Effect of Drought Stress and Mineral Nitrogen Supply on Growth and Seed Yield of White Clover in Mediterranean Conditions

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ABSTRACT

Water and nitrogen needs of white clover (*Trifolium repens* L.) were studied to improve seed yield under two irrigation systems, based on tensiometric measurements, and three levels of nitrogen nutrition. For each combination of these two factors, dry matter accumulation in the different parts of the plant, water consumption (measured with a neutron probe), nitrate reductase activity and grain yield components were measured.

White clover seed production seems to be very sensitive to water supply. An excess of 25 mm in water supplied was responsible for a 30% decrease in seed yield mainly due to a lower number of ripe flowering heads. This could be explained by the stimulation effect of water on growth (leaf DM production and leaf NRA) which increased the competition between vegetative and reproductive parts, and inhibited the re-mobilization process. On the other hand, in the more stressed treatment, the increase in seed yield was associated with an increase in root and stolon biomass.

Mineral N supply during the vegetative phase (50 kg N ha^{-1}) or throughout the whole cycle (150 kg N ha^{-1}) had no significant effect on seed yield components. Nevertheless it seemed to increase seed yield in the more limiting treatment.

These results show the importance of the scheduling of irrigation for white clover seed production. A certain level of water stress is necessary to obtain maximum seed yield. Tensiometers appear to be a very useful tool for achieving this goal.

The role of mineral N supply in improving seed yield in the situation of slight drought stress needs to be reassessed in a soil with a lower level of N.

Additional index words: irrigation, *Trifolium repens*, growth.

INTRODUCTION

White clover (*Trifolium repens* L.) is one of the most important forage crops in temperate zones, particularly in North America, New Zealand and Europe where it represents 6 to 8 million ha (Gayraud, 1983). In France it is sown on 300 or 400,000 ha each year (Dattee, 1983) and is common in most of the natural pastures in central and western France. Although several cultivars adapted to European conditions were selected, they are not grown for forage because of lack of seed.

As for many other legumes the main problem for white clover seed production is the low level and the irregularity of seed yield. In addition, in the regions where climate is favorable to forage production, the conditions for flowering, pollination, maturation and harvest are frequently bad because of excess of water.

For this reason, irrigated fields in the Mediterranean zone, similar to California or NZ, represent a good potential for seed production. Previous experiments on grain legumes in these climatic conditions have shown the role of plant water status on seed yield (Wery et al., 1983), and the strong interaction of these two processes with N nutrition (Wery, 1987a). The same approach was developed on white clover to study the possibilities of water and N management to improve seed yield and quality.

MATERIAL AND METHODS

This experiment was conducted during the 1986 growing season, in Montpellier (southern France) on a deep sandy-clayey-silty soil. White clover cv Olwen (Hollandicum type), was sown on September 24, 1985 with a 30 cm inter-row spacing. On May 10, 1986 we made a pre-cutting at the beginning of the flowering buds appearance in order to slow growth. We used a split-plot design, with three Fischer blocks and two factors: water alimentation (as sub-blocks) and N nutrition (as primary plot):

Water alimentation: we compared two treatments based on tensiometric measurements defined according to previous experiments on maize (Wery et al., 1981) and soybean (Wery et al., 1983):

W1: first and second irrigation based on tensiometer inserted at 30 cm depth, third irrigation based on tensiometer at 45 cm depth, fourth irrigation (and others if necessary) based on tensiometer at 60 cm depth.

W2: first irrigation based on tensiometer inserted at 45 cm depth, second irrigation based on tensiometer at 60 cm depth, third irrigation (and others if necessary) based on tensiometer at 90 cm depth.

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For each treatment and each depth we used the average value of two tensiometers. Each irrigation began when the hydraulic charge reached -0.06 MPa for the reference tensiometer. The amount of water applied was 25 mm, that was normally enough to rewet the soil surrounding the tensiometers inserted at 30 or 45 cm depth but not enough to entirely cover the plant water consumption. The irrigation was stopped 10 days before the expected harvesting date. Treatment W2 represented a strategy of severe limitation of irrigation, in order to force the crop to use, as much as possible, the water stored in the soil. Treatment W1 was probably more adapted to grain legumes such as soybean (Wery et al., 1983).

Each irrigation treatment was combined with three levels of N nutrition by applications of ammonium nitrate:

N1: no application (i.e. only N fixation plus soil N assimilation).
N2: 50 kg N ha^{-1} during vegetative phase (i.e. before pre-cutting).
N3: 150 kg N ha^{-1} in three applications during the crop cycle (the first one was at the same time as that of N2).

Treatment N3 represented a strategy of adequate N nutrition, particularly during the seed filling phase when N fixation is known to decrease (Wery, 1987b).

Volumetric soil water content was determined with a neutron probe each 10 days on two replications per treatment at each 10 cm to 1.60 m depth, and converted to volumetric humidity by using the calibration curves previously established for this soil (Wery et al, 1983). Crop water consumption between two dates was calculated as the sum of the variation of the soil water content, plus irrigation and rainfall (100 mm from the beginning of growth to harvest).

Accumulation of dry matter (DM) in the different components of the plant was measured on average each 10 days on samples of 400 cm^2. The plants were divided in four parts: roots (in the first 25 cm of soil), stolons, leaves, and flowering heads. The number of leaves and flowering heads were also measured.

The effect of mineral N supply was studied by following the evolution of nitrate reductase activity (NRA) measured with the *in situ* method of Robin et al. (1983). For each treatment and each replication we measured seed yield and its main components (i.e. number of flowering heads per m^2, number of florets per flowering head, number of seeds per floret and 1000 seed weight). The crop was harvested on July 18, 1986.

RESULTS AND DISCUSSION

Water consumption

White clover grown for seed production receiving two irrigation treatments used a significant amount of water (Table 1). From the beginning of growth to maturity, the total water consumption averaged 463 mm for W1 (125 mm from irrigation) and 446 mm for W2 (100 mm from irrigation). However, the two irrigation treatments supplied less water than required for either high grain legume production or high white clover forage yields, in this environment. White clover has a high transpiration rate associated with a poor stomatal regulation and so is poorly adapted to drought. The adaptation of the transpiration is mainly obtained by the acceleration of leaf senescence (Haynes, 1980). During the crop cycle, the evolution of water consumption was very similar for the two irrigation treatments and for the three N treatments. We observed no significant difference between the 6 combinations, for total water consumption during the 7 measurements we made between precutting and harvest (data not shown). The amount of water used before precutting was very low (16% of the total amount), then water consumption increased significantly during flowering and pod filling. This shows that, as for grain legumes, white clover has its greatest need for water at the beginning of the reproductive phase. On the other hand, we found no difference in the amount of water extracted from the different soil layers, under the two irrigation treatments.

Table 1. Effect of irrigation and N supply on water consumption.

Treatment		Total water Consumption		Soil Water
Irrigation[a]	N			
	kg ha^{-1}	------- mm		-------
W1	0	471	(20.5) [a]	231
	50	451	(11.8)	235
	150	467	(13.1)	242
	Mean	463	(10.6)	236
W2	0	460	(1.6)	260
	0	442	(8.0)	240
	150	435	(0.8)	236
	Mean	446	(12.9)	246

[a] Irrigation treatment W1 125 mm; Irrigation treatment W2 100 mm

Value in parenthesis is standard error of the mean

White clover did not have a shallow root system because water consumption was substantial to 1.0 m and continued to 1.5 m depth. This was confirmed by observation of the rooting profile; although most of the roots were in the upper part of the soil (0.05 to 0.30 m), we found roots to 0.90 m depth (data not shown).

Finally, the amount of water extracted from the soil and its distribution along the profile were not affected by the irrigation or by the nitrogen treatments (Table 1). In conclusion we can assume that the water consumption of the different treatments differed only from the amount of water applied by irrigation: 4 irrigations of 25 mm for W_2 and 5 irrigations of 25 mm for W_1. Nevertheless, this difference (25 mm) and the difference in the distribution of the irrigations during the reproductive phase were sufficient to affect the physiological processes involved in seed production.

DM production and distribution

N had no significant effect, but irrigation influenced DM production in the different components of the crop: roots, stolons, leaves and flowering heads. For the three vegetative parts the biomass increase was only temporary stopped by pre-cutting and increased until the end of flowering (Figures 1, 2, 3).

The root mass was greater (+22%) in treatment W2 compared with treatment W1 (Figure 1). As we have seen previously, this increase in the root system of the low-irrigation treatment was not translated to an increase in the soil water consumption, probably because it had not changed the rooting pattern (data not shown) and did not allow the plant to use more water particularly in the lowest part of the profile. Most of this additional Dry Matter was probably stored in the taproot.

The stolon biomass followed the same trend and finally, it was again more important for the more stressed treatment (W2) (Figure 2). This can be explained, at least partially, by an increase in the number of stolons (+29%). On the contrary, leaf biomass was greater in the heavier irrigated treatment (W1) and did not develop a plateau - which is visible on W2 - at the end of the cycle (Figure 3). This was due to the continuous formation of new leaves translated in a higher final number of leaves in W1 (+11%).

Taking into account the biomass of the three vegetative components we can conclude that treatment W2 created a typical reaction to drought stress: it increased the ratio (absorbtion and storage organs = roots + stolon) / (transpiration organs = leaves) from 1.01 to 1.52. This increase can be explained by the fact that translocations and photosynthesis are less sensitive to drought stress than is leaf growth, creating an excess of carbohydrates stored into the roots and stolons, as previously observed in alfalfa (Durand, 1987).

Evolution of NRA

For the two irrigation treatments, leaf NRA increased until June 6, near the end of the flowering period (Table 2). The activity was stable during a period whose duration was dependent on the level of N applied and above all on the amount of water received. This stimulation of NO_{3-} assimilation by irrigation can be explained by an increase in nitrate supply associated with the transpiration rate arriving at the leaves (Wery, 1987a). In treatment W1 NRA decreased later than for W2 (July 3) due to the prolongation of leaf growth by irrigation previously shown in Figure 3. The supply of N increased the NRA, mainly during the period of maximum activity and has probably strongly decreased the N-fixing activity because it had no positive effect on growth (Wery, 1987b).

Evolution of flowering

The evolution of DM and number of flowering heads (Table 3) shows that on treatment W2, flowering began earlier and was heavier. The difference in earliness and number of flowers could be explained by the fact that, in treatment W1, the canopy was more dense (more leaves with larger size) and higher, (the average length of the leaf peduncle was significantly higher for W1 (26.8 cm) than for W2 (22.8 cm)), decreasing the solar radiation at the flowering nodes which is important for floral induction. According to Clifford (1985), drought stress has a negative effect on white clover leaf area providing a good tool for delaying canopy closure and increasing floral induction. In treatment W2, this beneficial effect on the number of inflorescences was probably accentuated by the limitation of the competition between leaf growth and accumulation of DM in the flowering heads.

Seed yield

The only significant effects were that of irrigation treatment on seed yield ($F \geq 0.01$) and on number of inflorescences m^{-2} ($F \geq 0.02$), which is known to be the yield component most closely correlated with seed yield. The increase of 25 mm in water supply (treatment W1 vs W2) decreased the number of flowering heads m^{-2} by 12% (Table 4). Other yield components were not affected (number of florets per inflorescence and number of seeds per pod) or showed a tendency to decrease in the more limited treatment (1000 seed weight). This can be explained by a compensation effect due to an excessive number of flowers in W2, in a situation of slight drought stress at the end of the reproductive phase. Similar results were obtained by Clifford (1985). Finally seed yield was, on average, 35% greater in W2 than in W1 (Table 4). Moreover, if we take all the plots into account, white clover seed production was negatively cor-

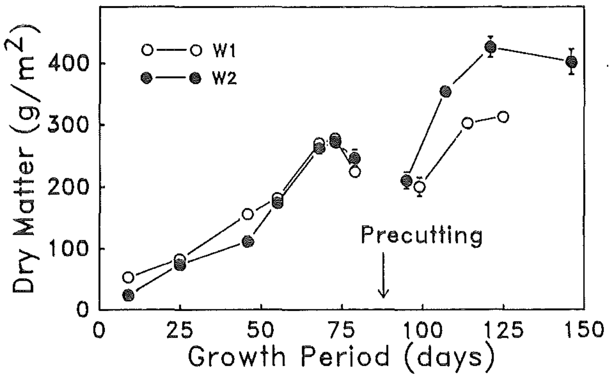

Figure 1: Root dry matter accumulation.

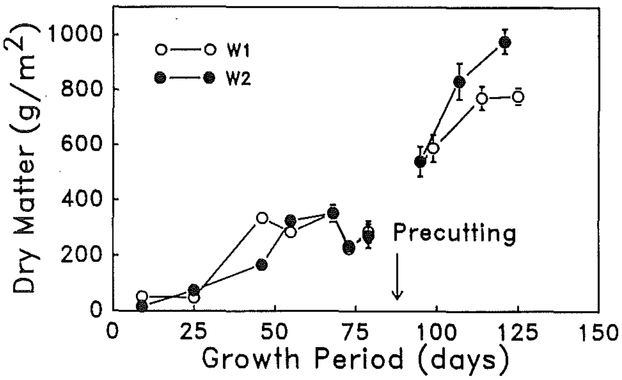

Figure 2: Stolon dry matter accumulation.

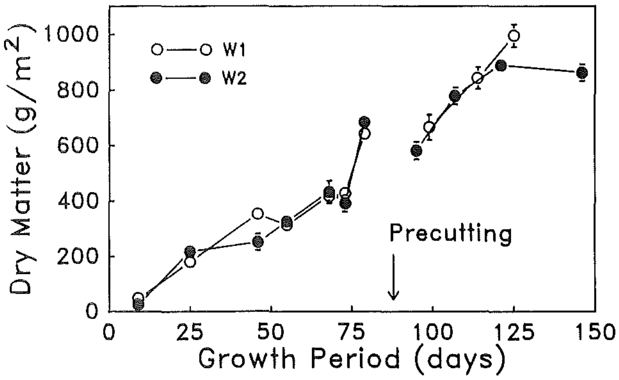

Figure 3: Leaf dry matter accumulation.

(Each value is the mean of the 3 replications and the 3 nitrogen treatments. The standard deviation is represented by a vertical bar when it is larger than the symbol). (Day 0 is February 15, which is the beginning of the growing period.) Irrigation treatment W_1: 125 mm; Irrigation treatment W_2: 100 mm.

Table 2. Seasonal nitrate reductase activity.

Treatment		Nitrate Reductase Activity					
Irrigation[a]	N	May 25	May 30	June 6	June 18	June 27	July 3
	Kg ha^{-1}	-------umoles $NO^-_2.h^{-1}.g^{-1}$DM-------					
	0	0.11	1.13	2.16	1.65	1.49	0.69
W_1	50	0.50	1.86	3.09	3.31	3.04	0.79
	150	0.54	2.86	3.65	3.41	3.02	0.89
	0	0.13	0.83	1.14	1.05	0.70	0.10
W_2	50	0.54	0.95	2.98	1.62	1.26	0.00
	150	0.55	1.36	3.34	2.80	0.80	0.00

[a] Irrigation treatment W_1: 125 mm; Irrigation treatment W_2: 100 mm.
Nitrate reductase activity is the mean of two samples of 10 leaves.

Table 3. Number and dry matter of flowering heads.

		Number of flowering heads m^{-2}		Flower DM/m^2	
Date	Treatment[a]	W_1	W_2	W_1	W_2
May 5		4.2 (2.5)	41.6 (15.0)	-- --	5.5 (5.0)
May 21		-- --	281.7 (33.3)	-- --	81.8 (18.2)
May 25		151.6 (25.0)	-- --	54.5 (13.6)	-- --
June 2		-- --	490.0 (35.0)	-- --	186.3 (13.6)
June 9		320.8 (58.3)	-- --	216.4 (19.1)	-- --
June 16		-- --	675.0 (35.0)	-- --	400.0 (22.7)
June 20		589.2 (37.5)	-- --	419.1 (18.2)	-- --
July 3		-- --	-- --	-- --	538.2 (24.5)

[a] Irrigation treatment W_1: 125 mm; Irrigation treatment W_2: 100 mm.
Each value is the mean of the 3 replications and the 3 nitrogen treatments. The standard deviation is in parentheses.

Table 4. Seed yield and its main components

Treatment						
Irrigation[a]	N	Flowering heads	Florets per inflorescence	Seeds per pod	1000 Seed weight	Seed yield
	(kg ha^{-1})	(m^{-2})			(g)	(kg ha^{-1})
	0	708	81	3.4	0.57	478
W1	50	692	82	3.2	0.59	497
	150	743	82	3.2	0.59	423
	Mean	714	82	3.3	0.58	466
	0	722	77	3.1	0.59	578
W2	50	886	80	3.3	0.57	630
	150	836	80	3.1	0.55	680
	Mean	815	79	3.2	0.57	629

[a] Irrigation treatment W1 125 mm; Irrigation treatment W2 100 mm

related with the amount of water used by the crop, particularly for a given irrigation treatment: for W_2 $X = -0.24\ Y + 596.6$ ($R^2=0.9216$) and for W_1 $X = -0.20\ Y + 552$ ($R^2=0.9801$). The low and non-significant differences observed in water consumption (see Table 1) show how white clover seed production is sensitive to an excess of irrigation. Nevertheless, we can suppose the existence of an optimum water supply, because if we had reduced the amount of water applied too much, we would have probably affected pollination, pod formation and seed filling, three processes which are very sensitive to drought stress (Clifford, 1985).

On the other hand, the efficiency of water consumption for white clover seed production was very low if we compare with annual crops. For white clover we found, on average, 0.10 kg of seeds m^{-3} of water used, that is about 10 fold lower than for soybean (Wery et al., 1983) and 20 fold lower than for maize (Wery et al., 1981). This element needs to be taken into account for the evaluation of economic feasibility of white clover seed production in Mediterranean conditions.

The supply of N seemed to increase seed yield only in the more stressed treatment (W2) (Table 4). This could be explained by a slight N stress created by the limitation of water consumption in this treatment which had probably a more inhibiting effect on N fixation than on flowering, photosynthesis and translocation (Wery, 1987a). Nevertheless, this effect is too weak to be significant in our experimental design and it needs to be reassessed in a poorer soil with more replications.

CONCLUSIONS

These results show that the management of white clover seed crops at a certain level of drought stress during the reproductive phase can be a useful tool for improving seed production. A 20% decrease in the amount of water received by irrigation was sufficient to limit the vegetative growth (leaves number and biomass) and to induce and support more flowering heads. It is interesting to notice the association of this seed yield increase with an increase of the roots and stolons biomass versus leaf biomass, which is a very typical reaction to drought stress. Finally we found a very good negative correlation between seed yield and the total amount of water used; however, a severe drought would have probably reduced seed yield by limiting pod formation or pod filling (Clifford, 1985). It is so necessary to define and create in the farmers fields an optimal drought stress in order to obtain the maximum seed yield. In Mediterranean regions optimal stress can be obtained by a good management of irrigation, based for example, on tensiometers (see treatment W2) which are inexpensive, simple, and already used by the farmers on other crops in southern France.

In comparison with irrigation, N nutrition did not appear to be an important problem for white clover seed production under our conditions. Nevertheless, the fact that N fixation is more sensitive to drought than is photosynthesis and seed yield (Wery, 1987a), can explain why mineral N supply had a positive effect only on the more stressed treatment (Table 4). This N starvation induced by drought stress needs to be reassessed in soils with lower level of mineral N and phosphorus, which are probably better for white clover seed production (Clifford, 1985).

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