Response of alfalfa (*Medicago sativa* L.) to diurnal and nocturnal saline sprinkler irrigations.

II: Shoot ion content and yield relationships

Isla R., Aragüés R.

Unidad de Suelos y Riegos (asociada al CSIC), Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Gobierno de Aragón. Avda. Montañana 930, 50059 Zaragoza, España. Email: risla@aragon.es

Abstract

When using saline waters, sprinkling irrigation at night is a recommended practice to reduce evaporation, salt absorption by the wetted leaves and its negative effects on crops. We measured shoot ion concentrations (Cl^-, Na^+ and K^+) and total dry matter in alfalfa subject to diurnal and nocturnal saline sprinkler irrigations and established potential relationships among them. The work was carried out along the 2004 to 2006 growing seasons using EC waters from 0.5 to 5.6 dS m^{-1}. Saline sprinkling irrigations linearly increased shoot Cl^- and Na^+ and decreased shoot K^+. Even though daytime evaporation was much higher than nigh-time, shoot ion accumulation and total dry matter were similar in the diurnal and nocturnal irrigations. The salinity tolerance of alfalfa decreased in year 2006 due to increases in shoot Cl^- and, particularly, shoot Na^+. The lower threshold for shoot Na^+ (276 meq kg^{-1}) than for shoot Cl^- (726 meq kg^{-1}) shows that alfalfa is more sensitive to Na^+ than to Cl^-, and that Na^+ accumulation is the preponderant cause of alfalfa yield decline after three years of sprinkling with saline waters.

Keywords: alfalfa, soil salinity, sprinkler irrigation, sodium, chloride, potassium, saline water, ion toxicity

Introduction

The use of low-quality waters in above-canopy sprinkler systems poses the potential problems of foliar salt absorption, specific ion toxicity and decreased yields (Ayers and Westcot 1985; Shalhevet 1994). Whereas the salinity tolerance of crops in surface-irrigated systems is well established (Maas and Hoffman 1977; Maas 1986; Maas and Grattan 1999), the potential deleterious effects of saline sprinkler irrigations on crop yield are not well documented, and few field studies on only a limited number of crops have been conducted (Bernstein and Francois 1975; Hoffman et al. 1983; Isla et al. 1997; Maas et al. 1982). Most reports are based on studies that demonstrate the higher accumulation of Na^+ and Cl^- in plant tissues exposed to sprinkler saline waters (Francois and Clark 1979; Gorham et al. 1994; Grattan et al. 1994; Grieve et al. 2003; Maas et al. 1982), but only some studies report the corresponding losses in crop yields (Benes et al. 1996; Busch and Turner, 1967; Royo and Aragüés 1999).

Maas (1985) showed the lack of correlation in different crop species between foliar injury due to toxic ion accumulation and yield losses. However, recent works (Anand et al. 2000; Boughanmi et al. 2005; Chaudhary et al. 1996; Essechie and Rodriguez 1999; Mckimmie and Dobrenz 1991; Mohammadi et al. 2008) have established several relationships between salinity tolerance and Na^+, Cl^- and K^+/Na^+ concentrations in alfalfa shoots, indicating that the accumulations of Na+ and Cl^- have detrimental effects in yield.

In a two-year study of cotton sprinkled with saline water (EC = 4.4 dS m^{-1}; SAR = 17.8), Busch and Turner (1967) showed that daytime evaporation losses from sprinkling markedly affected both sodium content in plant leaves and crop yield. However, the results obtained in part I of this work (Isla and Aragüés 2009) demonstrate that the yield of alfalfa under overhead saline sprinkler irrigations was independent of the timing of irrigation (i.e., diurnal or nocturnal). Thus, the recommended practice of irrigating at night when using saline waters in sprinkler irrigation (Ayers and Westcot 1985; Garg and Gupta 1995; Maas and Grattan 1999) was not supported by our results in alfalfa.

This unexpected finding should be substantiated by the analysis of shoot ion accumulation under diurnal and nocturnal irrigation, since our similar yields for alfalfa subject to diurnal and nocturnal saline sprinkler irrigations should correspond with similar plant ion contents in both irrigation treatments.

The objectives of part II of this work were to (1) compare the accumulation of shoot Cl^-, Na^+ and K^+ in alfalfa sprinkler irrigated with saline waters under diurnal and nocturnal irrigations, and (2) examine potential relationships between accumulation of these ions and alfalfa yield subject to saline sprinkler irrigations.

Materials and methods

The details of the experimental set-up were given in part I of this work (Isla and Aragüés 2009). The trial was carried out during the 2004 to 2006 alfalfa growing seasons using a triple line source sprinkler system (TLS) (Aragüés et al. 1992). The design of the modified TLS allows creating a continuous gradient in the salinity of the irrigation water in two areas that were irrigated at daytime and at night. Table 1 shows the general characteristics of the irrigation events and the T1-T7 intervals of the season-average irrigation water EC, Na^+, Cl^-, Ca^{2+} and SAR concentrations in each experimental year. In 2004, the saline solution was made up with a mixture of sodium and calcium chloride with a final SAR close to 4 (treatment T7). In 2005 and 2006 the sodium chloride was increased to better ascertain the potential toxic effects of Cl^- and Na^+ in alfalfa. Irrigation water K^+ concentrations were around 0.30 meq L^{-1} and similar in all years

Alfalfa was sown in fourteen strips (1.55 m wide by 30 m long), seven in the diurnal and seven in the nocturnal irrigated areas, corresponding to the seven irrigation water saline treatments designated in this trial (Table 1). Alfalfa was cut five times in 2004 and six times in 2005 and 2006 in three replicates of each salinity treatment of the diurnal and nocturnal irrigations. The harvested material was oven dried at 65ºC and the yield of alfalfa was given in Mg ha^{-1} of total dry matter (TDM).

A portion of the alfalfa dry matter sampled in the ten cuts taken during the summer periods (three in 2004 and 2006 and four in 2005) was finely ground using a 0.5-mm sieving mill. A sample of 0.25 g was extracted with 50 mL of an extracting solution made of 900 mL of deionized water, 100 mL of acetic acid, and 6.4 mL of nitric acid. Chloride was analyzed using a chloridometer (Cotlove 1963) and sodium, potassium and calcium were analyzed by flame photometry using a continuous flow auto analyzer (AA3- Bran Luebbe). Shoot ion concentrations are given in meq kg^{-1}.

The statistical analyses were performed using the SAS 9.1 software. Comparison of regression lines was made using an F-test, taking the root mean error (RME) of the overall regression as the error for the pairwise comparisons. The Marquardt algorithm (proc NLIN, procedure in the SAS software) was used to estimate the non-linear models. The significance of the regression analyses was indicated as **, *, and NS at probability levels $P < 0.01$, < 0.05, and > 0.05, respectively. Relative total dry matter values (TDMr) were obtained dividing the aboveground TDM of each plot by the average of the two highest TDM plots of each cut. When it was necessary to pool the data from different samplings, standardized values of ion concentrations and TDM were obtained by transforming the data from each sampling to a population with zero mean and one standard deviation.

Results and discussion

Effect of diurnal vs. nocturnal saline sprinkler irrigations on shoot Cl^- and Na^+

Diurnal irrigations were given at higher average temperature, wind speed and solar radiation, and lower relative humidity than nocturnal irrigations (Isla and Aragüés, 2009). Based on these values, the water evaporation rate (WER) in the 60-min period following each irrigation was between 4 and 29 times higher at daytime than at night. This higher daytime water evaporation rate will concentrate the ions in the water standing in the wetted leaves after each sprinkler event. Thus, leaf ion accumulation could be higher in the leaves wetted during the day, and this is the basis for recommending irrigation at night, when both evaporation and salt absorption are reduced (Maas and Grattan 1999).

Shoot Cl^- and Na^+ tended to be more closely correlated with applied water salinity (ECaw) than with soil salinity (ECe). Thus, the average R^2 for the individual cuts increased from 0.57 to 0.81 for Na^+ regressed on ECaw rather than ECe, and likewise from 0.58 to 0.84 for Cl^- regressed on ECaw rather than ECe, indicating that the accumulation of these ions was due primarily to leaf ion uptake. These R^2 values, significant at $P < 0.01$, are consistent with previous findings in that Cl^- and Na^+ are positively correlated with irrigation water salinity (Benes et al. 1996). The review by Maas (1985) compiling data from different studies shows that sprinkling alfalfa with waters of EC > 2 dS m^{-1} and Na^+ and Cl^- of about 10-20 meq L^{-1} resulted in foliar injury to the crop. In contrast, under our experimental conditions marginal leaf necrosis was only visually apparent in the higher salinity treatments (T6 and T7), corresponding to ECiw > 5 dS m^{-1} and Na^+ and Cl^- of about 32 to 38 meq L^{-1} (Table 1). Shoot Na^+ and Cl^- increased to values of about 800 meq kg^{-1} after three years of sprinkling with saline waters of 5 dS m^{-1} (Fig. 1). In contrast, these high concentrations of Na^+ and Cl^- were only attained in surface irrigation systems when alfalfa was subject to waters of 17 dS m^{-1} (Rogers et al. 1998), indicating the enhanced absorption of ions by the alfalfa shoots under saline sprinkling.

Of the ten regressions calculated for each alfalfa cut, eight Cl^- vs. ECaw and nine Na^+ vs. ECaw regressions were not significantly different ($P > 0.05$) between the diurnal and nocturnal irrigations. Thus, irrigating at night did not decrease foliar ion uptake as compared to irrigating at daytime. Similar results were obtained when pooling together all the cuts of each experimental year (Fig. 1). Although in all cases the slopes of the diurnal regressions were higher than the slopes of the nocturnal regressions, they were not significantly different ($P > 0.05$). These results demonstrate the absence of significant differences in Cl^- and Na^+ accumulation in alfalfa subject to diurnal and nocturnal irrigations for the ECaw values imposed in this study. This finding disagrees with the recommended practice of irrigating at night to decrease leaf ion uptake under saline sprinkler irrigations.

Even though Cl^- and Na^+ in the applied water were similar (Table 1), shoot Cl^- was much higher than shoot Na^+, especially for low saline waters (Fig. 1). Thus, the average intercepts were, depending on years, between 6 and 3.6 times higher for Cl^- than for Na^+. These differences in shoot Cl^- and Na^+ in non saline conditions were also described by Noble et al. (1987)

Since the regressions of the diurnal and nocturnal irrigations were not significantly different, they were pooled together to calculate the average intercepts and slopes for each of the ten summer cuttings performed along 2004-2006 (Fig. 2). The Cl^- intercepts and slopes did not change significantly ($P > 0.05$) along the study period. In contrast, both the Na^+ intercepts ($P < 0.05$) and the Na^+ slopes ($P < 0.01$) significantly increased over time, indicating that the ability to restrict Na^+ uptake decreased with time of exposure to salts. Based on the regression equations for the slopes (Fig. 2), the rate of Na^+ accumulation with time (cut number) was 9.4 as compared to 2.6 for Cl^- (i.e., 3.6 times higher for Na^+ than for Cl^-). Differences in irrigation water Cl^- and Na^+ in 2004 ($Cl^- > Na^+$) and 2005-2006 ($Cl^- = Na^+$) (Table 1) could have also a minor impact on these different accumulation rates.

No consistent relationships were found between shoot Ca^{2+} and ECaw along the three experimental years. Thus, only in one out of the ten alfalfa cuts a negative and significant ($P < 0.05$) linear correlation was found between shoot Ca^{2+} and ECaw. Shoot Ca^{2+} was higher in 2004 (mean = 453 meq kg^{-1}) than in 2005 (345 meq kg^{-1}) and 2006 (355 meq kg^{-1}), reflecting the higher irrigation water Ca^{2+} in 2004 than in the following years (Table 1).

Effect of diurnal vs. nocturnal saline sprinkler irrigations on shoot K^+

Since irrigation water K^+ was low (0.3 meq L^{-1}) and similar in the T1 to T7 saline treatments, shoot K^+ measured in each alfalfa cut was regressed against soil salinity (ECe) (Fig. 3). The data from cut #4 (June 06, 2005) are not presented in this figure because shoot K^+ and ECe were not significantly correlated ($P > 0.05$). Seven out of the nine regressions calculated for each alfalfa cut were not significantly different ($P > 0.05$) for the diurnal and nocturnal irrigations. Pooling the diurnal and nocturnal treatments of these seven K^+-ECe regressions together showed that six of them were significant at $P < 0.01$ and one at $P < 0.05$ (Fig. 3). Thus, shoot K^+ decreased linearly with soil salinity despite the high doses of potassium fertilizer applied to the soil (400 kg ha^{-1} $year^{-1}$of K_2O).

These decreases in shoot K^+ with increases in soil salinity have also been shown in other alfalfa studies (Grieve et al. 2004; Mohammadi et al. 2008; Rogers 2001) and are of the same order of magnitude than those reported by Noble et al. (1987). Decreases in shoot K^+ were associated with increases in shoot Na^+, as indicated by the negative and significant correlations among them (2004: R^2=0.10*, 2005: R^2=0.73**, 2006: R^2=0.62**). This K^+-Na^+ competition and the corresponding decreases in the K^+/Na^+ ratio with increases in soil salinity have been demonstrated in many studies and is considered a relevant indicator of salt sensitivity and nutrient imbalances associated to salinity (Anand et al. 2000; Rogers et al. 1998).

Relationships between alfalfa yield (Total Dry Matter, TDM) and shoot Cl^-, Na^+ and K^+

Since TDM and shoot ion concentrations were not significantly different for the diurnal and nocturnal irrigations, they were pooled together to obtain a larger population for analysis. The linear regressions between shoot ion and relative total dry matter (TDMr) for each alfalfa cut were not significant, except in one cut for Cl^- and Na^+ and four cuts for K^+ (Table 2). Pooling together all the cuts of each year, none of the shoot ions were significantly ($P < 0.05$) correlated with TDMr in 2004 and 2005. In contrast, TDMr in 2006 was negatively correlated with Cl^- and Na^+ and positively correlated with K^+ and K^+/Na^+ ($P < 0.01$).

When all the data for the three experimental years (n = 135) were analyzed together after standardizing both TDMr and shoot ions to eliminate possible differences attributable to sampling date, the results show that alfalfa yield was negatively correlated ($P < 0.01$) with shoot Cl^- and Na^+, and positively correlated with the K^+/Na^+ ratio (Table 2). The positive correlation between TDMr and K^+/Na^+ suggests that this ratio rather than shoot K^+ is related to alfalfa yield.

This result is consistent with those of Anand et al. (2000) and Mohammadi et al. (2008) in alfalfa showing that tolerant genotypes had higher K^+/Na^+ ratios than susceptible genotypes.

Relative alfalfa yield (TDMr) and shoot Cl^-, Na^+ and K^+/Na^+ for the three cuts of year 2006 were adjusted using a piecewise linear response model (Maas and Hoffman 1977) (Fig. 3). This analysis was not performed for years 2004 and 2005 because alfalfa yield and shoot ions were generally not correlated (Table 2). The model was significant ($P < 0.05$) due to the data of cut #10 (end of the study period), when TDMr decreased, shoot Cl^- and Na^+ increased, and shoot K^+/Na^+ decreased. This result is consistent with the increasing slopes of Cl^- and Na^+ with time shown in Fig. 2, indicating that the salinity tolerance of alfalfa decreased with time of exposure to salts and the concomitant increases in Cl^- and, in particular, Na^+ in the shoots.

The adjusted model gives threshold values (i.e., values above which yield declines) of 726 meq Cl^- kg^{-1} (equivalent to 2.6 %), 276 meq Na^+ kg^{-1} (equivalent to 0.6 %) and 1.4 for the K^+/Na^+ ratio. Although these thresholds should be taken as approximate due to scattering of the data (Fig. 4), they show that alfalfa was more sensitive to Na^+ than to Cl^- accumulation. The higher toxicity of Na^+ than Cl^- in alfalfa seedlings has been suggested by McKimmie and Dobrenz (1991) based in the higher root/shoot exclusion of Na^+ relative to Cl^- in tolerant plants.. Although the relative toxicities of Na^+ and Cl^- remain uncertain (Maas and Grattan 1999) for many crops, our results suggest that Na^+ accumulation was the preponderant reason for the yield decline of alfalfa after three years of sprinkling with saline waters.

Conclusions

The accumulation of Cl^- and Na^+ in alfalfa sprinkler-irrigated with saline waters was positively and linearly correlated with irrigation water salinity and was primarily due to leaf rather than root ion uptake.

Although daytime evaporation was about eleven times higher than nighttime evaporation (average for the 2004-2006 experimental years), shoot Cl^- and Na^+ accumulation were similar for the diurnal and nocturnal saline sprinkler irrigations. This result substantiates the lack of significant differences in alfalfa yield reported in our preceding publication (Isla and Aragüés, *in press*) for these irrigation treatments. Shoot K^+ decreased significantly with increases in soil salinity but it was also similar for the diurnal and nocturnal irrigations.

Shoot Na^+ was initially much lower than shoot Cl^-, especially for low saline irrigation waters, but the rate of Na^+ accumulation along the three years examined was about 3.6 times higher than the Cl^- accumulation rate. Hence, the salinity tolerance of alfalfa decreased with time of exposure to salts due to concomitant increases in Cl^- and, particularly, Na^+ in the shoots.

The fitting of the 2006 relative yield and shoot ion data to a piecewise linear response model show that the yield of alfalfa declined at shoot Na^+ and Cl^- concentrations of 276 and 726 meq kg^{-1}, respectively. The lower shoot Na^+ threshold indicates that alfalfa is more sensitive to Na^+ than to Cl^-, so that Na^+ accumulation is the preponderant reason for yield decline of alfalfa after three years of sprinkling with saline waters.

Acknowledgments: This study was supported by the Ministry of Education and Science of Spanish Government (Project AGL2003-01942). Thanks are also given to field and laboratory personnel of the Soils and Irrigation Department of C.I.T.A.

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Table 1 General characteristics of the irrigation events given in the 2004 to 2006 experimental years of the alfalfa trial.

Experimental year	2004	2005	2006
Number of irrigations	43	39	38
First saline irrigation	May 28	May 10	May 11
Last saline irrigation	October 6	October 5	October 10
Saline treatments T1 to T7			
T1 – T7 ECiw interval (dS m^{-1})	0.5 – 4.5	0.5 – 5.6	0.5 – 5.5
T1 – T7 Na^+ interval (meq L^{-1})	2.0 – 14.0	2.6 – 45.1	2.0 – 42.5
T1 – T7 Cl^- interval (meq L^{-1})	1.8 – 28.8	4.1 – 45.5	3.2 – 44.5
T1 – T7 Ca^{2+} interval (meq L^{-1})	2.2 – 25.6	2.2 – 10.1	2.2 – 10.6
T1 – T7 SAR interval (mmol $L^{-1})^{0.5}$	2.0 – 3.6	3.0 – 19.9	3.1 – 21.5
T1 – T7 ECaw interval [a] (dS m^{-1})	0.4 – 4.4	0.4 – 4.7	0.4 – 4.3

[a] Electrical conductivity of applied water = volume-weighted average of irrigation EC plus rainfall EC.

Table 2 Significance of the correlation coefficients of the linear regressions between plant Cl^-, Na^+, K^+ and K^+/Na^+, and the relative alfalfa total dry matter (TDMr) for each cut, the yearly pooled cuts and the standardized values of the 2004 to 2006 experimental years; n = number of data.

Year	cut	n	Cl^-	Na^+	K^+	K^+/Na^+
2004	1 (July 23th)	13	(ns)	(ns)	0.71**	0.58*
	2 (Aug. 24th)	13	(ns)	-0.49[b]	(ns)	(ns)
	3 (Sep. 28th)	14	(ns)	(ns)	(ns)	(ns)
	Pooled cuts	40	(ns)	-0.29[b]	(ns)	0.34*
2005	4 (June 6th)	14	(ns)	(ns)	(ns)	(ns)
	5 (June 30th)	14	(ns)	(ns)	(ns)	(ns)
	6 (Juy 28th)	13	(ns)	(ns)	(ns)	(ns)
	7 (Aug. 31th)	13	(ns)	(ns)	0.57*	0.50*
	Pooled cuts	54	-0.24[b]	(ns)	(ns)	(ns)
2006	8 (July 3 th)	14	(ns)	(ns)	-0.57*	(ns)
	9 (July 31 th)	14	(ns)	(ns)	(ns)	(ns)
	10 (Sep. 5th)	13	-0.79**	-0.77**	0.78**	0.82**
	Pooled cuts	41	-0.46**	-0.53**	0.49**	0.42**
All years, Standardized[a]		135	-0.28**	-0.28**	(ns)	0.26**

[a] Correlations performed using ion concentration standardized (mean = 0 and standard deviation = 1) for each individual cut. [b] Significant at $P < 0.1$

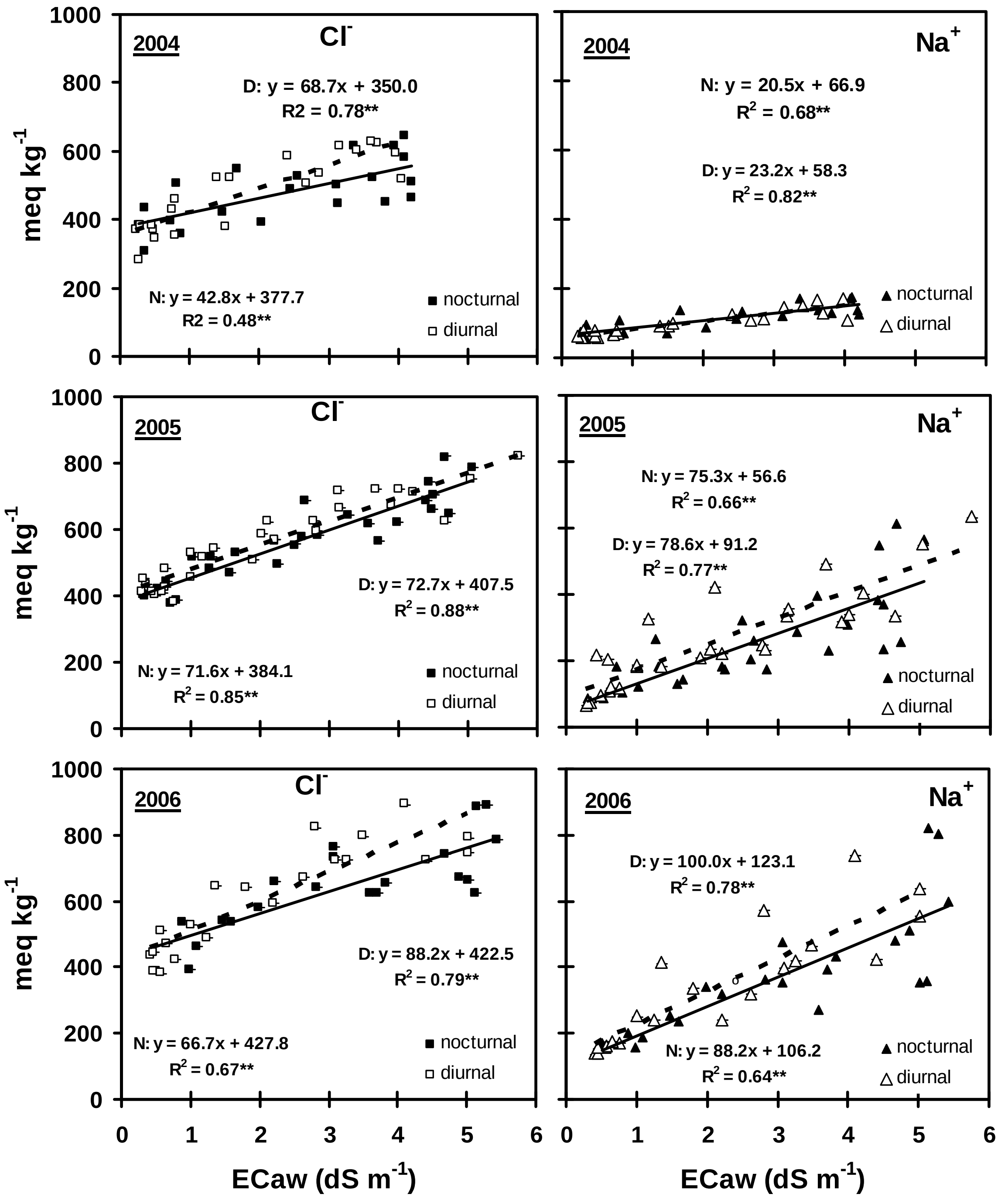

Fig. 1 Relationships between shoot Cl^- and Na^+ and the average electrical conductivity of the applied water (ECaw) for the diurnal and nocturnal irrigations of the 2004 to 2006 experimental years. Data from all cuttings for a given year were pooled together for obtaining the linear regression equations for the diurnal (dotted line) and nocturnal (solid line) irrigations

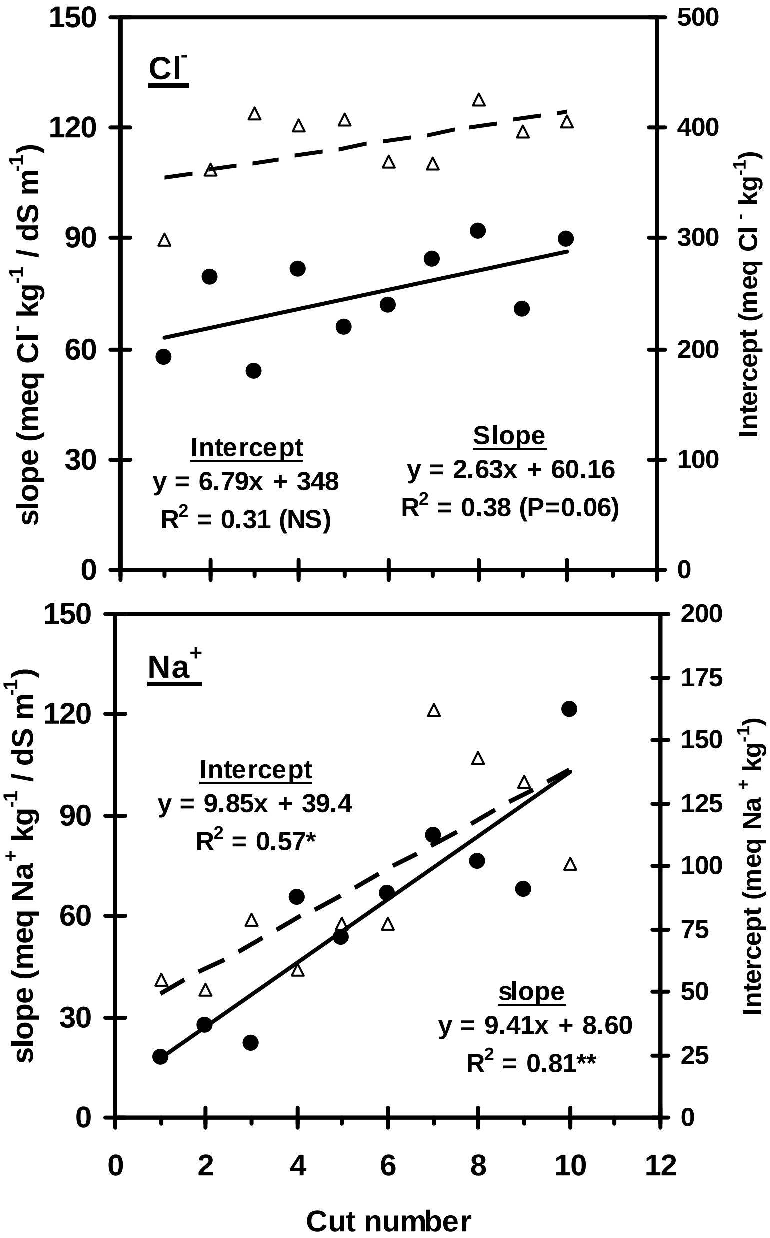

Fig. 2 Temporal evolution of the average diurnal and nocturnal slopes (solid circles) and intercepts (open triangles) of the shoot Cl^--ECaw and shoot Na^+-ECaw regressions established for each of the ten alfalfa samplings (cuts) performed along the 2004 to 2006 experimental years

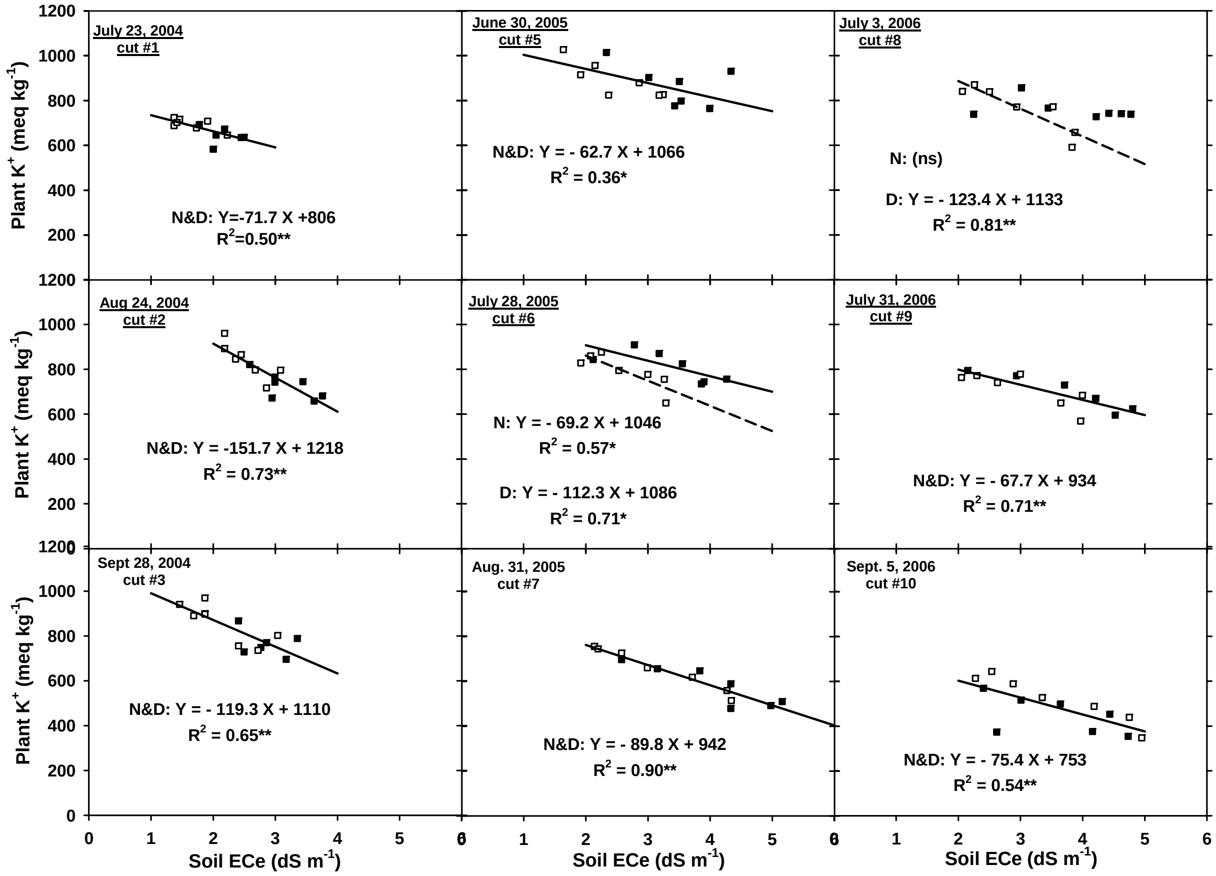

Fig. 3 Effect of soil salinity (ECe) on shoot K^+ in alfalfa sprinkler irrigated during the day (open squares) and at night (solid squares) for the different cuts. The nocturnal (thin line) and diurnal (dashed line) linear regressions fitted for each cut were pooled (thick line) when they were not significantly different (P > 0.05)

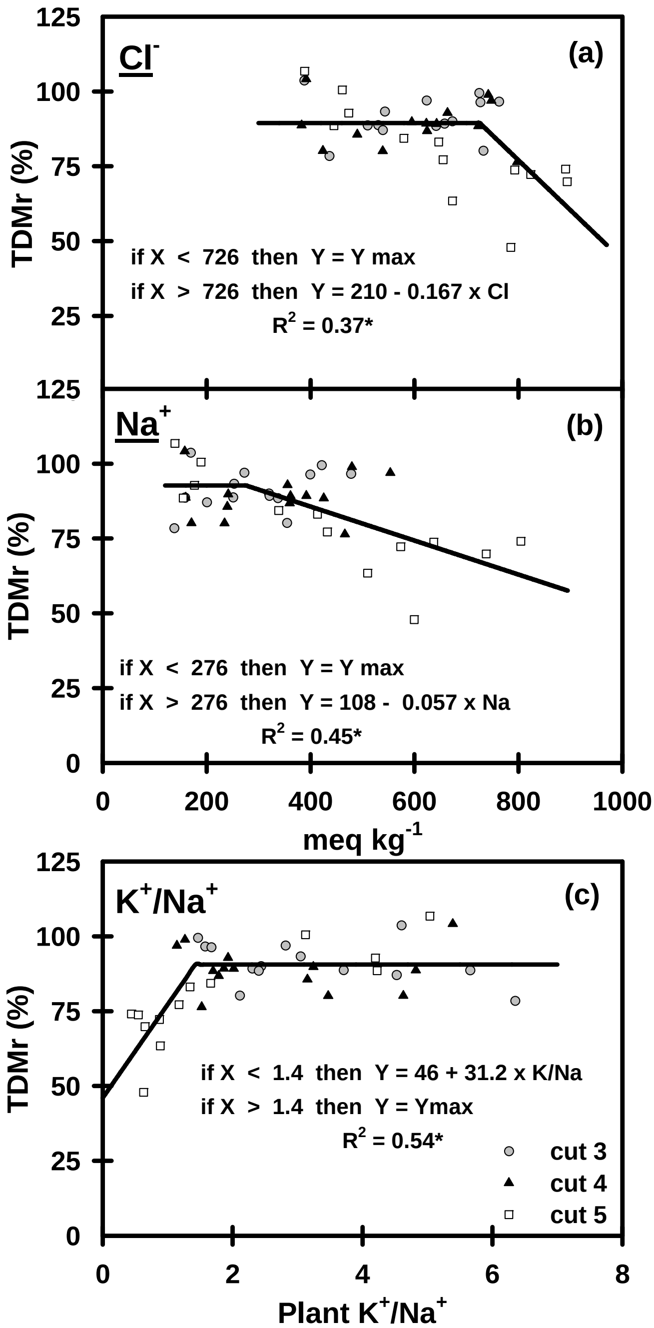

Fig. 4 Relationships between relative alfalfa Total Dry Matter (TDMr) and (a) shoot Cl^-, (b) shoot Na^+ and (c) shoot K^+/Na^+ established for the three cuts harvested in 2006. Data adjusted to a piecewise linear response model