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EFFECTS OF SOIL DISTURBANCE AND DISEASE ON THE GROWTH AND REPRODUCTION OF *LOLIUM PERENNE* (POACEAE) INTRODUCED TO SEMI-NATURAL GRASSLANDS

ABSTRACT: We performed manipulative field experiments to investigate the effects of soil disturbance and exposure to a fungal plant pathogen, *Puccinia coronata* (Corda), on the establishment and spread of two introduced, cultivated genotypes of perennial ryegrass, *Lolium perenne* (L.). The two cultivars of *L. perenne* with different levels of susceptibility to *P. coronata* were introduced to grassland sites to investigate whether a plant cultivar selected to resist a pathogen shows better establishment in semi-natural plant communities than a susceptible cultivar. At two sites where *L. perenne* was already present, the addition of *L. perenne* seeds had no significant effect on the shoot biomass of the species, indicating that these populations were not seed limited. Exposure to the pathogen resulted in disease, and infected *L. perenne* populations showed increased shoot biomass over the course of the 3 year experiment and at harvest the final year, but no effect on seed production. Reproductive allocation was not affected by disease exposure in disturbed plots, but decreased in the presence of disease in undisturbed plots. The increased biomass observed in the semi-natural plant communities when exposed to the pathogen contrasts with the reduced biomass observed in garden experiments when the two cultivars of *L. perenne* were exposed to pathogen attack. The surprising positive effect of *P. coronata* on biomass in semi-natural communities indicates that processes here are more complex than in more intensively managed production systems.

KEY WORDS: crown rust, plant-pathogen interaction, perennial ryegrass, plant competition, plant establishment, plant introduction, *Puccinia coronata*

1. INTRODUCTION

Many cultivated plant species are able to grow and persist in non-cultivated habitats and this has given rise to speculations about their effects on the dynamics of natural plant communities (Mack and Erneberg 2002). The introduction and cultivation of non-native genotypes may be undesirable if such genotypes are able to escape from cultivation and modify the recipient native plant community by out-competing natives (Braithwaite *et al.* 1989), changing ecosystem processes (Walker and Vitousek 1991), or facilitating the invasion of other invasive plants (Simberloff and Von Holle 1999). In addition, genetic changes in the flora may occur if genes from introduced plant species introgress into natural populations of closely related cross-fertile plant species (Rogers and Parkers 1995, Hansen *et al.* 2001).

After many years of research on crop diseases, we have some understanding of the effects of plant pathogens on crops in agricultural fields. Much less is known about the

effects of pathogens on growth, fitness and competitive ability of their hosts in non-cultivated habitats, and even less is known about long-term pathogen-mediated effects on plant population dynamics. Most of the published research on plant-pathogen interactions in natural systems has confirmed that pathogens have negative effects on host growth and reproduction, although there are often important interactions with environmental conditions (Paul and Ayers 1987, Wennström and Ericson 1990, García-Guzmán *et al.* 1996b, Morrison 1996). In some cases pathogen attack appears to increase the vegetative vigour of host plants (Bradshaw 1959, Catherall 1966, Clay 1984, Wennström and Ericson 1991), and the mechanisms behind such effects are not generally understood. In one study, a pathogen weakened the seed heads of the host, leading to accelerated germination and increased plant establishment (Eviner and Chapin 2003).

In perennial ryegrass, *Lolium perenne*, resistance to the pathogen *Puccinia coronata* is an important aspect of current breeding programmes (Dumsday *et al.* 2003). Will increased resistance increase the likelihood that these populations or genotypes will spread beyond the fields in which they are sown into natural and cultural landscapes?

L. perenne was intentionally introduced into Denmark in the late 1700, presumably for cultivation (Christiansen 1977). We investigated the effects of soil disturbance and disease and the introduction of two cultivars of *L. perenne* in field experiments in grassland communities, where the species was already either present or absent, and compared these with non-treated plots. In treated plots the soil was disturbed before seed addition, since disturbances are known to be of crucial importance for the establishment of plants from seeds in perennial vegetation (Crawley 1987, Burke and Grime 1996). Finally, spores of the biotrophic fungus, *Puccinia coronata*, were applied to pathogen-treated plots. The experimental set-up enabled us to evaluate 1) whether *L. perenne* is seed and microsite limited, 2) the effect of soil disturbances, 3) the effect of pathogen attack on two cultivars with different levels of susceptibility.

2. MATERIALS AND METHODS

2.1. Study organisms

Lolium perenne L. (perennial ryegrass) is a 20-50 cm tall, perennial turf grass. In Denmark, as in much of the world, it is among the most frequently sown fodder grasses and is also an important component of grass seed mixtures used to seed pastures for grazing, golf courses, lawns and roadsides (Pedersen 1974). It is commonly found naturalised in semi-natural grasslands and along roadsides in Denmark (Frederiksen 1981).

Crown rust, caused by the fungus *Puccinia coronata* f. sp. *lolii* (Corda), is one of the most important diseases of cultivated *L. perenne*. This form of the fungus only infects *L. perenne* and a few other closely related grasses (Eshed and Dinoor 1980). Uredospores formed by *P. coronata* f. sp. *lolii* repeatedly infect and re-infect *L. perenne* until the onset of winter (Gäuman 1959, Simons 1970). Long distance wind dispersal of uredospores is well known, but a steep inoculum gradient near the source is observed in plant canopies (Roelfs and Martell 1984). The fungus ruptures the leaf epidermis of the grass host plants, resulting in rust-coloured pustules. Infection has been reported to result in increased respiration and reduced photosynthesis (Lancashire and Latch 1966, Potter 1987, Plummer *et al.* 1990) and hence a highly reduced quality and quantity of leaves and seeds of attacked crops (Simons 1970).

We investigated introduction of a partially resistant (Fanda) and a susceptible cultivar (Meradonna) of *L. perenne* (DLF Trifolium, Store Heddinge, Denmark). We used a field isolate of *P. coronata*, collected as a bulk sample of uredospores of *P. coronata* from a *L. perenne* population at Store Heddinge, Denmark, in the fall of 1997, to mimic a likely scenario under natural conditions. Inoculum was produced on the susceptible *L. perenne* cultivar of which seeds were sown in peat in $5 \times 5 \times 5$ cm pots, and three weeks later inoculated with a spore-talcum-mixture (1:10) using a brush. The inoculated plants were incubated in dew chambers in the dark for 24 hours at 20°C and then moved back into the

greenhouse for another two weeks. Spores were regularly collected with a cyclone spore collector and stored in a dessicator at 5°C.

2.2. Design of field experiments

The experiments were set up in May 1998 at four permanent grassland sites situated 10-15 km south of Viborg, Denmark (Table 1): Hald (site I), Brattingsborg "up-slope" (site II), Brattingsborg "down-slope" (site III), and Dollerup (site IV). Site I and II had naturalised populations of *L. perenne* prior to the experiments, while the species was not found at sites III or IV. All sites had sandy soils and pH values between 5.4 and 5.6. Site IV was the most pristine site with no records of cultivation or grazing for at least 50 years. The other sites were cultivated up to the late 1980s and then turned into grassland. The experiments were set up in a split-block design (Kuehl 2000) with four blocks per site and 12 experimental plots (75 × 75 cm^2) per block. The plots were given factorial combinations of soil disturbance (+/- disturbance), seed addition (addition of a susceptible cultivar of *L. perenne*, addition of a partially resistant cultivar, or no seed addition) and pathogen exposure (+/- addition of pathogen). Thus there were 4 (sites) × 2 (soil disturbance) × 3 (seed addition) × 2 (pathogen exposure) × 4 (replicates) = 192 plots. Plots within a block were arranged in two rows separated by 4.5 m: one row with six pathogen-exposed plots and the other with six unexposed plots to

Table 1. Characteristics of the permanent grassland areas used as the experimental sites in the study.

Site	Location	N - P -K[1]	sand - silt - clay - humus[2]	Present management practices	Disturbances	Density of vegetation	Three most dominaant plant species	Abundance of *L. perenne* prior to the experiment
Site I	56°24'N, 9°21'E Adjacent to a forest	2.7 - 3.1 - 12.3	85.7 - 4.6 - 7.1 - 2.6	Grass is mowed annually in July No pesticides and fertilisers	People walking with dogs and horse riding	Medium	*Festuca rubra* *Taraxacum* spp. *Hypochoeris radicata*	Low
Site II	56°23'N, 9°21'E Same field as site III but upslope	3.1 - 3.1 - 15.7	89.9 - 3.2 - 4.3 - 2.6	Extensive cattle grazing No pesticides and fertilisers	Grazing cattle	Medium	*Lolium perenne* *Rumex acetosella* *Taraxacum* spp.	High
Site III	56°23'N, 9°21'E Same field as site II but downslope	5.1 - 2.5 - 12.3	91.2 - 2.8 - 5.9 - 2.1	Extensive cattle grazing No pesticides and fertilisers	Grazing cattle Molehills	Low	*Festuca rubra* *Plantago lanceolatum* *Rumex acetosella*	None
Site IV	56°22'N, 9°19'E Adjacent to a lake	5.2 - 1.2 - 5.6	80.9 - 7.8 - 7.4 - 3.9	None No pesticides and fertilisers	Molehills	High	*Poa pratensis* *Arrhenaterium elatior* *Elytrigia repens*	None

[1] Plant available N, P and K contents were determined from dried soil samples; nitrate-N (mg kg^{-1}), phosphate (mg $100g^{-1}$), potassium (mg $100g^{-1}$).
[2] The soil texture components are given in percent. Sand, silt and clay contents were determined by the particle size: sand: 2.00-0.02 mm; silt: 0.020-0.002 mm; clay: <0.002 mm.

minimize spread of spores of the airborne pathogen from the pathogen-exposed plots to the unexposed plots. Pathogen-exposed rows of neighbouring blocks were set up as mirror images. The blocks were separated by 1.5 m, and plots within rows by 1.0 m. The disturbance and seed addition treatments were randomly assigned to the plots.

The experimental areas were fenced to protect from damage from deer and cows. At sites II and III, the vegetation was cut with a grass trimmer and removed every autumn to reduce the build-up of plant litter in the absence of usual grazing. At site I, the grass was cut according to this practice in 1998 and 1999, but in 2000 cutting was carried out later than usual to assess seed production.

Disturbances were established in May 1998 before seed addition. Four large (25×25 cm^2) and four small (5×5 cm^2) disturbances were created in each of the disturbance-treated plots (approx. one third of the topsoil) by over-turning the soil to a depth of 20 cm in a specific pattern using a spade and a soil corer, respectively. Either no seeds or 200 seeds of the partially resistant or the susceptible cultivar were broadcast by hand onto the plots at a rate of 356 seeds m^{-2}.

The plots were exposed to *P. coronata* by transplanting one pot with infected, sporulating plants into the centre of each plot (n = 96) in May 1998. The procedure was repeated in May 1999 and 2000. There were no visual crown rust symptoms on the *L. perenne* individuals at the experimental sites at the onset of the experiment.

At the two sites (I and II) with an existing population of *L. perenne*, the point intercept method, based on the number of point contacts to plant leaves, was used to provide a non-destructive estimate of the biomass of *L. perenne* within a plot (Jonasson 1983, 1988). A 75×75 cm frame with 80 equally spaced 1.6 mm diameter needle positions was placed in each plot. In each position the needle was passed through the vegetation and the total number of hits of *L. perenne* leaves on each needle was recorded and summed up for all 80 positions within each plot. At the two remaining sites with no natural population of *L. perenne* (sites III and IV), the number of individual plants per plot was counted. At all four sites, the assessments were carried out twice a year (May and August) in 1998-2000. At all four sites, disease incidence was assessed in August 1999 and 2000, also using the point intercept method: The number of point contacts on rust-infected *L. perenne* leaves out of the total number of hits on *L. perenne* leaves was used to calculate disease incidence (percentage). Seeds were harvested and weighed in July 2000. Aboveground biomass was harvested at all plots in late August 2000, dried at 60°C for 48 hours, and the dry mass determined. The relationship between number of point contacts recorded in August 2000 and dry mass was analysed. Reproductive allocation was estimated as seed mass / total aboveground dry mass.

2.3. Test of cultivar susceptibility

A test was carried out to assess differences in growth and disease severity of the partially resistant and the susceptible cultivar of *L. perenne* when grown under uniform conditions in an experimental garden. Seeds of both cultivars (Fanda and Meradonna) were sown in May 1998 in four 60×60 cm wooden boxes. Germinating plants were thinned to 25 individuals per box, and eight weeks later, in July, two of the boxes of each cultivar were inoculated with uredospores of *P. coronata* as described above, the other two boxes were included as controls. Disease severity was estimated visually in August as the percentage of leaf area with symptoms, and all aboveground parts of the plants were harvested and the dry weights were determined as described above.

2.4. Statistical analyses

While the experiment was set up as a split-block experiment (Kuehl 2000), this design only allowed a weak test of the effect of pathogen addition due to the low number of degrees of freedom in the error term. There was no indication of larger differences among plots within rows than among plots between rows in the blocks for the variables studied, so we chose to analyse the data with a model for randomised complete blocks. Means of the square-root

transformed (to improve homogeneity of variance) number of point contacts (biomass estimate) over the course of the experiment, harvested dry leaf biomass and seed production were analyzed with general linear models with site, soil disturbance, seed addition, and pathogen exposure as fixed effects and block nested within site as a random effect. We analyzed the number of point contacts (biomass estimate) at each measurement with repeated measures MANOVA, with soil disturbance, seed addition, pathogen exposure, site and block nested within site as factors. While analyses were performed on transformed variables, we show untransformed variables in figures. Finally, we analyzed disease incidence with logistic regression on site, soil disturbance, seed addition, pathogen exposure and final total dry biomass. Establishment of *L. perenne* at site IV was extremely low and the site was not included in the statistical analyses. Establishment at site III was also quite low, and included many zero values. When the data from site III were consistent with the assumptions of an analysis, site III was included. In other cases, only data from sites II and I were analysed statistically. In one case (analysis of reproductive effort), inclusion of plots with no seeds added violated the assumptions, so we analyzed only plots to which seeds were added.

3. RESULTS

3.1. Test of cultivar susceptibility

The garden experiment testing the susceptibility of the two *L. perenne* cultivars to *P. coronata* resulted in a mean disease severity on the partially resistant (Fanda) and susceptible plants (Meradonna) of 3% and 19%, respectively. Crown rust was not found on unexposed plants. When not exposed to the pathogen, the partially resistant cultivar of *L. perenne* grew more vigorously (mean aboveground dry mass = 2.75 g; SE = 0.53) than the susceptible cultivar (1.57 g; SE = 0.02). When exposed to the pathogen, the resistant cultivar was less suppressed by attack of *P. coronata* (34% reduction in aboveground dry mass) than the susceptible cultivar (58% reduction in aboveground dry mass).

3.2. Relationship between point contacts (biomass estimate) and total dry mass at harvest

The slope of the regression of log (dry mass) at harvest in August 2000 on number of log (number of point contacts) just previous to harvest was not significantly different from 1. The regression accounted for 73% of the variation in log (dry mass) and the residuals were homogeneous, so point contacts can be considered a reasonably good estimate of biomass in this study.

3.3. Plant establishment and growth

At both of the experimental sites without a pre-existing *L. perenne* population (site III and IV), seedlings of *L. perenne* established from sown seeds. Establishment success and the subsequent seedling mortality were site-specific. At site III, assessments in August 1998 showed seedling recruitment in 38% of the disturbed plots and 25% of the undisturbed plots. At site IV, seedling establishment occurred in 69% and 0% of disturbed and undisturbed plots, respectively. At site IV, only two plants survived to 1999, the second growing season, so all further experimental activities were stopped at this site. The small, established population of *L. perenne* persisted at site III, but there were no apparent effects of the pathogen or disturbance treatments. Assessments made in August 2000 at the end of the experimental period showed that a total of 54 plants had established in the disturbed and 45 in the undisturbed plots after seed addition.

Aboveground dry mass (square-root transformed) of *L. perenne* determined at the end of the experiment in 2000 was similar at sites I and II, but significantly lower at site III (Fig. 1). The aboveground dry mass was significantly higher when plots were exposed to the pathogen ($F = 6.16$, $P = 0.02$), while soil disturbance, seed addition and treatment interactions had no significant effects.

Repeated measurements analysis of the number of point contacts (square-root transformed) over the course of the experiment gave significant effects of site, block and a positive effect on biomass of pathogen exposure (Table 2, Fig. 2).

Table 2. Repeated measures MANOVA of the effects of site, soil disturbance, pathogen exposure and seed addition on number of *L. perenne* point contacts (biomass estimate, square-root transformed) over 3 years. For treatments – see Fig. 1.

Factor	DF	F	*P*
Site	1	232.37	<.0001
Block (within site)	6	4.76	0.0003
Soil disturbance	1	0.002	0.9665
Pathogen exposure	1	10.99	0.0014
Seed addition	2	0.28	0.7570

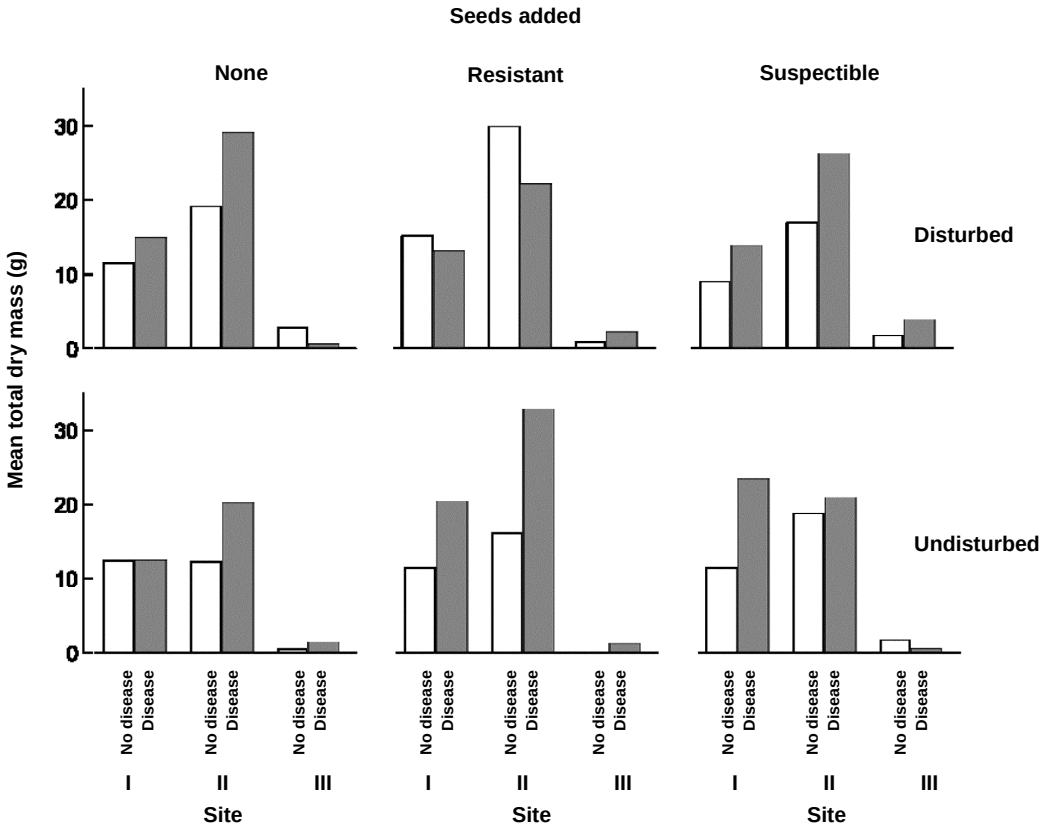

Fig. 1. Total aboveground dry mass of *Lolium perenne* per plot harvested at three sites at the end of the experiment (August 2000).

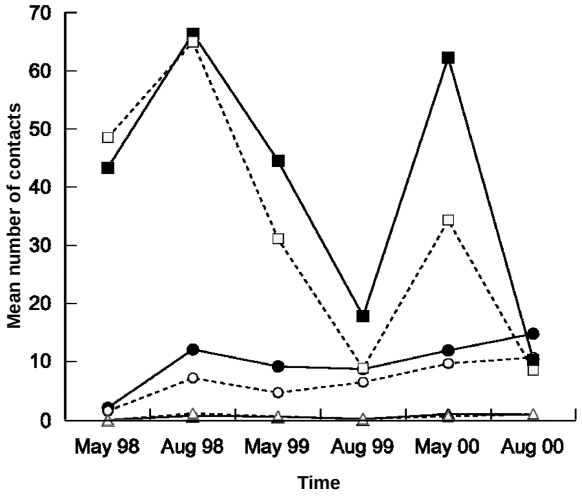

Fig. 2. Mean number of point contacts (biomass estimate) with *Lolium perenne* leaves per plot at three sites (Table 1) over the course of the experiment. Site I: circles; Site II: squares; Site III: triangles; exposed to *Puccinia coronata*: filled symbols and solid lines; not exposed to the pathogen: open symbols and dashed lines.

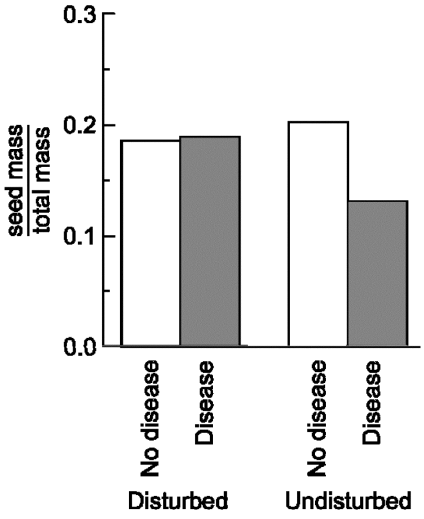

Fig. 3. Mean reproductive allocation (seed mass / total dry mass) with and without soil disturbance and pathogen exposure when seeds were added. The interaction between soil disturbance and pathogen exposure on reproductive allocation (log transformed) is significant (P <0.02).

3.4. Plant reproduction

Seed production by *L. perenne*, estimated by dry mass, was only affected by site (Table 3). The seed dry mass was more than three times higher at site II relative to site I (site II = 4.99 g; SE = 0.544, n = 48; site I = 1.478 g; SE = 0.148, n = 47). When seeds were added, reproductive allocation was significantly affected by site, seed addition and the interaction between soil disturbance and pathogen exposure (Table 3). Reproductive allocation was not affected by disease exposure in disturbed plots, but decreased in the presence of disease in undisturbed plots (Fig. 3).

3.5. Presence of disease

The spread of the pathogen from pathogen-exposed to unexposed plots was very limited. No needles touched infected leaves in the unexposed plots at site I and II in 1999. In 2000, mean disease incidence in plots without pathogen exposure was 1% at site I and II. Only exposure to the pathogen had an effect in a logistic regression of the presence of disease on point-contacted leaves in either year (Table 4). In 2000, disease incidence was highest on *L. perenne* in disturbed vegetation at site II while there was no significant difference in disease incidence

in disturbed and undisturbed vegetation at site I (Fig. 3).

None of the few plants established at site IV became infected by *P. coronata*. At site III, few diseased leaves were observed in pathogen-exposed plots, but none were sampled by point contacts. We were therefore not able to investigate how a difference in cultivar susceptibility to *P. coronata* would affect disease development in small host populations. The importance of host susceptibility could likewise not be established in the two naturalised populations (site I and II) due to the lack of effect of seed addition (no seed vs. seed added, susceptible cultivar vs. partially resistant cultivar) on disease incidence or shoot growth. The survival of the pathogen from one season to the next was low at all four sites. Small pustules were observed in few plots at site I and II in the spring of 2000.

4. DISCUSSION

4.1. Seed and microsite limitation of *L. perenne* in grasslands

Addition of *L. perenne* seeds to grassland sites without an established population of the species (site III and IV) resulted in seedling establishment, suggesting that dispersal-limitation is a likely explanation for the absence of the species. At site III, the population persisted throughout the experimental period. At site IV, however, vegetative spread of competitive, perennial grasses from the undisturbed surrounding vegetation eliminated the gaps of disturbed soil and appeared to out-compete the *L. perenne* seedlings, which died before reaching the adult life stage. Failure of seedling establishment is commonly seen in established perennial vegetation due to the strong asymmetric competition between adult plants and seedlings (Fenner 1978, Fowler 1986). This may in particular be an important factor for establishment of cultivated plants in natural ecosystems, because their competitive ability may become reduced during breeding programs, as has been shown for wheat (Vandeleur and Gill 2004).

At the two sites with well-established *L. perenne* populations (site I and II), seed production was close to zero for a number of years prior to the experiment due to the management practices. At site II, grazing severely reduced the number of mature spikes of *L. perenne,* and at site I most spikes were cut before the seeds reached maturity. Experimental seed addition did not result in increased biomass, indicating that the maintenance of these well-established populations were largely independent of sexual reproduction. This is in accordance with previous findings revealing that most grassland populations are maintained by vegetative propagation, with episodic recruitment from seeds (Lauenroth *et al.* 1994).

4.2. Effect of soil disturbance on *L. perenne* performance

Shoot biomass of *L. perenne* populations at site I and II was positively influenced by soil disturbance in the first year of the experiment, presumably because of increased growth following the release of limiting resources. The increased growth was a result of growth of established plants rather than recruitment of seeds from the seed bank since very few seedlings of *L. perenne* were observed, even in the gaps of disturbed soil. Unlike the pathogen treatment, which was applied annually, soil disturbance was only created at the onset of the experiment. Overall, disturbance had no effect on the shoot biomass of *L. perenne* measured three years after the soil was disturbed or averaged over the whole course of the experiment.

4.3. Effect of disease on plant performance

In agreement with earlier studies on other host-pathogen systems (Burdon and Chilvers 1982, García-Guzmán *et al.* 1996a), we observed an increase in plant biomass with disease incidence ($P < 0.05$; Table 3). Spores of *P. coronata* are passively dispersed by wind, and if the host biomass is decreased, a larger proportion of the spores will land on non-susceptible plant tissue and perish. In natural plant communities, where many of the plant species have a stature that is different from that of *L. perenne*, the sizes and shapes of neighbouring non-host plants also play an important role for inoculum dis-

Table 3. F and P values for analysis of variance on various dry mass components of *L. perenne*. Results of reduced models containing main effects as well as interactions for which P <0.10 are shown. Because of the large number of zero values at site III, only site I and II are analyzed. Analysis of seed mass/total aboveground dry mass was restricted to plots with seed addition.

Dependent Variable	Site	Pathogen exposure	Seed addition	Soil disturbance	Pathogen × soil disturbance
Total dry mass (log transformed)	$F_{(1, 6)} = 1.22$ $P = 0.31$	$F_{(1, 81)} = 6.16$ $P = 0.02$	$F_{(2, 81)} = 0.30$ $P = 0.74$	$F_{(1, 81)} < 0.01$ $P = 0.98$	n.s.
Seed mass (log transformed)	$F_{(1, 6)} = 10.53$ $P < 0.02$	$F_{(1, 83)} = 2.75$ $P = 0.10$	$F_{(2, 83)} = 0.12$ $P = 0.89$	$F_{(1, 83)} = 0.32$ $P = 0.57$	n.s.
Reproductive allocation: Seed mass / total dry mass (log transformed)	$F_{(1, 6)} = 10.89$ $P<0.02$	$F_{(1, 51)} = 3.49$ $P = 0.07$	$F_{(1, 51)} = 4.46$ $P = 0.04$	$F_{(1, 51)} = 2.08$ $P = 0.16$	$F_{(1, 51)} = 6.23$ $P < 0.02$

Table 4. Logistic regression of disease incidence on *L. perenne* leaves sampled by contact points (point intercept method), on site (Table 1), soil disturbance, pathogen exposure and seed addition.

Factor	DF	Wald χ^2	*P*
Site	2	3.63	0.163
Soil disturbance	1	0.02	0.878
Pathogen exposure	1	29.79	<0.0001
Seed addition	2	2.26	0.323

semination, as large plants may act as physical barriers for the dispersal of inoculum (Chin and Wolfe 1984, Morrison 1996). Differences in the height of the co-occurring vegetation may for instance explain the site-specific relationship between host biomass and disease incidence observed in this study. Other possible explanations include differences in the susceptibility of the host populations, and differences in the local environment that affect the development of the pathogen, for example humidity or wind-exposure.

In agricultural production systems *P. coronata* can be a major problem in *L. perenne* crops and growth and reproduction is negatively affected by attack (Lancashire and Latch 1966, Potter 1987, Plummer *et al.* 1990). This is consistent with results from our garden experiment. In more natural plant communities, however, *P. coronata* can influence the growth and thus the competitive ability of *L. perenne* quite differently: In our field experiment plants in the pathogen exposed plots at site I and II grew more vigorously than unexposed plants, and this result was supported by all our analyses. Evidence of enhanced growth mediated by attack of pathogenic organisms has been reported in other cases (Bradshaw 1959, Catherall 1966, Clay 1984, Wennström and Ericson 1991). Another study of *L. perenne* also showed that some plant cultivars produced more tillers when they were infected with the barley yellow dwarf virus compared to healthy individuals (Catherall 1966, Catherall and Parry 1987).

Why does *P. coronata* affect the shoot biomass of *L. perenne* negatively when it is a crop but positively in more natural systems? There are several possible explanations, which can serve as hypotheses in future studies:

1) Changes in allocation. The increase in leaf biomass we observed in the presence

of disease could reflect a change in allocation. Pathogen attack may lead to a change in the allocation patterns of plants, so that more biomass is invested in leaves and less in roots or seeds (Bradshaw 1959, Clay 1984, Catherall and Parry 1987, Wennström and Ericson 1991). While this possibility cannot be excluded, it seems unlikely, since disease also had a positive effect on seed production in our plots, and root biomass is probably as important as shoot biomass for reproduction.

2) Indirect effects. The performance of a plant population in the field is a function of many interacting factors. While disease may have negative direct effects, indirect effects can be positive. *P. coronata* does not infect a wide range of species, so competition-mediated effects are unlikely to explain the improved performance of *L. perenne* in the presence of the disease. Infection by *P. coronata* has been shown to affect the allelopathic effects of *L. perenne* (Mattner and Parbery 2001). Uninfected plants of *L. perenne* release allelochemicals into the soil (Takahashi *et al.* 1988, Takahashi *et al.* 1991) but the allelopathic potential is lost as the plants age (Mattner and Parbery 2001). Infected plants have a prolonged allelopathic potential and an enhanced allelopathic effect (Mattner and Parbery 2001). When clover was grown in soil in which rust-infected *L. perenne* plants had grown, the biomass of clover was 36% lower than when it was grown in soil that previously had healthy *L. perenne* plants. Similarly, clover biomass was reduced by 27% when watered with leachate from soil with diseased, compared to healthy, *L. perenne* plants. This could be an explanation for differences in the vigour and competitive ability of rust infected *L. perenne* grown in agricultural systems, where most or all of the plants are conspecifics, and in more natural multi-species communities. The ability to release allelochemicals into the soil and thereby negatively affect the growth and recruitment of neighbouring plants has been demonstrated for other non-indigenous plants and may have important implications for their invasion potential in natural communities (Wardle *et al.* 1998, Goslee *et al.* 2001, Hierro and Callaway 2003, Callaway *et al.* 2004).

Another uncontrolled factor in our study, which may have influenced the results, is infection of *L. perenne* by endophytes such as *Neotyphodium lolii*. Under controlled conditions, plant growth and photosynthesis was independent of endophyte concentration (Spiering *et al.* 2006). However, endophyte concentrations may affect insect herbivores, (Krauss *et al.* 2007) and pathogens. Very little is known about endophyte-pathogen interactions (Faeth 2002), but in one study *Acremonium lolii*-infected *L. perenne* plants were more resistant to *P. coronata* than uninfected plants (Clay 1990).

3) Overcompensation. There is evidence that plants sometimes respond positively to low levels of herbivory (Paige 1999, Agrawal 2000). If this is the case, the same may be true for disease attacks. This could also explain the difference between production and more natural systems if disease severity is higher in the former.

Our results suggest that there are important differences in the effects of disease on *L. perenne* in production systems *versus* in more natural communities. In production and in our test of cultivar susceptibility, the partially resistant cultivar suffers less from disease, but in a more natural plant community, where plant density of one species normally is much lower than in production systems, there was no detectable difference in the performance of these 2 cultivars, even in analyses restricted to plots with seeds added.

We have shown that a biotrophic fungus can have positive effects on the host plant in semi-natural plant communities, at least in the short term, even though it has negative effects in more intensively managed production systems. The role of disease in more complex and variable natural ecosystems may be strongly influenced by multiple indirect effects. Generally, *P. coronata* had relatively small effects on the growth and fitness of *L. perenne* and the effect appeared to be reduced over time. This suggests that the fungus may not influence the longer-term dynamics of naturalised populations of *L. perenne* to a great extent.

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