supplemented summer rain and winter warming (after CCIRG 1991; CCIRG 1996; Houghton *et al.* 1996) will result in a reduction in the number of viable seeds in the seed bank. This hypothesis was not supported: there was no significant difference in the percentage of intact seeds between the supplemented summer rain/winter warming plots and the control plots, for any of the four species. The third hypothesis was that any reduction in the number of viable seeds in the seed bank under simulated climate change could be attributed to enhanced fungal pathogen activity. As there was no reduction in the number of intact seeds under the supplemented summer rain/winter warming plots compared to the control plots, and no significant climate × fungicide treatment interactions found for any of the four species, this hypothesis was also rejected. Thus, it does not appear that the additional summer rain and warmer winter provided in the simulated climate plots favoured the growth and survival of fungal pathogens that affect the soil seed bank of the four species studied here.

One possible explanation for the lack of a predicted effect of a warmer and wetter climate on seed fungal pathogens is that the simulated climate conditions were not sufficiently warm and wet to enhance fungal activity. The simulated winter warming is provided by heating cables on the soil surface. These heating cables may act to dry out as well as warm the soil, thus making conditions potentially less favourable for fungal pathogens. Increased soil temperatures may also result in increased plant growth and thus increased evapotranspiration, resulting in reduced soil moisture. Secondly, the summers of 1995 and 1996 were particularly hot, so that the provision of extra summer rainfall may not have increased soil moisture sufficiently (due to high evaporation rates) to affect fungal activity. Thus, it is possible that the experimental conditions simply did not provide a sufficiently moist environment.

There have been few previous studies on the relationship between climate and seed bank density. Pakeman *et al*. (1999) found smaller seed bank densities for *Calluna vulgaris* in the drier and warmer regions of Great Britain. However, their study was unable to separate the effects of climate on seed output and seed mortality. Two studies have examined the effect of manipulated climates on soil seed banks, in meadow communities (Akinola, Thompson & Hillier 1998b) and calcareous grassland communities (Akinola, Thompson & Buckland 1998a). Both studies found little effect of climate treatment on seed banks of a range of species. However, again, the separate effects of climate on vegetation (and hence input to the seed bank) and seed mortality were not explicitly addressed.

Similarly, there have been few previous studies which have attempted to study the impact of fungal pathogens on seed viability in the soil. Crist & Friese (1993) studied the role of fungi in seed persistence in the soil of five species in a shrub-steppe system in Wyoming. They reported that fungal attack was responsible for a decrease in seed survivorship of up to 35·2%, with the greatest decrease occurring from autumn to winter, although this varied substantially across species. Kirkpatrick & Bazzaz (1979) studied the influence of fungi on germination and survival of four annual species. They found that seed-borne mycoflora were abundant, although variable among the species, and that fungal isolates affected seed germination and seedling development. Neither of these studies attempted to use chemical exclusion methods to manipulate fungal activity. In fact, there are very few reports on the use of chemical exclusion for fungi (Paul *et al*. 1989). In three studies where fungicide treatments have been used, results ranged from almost no effect of fungal pathogens on seed mortality (Jansen & Ison 1995) to an increase in mortality up to 47% (Lonsdale 1993; Dalling *et al*. 1998). One potential problem with the use of fungicides is that a mixture of compounds is required to ensure efficacy against a range of fungi, and the use of combinations of compounds may result in unintended side-effects (Paul *et al*. 1989). Gange, Brown & Farmer (1992) tested the effects of two insecticides and one fungicide on 20 herbaceous species in the laboratory and found that, although there was some evidence of interactive effects, the fungicide used (Roveral, also used in this study) showed little effect on seed germination. Paul *et al*. (1989) state that metalaxyl (contained in two of the fungicides used in this study) has rarely been reported as having phytotoxic effects. In this study, the application of fungicide increased germination in three of the four species (*M. lupulina*, *C. arvensis* and *L. corniculatus*). In contrast, Gange *et al*. (1992) found that where insecticide application did have an effect on seeds, it was to reduce rather than to increase germination.

Finally, this study found an effect of simulated climate change on percentage germination in two of the four species. Supplemented summer rainfall plus winter warming reduced the percentage germination of *M. lupulina* and *L. corniculatus*. This result may also be due to the relatively dry soil conditions that resulted from the increased soil temperature under the supplemented summer rain plus winter warming climate treatment. However, surprisingly, Clarke (1998) found that the emergence of seedlings of *M. lupulina* and some annual species was increased by these climate treatments, though no records of pre-emergence mortality were made.

In conclusion, studies which attempt to simulate the effect of predicted climate change on vegetation communities provide a valuable contribution to our understanding of future global change. In this study, we tested several simple predictions concerning the influence of warmer winters plus increased summer rain on the effect of fungal pathogens on seed persistence in the soil, which may have important consequences for vegetation dynamics. The predictions were not fulfilled, probably due to the side-effect of warmer winters creating drier soils, thus reducing any increased rainfall effect on fungal activity. Without field experiments such as the one reported here, such side-effects may not be recognized, and seemingly simple predictions be mistakenly accepted.

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