

The influence of edaphic and competitive factors on productivity of marsh reed grass (*Calamagrostis canadensis*) in a cooperative pathosystem

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Abstract: *Calamagrostis canadensis* (Michx.) Beauv., marsh reed grass, hinders the regeneration of conifer seedlings. The influence of nutrients, autoallelopathy, and intraspecific competition on the productivity of greenhouse-grown marsh reed grass inoculated with a combined *Fusarium avenaceum* (Fr.:Fr.) Sacc. – *Pseudomonas* sp. pathosystem was studied. The productivity of uninoculated plants increased following fertilization, but these increases were not observed in inoculated plants. Leaf damage in inoculated plants was enhanced by P and K fertilization, but shoot height was not affected. Autoallelopathic straw extract interacted with intraspecific competition to nearly double pathogenic inhibition of shoot growth at high levels of competition. The straw extract also interacted with N fertilizer to reduce shoot productivity. Shoot growth was inhibited at higher levels of intraspecific competition. Responses to nutrients, autoallelopathy, and competition may be useful in developing improved biological control strategies.

Key words: *Fusarium avenaceum*, rhizobacteria, biocontrol, bioherbicide, fertility, soil ecology.

Résumé : La calamagrostide du Canada (*Calamagrostis canadensis* (Michx.) Beauv.) nuit à la régénération par les plantules de conifères. Les auteurs ont étudié l'influence des nutriments, l'autoallélopathie et la compétition intraspécifique sur la productivité d'agrostides cultivées en serres, et inoculées avec le pathosystème *Fusarium avenaceum* (Fr.:Fr.) Sacc. – *Pseudomonas* sp. (FPCP). La productivité des plantes non-inoculées augmente suite à une fertilisation, mais on n'observe pas ces augmentations chez les plantes inoculées. Chez les plantes inoculées, les dommages foliaires sont accrus par la fertilisation avec le P et le K, mais la hauteur des tiges n'est pas affectée. L'extrait de paille autoallélopathique interagit avec la compétition intraspécifique pour presque doubler l'inhibition d'origine pathologique de la croissance des tiges, en fortes compétitions. Les extraits de paille interagissent également avec la fertilisation azotée pour réduire la productivité des tiges. La croissance des tiges est inhibée aux niveaux de compétition intraspécifique plus élevés. La réaction aux nutriments, l'autoallélopathie et la compétition pourraient être utiles dans le développement de stratégies de maîtrise biologique améliorées.

Mots clés : *Fusarium avenaceum*, rhizobactéries, maîtrise biologique, bioherbicide, fertilité, écologie du sol.

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Introduction

The microbial ecology of endemic weed pathogens is an important consideration in biological control research. Although many pathogens have been investigated for use as potential bioherbicides (TeBeest 1991), their success has been partially limited by constraining biological and environmental factors (Auld and Morin 1995). Induced resistance (Auld et al. 1990; Weete 1992; Winder 1997), biotypical resistance (Okoli et al. 1997), morphological adaptations (Auld and Morin 1995; Winder and Van Dyke 1990), endophytic antagonism (Winder 1997), and various

environmental factors (Auld and Morin 1995; Winder and Macey 1998) have all been cited as real or potential constraints to the successful use of endemic pathogens. It is well documented that these kinds of constraints can be used to control crop diseases, but it may also be possible to reduce these constraints to enhance the efficacy of biological control agents.

Marsh reed grass (*Calamagrostis canadensis* (Michx.) Beauv.) can be used to examine many of the above constraints. The grass hinders coniferous regeneration in boreal forests (Liefers et al. 1993) and is a potential target for biological control efforts (Macey and Winder 1997; Mallet et al. 2000; Winder 1992, 1997, 1999a, 1999b). In North America, biological control research concerning marsh reed grass has focused on the possible use of endemic agents because the plant itself is endemic to Canada and Alaska (Hultén 1968). Mature perennial plants are the proximate cause of straw accumulation and suppression of conifer seedlings, but seedlings represent a more vulnerable target for biological control. The seeds of marsh reed grass are

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very light and <2 mm long. Wind disseminates them through the air beginning in late summer and over the snow surface in winter. Despite the presence of some perennial plants prior to harvesting, genetic evidence suggests that seedlings are the primary mode of dispersal into newly harvested forest areas (Macdonald and Lieffers 1991). After colonizing a location, the grass completes spread and colonization via underground rhizomes and attains a substantial size and thatch that makes it difficult to control by any means. By hindering infestations at the earliest stage, biological control agents that attack seedlings could suppress the grass population long enough for conifers to achieve free-to-grow status.

Although it is possible to damage seedling shoots with common endemic fungi such as *Fusarium avenaceum* (Fr.:Fr.) Sacc. (Winder 1999b), the apical meristem and underground portions of marsh reed grass survive, allowing the seedlings to regenerate (Winder and Macey 1998). When *F. avenaceum* is combined with common rhizobacteria that are mildly pathogenic to the roots, the resulting cooperative infection causes more damage than either pathogen alone (Macey and Winder 1997; Winder and Macey 1998). However, the efficacy of this pathogen combination is variable. Potential constraints in this *F. avenaceum* – *Pseudomonas* sp. cooperative pathosystem (FPCP) need to be identified before proceeding with field tests. For example, variable soil fertility is known to alter the influence of rhizobacteria on plant growth (de Freitas and Germida 1992; Jensen and Nybroe 1999; Sato and Jiang 1996; Bethlenfalvai et al. 1997; Palaxo et al. 1997; Ruppel and Gransee 1998). Amendment of soil with plant residues also alters growth conditions for rhizobacteria, consequently affecting the physiological response and growth of the host (Jensen and Nybroe 1999). Fertility, allelopathy, and competition are edaphic factors that may also act to intensify or ameliorate the damage caused by microbial biological control agents and plant diseases (Winder and Van Dyke 1990; Paul and Ayres 1986a, 1986b, 1987; Winder 1997; DiTommaso et al. 1996; Guntli et al. 1999).

This study examines the influence of edaphic and competitive factors on the productivity of marsh reed grass exposed to the FPCP pathogens under greenhouse conditions. Although edaphic influences originate in the rhizosphere, we focused primarily on their foliar impacts. Foliar impacts were considered to be the most relevant because profuse accumulation of aboveground foliage retards soil thaw and causes damage when heavy snowfall causes straw to smother conifer seedlings. The shoots therefore represent the primary deleterious effect of this grass on conifer seedlings (Lieffers et al. 1993). Soil nutrients, autoallelopathy, and intraspecific competition were the particular factors examined. The specific objectives of this study were to determine the interactive effects of autoallelopathy versus competition, autoallelopathy versus nutrients, and competition versus nutrients on the efficacy of the FPCP in marsh reed grass seedlings.

Materials and methods

Procedures common to all experiments

Seeds of marsh reed grass were harvested in August 1996 from a clear-cut area near mile 73 of the Alaska Highway (56°33'27"N, 121°12'9"W, elevation 879 m). Collected seeds were stored in a

paper bag at room temperature and sown the following spring in 10-cm-diameter plastic pots (5 per pot) containing a 3:1 mixture of sterilized peat and vermiculite amended with 3.2 kg/m³ slow-release fertilizer (Osmocote 18–6–12, 8- to 9-month release rate, Sierra Chemical Co., Milpitas, Calif.). The pots were placed in plastic dishes to contain water and nutrients. The peat-vermiculite mixture was selected because it approximates the peat or litter found in the humus of boreal soils and because marsh reed grass roots are largely confined to that organic layer (Comeau et al. 1982; Haeussler et al. 1990; Younkin 1973, 1974). In experiments requiring nutrient manipulations, the slow-release fertilizer was not added. Three randomly chosen 500-mL lots of the unfertilized soil mix were submitted to the Chemical Services Laboratory of the Pacific Forestry Centre, Victoria, B.C., for analysis of nutrient levels. For comparison, soil samples of similar size were collected to a 15-cm depth (F and H organic horizons) from 84 field sites with dense populations of marsh reed grass located between Dawson Creek, B.C., and Fort Nelson, B.C. Sown pots were placed in a temperature-controlled (18 ± 3°C) greenhouse for 3 weeks to produce grass plants at the 2- to 3-leaf stage for use in experiments. Pots were watered biweekly (30 mL/pot) unless otherwise noted.

Conidia of *F. avenaceum* isolate PFC70, an isolate with stable performance in previous studies (Winder 1999a), were produced in bag cultures of puffed wheat (Winder 1999b) and harvested after 14 days. *Pseudomonas* sp. aff. *putida* (Trevisan) Migula isolate PFC-R328, a deleterious rhizobacterium isolated from boreal *C. canadensis*, was cultured in baffled flasks containing tryptic soy broth (TSB, Difco Laboratories, Detroit, Mich.) on a rotary shaker for 48 h at room temperature (22°C).

All experiments had a factorial design incorporating two levels of inoculation (inoculated and uninoculated) as a common factor. Plants inoculated with the FPCP pathogens were treated with both pathogens on the same day, subsequent to measurement of the shoot length (above the soil surface) for each seedling. For the foliar inoculation, a hand sprayer was used to apply 3 × 10⁷ conidia/mL in 0.1% organosilicone surfactant (Silwet L-77, Loveland Industries, Greeley, Colo.) until run-off. Immediately after this, the soil of each pot was drenched with an 8-mL aliquot of the bacterial culture. Owing to the large size of the experiments, it was necessary to sow the uninoculated plants on a different starting date. Conditions were similar in all other respects; therefore, the inoculation factor in these experiments was treated as a split-plot in time. The uninoculated plants were also treated with organosilicone surfactant and uninoculated TSB in amounts corresponding to the inoculated treatments. Following treatment, pots were temporarily incubated within supported plastic bags and shaded for 24 h to ensure the condensation of moisture needed for fungal germination and infection. Placement of pots was randomized at each step of culturing, and the initial watering regime was maintained throughout the experiments.

For each experiment, one person visually estimated the percentage of leaf area damaged (LAD) 4 days after inoculation. Length (above soil line), percent LAD, and fresh weight were also recorded for each plant shoot 21 days after inoculation. Necrotic leaves or leaf tips were not included in the height measurement. Owing to the large size of the experiments, fresh weight data were not collected for uninoculated plants. Each experiment was performed twice. For statistical analysis, plants within pots were treated as subsamples, and data were subjected to analysis of variance. If regression analysis indicated a significant relationship between initial height and a parameter, initial height was used as a covariate in analysis of covariance (ANCOVA). For damage ratings, only data from inoculated plants were subjected to this regression test because control plants were undamaged and this would obscure trends in the inoculated plants. The ANCOVA-adjusted data were used to improve resolution of differences and to compensate for pre-inoculation heterogeneity. For brevity of pre-

sensation, only adjusted results are shown where ANCOVA was performed. Statistical analyses were performed with computer software (Statistica 5.1, Statsoft Inc., Tulsa, Okla.).

Autoallelopathy versus competition

A $2 \times 4 \times 5$ factorial combination of treatments was used to assess the interactive effect of autoallelopathy and intraspecific competition (planting density) on the FPCP. Standing straw of marsh reed grass was collected at the time of seed collection and stored in plastic bags at 5°C. Competition and autoallelopathy were altered by planting 4, 8, 16, or 32 seeds per pot and watering biweekly (30 mL/pot) throughout the experiment with 0, 10, 20, 30, or 40 g straw/L extract (Winder 1997). There were six pots per treatment (total $n = 480$). For a balanced analysis, only four randomly chosen seedlings were measured in pots with 8, 16, or 32 seedlings per pot.

Autoallelopathy versus N, P, and K

A $2 \times 2 \times 2 \times 2 \times 2$ factorial combination of treatments was used to assess the interactive effect of autoallelopathy and soil nutrients on the FPCP. The watering regime incorporated two levels of reed grass straw extract (0 and 40 g straw/L extract), N fertilizer (0 and 1 mM NH_4NO_3), P fertilizer (0 and 1 mM H_2PO_4), and K fertilizer (0 and 6 mM K_2SO_4). There were seven pots per treatment combination (total $n = 448$).

Competition versus nutrients

A $2 \times 2 \times 5$ factorial combination of treatments was used to assess the interactive effect of intraspecific competition (plant density) and soil nutrients. Seedling culture was altered by planting either 3 or 30 seeds per pot and by watering with 20 mL/(pot-day) throughout the experiment. For each pot, the watering regime incorporated a biweekly 30-mL addition of aqueous fertilizer solution. The fertilizer (20–20–20, all-purpose, Plant Products Co. Ltd., Brampton, Ont.) was delivered at a rate of 0, 0.5, 1.0, 1.5, or 2.0 g fertilizer/L. To balance the design, only three randomly chosen seedlings were measured at the higher density. There were seven pots per treatment combination (total $n = 280$).

Results

Autoallelopathy versus competition

Inoculation with FPCP inhibited ANCOVA-adjusted growth ($p = 0.0001$), as did increasing intraspecific competition ($p = 0.0001$). Furthermore, a significant ($p = 0.0001$) interaction between the two factors indicated that most of the density-dependant inhibition was in the uninoculated plants (Fig. 1A). There was also an interaction ($p = 0.001$) between straw and inoculation, as increasing concentrations of straw extract slightly inhibited and then stimulated shoot growth in uninoculated controls but caused no change in inoculated plants (data not shown). A significant ($p = 0.04$) three-way interaction between inoculation, competition, and straw extract treatments caused plants at the highest (32 plants/pot) density to behave differently. For uninoculated plants, inhibition of growth caused by the higher density was exacerbated in low concentrations of straw extract but ameliorated in higher concentrations of straw extract. For inoculated plants, low concentrations of straw extract slightly reduced the density-related inhibition, but this was overcome with higher amounts of extract (Fig. 1B).

The 4-day ANCOVA-adjusted damage in inoculated shoots was relatively low ($17 \pm 1\%$ LAD), but this main effect was significantly greater ($p = 0.0001$) than for uninoculated controls ($1 \pm 1\%$ LAD). As a main effect, increased intraspecific

competition led to a corresponding increase in 4-day damage ($p = 0.0001$), but a significant interaction with inoculation ($p = 0.0001$) limited the effect to inoculated plants (Fig. 2). There was also a significant increase ($p = 0.0001$) in damage corresponding to the main effect of increased straw extract concentration. Significant ($p = 0.0001$) three-way interaction between competition, autoallelopathy, and inoculation limited the scope of this damage increase. Increased straw concentrations caused increased damage at the highest (32 plants/pot) density, but the highest increase was for intermediate (23–30 g/L) concentrations of straw. At intermediate (8–16 plants/pot) densities, low (10 g/L) straw concentration produced slightly lower damage, but higher amounts did not produce any change. At the lowest (4 plants/pot) density, damage was slightly lower with increased concentration of straw (Fig. 2). Although still significant 21 days after treatment, damage in treated shoots was lower (8% LAD), as plants overcame FPCP-induced disease. At this point, significant main effects, interactions, and trends were similar to those noted for 4-day damage. Plants experiencing greater competition, and therefore greater damage, recovered to a slightly (<2%) greater degree (data not shown).

In inoculated plants, increasing competition caused a slight but significant increase ($p = 0.0061$) in ANCOVA-adjusted shoot fresh weight (Fig. 3). This increase in fresh weight despite the observed decrease in height could have been due to an increase in the number of basal shoots, but this was not measured.

Autoallelopathy versus N, P, and K

Plants growing in unfertilized soil did not display any symptoms of nutrient deficiencies; the levels of N and K present in the unfertilized soil were comparable to the mid-range of levels sampled in the field. Levels of P were low, but the levels available in the water (Roxborough et al. 2000) were cumulatively comparable to the lower levels present in the field (Table 1). Overall, N fertilization produced taller seedlings, with an ANCOVA-adjusted 21-day height of 143.1 ± 1.6 mm in N-treated seedlings compared with an adjusted 21-day height of 110.8 ± 1.6 mm in seedlings not treated with N. Inoculation with FPCP pathogens inhibited growth, with an adjusted 21-day height of 99.7 ± 1.0 mm in inoculated plants versus one of 154.2 ± 1.9 mm in uninoculated plants. P fertilization did not produce a significant main effect. However, there was a significant interaction with inoculation ($p = 0.0001$), where P fertilization stimulated the growth of uninoculated plants but slightly inhibited the growth of inoculated plants. There was a similar interaction with respect to N fertilization ($p = 0.0001$), where the effects of N were much greater in uninoculated plants (Fig. 4A). K fertilization interacted ($p = 0.0001$) with P fertilization, as K fertilization slightly reduced adjusted 21-day height in the absence of P fertilization (118.6 ± 7.2 mm versus 130.7 ± 6.2 mm with P fertilization) and slightly increased height with P fertilization (137.4 ± 8.0 mm versus 121.2 ± 7.6 without P fertilization). A significant 3-way interaction ($p = 0.002$) with FPCP inoculation limited these growth effects to uninoculated seedlings (Fig. 4B), while a significant 3-way interaction ($p = 0.006$) with N fertilization limited these growth effects to N-fertilized plants (Fig. 4C). However, the combined effects of N, P, and K were only ap-

Fig. 1. (A) The effects of planting density and inoculation on the height of *Calamagrostis canadensis* (marsh reed grass) shoots 21 days after treatment. Error bars are \pm SE. (B) The interactive effects of planting density and straw extract concentration on the reduction of inhibition of shoot height in marsh reed grass shoots 21 days after treatment. All data (Figs. 1A and 1B) were adjusted by ANCOVA.

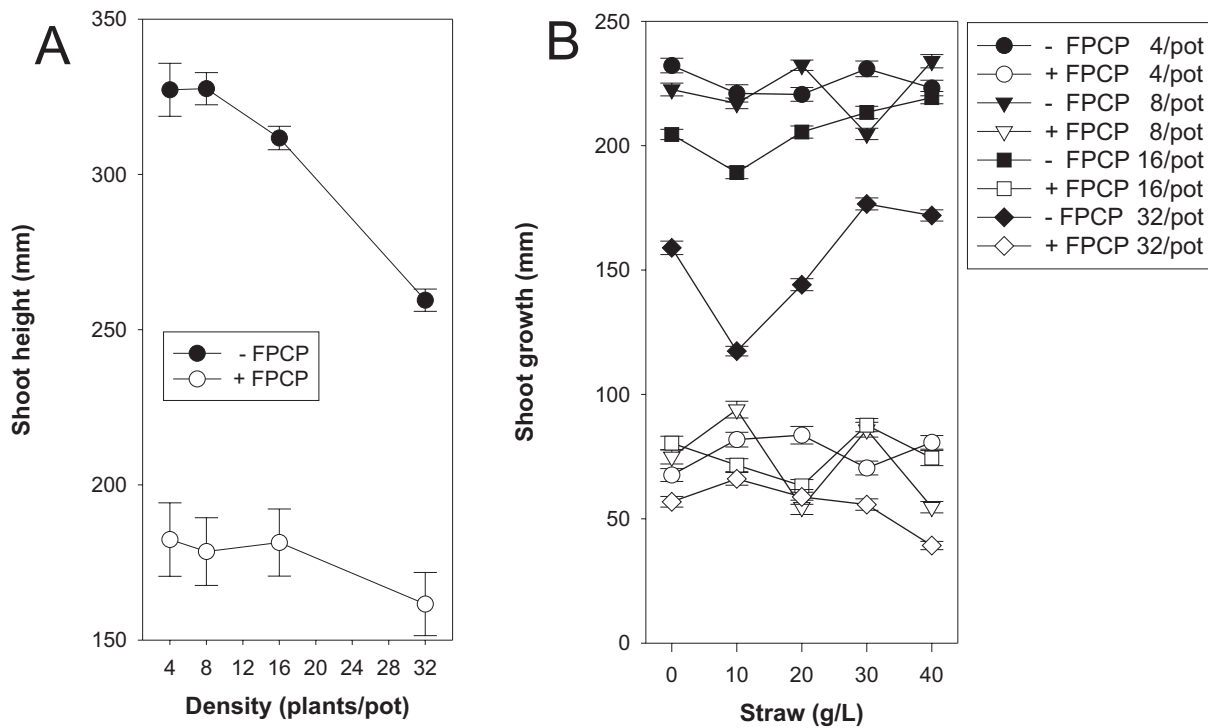


Fig. 2. The interactive effects of planting density and straw extract concentration on damage to *Calamagrostis canadensis* (marsh reed grass) shoots 4 days after treatment.

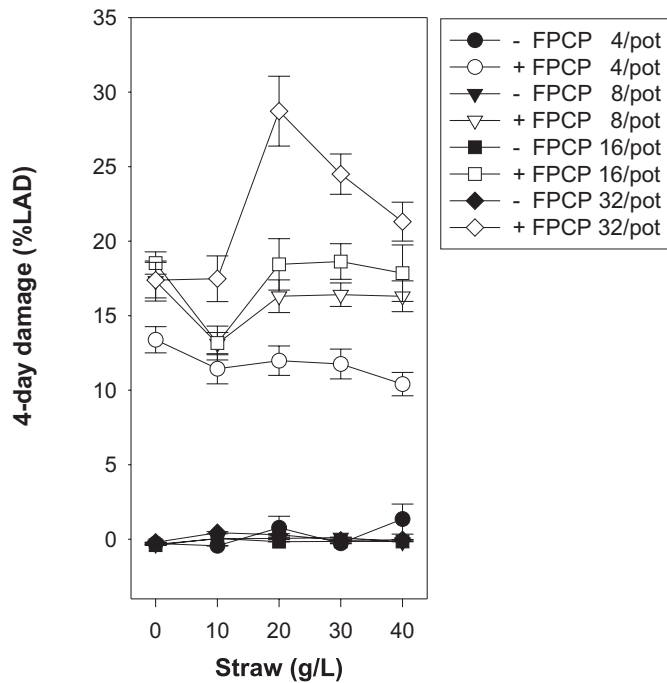
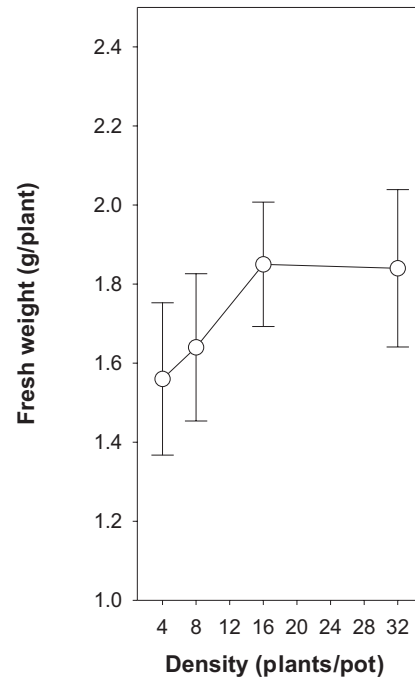


Fig. 3. The effect of density on fresh weight of FPCP-inoculated *Calamagrostis canadensis* (marsh reed grass) seedlings 21 days after inoculation (ANCOVA adjusted). Error bars are \pm SE.



parent in uninoculated plants because of a significant ($p = 0.005$) 4-way interaction with FPCP inoculation (Table 2). There was a significant 4-way interaction ($p = 0.04$) be-

tween inoculation, straw extract, N fertilization, and P fertilization, as there was slight growth stimulation in inoculated seedlings having both N and P fertilization, but only in the

Table 1. Comparison of the N, P, and K levels in greenhouse water, unfertilized peat-vermiculite soil medium, and representative soil samples collected throughout Northeastern B.C.

Nutrient source	N (ppm)	P (ppm)	K (ppm)
Water	0.01 – 0.89	0.01–0.2	0.06–0.49
Peat-vermiculite	7 510 – 8 720	<0.02	865–2485
Boreal soils	500 – 24 400	5.8–397.0	21–1362

Note: For the peat-vermiculite medium, a range of three replicates is shown. For boreal soils, the range of nutrient levels includes measurements from 84 sites in the boreal forest of Northern B.C. For water, the range reported by the Victoria Capital Regional District for a 9-year period (1990–1999) is shown.

absence of straw extract (Table 3). There was another significant 4-way interaction ($p = 0.002$) between straw extract, N fertilization, P fertilization, and K fertilization, as K fertilization in the absence of straw extract enhanced growth stimulated by N fertilization alone but limited growth stimulated by combined N and P fertilization (Table 4).

Damage in inoculated seedlings ($22 \pm 2\%$ LAD) was significantly greater ($p = 0.0001$) than damage in controls ($1 \pm 1\%$ LAD) 4 days after inoculation. Inoculation and P fertilization produced a significant ($p = 0.0001$) interaction, where P fertilization caused damage in inoculated plants to increase from $19 \pm 2\%$ LAD in plants without P fertilizer to $28 \pm 3\%$ LAD in P-fertilized plants, without affecting uninoculated seedlings ($1 \pm 1\%$ LAD for both controls and P-fertilized plants). Three-way interaction between P fertilization, FPCP inoculation, and straw extract was also significant ($p = 0.04$), with straw extract nearly doubling damage in combination with P fertilization (Fig. 5A). The 21-day ANCOVA-adjusted damage means and variances associated with inoculation, P fertilization, and their interaction were not appreciably different from the measurements taken after 4 days, but there was no longer a significant 3-way interaction between P fertilization, FPCP inoculation, and straw extract for 21-day damage. A main effect from straw extract ($p = 0.005$) became evident in the 21-day damage rating, with damage in inoculated seedlings ($13 \pm 2\%$ LAD) slightly increased from controls ($10 \pm 1\%$ LAD). There was also an additional interaction between inoculation, N fertilization, P fertilization, and K fertilization not seen in the 4-day measurements, as K fertilization caused increased damage to inoculated seedlings in the absence of other fertilizers but decreased damage in the presence of N fertilizer (Fig. 5B).

Increasing fresh weight of shoots from inoculated plants directly correlated with initial plant height ($\beta = 0.429$, $p = 0.00001$, $R^2 = 0.18$), but treatments produced no significant differences in adjusted fresh weights.

Competition versus nutrients

Increased competition produced significantly shorter seedlings ($p = 0.0001$), with 21-day ANCOVA-adjusted height decreasing from 154 ± 6 mm with 3 plants/pot to 139 ± 4 mm with 30 plants/pot. At the same time, increasing nutrient levels caused an increase in 21-day height (Fig. 6A). The competition effect was significantly modified ($p = 0.0001$) by interaction with FPCP inoculation. Height of uninoculated plants decreased from 179 ± 11 to 116.5 ± 5 mm while

height of inoculated plants increased from 130 ± 4 to 162 ± 4 mm when planting density increased from 3 plants/pot to 30 plants/pot, respectively. Fertilizer application interacted with inoculation, with inoculated plants showing substantial stimulation of shoot growth in the absence of fertilizer and less stimulation than uninoculated controls at higher rates (Fig. 6A). There was also a significant interaction ($p = 0.007$) between competition and application of fertilizer, with seedlings at the lowest density (3 plants/pot) being more responsive to the stimulatory effects of fertilizer than seedlings at the highest density (30 plants/pot). This density \times nutrient interaction was modified by a significant 3-way interaction ($p = 0.0007$) with inoculation. Uninoculated seedlings became taller than inoculated seedlings with increasing fertilizer at the lowest density or approached the height of inoculated seedlings with increasing fertilizer at the highest density. There was relatively less response to increasing fertilizer in inoculated seedlings at either density (Fig. 6B).

The 4-day damage in inoculated shoots ($16 \pm 1\%$ LAD) was significantly greater ($p = 0.0001$) than in control plants ($1 \pm 1\%$ LAD). There was a significant decrease ($p = 0.0001$) in damage with increasing fertilizer dose (Fig. 7). In the significant interaction ($p = 0.0001$) between inoculation and fertilizer application, it was apparent that the decrease in damage with respect to increasing fertilizer dose only occurred in inoculated plants (Fig. 7). ANCOVA-adjusted 21-day damage levels associated with inoculated seedlings ($8 \pm 2\%$ LAD) were greatly reduced from 4-day damage but were still significantly greater ($p = 0.0001$) than damage in controls ($3 \pm 1\%$ LAD), which had increased slightly during the same time period. Owing to a significant interaction ($p = 0.0001$) with competition levels, most of the damage reduction occurred at the higher (30 g/pot) planting density. In pots with three seedlings, 21-day damage was $1 \pm 1\%$ LAD (control) versus $10 \pm 2\%$ LAD (inoculated), while in pots with 30 seedlings it was $5 \pm 1\%$ LAD (control or inoculated).

The ANCOVA-adjusted fresh weight of shoots at the higher (30 plants/pot) planting density (0.7 ± 0.1 g/plant) was significantly greater ($p = 0.0001$) than that of shoots at the lower (3 plants/pot) density (1.9 ± 0.2 g/plant).

Discussion

Inoculation with the FPCP pathogens tended to override or buffer nutrient influences. Increased leaf damage in low nutrient conditions was accompanied by increased shoot growth. As measured by height and fresh weight, the productivity of inoculated plants was largely unaffected by changes in N, P, and K fertilization levels, although high rates of P caused a slight reduction in productivity. The increased damage associated with P and K nutrient imbalances was therefore not enough to substantially limit early seedling growth, particularly in the presence of higher levels of N. Despite the similar level of growth, the disease present in the seedlings presumably affects their competitiveness. Competition versus specific nutrient imbalances was not tested in this series of experiments, but if a competitive disadvantage under appropriate conditions can be confirmed, it could be a useful property to exploit in biological control. The stimulatory growth effects of single nutrients and N–P–K fertilizer have been noted in previous studies of *C. canadensis* (Landhäusser et al. 1996b; Laughlin et al. 1984;

Fig. 4. The interactive effects of N, P, and K fertilization on the shoot height of FPCP-inoculated and uninoculated *Calamagrostis canadensis* (marsh reed grass) seedlings 21 days after treatment. (A) The interactive effects of N and P fertilization. (B) The interactive effects of P and K fertilization. (C) The interactive effects of N, P, and K fertilization. All data are ANCOVA adjusted, and error bars are \pm SE. Overlapping bars indicate means that are not significantly different.

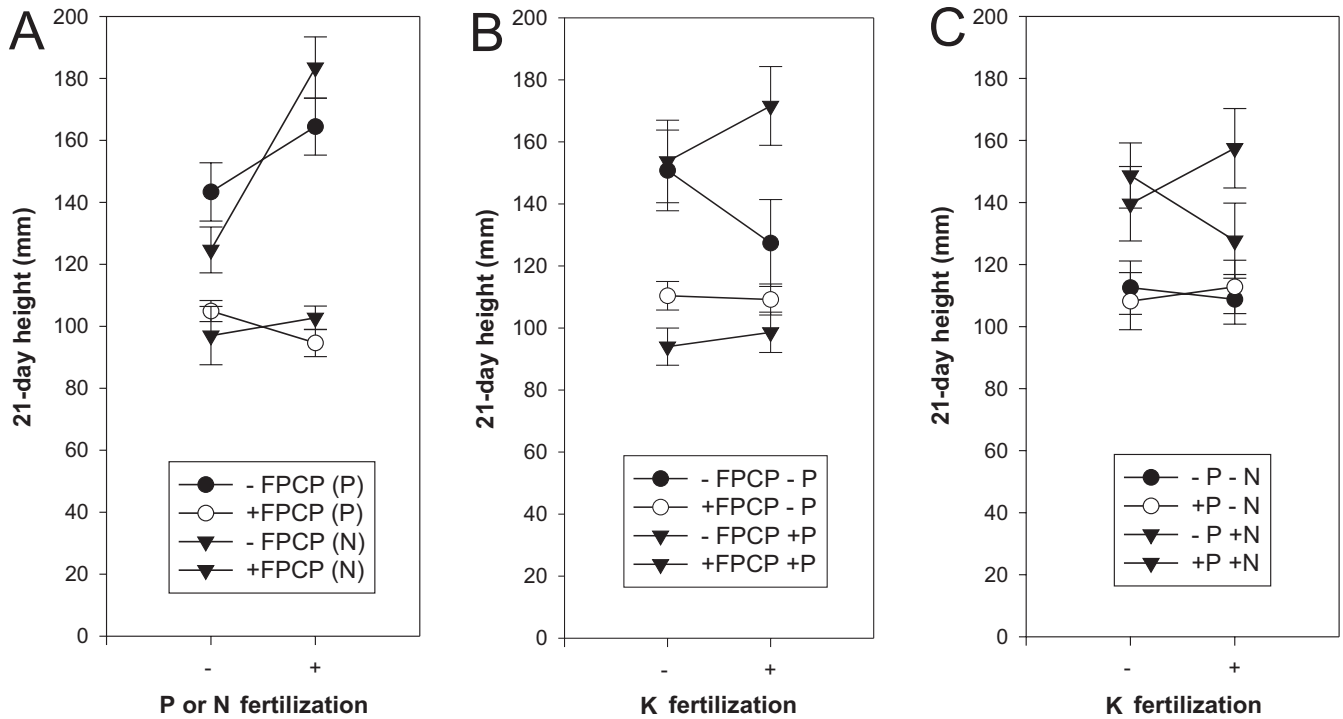


Table 2. The interactive effects of inoculation with FPCP and N, P, and K fertilization on the 21-day height of *Calamagrostis canadensis* (marsh reed grass) seedlings.

Fertilization			21-day shoot height (mm)*
N	P	K	
Uninoculated			
-	-	-	1140 \pm 17
-	-	+	109 \pm 15
-	+	-	125 \pm 16
+	-	-	187 \pm 17
+	+	-	183 \pm 20
+	-	+	150 \pm 23
-	+	+	129 \pm 13
+	+	+	214 \pm 18
Inoculated with FPCP			
-	-	-	111 \pm 5
-	-	+	109 \pm 7
-	+	-	92 \pm 10
+	-	-	110 \pm 8
+	+	-	96 \pm 7
+	-	+	110 \pm 7
-	+	+	96 \pm 10
+	+	+	110 \pm 9

*Values are ANCOVA-adjusted means \pm SE.

Table 3. The interactive effects of N and P fertilization and straw extract on the height (mm) of *Calamagrostis canadensis* (marsh reed grass) seedlings 21 days after inoculation with FPCP.

Straw extract	Fertilization			
	No N		With N	
	With P	No P	With P	No P
Uninoculated				
No extract	105 \pm 13	122 \pm 10	160 \pm 15	201 \pm 17
With extract	117 \pm 17	132 \pm 17	173 \pm 25	196 \pm 21
Inoculated with FPCP				
No extract	107 \pm 7	96 \pm 9	118 \pm 7	96 \pm 8
With extract	112 \pm 6	92 \pm 11	101 \pm 6	101 \pm 8

Note: Values are ANCOVA-adjusted means \pm SE.

Table 4. The interactive effects of N, P, and K fertilization and straw extract on the height (mm) of *Calamagrostis canadensis* (marsh reed grass) seedlings 21 days after inoculation with FPCP.

K fertilization	Straw extract	N and P fertilization			
		No N		With N	
		With P	No P	With P	No P
-	-	104 \pm 11	110 \pm 12	154 \pm 10	134 \pm 16
-	+	122 \pm 13	106 \pm 15	143 \pm 21	145 \pm 19
+	-	109 \pm 9	108 \pm 7	124 \pm 13	163 \pm 17
0	+	109 \pm 13	117 \pm 16	131 \pm 21	152 \pm 20

Note: Values are ANCOVA-adjusted means \pm SE.

Fig. 5. (A) The interactive effects of inoculation with FPCP, P fertilization, and straw extract on the 4-day damage (% LAD, \pm SE) to shoots of *Calamagrostis canadensis* (marsh reed grass) seedlings. (B) The interactive effects of inoculation with FPCP, N, P, and K fertilization on 21-day damage (% LAD, \pm SE).

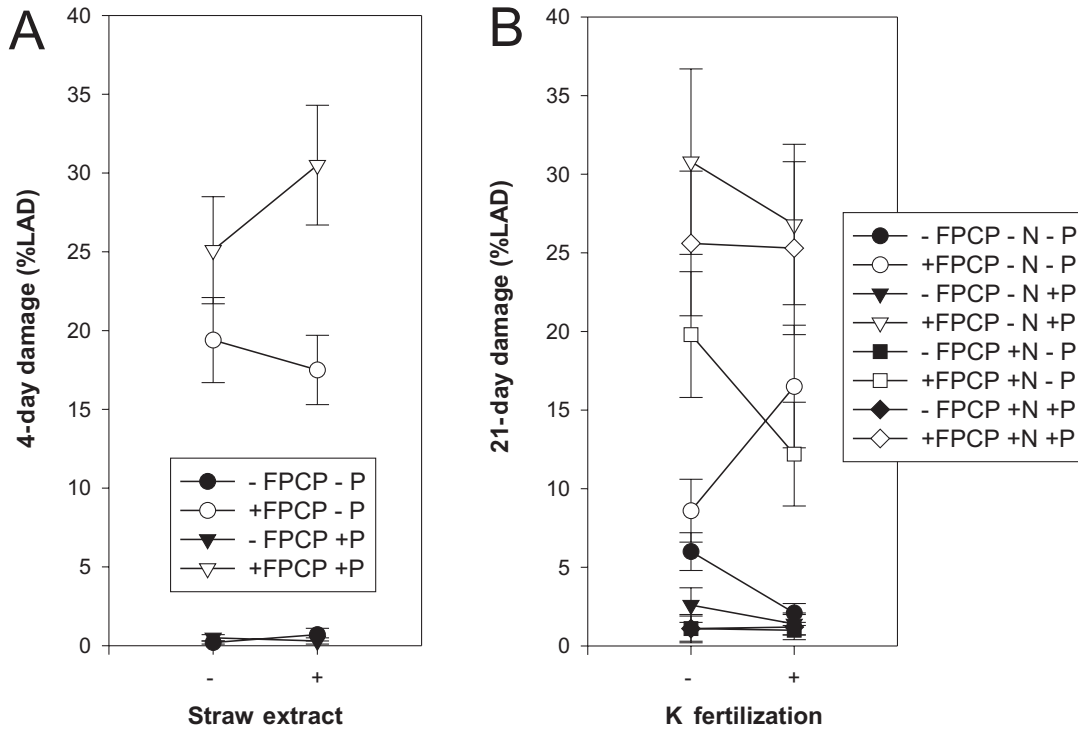


Fig. 6. (A) The effect of 20:20:20 (N-P-K) fertilizer on the 21-day height of *Calamagrostis canadensis* (marsh reed grass) seedlings. (B) The interactive effect of fertilizer, planting density, and inoculation with FPCP on 21-day shoot height. All data are ANCOVA adjusted, and error bars are \pm SE.

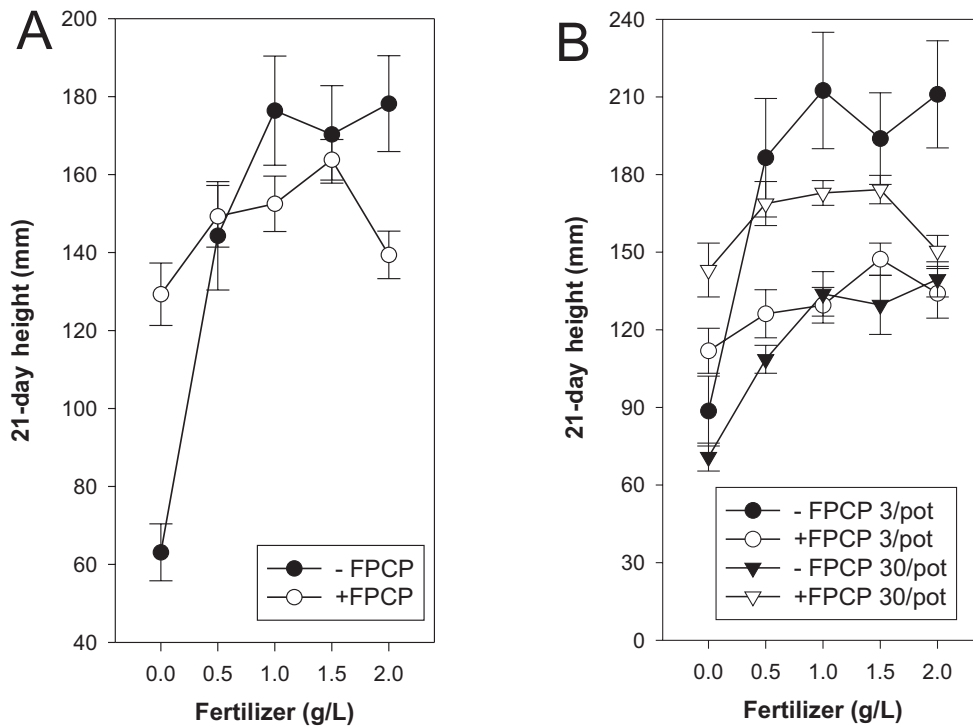
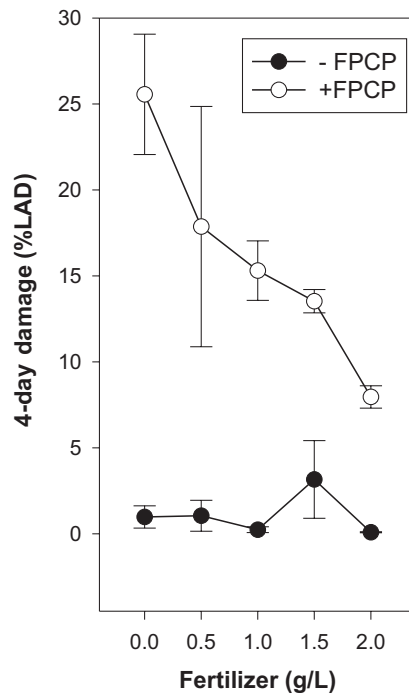


Fig. 7. The effect of 20:20:20 (N-P-K) fertilizer and inoculation with FPCP on 4-day damage (\pm SE) in *Calamagrostis canadensis* (marsh reed grass) seedlings.



Mitchell 1974) and a related Eurasian forest competitor, *Calamagrostis epigejos* (L.) Roth (Bergmann 1995). *Calamagrostis epigejos* is relatively insensitive to imbalances in P and K relative to N (Heinsdorf 1984), but *C. canadensis* productivity increases when N and K are combined (Laughlin et al. 1984). In this study, productivity increases caused by N and K fertilizers depended on the presence of sufficient P fertilizer.

Straw extract, primarily an inhibitor of seedling root growth (Winder 1997), did not exert a large influence on shoot growth. However, interaction between competition and allelopathy at their highest levels nearly halved the height of seedlings already severely inhibited by the FPCP pathogens (Fig. 1B). Straw extract also altered the effect of soil nutrients on shoot fresh weight and height of inoculated plants, possibly a result of its own nutrient content. Biological control with the FPCP pathogens could be more effective where more allelopathic straw has accumulated.

Increasing intraspecific competition inhibited seedling growth (Fig. 1A), but this could be moderated by the presence of nutrients and the FPCP inoculants (Fig. 6B). While the inhibitory effect of inoculation appears to have been of primary importance, the increased inhibition in interaction with the highest levels of allelopathy should not be overlooked. Biological control with the FPCP pathogens could be more effective in denser populations of the grass.

Higher soil fertility and, in particular, higher levels of N may be important constraints on the success of microbial herbicides. Ultimately, the speed of foliar pathogenesis is likely to be a key determinant of success for biological control with the FPCP pathogens. If shoot growth outstrips the spread of foliar diseases, seedlings may acquire sufficient photosynthate to maintain viable roots and shoots. On the

other hand, if shoot growth is impaired by edaphic factors, the speed of foliar pathogenesis relative to foliar growth could increase, resource allocation to roots and rhizomes would suffer, and efficacy of the biological control agents would be enhanced. In future studies, measurements of basal shoots and root characteristics over a longer term would aid our understanding of edaphic influences on the entire plant. Light should also be included as a nonedaphic factor in future studies because it interacts with nutrients to change the above- and below-ground partitioning of resources in marsh reed grass (Powelson and Lieffers 1992). Other species can compete effectively with marsh reed grass (Landhäusser et al. 1996a; Landhäusser and Lieffers 1994), so the effects of interspecific competition on disease progress should also be considered. Calcium levels are another factor that could be important, since they affect the productivity of *C. epigejos* in the field (Heinsdorf 1984). There are other common fungal pathogens such as *Puccinia coronata* Crda. var. *calamagrostidis*, *Lidophia graminis* (Sacc.) Walker & Sutton (Winder 1997), or an unnamed snow mold (low temperature basidiomycete) (Mallett et al. 2000), as well as common deleterious rhizobacteria such as *Pseudomonas fluorescens* Migula, *Pseudomonas chlororaphis* (Guignard & Sauvageau) Bergey et al., and *Pantoea agglomerans* (Beijerinck) Gavini et al. (Winder and Macey 1998), that could be used as alternatives to the FPCP microbes. Finally, the impacts of non-edaphic environmental factors such as temperature and humidity need to be considered; studies of these impacts are presently underway.

The results indicate several edaphic factors that will be important to consider in biological control of marsh reed grass. In forest vegetation management, biological control should aim to suppress competing vegetation rather than to kill it, because the vegetation still provides beneficial effects once the desired tree species are free to grow (Haeussler et al. 1990). So, while the FPCP pathogens did not produce an extremely high level of mortality, the level of suppression seen in plants exposed to the most promising edaphic stresses might be sufficient for successful management of the grass in some cases. Where soil fertility is higher, it may still be possible to limit the availability of a nutrient through changes in soil pH or the introduction of soil microbes able to sequester the nutrient. Opportunities exist to exploit edaphic stress as a method for suppressing competing vegetation with microbial agents.

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