Effect of Moisture Stress on Seed Yield and Quality in Four Annual Clovers

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ABSTRACT

Field experiments with four forage legumes, berseem (*Trifolium alexandrinum* L.), crimson clover (*T. incarnatum* L.), Persian clover (*T. resupinatum* L.) and squarrosum clover (*T. squarrosum* L.), were conducted in 1993 and 1994 at Foggia (Italy) to evaluate the influence of moisture stress during the seed filling period on seed yield and its components, and on germination and vigour (as measured by seedling length and dry matter, and accelerated ageing) of the harvested seed. An irrigated control was compared with a moisture stress treatment. The development of stress was monitored measuring leaf water potential (Ψw) at around midday. Seed yield was reduced by an average of 61% in 1993 and 35% in 1994 by moisture stress, with the decrease ranging from 24 to 68% among the species. The main seed yield components affected were stolon number and seeds per flower head (41 and 39% mean reduction, respectively). Standard germination percentage, germination rate index (GRI), seedling growth rate and accelerated ageing were mostly not influenced by moisture stress. Only seedling dry weight was significantly lowered in seeds ripening under moisture stress. Moisture stress occurring during seed development reduced seed yield and its components, but the seeds produced were viable and of high vigour.

Additional index words: **annual clovers, moisture stress, drought, seed yield, seed germination, seed vigour.**

INTRODUCTION

The Mediterranean environment is characterized by concentrated rainfall during the winter months, and prolonged drought periods during the spring and summer seasons. In these regions, annual legume forage crops with early spring growth habits are able to ensure a fairly long period of forage supply for hay production (Bullitta, 1976; Martiniello and Ciola, 1993). Clover *(Trifolium)* species can be utilized for this purpose, either as pure stands, or in a mixture with a grass species; their forage is of high quality and mainly used as hay and they are well suited to non-competitive grassland farming. However for seed production, these crops are frequently subjected to the detrimental effects of the environment, particularly high temperatures and water deficits, during the periods of seed formation and filling. The availability of quality seeds is a decisive factor for widespread, successful cultivation, particularly for crops with small sized seeds. It is broadly accepted that not only yield, but also seed quality, can be affected by climatic conditions. For example in soybean [*Glycine max* (L.) Merr.], Smiciklas, Mullen, Carlson and Knapp (1992) indicated that variable seed quality could be attributed to the timing of water stress, and pod position on the plant. Furthermore, Heatherly (1993) found that irrigation significantly increased seed yield and seed number, and that drought negatively influenced seed quality, by reducing germination. In contrast, Dornbos, Mullen and Shibles (1989) reported that the effect of drought on germination and vigour was substantially less than that on yield, while Vieira, TeKrony and Egli (1992) suggested that moisture stress during seed filling was unlikely to affect seed germination or vigour, in the absence of shrivelled seed.

The present study was therefore conducted with the aim of examining the effects of moisture stress during seed development and maturation on seed yield and its components, and on seed germination and vigour, in four annual clovers which are characterized by different morphological features and edaphic adaptation.

MATERIALS AND METHODS

Field experiments were conducted at the Forage Crop Institute farm located in Foggia (southern Italy: 15° 33' E; 41° 31' N; 76 m above sea level) during the 1992/93 and 1993/94 growing seasons. Four annual clovers were evaluated; berseem (*Trifolium alexandrinum* L.) cv. Sacromonte, crimson clover (*T. incarnatum* L.) ecotype Campano, Persian clover (*T. resupinatum* L.) cv. Accadia and squarrosum clover (*T. squarrosum* L.) ecotype Toscano-Laziale. Seeds were drilled on 19 October 1992 and 5 October 1993. Before sowing, nitrogen (16 kg N ha^{-1}) and phosphorus (70 kg P ha^{-1}) were broadcast and harrowed in, and diazinone [0,0-diethyl-0-(isopropyl-4-methyl-6-pyrimidil) thiophosphate] applied at 35 kg product ha^{-1}. Plots consisted of 8 rows, 5 m long and 18 cm apart, with a plant density rate of 1100, 1200, 3100 and 700 seeds m^{-2} for berseem, crimson, Persian and squarrosum clovers, respectively. The plots were arranged following a split-plot design with four replications, with the two irrigation treatments in the main plot and the four species in the subplot. Climate data during the experimental period are shown in Fig. 1. The plants were grown under two water regimes from early flowering to seed harvest: well watered (irrigated) as the control, and water stressed (non-irrigated). The irrigation treatment was applied when

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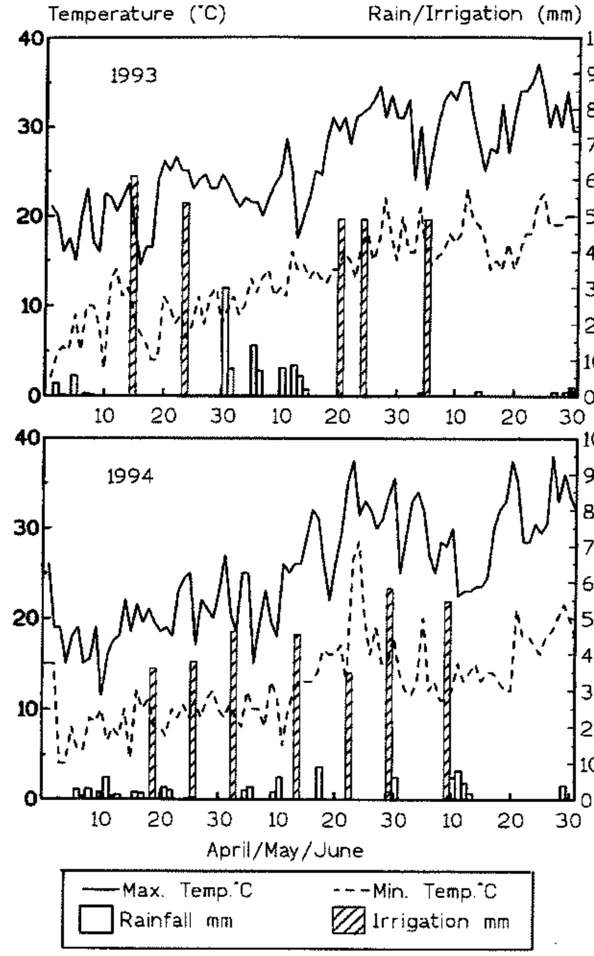

Fig. 1 Climate data (rainfall, maximum and minimum temperatures) and amount of water supplied by irrigation at Foggia, Italy, during April, May and June in 1993 and 1994.

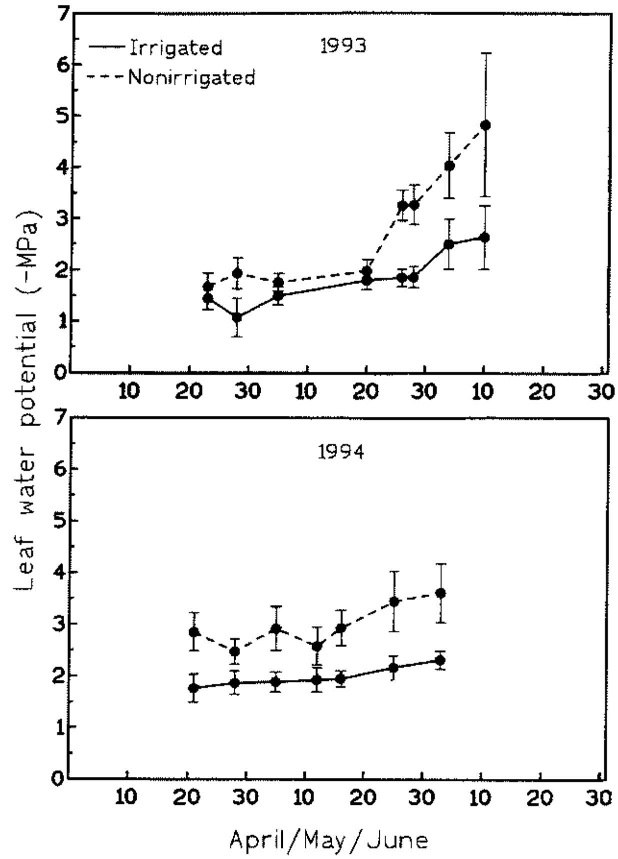

Fig. 2 Leaf water potentials at various times after 1st April under irrigated and non-irrigated conditions in 1993 and 1994. Values are means of the four clover species ± SD.

evapotranspiration, calculated on the basis of evaporation (derived from significant rainfall and using FAO's crop coefficient related to the species) reached 80 mm. The plots were watered by means of a horizontal bar 16 m long, 1.25 m from ground level and with a low nozzle pressure. In 1993 and 1994, 260 and 310 mm ha^{-1} were applied respectively (Fig. 1). Moisture stress was imposed by interrupting irrigation and its intensity was measured by leaf water potentials Ψw) of the uppermost fully expanded leaflets. The Ψw values were determined in all plots near midday (11.00-14.00 h) at approximately weekly intervals from April to June using the pressure chamber technique describd by Scholander, Hammel, Hemingsen and Bardstreet (1965). Stress history is shown in Fig. 2. Seed moisture content was monitored, and plots harvested when seed moisture content was 15% by cutting all plants within 1 m^2 per plot. A sample of 20 stolons taken at random, was used to assess seed yield components: number of stolons m^{-2}, flower heads per stolon, seeds per flower head and 1000 seed weight (g). Harvest index (HI, percentage ratio of seed weight to the total above-ground biomass) was calculated. The threshed seeds of each plot were stored at 4°C.

Seed quality was evaluated by standard germination and vigour tests. Germination tests at a constant temperature of 20°C were conducted without light according to ISTA specifications (ISTA, 1985); the first count (7th day) and final count (14th day) as well as speed of germination (GRI, germination rate index) according to Maguire (1962) were determined. For vigour tests, seeds were germinated in rolled towels in the dark at a constant temperature (20°C) and 100% RH; after 7 days shoot and root length (mm) and seedling dry matter (mg), after drying for 48 h at 60°C, were recorded (ISTA, 1987). The accelerated ageing (AA) test was conducted by exposing 100 seeds from each field plot to 40°C and 100% relative humidity for 72 h, and then testing germination as described using two 50-seed replicates. Data were subjected to analysis of variance and mean separations were performed using the LSD test at the 0.05 probability level when the F test was significant. The relationships between all characters were determined by correlation analysis for each species (n = 16).

RESULTS

Moisture stress imposed at early flowering had a significant effect on leaf water potential in both years (Fig. 2). At the last observation (early June) the non-irrigated treatment had a leaf water potential of 2.2 and 1.3 MPa

Table 1. Seed yield, harvest index, stolon, flower head and seed numbers and 1000 seed weight of four annual clovers with and without irrigation during seed filling at Foggia, Italy, in 1993 and 1994.

Treatment[1]	Clover Species	Seed yield (g m⁻²)		HI (%)		Stolon no. m⁻¹		Flower heads per stolon		Number of seeds per flowerhead		TSW[2] (g)	
		1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
I	Berseem	37.5	41.2	3.5	3.1	283.1	510.3	9.1	6.5	35.6	24.3	1.98	3.23
	Crimson	47.1	17.9	2.9	1.3	1396.7	962.7	1.0	1.0	117.9	24.6	2.73	3.33
	Persian	104.9	87.5	5.0	3.2	707.9	383.0	16.6	25.3	17.5	13.8	1.65	1.94
	Squarrosum	119.8	14.7	6.4	1.0	281.9	206.3	5.1	5.9	45.4	25.5	3.68	4.20
Mean		77.3	40.4	4.5	2.2	667.4	515.6	7.9	9.7	54.1	22.0	2.51	3.18
NI	Berseem	8.7	20.4	1.4	4.5	264.2	212.0	5.1	6.9	16.7	14.7	2.15	2.75
	Crimson	45.7	3.9	5.0	1.4	1084.6	273.6	1.0	1.0	57.3	23.5	2.30	3.04
	Persian	34.4	69.4	4.9	6.7	283.4	170.6	15.9	33.9	13.0	9.5	1.30	1.64
	Squarrosum	32.3	10.9	3.2	2.0	362.1	131.3	3.2	5.5	35.1	17.4	2.38	4.04
Mean		30.3	26.1	3.6	3.7	498.6	196.9	6.3	11.8	30.5	16.3	2.03	2.87
LSD(P<0.05)		9.10	NS	0.78	0.70	146.9	107.60	1.44	2.82	13.22	NS	0.428	NS
Significance between treatments		**	*	NS	**	**	**	*	**	**	**	*	NS
Significance between years		NS		*		*		**		**		**	

[1] *I = irrigated and NI = nonirrigated during seed filling.*
[2] *Thousand seed weight*
*, ** *Significant at P<0.05 and 0.01 respectively.*

Table 2. Standard germination and vigour tests for seeds of four annual clovers produced with and without irrigation during seed filling at Foggia, Italy, in 1993 and 1994.

Treatment[1]	Clover species	Percent germination: First count (%)		Percent germination: Final count (%)		GRI % day⁻¹		Seedling growth: Shoot length (mm)		Seedling growth: Root length (mm)		Seedling growth: Dry weight (mg)		Germination after accelerated ageing (%)	
		1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
I	Berseem	73	95	73	95	43.8	83.7	13.3	14.6	9.5	14.2	1.74	2.22	89	95
	Crimson	91	94	91	94	86.3	88.4	18.6	13.8	9.9	15.3	1.99	2.47	89	88
	Persian	94	74	94	74	90.8	73.1	14.7	6.4	14.4	9.4	1.37	1.29	94	60
	Squarrosum	50	77	65	84	18.0	32.2	9.9	13.7	7.4	10.2	2.82	2.82	72	82
Mean		76.9	84.8	88.8	86.7	59.7	69.4	14.1	12.1	10.3	12.3	1.98	2.20	85.8	81.3
NI	Berseem	72	86	75	86	46.3	77.3	10.8	13.4	7.8	13.2	1.64	2.02	82	83
	Crimson	92	95	93	95	88.6	92.7	14.4	12.4	10.2	8.5	1.93	1.95	94	81
	Persian	97	94	97	94	93.2	93.0	20.2	18.8	12.8	8.3	1.05	1.16	97	74
	Squarrosum	56	86	67	90	18.6	45.8	12.5	21.3	8.1	11.6	1.76	2.43	69	86
Mean		79.3	90.0	82.8	91.3	61.7	77.2	14.5	16.5	9.7	10.4	1.59	1.89	85.6	80.9
LSD(P<0.05)		NS	7.1	NS	6.2	NS	7.19	5.02	4.97	NS	4.04	0.274	NS	NS	8.5
Significance between treatments		NS	NS	NS	NS	NS	*	NS	*	NS	*	*	*	NS	NS
Significance between years			**		**		**		NS		NS		*		NS

[1] *I = irrigated and NI = non irrigated during seed filling.*
*, ** *Significant at P<0.05 and 0.01 respectively.*

lower than the irrigated treatment in 1993 and 1994, respectively. Leaf water potentials did not differ significantly among species, but in both years, Persian and squarrosum clovers had the lowest (0.75 MPa) and highest (1.11 MPa) decreases, respectively.

Seed yield and the yield components were significantly reduced by water deficits imposed during the seed filling period (Table 1). All characters, except harvest index and 1000 seed weight in 1993 and 1994, respectively, differed significantly between irrigation treatments. Seed yield reduction caused by moisture stress was higher in 1993 than in 1994 (mean of 61% and 35%, respectively), probably because of greater seed production under irrigated conditions in the first year. Persian clover proved the most productive species and berseem and crimson the least productive (74, 27 and 29 g m^{-2} on average, respectively). Harvest index did not differ between the two irrigation treatments in 1993, which may have been due to a similar rate of seed yield and biomass reductions under moisture stress conditions. By contrast in 1994, there was a greater biomass loss for the non-irrigated treatment (as harvest index values were higher). The decrease in the number of stolons was greater in the second year (62%), because of the considerable reduction in stolon density under moisture stress conditions compared with the first year. Crimson clover had the greatest stolon number in both years (1240 and 620 on average, in 1993 and 1994, respectively). The number of flower heads per stolon was more influenced by moisture stress in 1993, particularly in berseem and squarrosum clovers (Table 1). By contrast, Persian clover always had the highest number of flower heads and moisture stress did not influence the number in 1994. Seeds per seed head were reduced in the non-irrigated treatment, but by different numbers in the two years and for each species. In 1993, in which the decrease was higher (44%), crimson clover had the greatest number of seeds per seed head in both treatments. This may have been due to the early flowering of crimson clover, which was therefore less influenced by the moisture stress that occurred strongly towards the middle of May. Moisture stress affected the seed weight, reducing it by 19% in 1993; a non-significant reduction occurred in 1994 for all species. Squarrosum and Persian clovers were characterized by the heaviest and lightest seeds respectively.

The effects of moisture stress on seed germination and vigour were either very small or not statistically significant (Table 2). The germination percentages recorded on the 7th and 14th day did not differ significantly between irrigation treatments, but there was a significant species x irrigation treatment interaction in 1994, where berseem showed a 10% decrease in germination under non-irrigated conditions. Berseem, crimson and Persian clovers reached maximum values of germination at 7 days; but squarrosum clover increased 17% on average from 7 to 14 days. This latter species always had the lowest germination (66 and 87% in 1993 and 1994, respectively), and the lowest GRI (18 and 39% in the first and second year, respectively). In 1994 a significant difference for GRI values was found between irrigated and non-irrigated treatments. This was mainly due to the higher values recorded under moisture stress by Persian and squarrosum clovers. Seedling shoot length differed significantly between irrigation treatments in 1994, because of the higher values for the non-irrigated treatment recorded by Persian and squarrosum clovers; a similar trend was found for squarrosum clover in 1993. Moisture stress negatively affected seedling root length in 1994 only in crimson clover. In both years, seedling dry weight was the character most affected by drought (Table 2), with a 20 and 14% decrease for the first and the second years, respectively. Furthermore, in 1994, all species showed a decrease in seedling dry weight, while in 1993 Persian and squarrosum clovers were more greatly affected and showed the lowest and highest values (annual means of 1.21 and 2.46 mg, respectively). Seed vigour, as measured by accelerated ageing was high for all species. A statistically significant species x treatment interaction was found for seed vigour: berseem seed showed a vigour decrease when maturing under moisture stress conditions in both years (8 and 13% in 1993 and 1994, respectively).

DISCUSSION

Moisture stress developed during reproductive growth in the non-irrigated treatment in both years, as evidenced by a decrease in leaf water potentials. As a consequence, the reductions in seed yield recorded were related to water deficit during seed development. All yield components decreased, albeit at different rates, and each species was affected differently according to its own morphological characters. Thus, seed yield was correlated with different characters in each species *ie* the number of flower heads per stolon and seeds per flower head were important seed yield components in berseem (r=0.71** and r=0.74**, respectively) while stolon density was important in crimson and Persian clovers (r=0.80** and r=0.69**, respectively). For squarrosum clover the number of seeds per flower head was the most important yield component (r=0.82**). Oliva, Steiner and Young (1994a and b) found that the seed yield decrease following plant water stress was caused by reductions in different yield components for red (*T. pratense* L.) and white (*T. repens* L.) clovers. Under our experimental conditions, we found that to increase seed production when water is a limiting factor, it is impossible to improve the same seed yield component for all annual clovers.

Steiner (1993) found in lucerne (*Medicago sativa* L.) and in clover species that plants grown under very different water stress conditions may have different yields, but similar germination percentages and seedling vigour levels. Our data showed that seed germination was not greatly affected by moisture stress, but seedling dry weight was reduced. Yaklich (1984) found similar responses in soybean. Positive correlations between seed weight (measured as 1000 seed weight) and percentage germination (first and final count) and speed of germination, were found for berseem and squarrosum clovers (r=0.68**, 0.67**, 0.81** and 0.51*, 0.61*, 0.47*, respectively). These results indicated that, for these two species, the smaller seeds produced under moisture stress will have a lower percentage germination. The regression coefficient calculated over all species, relating to seed weight and seedling dry weight, was 0.91*** (n=64). According to McKersie and Tomes (1982) and Dornbos *et al.* (1989) these data support the existence of a relationship between seed size and initial seedling vigour when seed size is reduced by environmental stress. The results showed that seed vigour, measured by accelerated ageing, depends on the species of clover. Only berseem seed quality was negatively influenced by water deficit during seed maturation.

In conclusion, moisture stress imposed from early flowering to the maturity stage of plant development greatly reduced seed yield and its components, but had little effect on vigour, germination and speed of germination. Since seed weight reduction was small in comparison with seed yield and other yield components, a relationship may exist between seed size and seed vigour and germination. Of the annual clovers examined in the study, berseem was the most susceptible species, as it had the greatest decrease under water deficit for both seed yield and seed quality.

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