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LETTER

Pathogens promote plant diversity through a compensatory response

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Abstract

Pathogens are thought to promote diversity in plant communities by preventing competitive exclusion. Previous studies have focussed primarily on single-plant, single-pathogen interactions, yet the interactions between multiple pathogens and multiple hosts may have non-additive impacts on plant community composition. Here, we report that both a bacterial and a fungal pathogen maintained the diversity of a four-species plant community across five generations; however, significant interactions between the pathogens resulted in less plant diversity when the two pathogens were present than when the fungal pathogen was present alone. Standard models predict that pathogens will maintain plant diversity when they cause a disproportionate loss of fitness in the dominant plant species. In our experiment, however, pathogens maintained plant diversity because the rare species produced more seeds through a compensatory response to pathogen infection. Finally, we found that the influence of pathogens on maintaining plant diversity was 5.5 times greater than the influence of nutrient resource heterogeneity. Pathogens may be a major factor in maintaining plant diversity, and our findings emphasize the importance of investigating the roles of pathogens in natural plant communities.

Keywords

Brassica, compensatory response, model systems, plant community composition, plant–pathogen interactions, species diversity.

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INTRODUCTION

Theoretical models, field observations and experiments suggest several ways in which pathogens can promote diversity in plant communities by preventing competitive exclusion (Gillet 1962; Janzen 1970; Connell 1971; Chilvers & Brittain 1972; Burdon & Chilvers 1977; Augspurger 1983; Gilbert *et al.* 1994; Gilbert 2002; Bell *et al.* 2006). First, if pathogens are specialists and attack their hosts in a density-dependent manner, then rare species should increase in relative frequency (Gillet 1962; Janzen 1970; Connell 1971). Patterns of pathogen infection and tree distributions observed in both tropical and temperate forests support these predictions (Augspurger 1983; Gilbert *et al.* 1994; Wills *et al.* 1997; Packer & Clay 2000; Bell *et al.* 2006). Second, pathogens may promote species coexistence if there is a trade-off between a plant's competitive ability and its susceptibility to pathogen infection (Gillet 1962; Chilvers & Brittain 1972; Levin *et al.* 1977; Burdon & Chilvers 1982; Ayres & Paul 1990). Indeed, empirical studies reveal that pathogens can alter the outcome of competitive interactions and even allow species to coexist that could not in the absence of the pathogen (Burdon & Chilvers 1977; Ayres & Paul 1990; Alexander & Holt 1998). Finally, field and greenhouse experiments suggest that host-specific shifts in soil pathogen composition can generate negative feedbacks that differentially increase the survival of rarer plant species (Van der Putten & Peters 1997; Klironomos 2002; Bever 2003).

In natural plant communities, multiple plant species compete for resources and are simultaneously challenged by numerous pathogens. Despite evidence that pathogens can maintain plant diversity, experiments are lacking that evaluate the effects of *pathogen* diversity; multiple pathogens could have additive or multiplicative effects, or interfere with one another. We performed an experiment with multiple host species and multiple pathogens to test whether interactions between pathogens and among hosts, as well as

direct impacts of pathogens on their hosts, affect plant diversity over numerous plant generations.

There are many reasons to expect that plant–pathogen and pathogen–pathogen interactions could be important and have complex, non-additive effects on plant community composition. For example, pathogens may have cross-generational effects on plants that would not be detected in a typical one-generation experiment; diseased plants can produce inferior seeds thereby reducing the fitness of progeny (Jarosz *et al.* 1989), or induced defenses may be transmitted to offspring (Agrawal *et al.* 1999). Additionally, physiological responses by the host, such as reproductive overcompensation that can offset losses to mortality, could counteract pathogen impacts (Friess & Maillet 1997; Alexander & Holt 1998; Alexander & Mihail 2000) and may be influenced by the host's competitive environment (Korves & Bergelson 2003, 2004). Synergistic or competitive interactions between multiple pathogens can have positive or negative effects on plant fitness (Fernando *et al.* 1994; Morris *et al.* 2007). Finally, the fitness impacts of a pathogen on a host plant will likely differ depending on the competitive environment of the host (Lively *et al.* 1995).

In the same experiment, we evaluate the relative impacts and interactions between top–down pathogen impacts and bottom–up competitive effects by testing the hypothesis that the influence of pathogens on plant diversity is equal to or greater than the influence of nutrient resource heterogeneity. Perhaps more than any other ecological factor, the spatial heterogeneity of nutrient resources (Tilman 1982) is invoked to explain the maintenance of plant diversity (Miller *et al.* 2005). Evaluating the relative contribution of pathogens and nutrient heterogeneity, or interactions between the two, is critical in understanding how plant diversity is maintained in natural communities.

To address these questions, we used mesocosm communities consisting of four rapid-cycling *Brassica* species (*B. juncea*, *B. napus*, *B. nigra* and *B. rapa*). Each mesocosm was initiated with an equal number of seeds of the four plant species and exposed to a pathogen treatment (control, a fungal pathogen [*Alternaria brassicicola*], a bacterial pathogen [*Xanthomonas campestris*] or co-inoculated with both). In addition, each of these four pathogen treatments was grown on two resource treatments (homogenous or heterogeneous). Each community was followed for five generations.

Although it would be ideal to ask these questions in a naturally occurring community, a mesocosm approach follows in the tradition of using model systems to elucidate the key ecological principles, such as competitive exclusion (Gause 1934), predator–prey dynamics (Luckinbill 1973) and diversity–ecosystem function relationships (Tilman & Downing 1994). It is simply not feasible to finely manipulate pathogen diversity in the field, adding particular pathogens while preventing further infection by all other viruses, bacteria and fungi. The use of rapid-cycling plant species also allows us to consider the community-wide effects across five plant generations. At the same time, the composition of the mesocosms are relevant to field communities; *Brassica* species co-occur in nature along with other closely related mustard species such as *Raphanus* and *Barbarea* (Magee & Ahles 1999), and both pathogens, *Alternaria* and *Xanthomonas*, are widespread and cause economically important crop diseases (Williams & Hill 1986).

Our multi-generational community experiment yields a number of results that are not predicted by simpler models and experiments. We found evidence that while each pathogen helped maintain plant diversity, interactions between the two pathogens had unexpected interactive effects on diversity. Moreover, plant diversity increased in the presence of pathogens because the rare species produce more seeds through a compensatory response to pathogen infection, rather than through reduced seed set of the numerically dominant species. Finally, we found that the influence of pathogens on plant diversity in our experiment was 5.5 times greater than the influence of nutrient resource heterogeneity, suggesting that the role of pathogens in natural communities requires much further investigation.

MATERIALS AND METHODS

Mixed-species mesocosms

We established 48 replicate mixed-species plant community mesocosms (six replicates per treatment) consisting of four *Brassica* species: *B. juncea*, *B. napus*, *B. nigra* and *B. rapa*. These rapid-cycling species have been developed and maintained by the Crucifer Genetics Cooperative (University of Wisconsin, Madison, WI, USA) (Williams & Hill 1986) and were obtained from Wisconsin Fast Plants® (University of Wisconsin, Madison, WI, USA). Under standard growing conditions, the populations flower within 30 days and set seed in 60 days or less, and the variation in growth forms and reproductive timing among the species is well documented (Williams & Hill 1986).

Seeds of all four species were randomly planted in alternate cells of 8 × 8 divided-cell flats (25 × 25 × 10 cm) and grown in a walk-in growth chamber at constant temperature (26 °C), humidity (65%) and day-length (16 h). For the first generation, 92 seeds were planted randomly into each flat, with 3–4 seeds per cell (20 seeds of *B. rapa*, and 24 seeds of *B. juncea*, *B. napus* and *B. nigra*). Plants were hand-pollinated with pollen from haphazardly selected conspecific individuals within the same flat to avoid the transmission of pathogens among flats. Mature seeds were harvested, counted and weighed. New generations of each mesocosm were sown with seeds produced by that mesocosm in proportion to the seed set of each species in the

previous generation. After the first generation (generations 2 through 5), we randomly planted 200 seeds produced from the previous generation into each flat, with 7–8 seeds per cell. Fewer seeds were planted in the first generation because stock seeds have unusually high germination rates (98–99%). Each generation, from seed planting to seed harvesting, lasted 56 days, and the five generations were completed between November 2004 and December 2005.

Nutrient heterogeneity

Half of the communities were grown in a homogenous resource environment, and half in a heterogeneous resource environment. We added two types of slow-release fertilizers (Pursell Polyon™ Agrium Advanced Technologies, Sylacauga, AL, USA) to each cell within a flat: 12-0-42 (hereafter NK) and 10-48-0 (hereafter NP). The total amount of nutrients applied in each treatment was held constant (0.125 g per cell). For the homogenous treatment, a 50 : 50 mixture of the NK and NP fertilizers were added to each cell within a flat. In the heterogeneous treatment, the fertilizers were applied in different ratios and different clusters across the flat cells. The four clusters in each flat consisted of the following ratio of resources: 100NP : 0NK, 66NP : 33NK, 33NP : 66NK and 0NP : 100NK. Seeds were replanted into new flats with the same fertilizer pattern in each generation to maintain the resource ratio patterns across generations. This particular fertilizer pattern was chosen to represent a high degree of resource heterogeneity, as may be found in natural systems. In fact, our experimental design may exaggerate the degree of heterogeneity in individual plants experience because they are not able to forage across the mesocosm for more favourable nutrient patches.

To confirm that nutrient levels were enriched throughout a generation, we analysed the concentration of ammonium, phosphate and nitrate in soils treated with the slow-release fertilizer. We added 0.125 g of NP or NK fertilizer into 14 replicate cells that had no plants. After 56 days (the length of one plant generation), we collected 1 g of soil from each replicate cell, sifted the soil through a 1-mm mesh and extracted it in 2 M KCL (nitrate and ammonium) and 0.5 M $NaCOH_3$ (phosphate) for subsequent spectrophometric analysis performed with Hach™ colorimetric kits (Hach Company, Loveland, CO, USA). From these values, we calculated the average nutrient concentration across all cells in a mesocosm to be 0.22 ± 0.06 mg g^{-1} ammonium, 0.11 ± 0.03 mg g^{-1} nitrate and 0.02 ± 0.004 mg g^{-1} phosphate. Next, we used these values to estimate the nutrient levels in each cluster within a heterogeneity mesocosm (100NP : 0NK, 66NP : 33NK, 33NP : 66NK and 0NP : 100NK) in order to estimate the degree of resource heterogeneity within a community. As such, the spatial arrangement of these nutrients in the heterogeneity mesocosms varied among cells by an average factor of 1.5, 1.8 and 2 for phosphate, nitrate and ammonium, respectively.

Pathogens

Because of their agricultural and economic importance, the suite of pathogens associated with *Brassica* spp. has been well studied. We chose two pathogens for use in this system: the fungal pathogen, *A. brassicicola*, which causes dark leaf spot of crucifers, and the bacterial pathogen, *X. campestris* pv. *campestris*, which causes black rot of crucifers. Single cell and spore isolates were obtained from infected cabbages in New York (USDA permit No. 52879 to JBHM). In inoculations of each of the *Brassica* spp. grown individually (see description below), symptoms were observed in 61% of plants inoculated with *Alternaria*, and this pathogen infected an average of 23% (±3%) of leaf tissue. Similarly, 56% of plants inoculated with *Xanthomonas* expressed symptoms, and lesions were visible on 17.5% (±2%) of leaf area. Furthermore, each pathogen had differential impacts on the four species ($F_{3,15} = 10.1$, $P < 0.0001$), such that 61% of *B. rapa* plants were infected, compared with 47% of *B. juncea*, 44% of *B. nigra* and 39% of *B. napus*. Both pathogens are dispersed through seed and air and by splashing, most likely over a distance greater than the 25 cm width of our mesocosms. Thus, our experimental design mimics the case where pathogen spread is operating on a larger scale than our mesocosms.

Each community was inoculated with one of the following pathogen treatments: no pathogens, one fungal pathogen (*A. brassicicola*), one bacterial pathogen (*X. campestris*), or both *A. brassicicola* and *X. campestris*. In each generation, all communities were inoculated 12 days after planting, when plants were at the two to three leaf stages. Leaves of each plant were pin-pricked immediately prior to inoculation to facilitate pathogen penetration and infection. Plants were sprayed, until runoff, with a fungal spore suspension of 5.0×10^4 spores mL^{-1} and/or a 10^4 cells mL^{-1} solution of bacterial cells. After inoculation, the flats were held in plastic bags with wet paper towels to maintain moist conditions for 24 h. Both fungal and bacterial lesions were visible on leaves as early as four days post-inoculation.

The number of plant belonging to each species, as well as the number of plants showing symptoms, was assessed visually 12 days after inoculation for each mesocosm. After each generation, both pathogens were re-isolated from symptomatic leaves to confirm the cause of disease.

Single-species mesocosms

Additionally, we performed single-species inoculations to determine the effect of each pathogen treatment on each

plant species in the absence of neighbours. Single plants of each of the four plant species were grown and maintained in a manner consistent with that of the mixed mesocosm communities (in a walk-in growth chamber at 26 °C, 65% humidity and 16-h day-length), in flats with 25 × 25 × 10 cm cells, with each cell containing only one plant and no plants in neighbouring cells. Plants were grown in soil with an equal mix of NP and NK fertilizer (see above). Fifteen replicates of each plant species were inoculated as described above with one of the four pathogen treatments (*A. brassicicola*, *X. campestris*, both *A. brassicicola* and *X. campestris*, or a control), for a total of 240 plants. Plants were hand pollinated and seeds were collected as described above.

Statistical analyses

Species diversity was calculated from plant abundance data using the Shannon Diversity Index, $H' = -\sum p_i \ln p_i$, where p_i is the abundance of the ith species divided by total abundance. This index reflects the combined influence of richness and evenness (Shannon & Weaver 1949; Stirling & Wilsey 2001; Magurran 2004) and has been used in numerous experiments to evaluate the changes in diversity (Harms *et al.* 2000; Worm *et al.* 2002; Magurran 2004). We performed a three-way ANOVA on H' summed over the five generations, testing the separate and interactive effects of *Alternaria* presence, *Xanthomonas* presence and nutrient heterogeneity. To determine the relative importance of these three factors, we partitioned the variance from the three-way ANOVA on H' (Gotelli & Ellison 2004). To separately account for generation time, we used a three-way repeated measure MANOVA on H'. We performed the same analyses on species richness, and because the results were similar, H' is used for all subsequent analyses. We also used a three-way ANOVA to test the separate and interactive effects of pathogens, nutrient heterogeneity and species

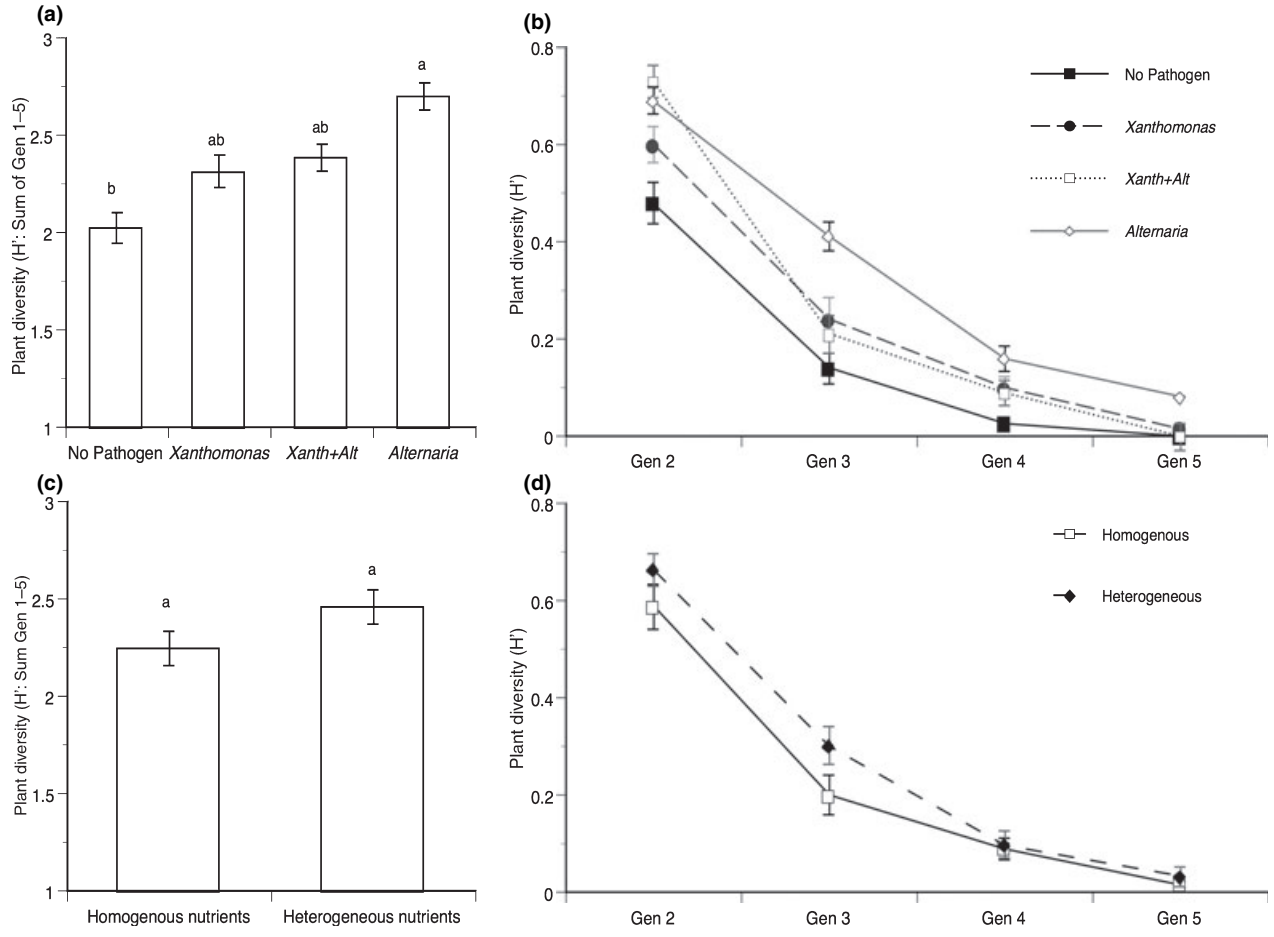

Figure 1 The presence of pathogens maintained plant diversity over multiple generations. (a) and (c) Sum of diversity (H') in generations 1 through 5. Values with different letters are significantly different at the $P < 0.05$ level. (b) and (d) Change in plant diversity over time. Error bars depict ±1 standard error.

identity on the number of seeds produced per plant, weight per seed and the proportion of plants infected, averaged over time. Where significant differences were found with ANOVA, we tested the difference between means using Tukey's test.

RESULTS

The presence of pathogens in the mesocosm communities maintained greater plant richness ($F_{7,47} = 4.88$, $P = 0.0005$) and diversity (H') over the five generations compared with communities grown in the absence of disease (Fig. 1; $F_{7,47} = 3.75$, $P = 0.0033$). Because the total number of seeds replanted in each generation was kept constant, and the plant species differ in their average seed set, diversity (both richness and evenness) necessarily decreases over the generations in all treatments. Thus, pathogens maintained species diversity by slowing the rate at which the three rarer species went extinct (Fig. 2; $F_{31,191} = 49.4$, $P < 0.0001$); both *B. rapa* and *B. napus* took a significantly longer time to go extinct in communities infected with the fungal pathogen than in communities that were not exposed to pathogens (Fig. 2; *B. napus*: $F_{1,160} = 55.6$, $P < 0.0001$; *B. rapa*: $F_{1,160} = 11.49$, $P = 0.0009$).

Interactions between the two pathogens also influenced plant diversity. Each of the pathogens independently prevented the loss of species diversity, although the fungal pathogen maintained a higher level of plant diversity than the bacterial pathogen (Fig. 1a; *Alternaria*: $F_{1,40} = 20.2$, $P < 0.0001$; *Xanthomonas*: $F_{1,40} = 3.7$, $P = 0.06$). However, communities co-inoculated with the two pathogens were not more diverse than those exposed to just one pathogen (Fig. 1a; $F_{1,40} = 0.9$, $P = 0.35$). Instead, plant diversity in the two-pathogen communities was maintained at a level intermediate to the communities inoculated with *Xanthomonas* or *Alternaria* alone (Fig. 1a; $F_{1,40} = 8.15$, $P = 0.007$).

In addition, the influence of pathogens on plant diversity changed over time (Fig. 1b; time × *Alt*: $F_{4,37} = 4.36$, $P = 0.005$; time × *Xc*: $F_{4,37} = 2.01$, $P = 0.11$; time × *Alt* × *Xc*: $F_{4,37} = 5.76$, $P = 0.001$). The ranking of diversity among the communities after one generation of exposure to pathogens (generation 2) did not predict the order of diversity among treatments in generations 3 through 5 (Fig. 1b).

What was the mechanism behind the pathogen effect on plant diversity? *B. juncea*, the species that consistently became dominant in the mesocosms (on an average over the five generations, representing 84% of a community), showed greater disease symptoms than did rarer species (Fig. 3; $F_{3,191} = 6.26$, $P = 0.0005$). The proportion of plants expressing symptoms in a mesocosm ranged from 28 (*B. rapa* plants infected with *Xanthomonas*) to 64% (*B. juncea* plants infected with *Alternaria*) (Fig. 3). It is often assumed that if pathogens preferentially infect the dominant plant species, then these plants will suffer a greater cost of infection (e.g. lower seed set and/or seed weight) (Gillet 1962; Burdon & Chilvers 1982; Ayres & Paul 1990). As expected, pathogens had a significant effect on both the average number of seeds produced per plant ($F_{3,191} = 3.9$, $P = 0.01$) and seed weight ($F_{3,191} = 2.1$, $P = 0.001$), and the influence of pathogens on seed set differed among the four plant species (Fig. 4; species: $F_{3,191} = 287$, $P < 0.0001$; species × pathogen: $F_{9,191} = 1.97$, $P = 0.046$) .

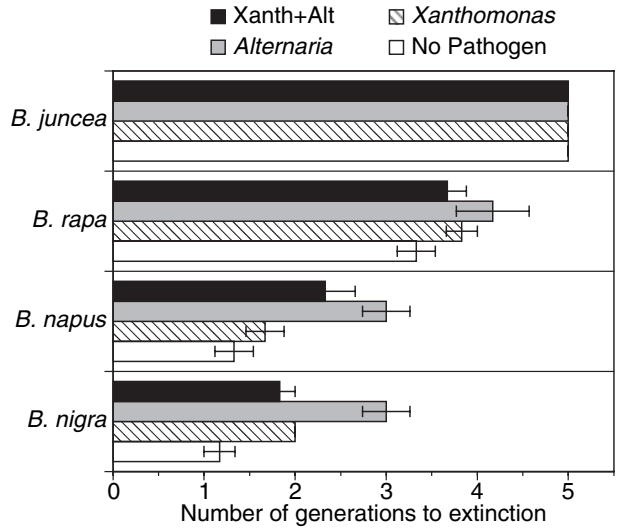

Figure 2 Mean number of generations before a species goes extinct from a mesocosm community. Pathogen treatments are ordered on the *y*-axis by increasing plant diversity. Error bars depict ±1 standard error.

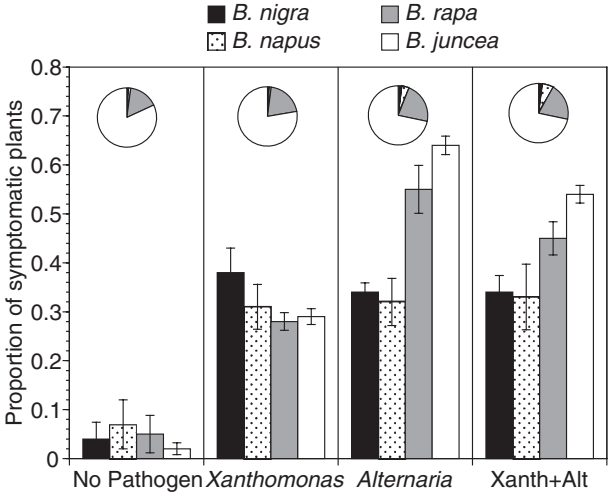

Figure 3 Mean proportion of plants showing disease symptoms over four generations. Inset pie charts represent the average abundance of each of the four plant species in generation 2, in each pathogen treatment. Error bars depict ±1 standard error.

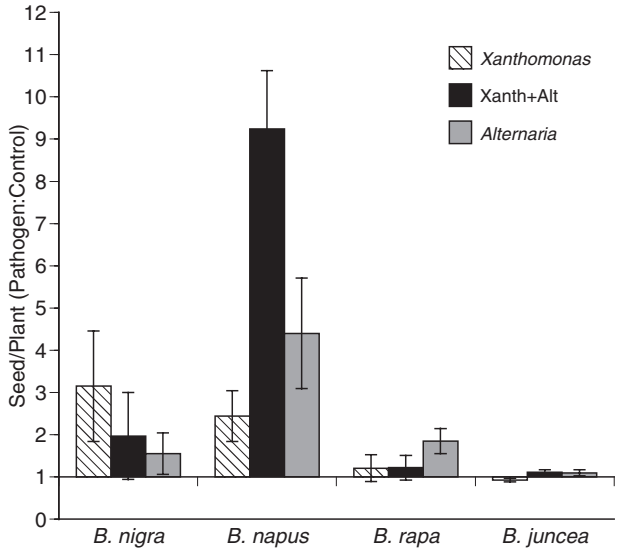

Figure 4 Average number of seeds produced per plant in pathogen vs. no pathogen treatments. The ratios of seeds produced in communities exposed to pathogens (*Alternaria*, *Xanthomonas* + *Alternaria*, or *Xanthomonas*) vs. no pathogens in the mixed-mesocosm experiment. Error bars depict ±1 standard error.

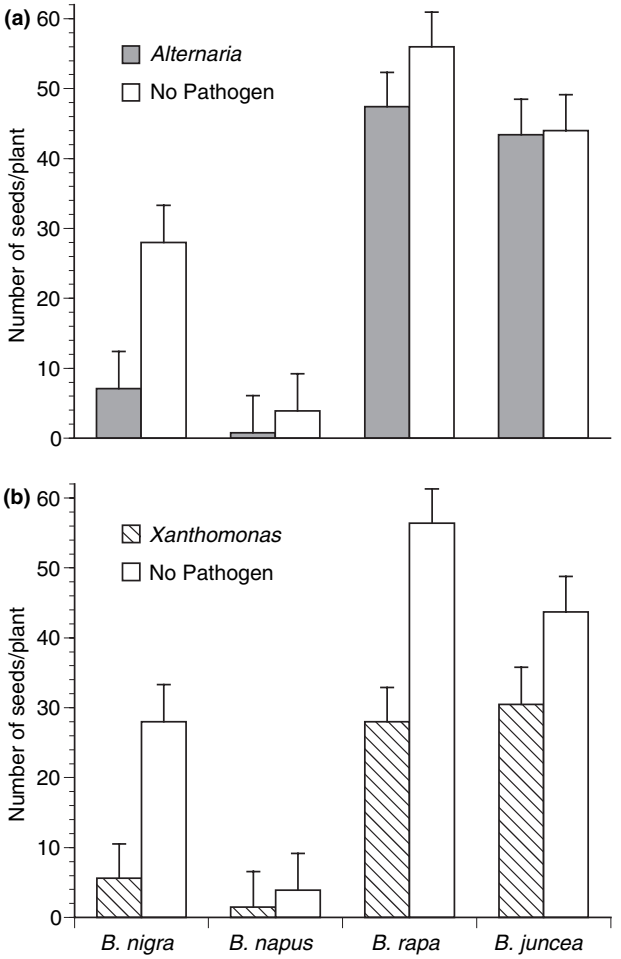

Figure 5 Mean number of seeds produced per plant in single-species mesocosms where individual plants were inoculated with either (a) *Alternaria* or (b) *Xanthomonas* or a no pathogen control. Error bars depict ±1 standard error.

Unexpectedly, however, the dominant species, *B. juncea*, produced the same amount of seeds whether or not it was exposed to pathogens ($F_{1,160} = 0.02$, $P < 0.90$), whereas two of the rarer species, *B. rapa* and *B. napus*, produced significantly more seeds per plant when exposed to pathogens (Fig. 4; *B. rapa*: $F_{1,160} = 4.5$, $P = 0.03$; *B. napus*: $F_{1,160} = 7.3$, $P = 0.007$). Similarly, seeds produced by plants exposed to pathogens were heavier than non-inoculated plants ($F_{1,191}$, $P = 0.02$). This compensatory response in the rarer species was only observed in the mixed-species mesocosm communities. When grown individually, without competitors, each of the four species produced significantly fewer seeds (on an average, 24% fewer) when inoculated with either the fungal or bacterial pathogen (Fig. 5; pathogen: $F_{2,166} = 11.5$, $P < 0.0001$; species: $F_{3,166} = 50.28$, $P < 0.0001$; pathogen × species: $F_{6,166} = 2.09$, $P = 0.06$).

Heterogeneous nutrients had a marginally significant positive effect on plant diversity (Fig. 1c, three-way ANOVA on a sum of diversity: $F_{1,40} = 3.44$, $P = 0.07$). The effect of nutrient heterogeneity on plant diversity did not change across generations (Fig. 1d; $F_{4,37} = 1.6$, $P = 0.19$) and did not change in the presence of pathogens (nutrient × *Alt*: $F_{1,40} = 0.015$, $P = 0.9$; nutrient × *Xcc*: $F_{1,40} = 1.22$, $P = 0.28$).

Overall, pathogens explain 78% of the difference in diversity among treatments whereas nutrient heterogeneity accounts for only 14% of the difference; this is a 5.5-fold greater impact from pathogens than from resource heterogeneity in this experiment. The fungal pathogen and the interaction between *Alternaria* and *Xanthomonas* explained the greatest proportion of variation in plant diversity (*Alternaria*: 47%, *Xanthomonas*: 0%, *Alternaria* × *Xanthomonas*: 31%). Interactions between pathogens and resource heterogeneity explain the remainder of variation (10%).

DISCUSSION

Here, we report the first experimental evidence that pathogens maintain plant diversity over multiple generations in a multi-species plant community. Our findings reveal details about the manner in which pathogens influence plant diversity that are not predicted by simpler models and experiments. First, we present evidence that while a bacterial and a fungal pathogen each independently maintained plant diversity, interactions between the two pathogens modify

the effects on plant diversity. We expected the communities co-inoculated with both pathogens to have the highest diversity; indeed, Levin *et al.* (1977) predict that each additional pathogen should either have no effect or increase host diversity. In contrast, we found that plant diversity was higher with the fungal pathogen alone compared with communities exposed to both pathogens together, suggesting interference between the two pathogens.

How often pathogen interactions such as these influence host coexistence remains an open question. In the field, plants are simultaneously infected by a diverse suite of pathogens whose interactions may have synergistic or antagonistic effects on their hosts. In a recent meta-analysis, Morris *et al.* (2007) found that, on an average, interactions between enemies did not influence plant performance. However, many individual studies showed large significant interaction effects, both positive and negative. The authors suggest that these interactions might be unique to particular enemy–enemy pairs; for instance, Oliver *et al.* (2001) showed that two different *Alternaria* species compete with each other on the mustard *Cakile maritime*, while Fernando *et al.* (1994) found that a bacterial pathogen, *Pseudomonas*, increased infection rates by a fungal pathogen on velvetleaf (*Albutilon theophrasti)*.

The effect of pathogens on plant diversity in this experiment is particularly striking because our mesocosms consist of four closely related species. Because closely related plants are more likely to share pathogens (Gilbert & Webb 2007), we would expect less differential effects across plant species. The pathogens used in this study infect all four, closely related plant species, but have differential impacts on the fitness of different plant species. Thus, any effects of pathogens on maintaining diversity are likely to be conservative estimates of what to expect in more phylogenetically diverse communities. Specifically, our experiment demonstrates that host-specificity is not required for pathogens to influence plant diversity. Models for the top–down maintenance of plant diversity often assume that each enemy is specific to just one plant host (Janzen 1970; Connell 1971) but most plant pathogens infect a range of host species (Farr *et al.* 1989). The results here suggest that these generalist pathogens may have a large impact on plant community composition.

Most plant pathogen experiments are followed for only one or two generations, yet multi-generational effects of a pathogen's presence could lead to spurious extrapolations. Indeed, we found that the effect of pathogens on plant composition changed between the second and third generations, indicating that maternal effects, vertical transmission of the pathogens or evolution of the host defenses might be involved (Jarosz *et al.* 1989; Burdon & Thompson 1995; Agrawal *et al.* 1999). Additional experiments are needed to tease apart the mechanisms behind this observation.

It is generally well accepted that increased spatial heterogeneity of resources will increase plant diversity (Tilman 1982; Miller *et al.* 2005). Of the studies that perform experimental manipulations of nutrient heterogeneity, most find support for the resource-ratio theory; Miller *et al.* (2005) reviewed 1333 papers that cite Tilman's model and find that predictions of the resource-ratio theory were supported 75% of the time. We found that plant diversity tended to be greater in heterogeneous communities, but this effect was only marginally significant. Moreover, the influence of pathogens was 5.5 times more important for the maintenance of plant diversity than the spatial heterogeneity of nutrient resources. These results suggest that, in some settings, pathogens may be as important a factor as resource heterogeneity on plant community diversity.

However, the importance of resource heterogeneity for a plant community will depend on the nutrient regime and the resource requirements of the particular species. We chose a fertilizer pattern to represent a degree of resource heterogeneity that may be found in natural systems. For example, Jackson & Caldwell (1993) found that nitrate and phosphate varied by a factor of 2.8 and 1.3, respectively, in soil around plants spaced 3 cm apart in the field (vs. 1.8 and 1.5 between cells in this experiment). In all mesocosms, root competition should be relatively intense; individual plants in a cell compete for resources with seven to eight other individuals under a locally (within cell) homogeneous nutrient environment. Further, the division of the cells within the mesocosm exaggerates the degree of nutrient heterogeneity, because the cells prevent foraging to more favourable nutrient patches. Thus, in this way, resource heterogeneity may be more intense than natural plant communities typically experience on this spatial scale. At the same time, the *Brassica* species are closely related and therefore may share more similar nutrient requirements than most species in a natural plant community; this might contribute to the weak effect of nutrient heterogeneity. Although as discussed above, the genetic similarity between the plant species also makes the effects of pathogens all the more surprising.

Perhaps the most unexpected finding of this experiment counters a basic assumption of all pathogen–host diversity models. The models assume that the most abundant (or competitively dominant) species will be the most heavily infected by pathogens, and should suffer a higher fitness cost than do competing species and thus provide a rare-species advantage that helps maintain plant diversity. In single-species experiments, the pathogens did reduce the fitness of infected host plants, and in multi-species experiments, the rate of infection of *B. juncea* did increase as it became more dominant in the community over the generations. Unexpectedly, however, the pathogens did not impose a fitness cost on their hosts when grown in mixed

communities. Instead, seed set of the most abundant host species, *B. juncea*, in multi-species communities was not affected by pathogen infection, whereas seed set of the less abundant plant species actually increased significantly in the presence of pathogens. Thus, we conclude that compensatory reproduction by the less abundant plant species resulted in the maintenance of the less competitive species in the system.

What type of mechanisms might account for this compensatory response when hosts are grown in competition with one another? We hypothesize that this compensatory response is due to the pathogens eliciting accelerated reproduction in the plants. Early seed production would increase the likelihood of producing viable seeds before the plant succumbs to disease. In another study, infection by three different pathogens (*Pseduomonas syringae*, *X. campestris* and *Peronospora parasitica*) reduced *Arabidopsis*' time to flowering and the number of aerial branches produced. Moreover, flowering time was even faster in the presence of competitors and depended on the total amount of pathogens to which the plants were exposed (Korves & Bergelson 2003, 2004). Although few studies have reported evidence of pathogen-induced overcompensation, this phenomenon has often been observed in response to herbivores (Paige & Whitman 1987; Lennartsson *et al.* 1998; Agrawal 2000).

Model systems have a long and successful history in ecology and are powerful tools for the study of ecological patterns and processes (Jessup *et al.* 2004). Controlled mesocosm experiments such as the one presented here have their limitations, but they can reveal potentially overlooked biological mechanisms and suggest directions for future study. They are particularly useful when critical manipulations (such as maintaining exactly zero, one, or two pathogens in a plant community over multiple generations) are logistically impossible under field conditions. The current experiment suggests that plant pathogens are potentially a large force in regulating plant community diversity (perhaps even larger than nutrient heterogeneity), but the magnitude of their effects cannot be extrapolated from single generation, single host-pathogen models and experiments. The work also raises the question of whether compensatory responses to pathogen infection are commonplace in natural plant communities. Given the high diversity of both fungal and bacterial communities in natural environments, the importance of complex, non-additive pathogen effects on plant community composition deserves more attention.

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