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Effect of Water Stress on Competition Between *Medicago truncatula* and Wheat in ^{15}N Labeled Soil

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Crop mixtures that include legumes increase yield and restore soil fertility. These mixtures are limited by drought, which is frequent in Morocco due to lack of and poor distribution of rain. However, annual Medicago species are very important to forage production, and they have a tolerance to water stress. The effect of water stress on N_2 fixation and N transfer by Medicago truncatula *cv. Jemalong (medic), when mixed with wheat (*Triticum durum *cv. Karim), was investigated in three greenhouse experiments. Annual medic and wheat were seeded in 20-L pots in pure and mixed stands. Three watering treatments were used: −0.9, −1.5, and −2.5 MPa in the first experiment; and −0.9, −2.5, and −3.5 MPa in the second and third experiments. Competition between medic and wheat was affected by hydric treatments; the medic was more competitive for soil water than the wheat, especially under severe water stress. Shoot dry weight of wheat grown with medic was higher than that of wheat grown in pure stands. Medic alone took up more soil N than wheat alone, while in the mixture, medic took up less soil N than wheat, indicating that the mixed medic was not competing for soil mineral N. Using the ^{15}N dilution technique, it was found that medic ^{15}N concentration was lower than that of the wheat in all treatments, suggesting active N_2 fixation. Apparently, the medic obtained from 20% to 73% of its N from the atmosphere, depending on the water regime. The atoms-percent excess ^{15}N in medic decreased significantly in the mixture, showing higher N_2 fixation (^{15}N dilution method) than in pure medic. Mixed medic derived 82% of its N from fixation at severe water stress (T_2) and 92% at the control (T_0), showing that N_2 fixation by medic was less affected by water stress in the mixed stand than in the pure stand. In the first experiment, N transfer was detected only in the control treatment, but in the second and third experiments, transfer of N was significant (*$p = 0.05$*).*

Keywords competition, isotope dilution, *Medicago truncatula*, nitrogen fixation, water stress

Mixtures of legumes and grasses often give greater dry matter and protein yields without requiring as high fertilizer N input as grass culture alone (Haynes, 1980). This was explained by a better competition for the soil N by the nonlegume than the legume plants (Vallis et al., 1967). Many other workers attributed the benefit of the mixture to N transfer from the fixing to the nonfixing plant (Ismaili & Weaver, 1987; Danso et al., 1993).

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In arid and semiarid areas, water is the greatest limiting factor in crop production. Therefore, Medicago species bred for drought tolerance must be introduced (Rumbaugh & Johnson, 1986). Water stress reduces biological nitrogen fixation (Sprent, 1972a), as nodules have a direct requirement for adequate moisture to maintain nitrogen fixing activity that is optimum at field capacity (Sprent, 1972b). Extensive research has been conducted on N management and N_2 fixation in cropping systems using ^{15}N fertilizers and the difference method (Henson & Heichel, 1984). Nitrogen balance in a grass-legume mixture versus pure stands was investigated using the dilution method, and direct transfer of N from legume to grass was demonstrated (Ismaili & Weaver, 1987).

The isotope dilution method and its limitations and problems are discussed in review articles (Witty, 1983; Vose & Victoria, 1986). The only requirement of this method is that the control and the legume take ^{15}N and ^{14}N from the soil in proportion to the N available (Fried & Broeshart, 1975). The N balance in the mixed systems should be investigated to improve management techniques. Our objectives in this study were to assess the effect of different levels of water stress on yield and N accumulation by medic and wheat grown alone or in a mixture, and to quantify N_2 fixation and the beneficial effect of annual medics on wheat.

Materials and Methods

A greenhouse experiment, repeated three times, was conducted in 20-L pots filled with well-mixed silty clay soil with 29% water holding capacity. Soil chemical characteristics are pH (H_2O) 7.60, organic matter 1.55%, total C 0.25%, total N 0.15%, and available P 17.5 $\mu g\ g^{-1}$.

Nitrogen 15 was made available to the plants as ammonium sulfate, $(^{15}NH_4)_2SO_4$, enriched with 4.89 atoms-percent excess ^{15}N in solution of distilled water. The ^{15}N solution was injected into the pots in very small increments every 3–4 days, 1 month after seeding. The injections were made into a tube placed in the center of each pot. The tubes were filled with sand and cotton and contained many holes to permit diffusion of the ^{15}N through the entire volume of soil.

Three moisture treatments were maintained by the gravimetric method, and determination of leaf water potential of the plants was made every 2–3 days with a Wescor HR-33T microvoltmeter and C-52 sample chamber. Leaf water potential was determined on leaf discs 5 mm in diameter that were collected from a leaf with a paper punch. Approximately five discs were placed in a sample holder. The discs were compressed in the holder so that most of the volume was occupied with leaf material. Equilibration required 5–20 min, depending on severity of water stress. The junction was cooled for 5 s in the read mode, which was consistently associated with a plateau in microvolt output. All microvolt readings were corrected for temperature if they had not been obtained at 25°C. The psychrometer unit had previously been equilibrated with standard NaCl solutions. All measurements of leaf water potential were made early in the morning. Two leaf samples of five discs each were collected from each plant. On sampling dates when watering was to be resumed, plants were sampled prior to subirrigation, to keep leaf water potential at the defined level.

The three watering treatments were as follows. Water treatment T_0 entailed keeping the moisture potential optimal for plant growth (leaf water potential –0.9 MPa) by regular irrigation. Water treatment T_1 allowed plant leaf water potential to reach –1.5 MPa in the first experiment and –2.5 MPa in the second and third experiments, after which the soil was irrigated to keep the leaf water potential at this level by oscillating around –1.5 MPa in the first experiment and –2.5 MPa in the second and third experiments. Water treatment T_2 allowed plant leaf water potential to reach –2.5 MPa in the first experiment and –3.5

MPa in the second and third experiments, after which the soil was irrigated to keep leaf water potential at this level, oscillating around –2.5 MPa for the first experiment and –3.5 MPa in the second and third experiments. The stress treatments were mild, as large pots were used and the soil had a high water holding capacity (29%). The stress treatments started 60 days after planting (DAP) in the first experiment and 45 DAP in the second and third experiments.

Three cropping systems were compared: two monocultures and one mixture. Monocultures were continuous wheat (*Triticum durum* cv. Karim) and continuous medic (*Medicago truncatula* Gaertn. cv. Jemalong). The mixture was a 50:50 mix of wheat and medic. Wheat and *Rhizobium*-inoculated medic seeds were planted 28 per pot for all cropping systems (pure or mixture). This operation was repeated for each new experiment. There were two experiments per year: one lasting from September to January and the other from January to May. From May to September the pots were left uncropped in the greenhouse without disturbing the soil. Before seeding the subsequent crop in experiment 2, the top 15 cm of soil were carefully mixed, avoiding displacement of the root litter of the previous wheat and medic of the first experiment. This procedure was repeated for the third experiment.

Aboveground biomass of all the plants in each pot was harvested for both wheat and medic at physiological maturity. The samples were oven dried at 70°C for 48 h, weighed for dry matter yield determinations, and then ground for total N and ^{15}N percent excess determinations. Total N was measured by Kjeldahl analysis (Nelson & Sommers, 1973), and ^{15}N was measured with an Isotope Micromass 602 D mass spectrometer (VG Isotopes Limited) (Porter & O'Deen, 1977). Wheat was used as a reference plant to determine isotopic dilution of the legume plants. Previous experiments indicated that wheat was a good reference plant for annual medics. To determine legume and wheat N derived from soil and N_2 fixation, the isotope dilution method and difference method were used (Talbott et al., 1982; Henson & Heichel, 1984).

The experiment was repeated three times on the same soils and pots. Data were analyzed using analysis of variance for a completely randomized design with four replications. Differences between the means, tested by Duncan's multiple range test, were reported significant for $p = 0.05$.

Biological nitrogen fixation by the legume ($\%N_{dfa}$) was calculated:

$$\%N_{dfa} = \left(1 - \frac{\text{medic atoms-percent excess } ^{15}N}{\text{wheat atoms-percent excess } ^{15}N}\right) \times 100$$

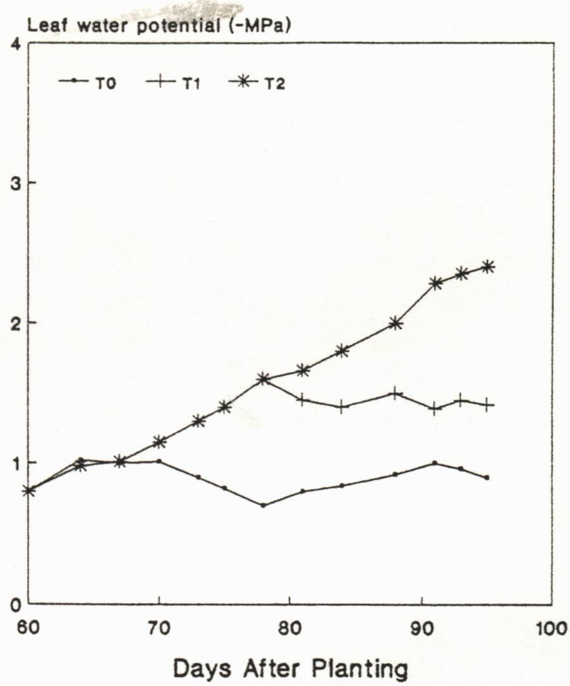
Nitrogen transfer was expressed as the percentage of wheat shoot N derived from legumes (%NT) according to the following calculations:

$$\%NT = \left(1 - \frac{\text{atoms-percent excess } ^{15}N \text{ of wheat in mixture}}{\text{atoms-percent excess } ^{15}N \text{ of wheat alone}}\right) \times 100$$

Results and Discussion

Water treatments affected leaf water potentials of medic and wheat. Treatment T_0 kept leaf water potential at –0.9 MPa. After withholding irrigation from the pots, leaf water potential decreased slowly because the soil had a high water holding capacity. Treatment T_1 kept leaf water potential at –1.5 MPa and –2.5 MPa, and treatment T_2 kept leaf water potential at –2.4 MPa and –3.5 MPa (Figure 1). Using large pots delayed the effect of

First experiment



Second experiment

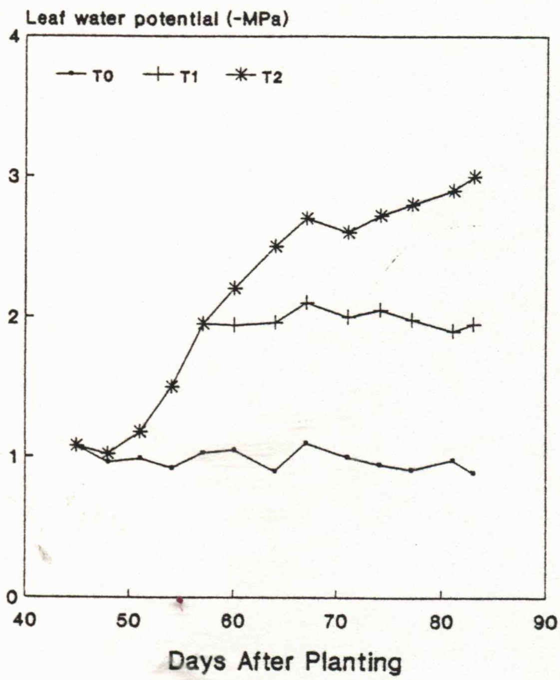


Figure 1. Leaf water potential (−MPa) in the first, second, and third experiments.

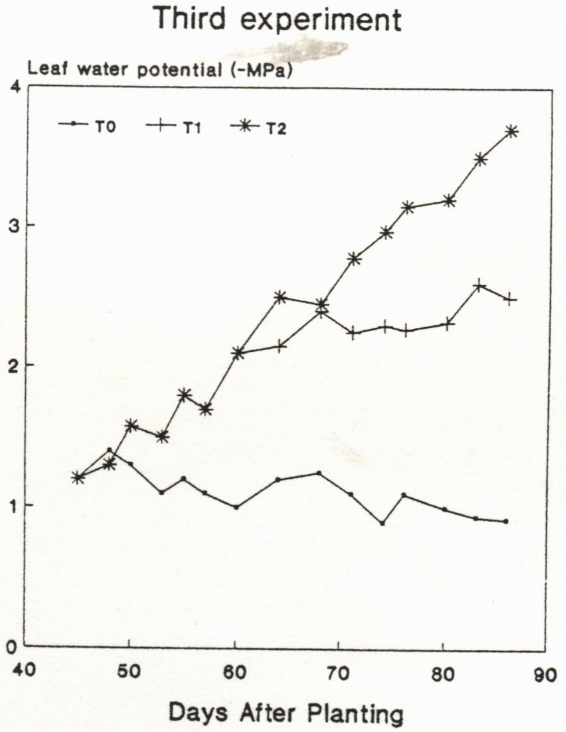


Figure 1. Leaf water potential (−MPa) in the first, second, and third experiments. *(Continued).*

stress on the plants. Therefore, in the second and third experiments, we started water stress treatment 45 DAP. Ismaili et al. (1983) used vermiculite and small pots and obtained highly stressed plants after withholding water from the pots for a few days.

In all watering treatments, dry matter production was improved from one experiment to the next (Table 1). This increase was more apparent for the third experiment, where dry matter production was 2–3 times higher than that of the first experiment. No fertilizer was applied during this experiment; thus the N nutrition of the plants became dependent on the cycle of the soil organic matter mineralization. Only 1 week separated the end of the first experiment and the beginning of the second, while a 3-month period came between the second and third experiments. This rest period allowed time for soil organic matter mineralization, resulting in a high increase in the dry matter and total N yields in the third experiment (Table 1).

Water stress treatment did not affect medic and wheat yields in the mixture as it did in the pure stands (Table 1). The water regime induced greater decrease in dry matter production of mixed wheat and pure medic than on pure wheat and mixed medic, respectively. Also, in all experiments, the medic water content was affected by the water treatments in both pure and mixed stands, unlike wheat, which was affected in the mixture but not in the pure stand. At T_2 the water content of mixed wheat was less than that of pure wheat, while water content of mixed medic was similar to that of pure medic. This indicates that the medic was more competitive for soil moisture than the wheat.

In the first experiment, mixed wheat yielded more dry weight per plant than pure wheat, showing that wheat benefited from intercropping (Table 1). Mixed wheat accumulated 2 times more N than pure wheat at T_0 and T_1, indicating that wheat was more

Table 1

Shoot dry weight, total N, and water content of wheat and medic in pure and mixed stands during three experiments

Component	Treat-ment	Experiment 1			Experiment 2			Experiment 3		
		Dry weight (g/plant)	Total N (mg/plant)	Water content (g H_2O/g dry weight)	Dry weight (g/plant)	Total N (mg/plant)	Water content (g H_2O/g dry weight)	Dry weight (g/plant)	Total N (mg/plant)	Water content (g H_2O/g dry weight)
Pure wheat	T_0	0.75	11.1	2.10	1.01	14.6	1.25	2.11	37.5	1.40
	T_1	0.73	9.6	1.77	0.91	14.3	1.19	1.88	29.6	1.39
	T_2	0.72	8.6	1.82	0.87	12.9	1.07	1.59	28.2	1.34
Mixed wheat	T_0	1.30	20.0	1.91	1.77	25.0	1.22	3.45	64.3	1.36
	T_1	1.41	19.3	1.73	1.60	21.43	1.03	2.93	53.4	1.27
	T_2	1.15	14.3	0.98	1.46	20.86	0.65	2.30	41.4	0.74
$LSD_{0.05}$*		0.12	0.5	0.29	0.10	2.37	0.30	0.19	5.0	0.31
Pure medic	T_0	1.33	51.4	3.94	1.41	52.50	4.18	1.84	55.0	3.59
	T_1	1.14	39.6	3.10	0.97	29.64	3.50	1.63	46.4	2.45
	T_2	1.13	37.1	2.04	0.90	27.00	1.43	1.59	44.3	1.98
Mixed medic	T_0	1.14	41.4	3.77	1.94	62.14	3.77	1.98	62.9	3.29
	T_1	1.13	38.6	2.95	1.61	49.28	3.17	1.87	62.1	2.25
	T_2	1.13	34.3	1.88	1.49	51.43	1.69	1.81	57.9	1.96
$LSD_{0.05}$*		0.15	3.7	0.34	0.27	11.98	0.67	0.23	7.9	0.62

*Least significant difference (LSD) values are for the interaction (water treatment) × (cropping system).

efficient in competing for soil mineral N than medic and/or that it was benefiting from N transfer. At T_2, wheat yielded only 1.68 times more N than pure wheat, showing that the beneficial effect of the mixture on wheat was reduced by the severe water deficit. Shoot dry weight and total N at T_0 were lower for mixed medic than for pure medic. But at T_1 and T_2, dry matter and total N production were similar for mixed medic and pure medic plants, showing that the medic was less water limited in the mixture than in the pure stand.

In the second and third experiments the dry weight and total N productions of both medic and wheat in the mixture were higher than their respective pure stands, indicating that the mixture favored the two species. This can be explained by better soil conditions in the second and third experiments than in the first one, consequently reducing the competition between the medic and wheat.

Percent ^{15}N enrichment was lower in medic than in wheat in all water treatments (Table 2), indicating N_2 fixation by medic. In our study, nitrogen fixation was measured by the difference method and the isotope dilution method (Table 3). The difference method gave higher estimates than the isotope dilution method. Nevertheless, results of N derived from soil (Table 4) indicated that the medic was more efficient than wheat in soil N uptake. The N difference method requires that both crops absorb equal quantities of N from the soil (Weaver, 1986). In our work this is not the case, and the difference method overestimated N_2 fixation. The ^{15}N method provided more accurate estimates of N_2 fixed, as was reported by Rennie et al. (1978). The main assumption in this method is that the reference plant and the legume plant utilize the same isotope ratio of ^{15}N and ^{14}N from the soil. It is not necessary that they take up the same quantity of N from the soil. In pot experiments, as in our study, the root systems of nonfixing and fixing plants sample the same soil volume. We ensured a uniform distribution of ^{15}N in the soil by adding the ^{15}N solution in small increments to keep the concentration of ^{15}N in the soil constant throughout the experiment. Ismaili and Weaver (1986) utilized labeled plant material as a fertilizer source to accomplish this objective. Depending on the water regime, pure medic obtained from 73% (at T_0) to 20% (at T_2) of its N from the atmosphere (Table 3), showing that water stress reduced N_2 fixation by medic. Water stress reduced photosynthesis, and this

Table 2

Shoot atoms-percent excess ^{15}N of the medic and wheat grown either in pure or mixed stands during three experiments

	Experiment 1		Experiment 2		Experiment 3	
Treatment	Pure	Mixed	Pure	Mixed	Pure	Mixed
Medic						
T_0	0.0431	0.0135	0.0509	0.0475	0.1240	0.1232
T_1	0.0912	0.0167	0.0582	0.0575	0.1297	0.1210
T_2	0.1153	0.0254	0.0834	0.0663	0.1382	0.1290
$LSD_{0.05}$	0.0120	0.0040	0.0234	0.0092	0.0086	NS
Wheat						
T_0	0.1597	0.1478	0.1307	0.1156	0.1363	0.1105
T_1	0.1503	0.1736	0.1508	0.1337	0.1288	0.1104
T_2	0.1434	0.1703	0.1554	0.1357	0.1293	0.1092
$LSD_{0.05}$	NS	0.0101	0.0203	NS	NS	NS

LSD, least significant difference; NS, not significant.

Table 3

Comparison of the isotope dilution and difference methods for measuring N_2 fixed

Treatment	Pure medic				Mixed medic	
	Isotope dilution		Difference method		Isotope dilution	
	N_{dfa} (mg/plant)	$\%N_{dfa}$	N_{dfa} (mg/plant)	$\%N_{dfa}$	N_{dfa} (mg/plant)	$\%N_{dfa}$
T_0	36.9	73.0	39.4	78.3	35.2	91.5
T_1	15.6	39.3	29.7	75.2	34.1	88.9
T_2	07.3	19.6	28.8	76.9	28.3	82.2
$LSD_{0.05}$	5.1	8.4	3.2	2.0	3.2	3.0

N_{dfa}, amount of N_2 fixed; $\%N_{dfa}$, proportion of N_2 fixed; LSD, least significant difference.

resulted in inadequate carbohydrate supply to the nodule and decreased nitrogen fixation (Chu & Robertson, 1974; Ruegg & Alston, 1978).

The atoms-percent excess ^{15}N was lower in the mixed medic than in the pure medic (Table 2), indicating higher N_2 fixation (Table 3). At T_0, pure medic and mixed medic fixed similar quantities of N, but at T_1 and T_2 this amount was much smaller in the pure stand than in the mixed crop, indicating again that the medic was less water limited in the mixed than in the pure stand.

In all water treatments, wheat took up more soil N when mixed than in pure form. Medic took up more soil N than wheat when grown separately (Table 4). However, soil N uptake was reduced in medic and increased in wheat when both species were grown together. Nitrogen uptake was higher in mixed wheat than in mixed medic, showing a reverse situation to the pure crops. Higher competitiveness of grasses than legumes for soil

Table 4

Proportion and amount per plant of N derived from soil and fertilizer in experiment 1 by medic and wheat under watering treatments T_0, T_1, and T_2

Treatment	N_{dfs} (mg/plant)		$\%N_{dfs}$		N_{dff} (mg/plant)		$\%N_{dff}$	
	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed
Medic								
T_0	13.1	3.2	26.1	8.2	0.44	0.11	0.88	0.28
T_1	23.2	4.1	58.8	10.8	0.73	0.13	1.86	0.34
T_2	29.2	5.9	78.0	17.2	0.88	0.17	2.35	0.52
$LSD_{0.05}$	03.1	0.8	8.2	2.9	0.09	0.02	0.26	0.09
Wheat								
T_0	10.6	18.3	96.7	89.6	0.36	0.62	3.3	3.0
T_1	9.5	18.5	96.9	96.4	0.30	0.68	3.1	3.6
T_2	8.3	13.9	97.1	96.5	0.25	0.50	2.9	3.5
$LSD_{0.05}$	1.0	1.7	NS	0.3	0.06	0.06	NS	0.2

N_{dfs}, amount per plant of N derived from soil; $\%N_{dfs}$, proportion of N derived from soil; N_{dff}, amount per plant of N derived from fertilizer; $\%N_{dff}$, proportion of N derived from fertilizer; LSD, least significant difference; NS, not significant.

Table 5
Percent of wheat N transferred from the medic during the three experiments

Treatment	Experiment 1	Experiment 2	Experiment 3
T_0	7.2	11.5	18.9
T_1	0	11.3	14.3
T_2	0	12.7	15.5
$LSD_{0.05}$	2.5	NS	NS

LSD, least significant difference; NS, not significant.

N uptake in mixed stands was found by other workers (Davidson et al., 1986; Morris & Weaver, 1987). The amount of N derived from soil increased in mixed and pure medic, and decreased in mixed and pure wheat with water deficiency (Table 4).

The ^{15}N enrichment in mixed and pure medic (Table 2) increased from one experiment to the next, probably due to a high soil N availability, which resulted in a decrease in nitrogen fixation. However, atoms-percent excess ^{15}N in mixed wheat decreased from experiment 1 to 3 due to the uptake of ^{14}N from decomposing legume root material.

In the first experiment, the wheat grown with medic had lower atoms-percent excess ^{15}N than wheat grown alone (Table 2). The dilution of ^{15}N in mixed wheat showed that about 7% of mixed wheat total N came from transfer of legume fixed N (Table 5). However, there was no evidence of N transfer in T_1 and T_2. In the second and third experiments, however, N transfer was detected at all water treatments and was higher than that obtained in the first experiment (Table 5). This supports the view that the release of N from breakdown and decomposition of dead legume tissues is the main process involved in nitrogen transfer from legume to associated grass (Henzell & Vallis, 1977; Goodman, 1988; Mallarino et al., 1990).

Conclusion

Mixtures of legumes and grasses show a higher tolerance to drought. The legume competes better for water, continues to fix N_2 from the air, and contributes to N nutrition of the grass. Legume-grass mixtures are good for alternative cropping systems in tropical agriculture and arid environments. The data allowed us to quantify the competition for water and soil N between the legume and the grass and showed N transfer and mutual beneficial effects of the two species in mixture. Those findings also apply to field conditions.

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Cattle Dungpat Microenvironmental Effects on Germination and Establishment of Crested Wheatgrass

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*A greenhouse study was conducted to determine the effects of ambient environmental conditions on cattle dungpat temperature, moisture, and crust formation dynamics, which in turn, influence ingested/passed-seed germination and plant establishment at different locations in dungpats. "Hycrest" crested wheatgrass [*Agropyron desertorum *(Fisch. ex Link) Schult. ×* A. cristatum *(L.) Gaert.] was used as a representative revegetation species for the Great Basin region of the western United States. After collecting feces from Holstein steers that had each been fed 60,000 seeds of crested wheatgrass, uniform dungpats were prepared and placed on loam and coarse sand soil types, in containers, under three initial watering treatments (field capacity, one-half field capacity, and no water). Dungpat and underlying soil microenvironmental factors were monitored over a 14-week period. Dungpat moisture and temperature conditions were favorable for germination during the first 4 weeks, but rapid crust development prevented most of the developing seedlings from emerging from the dungpats. Seedling emergence and development were greater at the periphery than at the interior of the dungpats, and greater on the underlying loam soil at one-half field capacity or higher than on the underlying sandy soil at similar moisture contents. Seedlings developing in dungpats on drier soils did not survive; however, some remaining ungerminated seeds in dungpats could germinate at a later date if ambient environmental conditions became more favorable.*

Keywords *Agropyron desertorum × A. cristatum*, crusting, moisture dynamics, revegetation, seed dispersal, seedling recruitment, temperature dynamics

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