RESEARCH

Emergence, Forage Production, and Ion Relations of Alfalfa in Response to Saline Waters

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is an important crop utilized in irrigated regions that are commonly impacted by salinity. We evaluated the effect of salinity continually from emergence to mature plant growth in successive harvests. We studied emergence, biomass production, salt tolerance, and shoot ion composition as potential physiological mechanisms in four nondormant salt-tolerant cultivars: Salado, SW 8421S, SW 9720, and SW 9215. Irrigation water salinity dominated by sodium sulfate ranging from 3.1 to 30 dS m^{-1} of electrical conductivity (EC_{iw}) was imposed at planting date. Plants were grown in outdoor sand tanks in Riverside, CA for 300 d. Relative emergence (%) decreased above EC_{iw} 12.7 dS m^{-1} and was reduced to 53 and 13.4% at 18.4 and 24 dS m^{-1}, respectively. At EC_{iw} 30 dS m^{-1} there were no survivor plants. Absolute and relative accumulated biomass from 6 harvests significantly decreased for EC_{iw} above 12.7 dS m^{-1} (6.0 dS m^{-1} in the saturation extract [EC_e]). Plants grown at 18.4 and 24 dS m^{-1} produced 68% and 30% respectively of the biomass produced at 3.1 dS m^{-1}. Salado showed the least reduction in biomass at elevated salinity and, as with all the cultivars, exhibited yield increases in successive harvests from first through seventh. Increasing salinity increased shoot Na^+, total-S, Cl^-, Mg^{2+}, and P and decreased K^+ and Ca^{2+}. The ability of Salado to maintain low shoot Na was the mechanism most associated with salt tolerance. Saline waters with resultant EC_e values of up to 6 dS m^{-1} did not significantly reduce total forage production of the second through the seventh harvests. This suggests that irrigation with saline waters resulting in EC_e values less than 6 dS m-1 can be used throughout the production cycle (planting to multiple harvests) without significant yield loss for the cultivars examined.

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Abbreviations: DW, dry weight; EC, electrical conductivity; EC_e, saturation extract; EC_{iw}, electrical conductivity values of the irrigation water; EC_{sw}, average soil water electrical conductivity; E_{max}, asymptotic maximum of E taken as a maximum cumulative emergence at each specified salinity level; ET, evapotranspiration; ICPOES, inductively coupled plasma optical emission spectroscopy; t_{max}, the mean time to reach E_{max}.

Alfalfa is an important high-value forage crop suitable and utilized in semiarid regions of the world where sufficient rain or irrigation water is available. Alfalfa has a relatively high water consumption; thus, in irrigated regions, increasing water scarcity and alternative competing demands on water supplies makes continued alfalfa production in these regions uncertain. Utilization of more saline waters, including drainage waters and brackish ground waters, may be feasible for alfalfa production, thus conserving fresh water for other uses. In the San Joaquin Valley of Central California, especially on the west side, the shallow groundwater consists of mixed types of chemical compositions, often Na_2SO_4 dissolved as either the dominant salt or in equal proportions with NaCl. This groundwater composition as well as the resultant drainage waters are being evaluated for reuse to irrigate crops, such as alfalfa.

The response of plants to soil salinity has been linked with the stage of crop development (Shannon, 1997; Smith, 1994). The germination stage and the seedling stage in many crops have

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been reported as the most sensitive stages under salt-stress conditions (Ashraf and Foolad, 2005). Some authors have suggested that the performance at these growth stages is a good indicator of the future performance of the mature plants. However, the literature is varied and not clear about this general concept because different responses have been found depending on the species, cultivar, experimental conditions, type of dissolved salts, and levels of salinity (Bernstein, 1974; Carter and Grieve, 2008; Shannon, 1997; Steppuhn and Raney, 2005; Ungar, 1996). A critical commentary about this correlation between emergence rate and the potential salinity tolerance has been reported by Katerji et al. (2012), who concluded that the ability to emerge in saline conditions does not represent an indicator of tolerance, either for tolerant species (durum wheat [*Triticum durum* Desf.] and barley [*Hordeum vulgare* L.) or sensitive species (chickpea [*Cicer arienetum* L.] and broad bean [*Vicia faba* L.]). In alfalfa, the results of previous studies are inconclusive. Some studies have concluded that the ability of the plants to produce biomass is not necessarily related to germination (Al-Niemi et al., 1992; Johnson et al., 1992; Rumbaugh and Pendery, 1990) and emergence (Steppuhn et al., 2012). However, other studies have demonstrated a positive correlation between germination at severe salinity (342 mM of NaCl) with regrowth potential under salt stress (greenhouse experiment) and also with production under field conditions (Scasta et al., 2012).

The salt tolerance of alfalfa is reported as moderately sensitive to NaCl and intermediate in tolerance among forages (Maas, 1987; Maas and Hoffman, 1977) and very tolerant within legumes (Munns and Tester, 2008).

In general, the most tolerant plants are able to restrict higher accumulations of toxic ions like Na^+ and Cl^- and the toxicity of each ion specific to the plant species (Munns and Tester, 2008). Both Na^+ and Cl^- ions can limit the plant growth through different mechanisms simultaneously (Tavakkoli et al., 2010). Because Na^+ interferes with K^+ nutrition, high K/Na ratio has been reported as an important factor related with tolerance (Maathuis and Amtmann, 1999). Also, Na^+ limits the activity and availability of Ca^{2+} and reduces the Ca^{2+} uptake for the plant (Ashraf, 2004; Suarez and Grieve, 1988). Salt tolerance in alfalfa, when NaCl was used as the salinizing salt, was related with the capacity to limit the transport of both ions to the shoot (Ashraf et al., 1986). Thus, some studies have focused on salt tolerance as related to control of Cl^- uptake (Noble and Shannon, 1988; Noble et al., 1984) and others on the Na^+ exclusion (Khorshidi et al., 2009; Mezni et al., 2012). Also, the restriction of S transport to the shoots was suggested as a mechanism of tolerance to high external concentrations of Na_2SO_4 (Rogers et al., 1998).

Previous studies (Grattan et al., 2004; Scasta et al., 2012) have evaluated salt tolerance of some alfalfa cultivars such as Salado and SW 9720 where salinity was applied after establishment. However, this delay in salt stress application may not be pertinent to field conditions. Information regarding salt tolerance is needed in instances where saline water is the only water available and is utilized from seeding to mature stage.

Our objective was to evaluate the effect of salinity continually from alfalfa emergence to mature plant growth, including regrowth in successive harvests. We studied the ion composition as a potential physiological mechanism to compare the tolerance of four nondormant purported salt-tolerant cultivars of alfalfa currently in the market under salinity dominated by sodium sulfate.

MATERIALS AND METHODS

The experiment was conducted from 23 June 2011 to 17 Apr. 2012 in an outdoor lysimeter system at the United States Salinity Laboratory (USDA-ARS) in Riverside, CA. The experiment consisted of a factorial combination of salinity (six levels) and cultivars (four) arranged in a split-plot experimental design with four replications. The salinity levels (main plot) were 3.1, 7.2, 12.7, 19.4, 24.0, and 30 dS m^{-1} expressed in terms of EC_{iw}. The water ion compositions in this study were prepared to simulate the range in compositions of the drainage water in the Central Valley of California with SO_4^{2-} as the dominant anion in solution. The salinity levels were developed with subsequent concentrations of salts considering mineral precipitation (calcite and/or gypsum) using the UNSATCHEM model (Suarez and Simunek, 1997), which simulates typical soil water interactions. To reach these EC_{iw} values and specific ion compositions, salts of $MgSO_4$, Na_2SO_4, NaCl, and $CaCl_2$ were added to Riverside Municipal tap water. Table 1 provides the summary of the concentration of salts added to all treatments.

All reservoirs had modified Hoagland's solution consisting of the following micronutrients (in μmol L^{-1}): Fe (50) added as Fe-DTPA (Sprint 330), $ZnSO_4.7HO_2$ (0.4), $CuSO_4.5HO_2$ (0.2), H_2MoO_4 (0.1), H_3BO_3 (23), $MnSO_4$ (5), and macronutrients (mmol$_c$ L^{-1}) $MgSO_4.7H_2O$ (3.1), KNO_3 (5.0), KCl (1.0), KH_2PO_4 (0.34), and $CaCl_2$ (3.0). When considering the composition of Riverside tap water (electrical conductivity [EC] = 0.60 dS m^{-1}), which added (in mmol$_c$ L^{-1}) 3.4 Ca^{2+}, 0.8 Mg^{2+}, 1.6 Na^+, 0.1 K^+, 1.3 SO_4^{2-}, 0.83 Cl^-, and 0.486 NO_3^- and added salts (Table 1), all treatments had at least 6.45, 4, 6.4, and 5.5 mmol$_c$ L^{-1} of Ca^{2+}, Mg^{2+}, K^+, and NO_3^-, respectively. The lowest EC_{iw} served as a control. This control also had a small amount of salt added to reach 3.1 dS m^{-1} considered as our lowest target. This salinity level will probably not result in yield loss, as previous research has established that there is no decrease in alfalfa production if the soil water extracts are below EC_e 2.0 dS m^{-1} (Maas and Hoffman, 1977). In our system, an irrigation water of EC 3.1 dS m^{-1} corresponds to an EC_e of 1.46 (the relation will be explained below). Salts and nutrients were added to the irrigated test waters before seeding. The pH was adjusted and maintained between 7 and 7.8.

The four subplots included nondormant cultivars of alfalfa, all of them purportedly salt tolerant according to the last report of Alfalfa Variety Ratings (NAFA, 2014). Salado was classified as salt tolerant in both germination and forage production; SW

Table 1. Concentration of salts added to the irrigation waters and electrical conductivities (EC_{iw}) reached at each salinity level. Electrical conductivity includes nutrients and ion composition of the City of Riverside municipal tap water.†

EC_{iw} dS m^{-1}	$MgSO_4.7H_2O$	$CaCl_2$	NaCl	Na_2SO_4
	——— mmol$_c$ L^{-1} (g L^{-1} per salt) ———			
3.1			3.0 (0.17)	10.9 (0.77)
7.2	10.4 (1.28)	12.8 (0.71)	14.1 (0.83)	38.5 (2.73)
12.7	20.2 (2.49)	18.6 (1.02)	39.4 (2.30)	60.3 (4.28)
18.4	36.8 (4.54)	23.0 (1.26)	76.6 (4.48)	91.1 (6.47)
24	54.6 (6.73)	23.0 (1.26)	105.1 (6.14)	122.1 (8.67)
30	59.1 (7.28)	22.1 (1.21)	140.7 (8.22)	124.7 (8.85)

† The nutrients added in all levels were in mmol$_c$ L^{-1}: 0.34 KH_2PO_4, 5 KNO_3, 3.1 $MgSO_4.7H_2O$, 3.0 $CaCl_2$, and 1 KCl. Municipal tap water contained, in mmol$_c$ L^{-1}: 3.4 Ca^{2+}, 0.8 Mg^{2+}, 1.6 Na^+, 0.1 K^+, 1.3 SO_4^{2-}, 0.83 Cl^-, and 0.486 NO_3^-.

9215, SW 9720, and SW 8421S only salt tolerant in forage production. The fall dormancy is rated on a scale of 1 to 11 (1 being very dormant to 11 very nondormant) with Salado, SW 9215, and SW 9720 ranking 9 and SW 8421S ranking 8.

Plants were grown in 24 sand tanks, each measuring 82 cm wide by 202 cm long by 85 cm deep. The tanks were divided in four equal parts by plastic barriers and the area sown was 0.32 m^2 per cultivar per tank. We adopted a seeding rate of 330 seed m^{-2}, recommended for alfalfa (Mueller et al., 2008). A total of 108 seeds per cultivar per replication were sown, arranged in 9 rows and 12 seeds per row. The seeds were placed at a depth of 10 mm into the sand and positioned 3.7 cm apart within the rows with spacing of 8 cm between rows. The tanks contain river sand, allowing for good drainage and providing essentially no exchange of soil water inorganic constituents with the solid phase, thus simplifying calculation of in situ water chemistry. Each tank was irrigated by pumping approximately 1100 L of irrigation water from the reservoirs (1740 L) to the sand tanks, equivalent to the unit volume height of 60 cm per tank, an amount estimated to thoroughly leach the sand with each irrigation. The leached water drained back into the reservoirs during and after the irrigation. At saturation, the sand has an average volumetric water content of 0.36 cm^3 cm^{-3}, corresponding to approximately 500 L of water stored per tank and 240 L of stored water per tank at field capacity of the sand. Each irrigation flushed the tank with at least two pore volumes. The water lost by evapotranspiration was replenished in the reservoirs to maintain essentially constant osmotic potential in the irrigation water and in the sand tanks. Refilling the reservoirs was done by adding Riverside Municipal tap water through an automated refill system. Irrigation was twice daily. We calculated the relationship between EC_{iw} and EC_e on the basis of twice-daily irrigation, saturation water content, and field capacity of 0.36 and 0.17 cm^3 cm^{-3} respectively, root depth of 40 cm, and ET_0 always less than 0.8 cm d^{-1}. The root zone held 120 L of water at field capacity (and 240 L to a depth of 84 cm). Just before the next irrigation, the crop consumed less than 6.4 L of water since the previous irrigation. We consider that there was no water stress as the plants were always well watered and the sand near field capacity at all times. The EC just before an irrigation is calculated as being not more than 5.3% (6.4 L/120 L) greater than EC_{iw}, and thus the average soil water EC (EC_{sw}) between irrigations = 1.03EC_{iw} (EC_{sw} = EC_{iw} after irrigation and EC_{sw} = 1.053EC_{iw} just before the next irrigation). The volumetric saturation of our soil is 0.36 cm^3 cm^{-3} for the saturation extract EC_e = 0.472 EC_{iw}. Using this relationship, our salinity treatment from 3.1 to 24 dS m^{-1} may be calculated as 1.46, 3.40, 6.0, 8.68, 11.33 dS m^{-1} expressed as EC_e.

The EC_{iw} was measured every week and samples were taken twice during the experiment for analysis of Ca^{+2}, Mg^{2+}, Na^+, K^+, and PO_4^{3-} and total-S using Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) (PerkinElmer Corp., Waltham, MA). Chloride was determined by amperometric titration.

Measurements

In this study, emergence is defined as the appearance of the first unifoliate leaf. Observations of the emerged seedlings started the fourth day after planting and then were subsequently counted daily or on each second day in each subplot during the 3 wk after seeding. The emerged seedlings were counted in seven rows per cultivar per subplot in each of the four repetitions and then the cumulative emergence by day (%) was calculated as:

$$E = (\text{number of emerged plants} / 84)100 \quad [1]$$

where E is the percentage of cumulative emergence at any specific salinity by day and 84 is the number of seeds sown (12 seeds × 7 rows) in the area measured.

At the end of the establishment period (21 d from the sowing date), the surviving plants continued growing under the same treatments; we did not adjust the plant density.

We recorded growth measurements for seven harvests during 296 d from the planting date. Harvest dates in 2011 were 22 August, 15 September, 18 October, and 15 November; in 2012 they were 11 January, 22 February, and 17 April. The number of days of growth between first to seventh harvest were 60, 24, 33, 28, 57, 42, and 54, respectively. Until October, the harvests were done at the early flowering stage (10% flowering). After October, in the absence of flowering, the harvests were done when control plants were at a late vegetative stage. At harvest, all plants were cut approximately 5 to 7 cm above the crown of the plant. We measured the shoot fresh weight and dry weight (DW; dried at 70°C for 48 h). Shoot biomass production per harvest was expressed on the DW basis in g m^{-2}. The first harvest was analyzed comparing only the biomass from Salado, SW 8421S, and SW 9715. To compare the accumulated biomass among cultivars, the biomass from the second to seventh harvest were summed. The first harvest could not be included because of missing data from SW 9720. The relative biomass (%) was calculated as follows:

$$\text{Relative biomass (\%)} = (\text{biomass at any EC level} / \text{mean biomass at EC 3.1 dS m}^{-1})100 \quad [2]$$

We combined plants of each cultivar in each subplot (replication) taken in the second and seventh harvest. The samples were washed with deionized water immediately after harvesting and dried in a forced-air oven at 70°C for 72 h. Chloride was determined from nitric-acetic acid extracts by amperometric

titration. The concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, and total-S were determined from nitric acid digestions of the dried, ground plant material by ICPOES. There was insufficient plant material to analyze samples from the EC 24 dS m^{-1} treatment in the second harvest.

Data Analysis

Means per subplot of the cumulative emergence on successive days after seeding were analyzed in a manner described by the Gompertz function using the equation specified by Steppuhn and Raney (2005) and Steppuhn et al. (2012) as follows:

$$E = E_{max} \exp[-b \exp(-kt)] \quad [3]$$

where t is time from seeding (day), E is the percentage of cumulative emergence at any specific salinity level at time t (%), E_{max} is asymptotic maximum of E taken as a maximum cumulative emergence at each specified salinity level in percentage attained with each cultivar (%), b is empirical Gompertz shape parameter, and k is Gompertz time constant (d^{-1}).

In addition to these parameters, Gompertz function-derived indices were calculated:

R_i = rate of the plant emergence that occurs at the inflection point (i) of the Gompertz function (% per day);

t_{max} = time since seeding to reach 99% of the asymptotic maximum emergence, 0.99 E_{max} (day);

E_i = cumulative emergence by time t_i, at the Gompertz inflection point (%).

According to Eq. [3], these indices are defined as:

$$R_i = (k\ E_{max}) / e(\%\ d^{-1}) \quad [4]$$

$$t_{max} = [4.6 + \ln(b)] / k(d) \quad [5]$$

$$E_i = E_{max} / e(\%) \quad [6]$$

The relative emergence (%) with respect to the control was calculated at Day 21, considered as the end of the period in which the emerged plants stabilized as:

$$\text{Relative Emergence (\%)} = (E \text{ at any specific EC level} / E \text{ at EC 3.1 dS m}^{-1})100 \quad [7]$$

Cumulative emergence data for cultivar and EC were analyzed by regression applying Eq. [3]. Thus, we obtained the Gompertz parameters and indices that were analyzed by two-way ANOVA. In addition, the E_{max} was analyzed by one-way ANOVA within each salinity level, comparing among cultivars. The mean differences were determined by Fisher's LSD test at 0.05 probability. Analyses were conducted with InfoStat program (Di Rienzo et al., 2012). The initial and final absolute density for each cultivar at the same salinity level were analyzed by t test.

The data for relative emergence, plant density, biomass per harvest, absolute and relative accumulated biomass, and mineral composition per harvest were analyzed using a split-plot procedure, with the following statistical model:

$$Yijk = \mu + Sj + R(S)j(i) + Ck + SCjk + \varepsilon ijk$$

where R, S, and C represent the repetitions (i = 1,…4), salinity level (j = 1,… 6: from 3.1 to 30 dS m^{-1} to analyze relative emergence and j = 1,…5: from 3.1 to 24 dS m^{-1} to analyze the other dependent variables), and cultivars (k = 1,...4), respectively. All effects were considered as fixed effects. Thus, $Yijk$ is the response to repetition i in Sj and Ck, μ is the overall mean, and ε ijk represents the random error. The significance in the split-plot design was calculated by deriving the mean squares in the analysis of variance with a completely randomized design. The significance of the main plot (S) was tested by $S > R$ as an experimental error of the whole plot and the mean square error was used to test significance of the subplot (C) and the interaction $S \times C$, respectively. The mean differences were determined by Fisher's LSD test at 0.05 probability.

The calculated shoot K/Na, Ca/Mg, and $Na/(Ca/2)^{0.5}$ ratios were analyzed in the same way as described above for the other variables.

RESULTS

Emergence and Relative Emergence

Differences in the timing of emergence were related to the levels of salinity (Fig. 1). At Day 4 after seeding, there was 69% emergence at EC_{iw} 3.1 dS m^{-1}, while at 7.2 dS m^{-1} the emergence was 25% for all cultivars except for SW 9720, which had 85% emergence at both salinity levels. One week after seeding, plants from all cultivars had emerged from treatments of EC_{iw} 3.1 to EC_{iw} 18.4 dS m^{-1}. At this time, the EC_{iw} 24 dS m^{-1} treatment plants of Salado and SW 9720 had also emerged, but plants of SW 9215 and SW 8421S emerged 1 wk after that. No emergence was observed at EC_{iw} 30 dS m^{-1} from any cultivar, with the exception of a few plants in Salado after 2 wk, but they subsequently died. Significant differences were observed in the parameters and indices calculated from Eq. [1–6] among salinity levels ($P > 0.05$, Table 2). The E_{max} decreased significantly with increasing salinity above EC_{iw} 7.2 dS m^{-1} ($P < 0.001$). Not until EC_{iw} 18.4 dS m^{-1} did the mean E_{max} drop below 50%, while at 24 dS m^{-1} it dropped drastically to 13%. There were no significant differences in the Gompertz time constant (k) between the EC_{iw} 3.1 dS m^{-1} and the other EC levels except at 30 dS m^{-1} ($P < 0.01$). The values of R_i were variable for each cultivar at each EC_{iw} level. There were no significant differences in R_i between EC 3.1 dS m^{-1} and 12.7 dS m^{-1}, but significant differences were found between 3.1 dS m^{-1} and the other

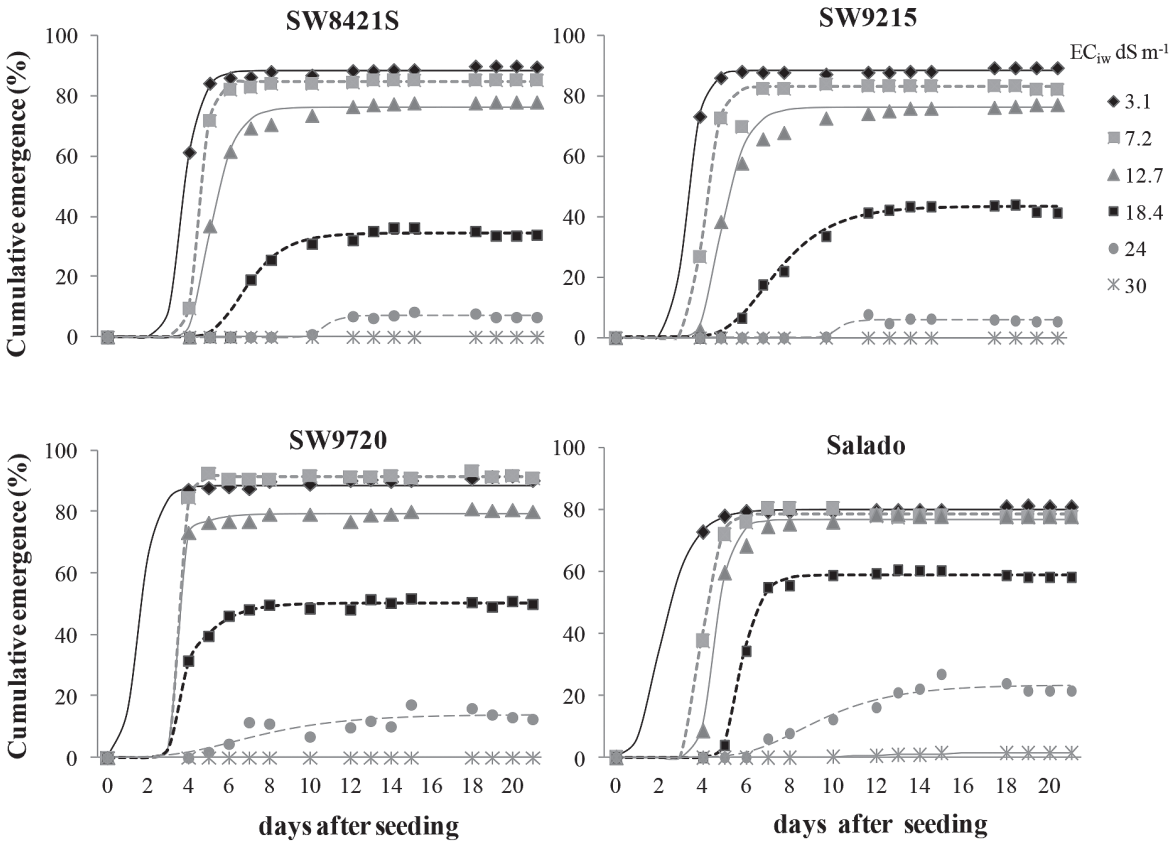

Figure 1. Average cumulative emergence by alfalfa cultivar evaluated at different electrical conductivity values of the irrigation water (EC_{iw}). The data are plotted as fitted curves as a function of days with observed means represented by points.

EC levels ($P < 0.001$). In both k and R_i, the highest values occurred at EC 7.2 dS m^{-1}, then the values tended to subsequently decrease with increasing salinity. The t_{max} (time required to reach 99% of the E_{max}) increased from 5 d at EC_{iw} 3.1 dS m^{-1} to 16 d at 24 dS m^{-1} ($P < 0.05$). However, from EC_{iw} 3.1 to 18.4 dS m^{-1} the t_{max} did not differ statistically. In addition, the E_{max} tended to decrease as salinity increased; the *Ei* had a similar trend ($P < 0.001$).

The relative emergence calculated with respect to the EC_{iw} 3.1 dS was influenced by salinity ($P < 0.001$), cultivar ($P < 0.05$), and the salinity × cultivar interaction ($P < 0.05$; Fig. 2). Besides the overall reduction in the relative emergence when the EC_{iw} exceeded 12.7 dS m^{-1}, significant differences among cultivars were observed at EC_{iw} 18.4 and 24 dS m^{-1}. At EC_{iw} 18.4 dS m^{-1}, Salado showed a significantly greater relative emergence than the other cultivars, and there were no significant differences between SW 9720 and SW 9215. At EC_{iw} 24 dS m^{-1}, Salado also showed the greatest relative emergence with significant differences with SW 9215 and SW 8421S but not SW 9720.

Initial and Final Plant Density

The plant density decreased with increasing salinity and time (Table 3). For all cultivars, the differences between initial and final density were statistically significant at EC_{iw} 3.1, 7.2, and 12.7 dS m^{-1} (*t* test, $P < 0.01$), except for the SW 9720 cultivar where the differences between both moments were not significant for any salinity level (*t* test, $P > 0.05$). Also, for all cultivars, the percentage of the density reduction tended to increase as salinity increased. At 3.1 dS m^{-1} (control) plant density was reduced by 10, 26, 30, and 37% for SW 9720, Salado, SW 8421S, and SW 9215, respectively. If we assume that these reductions were not affected by salinity but rather primarily from intracultivar competition or crowding effects, the differences between these reductions and the reductions at each EC_{iw} level give estimates of the salinity effects. From EC_{iw} 7.2 to 18.4 dS m^{-1}, the average reduction in plant density due to salinity ranged from approximately 5% for SW 8421, 5% for SW 9215, 10% for SW9720, and 15% for Salado; while at EC_{iw} 24 dS m^{-1}, the reduction in plant density increased to 20, 19, 29, and 17%, respectively. Assuming that the reduction in the density was linear throughout time (between Day 21 and 300), the effect of salinity is less than the crowding effect, except for SW9720 cultivar.

Biomass per Harvest

The shoot biomass per harvest was significantly influenced by increasing salinity, except in the seventh harvest (Table 3). Significant differences among cultivars were found in three of the six harvests and the salinity × cultivar interaction was significant only in the first and second

Table 2. Mean Gompertz parameters and indices for each alfalfa cultivar evaluated at different electrical conductivity values of the irrigation water (EC_{iw}).

Cultivar	Salinity levels (EC_{iw} dS m^{-1}) 3.1	7.2	12.7	18.4	24	30
	%					
Maximum emergence (E_{max})						
SW8421S	88.5	84.7	76.3	34.4	7.0	0
SW9215	88.3	83.1	74.8	43.2	6.0	0
SW9720	88.6	91.2	79.2	50.2	13.8	0
Salado	79.9	78.2	76.7	58.9	23.3	1.5
Mean†	86.3a***	84.3ab	76.8b	46.8c	12.5d	0.4e
	d^{-1}					
Gompertz time constant						
SW8421S	1.8	2.5	1.3	0.8	1.8	0
SW9215	2.1	2.1	1.1	0.6	2.3	0
SW9720	1.7	3.7	1.0	0.7	0.3	0
Salado	1.1	2.2	1.9	1.7	0.4	0.4
Mean	1.7ab**	2.5a	1.3b	0.9bc	1.2b	0.1c
	% d^{-1}					
Rate of emergence at the inflection point						
SW8421S	59.3	79.5	37.3	10.3	4.6	0
SW9215	67.2	64.2	30.3	8.9	5.0	0
SW9720	57.0	125.0	29.7	14.2	1.7	0
Salado	32.3	63.3	55.0	36.2	3.5	0.3
Mean	54.0b***	78.4a	38.1bc	17.4cd	3.7d	0.1d
	d					
Time to reach 99% E_{max}						
SW8421S	6.0	6.1	8.3	12.1	12.9	0
SW9215	5.4	6.2	9.0	15.3	12.4	0
SW9720	3.9	4.6	6.1	9.0	20.2	0
Salado	6.0	5.9	6.7	8.4	19.7	22.0
Mean	5.3b*	5.9b	7.5b	11.2b	16.3a	5.5b
	%					
Cumulative emergence at the inflection point						
SW8421S	32.6	31.2	28.1	12.7	2.6	0
SW9215	32.5	30.6	27.5	15.9	2.2	0
SW9720	32.6	33.6	29.1	18.5	5.1	0
Salado	29.4	28.6	28.2	21.7	8.6	0.6
Mean	31.8a***	31.0ab	28.2b	17.2c	4.6d	0.1e

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Means followed by the same letters among salinity treatments are not significantly different according to LSD (0.05). Means by cultivar based on four replications.

harvest. In both harvests, significant differences among cultivars were observed at 3.1 and 18.4 dS m^{-1} and also at 12.7 dS m^{-1} in the second harvest. At EC_{iw} 24 dS m^{-1}, there was insufficient biomass to harvest, as was also the case for the third and fifth harvest. In the first harvest at 3.1 dS m^{-1}, SW 8421S had the largest biomass and it was significantly different from Salado, while at 18.4 dS m^{-1}, Salado had significantly higher biomass than SW9215 and SW8421S ($P < 0.01$). A similar trend was observed in the second harvest, in which at 3.1 dS m^{-1}, SW8421S maintained the highest biomass. At EC_{iw} 12.7 dS m^{-1}, the

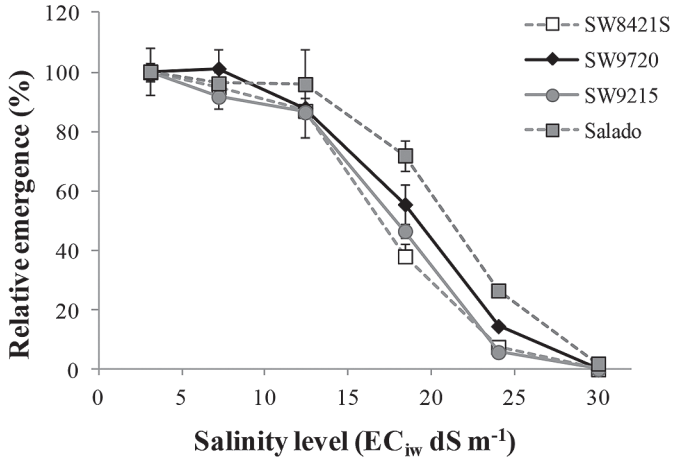

Figure 2. Relative emergence of four alfalfa cultivars at different electrical conductivity values of the irrigation water (EC_{iw}). Emergence observed at Day 21 after seeding in outdoor sand tanks in Riverside California (July 2011). Mean ± SE by cultivar based on four replications.

biomass of SW 9720 was significantly lower than that of the other three ($P < 0.05$). At EC_{iw} 18.4 dS m^{-1}, Salado had the largest biomass and SW 9720 had the lowest ($P < 0.01$). From the third to fifth harvest, there were significant differences in the biomass above EC_{iw} 12.7 dS m^{-1}, where the biomass decreased for the subsequent levels. At the sixth harvest, we found no significant differences in biomass up to EC_{iw} 18.4 dS m^{-1}, but there were no significant differences between EC_{iw} 3.1 and 24 dS m^{-1}. No significant differences between EC_{iw} levels were observed in the seventh harvest despite the lower biomass observed at 24 dS m^{-1} for SW cultivars.

The biomass per area harvested at each moment is a combined response of plants m^{-2} and g $plant^{-1}$ because the plant density was not constant across salinity levels at the beginning of the growth period. In addition, the density also decreased with time (crowding plus salinity effects). We calculated the biomass per plant for first (using initial plant density) and seventh harvest (using its density). In the first harvest the DW per plant decreased as salinity increased (Table 3). This means that the individual plant growth was affected by salinity. While in the seventh harvest, the DW per plant increased with increasing salinity, particularly at EC_{iw} 24 dS m^{-1} (the highest weight per plant). We assumed that the increase in g per plant was due to reduced plant competition and the ability of the best survivor plants to grow and develop larger crowns with time.

Absolute and Relative Accumulated Biomass

When the biomasses from all harvests were summed, absolute accumulated biomass was significantly influenced by both salinity and by the impact of plant density. Positive correlation between plant density and accumulated biomass per area was found, with higher correlation with the initial ($r = 0.81$; $P < 0.001$) than final plant density ($r =$

Table 3. Initial and final plant density, reduction in plant density, biomass per harvest, and dry weight per plant in the first and seventh harvest, by alfalfa cultivar and salinity levels.

	Plant density			Dry weight by harvest								
EC_{iw}	Initial	Final	Red.‡	1st	2nd	3rd	4th	5th	6th	7th	1st	7th
dS m^{-1}	— plant m^{-2} —		%	— g m^{-2} —							— g $plant^{-1}$ —	
SW8421S												
3.1	301	210	30	558	475	440	363	428	277	399	1.85	1.90
7.2	287	190	34	309	307	347	325	354	333	402	1.08	2.11
12.7	262	162	38	194	247	290	291	306	326	390	0.74	2.41
18.4	114	76	33	15	59	139	178	213	261	389	0.14	5.12
24	22	11	50	§	§	§	106	§	220	279		25.3
SW9215												
3.1	300	188	37	495	333	333	277	316	280	380	1.65	2.02
7.2	276	155	44	362	325	349	315	382	330	389	1.31	2.51
12.7	259	153	41	212	245	294	325	395	382	400	0.82	2.61
18.4	139	84	40	23	71	166	197	250	300	410	0.17	4.88
24	18	8	56	§	§	§	54	§	99	290		36.26
SW9720												
3.1	305	274	10	¶	402	365	262	298	245	375		1.37
7.2	308	244	21		264	300	211	295	249	376		1.54
12.7	268	210	22		159	273	238	310	326	389		1.85
18.4	169	139	18		25	153	154	185	234	403		2.90
24	44	27	39		§	§	34	§	139	331		12.3
Salado												
3.1	271	200	26	439	321	317	280	362	311	390	1.62	1.95
7.2	260	133	49	331	320	342	334	396	360	394	1.27	2.96
12.7	261	159	39	185	241	302	298	348	365	393	0.71	2.47
18.4	195	127	35	39	115	214	271	328	429	406	0.20	3.19
24	72	41	43	§	§	§	162	§	356	391		9.54
				ANOVA								
Salinity (S)	***	***		***	***	***	***	***	***	NS		
Cultivar (C)	*	**		NS	*	NS	**	NS	**	NS		
S × C	**	**		*	**	NS	NS	NS	NS	NS		
Lsd (0.05) SxC	34	43		92	65	75	95	116	159	95		

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† EC_{iw}, electrical conductivity values of the irrigation water.

‡ Red., percentage of reduction in the plant density between initial (Day 21) and final (Day 300).

§ No growth.

¶ Missing data of first harvest.

0.69; $P < 0.001$). Absolute and relative accumulated biomass was significantly influenced by salinity ($P < 0.001$) and cultivar ($P < 0.01$) but not by the salinity × cultivar interaction ($P > 0.05$).

Absolute accumulated biomass for all cultivars averaged 2000 g m^{-2} at EC_{iw} 3.1 dS m^{-1} and there were no significant differences in average biomass with increasing salinity until EC_{iw} > 12.7 dS m^{-1}, when the biomass significantly decreased (Fig. 3, $P < 0.001$). The biomass was significantly reduced to 68% at 18.4 dS m^{-1}, while at 24 dS m^{-1} it was further reduced to 30%. When the accumulated biomass is separated by year, considering three harvests for 2011 (second to fourth harvest) and the other three for 2012, we observed a tendency of biomass increasing under moderate to severe salinity (from 12.7 dS m^{-1} to 24.dS m^{-1}; Fig. 3). This observation may suggest increasing salt tolerance with time for all cultivars. Across salinity levels, Salado and SW 8421S cultivars had the highest accumulated biomass and SW 9720 the lowest. The accumulated biomass of SW 9215 was intermediate and did not significantly differ from the others. The relative biomass of Salado and SW 9215 were significantly higher than SW 9720 and SW 8421S (Fig. 4).

Mineral Ion Concentrations and Ion Ratios

Shoot Na^+ concentrations increased significantly as Na^+ increased in the external solution in both harvests ($P < 0.0001$, Fig. 5). In the second harvest, shoot Na^+ concentrations in SW 9720 and Salado were higher and lower, respectively, than in the other two cultivars ($P < 0.0001$).

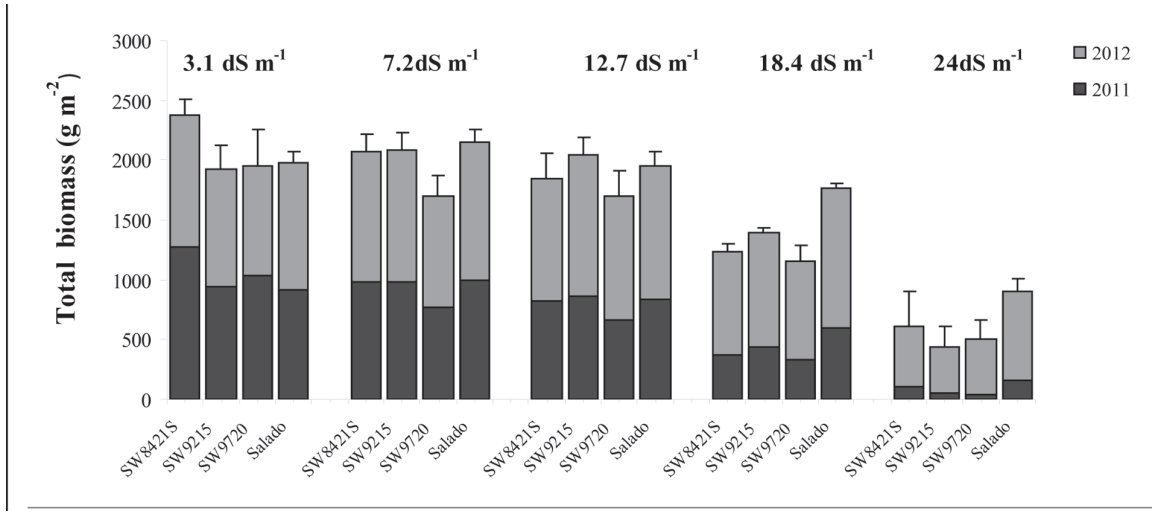

Figure 3. Total absolute biomass (dry weight) from six harvests during 2011 to 2012 of four alfalfa cultivars at different electrical conductivity values of the irrigation water. Mean + SE by cultivar based on four replications.

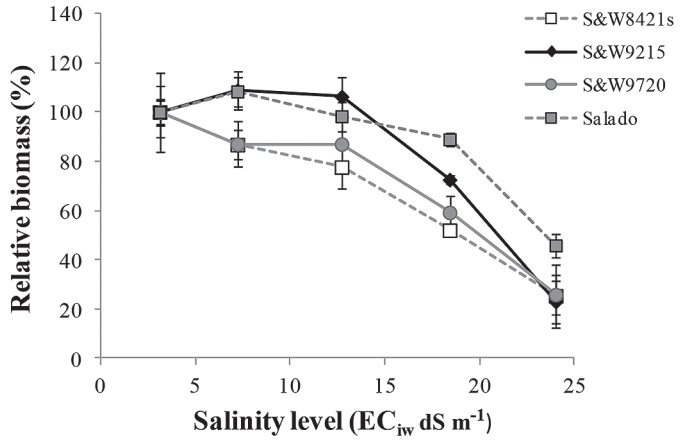

Figure 4. Relative biomass of four alfalfa cultivars at different electrical conductivity values of the irrigation water (EC_{iw}). Mean ± SE by cultivar based on four replications.

Salado accumulated up to 40% less Na^+ at EC_{iw} 18.4 dS m^{-1} compared with the average of the other three cultivars ($P < 0.01$). In the seventh harvest, the shoot Na^+ increased up to EC_{iw} 12.7 dS m^{-1} and remained constant after that ($P < 0.0001$). Also, Salado showed significantly different (lowest) Na^+ accumulation up to EC_{iw} 18 dS m^{-1} ($P < 0.01$). Shoot K^+ concentrations decreased with increasing salinity in both harvests and all cultivars ($P < 0.0001$, Fig. 5). In the second harvest, the K^+ concentrations decreased from 1200 to 900 mmol kg^{-1} DW with significant differences among salinity levels. In the seventh harvest, K^+ also decreased with increasing EC_{iw} with a sharp drop in K^+ between EC_{iw} 3.1 dS m^{-1} and the other EC_{iw} levels. Significant differences among cultivars were found only in the second harvest, in which shoot K^+ concentrations in SW 9720 were the lowest at all salinity levels ($P < 0.0001$). In the second harvest, the total S concentrations significantly increased as this ion increased in the external solution ($P < 0.0001$, Fig. 5). Higher total S concentrations in SW 9720 were detected compared with other cultivars ($P < 0.001$). In the seventh harvest, the total S concentrations tended to increase but no significant differences were detected among EC_{iw} levels or cultivars ($P < 0.05$). The shoot Cl^- concentrations increased with respect to the control but remained constant from EC_{iw} 7.2 to 18.4 dS m^{-1} and SW 9720 had the lowest concentration at 3.1 dS m^{-1} and the highest at 18 dS m^{-1} ($P < 0.01$, Fig. 5). In the seventh harvest, the shoot Cl^- concentrations increased from 300 to 400 mmol kg^{-1} DW but the differences among salinity levels and cultivars were not significant ($P < 0.05$). In both harvests, the shoot Ca^{2+} concentrations did not decrease until the salinity exceeded 12.7 dS m^{-1} ($P < 0.05$), while that the shoot Mg^{2+} concentrations did not increase until the salinity exceeded 7.2 dS m^{-1} ($P < 0.0001$, Fig. 5). In the second harvest, the shoot Ca^{2+} and shoot Mg^{2+}concentrations in SW 9720 were higher from 3.1 to 12.7 dS m^{-1} when compared with the other cultivars ($P < 0.0001$). In the seventh harvest, Salado had the highest Ca^{2+} concentrations ($P < 0.05$). In both harvests, the shoot P concentrations remained constant up to 12.7 dS m^{-1} (average was 94 mmol kg^{-1} DW) and significantly increased until 120 and 140 mmol kg^{-1} DW at 18.4 and 24 dS m^{-1} in the second and the seventh harvest, respectively ($P < 0.05$, data not shown).

In the second harvest, the K/Na ratio in the shoots significantly decreased ($P < 0.01$) as salinity increased from EC_{iw} 3.1 to 12.4 dS m^{-1} (9.6 to 4.02, respectively). In this harvest, the K/Na ratios in Salado were consistently the highest, whereas the ratios in SW 9720 were the lowest ($P < 0.0001$). In the seventh harvest, the ratios significantly decreased from 3.1 dS m^{-1} and there were no differences among other levels (5.4, 2.8, 2.1, 1.7, and 1.8, respectively). In both harvests, the shoot Ca/Mg ratios decreased significantly when the EC_{iw} was >7.2 dS m^{-1} ($P < 0.01$). In the second harvest the ratios decreased from 3.3 to 1.5 and in the seventh harvest the ratio decreased

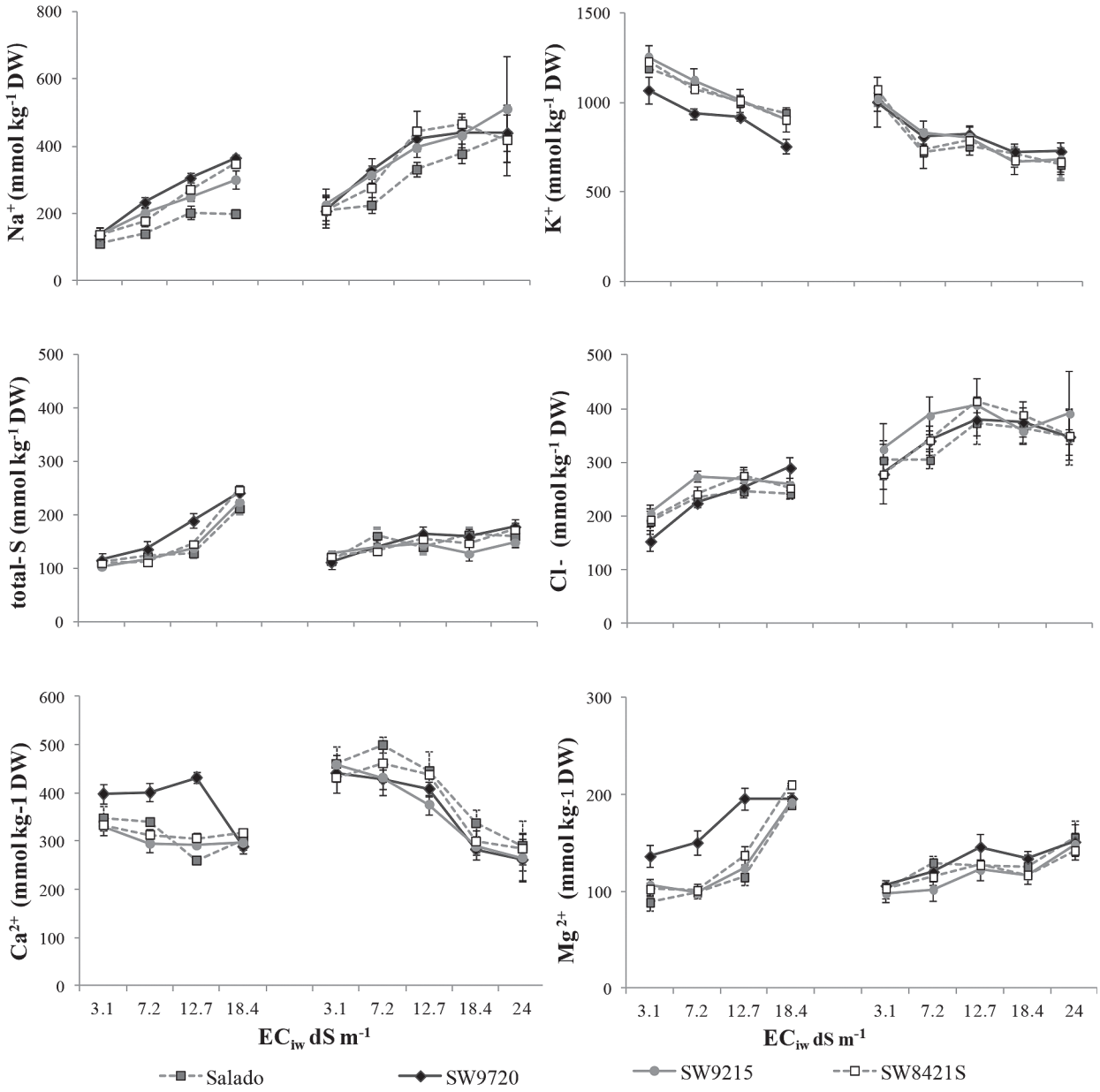

Figure 5. Shoot Na^+, total-S, Ca^{2+}, K^+, Cl^-, and Mg^{2+} concentrations of four cultivars at different electrical conductivity values of the irrigation water (EC_{iw}). For each ion, the data on the left represents the second harvest and the data on the right represents seventh harvest. For each harvest, the mean + SE by cultivar is based on four replications. DW, dry weight.

from 4.5 to 1.9 across all salinity levels, respectively. In both harvests, the ratios in Salado were the highest and in SW 9720 were the lowest ($P < 0.01$). The $Na/(Ca/2)^{0.5}$ ratios increased significantly across of the salinity levels from 9.9 to 24.8 in the second harvest and from 14.5 to 42.5 in the seventh harvest ($P < 0.01$). In both harvests, the ratios in Salado were the lowest ($P < 0.01$).

The ion concentrations and accumulated biomass across all cultivars and salinity levels were significantly correlated ($P < 0.0001$). The highest negative correlations occurred with shoot S, shoot Na^+, and shoot Mg^{2+} and the highest positive correlations occurred with shoot K^+ and shoot Ca^{2+} (only in the seventh harvest). No significant correlations were found with shoot Cl^- at any harvest ($P > 0.05$). The coefficients (r) were greater with the ion concentrations of the second harvest than seventh harvest (shoot S $r = -0.69$, shoot Na^+ $r = -0.62$, shoot Mg^{2+} $r = -0.71$, shoot K^+ $r = 0.56$ in second harvest and shoot Ca^{2+} $r = 0.73$ in seventh harvest).

DISCUSSION

Salinity causes a general delay in alfalfa emergence (Ashraf and Foolad, 2005; Assadian and Miyamoto, 1987; Steppuhn et al., 2012) and our results confirm that. Rapid emergence is favorable because it reduces the time for salts to accumulate in plant tissue (Assadian and Miyamoto, 1987). The mean time to reach the E_{max} (t_{max}) tended to increase by 0.6, 1.6, 3.7, and 5.1 d for the respective EC_{iw} increments from 3.1 to 7.2, 7.2 to 12.7, 12.7 to 18.4, and

18.4 to 24 dS m^{-1}, although only the 18.4 to 24 dS m^{-1} increase in t_{max} was significantly different from the others. An approximately similar delay between salinity levels was found by Steppuhn et al. (2012) in tolerant alfalfa cultivars (dormant type) wherein the time to reach E_{max} increased from 0.7 to 2.18 d for the respective increments from 1.5 to 8.0 and 8.0 to 15.6 dS m^{-1}. However, they reported greater means of E_{max} percentages (95.8, 97.3, and 89.9%), and greater t_{max} (6.96, 7.68, and 9.86 d), respectively, at 1.5, 8.0, and 15.6 dS m^{-1} as compared with our results (Table 2). From this comparison, it would seem that dormant types emerge better than nondormant types, both with relatively high tolerance to salinity, contrary to expectations that nondormant types would emerge better. These data are probably explained by the higher average of daily maximum temperature that occurred under our outdoor conditions during the emergence period (32°C, June–July) as compared with their greenhouse condition (daily temperature maximum 20°C ± 2°). If the emergence is considered an extension of germination, then we can compare emergence and germination (%). These temperature differences could have influenced the germination and thus the comparatively lesser emergence that we observed (compared with the data of Steppuhn et al. [2012]). As was earlier reported by Ungar (1967), the temperature affected the seed germination when the salinity increased. Ungar (1967) found that alfalfa has a small variation in germination in the temperature range of 13 and 21°C, while at 32°C the germination decreased 20% when the salinity increased from 0 to 0.5% NaCl and no germination was recorded when the salinity was increased to 1% (171 mM). Scasta et al. (2012) indicated that the germination mean (evaluated after 7 d in petri dishes at 25°C) from 16 cultivars was 78% at 256 mM NaCl (approximate EC of 25 dS m^{-1}) and at 342 mM the mean germination decreased to 31%. Among the 16 cultivars, Salado had the most germination (86%). These data suggest that one would expect higher germination values than emergence values and that, comparatively, Salado plants germinated and emerged better under saline conditions than the plants of other cultivars.

Part of the decrease in plant density that we observed is explainable as a typical behavior of alfalfa during the first year of production due to intraspecific competition (Stout, 1998). A previous study with alfalfa (but without salinity stress) showed that after 1 yr following sowing, the reductions in plant densities were greater in those treatments with greater initial plant densities (Mueller et al., 2008). In agreement with that, our results showed that the density decreased over time and more plants died when the initial density was greater, which in our case occurred at EC_{iw} 3.1, 7.2, and 12.7 dS m^{-1} in comparison to EC_{iw} 18.4 and 24 dS m^{-1}. At the same time, the greatest survival occurred at 3.1 dS m^{-1} along with the greatest plant density among the salinity treatments. This contrasted with the opposite results recorded for alfalfa growing in nonsaline environments, where the percentage of survival decreased with the greater initial plant densities during the first (Mueller et al., 2008) and second growing seasons (Stout, 1998). This suggests that the survival decreased as salinity increased due to competition and salinity effects interacting together.

Crop production under salinity can be calculated from the plant density (plant m^{-2}) multiplied by the biomass per plant (g $plant^{-1}$). Both density and biomass depend on the crop tolerance at emergence and plant growth stages (Katerji et al., 2012). Alfalfa plants can compensate for the effect of low density over time by an increase in the number of shoots (and width) per plant (Askarian et al., 1995; Bagavathiannan et al., 2011). It is probable that the effect of the lower initial density at high salinity could have, over time, been compensated by a larger number of shoots, reducing the impact of lower density on biomass per area mainly at EC_{iw} 18.4 and 24 dS m^{-1}. We consider that this compensation could also have happened at 7.2 and 12.7 dS m^{-1} but to a lesser extent. The compensation for density might partially explain the absence of reduction in the biomass per area and the high tolerance in the last harvest evaluated.

In perennial crops such as alfalfa, where plants can grow all year if the environmental conditions permit, it is reasonable that climate influences plant response to salinity. In this experiment, the cultivars studied are nondormant and the harvests were done during almost 1 yr across all seasons with different cutting time intervals according to growth (first harvest: middle summer, second: late summer, third and fourth: fall, fifth and sixth: winter, and seventharvest: early spring).

The seasonal environmental conditions during the experiment are presented in Table 4. In general, it is known that plant salt tolerance decreases under hot and dry conditions where the evaporative demand is high (Bernstein, 1974). In this experiment, the quantity and frequency of the irrigation was constant during all experimental periods and greatly exceeded the daily evapotranspiration (ET) for all seasons. The ET in the first and second harvest (summer) was 2.6 times that in the fifth and sixth harvest (winter). We consider that the sharp decline in biomass (g m^{-2}) in response to increasing salinity in the first and second harvest was aggravated by factors such as high temperature and high water demand. The environmental conditions of the seventh harvest as compared with the first and second harvest were cooler temperatures (13.8 and 23.3–25.2°C, respectively), lower ET, and comparatively lower solar radiation.

An additional factor potentially impacting biomass is the seeding time, which in this experiment was not the optimal time for alfalfa. Planting in early summer (23 June) probably increased the adverse response to salinity due to a lesser development of the root system under the warmer conditions. Root growth was found to be less affected than

Table 4. Summary of environmental conditions during the experimental period.†

Parameter	Harvest						
	1st	2nd	3rd	4th	5th	6th	7th
Harvest date (2011–2012)	22 Aug.‡	15 Sep.	18 Oct.	15 Nov.	11 Jan.	22 Feb.	17 Apr.
Growth period, d	60	24	33	28	57	42	54
Variable							
Avg. ET_o mm.d^{-1}	6.4	5.7	3.9	2.6	2.3	2.3	4.1
Total precipitation, mm	7.3	0	9.5	25.6	24.9	16.2	48.2
Avg. vapor pressure, kPa	1.4	1.4	1.3	0.9	0.6	0.6	0.7
Max. air temperature, °C	32.0	34.1	29.2	23.5	20.4	19.6	21.2
Min. air temperature, °C	16.5	17.8	14.1	9.7	6.6	6.7	7.4
Avg. air temperature, °C	23.3	25.2	20.6	15.8	13.0	12.8	13.8
Avg. relative humidity, %	50.0	43.4	52.6	49.9	40.0	43.1	48.6
Wind run, km	158	138	125	122	149	154	161
Net radiation avg.,Wm^{-2}	320	271	208	154	128	146	236
Min. photoperiod, light hours	13:14	12:25	11:17	10:25	9:53	10:05	11:12
Max. photoperiod, light hours	14:24	13:12	12:23	11: 15	10:15	11:10	13:07

† Data from California Irrigation Management Information System. Weather station 44 = University of California, Riverside (CIMIS, 2012).

‡ From 23 June.

shoot growth with increasing salinity (Serraj and Drevon, 1998). It suggests that over time, the roots developed greater biomass relative to shoots (that were harvested), enabling better extraction of water against the osmotic gradient at the same EC level. This would result in greater salt tolerance for mature alfalfa plants (as observed in seventh harvest).

Winter conditions seemingly decreased the plant growth in the controls (mainly at harvest 6), but the response to salinity up to EC 12.7 dS m^{-1} continued without significant differences among treatments. More biomass was produced in the winter at EC 18.4 dS m^{-1} compared with the previous harvests. The low temperature and low radiation affected the growth rate during the long interval between cuts. In early spring, better conditions for biomass production are evidenced by a large increase in radiation, longer daylength, and a slight increase in temperature, probably enabling the plants to better endure salinity stress.

Increasing biomass in successive harvests with EC_{iw} treatments of 8 to 24 dS m^{-1} was also observed in a dormant-type cultivar evaluated under greenhouse conditions where plants were thinned to equal plant density for the various EC treatments (Steppuhn et al., 2009). Thus, it is likely that not all of the increased tolerance evidenced in our results for the later harvests is attributable to climatic conditions.

Our relative biomass results are similar to those reported under controlled conditions, with saline solution applied from the beginning of the growth stage (Rogers et al., 1998). These authors reported that at 17 dS m^{-1} of EC_{iw} dominated by Na_2SO_4, the biomass of 16 lines of nondormant alfalfa was reduced to 66% in comparison with the biomass produced at EC 2.1 dS m^{-1}.

The concentration of the shoot ions for both analyzed harvests (second and seventh) and all salinity treatments indicated that the plants were growing with adequate levels of nutrients (data in Fig. 5 for K^+, Ca^{2+}, total S, Mg^{2+}; data P not shown). Comparisons between the ion concentrations (in % of DW) of the nutrients in the top 15 cm of alfalfa at the first flowering were made, with those concentrations considered nutritionally sufficient (Undersander et al., 2011). The shoot K^+ concentrations ranged from 4.64 to 2.65%; all were in the range or higher than the sufficient level for alfalfa (2.25–3.4%). The higher concentrations indicate that a luxury consumption occurred since alfalfa may take up more K^+ that required (Marschner, 1986). Shoot P, shoot Ca^{2+}, and shoot Mg^{2+} concentrations were all within the sufficient range for alfalfa (0.25–0.45%, 0.70–2.5%, and 0.25–0.70%, respectively). The concentrations of total S ranged from 0.35 to 0.74% and only the concentrations at 18 and 24 dS m^{-1} were above the sufficient range cited for alfalfa (0.25 to 0.50%). The shoot N concentrations did not indicate deficiency of this nutrient under any salinity level (the average ranged between 3.41 and 5.05% for both harvests and all salinity treatments (data not shown) as the range cited as sufficient for alfalfa is 2.5 to 4%.

The cultivars evaluated in our study were able to restrict Na^+ transport in both harvests analyzed for ion uptake, as the concentrations were below those cited by Rogers et al. (1998), with similar amounts of Na^+ in solution. The concentrations of shoot Na^+ were higher than 600 mmol kg^{-1} DW at EC 17.2 dS m^{-1} (Rogers et al., 1998). Our results obtained from Salado and SW 9720 cultivars are in agreement with those reported previously under two salinity levels (EC_{iw} 15 and 25 dS m^{-1}), which also did not exceed that value (Grieve et al., 2004). Alfalfa ion analyses of successive harvests have proven that the ability to restrict Na^+ uptake decreases with time under salinity treatments (Grieve et al., 2004; Isla and Aragüés, 2009). Although our results showed that the plants accumulated more Na^+ in the shoots, no significant reduction

in biomass was observed in the seventh harvest. At this moment, the shoot Na^+ concentrations tended to plateau around EC_{iw} 12.4 dS m^{-1} (approximately at 100 $mmol_c$ L^{-1} in the irrigation water), in contrast with the second harvest. Overall the data suggest that the current salt tolerance cultivars have been improved for this Na^+ trait.

The K^+ concentrations are reduced in the presence of Na^+ at higher concentrations because Na^+ competes for binding sites, inhibiting important metabolic pathways that depend on K^+ (Maathuis and Amtmann, 1999; Tester and Davenport, 2003). Concentrated solutions of Na^+ cause disruptions of the root membrane integrity and changes ion selectivity in the root system because K^+ is replaced by Na^+ (Marschner, 1986). According to this consideration, changes in the selectivity of K^+ are related with the decreasing shoot K^+ and decreasing K/Na ratio in the shoots when salinity increases. This decreasing K^+ with increasing salinity in alfalfa has also been reported in other studies (Ashraf et al., 1986; Grieve et al., 2004; Mohammadi et al., 2008; Rogers et al., 1998). Among the test cultivars, Salado was particularly efficient in maintaining the lowest shoot Na^+ concentrations, especially up to EC_{iw} 18.4 dS m^{-1} and consequently the highest K/Na ratio among the cultivars. This attribute probably played an important role in enabling Salado plants to maintain biomass production at EC_{iw} 18.4 and 24 dS m^{-1}.

As a dominant anion used in our experiment, SO_4^{2-} increased in the solution with increasing salinity. In the second harvest, the total S concentrations in the shoots increased from 110 to 232 mmol kg^{-1} DW with increasing salinity, while at the seventh harvest the increase was much less. Previous studies have demonstrated that S accumulation in alfalfa could be greater than its nutritional requirements when the crop is exposed to large concentrations of S (Martin and Walker, 1966). In agreement with these authors (Mayland and Robbins, 1994), our results suggest that the plants have taken more S than needed because the ion was available in solution. Suyama et al. (2007) reported a similar high range of shoot S accumulation (158–218 mmol kg^{-1} DW) with no trend in shoot S with increasing salinity in the periods March to August and September to December, similar to our results from the seventh and second harvest, respectively.

Shoot Cl^- concentrations remained relatively constant across the salinity levels, although concentrations tended to increase between both harvests and with increasing salinity. Similar results were reported by Grieve et al. (2004), in which the shoot Cl^- concentrations also tended to increase with the harvest time until the seventh harvest. As with Na^+, the plants were able to accumulate more Cl^- without detrimental effects on the biomass and also tended to plateau around EC_{iw} 12.4 dS m^{-1} (approximately 63 mmol L^{-1} Cl^- in the irrigation water) compared with the second harvest. Even higher concentrations of Na^+ and Cl^- in the alfalfa shoots can be expected than observed in our results (Ashraf et al., 1986; Khorshidi et al., 2009; Mezni et al., 2012; Noble and Shannon, 1988;) when the alfalfa is grown exposed to NaCl solutions.

At high levels of salinity, the plant root membranes lose their ability to discriminate between Ca^{2+} and Na^+, resulting in plants with decreased plant Ca^{2+} concentrations (Ashraf, 2004; Grattan and Grieve, 1999; Suarez and Grieve, 1988). Our results showed that although the Ca^{2+} concentrations in solution varied only slightly among the salinity levels, the shoot Ca concentrations decreased when EC_{iw} was above 12.7 dS m^{-1} (especially in the seventh harvest). The $Na/(Ca/2)^{0.5}$ ratio in the solution increased as well as in the shoot with the increase in salinity. This ratio is useful for predicting Na–Ca relations in plants (Suarez and Grieve, 1988). A smaller decrease in Ca^{2+} with increasing salinity and smaller increase in Na^+ is related to the salt tolerance (Ashraf, 2004). This trend was showed by Salado, when compared with the other cultivars, consistent with its relative salt tolerance. Elevated soil solution Ca^{2+} can affect Mg^{2+} uptake because it competes for binding sites on the root plasma membrane (Marschner, 1986). The Ca/Mg ratio in our salinity treatments decreased and the ratio also decreased in shoots in both harvests with increasing salinity. Antagonistic effects between both ions have been reported in alfalfa shoots with increasing salinity (Grieve et al., 2004; Khorshidi et al., 2009).

Earlier studies (Noble and Shannon, 1988; Noble et al., 1984) have related salt tolerance of alfalfa with the capacity to limit Cl^- transport. In our results, there was no correlation between biomass and shoot Cl^- concentrations. Although the greatest correlations were found with total S and Na^+, the results with respect to total S were not useful because the shoots of all the cultivars revealed similar results, in contrast with the results of Rogers et al. (1998).

CONCLUSIONS

This study showed that when the salinity was present from planting time, it delayed and decreased the emergence, especially at EC_{iw} 18.4 and 24 dS m^{-1}. The total biomass production was adversely impacted by salinity, at least in part due to reduction in plant density with increasing salinity.

The plants representing each of the four test cultivars exhibited yield increases in successive harvests from first to seventh. The plants also exhibited changes in the salt tolerance through the harvest time, which is partially explained with changes in the environmental conditions.

The advantage showed by SW 9720 with rapid emergence and highest plant density did not turn into a benefit in terms of greater biomass or salt tolerance for mature plants in the period of time evaluated. The reductions in biomass production with increasing salinity are comparable to those in published experiments where salinity was imposed after seedling establishment. This result is also consistent with

our emergence response to salinity, as the emergence stage was not more sensitive than the subsequent growth stages.

The ability of Salado to maintain low shoot Na^+, which induced differences in the shoot ion concentrations and ratios, appears related to salt tolerance as determined by biomass production. We conclude that Salado was the most salt-tolerant cultivar as well as producing the greatest biomass, especially at high salinity levels (18.4 and 24 dS m^{-1}). The low emergence and slow growth rate at EC_{iw} 24 dS m^{-1} (EC_e 11.3 dS m^{-1}) causes this salinity level to have extremely severe impacts on production, despite use of tolerant cultivars. Our results suggest that saline waters resulting in EC_e values of up to 6 dS m^{-1} can be used throughout the total production cycle (planting to multiple harvests) without significant yield loss for the cultivars evaluated.

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