The Effect of Water Deficits during Flowering and Seed Production on Cultivars of Subterranean Clover and Annual Medic

E. C. Wolfe
Charles Sturt University, Australia

W. J. Collins

R. C. Rossiter

W. R. Stern

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Wolfe EC (Ted)*, Collins WJ[†], Rossiter RC[#] and Stern WR[†]

[*]Graham Centre, Charles Sturt University and NSW Department of Primary Industries, Wagga Wagga NSW 2678, twolfe@csu.edu.au; [†]formerly University of Western Australia, Nedlands WA; [#]formerly CSIRO, Floreat Park WA

Abstract

In improved pastures in inland southern Australia, the persistence and growth of annual pasture legumes depends in part on their ability to produce and conserve abundant seed for regeneration and production. For near-maximum seed production in spring, adequate soil water is needed for at least 70 days in subterranean clover (*Trifolium subterraneum*) and medic (*Medicago*) species. Water deficits during spring are a common occurrence, and they appear to be increasing in frequency. The effect of relatively short periods of water deficit during reproductive development has received some attention but the findings conflict. The present experiment was conducted to examine further the responses of subterranean clover to water deficits imposed during the reproductive phase, and to compare the response of medic to that of subterranean clover. The flowering of two early strains of subterranean clover (Northam, Daglish) was synchronised with two early-flowering cultivars of annual medic (Cyprus barrel medic and Harbinger strand medic). Seed production parameters were observed on four watering treatments (nil, early, mid and late deficits during the reproductive phase). Notable differences occurred between the two genera and between the water treatments in their effect on reproductive development. A highly significant interaction favouring medic was recorded between the legumes (clover, medic) and the early deficit treatment, in that the individual seed weight of medic was slightly enhanced by water stress and clover was significantly depressed. In the mid- and late-stress treatments, a significantly lower proportion of viable seed was recorded with clover versus medic. The implications of the findings for the use of adapted annual legumes are discussed.

Additional keywords: drought, annual legumes

Introduction

In improved pastures in southern Australia, the persistence and growth of annual legumes, notably subterranean clover and medics, depends in part on their ability to produce and conserve abundant seed (Rossiter 1978) for regeneration and production. For near maximum seed production, adequate soil water is needed from the beginning of flowering for at least 70 days in subterranean clover (Rossiter 1978) and longer in some medic species (Clarkson and Russel 1976). Water deficits are a common occurrence in Australian agriculture. In recent years, the reproductive development of winter-growing annual legumes has been increasingly disrupted by apparent changes to the reliability and predictability of soil water in early spring, a possible manifestation of climate change. In subterranean clover, moderate and severe deficits throughout the reproductive phase depress the rate of flowering (Andrews et al. 1977), shorten its duration (Andrews et al. 1977) and reduce seed production (Andrews et al. 1977, Rossiter 1978). In some species of medic, a significant reduction in the duration of flowering occurs in response to prolonged water stress (Clarkson and Russell 1976), but at the time of the investigation (1980) the comparative reaction of these species to moisture stress during flowering and seed production has not been compared under controlled conditions. The present experiment examined further the responses of subterranean clover to water deficits imposed during the reproductive phase, and compared the response of medic to that of subterranean clover.

Methods and Study Site

The experiment was a factorial with four legume cultivars, four water deficit treatments and two replications (= 32 microswards) grown in open-sided glasshouses at CSIRO, Floreat Park. The subterranean clover genotypes were Northam (an early-flowering cultivar with a relatively long duration of flowering) and Daglish (a very early-flowering, early-maturing strain, one of the parents of cv. Nungarin, the earliest maturing subclover cultivar (Gladstones and Collins 1984). The medics were also very early-flowering, Cyprus (a cultivar of barrel medic *Medicago truncatula* var. *truncatula*) and Harbinger (a cultivar of strand medic *M. littoralis*). In May 1980, sieved seeds of each legume (clovers 7.5-7-7 mg, medics 3.0-3.4 mg) were sown at the rate of 25 viable seeds dm^{-2} (1 dm^2 = 100 cm^2) into soil in insulated wooden boxes (60 cm x 40 cm x 26 cm deep) on benches. Each box contained 90 kg of a 4/1 sand/loam mixture (bulk density 1.45). Before sowing, commercial fertilisers (g per box) were incorporated into the top 5 cm of soil: Cu-Zn-Mo superphosphate 11.5, potassium chloride 5.8, ammonium nitrate 1.4 and magnesium sulfate 0.35. The sowing dates of these strains were varied to overcome slight differences between these strains in the usual number of days from sowing to flowering (Gladstones and Collins 1984), in a successful attempt to synchronise their start of flowering (mid-August). The boxes were rotated and re-randomized every two weeks. After emergence, the seedlings were thinned to a density of 20 plants dm^{-2} in early June, and from mid-June to mid-August the microswards were defoliated weekly to a height of 2 cm. During this period, they were watered adequately by a hand-held sprinkler or by filling trays (71 cm x 51 cm x 7.5 cm deep) placed permanently under each box. All of the microswards grew healthily during the vegetative phase. During the reproductive phase, blue-green aphids (*Acyrthosiphon kondoi*) were controlled by an appropriate insecticide at double the recommended rate. After flowering began in mid-August, the following water management treatments were applied to each strain (note the 21-d wilting period in WE, WM and WL):

- W0 – an unstressed treatment. The water trays were filled late on Sunday, Tuesday and Thursday each week. Excess water was drained away the following morning. Watering ended on 2 November.
- WE – Early stress. Water was withheld from 18 August until 22 September. These microswards began to wilt on 1 September. Until full watering resumed on 22 September, small applications of water (500 mL per box per occasion, total applied 5.0 L per box) were sprinkled on each WE microsward three to four times weekly, to prevent the death of the legumes. Thereafter, as for W0.
- WM – Mid-period stress. Water was withheld from 5 September. Restricted watering (total 5.0 L per box, as above) was applied from wilting (15 September) until 6 October. Thereafter as for W0.
- WL – Late stress. Water withheld from 21 September. Swards began wilting on 30 September. Restricted watering (a total of 6.0 L per box) was applied on four occasions per week during the three-week wilting period until 20 October. Thereafter as for W0.

Measurements

At 0900-1000 hours every Tuesday from pre-flowering until maturity, soil water was determined gravimetrically from cores taken to a depth of 20 cm in each box. The moisture characteristic of the soil was determined using a pressure plate (-0.1 and -0.6 kPa) and pressure membrane (-5 and -15 kPa) equipment. Leaf water stress was measured on green leaflets between 0700 and 0900 hours each Tuesday with an hydraulic press (Campbell Scientific, Model J14). On each occasion, 2-6 leaflets were cut just above the junction of the leaf petiolules, placed between a folded cigarette paper and pressed to an end-point where water clearly exuded from the leaf surface. In late October, leaflet readings on the hydraulic press were calibrated with those made on excised leaves suspended by the petiole in a pressure chamber. Once flowering began, inflorescences within a fixed quadrat (2.25 dm^2) in each box were counted and tagged three times a week during the first half of the flowering period, and thereafter they were counted before the petals withered. At harvest (early December, after senescence), subterranean clover burrs were removed from a 5 dm^2 quadrat in each box and all medic burrs and tops were collected. These samples were dried for 3-4 days at 40°C and from them were derived values for the total number of burrs produced, the number and individual weight of mature seeds (separated from immature seeds by colour) and seed yield. Finally, 200 seeds were subsampled from the air-dried seed collected from each box and allocated to petri dishes with moistened filter paper to determine the proportions of seed in four categories: hard seed, soft seed producing normal seedlings, soft seed producing abnormal seedlings and dead seed (that disintegrated in water).

Results

Soil water, leaf water and flowering. When water was withheld successively from each of the water management treatments (WE, WM, WL), soil moisture fell from 0.16-0.20 $cm^3.cm^{-3}$ (field capacity) to reach wilting point (0.04-0.05 $cm^3.cm^{-3}$) after 13 d (WE), 9 d (WM) or 7 d (WL). Leaf water readings on the hydraulic press, initially -5 to -8 kPa (equivalent to pressure chamber readings of -5 kPa for both legumes), declined slowly for two weeks and then more rapidly during the next week as the soil dried towards wilting point (-15 kPa), at which point the leaf water deficits were -18 kPa (subterranean clovers) and -22 kPa (medics) on the hydraulic press (equivalent to pressure chamber readings of -15 kPa). Nearing the trough of water status, the leaves began to turn yellow and senesce. At the end of each 21-d period of water deficit, most leaves on the stressed microswards were dead, but restricted surface watering had maintained turgor and a green colour in the stems and some leaves. On the W0 treatment, all of the cultivars reached a peak rate of inflorescence production 14-21 days after all legume cultivars began flowering on 18 August. Northam subterranean clover peaked at 26 inflorescences/week/dm^2 compared with Daglish subterranean clover (31), Cyprus medic (53) and Harbinger medic (60). The duration of flowering on W0 (taken when inflorescence production >1/dm^2/ week) was shortest with Daglish (42 days) compared with Harbinger (63 days) and then Northam and Cyprus (70 days). The cumulative total of inflorescences produced ranged from about 100 per dm^2 (Daglish) to >200 (Cyprus and Harbinger). Average inflorescence production was 13 flowers/dm^2/day on W0 and WE at 0-7 days after regular watering ceased on WE, 39 (W0) vs 35.5 (WE) at 7-14 d, 38 (W0) vs 27 (WE) at 14-21 d, declining to 19 (W0) vs 1 (WE) at 28-35 d, before the WE treatment was re-watered. Upon re-watering WE, the recovery of vegetative growth and flower production was initially more rapid for the medics than for the clovers; the peak rate of re-flowering was 19, 9, 28 and 23 inflorescences per dm^2 per week for Northam, Daglish, Cyprus and Harbinger respectively. The cumulative total number of inflorescences produced on WE was comparable with W0 for the clovers (W0 = 132 per dm^2 for Northam, 92 for Daglish), but WE was 25-30% less than W0 for the Cyprus and Harbinger medics. After re-watering the WE treatment, the burrs on the medic microswards continued to swell as they matured. On WM and WL, water stress (indicated by wilting) terminated flowering within 5-7 days. Over the next three weeks, leaf death was considerable but stolons remained green, as did a few leaves. On WM, the flowering rates after re-watering were much lower than on WE after re-watering. On WL, the re-watered microswards failed completely to recover. After watering ended on all treatments (2 November), about 50% of the flowers aborted at maturity on Cyprus medic and 10-20% on Harbinger medic. At harvest (early December), the components of seed yield are given in Table 1.

Table 1. The effect of the water deficit treatments on burr and seed production from microswards of subterranean clover (Northam-N and Daglish-D) and annual medic (Cyprus-C and Harbinger-H).

Legume	Water treatment	Burrs.dm^{-2} x	Burrs.dm^{-2} √x	Seeds.dm^{-2} x	Seeds.dm^{-2} √x	Seeds/burr #	Seed weight mg	Seed yield g.dm^{-2}
Subterranean clover (Sc)	W0	106.3	10.31 bc	305	17.47	2.88 c	6.31 a	1.915 a
	WE	100.4	10.02 bc	244	15.61	2.43 d	4.47 c	1.096 b
	WM	62.6	7.91 d	125	11.18	1.99 e	5.21 b	0.673 c
	WL	84.6	9.20 c	239	15.45	2.84 c	5.20 b	1.248 b
Medic (Me)	W0	149.5	12.28 a	642	25.33	4.48 a	3.21 d	2.023 a
	WE	117.3	10.83 b	534	23.10	4.68 a	3.48 d	1.848 a
	WM	68.7	8.29 d	286	16.90	4.24 b	2.84 e	0.804 c
	WL	116.9	10.81 b	471	21.72	4.16 b	2.46 e	1.156 b
Interactions:	LSD (P=0.05)		0.93		-	0.31	0.28	0.249
Sc v Me x W	Significance		P<0.05		n.s.	P<0.01	P<0.01	P<0.01
N vs D x W:	Significance		n.s.		n.s.	n.s.	n.s.	n.s.
C vs H x W	Significance		n.s.		n.s.	P<0.01	P<0.01	P<0.05

Burr and seed production. On the WE treatment, which began just after the start of flowering (mid-August), the harvested seed yields of the medics were not reduced significantly but clover seed yields were 40% less than the control (W0). The different responses of the clovers and medics to the WE treatment is seen further in the analysis of the seed yield components (Table 1). In summary, the effect of WE on medic was to produce fewer burrs compared with W0, but these burrs contained slightly more seeds (n.s.) that were slightly heavier (n.s.); in contrast, the clover burrs from the WE treatment compared with W0 were lower in terms of all the parameters, significantly so (in most cases. However, when the water stress was applied during the peak of burr and seed development (WM), the seed yields of both the clovers and the medics were reduced considerably compared with W0 (-65% for subterranean clovers and -60% for the medics), while seed production on WL was curtailed only moderately so (-35% for the subterranean clovers, -43% for the medics). The Northam vs Daglish interaction with water, which was of interest due to the better seed production of Northam vs Geraldton at WE in the study of Andrews et al. (1977), was not significant. The Cyprus vs Harbinger medic interaction with water treatment was less important than the main effects of water stress.

Seed quality. The data obtained on the quality of the seed produced by each of the clovers and medics revealed another important interaction between clovers and medics (Table 2) in their response to the water treatments. The main differences in seed quality were the higher proportions of dead seed in the WM and WL treatments in the case of the clovers compared with the medics, and a high proportion of abnormal seedlings for the Daglish subterranean clover in these later-imposed water stress treatments.

Table 2. The effect of the water deficit treatments on the quality of the seed produced from microswards of subterranean clover (Northam and Daglish) and annual medic (Cyprus and Harbinger).

Water treatment	Cultivars	Quality of the seed produced on each treatment (%) Hard seed	Normal seedlings	Abnormal seedlings	Dead seed
No stress W0	Northam	91.0	7.0	0.5	1.5
	Daglish	95.0	4.0	0.5	0.5
	Cyprus	78.5	15.0	2.0	4.5
	Harbinger	78.5	16.5	1.5	3.5
Early stress WE	Northam	75.5	17.0	1.5	6.0
	Daglish	89.5	6.0	2.0	2.5
	Cyprus	70.0	17.0	5.0	8.0
	Harbinger	70.0	22.5	1.5	6.0
Mid-stress WM	Northam	65.0	10.0	4.5	20.5
	Daglish	34.5	11.5	18.0	36.0
	Cyprus	82.5	12.0	1.0	4.5
	Harbinger	87.0	8.5	0.5	4.0
Late stress WL	Northam	42.5	8.5	9.5	39.5
	Daglish	20.5	36.5	17.5	25.5
	Cyprus	92.0	3.0	1.0	4.0
	Harbinger	88.0	6.5	1.5	4.0
Cultivar x Water	LSD (P<0.05)	8.6	6.7	4.3	10.0
Interaction	Significance	P<0.01	P<0.01	P<0.01	P<0.10

Discussion

The experiment confirmed the serious impact on flowering and seed development of short periods of drought. The effects depended on the timing of these periods in relation to flowering (0-40 days with a peak at about day 20) and seed development (30-70 d, peaking around day 50). The results were notable from *three* perspectives:

- *First*, there was no significant interaction between the clover strains and the water treatments in terms of seed yield or any of its components. This result contrasted with the investigation of Andrews et al. (1977), who reported that short periods of water stress imposed on the Northam cultivar either early (days 21-46 after first flower) or late (days 32-53 after first flower) had considerably less effect on seed yield than the early and late stresses applied to the Geraldton cultivar, which developed later due to a delay caused by high night temperatures. The Northam/Geraldton water stress treatments were also confounded by a decision to vary the final date of watering of the cultivars. Our results with annual legumes that were synchronised in reproductive development demonstrated similar patterns of seed production of all four strains on the W0, WM and WL treatments, and for both subterranean clover cultivars on WE. Hence, we conclude that the different responses of Northam and Geraldton to short periods of water stress applied by Andrews et al. (1977) were an artefact of their technique. Any expectation that some subterranean clover cultivars might be more adversely affected than others by a short period of water stress during reproductive development or, conversely, certain clover strains might have a greater ability to recover than other strains after the stress, was not supported by our results from the W0 and WE treatments.
- The significant clover vs medic interactions in seeds/burr, seed weight and seed yield that occurred in response to the WE treatment comprised the *second set* of notable results. These interactions collectively featured the ability of annual medics on the early-stressed treatment to compensate for a lower number of burrs by producing slightly more seeds per burr (n.s.) of greater weight (n.s) compared with significant depressions (P<0.05-0.01) recorded in these parameters for WE subterranean clover.
- The late water stress treatments revealed a *third* notable finding in the response of the medics and subterranean clovers to water stress, this time in seed quality. Again, the clovers were disadvantaged.

Overall, this investigation highlighted the sensitivity of subterranean clover and the superior tolerance mechanisms of Cyprus barrel medic when exposed to short droughts during reproductive development, phenomena that were reported in another study undertaken in 1986 at UWA by S Amoabin (Turner 1990). The lower sensitivity of some legumes to early water stress has been noted also in lentil (Shrestha et al. 2006) and in chickpea (Fang et al. 2011). Fang et al. (2011) reasoned that a recovery of chickpea growth and photosynthesis after re-watering (as in WE) provides a source of carbon for the developing seeds, whereas with terminal drought (as in WM and WL) the later-formed seeds have to compete with early seeds for a diminishing supply of carbon. Since 2000, the range of adapted annual legume species for the Australian wheatbelt has expanded, with the commercialisation and adoption of cultivars of biserrula (*Biserrula pelecinus*), bladder clover (*Trifolium spumosum*), gland clover (*Trifolium glanduliferum*), yellow serradella (*Ornithopus compressus*) and French serradella (*Ornithopus sativus*). These recent pasture legumes have proved successful for several reasons in Western Australia (Loi et al. 2005) and New South Wales (Hackney et al. 2015). Studies of the response of these species to water stress during flowering and seedset may reveal cultivars that, like the medics in this report, have adaptive mechanisms for coping with drought.

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