
CHAPTER 7

PLANT WATER RELATIONS

“The effects of prolonged water stress on a plant may be pronounced, yet changes in any single process at a given time during the stress may only be very minor and difficult to measure.”

Bradford and Hsiao, 1982

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7.1. Introduction

The maintenance of turgor by the accumulation of solutes, referred to as osmoregulation in this chapter, is considered to be an important adaptation to drought (see reviews by Hsiao et al., 1976; and Morgan, 1984). Positive associations between osmoregulation and yield have been shown in wheat, with an average yield increase of 11% over 56 field trials carried out over a period of 4 years (Morgan et al., 1986). Further, when isopopulations were used under extreme stress, yield increases of up to 50% were observed (Morgan, 1983). Differences in osmoregulation between cultivars of mustard have been reported (Kumar et al., 1984), and these differences have been positively associated with yield (Singh et al., 1990b). There is evidence of wide variation in plant water relations within Brassicaceae (Chaudhary et al., 1989; Singh et al., 1990a, 1990b). However, despite the large differences between mustard and canola in growth (Chapter 4) and yield (Chapter 5) when grown under stress, direct comparisons of mustard and canola for water relations in general and osmoregulation in particular are rare. Lewan (1988) compared water potentials (ψ) in the two species. However, the cultivars used were not matched for maturity type and neither osmotic potential (π) nor relative water contents (ζ) were measured. Kumar and Elston (1992), in a series of glasshouse trials, found that mustard was able to maintain positive turgor pressure (P_t) at ψ as low as -2.4 MPa while *B. napus* P_t reached zero at ψ of -1.8 MPa. However, the extent of osmoregulation was not measured in that study.

In this chapter the plant water relations and extent of osmoregulation in mustard and canola are reported for those same experiments used elsewhere in this thesis.

7.2. Results

7.2.1. Leaf water potential

Compared at the same stage of phenology, there was only one occasion in Experiment 3 where there was a leaf ψ difference, with mustard having a lower midday value than canola 151 days after sowing (Table 7.1). No differences in ψ occurred between the species in Experiment 4, where measurements were taken only twice in the season (Table 7.1). In the more extensively sampled Experiment 5, no differences were found at the low deficit site. At the high deficit site, early morning ψ readings of mustard were greater ($P < 0.05$) than those of canola on two occasions, 90 and 118 days after sowing (Fig. 7.1) and they were also greater at midday on two occasions, 48 and 62 days after sowing. Leaf water potentials remained relatively stable with time at the low deficit site with midday ψ being 0.2 to 0.6 MPa lower than at dawn. In contrast, at the high deficit site, there was a steady

decline in both dawn and midday ψ readings to values much lower than those occurring at the low deficit site (Fig 7.1). There was also a tendency for dawn and midday ψ readings to converge at the high deficit site towards the end of the season. No measurements of plant water relations were made in either Experiment 1 or 2.

The occasions when ψ was found to be significantly greater in mustard indicate a trend for that crop to have slightly greater values than canola except early in the morning under low deficit conditions (Fig 7.1.). However, this trend was not clear in Experiments 3 and 4 (Table 7.1). Generally, under the field conditions of the present experiments, it would appear that the variation in water potential between the species is relatively small with a tendency for leaves of mustard to have slightly higher values than those of canola.

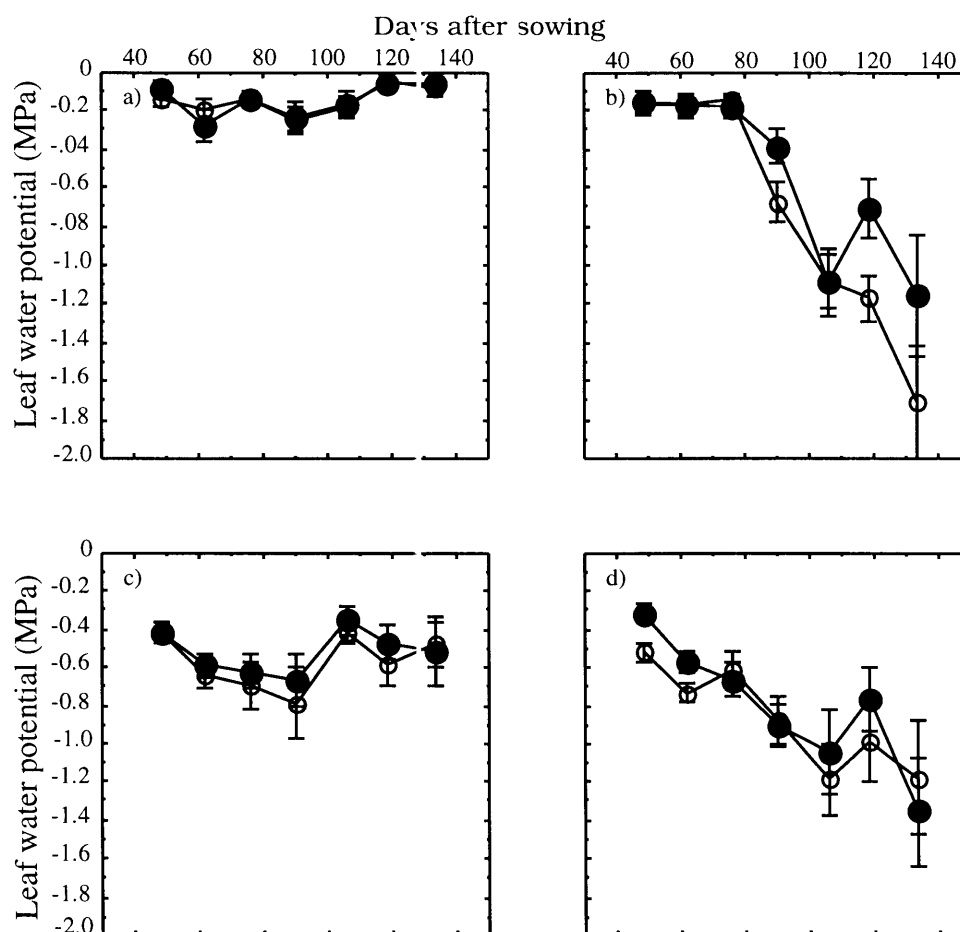


Figure 7.1. Changes in leaf water potential (\circ canola and \bullet mustard) with time in Experiment 5 under low deficit conditions a) early morning and c) midday and under high deficit conditions b) early morning and d) midday. Bars represent standard errors.

Table 7.1. Water potential (ψ), turgor pressure (P_t) and relative water contents (ζ) of leaves of canola and mustard sampled early in the morning (EM) or at midday (MD) in Experiments 3 and 4.

	ψ						P_t						ζ					
	Low deficit		High deficit		Low deficit		High deficit		Low deficit		High deficit		Low deficit		High deficit			
	EM	MD	EM	MD	EM	MD	EM	MD	EM	MD	EM	MD	EM	MD	EM	MD		
Exp. 3																		
112 DAS†																		
Canola	-0.04	-0.47	-0.10	-0.45	0.50	0.33	0.48	0.17	0.90	0.87	0.88	0.85						
Mustard	-0.16 ns	-0.61 ns	-0.14 ns	-0.53 ns	0.58 ns	0.47 ns	0.51 ns	0.47 ns	0.86 ns	0.87 ns	0.88 ns	0.84 ns						
139 DAS																		
Canola	-0.15	-0.49	-0.13	-0.33	0.41	0.32	0.31	0.33	0.96	0.79	0.94	0.81						
Mustard	-0.06 ns	-0.33 ns	-0.13 ns	-0.31 ns	0.65 †	0.50 †	0.67 **	0.57 *	0.96 ns	0.81 ns	0.94 ns	0.78						
151 DAS																		
Canola	-0.34	-0.42	-0.29	-0.96	0.14	0.00	0.10	0.00	0.95	0.81	0.93	0.75						
Mustard	-0.26 ns	-0.72 *	-0.49 ns	-0.87 ns	0.22 ns	0.10 ns	0.29 †	0.17 *	0.89 *	0.84 **	0.89 ns	0.79 †						
Exp. 4																		
44 DAS																		
Canola			-0.23	-0.49			0.38	0.37			0.82	0.81						
Mustard			-0.08 †	-0.63 ns			0.53 *	0.44 ns			0.84 ns	0.81 ns						
57 DAS																		
Canola			-0.07	-0.52			0.48	0.31			0.95	0.75						
Mustard			-0.14 ns	-0.66 ns			0.35 ns	0.22 ns			1.02 ns	0.73 ns						

† Days after sowing for the midday measurement, early morning readings were taken 114,140 and 153 DAS in Experiment 3 and 43 and 59 DAS in Experiment 4.

‡ P<0.1, * P<0.05, ** P<0.01

7.3.2. Leaf turgor pressure

At the low deficit site in Experiment 3, the leaf turgor (P_t) of mustard exceeded ($P < 0.1$) that of canola both early in the morning and at midday when measured 139 days after sowing (Table 7.1). While at the high deficit site it was greater 139 and 151 days after sowing at both times of day. In Experiment 4, mustard's leaf turgor was greater early in the morning 44 days after sowing.

In Experiment 5, under low deficit conditions, mustard's early morning leaf turgor exceeded that of canola at, 43 ($P < 0.1$), 106 ($P < 0.05$) and 133 ($P < 0.1$) days (Fig. 7.2) and at midday 106 ($P < 0.1$) days after sowing. Under high deficit conditions, mustard had greater ($P < 0.1$) P_t early in the morning at 76, 118 and 133 days and at midday, 48 and 106 days after sowing. On no occasion was canola found to have a higher P_t than mustard. These differences display a capacity in mustard to have higher P_t than canola, particularly evident in the early morning under high deficit conditions (Fig. 7.2. b).

7.3.3. Relative water content

In Experiment 3 (Table 7.1), the species exhibited no differences in relative water content except at the last sampling. On that occasions, at midday, mustard leaves had larger relative water contents than those of canola under low ($P < 0.05$) and high ($P < 0.1$) deficit conditions. In contrast, at dawn, mustard leaves had lower ζ at the low deficit site. In Experiment 4 no significant differences were present (Table 7.1).

In Experiment 5, leaves of mustard plants had a larger ($P < 0.005$) dawn ζ than canola at the low deficit site measured at 48 days after sowing and a larger ζ ($P < 0.05$) at midday 62 and 106 days. At the high deficit site, mustard had greater early morning ζ ($P < 0.05$) at 62 and 133 days after sowing and at midday 133 days. On no occasion was canola found to have a greater ζ than mustard which displayed a tendency to have slightly higher ζ on most occasions sampled (Fig. 7.3). Relative water contents at the low deficit site remained largely stable throughout the season with dawn values ranging between 0.88 and 0.97 and midday values between 0.84 and 0.89. However, at the high deficit site, there was a steady decline in ζ with time, particularly in canola where values reached as low as 0.55, 133 days after sowing. Conversely, mustard did not decline below 0.68 and stabilised its ζ at approximately this value over the last three samplings.

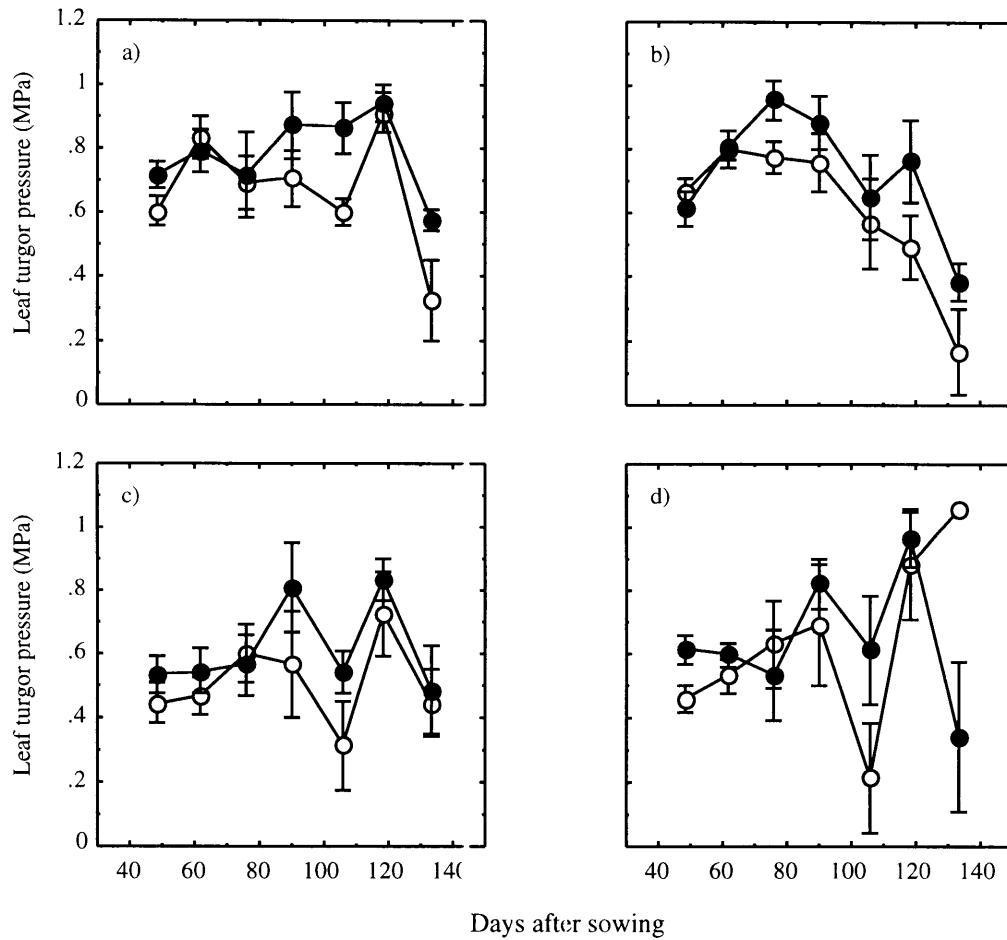


Figure 7.2. Changes in leaf turgor pressure (○ carola and ● mustard) with time in Experiment 5 under low deficit conditions a) early morning and c) midday and under high deficit conditions b) early morning and d) midday. Bars represent standard errors.

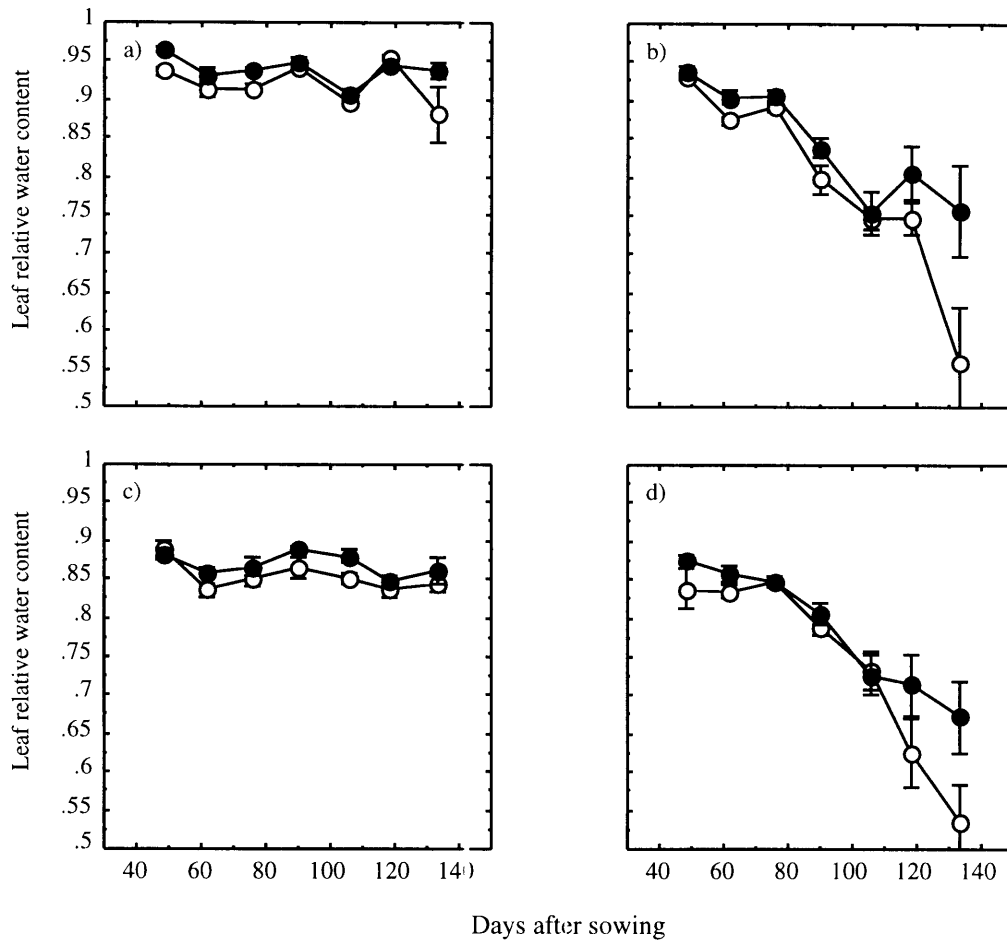


Figure 7.3. Changes in relative water content of leaves (○ canola and ● mustard) with time in Experiment 5 under low deficit conditions a) early morning and c) midday and under high deficit conditions b) early morning and d) midday. Bars represent standard errors.

7.3.4. Interrelations

Plots of ψ with π for each sampling date in Experiment 5, are presented in Figure 7.4. Linear regression was used to establish relationships between ψ and π at each sampling date in Experiments 3, 4 and 5. A straight line of the form

$$\pi = \pi_t + b\psi \quad (\text{Eqn. 7.1})$$

was fitted where π_t = osmotic potential at full turgor and b = change in π with change in ψ . Parameters of these fits are presented in Table 7.2. It is likely that the response is more complex than the straight line fitted, as plots of ψ with π , pooled across all sampling dates (not shown), while noisy, did resemble those found in wheat (Morgan 1977a). However, with the limited number of points available at each sampling, a more complex fit was not justified.

Comparisons of the water potential at zero turgor (ψ_0) at a corresponding stage of phenology showed that mustard reached zero turgor at lower ψ than did canola, with one exception which occurred in Experiment 5, 76 days after sowing.

This dissimilarity between the species when understood in terms of Equation 7.1, must stem from differences in either π_t and/or b . Standard errors of π_t overlapped at each sampling date in each experiment, with but two exceptions, at 57 days after sowing in Experiment 4 and at 106 days in Experiment 5. Furthermore, there was no consistent trend for one species to have a higher (or lower) π_t than the other, both mustard and canola having a higher π_t 6 out of the 12 times measured. In contrast, b was larger in mustard on 11 out of the 12 occasions it was determined and these differences reached statistical significance ($P < 0.05$) on two occasions in Experiment 5 at 90 and 118 days after sowing, on one occasion in Experiment 3, 151 days after sowing, and on the two occasions measurements were taken in Experiment 4. On a further two occasions, 106 days after sowing in Experiment 5 and 112 days after sowing in Experiment 3, mustard had a higher b at the ten percent level.

In Experiment 5, b remained relatively stable in mustard for the first three samplings and then increased with ontogeny from 76 days to the last occasion of sampling, 133 days after sowing (Table 7.2). This trend is less evident in canola. In the other experiments, which were sampled over a smaller range of phenology, b increased in both species in Experiment 4, while in Experiment 3 its values remained relatively stable, particularly for mustard.

Relationships of ζ on ψ and ζ on π for each sampling date were not clear under the field conditions of the present experiments as the range of ζ at each sampling date was relatively small. When the data were pooled across sampling dates in Experiment 5, the ζ of mustard declined at a significantly ($P < 0.05$) slower rate than that of canola as water potential declined (Fig. 7.5). There appears to be larger differences between the species in the response of ζ to declining osmotic potential (Fig. 7.6). Second order polynomials were fitted to the data reflecting the two phase nature of this response i.e. a phase of relatively slow decline in ζ with π followed by a more rapid fall. The divergent behaviour of the two species can be seen from the predicted relative water contents at an osmotic potential of -2.5 MPa, being 0.43 for canola and 0.61 for mustard.

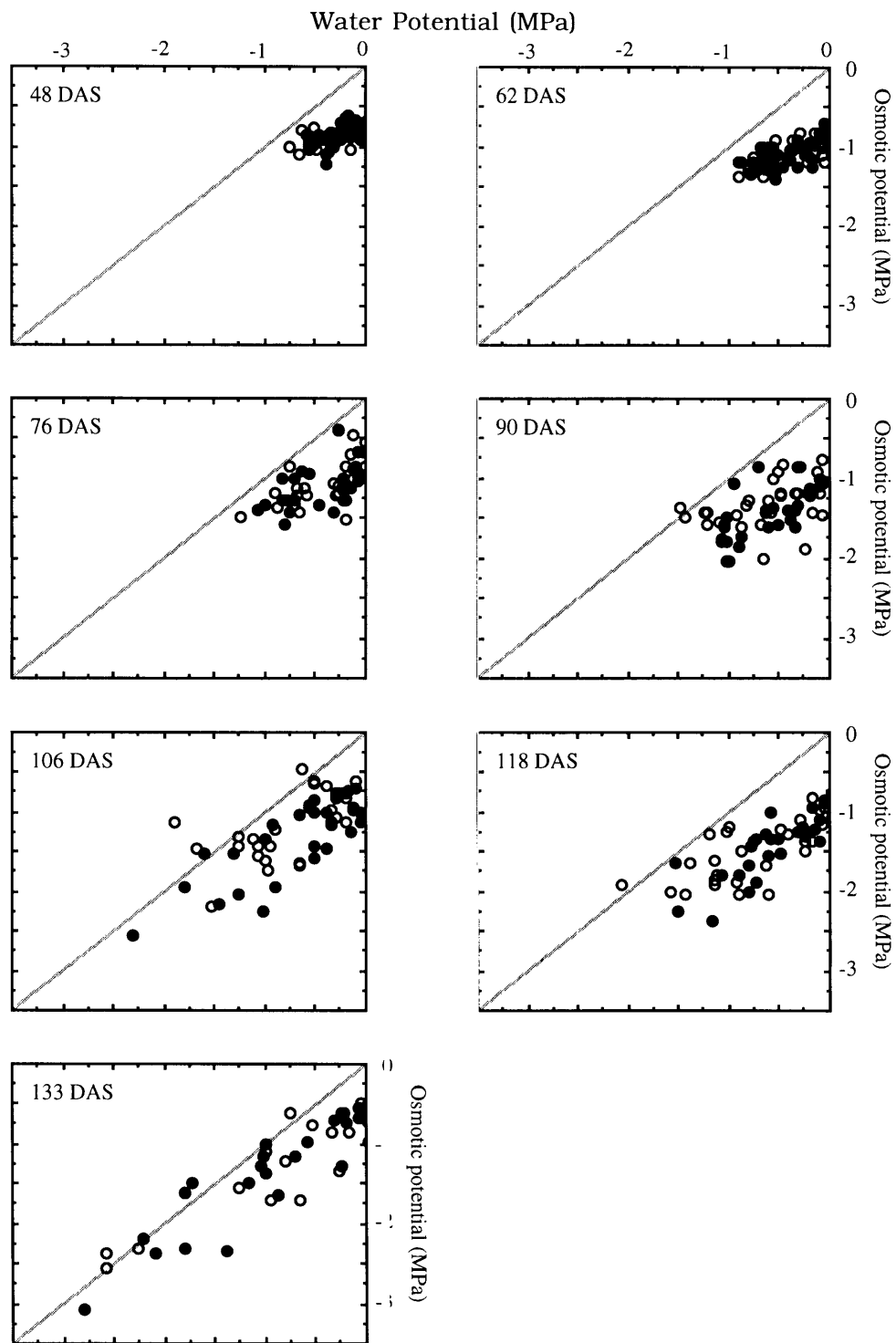


Figure 7.4. Relationship between leaf water potential and osmotic potential at different stages of ontogeny for canola (○) and mustard (●). The solid line represents the 1:1 relationship, where $y = x$ and hence $P = 0$.

Table 7.2. Osmotic potential at full turgor (π_t), the change in osmotic potential with change in water potential (b), water potential at zero turgor (ψ_0), and the coefficient of determination for relationships established at each sampling date for three experiments.

Days after sowing		π_t	b	ψ_0	r^2
Experiment 3					
Canola	112	-0.61±0.08	0.29±0.18	-0.9	0.173
	139	-0.55±0.07	0.47±0.15	-1.0	0.300***
	151	-0.33±0.06	0.41±0.08	-0.6	0.595***
Mustard	112	-0.57±0.10	0.69±0.24	-1.9	0.351*
	139	-0.65±0.06	0.63±0.16	-1.8	0.422***
	151	-0.44±0.06	0.70±0.11	-1.5	0.684***
Experiment 4					
Canola	44	-0.64±0.06	0.41±0.12	-1.1	0.420***
	57	-0.55±0.05	0.51±0.12	-1.1	0.551***
Mustard	44	-0.61±0.06	0.68±0.10	-1.9	0.751***
	57	-0.39±0.07	0.75±0.10	-1.5	0.762***
Experiment 5					
Canola	48	-0.73±0.03	0.39±0.09	-1.2	0.361***
	62	-0.93±0.04	0.36±0.09	-1.4	0.381***
	76	-0.88±0.06	0.49±0.08	-1.7	0.394***
	90	-1.11±0.09	0.34±0.12	-1.7	0.220**
	106	-0.72±0.11	0.58±0.11	-1.7	0.456***
	118	-1.08±0.08	0.53±0.09	-2.3	0.550***
	133	-0.62±0.09	0.73±0.08	-2.3	0.826***
Mustard	48	-0.76±0.03	0.44±0.09	-1.4	0.416***
	62	-0.92±0.04	0.43±0.09	-1.6	0.438***
	76	-0.93±0.09	0.40±0.16	-1.6	0.239*
	90	-1.04±0.08	0.65±0.12	-3.0	0.514***
	106	-0.87±0.08	0.68±0.09	-2.8	0.658***
	118	-1.01±0.06	0.73±0.09	-3.8	0.675***
	133	-0.64±0.08	0.77±0.07	-2.7	0.845***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$

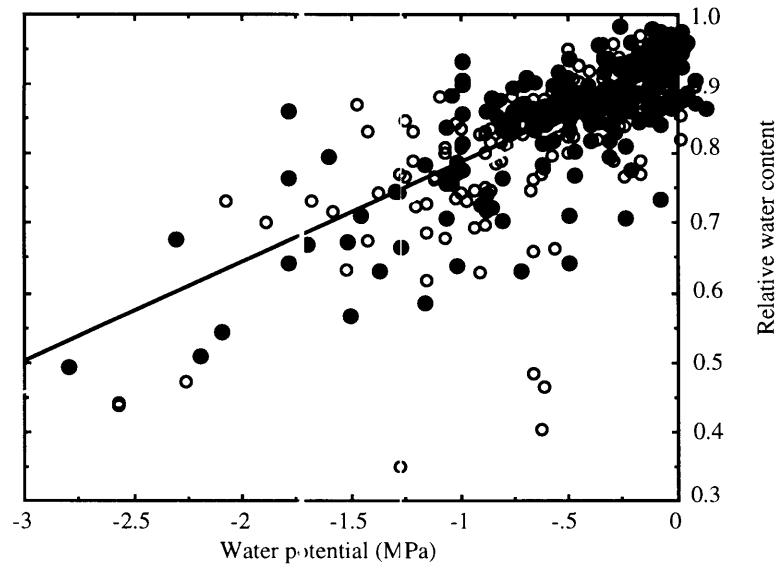


Figure 7.5. Change in relative water content with change in water potential (○ canola, ● mustard).
 Canola $y=0.922+0.155x$ ($r^2=0.50$, $P<0.001$), mustard $y=0.931+0.143x$, ($r^2=0.58$, $P<0.001$).

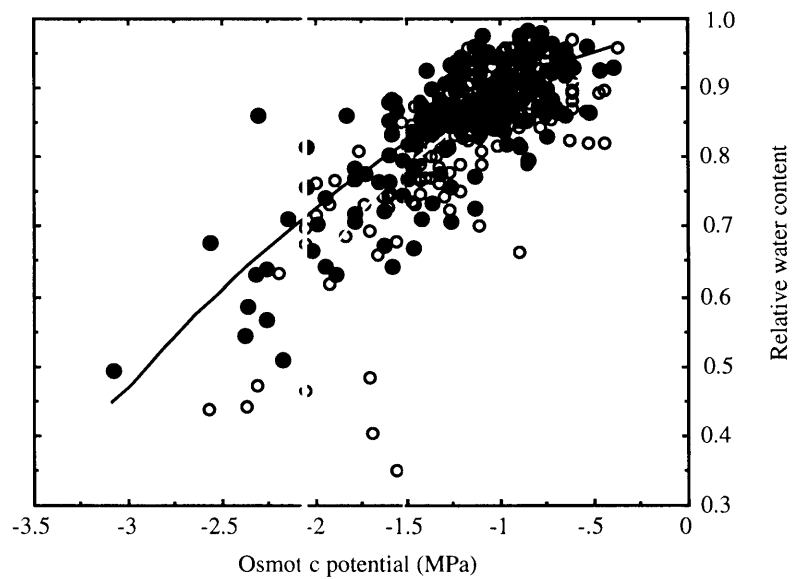


Figure 7.6. Change in relative water content with change in osmotic potential (○ canola, ● mustard).
 Canola $y=0.885-0.11x-0.1178x^2$ ($r^2=0.61$, $P<0.001$), mustard $y=0.985+0.05x-0.0411x^2$, ($r^2=0.65$, $P<0.001$).

Using pressure chamber techniques (Tyree and Hammel, 1972; Turner, 1988) under glasshouse conditions (Experiment 2), relationships between ψ and ζ were less variable than under field conditions. Parameters derived from the analyses are presented in Table 7.3 and typical response curves for canola and mustard are presented in Figure 7.7. As in the field data (Table 7.2), pressure chamber data under glasshouse conditions showed that ψ_0 was lower in mustard than in canola at corresponding stress levels. There was a tendency for increasing stress to cause a lowering of ψ_0 in both species. Osmotic potential at full turgor likewise appeared to be stress affected with mustard having a lower π_t than canola. Bulk modulus of elasticity was higher in mustard regardless of stress level and stress caused both species to have an increased ϵ at high compared to low deficits.

Osmotic adjustment can be calculated from the difference between the low and high deficit π_t . Using this method, osmotic adjustment in canola is 0.3 MPa and 0.1 MPa in mustard, despite the fact that mustard P_t was always lower than that of canola. The discrepancy between these findings and those for osmoregulation measured under field conditions (7.3.5) may arise because osmotic adjustment is a measure of the solutes present after tissue rehydration while osmoregulation is a measure of the solutes present during the stress. A further problem with the osmotic adjustment measurements were that leaf water potentials were not taken before sampling. Hence it is not possible to know if the plant sampled were experiencing the same stress level and this in turn calls into question the values of osmotic adjustment found here. Further, the estimates of ψ_0 were consistent with the field findings of greater adaptation in mustard.

Table 7.3. Water potential at zero turgor (ψ_0), osmotic potential at full turgor (π_t) and the bulk modulus of elasticity (ϵ) under low or high soil water deficits in Experiment 2. (n=7 for canola and n=6 for mustard).

	Canola		Mustard	
	Low deficit	High deficit	Low deficit	High deficit
ψ_0	-1.1±0.04	-1.4±0.06	-1.3±0.10	-1.6±0.10
π_t	-0.7±0.04	-1.0±0.06	-1.2±0.11	-1.3±0.11
ϵ	3.5±0.35	5.4±0.49	7.1±1.97	9.2±1.41

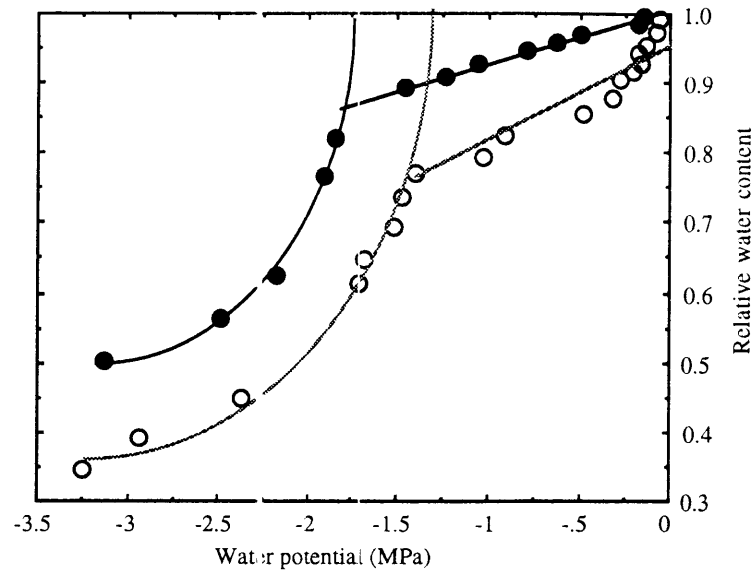


Figure 7.7. Change in relative water content with change in xylem water potential for canola (○) and mustard (●) under high soil water deficit. Zero turgor occurs at the intersection of the two fitted lines, and the osmotic potential at full turgor is given by the extrapolation of the curved line to a relative water content of 1.

7.3.5. Solute accumulation

Osmotic potential due to solute accumulation (π_a) was calculated as by Morgan (1980; 1991)

$$\pi_a = \pi_v - \frac{\pi_t \zeta_t}{\zeta} \quad (\text{Eq. 7.2})$$

where π_v is the osmotic potential at ζ , π_t the initial osmotic potential and ζ_t the relative water content at full turgor (assumed to be 1 for data in Figure 7.8) with $(\pi_t, \zeta_t)/\zeta$ being the osmotic potential arising from concentration with water loss. Solute accumulation, $\Delta\Pi$, is equal to $-\pi_a$ in this study. Plots of $\Delta\Pi$ with ψ are presented in Figure 7.8 for Experiment 5. Linear regression was used to establish relationships between $\Delta\Pi$ and ψ at each sampling date from each experiment where data was collected. A straight line of the form

$$\Delta\Pi = b\psi \quad (\text{Eq. 7.3})$$

was fitted through the origin, as the intercept of the relationship should theoretically equal zero and b = the rate of solute accumulation. In Experiment 5, when the species were compared at the same stage of ontogeny, b for mustard was significantly ($P < 0.05$) larger than for canola on three occasions, 90, 118 and 133 days after sowing. It was larger on both occasions when measurements were taken in Experiment 4 and on one occasion in Experiment 3, 151 days after sowing. For mustard in Experiment 5, the rate of change in $\Delta\Pi$ with water potential (b) remained stable for the first three samplings afterwards increasing while b in canola behaved much more erratically (Table 7.4). Similar trends appear to be present in the other

experiments but they were sampled less frequently and over a narrower range of phenology. The largest z observed for mustard was -0.58 at the last sampling in Experiment 3 and again at the last samplings in Experiments 4 and 5, when z was equal to -0.57 . Indicating that for every unit drop in water potential, solutes were accumulated by mustard that account for approximately 0.6 MPa of turgor.

Table 7.4. Rate of solute accumulation (z) and coefficient of determination for straight line fits of the form given in equation 7.3.

Days after sowing	z	r^2	
Experiment 3			
Canola	112	-0.08 ± 0.11	0.041
	139	-0.09 ± 0.09	0.030
	151	-0.32 ± 0.05	0.454***
Mustard	112	-0.20 ± 0.21	0.003
	139	-0.19 ± 0.12	0.105
	151	-0.58 ± 0.06	0.623***
Experiment 4			
Canola	44	-0.15 ± 0.07	0.212*
	57	-0.23 ± 0.07	0.245*
Mustard	44	-0.50 ± 0.07	0.635***
	57	-0.57 ± 0.06	0.662***
Experiment 5			
Canola	48	-0.14 ± 0.05	0.106†
	62	-0.08 ± 0.06	0.060
	76	-0.26 ± 0.08	0.251**
	90	-0.07 ± 0.05	0.052
	106	-0.35 ± 0.06	0.347***
	118	-0.14 ± 0.06	0.154*
	133	-0.39 ± 0.06	0.618***
Mustard	48	-0.20 ± 0.06	0.214**
	62	-0.19 ± 0.05	0.227**
	76	-0.20 ± 0.09	0.132†
	90	-0.40 ± 0.08	0.245**
	106	-0.41 ± 0.06	0.557***
	118	-0.32 ± 0.04	0.519***
	133	-0.57 ± 0.04	0.797***

† $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$

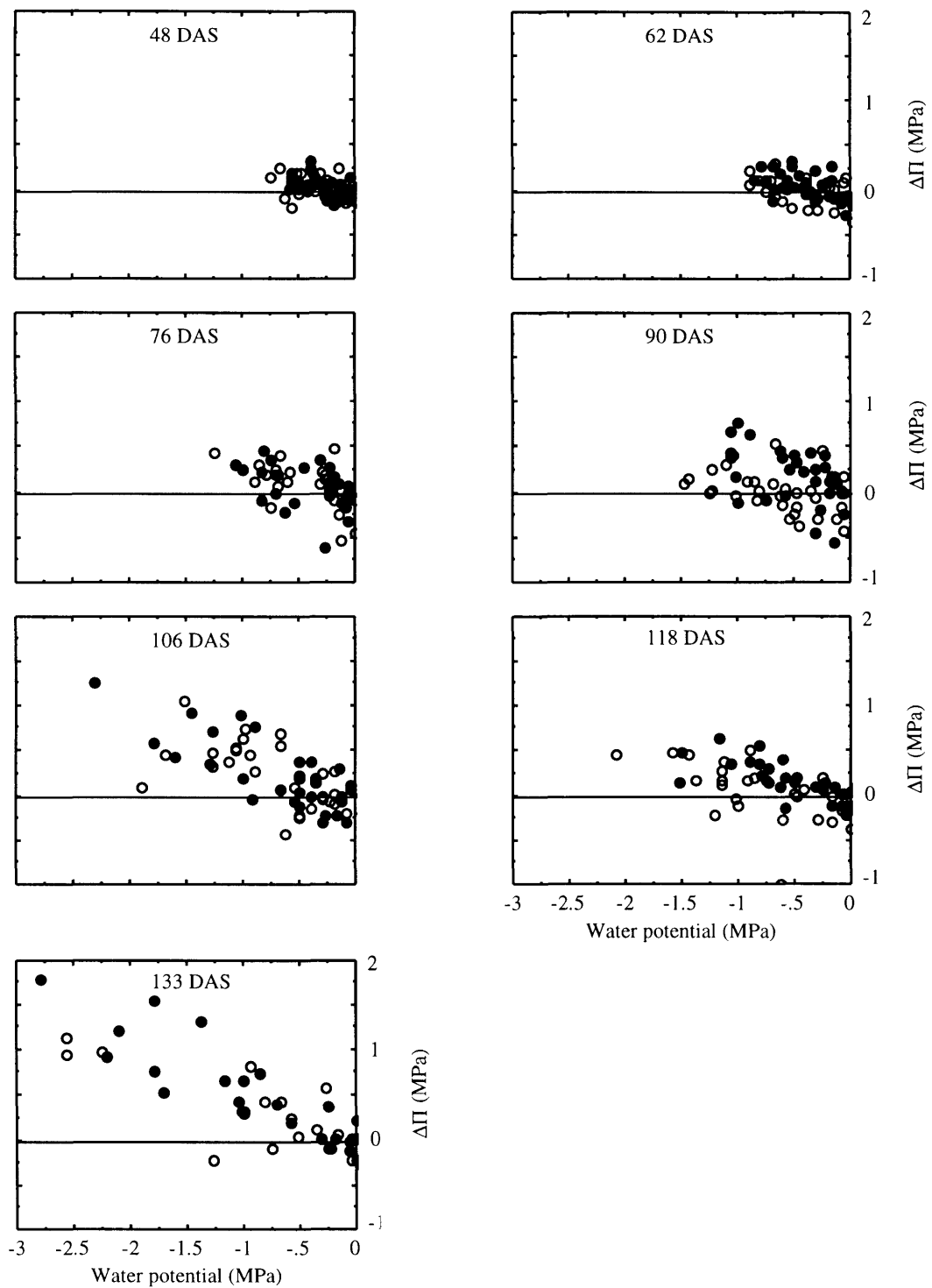


Figure 7.8. Change in solute accumulation with water potential at different stages of phenology for canola (○) and mustard (●).

7.4. Discussion

The results provide clear evidence of differences between the species in plant water relations and the capacity to osmoregulate.

Frequent measurements of leaf water potential, relative water content and turgor pressure were made over a wide range of phenology in Experiment 5 and over a more restricted range in Experiments 3 and 4. The data suggest an underlying trend for the leaves of mustard to have greater relative water contents at the end of the season (Fig. 7.3, Table 7.1) and slightly higher water potentials (Fig 7.1, Table 7.1). However, what the data demonstrate most clearly is that the leaves of mustard have greater turgor pressure than those of canola. Lewis (1992), using two *B. napus* lines and one *B. juncea* under controlled environment conditions, found higher water potentials in mustard under adequate water conditions and, under stress, higher turgor in its leaves. He did not find differences in relative water contents. The adaptive nature of the differences between the species in their midday leaf turgor can be seen in Figure 7.9. At both sites of Experiment 5 mustard had similar or slightly higher turgor until 78 days after sowing. After that, the turgor in the mustard leaves was clearly greater than in those of canola, being 1.7 times greater by 106 days after sowing at the low deficit site and 2.9 times greater at the high deficit site. These differences then declined towards the end of the season when leaf areas were also declining.

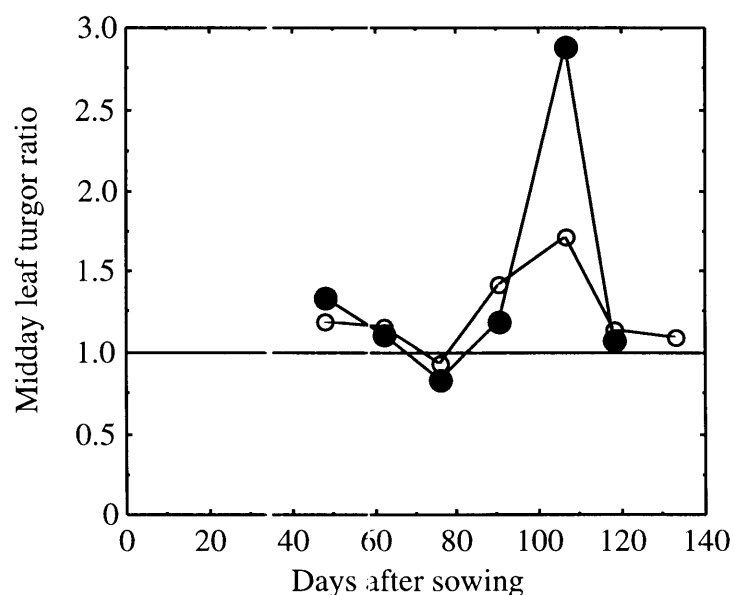


Figure 7.9. Relative difference in midday leaf turgor of mustard compared to canola (mustard/canola) in Experiment 5, open symbols for the low deficit site and closed for the high.

There is further evidence of adaptive differences under field conditions in that the water potential at which leaves reached zero turgor (ψ_0) varied between the species with mustard maintaining positive turgor at lower water potentials than canola. Kumar and Elston (1992) found ψ_0 of mustard as low as -2.4 MPa while ψ_0 of *B. napus* was -1.8 MPa in a series of glasshouse trials. The results reported from the current experiments accord with these findings. In Experiment 5, mustard leaves had an average ψ_0 of -3.1 MPa compared with -2.0 MPa for canola for the period from 90 days after sowing to the end of the season. Similar differences were present in the other field experiments with an average ψ_0 of -1.7 MPa in mustard and -0.8 MPa in canola in Experiment 3 and -1.7 MPa for mustard and -1.1 MPa in canola in Experiment 4. In Experiment 5, in the early period up to 90 days after sowing, ψ_0 differences were small (Fig 7.10). This suggests that the process that causes ψ_0 to later diverge is switched on either by phenology or more likely by the lowering of water potential, as was found to be the case with osmotic adjustment between cultivars of pearl millet (Henson, 1982) and rice (Turner et al., 1986). Further evidence of differences in ψ_0 between mustard and canola was found in the glasshouse experiment using pressure chamber techniques. The present experiments provide evidence that mustard is better adapted to water limiting environments, maintaining higher levels of leaf turgor than canola and an unequivocal advantage in the water potential at which turgor is lost under both field and glasshouse conditions. The magnitude of this advantage in water potential at the point of turgor loss of approximately 0.6 to 1.1 MPa, is consistent with the differences reported between high and low osmoregulating lines of wheat (Morgan, 1983) and legumes such as cowpea and soybean (Morgan, 1992a)

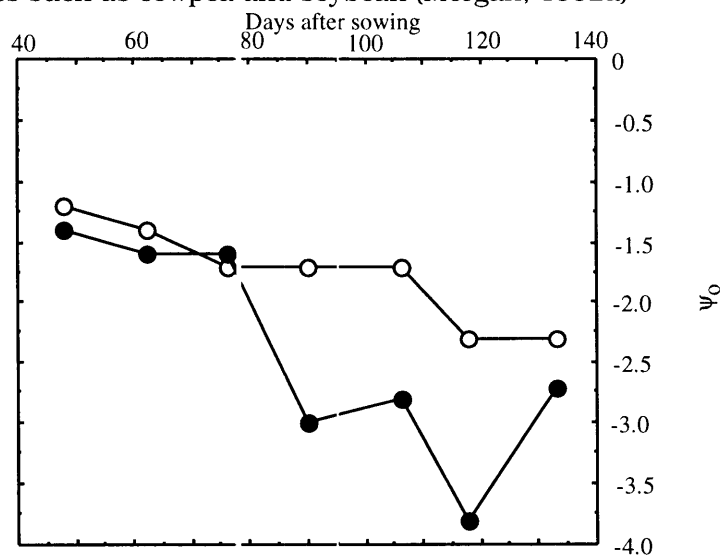


Figure 7.10. Change in the water potential at which turgor reaches zero with time for canola (○) and mustard (●) in Experiment 5.

Differences in ψ_0 can arise from differences in the osmotic potential at full turgor (the level of background solutes, often used to assess osmotic adjustment in the literature e.g. Jones and Turner, 1978) or from differences in the rate of decline in osmotic potential with declining water potential (equation 7.1). In the present studies there is no evidence, under field conditions, of differences in the level of background solutes (Fig 7.11) and strong evidence that the π of mustard leaves declines at a faster rate than is the case in canola (Table 7.2 and Fig 7.4). This explains the tendency of mustard to have higher turgor, both at midday and at dawn, during the season as $P_t = \psi - \pi$.

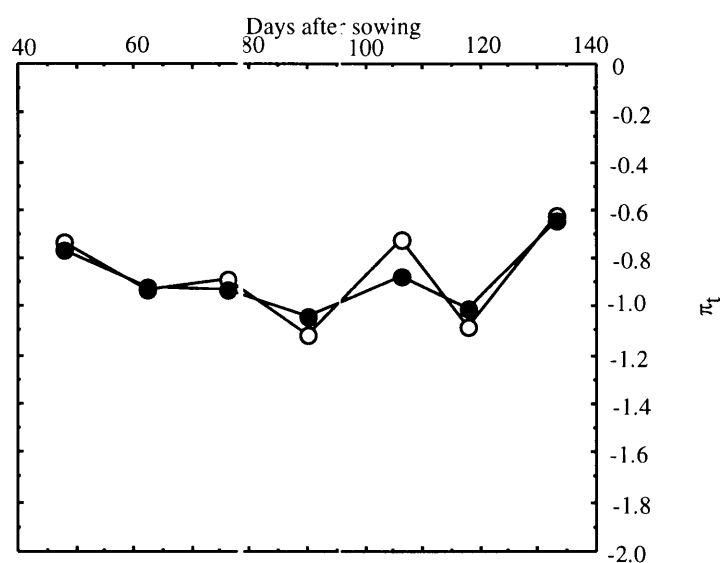


Figure 7.11. Change in the osmotic potential at full turgor with time for canola (○) and mustard (●) in Experiment 5.

Mustard's greater decline in π with declining ψ , found in these studies, results from its greater ability to accumulate solutes (Table 7.3 and Fig. 7.8). This finding is in agreement with work in India, where Singh et al. (1990a) classified cultivars of each species into high, medium or low osmoregulation (solute accumulating) groups. They found that of the 19 mustard lines classified, 37, 32, and 32% were in the high medium and low osmoregulation groups respectively while of the 8 *B. napus* lines none were in the high group, 25% were in the medium group and the remaining 75% in the low group. They also classified a range of Brassicas according to leaf relative water content at a water potential of -2.5 MPa and found that ζ in *B. juncea*, *B. carinata*, *B. chinensis* and *Eruca sativa* was relatively high while in *B. campestris*, *S. alba* and *B. tournifortii* it was intermediate and low in *B. napus*.

There is an association between osmoregulation and grain yield in wheat (Morgan et al., 1986) with those lines having high osmoregulation yielding on average 11% more than those in the low group. The osmoregulation differences between cultivars appears to be controlled by alternative alleles at a single locus on chromosome 7A (Morgan, 1991). In *Brassicaceae* Kumar et al. (1984) showed that a cultivar of *B. carinata* had twice the yield of a cultivar of *B. napus* under drought conditions and that this was associated with a greater degree of osmoregulation; these findings, were however, based on only one cultivar of each species.

Morgan (1991) used values from the fitted responses of ζ on ψ , and π on ψ to predict solute accumulation in wheat using Equation 7.2. These plots indicated that lines with a high capacity to osmoregulate accumulated solutes as soon as water potential began to decline while lines with low osmoregulation did not accumulate solutes until zero turgor was reached. In mustard and canola the response appears to be otherwise (Fig. 7.12) with both species accumulating solutes as soon as water potential falls. However, mustard does this to a greater extent than canola with the differences between them becoming more pronounced as water potential declines. Peak solute accumulation occurred in canola at a water potential of -2.4 MPa (0.4 MPa below the average point turgor loss) while mustard continued to accumulate solutes at water potentials as low as -3.0 MPa. This pattern of osmoregulation is more akin to that found in sorghum (Basnayake et al., 1993).

In the present studies, mustard produced more dry matter and yield (Chapters 4 and 5) under high deficit conditions. While it would require the use of near isogenic lines to obtain unequivocal proof that such a difference is linked to differences in osmoregulation, the use of at least two randomly selected cultivars from each species gives some confidence that the results reflect real differences. Furthermore, the difference between the species is consistent with evidence of this nature in other crops such as wheat (Morgan, 1983; Morgan et al., 1986) sorghum (Ludlow et al., 1990; Santamaria et al., 1990) and chickpea (Morgan et al., 1991) suggesting that mustard's advantage in growth and yield under water limited conditions can be at least partly explained by differences in solute accumulation. The fact that osmoregulation is present in both species but to a greater extent in mustard, may be an indication of complex genetic control. Though an inheritance study (Chaudhary et al., 1989) concluded that osmoregulation in *Brassicaceae* is controlled by either a single gene or by a block of interlinked genes acting as a single gene. However, these same studies also concluded that transpirational cooling was under single gene control which appears to be unlikely!

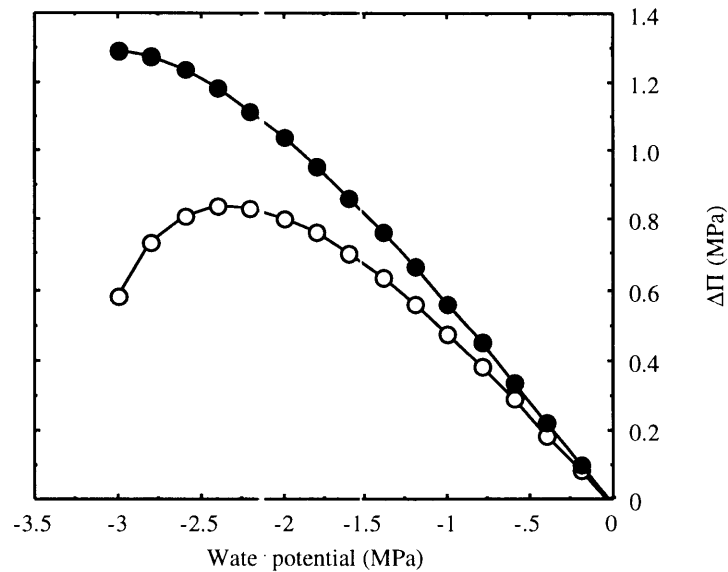


Figure 7.12. Calculated change in solute accumulation with declining water potential for leaves of canola (○) and mustard (●) from Experiment 5.

From the glasshouse work it is apparent that mustard has a higher bulk modulus of elasticity than canola and that both species increase ϵ with increasing stress. In sorghum (Jones and Turner, 1978) and wheat (Melkonian et al., 1982) ϵ was also found to increase with increasing stress while the opposite was found in field beans (Elston et al., 1976). Values of ϵ found in the present work were lower than those reported for sorghum (16 to 34 MPa, Jones and Turner, 1978) rice (average of seven cultivars 13.6 MPa, Turner et al., 1986) and wheat (22 to 40 MPa, Melkonian et al., 1982). However, they were similar to those reported for mustard and *B. napus* (Kumar and Elston 1992) but opposite in direction as they found mustard to have a average ϵ of 3.5 MPa compared to 8.2 MPa for *B. napus*. Average ϵ levels across stress levels in the present studies were 8.2 for mustard and 4.5 MPa for canola. This discrepancy is not easily explained even when taking account of the different techniques used. Kumar and Elston (1992) rewatered plants before measuring change in the length of leaf strips in mannitol solutions, while leaves rehydrated in the pressure chamber were used in the present studies with ϵ estimated from the pressure volume relationship using the technique of Turner et al. (1986). The discrepancy may reflect the instability of ϵ as a water relations parameter as it is both turgor pressure and relative water content dependent (Tyree and Jarvis, 1982). Decreases in π through osmoregulation can cause increases in P_t and thus increased ϵ and, for that reason the differences between the two studies may be the result of different levels of turgor in field and glasshouse plants. The significance of higher ϵ in plants is that at any given water potential such plants have a higher relative water content. Under low deficit conditions the bulk modulus

of elasticity for mustard was twice that of canola, with cell walls twice as rigid, while, under high deficits, ϵ was just less than twice as large. This small difference in response is unlikely to be responsible for the adaptive advantage of mustard under high stress.

7.5. Conclusion

Leaves of mustard plants have higher turgor than those of canola particularly under high soil water deficits. This difference arises because of the greater capacity of mustard leaves to accumulate solutes. Differences in bulk modulus of elasticity were noted but these are unlikely to explain differences in drought adaptation as the difference did not vary greatly between low and high deficit conditions.

Differences in turgor and osmoregulation are important adaptations to high water deficit conditions and may well explain part of the adaptive advantage mustard has under high deficit conditions in terms of growth (Chapter 4) and yield (Chapter 5). The relationship between these factors and those of water use efficiency will be discussed in the following chapter.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

*"I'm mad about mustard-
Even on custard."
Ogden Nash*

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8.1. Introduction

The principal aim of this project, as outlined in Chapter 1, has been to characterise the adaptation of mustard and canola at different levels of soil water deficit. It is clear from the work reported in previous chapters that mustard is better adapted to high water deficits and it is not disadvantaged at low deficits. Early growth is more vigorous, leaf area durations are longer and become relatively more so as stress increases (Chapter 4) with the result that mustard can produce more than twice the dry matter and seed yield of canola under certain stress conditions (Chapter 5). An examination of physiological and morphological adaptations within the crops has been undertaken (Chapters 6 and 7) in order to provide a preliminary understanding of their relative yield performance. These several matters are discussed and conclusions drawn in the preceding chapters of the thesis.

In this final chapter the interrelation of traits and their effects on yield and dry matter production are further explored and recommendations are made on areas requiring additional research.

8.2 Discussion

The adaptive advantage of mustard is related to its greater ability to accumulate dry matter rather than to differences in yield structure (Chapter 5). Greater early vigour is in part responsible for the greater dry matter production. However, it does not explain the adaptive differences, such as mustard's greater ability to maintain mid-season leaf area and hence canopy photosynthesis under high deficits (Chapter 4). Differences in both water use efficiency and osmoregulation were found in these studies. How these relate to differences in adaptation needs to be considered. The importance of the more mechanistic trait of osmoregulation and the role of water use efficiency and the links between these traits are examined in that order.

8.2.1. Osmoregulation and adaptation

A greater capacity for osmoregulation was found in mustard (Chapter 7) with the difference between the species increasing with increasing stress (as assessed by leaf water potential). What are the consequences of this finding for leaf area duration, dry matter production and yield and to what extent is it consistent with findings in other species?

Osmoregulation acts to maintain turgor and this has been shown to be the case in the present studies with ψ_c , the water potential at which a leaf loses turgor, being consistently lower in mustard than in canola. Mustard also had an increasing advantage in both dry matter production and yield as water deficits increased. There is need for care, however, in directly associating these occurrences as the role of turgor in the growth process is to some extent controversial (see sections 2.3. and

2.4.3). For example there is some evidence showing that root signals rather than leaf turgor control tissue expansion (Munns, 1987; Passioura, 1988b). Nonetheless, it is certain that tissue expansion cannot occur without positive turgor and, moreover, the evidence for root signals controlling leaf expansion is frequently not found (Sadras et al., 1993; Gallardo et al., 1994). Given the above and the compelling evidence of earlier work showing direct relationships between turgor and leaf expansion (Hsiao, 1973; Bunce, 1977), turgor must be considered an important factor in leaf expansion under water deficit.

Given that turgor is important in leaf expansion, and the functioning of the expanded leaf, there remains a difficulty in establishing an absolute link between it and dry matter production and yield. To establish such an unqualified association from field studies would require the use of near isogenic lines or a large number of random recombinant inbred lines. Suffice it to say that in the present studies correlative relationships are consistent with the processes being linked. For example, total dry matter production in the main field experiments was significantly related to the average of early morning and midday turgor (Fig. 8.1) and the crop growth rate for different phenological periods was also related to average turgor for the corresponding periods, particularly during the period of rapid growth (Fig. 8.2). These findings agree with correlative studies based on a large number of wheat lines where high osmoregulating lines were shown to average 11% more yield than low osmoregulating lines over 56 field trials (Morgan et al., 1986). Similarly in sorghum, high osmoregulating lines had greater yields than low lines under water deficit conditions (Ludlow et al., 1990; Santamaria et al., 1990). More importantly, using recombinant inbred lines of wheat differing in this trait, Morgan (1983) found an approximately 50% yield advantage in the high osmoregulating group compared to the low under severe water deficits. This work went beyond showing just an association. It showed that selecting or breeding for this trait had positive effects on yield where the stress environment sufficiently reduced leaf ψ . Later experiments further confirmed this (Morgan, 1995).

In other species, the higher dry matter production and grain yields found in high osmoregulating lines were associated with higher leaf area indices (Karami et al., 1980; Morgan, 1995) and greater net photosynthesis per unit leaf area (Karami et al., 1980; Morgan, 1984; Gupta and Berkowitz, 1987; Santakumari and Berkowitz, 1990). Present results are consistent with these findings. They show a positive association between total leaf area duration and average turgor over the whole growing season (Fig. 8.3a) and for various phases of growth (Fig. 8.3 b, c & d). The net rate of photosynthesis per unit area was found to be greater in mustard than in canola on two out of the three occasions where measurements were taken (Chapter 6). Both these characters act to increase carbon assimilation. While it

cannot be categorically stated that a greater capacity for osmoregulation is the reason for mustard's dry matter and yield advantage over canola the evidence here presented is consistent with this conclusion.

Water use has been found to be greater in high compared to low osmoregulating lines of wheat (Morgan and Condon, 1986). This was probably a consequence of the increased leaf area and stomatal conductance associated with turgor maintenance by osmoregulation. In the present work no increase in water use was found in mustard and this is surprising since increased water use is regarded as one of the main consequences of higher osmoregulation (Morgan, 1984). However, in at least one study with sorghum, yield increases were associated with osmoregulation in the absence of increased water use (Tangpremsri et al., 1995). In that instance the influence of higher osmoregulation was thought to lie in a reduction of the impact of water deficits on seed set. High osmoregulating lines of sorghum were found to suffer only a 6% reduction in grain numbers compared to an 11% reduction in the low group when comparing high and low deficit treatments. In the present studies the seed number of canola was likewise much more effected by water deficit than that of mustard. For example in Experiment 5, comparing the low deficit to the rain fed site, canola seed numbers were reduced by 63% and mustard by only 50%, while comparing the low deficit with the rain-exclusion site, canola seed numbers were reduced by 80% and mustard numbers still by only 50%. The maintenance of seed set in high osmoregulating lines of wheat is thought to be the consequence of turgor maintenance acting to delay the increase in concentration of ABA, a process associated with turgor declining to zero (Morgan, 1980b; Morgan, 1984; Morgan and King, 1984). High concentrations of ABA have been reported to increase abortion of reproductive tissue (Morgan, 1980a, 1980b; Saini and Aspinall, 1982). This may be part of the explanation for the less severe effect of water deficits on seed numbers in mustard. However, differences in dry matter accumulation were already present at flowering and much of the differences in seed number would be due to mustard's greater supply of assimilate. Hence, the differences in leaf area, dry matter production and yield in the absence of differences in water use remain, to some extent, puzzling.

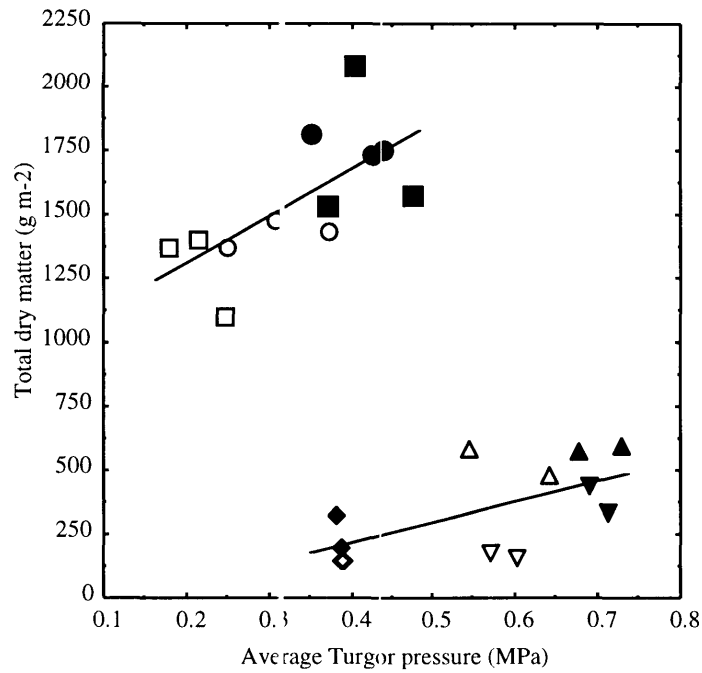


Figure 8.1. Relationship of total above ground dry matter production with average leaf turgor pressure for Experiment 3 (irrigated site, ○ canola, ● mustard; rainfed site □ canola, ■ mustard, $y=925+1810x$, $r^2=0.435$, $p<0.05$), and Experiments 4 (◇ canola, ◆ mustard) and 5 (irrigated site, △ canola, ▲ mustard; rain-exclusion site, ▽ canola, ▼ mustard, $y=-119+830x$, $r^2=0.400$, $P<0.05$).

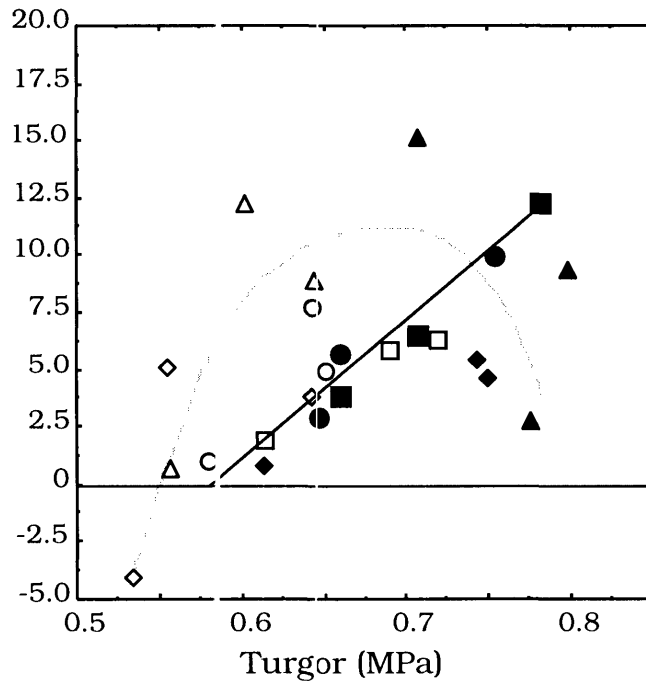


Figure 8.2. Relationship of crop growth rate for phases of growth with average leaf turgor pressure for each phase in Experiment 5 early growth phases, 0-4.3 (irrigated site ○ canola, ● mustard and rain-exclusion site □ canola, ■ mustard, $y=-28.77+51.21x$, $r^2=0.807$, $p<0.001$), and late growth phases 4.3-5.1 (rain-exclusion site △ canola, ▲ mustard; and rain-exclusion site ◇ canola, ◆ mustard, $y=170.13+513.54x-368.58x^2$, $r^2=0.343$, $P=0.15$).

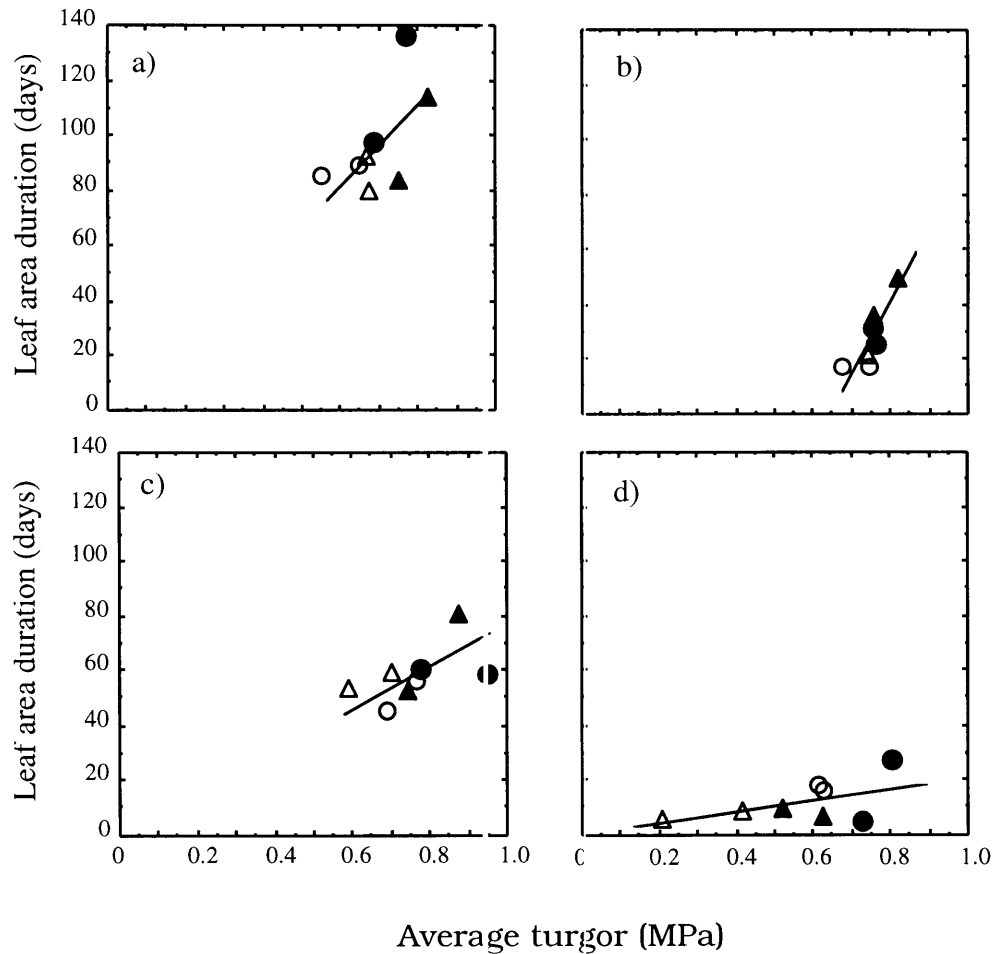


Figure 8.3. Relationship of leaf area duration and turgor pressure for Experiment 5 for different growth phases a) total 0-5.5 b) early 0-3.1, c) mid 3.1-4.4, and d) late 4.4-5.5. (irrigated site ○ canola, ● mustard, rain-exclusion site △ canola, ▲ mustard). a) $y=3.12+135.99x$, $r^2=0.355$, $P=0.12$. b) $y=-146.02+232.28x$, $r^2=0.694$, $P<0.05$, c) $y=18.30+53.17x$, $r^2=0.33$, $P=0.14$, d) $y=-1.81+25.12x$, $r^2=0.33$, $P=0.13$

8.2.2. Water use efficiency and adaptation

Passioura (1977) presented a model for understanding yield under water limiting conditions in which yield is considered to be the product of three factors, namely the amount of water used, the efficiency of water use and the harvest index. These factors are considered to be independent of each other under most conditions (Passioura, 1977).

In the present studies water use efficiency was consistently greater in mustard than in canola, while water use was either similar (Experiment 4) or less (Experiment 5) and harvest index was generally lower averaging 27% against 37% for canola when compared across sites and field experiments. In terms of Passioura's model, mustard's yield advantage must then derive from its greater water use efficiency.

Increased water use efficiency can arise from either a decrease in the proportion of soil evaporative losses relative to transpiration or from an increase in transpiration efficiency. Mustard appears to have an advantage over canola in both these respects. It has greater early vigour which would tend to reduce soil evaporation because of the greater leaf cover, which is a character found to be associated with increased early season water use efficiency (Chapter 6); mustard plants also tended to have a higher leaf level transpiration efficiency though this was not as clearly present at a canopy level (Chapter 6). Differences in leaf level transpiration efficiency can arise through reduced stomatal conductance or increased photosynthetic rates per unit leaf area. In this work both mechanisms were present to some extent with higher photosynthetic rate appearing to be the more important.

The relative yield advantage of mustard over canola increases with increasing stress and this is matched by an increasing advantage in water use efficiency of seed production and, less consistently, by an increasing advantage in water use efficiency of dry matter production (Chapter 6). Hence, water use efficiency is likely to have an important bearing on the comparative performance of the crops.

8.2.3. Osmoregulation and water use efficiency

Water use efficiency, like yield, is a highly integrated character influenced by many factors. The differences in osmoregulation between the species has been discussed in the context of how this may influence leaf area maintenance and hence canopy assimilation and yield. However, as reported earlier, the differences in osmoregulation were not associated with increased water use. Therefore it is pertinent to examine if osmoregulation and water use efficiency are linked.

Mustard's increased rates of photosynthesis per unit leaf area, a possible consequence of its greater turgor maintenance, may have relevance. However, in the present studies no clear relationships were found between net photosynthesis and leaf water potential or leaf turgor nor was any relationship found between stomatal conductance and either water potential or leaf turgor when assessed by gas exchange measurements (appendix 8.1). This finding may be a reflection of the limited number of gas exchange measurements made. Examination of the connections between g_s as measured by porometry and ψ or P_t , in Experiment 5, did show more evidence for these relationships (Fig. 8.4). Interestingly the clearest trend in the data occurring late in the season, was a general soil water deficit effect with high deficits greatly reducing stomatal conductance (Fig 8.4 e & f). These results are consistent with root signals influencing stomatal conductance. Earlier in the season there was no evidence of a general soil water deficit effect and conductance did appear to be related particularly to leaf turgor (Fig. 8.4 b). Hence the greater maintenance of turgor in mustard through osmoregulation may act to maintain leaf

function except perhaps at the end of the season. The failure of the gas exchange measurements to pick up such an effect may be the result of their being taken late in the season (133 days after sowing) at a stage when root signals rather than leaf turgor may have been more important.

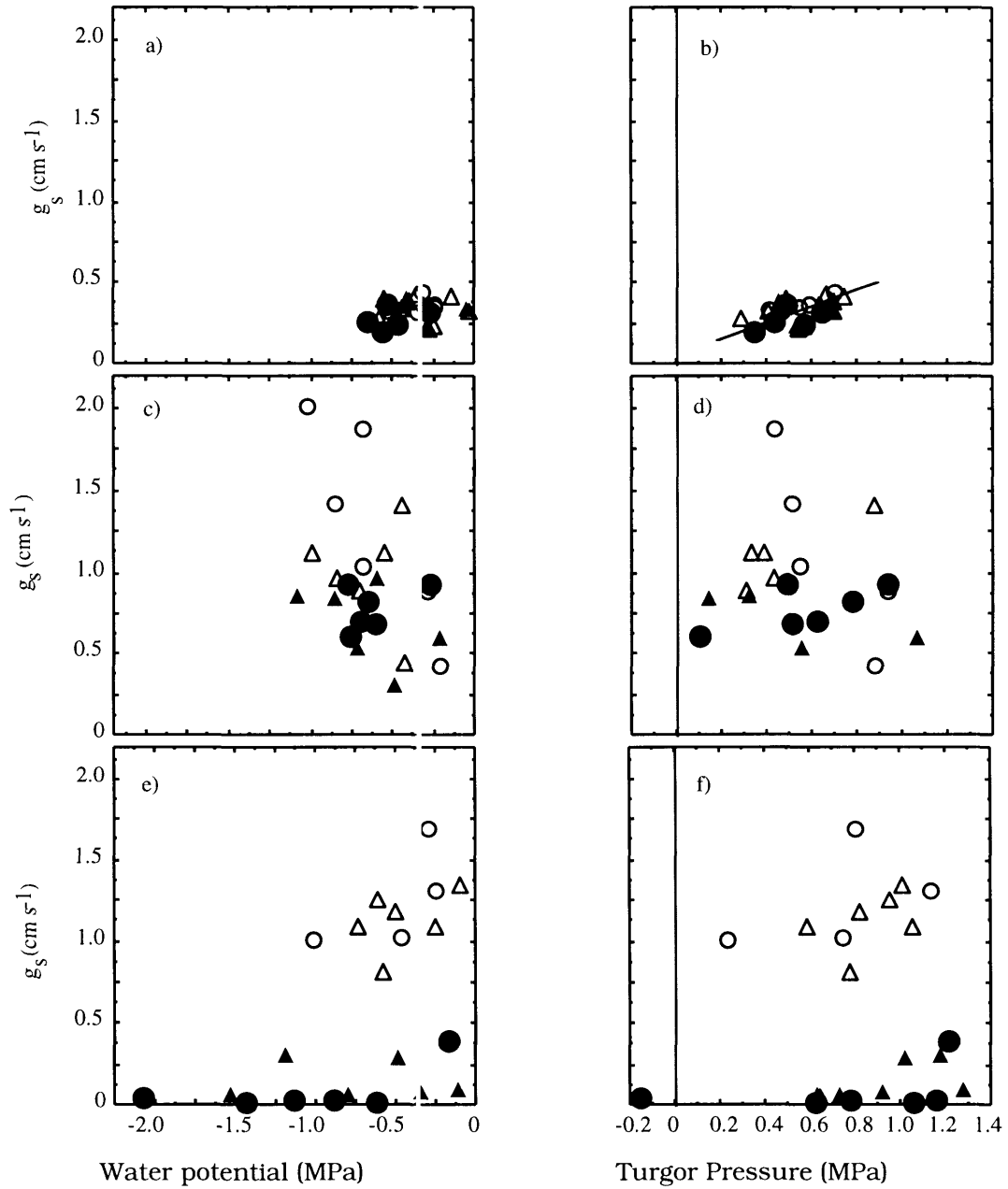


Figure 8.4. Relationship of stomatal conductance with leaf water potential (left hand column) or leaf turgor pressure (right hand column) on three different occasions in Experiment 5, a & b 48 days after sowing c & d 78 days after sowing, and e & f 118 days after sowing. Irrigated site ○ canola, △ mustard; rain-exclusion site ● canola, ▲ mustard. b) $y=0.18+0.27x$, $r^2=0.26$, $P<0.05$

Differences in photosynthesis per unit area were reflected, albeit weakly, in an adaptive difference in canopy level water use efficiency (Chapter 6). Singh et al. (1986), working with mustard, reported an association between water use efficiency and osmoregulation. Hence, in Brassicaceae osmoregulation may have a role in maintaining water use efficiency. Although it should be noted that in other species differences between cultivars in osm regulation were not found to be associated with differences in water use efficiency (Morgan and Condon, 1986; Ludlow et al., 1990; Santamaria et al., 1990).

While some questions remain, the results of the present field studies are generally consistent with the view that osmoregulation is an important adaptation to drought. The exact mechanism by which growth is controlled is not clear but is in part likely to be due to osmoregulation maintaining turgor under water deficit conditions and thus allowing mustard to maintain greater leaf area durations, resulting in higher canopy photosynthetic rates, and possibly higher canopy photosynthetic efficiency and water use efficiency, therefore greater dry matter production, increased assimilate supply per seed and increased seed set. The increased seed set may arise in part from high leaf turgor levels in mustard acting to delay the production of ABA but this was not measured.

8.3. Conclusions and Recommendations

The work presented in this thesis provides clear evidence of high yield potential in mustard with no yield disadvantage compared to canola when grown under low water deficits. Under high deficits a substantial yield advantage both in seed and oil yield is present. The differences in seed yield under deficit conditions is primarily explained by a difference in dry matter production rather than by marked species difference in yield structure. Mustard's advantage in dry matter production is hypothesised to arise from its greater capacity to osmoregulate and thereby maintain leaf turgor and, in turn, leaf area and canopy photosynthesis. Differences in yield structure may contribute to differences in water use efficiency as the larger number of pods of mustard may be more water use efficient than the canopy of canola with its fewer but larger pods.

It is therefore recommended that research be continued into the development of mustard as crop in its own right as it appears to be an appropriate alternative oilseed crop. However, lines will need to be developed that fully meet canola quality standards.

A greater understanding of the role of late season canopy structure needs to be developed for both crops as there may be scope for manipulating water use efficiency via this route.

Differences in yield were not directly related to yield structure hence, breeding for fewer pods and a greater number of seeds per pod appears to be an appropriate ideotype for mustard conferring the potential to increase harvest index. However, as noted above, there is a need to determine whether this approach adversely affects water use efficiency or not.

The extent of the variation in osmoregulation in both crops should be explored with the long term aim of screening for this trait in breeding programs. A method similar to that developed by Morgan (1988) for wheat, using coleoptile extension under deficit conditions in Petrie dishes may be appropriate.