Tiller and Rhizome Growth of Water-Stressed *Poa pratensis* 'Merion' Infected by *Ustilago striiformis* or *Urocystis agropyri*

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ABSTRACT

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Growth of tillers and rhizomes of *Poa pratensis* 'Merion' infected by *Ustilago striiformis* (cause of stripe smut) or *Urocystis agropyri* (cause of flag smut) was evaluated in nutrient solution alone and amended with polyethylene glycol to provide increasing levels of osmotically induced water stress. *U. striiformis* had no effect on total dry weight of rhizomes per shoot but decreased the percentage of rhizome dry weight as a portion of total shoot dry weight. Dry weight of tillers per shoot increased and was a greater percentage of total shoot dry weight than the percentage of total shoot dry weight in healthy plants. *U. agropyri* decreased rhizome growth and increased tiller growth expressed as total dry weight per shoot or as the percentage of total shoot dry weight when compared to healthy plants. Stimulation of tiller growth induced by *U. striiformis* was negated with increased water stress, but tiller stimulation induced by *U. agropyri* persisted under severe water stress.

Poa pratensis L. (Kentucky bluegrass) reproduces vegetatively by producing intravaginal (tillers) and extravaginal (rhizomes) branches. Rhizome and tiller production is at a maximum in spring, late summer, and fall and is minimal during periods of environmental stresses including heat, cold, and drought (1). Development of tillers and rhizomes on *P. pratensis* is sensitive to osmotically induced water stress, and such conditions ultimately contribute to increases in the root-shoot ratio of the plant (18).

Ustilago striiformis (Westend.) Niessl and *Urocystis agropyri* (G. Preuss) Schröt. are the causal organisms of stripe and flag smut, respectively. They are serious pathogens of several grass species including *P. pratensis* (3). Fungal hyphae infect the leaves, sheath, and floral parts of the host inducing morphological changes (6,7) and water stress as evidenced by lower leaf water and turgor potentials (19). Because the pathogens are systemic and the hyphae grow with developing axillary buds (10), tillers and rhizomes are almost always infected as they develop from infected plants (9). Factors such as irrigation (8) and nitrogen fertilization (13) that increase the survival of infected plants and favor tiller and rhizome production can enhance the spread of the disease. Irrigation may enhance survival by reducing the severity of the water stress induced by either pathogen (19).

The term "tillering" is commonly used to describe lateral bud growth into shoots without differentiating between intravaginal and extravaginal shoot development or the physiology of their selective development. Physiological control of tillering is subject to auxin and carbohydrate concentrations in the grass plants (14). Pathogenic Basidiomycetes induce hormonal imbalances in host plants (26), and infection of *P. pratensis* by *U. striiformis* or *U. agropyri* affects soluble carbohydrate levels in the leaves and roots (11,16). Altered hormonal and carbohydrate balance may affect tiller and rhizome production of infected *P. pratensis* and ultimately the morphology of the host and the epidemiology of the disease. This study was initiated to determine the effect of *U. striiformis* or *U. agropyri* on tiller and rhizome production by *P. pratensis* grown in nutrient solution, and to determine if the pathogen-induced effects on rhizome and tiller production are modified by increasing levels of osmotically induced water stress.

MATERIALS AND METHODS

Healthy plants and plants infected by *U. striiformis* or *U. agropyri* were vegetatively propagated from individual shoots of a single clone of *P. pratensis* 'Merion' in a loam:peat:perlite (1:1:1) mix under greenhouse conditions. Several pots of healthy and systemically smutted plants were grown to produce healthy and infected shoots for various experiments. Individual shoots for experiments were removed from the soil mix, roots were washed, and all but the two youngest fully expanded leaves were stripped from the shoot. These two-leaf shoots were then selected for size uniformity and placed individually in specially designed nutrient culture jars (20) by sliding the shoots through the hole in the rubber stopper and applying silicone grease around the shoot to provide support. The nutrient solution was continuously aerated from a manifold designed to provide equal saturated air flow to each nutrient culture jar. The nutrient solution used (Table 1) was similar to that used by Pellet and Roberts (22), except that higher rates of N (156 vs. 102 mg L^{-1}) were used to favor symptom development (13), less K was used (50 vs. 63 mg L^{-1}), and MoO_3 was used as the Mo source instead of $(NH_4)_6Mo_7O_{24}4H_2O$. Nutrient solution experiments were initiated to determine the effect of infection by *U. striiformis* and *U. agropyri* on growth of tillers and rhizomes of *P. pratensis* through a 4-wk period. In addition, water stress experiments were initiated to test if pathogen-induced differences in rhizome and/or tiller production were maintained as plants were exposed to increasing levels of water stress.

Nutrient solution studies. Twenty-five two-leaf plants were selected for size uniformity from each disease group (stripe-smutted, flag-smutted, and healthy). Nutrient culture jars containing one plant each were placed in a growth chamber according to a completely randomized design with three treatments (disease groups) and 25 plants per treatment. Growth chamber conditions included 12-hr photoperiods, 50% RH, 650 ($\pm$ 100) $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) spectral irradiance, and 23 $\pm$ 2 C. Plants were grown for 4 wk and the solutions were replaced with new solution twice per week. Five plants from each disease group were sampled for total dry weight (dried at 60 C for 48 hr), rhizome dry weight, and tiller dry weight at 0, 1, 2, 3, and 4 wk. The experiments were repeated three times. Similar results were obtained for each experiment, so data were combined for the final analysis. Data are presented as weekly means (n = 15) of tiller and rhizome dry weight per shoot and the percentage of total dry weight contributed by tillers and rhizomes for each disease group. Least significant differences ($P = 0.05$) between disease groups were calculated for each week.

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Water stress studies. Sixteen two-leaf plants were selected for size uniformity from each health group and evaluated for tiller and rhizome growth in response to infection and progressively greater drought stress. Plants were preconditioned for 10 days by growing them in unamended nutrient solution. The plants were then placed in nutrient solution containing polyethylene glycol (PEG 8000, Fisher Scientific, Pittsburgh, PA) at concentrations of 0, 148, 212, and 252 g L^{-1}. These PEG concentrations provided initial nutrient solution osmotic potentials of −0.06, −0.4, −0.6, and −0.8 MPa (20). Osmotic potential of nutrient solution without PEG added was calculated from a modified van't Hoff equation (17) where total nutrient solution osmolarity equaled 8.48×10^{-3}M and the weighted correction factor equaled 2.66. Nutrient solutions were replaced with fresh solution twice per week, and growth chamber conditions were as previously stated. It should be noted that osmotic potentials reported here reflect only initial values. Actual values between nutrient solution replacements were allowed to become more negative with water usage.

Plants were grown for 4 wk under applied water stress after which four plants from each treatment combination (disease group by water stress level) were sampled for total, tiller, and rhizome dry weights. The experiment was repeated three times, and because each experimental run produced similar results, data were combined for the final analysis. Data are presented as means ($n = 12$) for all disease groups (healthy, stripe-smutted, and flag-smutted) for rhizome and tiller dry weight per shoot and the percentage of total shoot dry weight contributed by tillers or rhizomes for −0.06, −0.4, −0.6, and −0.8 MPa initial nutrient solution osmotic potentials. Least significant differences ($P = 0.05$) between disease groups were calculated for each stress level.

RESULTS

Nutrient solution studies. Infection by *U. striiformis* had no effect on total dry weight of rhizomes per shoot on *P. pratensis* grown in nutrient solution during the 4-wk period (Fig. 1A). The contribution of rhizomes to the total dry weight of the shoot, however, decreased between the second and third week of the study compared to healthy plants (Fig. 1B). Infection by *U. striiformis* increased the total dry weight of tillers per shoot (Fig. 1C) and their contribution to the total dry weight of the shoot (Fig. 1D) compared to healthy plants grown in nutrient solution.

Plants infected by *U. agropyri* exhibited a sharp decrease in rhizome dry weight per shoot (Fig. 1A) and rhizome contribution to the total shoot dry weight (Fig. 1B). Conversely, plants infected with *U. agropyri* exhibited sharp increases in tiller dry weight per shoot (Fig. 1C) and the percentage of the total shoot dry weight contributed by tillers (Fig. 1D).

Water stress studies. Progressively decreasing the osmotic potential of the nutrient solution in which healthy plants and plants infected with *U. striiformis* or *U. agropyri* were growing generally decreased the mean dry weight of rhizomes and tillers per shoot and their respective contribution to the total shoot dry weight (Fig. 2). Rhizome growth of the host was decreased by both pathogens under minimal water stress but more severely by *U. agropyri* (Fig. 2A). Increasing water stress reduced the differential decrease in rhizome growth between healthy plants and plants infected with *U. striiformis* but plants infected with *U. agropyri* showed severe decreases in rhizome growth at all levels of water stress (Fig. 2A,B).

Tiller dry weight per shoot of diseased plants did not differ from that of healthy controls under minimal stress (Fig. 2C). When tiller growth was expressed as a percentage of total shoot weight, however, plants infected with *U. agropyri* exhibited pronounced tiller growth compared to plants infected with *U. striiformis* or healthy plants (Fig. 2D). Tiller dry weight per shoot (Fig. 2C) and the percentage of total shoot dry weight

Table 1. Nutrient solutions with nutrient sources and concentrations

Nutrient	Source	Concentration (mg L^{-1})[a]
N	$Ca(NO_3)_2 \cdot 4H_2O$	78.00[b]
	$(NH_4)_2SO_4$	78.00
P	H_3PO_4	25.00
K	KOH	50.00
Mg	$MgSO_4 \cdot 7H_2O$	19.00
Fe	$Fe_2(SO_4)_3$	1.20
Mn	$MnSO_4 \cdot H_2O$	0.25
B	H_3BO_3	0.10[b]
Zn	$ZnSO_4 \cdot 7H_2O$	0.10
Cu	$CuSO_4 \cdot 5H_2O$	0.01
Mo	MoO_3	0.01[b]

[a] Nutrient solution contains 117 mg L^{-1} S and 112 mg L^{-1} Ca.
[b] Modified from Pellet and Roberts (22).

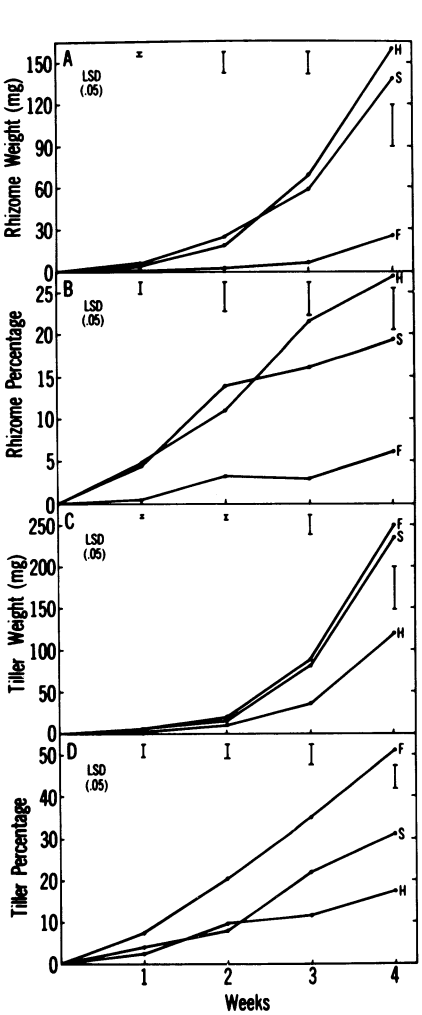

Fig. 1. Mean rhizome **(A)** and tiller dry weights **(C)** and percentage of total dry weight from rhizome **(B)** and tillers **(D)** of healthy (H), stripe-smutted (S), and flag-smutted (F) *Poa pratensis* 'Merion' shoots grown in nutrient solution. LSD ($P = 0.05$) values are shown for each week.

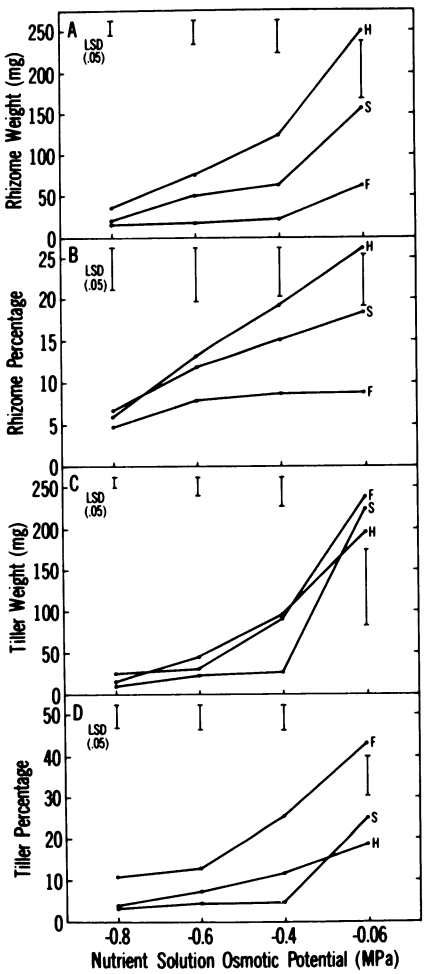

Fig. 2. Mean rhizome **(A)** and tiller dry weights **(C)** and percentage of total dry weight from rhizomes **(B)** and tillers **(D)** of healthy (H), stripe-smutted (S), and flag-smutted (F) *Poa pratensis* 'Merion' shoots grown for 4 wk in nutrient solution with progressively lower osmotic potentials. LSD ($P = 0.05$) values are shown for each initial nutrient solution osmotic potential.

contributed by tillers (Fig. 2D) decreased for all disease groups as the level of water stress increased. At the most severe level of water stress, however, plants infected with *U. agropyri* exhibited greater tiller dry weight per shoot (Fig. 2C) and a greater proportion of total shoot dry weight as tillers (Fig. 2D) than healthy plants or plants infected with *U. striiformis*.

DISCUSSION

Previous research has shown that tiller and rhizome growth of *P. pratensis* is very sensitive to water stress (18) and that infection by *U. striiformis* and *U. agropyri* results in lower leaf water and turgor potentials compared to healthy plants (19). This suggests that diseased plants should show decreases in both tillering and rhizome production compared to healthy plants. The results of this study show, however, that systemic infection by *U. agropyri*, and to a lesser extent by *U. striiformis*, stimulates the growth of tillers at the expense of rhizome production. We cannot rule out the possibility that stripping leaves from the shoot in order to obtain uniform experimental units may have affected the total number of lateral buds breaking dormancy during the 4-wk experimental period. However, increases in tillering and decreases in rhizome production have also been observed in previous greenhouse studies with pot-grown *P. pratensis* infected with *U. agropyri* (6,7). This shift in dry matter partitioning from rhizomes to tillers resulting from infection by *U. agropyri* or *U. striiformis* strongly suggests metabolic imbalances in diseased plants. Growth of tillers in grasses uses large reserves of stored carbohydrates (24,29). The stimulation of tillering in plants infected with *U. agropyri* or *U. striiformis* may account for some of the decrease in soluble carbohydrates (11,16) and reduced root growth of leaf-smutted plants (20). Carbohydrate starvation would also explain the dramatic decrease in rhizome growth of infected grasses, but it cannot explain the stimulation of tillering.

It is possible that shifts in dry-matter partitioning are a result of hormonal disturbances either as a direct consequence of infection (26) or through the additional water stress that accompanies infection (12,19,21). Plant pathogens of the Basidiomycetes often induce hormonal imbalances in their hosts (26), including increases in gibberellins (2). Gibberellins have been shown to stimulate dormant buds to develop in many species (25). Epidemiologically, the spread of leaf smuts may be enhanced by pathogen-induced stimulation of the host to proliferate tillers. The proliferation of tillers provides more systemically infected leaves for the production of teliospores and more inoculum for infection of seedling coleoptiles, axillary buds on crowns, and rhizomes (9). It also provides for more teliospores to lie dormant in the soil.

Hormonal disturbances in water-stressed plants include increases in abscisic acid (23) and decreases in cytokinins (15). Increased IAA oxidase activity has also been observed in response to water deficits (4). Tiller and rhizome growth in grasses depends on breaking dormancy of lateral buds (1). This may be induced by auxin produced in the apical meristem (14,27,28) or may require growth promoters such as gibberellins (5) to stimulate development. Increases in IAA oxidase activity or loss of auxin or gibberellin activity may account for the observed loss of tiller and rhizome production at more severe levels of water stress. However, the proliferation of tillers by plants infected with *U. agropyri* at the expense of rhizome production implies differential control of intravaginal and extravaginal branching.

Differential control of tiller and rhizome production would be a valuable tool for turf management. The ability to chemically stimulate rhizome production would speed the recovery of turf from damage and improve the sod quality of turf species with bunch-type growth habits. There is little knowledge, however, relating to the differential control of intravaginal and extravaginal branching in grasses. Determination of the physiological and hormonal factors controlling the branching of *P. pratensis* in response to infection by *U. agropyri*, and to a lesser extent by *U. striiformis*, might provide an understanding of such control provided that host genotypic variation is eliminated and environmental effects are tightly controlled.

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