Ecology, 82(12), 2001, pp. 3285–3294

MECHANISMS OF PLANT SPECIES COEXISTENCE: ROLES OF RHIZOSPHERE BACTERIA AND ROOT FUNGAL PATHOGENS

KRISTI M. WESTOVER[1,2] AND JAMES D. BEVER[3]

[1]*Department of Botany, Duke University, Durham, North Carolina 27708 USA*
[3]*Department of Biology, Indiana University, Bloomington, Indiana 47405 USA*

Abstract. Two independent experiments were conducted to investigate the influence of rhizosphere bacteria on the growth of *Anthoxanthum odoratum* and *Panicum sphaerocarpon.* We tested whether host-specific populations of *Bacillus mycoides* affected the growth of their *Anthoxanthum* and *Panicum* hosts and whether host-specific differences in *Bacillus* populations modified the strong detrimental effect of the root fungal pathogen, *Pythium macrosporum.* Our results showed both positive and negative effects of *Bacillus* inoculation and that *Anthoxanthum* and *Panicum* plants responded differently to *Bacillus* isolates that originated from different host plants. *Anthoxanthum* grew relatively better with isolates from *Panicum,* while *Panicum* grew relatively better with isolates from *Anthoxanthum,* consistent with a negative feedback. In both experiments *Pythium* infection was detrimental to plant growth, and *Panicum* was more negatively affected by inoculation with *Pythium.* Overall, *Bacillus* ameliorated the pathogenic effect of *Pythium.* However, there was no evidence that host-specific *Bacillus* populations had different effects on the interaction between these plant species and *Pythium.* Both host-specific differences in rhizosphere bacteria and host-specific accumulation of a fungal pathogen can generate negative feedback between these two plant species.

Key words: Anthoxanthum odoratum; Bacillus mycoides; *host specificity; negative feedback;* Panicum sphaerocarpon; *perennial grasses; plant community diversity;* Pythium macrosporum; *rhizosphere bacteria; root fungal pathogens.*

INTRODUCTION

A growing body of work indicates that soil organisms are key to coexistence among plants (e.g., Bever 1994, Mills and Bever 1998, Van der Heijden et al. 1998, Packer and Clay 2000). Fungal root pathogens (Mills and Bever 1998, Packer and Clay 2000) and fungal root symbionts (Grime et al. 1987, Van der Heijden et al. 1998) have been shown to make contributions to plant species coexistence. However, other components of the soil community may also exert strong effects on plant growth. These include rhizosphere bacteria, which have been shown to have positive and negative effects on plant growth (Albrecht et al. 1981, Neitko and Frankenburg 1989, Handman et al. 1991, Derylo and Skorupska 1992). Rhizosphere bacteria have also been shown to modify or eliminate the effects of other components of the soil community (e.g., Harris et al. 1997, Kim et al. 1997).

In experiments with soil organisms from a North Carolina grassland we found strong negative feedbacks on plant growth following changes in the composition of the soil community (Bever 1994, Bever et al. 1997). Negative feedback between two plant species occurs when both plant species change their respective soil communities in ways that are detrimental to themselves relative to the other species. Negative feedback can therefore contribute to the coexistence of competing plant species (Bever et al. 1997). For example, *Anthoxanthum odoratum* and *Panicum sphaerocarpon* change soil communities and these changes are detrimental for the respective host plant species (Bever 1994, Bever et al. 1997; K. Westover and J. Bever, *unpublished data*). Accumulation of *Pythium,* a fungal root pathogen, in the soil may contribute to the negative feedback between *Anthoxanthum* and *Panicum* (Bever 1994, Mills and Bever 1998). Additionally, the composition of bacterial (Westover et al. 1997) and arbuscular mycorrhizal fungal (Bever et al. 1996) communities in the rhizosphere can change due to influence of the host plant species, and differentiation of these communities may also generate negative feedback (Bever et al. 1997, Bever 1999). Moreover, the deleterious effect of *Pythium* may be ameliorated by interactions with rhizosphere bacteria (Harris et al. 1997).

Here we investigated the influence of rhizosphere bacteria on the growth of *Anthoxanthum odoratum* and *Panicum sphaerocarpon.* Specifically, we tested whether host plant mediated changes in populations of *Bacillus mycoides* affected growth of their respective *Anthoxanthum* and *Panicum* hosts. *Bacillus mycoides* was very common at our site (Westover et al. 1997)

Manuscript received 10 April 2000; revised 22 December 2000; accepted 28 December 2000; final version received 16 January 2001.

[2] Present address: Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208 USA. E-mail: westover@biol.sc.edu

PLATE 1. Late-summer view of the long-studied old-field site on the Duke University Campus, Durham, North Carolina. Photograph by Kristi Westover.

and through characterization of strains using restriction fragment length polymorphisms, we have found that the composition of *Bacillus mycoides* populations was influenced by host plant species (K. Westover, *unpublished data*). Members of the genus *Bacillus* are generally thought to be beneficial to plants (Claus and Berkeley 1996). *Bacillus* has been shown to enhance plant growth by producing phytohormones (Srinivasan et al. 1996) as well as by protecting plants from insects (Hofte and Whiteley 1989) and fungal pathogens (Faull and Campbell 1979, Fiddaman and Rossall 1993, Silo-Suh et al. 1994). Therefore, we also tested whether host-specific differences in *Bacillus* populations modified the strong effects of *Pythium*, an important crop pathogen (e.g., Abad et al. 1994, Pankhurst et al. 1995) which has been shown to have substantial effects on seedling dynamics in eastern deciduous forests (Packer and Clay 2000).

METHODS

Study system

The plants, microorganisms, and soil used for these experiments were obtained from a field between the east and west campuses of Duke University, Durham, North Carolina (35°54′ N, 78°54′ W; see Plate 1). This field has been mowed at least once a year for the last 60 yr. The plant community consists of a diverse assemblage of herbs and grasses, with no clearly dominant species (e.g., Fowler and Antonovics 1981, Fowler 1982). The soil is a sandy loam of the White Shore series with a high organic content, overlying a sand/clay hardpan (Fowler 1978, Fowler and Antonovics 1981). We used two common, short-lived perennial grass species, *Anthoxanthum odoratum* L. and *Panicum sphaerocarpon* Ell. Hereafter, both species will be referred to by generic name. *Anthoxanthum* and *Panicum* have been shown to change their respective soil communities in several independent laboratory and field experiments and these changes were detrimental for host-specific plant growth, consistent with negative feedback (Bever 1994, Bever et al. 1997; K. Westover and J. Bever, *unpublished data*).

Experimental overview

We used two parallel, but independent, experiments to test the joint effect of *Bacillus* and *Pythium* on the growth of *Anthoxanthum* and *Panicum.* In the first experiment, isolates of *Bacillus* were obtained from the rhizospheres of these two species growing in the field. We used isolates of *Bacillus* obtained from the rhizospheres of greenhouse grown *Anthoxanthum* and *Panicum* for the second experiment. Because plant species in close proximity to *Anthoxanthum* and *Panicum* in the field may also be influencing soil communities, we might expect *Anthoxanthum* and *Panicum* influence on field soil microbes to be less defined compared to the effect of only *Anthoxanthum* or *Panicum* on soil in pots. This effect, in turn, would be reflected by a larger difference in plant growth response to host-specific *Bacillus* isolates collected from the individual pots. To test this we evaluated the effect of both greenhouse and field-collected isolates of *Bacillus* on plant growth.

Both experiments shared a similar design. Specifically, the experiments were full factorial tests of the effects of five independent isolates of *Bacillus* (obtained from each of the two plant species; i.e., ten isolates total plus the sterile control) and *Pythium* on the growth of the two plant species. The treatments were replicated five times each, except for the uninoculated *Bacillus* combinations, which were replicated ten times (in order to increase the power of testing the net effects of *Bacillus*).

Isolation and preparation of Bacillus *for soil inoculation*

For the first experiment, isolates of *Bacillus* were collected directly from *Anthoxanthum* and *Panicum* in the field. These plants had been originally surface ster-

ilized (see Bever 1994) and planted into the field in a randomized block design as part of a larger experiment. Due to their random placement in the field, the differences in *Bacillus* isolates are attributed to host effects. After the plants had been established for 24 mo, isolates of *Bacillus* were obtained from all *Anthoxanthum* and *Panicum* plants (16 plants of each species). Isolates used for inoculation were obtained by assigning all a number and then randomly choosing five from each plant species. We allowed only one *Bacillus* isolate from any particular plant. All isolates were therefore considered to be independent.

Isolates for the second experiment were obtained from *Anthoxanthum* and *Panicum* plants potted individually in the greenhouse. Before potting, these plants were de-rooted and surface sterilized to remove microbes and then planted into pots containing a field soil mixture (as in Bever 1994). Therefore, the plants grew roots into an initially similar soil community. The soil communities were allowed to differentiate for six months and were then sampled for *Bacillus mycoides.* Isolates were again obtained from all plants (10 plants of each species). Five isolates from each plant species were randomly chosen as described for the first experiment.

Bacillus mycoides was isolated from soil as follows. Soil was collected from a plant by gently lifting and shaking ~2 g of rhizosphere soil from the roots (Tate 1995). Each soil sample was homogenized and serially diluted using 0.1% $CaSO_4$. Small (25 μL) aliquots from dilutions (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) of each sample were plated onto tryptic soy agar (TSA) (Wollum 1982). Colonies were counted after 24 h at 24°C and were re-plated onto TSA. *Bacillus* inoculum was prepared by transferring isolates to 10-mL tryptic soy broth tubes. The liquid cultures were incubated for 48 h at 24°C before inoculation. Aliquots of extra liquid cultures were serially diluted and plated onto TSA to estimate initial inoculum densities (colony-forming units per mL) of *Bacillus* cultures. There were no significant differences between estimated inoculum densities of *Bacillus* cultures in either experiment. The mean inoculum density was 10^7 colony-forming units per mL tryptic soy broth.

Preparation of Pythium *for soil inoculation*

The *Pythium* isolate was obtained from the roots of *Panicum* as described in Mills and Bever (1998). *Pythium* inoculum containing active mycelia and oospores that could survive in and infest the soil of treatment plants was prepared by growing the *Pythium* isolate in sterile grass blade culture (Martin 1992). Two plugs (each 0.5 cm^3) of corn meal agar with mycelium were placed in separate sterile tissue culture dishes (Falcon 3025, 150 × 25 mm style). Sterile deionized water was added to the dishes to a level where the water just covered the agar plugs, and 50 pieces (each 1.0–1.5 cm long) of autoclaved tall fescue grass leaves were placed in each dish. Five replicate dishes containing grass blade cultures were incubated at room temperature under continuous light for four days (Abad et al. 1994). Infection of grass blades by *Pythium* mycelia was monitored under the dissecting scope. Four days after the cultures were started, at which time most of the grass blades were infected, the grass blades were ready for inoculation of soil.

Planting, soil inoculation, and harvesting processes

Seedlings of *Anthoxanthum* and *Panicum* were started from surface-sterilized seeds of plants grown in the greenhouse. All seeds were planted in sterile seedling mix and allowed to grow until small seedlings were available. In June 1997, five replicate plants of each species were grown with each of the 10 field *Bacillus* isolates (five from *Anthoxanthum* and five from *Panicum*), alone, and with *Pythium.* All plants for this experiment were grown in 120-cc cone-tainers filled with sterile background soil. To prepare the background soil, field soil was first homogenized and passed through a 1-cm mesh. Equal parts of the sieved field soil and sand were then thoroughly mixed and autoclaved for 1.5 h. In January 1998, an experiment of similar design was planted using the greenhouse isolates of *Bacillus.*

Bacillus was added as 48-h 10-mL tryptic soy broth liquid cultures. A hole was made in the cone-tainer soil using a sterile glass rod and the liquid culture was well mixed and poured into the center. The plant was placed in this opening using sterile forceps. An additional set was planted in the same manner with sterile 10 mL of uninoculated media to serve as controls. Two *Pythium* infected grass blades from the grass blade cultures served as *Pythium* inocula. The infected grass blades were added to the pots using a sterile forceps and placed beneath the top 40 mL of soil in each pot. Another set of controls was planted in the same manner with sterile uninoculated grass blades instead of *Pythium* infected grass blades.

In both experiments, all cone-tainers were randomized and placed in a phytotron C chamber with 12-h day lengths and temperatures ranging from 10° to 25°C (Duke University Phytotron, Durham, North Carolina). The cone-tainers were spaced in racks with 5 cm between them to prevent cross contamination. All containers were kept well watered with distilled water during the first few days to allow initial establishment of the seedlings and *Pythium.* After the first week, plants were watered once daily with distilled water without application of nutrients. To prevent cross contamination, each cone-tainer was watered individually without splashing.

The experiments were harvested after ~8 wk and before the plants became ''root bound.'' Plants were removed from the soil and washed. Roots and leaves were separated, dried, and weighed. Estimates of plant size (e.g., leaf and tiller number) and fitness (e.g., number of reproductive inflorescences and seed set) are

correlated with plant biomass for *Anthoxanthum* and *Panicum* (A. Pringle, *unpublished data*). Therefore, dry mass of plant leaves and roots from each pot were summed for the subsequent analysis. Samples of soil and roots were also taken from select plots to ensure the presence of *Bacillus* and *Pythium* using the same procedures. There was no evidence of *Pythium* or significant *Bacillus* populations in the controls at harvest and as at the beginning of experiments, there were no significant differences between estimated densities of *Bacillus* in the *Bacillus* treatments. Mean *Bacillus* density was 10^7 colony-forming units per gram of soil.

Statistical analysis

Total plant dry mass values were $\log(x + 1)$-transformed to improve the homogeneity of variance. The effect of plant species, *Bacillus* inoculation treatment (including the presence of *Bacillus* and source of *Bacillus* isolate; i.e., three levels), *Pythium* inoculation treatment (two levels), *Bacillus* isolate nested within *Bacillus* inoculation treatment, and the appropriate interaction terms were analyzed using the General Linear Models Procedure of SAS (SAS Institute 1986). We analyzed the results in two ways. First, we treated *Bacillus* isolates as fixed effects. Second, we treated *Bacillus* isolates as random effects. The fixed-effects model gave us our most powerful test of differences among the ten particular *Bacillus* isolates used in each experiment. The mixed-model analysis (treating the *Bacillus* isolates as random effects) assumes that the particular *Bacillus* isolates are independent representatives of the host-specific soil communities. Testing effects with the mixed model allowed us to test for robust patterns, patterns that were likely to hold up even if we went back and randomly chose another five independent isolates from each plant species and repeated the experiment. For example, we could test whether the overall effect of *Pythium* would be negative even in association with ten different *Bacillus* isolates.

Within our design, the *Bacillus* inoculation treatment included the control along with the isolates from the two sources. This treatment factor was decomposed into two orthogonal components to test both the average effect of *Bacillus* (inoculated vs. controls), and the difference between the two sources of *Bacillus* (*Anthoxanthum* vs. *Panicum*). These effects were separated in all higher level interaction terms.

We were particularly interested in evaluating three sets of hypotheses relating to our prior observations of negative feedback between these two plant species. First, we wanted to test whether host-specific differences in *Bacillus* populations affected plant growth and therefore feedback between *Anthoxanthum* and *Panicum* (e.g., in Bever 1994). We tested this by comparing *Anthoxanthum* and *Panicum* growth with their own *Bacillus* populations compared to growth with each other's *Bacillus* populations (a ''home'' vs. ''away'' contrast as derived in Bever et al. [1997]).

Our second specific interest was to test whether the presence of *Bacillus* modified our interpretation that accumulation of *Pythium* on *Panicum* contributed to the negative feedback between these two plant species (from Mills and Bever 1998). We evaluated this by testing whether the presence of *Bacillus* altered plant response to *Pythium*, explicitly testing the two-way interaction of *Pythium* by presence of *Bacillus* and the three-way interaction of test plant species by *Pythium* by presence of *Bacillus*.

Our final specific interest was to evaluate the more subtle possibility that differences in *Bacillus* populations modify or ameliorate the deleterious effect of *Pythium*, and therefore modify negative feedback through accumulation of host-specific fungal pathogens. We evaluated this possibility by testing the two-way interaction of *Pythium* × *Anthoxanthum* vs. *Panicum* source of *Bacillus* contrast and the three-way interaction of Test plant species × *Pythium* × *Anthoxanthum* vs. *Panicum* source of *Bacillus* contrast.

RESULTS

Plant response to Bacillus *inoculation*

The mass of plants grown in soil inoculated with field and greenhouse isolates of *Bacillus* was significantly different compared to plants grown in uninoculated soil (Uninoculated vs. *Bacillus* contrast, Tables 1 and 2). There was evidence for both positive and negative effects of *Bacillus* inoculation. Plants grown in soil inoculated with field isolates of *Bacillus* were smaller than those grown in uninoculated soil, while plants grown in soil inoculated with greenhouse isolates of *Bacillus* were larger (Fig. 1a, 1b, negative and positive effects, respectively). The negative effect of the particular field isolates used in this experiment may not be representative of all *Bacillus* isolated from the field, as this effect was not significant in the mixed model (Table 1). However, the growth promotion by the greenhouse isolates was representative of the population of *Bacillus* that accumulated in the greenhouse (Table 2).

There was no significant difference between the overall effect of field-collected *Bacillus* isolates from *Anthoxanthum* vs. those from *Panicum* on plant mass (AN vs. PA isolate contrast, Table 1, Fig. 1a). But there were significant differences among individual *Anthoxanthum* isolates and among individual *Panicum* isolates collected from both the field and the greenhouse (Rhizosphere × Isolate interaction, Tables 1 and 2). Plant mass varied depending upon the particular *Anthoxanthum* or *Panicum* isolate used to inoculate the soil in both experiments (Fig. 1a, 1b). There were differences between the overall effect of greenhouse cultured *Bacillus* isolates from *Anthoxanthum* vs. those from *Panicum* on plant mass (AN vs. PA isolate contrast, Table 2, Fig. 1b). Plants inoculated with isolates of *Bacillus* from *Anthoxanthum* were larger than plants inoculated

TABLE 1. Field isolates of *Bacillus*: tests of hypotheses for fixed and mixed-model ANOVA of total plant dry mass.

Source of variation	df	SS	Fixed model *P*	Mixed model *P*
Test plant species†	1	2.200	0.0001	0.0001
Rhizosphere‡	2	0.057	0.0005	NS
Uninoculated vs. *Bacillus*	1	0.046	0.0004	NS
AN vs. PA isolates	1	0.011	NS	NS
Test plant species × Rhizosphere†	2	0.175	NS	NS
Test plant species × Uninoculated vs. *Bacillus*	1	0.002	NS	NS
Test plant species × AN vs. PA isolates	1	0.015	0.04	0.04
Pythium§	1	0.240	0.0001	0.004
Test plant species × *Pythium*‖	1	0.001	NS	NS
Pythium × Rhizosphere§	2	0.002	NS	NS
Pythium × Uninoculated vs. *Bacillus*	1	0.001	NS	NS
Pythium × AN vs. PA isolates	1	0.001	NS	NS
Test plant species × *Pythium* × Rhizosphere‖	2	0.005	NS	NS
Test × *Pythium* × Uninoculated vs. *Bacillus*	1	0.001	NS	NS
Test × *Pythium* × AN vs. PA isolates	1	0.001	NS	NS
Rhizosphere × Isolate¶	8	0.112	0.0003	NS
Test plant species × Rhizosphere × Isolate‖	8	0.022	NS	NS
Pythium × Rhizosphere × Isolate‖	8	0.086	0.003	NS
Test plant species × *Pythium* × Rhizosphere × Isolate#	8	0.043	NS	NS
Error	169	0.600		

Note: Test plant species in this study are *Anthoxanthum odoratum* (AN) and *Panicum sphaerocarpon* (PA).

† Error term in mixed-model analysis was the mean square for Test plant species × Rhizosphere × Isolate.

‡ Error term in mixed-model analysis was the mean square for Rhizosphere × Isolate.

§ Error term in mixed-model analysis was the mean square for *Pythium* × Rhizosphere × Isolate.

‖ Error term in mixed-model analysis was the mean square for Test plant species × *Pythium* × Rhizosphere × Isolate.

¶ Error term in mixed-model analysis was the sum of mean squares for Test × Rhizosphere × Isolate, *Pythium* × Rhizosphere × Isolate, and Test × *Pythium* × Rhizosphere × Isolate.

Error term in mixed-model analysis was the mean square for Error.

with *Bacillus* isolates from *Panicum* (Fig. 1b). This difference, however, was not significant when tested over the substantial variation among greenhouse *Anthoxanthum* and *Panicum* in the mixed-model analysis (Table 2), indicating that the difference observed between these five particular isolates from *Anthoxanthum* and *Panicum* may not be representative of the populations of *Bacillus* associated with these species in the greenhouse.

Anthoxanthum and *Panicum* test plants responded similarly to being gown in soil inoculated with *Bacillus* compared to uninoculated soil in both experiments (Test plant species × Uninoculated vs. *Bacillus* contrast, Tables 1 and 2). *Anthoxanthum* and *Panicum* also responded similarly to the presence of isolates of *Bacillus* collected from greenhouse pots of *Anthoxanthum* and *Panicum* isolates (Test plant species × AN vs. PA isolates contrast, Table 2). However, *Anthoxanthum* and *Panicum* responded differently to the presence of field-collected *Anthoxanthum* vs. *Panicum* isolates (Test plant species × AN vs. PA isolates contrast, Table 1). This contrast remained significant ($P < 0.04$) when the variation among *Anthoxanthum* and *Panicum* isolates was taken into consideration by using the mean square for the Test × Rhizosphere × Isolate interaction as the error term in the mixed-model analysis (Table 1). This again indicates that these differences are representative of the populations of *Bacillus* associating with these two species in the field. *Anthoxanthum* test plants were significantly larger when grown in soil inoculated with *Panicum* isolates of *Bacillus*, and *Panicum* test plants were significantly larger when grown in soil inoculated with *Anthoxanthum* isolates of *Bacillus* (Fig. 2), suggesting that the differentiation of *Bacillus* on different host plant species can contribute to negative feedback between *Panicum* and *Anthoxanthum*.

Plant response to Pythium *inoculation*

The presence of *Pythium* resulted in significant reductions in plant mass in both experiments (*Pythium* effect, Tables 1 and 2). Plant mass was reduced from a mean of 0.26 ± 0.01 g to 0.20 ± 0.01 g in the experiment where field isolates of *Bacillus* were used and from a mean of 0.23 ± 0.01 g to 0.15 ± 0.01 g in the experiment where greenhouse isolates of *Bacillus* were used. In both experiments, this deleterious effect of *Pythium* was consistent across multiple *Bacillus* isolates, as confirmed in the mixed-model ANOVA (Tables 1 and 2).

Panicum was more susceptible to *Pythium* than *Anthoxanthum* in both experiments, although this was only significant in the experiment where greenhouse

TABLE 2. Greenhouse isolates of *Bacillus*: tests of hypotheses for fixed and mixed-model ANOVA of total plant dry mass.

Source of variation	df	SS	Fixed model *P*	Mixed model *P*
Test plant species†	1	0.081	0.0001	0.0001
Rhizosphere‡	2	0.012	0.0002	NS
Uninoculated vs. *Bacillus*	1	0.008	0.0005	0.05
AN vs. PA isolates	1	0.004	0.02	NS
Test plant species × Rhizosphere†	2	0.001	NS	NS
Test plant species × Uninoculated vs. *Bacillus*	1	0.001	NS	NS
Test plant species × AN vs. PA isolates	1	0.001	NS	NS
Pythium§	1	0.085	0.0001	0.0002
Test plant species × *Pythium*‖	1	0.061	0.0001	0.0005
Pythium × Rhizosphere§	2	0.008	0.002	NS
Pythium × Uninoculated vs. *Bacillus*	1	0.107	0.001	0.01
Pythium × AN vs. PA isolates	1	0.001	NS	NS
Test plant species × *Pythium* × Rhizosphere‖	2	0.001	NS	NS
Test × *Pythium* × Uninoculated vs. *Bacillus*	1	0.001	NS	NS
Test × *Pythium* × AN vs. PA isolates	1	0.001	NS	NS
Rhizosphere × Isolate¶	8	0.012	0.02	NS
Test plant species × Rhizosphere × Isolate‖	8	0.007	NS	NS
Pythium × Rhizosphere × Isolate‖	8	0.006	NS	NS
Test plant species × *Pythium* × Rhizosphere × Isolate#	8	0.009	NS	NS
Error	176	0.117		

Note: Test plant species in this study are *Anthoxanthum odoratum* (AN) and *Panicum sphaerocarpon* (PA).

† Error term in mixed-model analysis was the mean square for Test plant species × Rhizosphere × Isolate.

‡ Error term in mixed-model analysis was the mean square for Rhizosphere × Isolate.

§ Error term in mixed-model analysis was the mean square for *Pythium* × Rhizosphere × Isolate.

‖ Error term in mixed-model analysis was the mean square for Test plant species × *Pythium* × Rhizosphere × Isolate.

¶ Error term in mixed-model analysis was the sum of mean squares for Test × Rhizosphere × Isolate, *Pythium* × Rhizosphere × Isolate, and Test × *Pythium* × Rhizosphere × Isolate.

Error term in mixed-model analysis was the mean square for Error.

isolates of *Bacillus* were used (Test plant species × *Pythium* interaction, Tables 1 and 2). Although not significant, in the first experiment (field isolates of *Bacillus*), *Panicum* mass was reduced by 52% and *Anthoxanthum* mass reduced by 23%. In the second experiment (greenhouse isolates of *Bacillus*), *Panicum* mass was reduced by 71% while *Anthoxanthum* mass was reduced by only 4% (Fig. 3). Again, this effect was also significant in the mixed-model ANOVA (Table 2).

Plant response to co-inoculation with Bacillus *and* Pythium

Plants grown in soil inoculated with *Pythium* and greenhouse *Bacillus* isolates were larger than those grown in soil inoculated with *Pythium* alone (*Pythium* × Uninoculated vs. *Bacillus* contrast, Table 2, Fig. 4). This contrast remained significant ($P < 0.01$) when the variation among *Anthoxanthum* and *Panicum* isolates was taken into consideration by using the mean square for the *Pythium* × Rhizosphere × Isolate interaction as the error term in the mixed-model analysis (Table 2). However, this effect was not significant in the experiment where field isolates of *Bacillus* were used (Table 1).

There was no evidence that plants responded any differently to being grown in soil inoculated with *Pythium* and isolates of *Bacillus* associated with *Anthoxanthum* or *Panicum* in either experiment (*Pythium* × AN vs. PA isolate contrast, Tables 1 and 2). There was also no indication from either experiment that *Anthoxanthum* and *Panicum* test plants responded differently to soil inoculated with *Pythium* and *Bacillus* (Test plant species × *Pythium* × Uninoculated vs. *Bacillus* contrast, Tables 1 and 2). Furthermore, *Anthoxanthum* and *Panicum* test plants did not respond differently to soil co-inoculated with *Pythium* and *Bacillus* isolates associated with *Anthoxanthum* or *Panicum* (Test plant species × *Pythium* × AN vs. PA isolate contrast, Tables 1 and 2). However, the ten individual isolates of *Bacillus* isolated from the field differed in their ability to alleviate the deleterious effect of *Pythium*, as confirmed by the significance of the Pythium × Rhizosphere × Isolate interaction in the fixed model (Table 1).

DISCUSSION

Our results suggest that plant species-specific *Bacillus* isolates contribute to the maintenance of plant diversity in a North Carolina old field community. Differentiation of *Bacillus* isolates on plant hosts is consistent with an analysis of restriction fragment length polymorphisms using these *Bacillus* isolates (K. West-

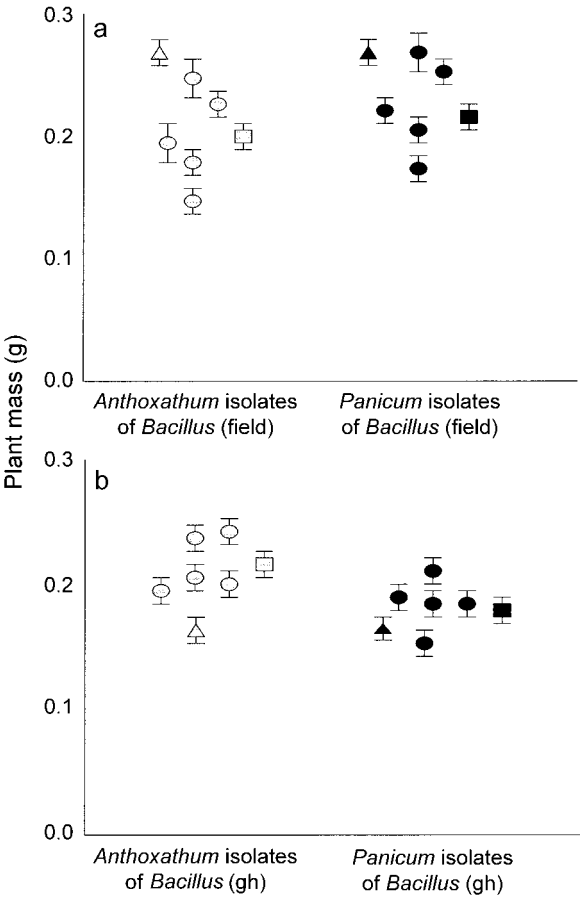

FIG. 1. Mean plant mass when inoculated with *Bacillus* isolates from *Anthoxanthum* and *Panicum* originally collected from the field (a) or greenhouse (b). Squares represent mean plant mass when inoculated with isolates from *Anthoxanthum* and *Panicum.* Circles represent mean plant mass when inoculated with each of the five individual isolates from *Anthoxanthum* and *Panicum* (showing variation among *Bacillus* isolates). Triangles represent mean plant mass for uninoculated controls. Error bars are ± 1 SE.

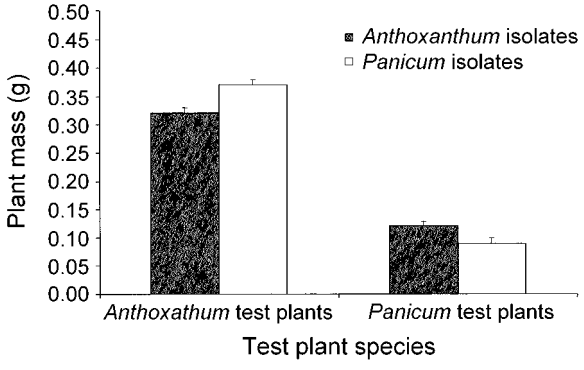

FIG. 2. Mean *Anthoxanthum* and *Panicum* test plant mass when grown in soil inoculated with *Bacillus* isolated collected from either *Anthoxanthum* or *Panicum* hosts. Error bars are ± 1 SE.

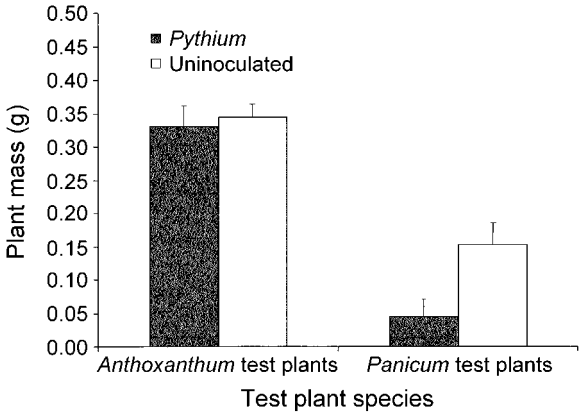

FIG. 3. Mean *Anthoxanthum* and *Panicum* test plant mass when grown in soil inoculated with *Pythium* compared to uninoculated controls. Error bars are ± 1 SE.

over, *unpublished data*) and with host-specific patterns of substrate utilization for entire rhizosphere bacterial communities (Westover et al. 1997). In the present study, we also found evidence of this host-specific differentiation among the field isolates as demonstrated by the significant interaction between test plant species and contrast of AN vs. PA isolates (Table 1). Such differentiation was suggested in the greenhouse isolate experiment as well by the significance of the AN vs. PA isolate effect within the fixed model (Table 2, Fig. 1b). *Anthoxanthum* and *Panicum* plants then responded differently to *Bacillus* isolates depending on their host of origin (Table 1). Specifically, *Anthoxanthum* grew relatively better with isolates from *Panicum*, while *Panicum* grew relatively better with isolates from *Anthoxanthum* (Fig. 2). This pattern held true for both experiments, though only statistically significant for field-collected isolates. This result demonstrates that host-specific differences in *Bacillus* populations can generate negative feedback on host growth. This is consonant with repeated observations of negative feedback between these same plant species due to changes in soil community composition (both in the lab, Bever 1994,

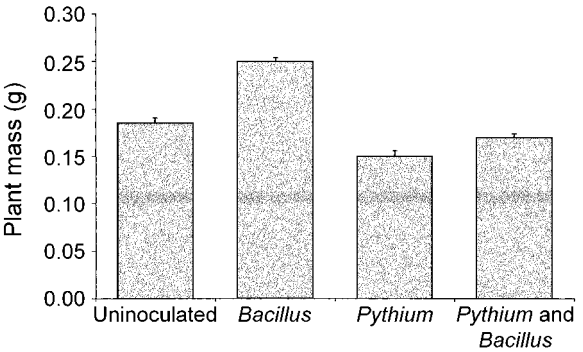

FIG. 4. Mean test plant mass when grown in soil inoculated with *Bacillus* alone, with *Pythium* alone, and with both, compared to uninoculated controls. Error bars are ± 1 SE.

Bever et al. 1997; and in the field, K. Westover and J. Bever, *unpublished data*). Together, these works suggest that the dynamics within *Bacillus* populations can contribute to the coexistence of these two plant species.

Previous results suggested that accumulation of *Pythium* on *Panicum* also contributed to the negative feedback observed between these two species (Mills and Bever 1998). We have previously found that *Pythium* accumulates in greater abundance on *Panicum* and that growth of *Panicum* was more sensitive to *Pythium* compared to *Anthoxanthum.* The present work also reinforces this hypothesis. In both experiments, *Pythium* infection was detrimental to plant growth and *Panicum* was more negatively affected by inoculation with *Pythium* than was *Anthoxanthum.* The difference in plant response (Test plant species × *Pythium* interaction) was highly significant in the greenhouse isolate experiment, but not in the field isolate experiment (Tables 1 and 2, Fig. 3).

Bacillus ameliorated the pathogenic effect of *Pythium. Bacillus* protects plants from *Pythium* infection in agricultural contexts and has been used in biological control efforts (Kim et al. 1997). Members of the *Bacillus* genus are known to produce antifungal compounds (Faull and Campbell 1979, Fiddaman and Rossall 1993, Silo-Suh 1994). Individual *Bacillus* isolates altered the response of plants to *Pythium* in the experiment with field-derived isolates where there was greater heterogeneity among the ten *Bacillus* isolates tested (Table 1). Such variation in the ability of *Bacillus* isolates to protect plants from fungal pathogens has been demonstrated in agricultural contexts as well (Landa et al. 1997).

Host-specific populations of *Bacillus* were important for plant growth, but there was no evidence that this had a net effect on the interaction between these plant species and *Pythium* (Test plant species × *Pythium* × AN vs. PA isolate term, Tables 1 and 2). Therefore we reject the hypothesis that the negative feedback that occurs through changes in the composition of the *Bacillus* populations is mediated by *Bacillus* protection from *Pythium.* However, given the level of variability in the protection from *Pythium* provided by different *Bacillus* isolates, this hypothesis warrants further exploration. In addition, some interactions among pathogens and mutualists can depend on life stage (Smith and Read 1997). Given that the plants used in the current experiment were seedlings, a potential temporal aspect to the interaction of these plant species with *Bacillus* and *Pythium* might also be expected.

The effect of individual *Bacillus* isolates on plant growth varied both within and between the two experiments, with evidence for both positive and negative effects of *Bacillus* inoculation. There was also slight variation in the growth of uninoculated plants between experiments. Uninoculated plants in the experiment where field isolates were used were ~0.1 g larger than uninoculated plants in the experiment where greenhouse isolated were used (Fig. 1a, 1b). In the field isolate experiment, *Bacillus* generally had negative impacts on plant growth, while the isolates in the greenhouse experiment had significantly positive effects (Fig. 1a, 1b, Tables 1 and 2). Moreover, the variation among isolates within the experiments was lower in the greenhouse experiment than in the field experiment (Fig. 1a, 1b, Tables 1 and 2). This suggests that while growing with plants in the greenhouse prior to collection of isolates for the current study (6 mo), the composition of the *Bacillus* populations narrowed and shifted from a generally pathogenic relationship towards a generally mutualistic one. Perhaps this indicated some level of adaptation to the greenhouse conditions, which benefited the plant. Or we might postulate that the positive greenhouse results are unrealistic. However, results from the mixed-model analysis indicated that the negative effect of the field isolates may not be representative (Table 1). It is also possible that strong host-specific mediated responses are moderated by diffuse interactions in the field or that *Bacillus* may have positive and negative effects depending upon physical conditions or the presence of diverse microbial communities.

A similar shift in variability and benefit was observed with respect to the ability of *Bacillus* to protect plants against *Pythium* (Tables 1 and 2). This result is surprising given our observation that host-specific shifts in *Bacillus* populations result in decreased performance of the host with their own *Bacillus* isolates. However, as we have previously demonstrated (Bever et al. 1997, Bever 1999), the direction of feedback through community differentiation (i.e., positive or negative) can be independent of the direction of direct ecological effect (i.e., mutualistic or pathogenic). For example, a mutualistic organism can still act as an agent of negative feedback if it provides a relatively greater benefit to a neighboring species.

In this paper, we demonstrated different effects in populations of *Bacillus mycoides* associated with *Anthoxanthum* and *Panicum* suggesting that previous observations of differentiation of rhizosphere bacterial communities associated with these and other plant species in the North Carolina old field (Westover et al. 1997) were likely an underestimation. Not only did previous methods count only culturable bacteria, but also the method used could not have detected host-specific ''genotype'' or ''strain'' differences among bacterial populations of the same species. In the present work, we were able to demonstrate the importance of this host-specific differentiation for negative feedback between *Anthoxanthum* and *Panicum.* Clearly, a complete understanding of how feedback through the soil organisms can contribute to the maintenance of diversity within plant communities must include not only evaluation of the role of community differentiation, but the role of population genetic differentiation within species as well. Conversely, the negative feedback ob-

served through differentiation of *Bacillus mycoides* populations is likely itself to be a subset of the larger negative feedbacks generated by community level differentiation on rhizospheres (as observed in Westover et al. 1997) and differentiation within other populations.

Together with the previous work of Mills and Bever (1998), a summary of current research indicates that two mechanisms generate the negative feedback observed between *Anthoxanthum* and *Panicum*, namely the accumulation of *Pythium* in *Panicum* soil and the differentiation of host-specific *Bacillus* populations. Note, however, that while the accumulation of *Pythium* can explain the reduced growth of *Panicum* when grown in *Panicum* vs. *Anthoxanthum* soil, this mechanism cannot explain the reduced growth of *Anthoxanthum* in *Anthoxanthum* vs. *Panicum* soil (also observed in Bever 1994; and J. Bever and K. Westover, *unpublished results*). The host-specific differentiation within *Bacillus mycoides* populations, however, can contribute to the reduction in growth of both species in their respective soil communities. By identifying the complementary roles of *Pythium* and *Bacillus*, we would not wish to claim to have identified all agents for the observation of negative soil community feedback between *Anthoxanthum* and *Panicum*. Rather this body of work provides illustration of the multidimensionality of the interaction between plants and their soil community. That is, we have tested two potential agents and found them both capable of generating negative feedback. There are numerous other potential agents of negative feedback between *Anthoxanthum* and *Panicum*. For example, other members of the rhizosphere bacterial (Westover et al. 1997) and mycorrhizal fungal communities (Bever et al. 1996) also differentiate on these host plants, and may contribute to the negative feedback we observed. Other potential root pathogens have been observed in this system as well, including *Fusarium* and root-feeding nematodes (J. Bever, *personal observation*). It therefore seems likely that there are multiple complementary mechanisms within the soil community that can contribute to the coexistence of competing plant species.

In summary, both the host-specific differentiation of rhizosphere bacteria and the host-specific accumulation of a fungal pathogen can generate negative feedback between these two plant species. Moreover, while *Bacillus* may ameliorate the detrimental effect *Pythium* on plant growth, this does not appear to alter the *Bacillus* role in negative feedback and therefore in plant species coexistence. Both beneficial and pathogenic members of the soil community can play an important role in the maintenance of plant species diversity.

Acknowledgments

Earlier versions of this manuscript were improved by the comments and suggestions of A. Pringle, P. Schultz, L. Moyle, J. Antonovics, R. Callaway, and two anonymous reviewers. C. Hall, M. Daymont, S. Johnson, and C. Tang provided technical assistance. We are particularly grateful to K. Mills and G. Abad for their advice and assistance in the isolation, culturing, and identification of the *Pythium* isolate used in the experiments. We acknowledge help from the Duke University Phytotron staff as well as funds awarded to the facility for research (NSF grant DEB-94-15541). The research was supported by USDA grant 96-35107-3311 and NSF grant DEB-96-15941.

Literature Citations

Abad, Z. G., H. D. Shew, and L. T. Lucas. 1994. Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. Phytopathology **84**:913–921.

Albrecht, S. L., Y. Okon, J. Lonnquist, and R. H. Burton. 1981. Nitrogen fixation by corn—*Azospirillum* association in a temperate climate. Crop Science **21**:301–306.

Bever, J. D. 1994. Feedback between plants and their soil communities in an old field community. Ecology **75**:1965–1977.

Bever J. D. 1999. Dynamics within mutualism and the maintenance of diversity: inference from a model of interguild frequency dependence. Ecology Letters **2**:52–62.

Bever, J. D., J. B. Morton, J. Antonovics, and P. A. Schultz. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology **84**:71–82.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561–573.

Claus, D., and R. C. W. Berkeley. 1996. Genus *Bacillus*. Pages 1105–1139 *in* P. H. A. Sneath, N. A. Mair, M. E. Sharpe, and J. G. Holt, editors. Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, Maryland, USA.

Derylo, M., and A. Skorupska. 1992. Rhizobial siderophore as an iron source for clover. Physiology of Plants **85**:549–553.

Faull, J. L., and R. Campbell. 1979. Ultrastructure of the interaction between take-all fungus and antagonistic bacteria. Canadian Journal of Botany **57**:1800–1808.

Fiddaman, P. J., and S. Rossall. 1993. The production of antifungal volatiles by *Bacillus subtilis*. Journal of Applied Bacteriology **74**:119–126.

Fowler, N. L. 1978. Competition and coexistence in an herbaceous plant community. Dissertation. Duke University, Durham, North Carolina, USA.

Fowler, N. L. 1982. Competition and coexistence in a North Carolina grassland. III. Mixtures of component species. Journal of Ecology **70**:77–92.

Fowler, N. L., and J. Antonovics. 1981. Competition and coexistence in a North Carolina grassland. I. Patterns in undisturbed vegetation. Journal of Ecology **69**:825–841.

Grime J. P., J. M. Macky, S. H. Hillier, and D. J. Read. 1987. Mechanisms of floristic diversity: evidence from microcosms. Nature **328**:420–422.

Handman, H., D. M. Weller, and L. S. Thomashow. 1991. Relative importance of fluorescent siderophores and other factors in biological control of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas flourecens* 2-79 and M4-80R. Applied Environmental Microbiology **57**:3270–3277.

Harris, A. R., K. Siwek, and B. M. Wiseman. 1997. Interactions between damping-off fungi, antagonists and *Capsicum* seedlings. Applied Soil Ecology **6**:251–263.

Hofte, H., and H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiological Review **53**:242–255.

Kim, D., R. J. Cook, and D. M. Weller. 1997. *Bacillus* sp. L324–92 for biological control of three root diseases of

wheat grown with reduced tillage. Phytopathology **87**:551–558.

Landa, B. B., A. Hervas, W. Bettiol, and R. M. Jimenez-Diaz. 1997. Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusarium oxysporum* f. sp. *ciceris*. Phytoparasitica **25**:305–318.

Martin, F. N. 1992. *Pythium*. Pages 39–49 *in* L. L. Singleton, J. D. Mihail, and C. M. Rush, editors. Soil borne phytopathogenic fungi. American Phytopathological Society Press, St. Paul, Minnesota, USA.

Mills, K. E., and J. D. Bever. 1998. Maintenance of diversity within plant communities: soil pathogens as agents of feedback. Ecology **79**:1595–1601.

Neitko, K. F., and W. T. Frankenberg, Jr. 1989. Biosynthesis of cytokinins in soil. Journal of the Soil Science Society of America **53**:735–740.

Packer, A., and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature **404**:278–280.

Pankhurst, C. E., H. J. McDonald, and B. G. Hawke. 1995. Influence of tillage and crop rotation on the epidemiology of *Pythium* infections of wheat in a red-brown earth of South Australia. Soil Biology and Biochemistry **27**:1065–1073.

SAS Institute. 1986. SAS System for Linear Models. SAS Institute, Cary, North Carolina, USA.

Silo-Suh, L., B. J. Lethridge, S. J. Raffel, H. He, J. Clardy, and J. Handelsman. 1994. Biological activity of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Applied and environmental microbiology **60**:2023–2030.

Smith, S. E., and D. J. Read. 1997. Mycorrhizal symbiosis. Second edition. Academic Press, San Diego, California, USA.

Srinivasan, M., D. J. Petersen, and F. B. Holl. 1996. Influence of indoleacetic-acid-producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gnotobiotic conditions. Canadian Journal of Microbiology **42**: 1006–1014.

Tate, R. L. 1995. Soil microbiology. John Wiley & Sons, New York, New York, USA.

Van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutogolis, R. Strietwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature **396**:69–72.

Westover, K. M., A. C. Kennedy, and S. E. Kelley. 1997. Patterns of rhizosphere microbial structure associated with co-occurring plant species. Journal of Ecology **85**: 863–873.

Wollum, A. G., II. 1982. Cultural methods for soil microorganisms. Pages 781–802 *in* A. L. R. H. Miller and D. R. Keeney, editors. Methods of soil analysis. Part 2: Chemical and microbiological properties. American Society of Agronomy, Madison, Wisconsin, USA.