EFFECTS OF INSECT HERBIVORY AND FUNGAL ENDOPHYTE INFECTION ON COMPETITIVE INTERACTIONS AMONG GRASSES[1]

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Abstract. Interactions among plants may be influenced by pests or parasites that differentially affect one competitor. The purpose of this study was to determine the effects of fungal parasitism and insect herbivory, alone and in concert, on plant competitive interactions. The effects of fungal endophyte (*Acremonium* spp.) infection and fall armyworm (*Spodoptera frugiperda*) herbivory on competitive interactions in one- and two-species mixtures of the grasses tall fescue (*Festuca arundinacea*), red fescue (*F. rubra*), and perennial ryegrass (*Lolium perenne*) were examined in greenhouse experiments. In general, herbivory reduced plant biomass whereas endophyte infection increased plant biomass. Endophyte-infected (E+) plants were less damaged by herbivory than uninfected (E−) plants of the same species. Studies on fall armyworm larval feeding and choice tests with the five grass species were generally consistent with the outcome of the competition experiments; E+ plants were less nutritious and less preferred than E− plants of the same species. There were significant interactions among factors so that the outcome of competition depended on the species identities and the presence or absence of endophytes and herbivores. In competition with Kentucky bluegrass (*Poa pratensis*), E+ and E− perennial ryegrass produced similar biomass in the absence of herbivory, but E+ perennial ryegrass had nearly twice the biomass of E− plants when herbivores were present. E+ and E− tall fescue were poor competitors with orchard grass (*Dactylis glomerata*) when herbivores were absent, but E+ tall fescue was a better competitor than E− plants and orchard grass when herbivores were present. This study indicates that competitive hierarchies among grasses are altered by interactions with insect herbivores and fungal endophytes, which have typically been ignored in past studies.

Key words: communities; competition; endophytes; fungi; grasses; herbivory; insects; interactions; Malathion; symbiosis.

INTRODUCTION

The effects of competition and insect herbivory on plant populations and communities have been major areas of ecological research over the past two decades, yet surprisingly the interaction of these two processes has rarely been considered experimentally (but see Bentley and Whittaker 1979, Windle and Franz 1979, Cottam et al. 1986, Louda et al. 1990). Competitively dominant species may be more apparent and subject to more herbivory than less frequent species, allowing weaker competitors to persist (Harper 1977, Windle and Franz 1979, Crawley 1983, Rai and Tripathi 1985). Differential herbivory can provide a competitive advantage to the least damaged species by reducing biomass and growth potential of the more damaged species (Crawley 1983, Cottam 1986). Studies where

herbivory is manipulated have demonstrated compositional changes in plant communities (Huntly and Inouye 1988, Brown and Gange 1989).

Many grasses are systemically infected by clavicipitaceous fungal endophytes that occur in aboveground plant tissues (Clay 1988, Clay 1990b). Most infections are asymptomatic, and the endophyte is transmitted maternally through the seeds of infected plants. Previous research has revealed that endophyte-infected (E+) grasses often are more vigorous and resistant to herbivory than uninfected (E−) conspecifics (Clay et al. 1985, Bacon et al. 1986, Read and Camp 1986, Clay 1990a). The proportion of E+ plants within both a population and a grassland community have been shown to increase over time (Large 1952, Clay 1984, Saha et al. 1987, Clay 1990a). These data, along with results of field and greenhouse competition studies (Kelley and Clay 1987, Marks et al. 1991), suggest that E+ grasses can competitively displace E− grasses.

The purpose of this study was to examine the interaction of insect herbivory, fungal endophyte infection, and plant competition on the growth of three grasses in greenhouse experiments. Our primary objective was to determine whether resistance to herbivory is an im-

portant mechanism in the documented competitive success of endophyte-infected grasses.

METHODS AND MATERIALS

Experimental organisms

Three perennial grasses were utilized in this study. Perennial ryegrass, *Lolium perenne* L. (abbreviated as PRG), is infected by the systemic fungal endophyte *Acremonium lolii* Latch, Christensen & Samuels (Latch et al. 1984). Seeds of the cultivar 'Repell,' which is highly infected (>95% of the seeds), were obtained from Loft's Seed Company (Bound Brook, New Jersey USA). Uninfected plants were obtained by heat-treating the seeds following the methods of Marks et al. (1991) in one experiment whereas uninfected plants in a subsequent experiment were obtained from the seeds produced by plants from heat-treated seeds. There is no difference in the growth of seedlings from heat-treated and untreated seeds (Marks et al. 1991). Tall fescue, *Festuca arundinacea* Schreb. (TF), is infected by *A. coenophialum* Morgan-Jones & Gams, which is similar to the endophyte of perennial ryegrass (Morgan-Jones and Gams 1982). Endophyte-infected (E+) and uninfected (E−) seed lots of cultivar 'Kentucky-31' were obtained from a common garden of E+ and E− plants located on the Indiana University campus. The third grass species utilized was red fescue, *Festuca rubra* L. (RF), which is often infected by the fungus *Epichloe typhina* (Pers.) Tul. (anamorph = *Acremonium typhinum*) (White and Morgan-Jones 1987). E+ and E− seed lots of cultivar 'Ensylva' were also obtained from a common garden of E+ and E− plants at Indiana University. In all three species the fungal endophyte infection is maternally transmitted through the seeds (Clay 1990b). This is the only mechanism of transmission in PRG and TF, but *E. typhina* infecting RF, in addition to maternal transmission, can fruit and produce ascospores (see Sampson 1933). However, fruiting did not occur on RF in this study.

For all three grasses the E+ and E− seed lots were not absolutely pure. E− seed lots contained some infected seeds but at infection rates of <10%; E+ seed lots contained >90% infected seeds. The levels of infection in plants from different seed lots were checked again after most of the experiments described below. Variation in the infection frequency of seed lots arises because (1) some of the maternal plants were not of the correct infection status and (2) the viability of endophytes in seeds declines with time more rapidly than does seed viability (Welty et al. 1987). It is difficult to obtain absolutely pure samples in experiments with large sample sizes, such as described here, because of the intensive labor required and because assessing the infection status of seeds is destructive. Statistical tests comparing mean values of E+ and E− categories are more conservative as a result of the low levels of contamination.

Two other grasses were used in this study. Kentucky bluegrass, *Poa pratensis* L. (KBG), served as an interspecific competitor against E+ and E− PRG, while orchardgrass (*Dactylis glomerata* L., OG) served as an interspecific competitor against E+ and E− TF. Neither KBG nor OG were infected. Seeds were obtained commercially. These grasses were chosen because they were similar in size, growth habit, phenology, and habitat requirements to their interspecific competitors and they often co-occur. While only KBG is native to North America, all of the species are now common, co-occurring components of many grassland communities.

The insect herbivore utilized was the fall armyworm (*Spodoptera frugiperda* Smith, Noctuidae, abbreviated as FAW), a generalist lepidopteran herbivore of grasses. Past research has shown that FAW larvae prefer to feed upon E− grasses and/or exhibit reduced survival and development on E+ grasses (Clay et al. 1985, Hardy et al. 1985, Cheplick and Clay 1988).

Preliminary experiments

Two preliminary experiments were conducted to determine the efficacy of insect releases and their effect on plant growth. In the first, equal numbers of PRG seedlings were transplanted into replicated pots, and when the plants were well established and tillering rapidly, neonate FAW larvae were released at a density of either 0, 2, or 4 individuals/pot. All larvae were removed after 10 d. A subset of pots in each herbivore treatment were harvested after 4, 6, 8, and 10 wk of regrowth, and their total aboveground dry biomass was determined. Analyses indicated a significant effect ($P < .02$ or less) of herbivory treatment at all four harvests. After 10 wk of regrowth, pots that initially had two or four larvae released produced only 85% and 58%, respectively, of the biomass of control pots. These results indicated that releasing neonate FAW larvae in pots was an effective technique for generating herbivore damage. A second, similar preliminary experiment with TF with two levels of herbivory (0 and 10 FAW/16 plants) resulted in a significant, 50% decrease in biomass when herbivores were present.

Greenhouse experiments

In all experiments the experimental design was based on a DeWit-type replacement series (Harper 1977) where density was held constant (four plants per pot) and only three proportions of plants were present (4:0, 2:2, 0:4), including interspecific mixtures. We recognize the limitations of this design and the criticisms it has received (Connolly 1986). However, our goal in these experiments was not to examine how intra- and interspecific competition varied with density and frequency but rather to understand how endophyte infection and insect herbivory altered the outcome of competition. Therefore, competitive design was held constant while infection, herbivory, and species composition were varied.

A total of six greenhouse experiments was conducted in 1987, 1988, and 1989. In all experiments seeds were initially planted in flats, and seedlings were transplanted to 10-cm^2 plastic pots filled with a steam-sterilized soil mixture (2 parts top soil : 1 part peat : 1 part perlite) at a constant density of 4 plants/pot. In mixtures of E+ and E− plants, the E+ plants were marked with a plastic ring. Plants were watered as necessary and fertilized biweekly. Environmental conditions in the greenhouse, which was unheated and received only natural light, were not identical each year, and no statistical comparisons are made between years.

The first two experiments examined intraspecific competition in PRG and interspecific competition with KBG. For the intraspecific competition experiment, 50 pots of four E+ plants, 50 of four E− plants (pure pots), and 100 pots with two E+ and two E− plants (mixed pots) were set up in early May 1987. The pots were arranged into four blocks each consisting of 12 or 13 pure E+ or E− pots and 25 mixed pots randomly arranged with respect to each other. Alternate blocks were designated as either herbivory or no-herbivory treatments and were separated by a 25-cm gap on the bench from the next block. After 9 wk of growth, when the plants were well established and growing vigorously, eight neonate FAW larvae were added per pot (2 larvae/plant) in the two herbivory blocks. A second release of 8 larvae/pot was at 15 wk. At 20 wk all plants were harvested and the aboveground dry biomass of each plant determined as before.

Our objective in the insect releases was to create visible herbivory damage—but not complete defoliation—on the experimental plants based on the results of the preliminary experiments, within the constraints imposed by egg production in our FAW colony. Actual damage would vary with insect survival, host plant suitability, host plant size and growth rate, temperature, and other environmental factors beyond our control. The use of insect releases to create damage was essential, despite the variable effects, given that insect choice represented a central part of our design. The success of our approach is evidenced by the significant effects of the herbivory treatments shown in the *Results* section.

In early June 1987, 50 pots of E+ PRG plants, 50 pots of E− PRG plants, 50 pots of KBG, 100 pots of E+ PRG and KBG mixture, and 100 pots of E− PRG and KBG mixture were planted, blocked, and arranged as in the intraspecific experiment. After 10 wk eight neonate FAW larvae were added to each pot in the two herbivory blocks. Another release of eight larvae per pot was made at 17 wk. At 20 wk a third insect release was made that differed from previous releases in that larvae were reared in culture on mixed grasses to the fourth or fifth instar and only 1 larvae/pot was released. Concerns about neonate survival prompted this change. After 24 wk all plants were harvested and their aboveground biomass was determined as before.

The following growing season two more greenhouse experiments using different grasses were conducted following the general procedures described above. In late June an intraspecific competition experiment with TF was set up consisting of 80 pots with E+ plants, 80 with E− plants, and 160 pots with 2 E+ and 2 E− plants. After 7 wk of growth, 1-wk-old FAW larvae (third to fourth instar) were released at a rate of 1–2/pot. Fewer numbers of larvae were available than desired resulting from problems with the insect colony. At 10 wk another group of 1-wk-old larvae were released at a rate of 1 larvae/pot. Towards the end of September and early October (weeks 14–16), a severe aphid infestation began to develop on the experimental plants. Several unidentified species appeared to be present and often reached densities of several hundred individuals per plant. Rather than making further FAW releases, we decided to reduce the populations of aphids in the no-herbivory blocks by spraying an insecticidal soap twice at weeks 14 and 16. Prior work has shown that many aphid species can strongly discriminate between E+ and E− plants (Clay 1991). Herbivory blocks were sprayed with water to serve as a control. All plants were harvested in week 20 and their aboveground biomass determined.

A parallel interspecific competition experiment consisted of 80 pots of E+ TF plants, 80 pots of E− TF plants, 80 pots of OG plants, 160 pots with two E+ TF and two OG plants, and 160 pots with two E− TF and two OG plants. Pots were set up on 5 July, a week after the intraspecific competition experiment. After 9 wk a single 1-wk-old (third or fourth instar) FAW larva was released per pot and after 12 wk two additional larvae of the same age were released per pot in the two herbivory blocks. The aphid infestation also affected the interspecific experiment and, as before, water and insecticidal soap was sprayed on the herbivory and no-herbivory blocks, respectively, in weeks 13 and 15. All plants were harvested and biomass determined after 20 wk.

In 1989 two more experiments were conducted with RF. To examine intraspecific competitive interactions, 40 pots of E+ RF, 40 pots of E− RF, and 80 pots of two E+ RF and two E− RF plants were set up in late April 1989. Based on the previous two years of research, which revealed substantial colonization of experimental plants in the greenhouse with a variety of insects, we elected to both increase herbivory by releasing FAW larvae and decrease herbivory in the no-herbivory blocks by spraying plants with the insecticide Malathion. A separate experiment designed to determine the effects of Malathion on plant growth was conducted (see *Effect of Malathion on plant growth,* below). Twelve weeks after planting, the no-herbivory blocks were sprayed with Malathion while the herbivory blocks were sprayed with water as a control. Two days later four neonate FAW larvae were released on each pot in the herbivory blocks. After 15 wk the no-

herbivory plots were sprayed again and 5 neonate larvae/pot were released the following week. At 25 wk pots in the no-herbivory blocks were sprayed with Malathion a third and final time and the following week five additional neonate larvae per pot were released in the herbivory blocks. All plants were harvested, dried, and weighed at 31 wk.

A parallel interspecific competition experiment was established at the same time as the intraspecific experiment using RF and PRG. Because both species were E+ or E−, four combinations of mixtures were planted in all permutations of E+ and E− RF with E+ and E− PRG (80 pots each per combination). In addition, 40 pots each were planted with all E+ RF, all E− RF, all E+ PRG, and all E− PRG. Dates for Malathion spraying, insect release, insect numbers, and plant harvest were the same as for the intraspecific competition experiment.

For all greenhouse experiments only biomass data were analyzed. PRG, RF, and KBG all require vernalization to flower so that no plants flowered in the greenhouse. In contrast, both TF and OG flowered profusely in the greenhouse towards the end of the experiments. The numbers of flowering culms produced were recorded but were highly correlated with biomass. In no interspecific competition experiments did one species flower while the competing species did not.

Fall armyworm feeding and preference tests

In order to compare the food quality of the various grasses used in this study, a series of feeding trials was conducted with FAW following the techniques of Hardy et al. (1985). In brief, neonate larvae were placed individually in petri plates and continuously provided with a supply of fresh leaves from one type of plant only. Forty replicates per plant–infection category were used. There were eight categories of plants: E+ and E− PRG, TF, and RF, plus E− KBG and OG. Developmental parameters measured included survival to pupation, 10-d-old larval mass days to pupation, and pupal mass. These are standard measures of food quality for lepidopteran herbivores and reflect the potential for population growth in the field (Clay et al. 1985, Hardy et al. 1985).

Preference tests were also conducted (Hardy et al. 1985) where two categories of grass were offered simultaneously to larvae. Only those 11 combinations relevant to the greenhouse experiments were used; for example, E+ vs. E− TF, E+ TF vs. OG, and E− TF vs. OG. Each test consisted of 30 petri plates with two grass leaves, one from each type of grass. Five neonate larvae were placed in each petri plate between the leaves. The plates were kept in a growth chamber at 22°C in the dark for 24 h until they were scored. The number of larvae on each type of leaf, or off the leaves, was noted and a subjective ranking of feeding damage to each type of leaf was made where 0 = no damage, 1 =

0–5% of leaf damaged, 2 = 5–20% of leaf damaged, and 3 = >20% of leaf damaged (Hardy et al. 1985).

Effect of Malathion on plant growth

In order to control for the possible effect of Malathion spraying on plant growth, an experiment was conducted with E+ and E− PRG, TF, and RF and E− OG. Previous research has shown little or no effect of Malathion on plant growth (Brown et al. 1987). Twenty plants in each category were planted individually in 15 cm deep tubes (volume: 72 cm³) and randomly arranged on the greenhouse bench. After 14 wk of growth the plants were randomized into two blocks, and plants in one block were sprayed with Malathion while plants in the other block were sprayed with distilled water. At 16 and 18 wk the blocks were randomly arranged again on the bench and sprayed as before. At 24 wk all plants were harvested and their aboveground dry biomass was determined.

Statistical analyses

All analyses were conducted using the SAS statistical packages (Helwig and Council 1985) and all biomass data were log-transformed before statistical analyses to meet the assumptions of normality for the analysis of variance. Data from each greenhouse experiment with PRG, TF, and RF were analyzed separately.

Biomass per plant data from the greenhouse intraspecific competition experiments were analyzed with two-way ANOVA, where herbivory and planting combination were the two fixed main effects. There were three planting combinations (all E+, all E−, and the E+/E− mixture); because the grass species composition was held constant, planting combination reflects the effect of endophyte infection. The interspecific competition experiments were analyzed using three-way ANOVA following Marks et al. (1991), where herbivory, identity of the target plant, and identity of the competitor were fixed main effects. "Target" refers to the identity of the plants whose biomass was analyzed (e.g., E+ or E− PRG, KBG) while "competitor" refers to the identity of the plants that the target was grown with (again, E+ or E− PRG, KBG). Significant target effects indicate differences in a plant's ability to grow under various planting combinations, while competitor effects indicate a plant's ability to interfere with other plant's growth. Block effects were analyzed initially but were never found to be significant, so subsequent analyses did not include this variable.

FAW feeding experiments were analyzed using one-way ANOVA where the identity of the food grasses was the main effect and insect developmental parameters were the dependent variables. Both the overall effects of the plant species–infection combination and specific E+ vs. E− contrasts within a grass species were analyzed. Larval preferences were analyzed using the Wilcoxon matched-pairs signed-ranks test (Siegel 1956)
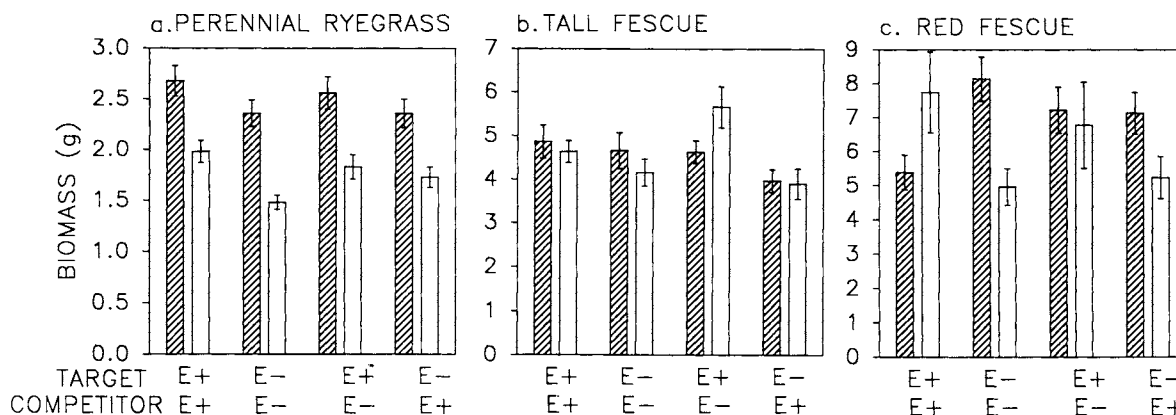
FIG. 1. Biomass production of E+ (endophyte-infected) and E− perennial ryegrass (PRG), tall fescue (TF), and red fescue (RF) in intraspecific combinations. Hatched bars represent no-herbivory treatments, open bars indicate herbivory treatments. Means ± 1 SE are shown.

and subjective feeding damage ranks were analyzed with t tests.

The effect of Malathion on the growth of grasses was analyzed with one-way ANOVA, where insecticide treatment was the fixed main effect and each grass species–infection combination was analyzed separately.

RESULTS

Greenhouse experiments

Intraspecific competition.—For perennial ryegrass (PRG), two-way ANOVA indicated significant effects of both herbivory ($P < .005$) and plant combination ($P < .05$) but not their interaction. Endophyte-infected (E+) plants in pure stands (target and competitor are the same) had the largest biomass both with and without herbivory (Fig. 1a). Uninfected (E−) plants in mixture or in pure stands had the lowest biomass both with and without herbivory (Fig. 1a).

In tall fescue (TF) there was no effect of herbivory or its interaction with planting combination, but there was a highly significant effect of planting combination ($P < .005$). There were little differences in biomass in three of the four planting combinations between the herbivory and no-herbivory treatments, although the no-herbivory treatments had slightly higher means (Fig. 1b). In contrast, E+ plants in mixtures with E− plants

had more biomass in the herbivory treatment than in the no-herbivory treatments (Fig. 1b). E+ plants in mixture with E− plants subjected to herbivory produced significantly more biomass than any of the other seven combinations (Fig. 1b).

For red fescue (RF) there was a significant effect of herbivory ($P < .05$), and a significant interaction between herbivory and planting combination ($P < .05$) on aboveground biomass. In pure stands E− plants had more biomass than E+ plants in the absence of herbivory, but with herbivory the relationship was reversed (Fig. 1c). Herbivory increased the biomass of E+ plants in pure stands. E+ and E− plants had similar aboveground biomasses in mixtures without herbivory but E− plants exhibited a greater decline in biomass than E+ plants with herbivory (Fig. 1c).

Interspecific competition.—When E+ and E− PRG were grown in competition with Kentucky bluegrass (KBG) there were highly significant main effects of herbivory, target plant, and competitor (Table 1). Further, all two-way interactions were also significant. In pure stands E+ plants had greater biomass than E− plants both with and without herbivory (Fig. 2), paralleling

TABLE 1. *F* values from three-way ANOVA of interspecific competition experiments. Abbreviations defined in Table 2.

Effect	Experiment		
	PRG vs. KBG	TF vs. OG	RF vs. PRG
Herbivory (H)	27.6‡	0.2	20.1‡
Target plant (T)	178.3‡	29.2‡	22.7‡
Competitor (C)	15.1‡	15.2‡	40.2‡
H × T	13.0‡	6.7†	6.9‡
H × C	6.2†	12.6‡	7.1‡
T × C	14.4‡	5.2*	8.1‡
H × T × C	1.2	4.6*	1.5

* $P < .05$, † $P < .005$, ‡ $P < .0005$.

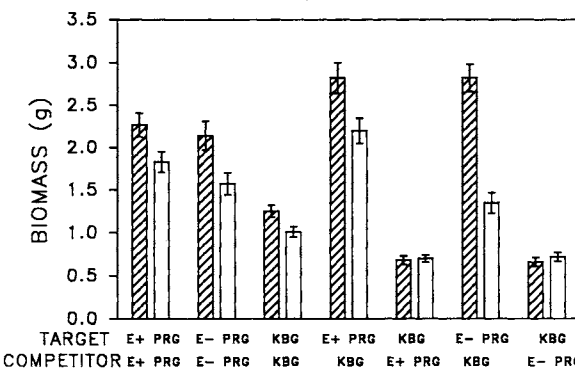PERENNIAL RYEGRASS vs. KENTUCKY BLUEGRASS

FIG. 2. Biomass production of E+ and E− PRG in competition with KBG (see Fig. 1 for explanation of abbreviations). Hatched bars represent no-herbivory treatments, open bars indicate herbivory treatments. Means ± 1 SE are shown.
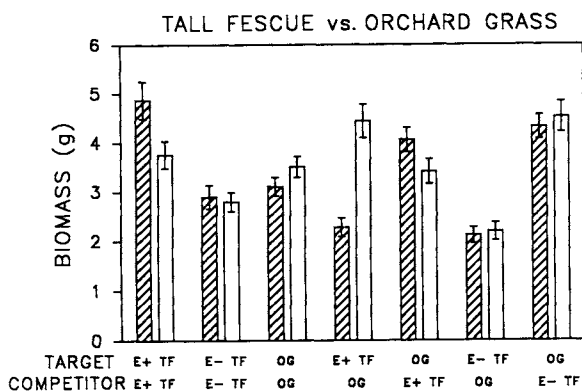
TALL FESCUE vs. ORCHARD GRASS

FIG. 3. Biomass production of E+ and E− TF in competition with OG (orchardgrass). Abbreviations defined in Fig. 1. Hatched bars represent no-herbivory treatments, open bars indicate herbivory treatments. Means ± 1 SE are shown.

the results of the intraspecific competition experiment (Fig. 1a). In all three types of pure stands, herbivory caused a significant decline in biomass (Fig. 2). In mixtures with KBG, E+ and E− PRG plants had the same biomass without herbivory, but with herbivory, E− produced only 61% of the biomass of E+ plants (Fig. 2). Overall, KBG had lower biomass in pure stands than either E+ or E− PRG, but in mixtures with PRG, herbivory had no impact on biomass production of KBG compared to a 20% reduction in biomass in pure stands (Fig. 2). The competitive advantage of PRG over KBG declined with herbivory, especially for E− PRG.

As in the intraspecific competition experiment, there was no significant effect of herbivory in the TF-OG (orchardgrass) competition experiment (Table 1). There were, however, significant main effects of target plant and competitor, and all two- and three-way interactions were significant (Table 1). In pure stands, E+ TF plants yielded more than E− plants both with and without herbivory (Fig. 3), again paralleling the results of the intraspecific competition experiment (Fig. 1b).

However, E+ plants suffered a significant reduction in biomass with herbivory, unlike E− plants (Fig. 3). The mean biomass of OG plants in pure stands was intermediate to E+ and E− tall fescue, and its mean biomass with and without herbivory was not significantly different (Fig. 3). In mixtures with OG, in the absence of herbivores, E+ and E− TF plants had similar biomasses; but when herbivores were present E+ plants had twice the biomass of E− plants and twice the biomass of E+ plants in the no-herbivory treatments (Fig. 3). In the absence of herbivores, E+ TF produced significantly less biomass than OG in mixture, but when herbivores were present E+ plants produced significantly more biomass than OG in mixture. Endophyte infection greatly increased competitive success of TF vs. OG when herbivores were present.

Mixtures in the RF–PRG competition experiment consisted of all permutations of E+ and E− plants since both species occurred in both E+ and E− forms. There were significant main effects of herbivory, target plant, and competitor (Table 1). In addition, all two-way interactions between the main effects were also significant.

Considering first pure stands of red fescue, E+ plants produced slightly more biomass than E− plants without herbivory, but the relationship was reversed with herbivory, E+ RF suffered a proportionally greater reduction in biomass with herbivory than E− RF (Fig. 4a). This result contrasted with the results from the intraspecific experiment (Fig. 1c). In pure stands, herbivory significantly reduced biomass production of both E+ and E− PRG but E− plants exhibited a greater proportional decline in biomass with herbivory than E+ plants (Fig. 4a). E+ PRG plants produced significantly more biomass than E− plants with herbivory.

RF was the inferior competitor to PRG, as indicated by the lower biomass per RF plant in mixtures compared to pure stands in all herbivory treatments and planting combinations (Fig. 4b). However, there was a tendency for herbivory to increase the biomass of RF
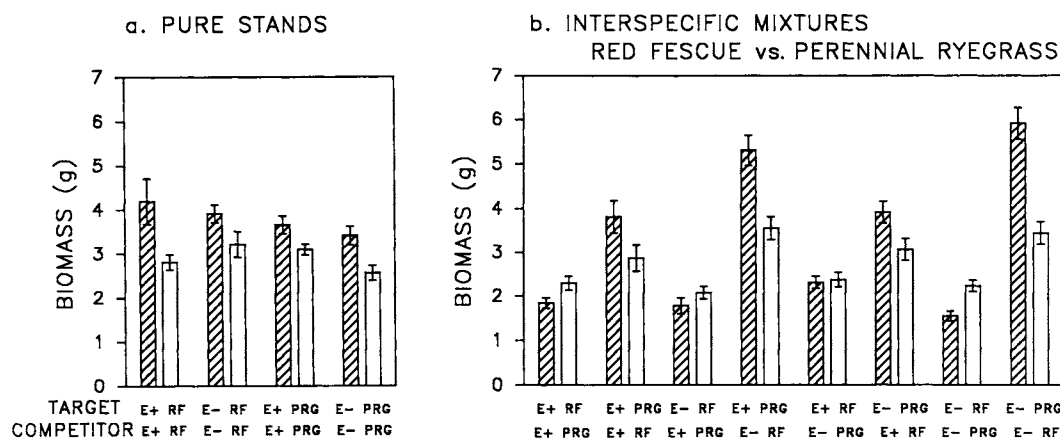
a. PURE STANDS

b. INTERSPECIFIC MIXTURES
RED FESCUE vs. PERENNIAL RYEGRASS

FIG. 4. Biomass production of E+ and E− RF (red fescue) in competition with E+ and E− PRG. Abbreviations defined in Fig. 1. Hatched bars represent no-herbivory treatments, open bars indicate herbivory treatments. Means ± 1 SE are shown.

TABLE 2. Survival and development of fall armyworm (FAW) larvae on grasses utilized in the competition experiments (means ± 1 SE). E+ = endophyte infected, E− = endophyte-free, PRG = perennial ryegrass, TF = tall fescue, RF = red fescue, KBG = Kentucky bluegrass, OG = orchardgrass.

Food plant	10-d mass (mg)	Survival to pupation (%)	Pupal mass (mg)	Days to pupation
E+ PRG*	26.3 ± 2.4	75	166.7 ± 2.3	18.4 ± 0.3
E− PRG	35.6 ± 2.9	65	155.4 ± 3.3	18.7 ± 0.3
E+ TF	18.0 ± 2.9	8	181.0 ± 12.3	23.7 ± 1.7
E− TF	37.0 ± 2.6	63	177.9 ± 5.3	21.0 ± 0.3
E+ RF	⋯	0	⋯	⋯
E− RF	33.4 ± 2.2	43	162.9 ± 4.8	20.9 ± 0.3
KBG	2.3 ± 0.0	0	⋯	⋯
OG	11.9 ± 2.3	33	164.6 ± 7.2	25.5 ± 0.5

in mixtures with PRG in all combinations relative to the no-herbivory mixtures, and these differences were significant where the infection status of the two species was the same (Fig. 4b). The difference in performance of E+ and E− RF was greatest when grown with E− PRG in the absence of herbivory (i.e., 2.31 g vs. 1.54 g).

In general, PRG performed better in mixtures with RF than it did in pure stands, especially in the absence of herbivory (Fig. 4b). Unlike the situation with RF, biomass of PRG declined in every planting combination when herbivores were present (Fig. 4a and b). E+ RF had a relatively greater inhibitory effect on both E+ and E− PRG than did E− RF, both with and without herbivory. There was no evidence that endophyte infection enhanced the competitive ability of PRG in mixture with RF.

At the conclusion of the competition experiments, the level of endophyte infection was checked again. For PRG, 18 of 20 E+ plants were observed to contain the endophyte (90%) while 0 of 20 E− plants were infected. In RF, 5 of 5 E+ plants were infected and 0 of 5 E− plants were infected. Inadvertently, the data for TF infection frequencies in the greenhouse experiments were lost. However, in field plots established

from the same seed lots, 9 of 13 E+ plants were infected (69%) while 4 of 16 E− plants contained endophyte hyphae (25%). The highly significant effects of combination in the intraspecific experiments and target in the interspecific experiments (Table 1) are therefore conservative given the differences in infection rate in E+ and E− TF seed lots.

FAW feeding and preference experiments

Feeding experiments conducted with FAW larvae demonstrated that there were large differences among the grasses with respect to the ability of the insects to survive and develop (Table 2). In terms of 10-d larval masses and survival to pupation, E− PRG, E− TF, and E− RF were the best food plants. KBG and OG were relatively poor food plants compared to E− plants of the other three species. Endophyte infection had the most detrimental effects on FAW larvae in RF where no larvae survived to 10 d, and the least detrimental effects in PRG (Table 2).

Larval preference tests showed that larvae discriminated between food grasses when two choices were offered simultaneously (Table 3). When E+ and E− leaves of the same species were offered together, more larvae were found on E− leaves than E+ leaves for all

TABLE 3. Preference and feeding damage of neonate fall armyworm larvae in paired plant samples. Abbreviations as in Table 2.

Paired sample		Percentage of larvae on leaf		Feeding damage to leaf (subjective ranks)	
A	B	A	B	A	B
E+ PRG	E− PRG	33	40	1.43	2.17*
E+ TF	E− TF	34	39	0.96	1.26
E+ RF	E− RF	20	41*	1.13	2.03*
E+ PRG	E− KBG	28	57*	1.26	2.37*
E− PRG	E− KBG	36	54*	1.10	1.77*
E+ TF	E− OG	9	62*	0.23	4.33*
E− TF	E− OG	22	55*	1.00	2.13*
E+ RF	E+ PRG	5	65*	0.27	2.87*
E+ RF	E− PRG	4	61*	0.30	2.93*
E− RF	E+ PRG	12	53*	0.40	2.57*
E− RF	E− PRG	9	65*	0.33	2.87*

* denotes a significant difference (P < .05) in the paired sample (larvae preferences: Wilcoxon matched-pairs signed-ranks test; feeding damage ranks: Student's t tests).

TABLE 4. Aboveground biomass (means ± 1 SE) of Malathion-treated and untreated grasses used in the competition experiments (except KBG). *F* and *P* values from the one-way ANOVAs. Abbreviations as in Table 2.

| | Mean biomass (g) | | | |
| | Malathion-treated | Untreated | *F* | *P* |
Plant				
E+ PRG	0.60 ± .07	0.53 ± .03	1.79	<.19
E− PRG	0.67 ± .05	0.59 ± .04	1.10	<.30
E+ TF	1.13 ± .07	1.02 ± .05	0.70	<.41
E− TF	0.87 ± .03	0.95 ± .06	0.38	<.55
E+ RF	0.49 ± .02	0.52 ± .02	0.59	<.45
E− RF	0.54 ± .04	0.48 ± .03	0.88	<.36
OG	1.08 ± .06	1.00 ± .06	0.54	<.47

three species, although significant differences occurred only in RF. When two species were offered in the choice tests, much stronger discrimination occurred, with species differences outweighing infection differences. FAW larvae significantly discriminated between species in all combinations tested, with KBG being preferred over PRG, OG over TF, and PRG over RF, regardless of their infection status (Table 3). The preference for OG and KBG occurred despite their poor food quality, as indicated by the feeding tests (Table 2). Patterns of feeding damage paralleled feeding location. Feeding damage was significantly less for E+ plants of PRG and RF in conspecific combinations, and was significantly less for PRG with KBG, TF with OG, and RF with PRG (Table 3).

Effect of Malathion on plant growth

The insecticide Malathion, which was utilized in experiments involving RF, ws found to have no significant effect on the growth of either E+ or E− RF (Table 4). Furthermore, there were no significant effects of Malathion on the growth of E+ and E− TF, E+ and E− PRG, and OG (KBG was not tested).

DISCUSSION

Recent investigations have demonstrated that endophyte infection is common in many grasses, especially in temperate grasses with the C-3 photosynthetic mechanism (White 1987, Clay and Leuchtmann 1989). Because most endophytes are seed-borne and incapable of contagious spread, the dynamics of infection within host populations is entirely a function of the relative survival, growth, and reproduction of infected vs. uninfected plants. Several studies have shown that the proportion of infected plants within populations increases over time, implying a fitness advantage for infected plants (reviewed in Clay 1990c). For example, in a 3-yr demographic study in a natural plant community where both competition and herbivory were present (but not quantified), the mean fitness of endophyte-infected (E+) tall fescue (TF) was twice that of infected (E−) TF (Clay 1990a). Two possible mechanisms for the success of E+ grasses are the direct

stimulation of plant vigor by endophyte infection (Clay 1990b, Marks et al. 1991) and enhanced resistance to herbivory (Clay et al. 1985, Cheplick and Clay 1988).

Our results support both possible mechanisms. The greenhouse experiments indicated that the performance of grasses in competition with other grasses can be significantly affected by insect herbivory, fungal endophyte infection, and their interaction. In both the intraspecific and interspecific competition experiments where herbivory was absent, E+ plants of perennial ryegrass (PRG), TF, and red fescue (RF) typically had higher yields in pure stands than E− plants. Fall armyworm (FAW) herbivory generally led to a reduction in plant biomass compared to plants grown in the absence of herbivory, but the magnitude of the reduction depended on infection status and the identity of its competitors. In natural communities, enhanced vigor and resistance to herbivores may interact synergistically to provide a greater benefit to host plants than either factor acting alone.

Despite differences among years and species in the rate of herbivory, as indicated by the numbers of larvae released and the final level of herbivory, we detected a significant effect of herbivory. Yields were reduced by herbivory compared to no-herbivory treatments in five of six comparisons in the intraspecific experiments and seven of eight comparisons in the interspecific experiments. However, biomass of E+ RF was nearly one-third higher in the herbivory vs. no-herbivory treatment in the intraspecific competition experiment (Fig. 1c). In contrast, pure stands of E+ RF exhibited lower yields in the herbivory treatment in the interspecific competition experiment with PRG (Fig. 4a), indicating that the increase in biomass with herbivory was not consistent. Equal numbers of larvae were applied to both groups of RF in the intraspecific and interspecific experiments, and both experiments occurred at the same time in the same greenhouse. We conclude that there was some source of unexplained variation leading to this inconsistent result. There was a slight, nonsignificant increase in orchardgrass (OG) biomass with herbivory in pure stands (Fig. 3). In mixtures of E+ and E− plants of the same species, herbivory reduced biomass in PRG and RF, but in mixtures of E+ and E− TF, herbivory was associated with greater biomass of E+ plants and total biomass per pot relative to the no-herbivory treatment (Fig. 1b). These data, from short-term greenhouse experiments, suggest that herbivory may increase biomass production in monospecific stands of mixed E+ and E− grasses of some species. Insect and vertebrate grazing have been shown, or hypothesized, to increase productivity of grasses and other plants (Dyer and Bokhari 1976, McNaughton 1983, Cargill and Jefferies 1984, Paige and Whitham 1987, Whitham et al. 1991).

In the feeding studies, endophyte infection had the greatest effect on resistance to herbivory in RF. No FAW larvae survived to 10 d on E+ RF although there

was 70% survival on E– RF. A previous study also found that FAW larvae were incapable of survival on E+ RF (Cheplick and Clay 1988). Feeding studies with other E+ and E– fescue species (*F. arundinacea, F. obtusa, F. versuta*) have indicated considerable variation in the effect of endophyte infection on plant resistance to herbivory (Clay et al. 1985, Cheplick and Clay 1988, this study). Knowledge of endophyte infection status does not in and of itself define the plant's resistance properties. FAW feeding studies with >15 grass species suggest that endophyte infection has the greatest effect on plant resistance to herbivory in grass species of intermediate food value to the herbivore (Clay et al. 1985, Cheplick and Clay 1988).

Increases in biomass with herbivory occurred more frequently in mixtures where FAW larvae could move between species or infection types growing in the same pot. Preference tests clearly showed that larvae were capable of discriminating among plants and that they preferred certain grasses over others. E– leaves were preferred over E+ leaves within a grass species, and there was a clear preference between grass species regardless of their infection status as follows: KBG > PRG, OG > TF, and PRG > RF (Table 3). In all combinations of RF and PRG, herbivory increased the yields of RF, relative to the same plants in the no-herbivory treatments, and decreased the yields of PRG. Similarly, herbivory enhanced yields of E+ TF growing with E– TF and with OG. The increasing yields in mixtures with herbivory probably resulted from behavioral responses by the larvae to feed on the more preferred species. Greater damage to the more preferred species (or infection type) would allow the less preferred species to capture proportionally more resources than it could in pure stands or when herbivory was absent.

Herbivore exclusion experiments, conducted primarily by erecting fences to prevent mammalian herbivores, have demonstrated that inferior competitors can rapidly disappear when competitively superior species are allowed to grow unchecked by herbivory (Tansley and Adamson 1925, Crawley 1988). Similarly, distasteful species can increase with heavy grazing as more palatable species are preferentially consumed (Smith et al. 1974). Several previous studies have shown that insect herbivory can also influence plant competitive interactions (Bentley and Whittaker 1979, Windle and Franz 1979, Cottam et al. 1986). Similar effects occur in marine systems where limpet grazing reversed the competitive hierarchy between two marine algae (Steneck et al. 1991). The results reported here suggest that species differ in their physiological response to herbivory, and the impact of herbivory in interspecific mixtures depends on the identities of the competitors, their response to herbivory, and the behavior of the herbivore.

Artificial defoliations have been used to examine the role of insect herbivory on plant competition (Lee and

Bazzaz 1980, Fowler and Rausher 1985). The resultant changes in competition may not be realistic given the variable impact on different species that herbivore choice creates. The results presented here clearly show that herbivore discrimination can occur even within a species as a result of endophyte infection. Further, previous work has shown that FAW feeding on grasses varies with leaf age and larval age, as well as with endophyte infection (Hardy et al. 1985). Although we were not able to quantify the amount of herbivory, and the impact of herbivory varied with species and planting combination, we believe that our results more accurately reflect natural processes than would more precise clipping experiments.

Despite the usual shortcomings of short-term greenhouse experiments, our results indicate the potential importance of the interactions of competition, herbivory, and endophyte infection. Results from other studies suggest that factors not considered here would not change the conclusions of this study. First, endophyte infection of PRG and TF seeds has been shown to stimulate seed germination, seedling growth, and resistance of seeds and seedlings to herbivory (Stewart 1985, Clay 1987, Cheplick and Clay 1988, Madej and Clay 1991). Bypassing the seed and seedling stage in this study probably underestimates the significance of herbivory and endophyte infection in plant competitive interactions. Second, studies where plant densities were explicitly altered gave results qualitatively similar to those here (K. Clay, *unpublished data,* Marks et al. 1991). Third, results of many longer-term field experiments with a variety of grasses were also consistent with those reported here (Bradshaw 1959, Harberd 1961, Clay 1984, Read and Camp 1986, Clay 1990*a*). Finally, the results of FAW feeding and preference were similar to those found for other herbivorous insects and other grasses (Clay 1991*a*).

An important conclusion of this study is that competitive ability in the grasses examined here—and by extrapolation in other plants—is conditional upon a variety of factors. The identity of the competitors, the infection status of the host plant, and the intensity of herbivory are all variable factors, the particular combination of which will alter competitive interactions. Additional careful experimental manipulations are needed to separate these effects and their interactions on plant growth.

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