

# Mycorrhizal Colonization of Crested Wheatgrass as Influenced by Grazing<sup>1</sup>

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## ABSTRACT

Vesicular-arbuscular mycorrhizal (VAM) fungi play a role in crop productivity and soil stability in agricultural ecosystems. This role is accentuated under adverse cultural or climatic conditions, such as grazing or drought. The purpose of this study was therefore to assess the effects of stress (grazing) on VAM-fungal colonization of a major forage species. Changes in root colonization of crested wheatgrass [*Agropyron desertorum* (Frisch.) Schult.] plants by VAM fungi were measured under three levels of grazing pressure, ranging from no grazing to heavy, continuous grazing. Percent colonization of root length was determined by microscopy, and VAM-fungal biomass spectroscopically. These findings were related to the changes in root/shoot ratios caused by grazing. The study site is characterized as a Wyoming big sage brush (*Artemisia tridentata* spp. *Wyomingensis*) -grassland community on an Abegese loam (fine-loamy, mixed, mesic Xerollic Haplargid) soil in central Nevada. Plants studied were introduced previously as part of a range improvement program. Colonization of root length declined significantly ( $P < 0.05$ ) and seasonally (40.2%, June and 50.6%, October) in plants under heavy grazing as compared to ungrazed plants. Spores (60.8% June and 89.5% October) and biomass (35.0% June and 61.5, October) of the VAM fungi also declined with grazing. Colonization of wheatgrass was favored when the amount of photosynthetic tissue relative to root mass was high. Phosphorus concentration in plant tissues was not significantly affected ( $P < 0.05$ ) by grazing. It is concluded that severe grazing adversely affects the colonization of crested wheatgrass by VAM fungi. This reduction in the fungal symbiont may have an effect on plant nutrition and soil structure and stability which needs further investigation.

*Additional index words:* *Agropyron desertorum*, *Glomus mosseae*, *Glomus constrictum*, Root/shoot ratio, Phosphorus nutrition.

EVIDENCE accumulating on the role of vesicular-arbuscular mycorrhizal (VAM) fungi in the ecophysiology of plants shows that an understanding of the symbiotic relationships between host plant and VAM-fungal endophyte is important for a complete and predictive analysis of the structure and function of an ecosystem (12,20,21,23,27,32). This may be particularly true for systems which are unusually fragile due to adverse environmental conditions or to overuse (1,6,7,24,30). In agricultural ecosystems grasses often play a dominant role. Grasses display considerable variability in their dependence (18) on VAM fungi for maximum growth (10,15,24). Growth responses by the symbiotic partners of the grass-fungus association to such management practices as grazing and fertilization are therefore difficult to ascertain and effects ranging from enhancement to inhibition are observed (6,22,26,33). The purpose of the present report was to investigate the effect of grazing pressure on VAM-fungal colonization of a grass introduced to a central Nevada range as part of a range improvement program.

## MATERIALS AND METHODS

The study area was at the Nevada Agricultural Experiment Station's Gund Research and Demonstration Ranch in Grass

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Valley, Central Nevada. The area is semiarid, with an average annual precipitation of 20 to 30 cm. The site of sample collection was an alluvial fan of 5 to 10% slope. Soil pH, organic matter, and plant-available  $\text{NO}_3\text{-N}$  and P (Olsen) were 7.4, 1.7%, 24 mg N/kg soil and 2.0 mg P/kg soil, respectively. Soil data were determined by standard analytical methods (25). The A1 and A2 horizons (to a depth of 30 cm) are of predominantly loamy texture of the Abegese series (Xerollic Haplargid). The vegetation, classified as a Wyoming big sagebrush (*Artemisia tridentata* spp. *Wyomingensis*) grassland community was degraded by a century of cattle (*Bos* spp.) grazing. Crested wheatgrass [*Agropyron desertorum* (Frisch.) Schult., cv. Nordan] was seeded on this site in 1979 with a deep-furrow rangeland drill, following plowing of the original plant cover. Soils, vegetation, and cultural practices at the collection site were characterized by Cluff et al. (13) and Young and Evans (34).

Samplings were made at three adjacent sites (Fig. 1) in June and October 1983. One site was not grazed in 1982 and 1983. Site two was subjected to an intermediate level of grazing pressure of 80% utilization (cattle in/out 26 May/23 June 1982 and 12 May/30 June 1983). The third site was heavily grazed during the entire growing seasons of 1982 and 1983. During 1983, weather conditions permitted approximately 9 weeks of growth prior to the June sampling and, due to unusually early fall rains, approximately 4 weeks of growth prior to the October sampling.

Twenty plant, soil, and VAM-fungal spore samples were taken at each site. Five sets of four pooled samples were evaluated statistically by Student's *t* test or Duncan's Mul-

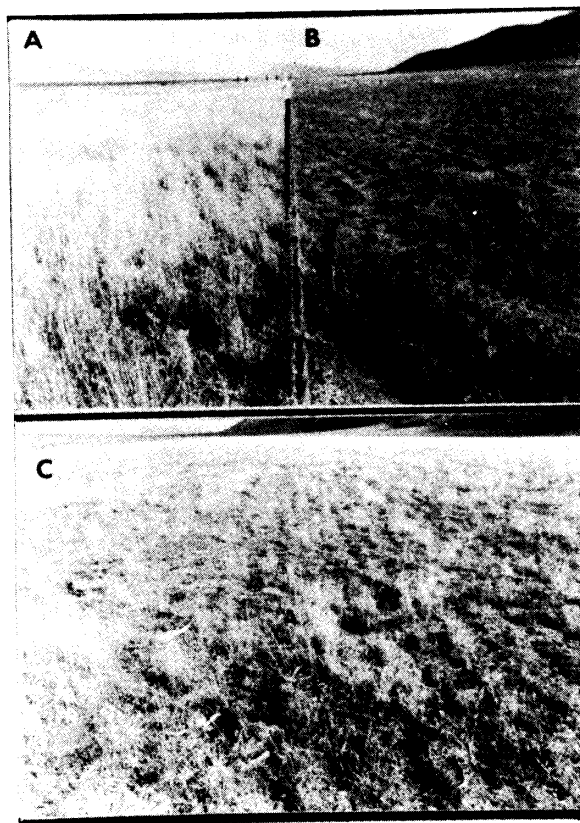


Fig. 1. Levels of grazing pressure in crested wheatgrass pastures at Gund Ranch, NV (A - ungrazed, B - severe grazing, C - intermediate grazing).

multiple Range Test (figure legends). The parameters evaluated by this pooling method were root/shoot ratios, fungal colonization of roots, fungal biomass, and the P contents of roots and shoots.

Crested wheatgrass plants were collected at each site by excavating roots to a depth of 30 cm. Plants were washed, and dried at 80 °C for 2 days. Root/shoot ratios were determined from dry weights. Prior to drying, roots were cut into 1-cm segments and dispersed in water to achieve a homogeneous distribution of segments. Samples of 100 segments were stained and evaluated for root colonization by VAM fungi (percentage of root length infected) visually (9). Root and shoot samples were ground in a Wiley mill (40 mesh screen). The intensity of VAM-fungal colonization (VAM-fungal biomass as percentage of colonized root) was determined by chitin analysis of the ground samples (9). The use of controls free of VAM fungi accounted for possible chitin contamination due to other chitin-containing organisms. Controls consisted of washed roots of crested wheatgrass plants grown in pot cultures in initially sterilized native soil which was reinoculated with a filtrate free of VAM fungal propagules. Phosphorus content of plant parts was determined according to Allen (2).

Taxonomic identification of VAM fungi was preceded by culturing the fungi in pots using native soil and sorghum [*Sorghum bicolor* (L.) Moench] as host plant. Roots and growth medium were assayed for VAM fungi after 4 months of growth in a greenhouse equipped with supplementary lighting providing photosynthetically active radiation of 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and extending day length to 16 h. Fungal spores isolated from soil and roots were identified according to Trappe (31).

Soil samples were taken from the root zone of crested wheatgrass plants in the field, pooled as described above, and wet-sieved according to Gerdemann and Nicholson (19) to determine VAM-fungal spore density. Smallest sieve pore diameter used was 43  $\mu\text{m}$ . Spores thus collected were counted microscopically.

## RESULTS AND DISCUSSION

Colonization of crested wheatgrass roots by VAM fungi was significantly ( $P < 0.05$ ) reduced by heavy grazing. At the intermediate level of grazing pressure neither colonized root length nor VAM-fungal biomass were significantly affected (Table 1). The two measures of colonization diverged seasonally. The percentage of host root length in which fungal hyphae were present was not significantly different for the summer and fall samplings, although the fall sampling was preceded by a shorter growth period. Intraradical VAM-fungal biomass, a measure of the intensity of colonization, however, was significantly reduced under grazing in October relative to that in June. As obligate biotrophs, VAM fungi depend on their host for reduced C. It is therefore reasonable to expect that any factor influencing photosynthesis will affect VAM colonization (8,14). Results, however, are often contradictory, and favorable effects of increased photosynthesis on VAM colonization have not always been confirmed experimentally (4,16, M.F. Allen, personal communication). It may be assumed that host-endophyte interactions, ranging from hormonal to source-sink effects, are affected differently at different times during the ontogeny of the symbionts and that such differences are further accentuated by the reactions of each symbiont to environmental conditions. The data base presently available in the literature dealing with grazing effects on VAM colonization is not sufficient

**Table 1.** Colonization of crested wheatgrass roots by vesicular-arbuscular mycorrhizal (VAM) fungi. Numbers reflect the percentage of root length containing fungal organs or fungal biomass as a percentage of the colonized root's dry weight. Numbers are means of five observations, each consisting of four pooled samples. Numbers in a column followed by the same letter are not significantly different ( $P > 0.05$ ) by Duncan's Multiple Range Test. Comparisons between June and October samplings were not significantly different by Student's *t* test unless so indicated (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).

Grazing pressure	VAM-fungal colonization			
	Root length		Biomass	
	6 June	5 October	6 June	5 October
	%			
Ungrazed	52.2a	58.9a	6.0a	5.2a
Medium	51.3a	49.3a	5.6a*	4.8a
Heavy	31.2b	29.1b	3.9b**	2.0b

**Table 2.** Root/shoot ratios of crested wheatgrass plants and spore counts of vesicular-arbuscular mycorrhizal (VAM) fungi. Numbers are the means of five replications, each consisting of four pooled samples. Numbers in a column followed by the same letter are not significantly different ( $P > 0.05$ ) by Duncan's Multiple Range Test. Significant differences between seasonal samplings are indicated as \*,  $P < 0.05$  and \*\*,  $P < 0.01$  by Student's *t* test.

Grazing pressure	Root/shoot ratio		VAM Spores/100 g soil	
	6 June	5 October	6 June	5 October
Ungrazed	0.35a*	0.67a	230a	190a
Medium	0.50b*	0.83ab	180ab	150a
Heavy	0.70c**	0.98b	90b**	20b

to explain contradictory observations. Increases or decreases in the incidence of VAM fungal structures in the roots of grazed relative to ungrazed plant may vary with the duration or severity of grazing pressure, with the taxonomic identity of both symbionts, environmental variables, and even with the method of observation. In the present case, reductions in root length colonized and in VAM-fungal biomass under heavy grazing pressure, as well as reduction in VAM-fungal biomass under grazing following a shorter growth period (October sampling) may be ascribed to limiting photosynthate availability.

The role of grazing in the availability and probably in the allocation of carbohydrates to the roots is also illustrated by the increase in root/shoot ratios with increasing grazing pressure (Table 2). As a result of the reduction in photosynthetic tissues, competition for reduced C between host and endophyte (11) is expected to increase until a threshold level of stress is reached. At that point soil permeation by the extraradical VAM-fungal mycelium may be adversely affected. This was suggested by the decreases in colonization (Table 1) and spore numbers (Table 2) which related to the decrease in shoot relative to root mass seasonally and as a result of severe grazing. The impact of such changes in VAM-fungal development on the plant-soil system goes beyond plant nutrition alone and is difficult to assess at this time due to a lack of sufficient information (32). While the importance of the extraradical mycelium to the host plant in nutrient uptake is well known (21) its effects on the soil microflora (3,23) and soil structure and stability (17,29) are just beginning to be appreciated.

The taxonomic identity of the VAM mycoflora is of interest in view of the host and soil preferences of these organisms (23) which may be an important factor in the selection of new plants to be introduced to an area. Compatibility of native VAM fungi with a prospective host should be determined prior to extensive utilization of the host. The two isolates of VAM fungi found at the sampling sites, *Glomus constrictum* Trappe and *Glomus mosseae* (Nicol. & Gerd.) Gerd & Trappe (Fig. 2) belong to the more ubiquitous species in the Endogonaceae. However, little is known about possible ecotypic adaptations (28) and the range of soil and host preferences of these organisms.

Inhibition of VAM fungal colonization by grazing was not reflected in the P concentration of the plants (Table 3). A decrease in P content of larger, unstressed plants has been noted earlier (4,8) and is attributed to dilution. An effect of the level of VAM-fungal colonization on P uptake as a function of grazing cannot be assessed at this time due to a lack of non-VAM

controls in the field. The low level of plant-available ( $\text{NaHCO}_3$ -extractable) soil P (2.0 mg P/kg soil) at the sampling sites, however, suggests that VAM fungi play a role in the P nutrition of their hosts (5).

It is concluded that severe grazing adversely affects the colonization of crested wheatgrass by VAM fungi.

Table 3. Phosphorus concentrations in crested wheatgrass colonized by vesicular-arbuscular mycorrhizal (VAM) fungi. Numbers are the means of five replications, each consisting of four pooled samples. Numbers in a column followed by the same letter are not significantly different ( $P > 0.05$ ) by Duncan's Multiple Range Test.

Grazing pressure	Phosphorus (%)			
	Shoots		Roots	
	6 June	5 October	6 June	5 October
	Phosphorus ( $\text{g kg}^{-1}$ )			
Ungrazed	2.0a	2.3a	1.0a	1.3a
Medium	2.2a	2.4a	0.9a	1.4a
Heavy	2.8b	2.5a	1.1a	1.3a

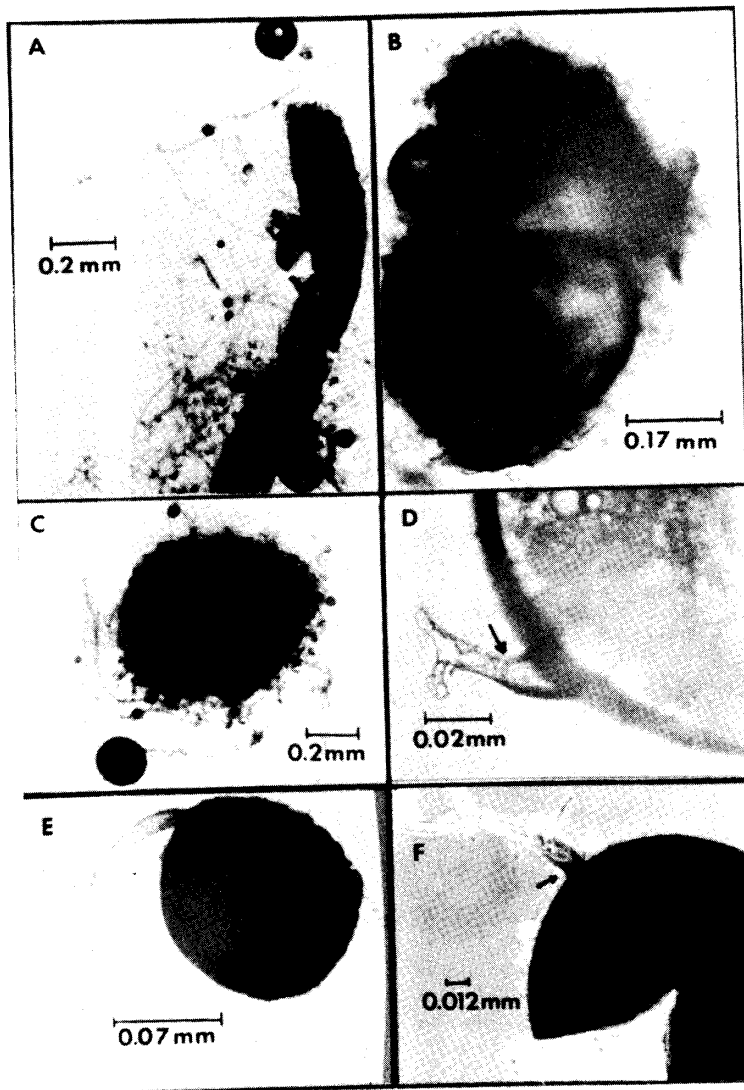


Fig. 2. Vesicular-arbuscular mycorrhizal fungi associated with wheatgrass at the Gund Ranch, central Nevada. A - Root segment with hyphae and spores of *Glomus mosseae*,  $\times 75$ . B - Macerated sporocarp of *Gl. mosseae* with internal spores,  $\times 140$ . C - *Gl. mosseae* sporocarp with external spore,  $\times 53$ . D - Some diagnostic characters of *Gl. mosseae*: flared hyphal attachment with curved septum (arrow)  $\times 800$ . E - Spore of *Gl. constrictum*  $\times 380$ . F - Some diagnostic characters of *Gl. constrictum*: narrow hyphal attachment with occlusion by spore wall thickening (arrow)  $\times 800$ .

This reduction of fungal mycelia and propagules may have an effect on plant nutrition and soil stability which need further investigation.

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