Leaf and Spikelet Primordia Initiation in Salt-Stressed Wheat

C. M. Grieve,* S. M. Lesch, E. V. Maas, and L. E. Francois

ABSTRACT

Salt stress is known to affect adversely shoot apex development in cereals. This study was conducted to determine rate and duration of leaf and spikelet primordia initiation as related to final leaf and spikelet numbers in salt-stressed wheat (*Triticum aestivum* L.). Early shoot development of two hard red spring wheat cultivars, Yecora Rojo and Anza, was studied in both greenhouse sand cultures and outdoor field lysimeters. In each case, two saline treatments were compared with a nonsaline control treatment. The sand cultures were irrigated with complete nutrient solutions to which NaCl and $CaCl_2$ (2:1 molar ratio) were added to achieve electrical conductivities (κ_{iw}) of 1.7, 12.2, and 15.1 dS m^{-1}. Lysimeters were irrigated with tap water salinized to κ_{iw} levels of 0.8, 11.4, and 17.1 dS m^{-1}. Timing of primordium initiation was expressed in accumulated thermal time measured in the soil (°C d_{soil}). A three-piece linear-spline model was developed to facilitate the statistical analysis and interpretation of primordium initiation. Parameters of the model corresponded to morphologically significant events. In both experimental locations, each genotype exhibited a similar response to increasing salinity. The rate of leaf primordium initiation decreased while the duration of this phase was unchanged. Salinity had no effect on the rate of spikelet primordium initiation of the genotypes, but the duration of this phase was shortened. As a result, the number of leaves on the main stem and the number of spikelets per spike were significantly reduced by salinity and the yield potential of both genotypes was severely limited.

WHEAT APICAL DEVELOPMENT is a continuous process that occurs in two distinct phases that are associated with specific developmental events (cf., Kirby, 1974). The first phase starts with imbibition and germination and continues until the total complement of main stem leaves is initiated. Initiation of the primordium destined to become the spike collar signals the onset of spikelet primordium initiation. Although the exact time of this transition may be determined only retrospectively, this event, rather than the formation of double ridges, marks the onset of the reproductive phase (Baker and Gallagher, 1983b). The transition from initiation of leaf primordium to spikelet primordium coincides with elongation of the apical dome and is characterized by an increase in initiation rate. Spikelet primordium initiation ceases with formation of the terminal spikelet. At this time the number of spikelets per spike, a critical yield determinant in wheat, has been established. Depending on the cultivar, spikelet primordia are initiated two to four times faster than leaf primordia (Kirby, 1974; Baker and Gallagher, 1983a; Delecolle et al., 1989).

Shoot organogenesis has been described quantitatively by regression of the number of primordia against accumulated thermal time in °C d (Kirby et al., 1987; Delecolle et al., 1989). This analysis is frequently based on air temperature measured 1.5 m above the soil surface. During leaf and spikelet primordia initiation, the site of temperature perception, the apical meristem, is located below or close to the soil surface and far removed from the site of air temperature measurement. Therefore, the temperature most closely correlated with that of the apex, at least prior to culm elongation, is undoubtedly that of the soil at a shallow depth (Hay and Wilson, 1982; Baker and Gallagher, 1983a). After jointing, development may be more closely correlated with air temperature than with soil temperature.

Although temperature is often the major environmental factor affecting apex development (Kirby, 1985), other variables may also influence primordium production. The two phases of primordium initiation may not be equally sensitive to the same environmental factor. For example, the rate of leaf initiation is not affected by photoperiod (Baker and Gallagher, 1983b), while the rate of spikelet primordium initiation decreases with increasing day length (Allison and Daynard, 1976; Kirby et al., 1987). Frank and Bauer (1982) observed that the duration of leaf initiation was insensitive to soil N levels, but that the length of the spikelet initiation phase increased with increasing soil N.

USDA, ARS, U.S. Salinity Laboratory, 4500 Glenwood Drive, Riverside, CA 92501. Received 12 Oct. 1992. *Corresponding author.

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Abbreviations: ANOVA, analysis of variance; df, degrees of freedom; κ_{iw}, electrical conductivity of irrigation water; κ_{sw}, electrical conductivity of soil water; *ld*, duration of leaf initiation; *lr*, leaf initiation rate; MSE, mean square error; *sr*, spikelet initiation rate; SSE, sum of squares error; *td*, total duration of primordia initiation.

Numerous investigators have addressed interactions between genotype and environment that influence final spikelet number. An inverse relationship between rate and duration of spikelet initiation has been observed in response to: photoperiod (Allison and Daynard, 1976; Rahman and Wilson, 1977b; Rahman et al., 1978), irradiance (Allison and Daynard, 1976), temperature (Friend, 1965; Rahman and Wilson, 1977a), nutrient supply (Whingwiri and Kemp, 1980; Frank and Bauer, 1984), and water stress (Frank et al., 1987). However, the relationship between rate and duration does not appear to be a strict inverse one and these factors may operate independently in the determination of spikelet number (Rahman and Wilson, 1977a).

An acceleration in shoot apex development and a progressive reduction in the time from planting to terminal spikelet formation, culm elongation, spike emergence and maturation in response to salinity was observed for two wheat species (Maas and Grieve, 1990). These investigators also noted that the final number of mainstem leaves and spikelets decreased in response to salt stress. They did not, however, study the effect of salinity on the parameters of leaf and spikelet primordia initiation by the shoot apex.

Our objective was to quantify the rate and duration of leaf and spikelet primordia initiation as a function of salinity for two hard red spring wheat cultivars. Ten three-piece linear-spline models with and without parameter restrictions were evaluated for their ability to describe leaf and spikelet primordia initiation. A model in which the duration of leaf primordia initiation and the rate of spikelet initiation are restricted was determined to be superior and was selected to analyze and compare the results for both greenhouse and field lysimeter conditions.

MATERIALS AND METHODS

Greenhouse Experiment

The semidwarf hard red spring wheat cultivars Yecora Rojo and Anza were grown in nine sand tanks in a greenhouse at the U.S. Salinity Laboratory at Riverside, CA. The tanks (1.2 by 0.6 by 0.5 m deep) contained washed sand having an average bulk density of 1.2 Mg m^{-3}. At saturation, the sand had an average volumetric water content of 0.34 m^3 m^{-3}. On 12 Jan. 1990, seeds of each cultivar were planted in two rows per tank. The rows were spaced 15 cm apart with 51 seeds per row. Sowing depth was approximately 2 cm. The plants were irrigated four times daily with a modified Hoagland's nutrient solution consisting of 2.5 m*M* Ca $(NO_3)_2$, 3.0 m*M* KNO_3, 0.17 m*M* KH_2PO_4, 1.5 m*M* $MgSO_4$, 50 μM Fe as sodium ferric diethylenetriamine pentaacetate (NaFeDTPA), 23 μM H_3BO_3, 5μM $MnSO_4$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, and 0.1 μM H_2MoO_4 added to local tap water. Each irrigation cycle continued about 15 min until the sand was completely saturated, after which the nutrient solution drained into 565-L reservoirs for recycling during the next irrigation. Water lost by evapotranspiration was replenished automatically each day to maintain constant osmotic potentials (Ψ) in the solutions. The solution pH was maintained between 5.5 and 6.0 by adding H_2SO_4 as required. Two saline treatments with $\Psi = -0.55$ and -0.70 MPa were imposed by adding NaCl and $CaCl_2$ (2:1 molar ratio to simulate saline soil conditions) to the nutrient solutions. The base nutrient solution served as the $\Psi = -0.05$ MPa control. Salination began at the time of seedling emergence, 3 d after planting. The osmotic potentials of the saline treatments were decreased to the desired levels by incremental additions of the salts over three consecutive days to avoid osmotic shock to the seedlings. The three treatment solutions had electrical conductivities of 1.7, 12.2, and 15.1 dS m^{-1}, respectively and are hereafter designated as (C)ontrol, (M)edium, and (H)igh salinities. The experimental design consisted of the three salinity treatments replicated three times in a randomized complete block, split-plot design, with salinity level as the main plot and cultivar as the subplot.

Maximum air temperature ranged between 23 to 36 °C (mean = 28 °C) during the day and minimum air temperature was between 6 to 19 °C (mean = 15 °C) at night. Relative humidity ranged from 96 to 42%, with a mean of 59% during the day and 81% during the night.

Field Lysimeter Experiment

Outdoor lysimeters (3.0 by 3.0 by 1.5 m deep) were located at the U.S. Salinity Laboratory, Riverside, CA. The lysimeters contained Pachappa fine sandy loam (mixed, thermic, Mollic Haploxeralf). Prior to planting, triple superphosphate was mixed into the top 0.25 m of soil at a rate of 73 kg P ha^{-1}. To ensure adequate N and K fertility throughout the experiment, $Ca(NO_3)_2$ (0.6 m*M*) and KNO_3 (1.0 m*M*) were added in every irrigation.

Seeds of both cultivars were planted in the center 2.4- by 2.4-m area of the level lysimeters on 11 Jan. 1990. Each lysimeter contained eight rows of each cultivar. Rows were spaced 15 cm apart, with the seeds placed 4.0 cm apart within the row to give a planting density of 167 plants m^{-2}. Sowing depth was approximately 1.5 cm. The planted area was surrounded by a wooden border extending 2.5 cm above and 12.7 cm below the soil surface to minimize lateral flow of water.

The experimental design was identical to that given for the greenhouse study. At planting, the soil profiles were still salinized from a previous experiment. The initial electrical conductivities of the soil water (K_{sw}) in the top 0.45 m of the soil for the three salinity treatments were 4.9, 14.4, and 18.5 dS m^{-1}. To facilitate germination, 25 mm of low-salinity water (0.9 dS m^{-1}) was applied to each lysimeter immediately after seeding. Twelve days after sowing, when the plants had just emerged through the soil surface, differential salination was initiated by applying irrigation water containing equal weights of NaCl and $CaCl_2$. The average κ_{iw} of the three saline irrigation waters were 0.8, 11.4, and 17.1 dS m^{-1} to give (C)ontrol, (M)edium, and (H)igh salinity treatments.

All lysimeters were flood-irrigated approximately every 7 to 10 d to keep the soil matric potential of the control treatment above -0.085 MPa at the 0.25-m depth. The total amount of irrigation water applied to each lysimeter between planting and 1 March 1990 was 100 mm within the 2.4- by 2.4-m area. To prevent dilution of the soil salinity by rain water, a clear plastic tarpaulin was pulled over each lysimeter on rainy days. A neutron probe and tensiometers were used to monitor soil water contents and soil matric potentials, and to guide irrigation frequency. Soil water status was measured before and after most irrigations at depths of 25, 45, 75, and 105 cm at two locations within each lysimeter.

Soil water salinity (κ_{sw}) was determined by extracting soil solutions with porous ceramic suction cups buried 25, 45, 75, and 105 cm below the soil surface in two locations within the lysimeters. Drainage effluent was collected after most irrigations. The absence of changes in soil water content below 45 cm indicated that the root system did not extend to the 75 cm level during the study period. Therefore, time- and depth-averaged κ_{sw}-values were calculated from salinity measurements at the 25- and 45-cm depths during the period of primordia initiation. The mean κ_{sw} values for the three salinity treatments averaged 4.4, 15.8, and 23.1 dS m^{-1} from planting until 14 Feb. 1990, and 3.8, 17.3, and 24.4 dS m^{-1} during the period from 14 Feb. to 1 March 1990.

Standard meteorological measurements were made with a Class I agrometeorological station adjacent to each experimental location. Soil/sand temperatures were measured at a depth

of about 1 cm; air temperatures were measured about 1 m above the crop canopy. Hourly mean temperatures were integrated over the 24-h period and summed to give cumulative thermal time, i.e., °C d_{soil} or °C d_{air} assuming a base temperature of 0 °C (Logan and Boyland, 1983). Maximum daytime air temperatures ranged from 10 to 27 °C (mean = 19 °C); nighttime from −2 to 11 °C (mean = 6 °C). Relative humidity ranged from 95 to 9%, with a mean of 24% during the day and 78% during the night.

Experimental Measurements

Experimental measurements were collected for two data sets. The first data set was comprised of mainstem leaf and spikelet measurements made on plants grown to maturity. Ten plants of each cultivar were randomly selected from each field lysimeter or greenhouse sand tank (30 plants of each cultivar per treatment). Main shoots were identified and leaves were tagged as they emerged. Mean numbers of mainstem leaves and spikelets were determined from these plants and are referred to as the observed leaf and spikelet counts in this paper.

The second data set was derived from destructive measurements made on plants sampled at specific time intervals after seedling emergence. Beginning shortly after seedling emergence, two seedlings of each cultivar were harvested three times weekly from each sand tank or lysimeter. The first samples from the greenhouse were taken 3 d (67 °C d_{soil}) after planting. At this time, seedling development of both cultivars was 0.2 to 0.3 on the Haun scale (Haun, 1973). The first samples from the field lysimeters were collected 12 d (133 °C d_{soil}) after sowing (Haun scale = 0.1). At each sampling, mainstem shoots were dissected to determine the number of fully emerged leaves, the number of expanding leaves, and the number of primordia present on the apex. A primordium was scored as present when a bulge protruded beyond the smooth flank of the apical dome (Kirby and Appleyard, 1987). At the double ridge stage, when both the leaf primordium and the spikelet bud could be seen, the double unit was counted as one (Kirby, 1974). Sampling was continued until the glumes of the terminal spikelet were clearly differentiated.

Statistical Analysis

The analysis of the mature leaf and spikelet data (first data set) was carried out with eight one-way ANOVA models. Each model was used to test for the effects of salinity on the final number of mainstem leaves or spikelets for either variety in the greenhouse or field plots. Additionally, Tukey's Studentized range test was used to differentiate between the observed mean leaf and spikelet counts across the three salinity levels.

The leaf and spikelet primordia data (second data set) were regressed against thermal time (°C d_{soil}) with a three-piece linear-spline model (Fig. 1). The linear-spline model was fit to these data with SAS (proc NLIN, method DUD) (SAS Institute, 1985). This model separates primordia production into distinct phases; initiation of leaf primordia occurs in phase 1, and spikelet primordia in phase 2 (Stern and Kirby, 1979). The third phase commences with the formation of the terminal spikelet at the end of primordia initiation. Mathematically, the linear-spline model may be expressed as:

$$\gamma = \begin{cases} \alpha_0 + \beta_1 t, & 0 < t \le \delta_1 \\ \alpha_0 + \beta_1\delta_1 + \beta_2 t, & \delta_1 < t \le \delta_2 \\ \alpha_0 + \beta_1\delta_1 + \beta_2\delta_2, & \delta_2 < t \end{cases} \quad [1]$$

where γ is the number of primordia, t is the thermal time (in °C d) after sowing, β_1 and β_2 are the leaf and spikelet initiation rates, respectively; δ_1 and δ_2 represent the end of the leaf and spikelet initiation phases, respectively; and α_0 represents the intercept with the vertical axis ($t = 0$). While fitting the linear-spline model to the apical morphogenesis data, α_0 was always constrained to equal 3, the number of leaf primordia already differentiated in the wheat seed at planting (Kirby and Appleyard, 1987). Starting values for the four remaining independent parameters were obtained by first averaging the primordia data across cultivars and then fitting simple linear regression equations to each phase separately. Convergence generally occurred after 14 to 24 iterations.

Modeling Considerations/Parameter Restrictions

One of the primary goals of the statistical analysis was to ascertain whether or not the estimates of the independent parameters in the linear-spline model changed across different salinity treatments. For example, would an increase in salinity levels result in decreasing initiation rates, yet not affect the duration estimates, or vise versa? The standard approach to test for changes in parameter estimates across treatment levels is to systematically compare the sum of squares error (SSE) estimates of each model encompassing one or more parameter restrictions to the full (or unrestricted) model SSE using an appropriate F test (Weisberg, 1985). However, in a strict mathematical sense a linear-spline model is discontinuous at every node that joins two lines together. Therefore, the 1st order derivatives do not exist for such a model, and hence the traditional statistical testing techniques are no longer valid.

One alternative is to qualitatively compare the mean square error (MSE) estimates of each restricted model to the MSE estimate of the unrestricted model. For example, to see if the leaf initiation rate estimates significantly changed across salinity levels, one would pool the data from all three salinity levels together and fit two models. The first model would require 12 parameters (four primordia parameters per salinity level × three levels) and the second model would require 10 parameters (since only one leaf rate parameter is required for all three salinity levels in the restricted model). One could then qualitatively compare the MSE estimate from the 10 parameter (restricted) model to the 12 parameter (unrestricted) model. If the magnitude of the MSE estimate from the restricted model was only a few percent greater than the full model's MSE, one might then tentatively conclude that the leaf initiation rates were not significantly changing across salinity levels.

A second, and more objective method for comparing one or more restricted models to an unrestricted model is by assessing the predictive capabilities of each model. Note that the three-piece linear-spline model shown in Eq. [1] can be used to predict both the total leaf and spikelet numbers through the following two formulas (assuming $\alpha_0 = 3$):

$$\text{Total expected leaf number} = L_p = 3 + \beta_1\delta_1 \quad [2]$$

$$\text{Total expected spikelet number} = S_p = \beta_2(\delta_2 - \delta_1) \quad [3]$$

The predicted leaf and spikelet counts from each model can then be compared to the observed leaf and spikelet counts from the data set of the mature plants. One standard comparison criterion would become the prediction variance estimate; e.g., the average of the squared differences between the observed and predicted leaf and spikelet counts across the three salinity levels (for each variety within each location). Another possible criterion could be a prediction bias test. For example, if the observed leaf and spikelet counts always decreased with increasing salinity, then it would be reasonable to expect the predicted estimates to do the same. If one or more of the models under consideration produced leaf or spikelet estimates whose orderings were inconsistent with the observed data, then those models could be judged to be biased.

For this investigation, both a qualitative comparison of the MSE estimates and a more quantitative comparison of the prediction capabilities were used to ascertain when and how the parameters in the three-piece linear-spline model were chang-

ing across the salinity levels. This in turn allowed us to infer which subset of the four developmental parameters seemed to be most influenced by salinity stress (leaf rate, spikelet rate, leaf duration, and/or spikelet duration).

RESULTS AND DISCUSSION

Observed Leaf and Spikelet Counts

The final number of leaves and spikelets on the main stems of both cultivars varied within as well as between treatments. For example, Yecora Rojo plants from the nonsaline control treatments produced eight or nine mainstem leaves and 18 to 21 spikelets. Unstressed Anza plants produced ten or eleven leaves and 21 to 24 spikelets. These findings are consistent with the general observation that within a given stand of wheat plants growing under similar environmental conditions there are subpopulations consisting of different leaf number and/or spikelet number classes. The timing of leaf and spikelet initiation and development may also vary among these subpopulations. Hay and Delecolle (1989) speculated that leaf appearance rate, as determined from successive, non-destructive measurements on the same plants, is modified to accommodate varying number of leaves. Since serial dissections are required to follow the progress of primordium initiation, it is difficult to assign an individual plant to a subpopulation based on leaf or spikelet number when the measurements are, by necessity, destructive.

The observed mean leaf and spikelet counts across the three salinity levels for mature mainstems of both wheat varieties within both locations are shown in Table 1. The same pattern appeared in both the leaf and spikelet data; the mean counts decreased with increasing salinity. The spikelet counts seemed to exhibit a more pronounced decrease. However, Tukey's Studentized range test revealed that the differences between medium and high salinity stress levels for either the leaf or spikelet numbers were generally not statistically significant at the 0.05 level. None the less, one should keep in mind that Tukey's test was applied individually (on each leaf or spikelet within a given variety and location). The consistency of the decreasing mean counts across the cultivars and locations strongly suggested the existence of an inverse relationship between salinity stress and the final numbers of leaves and spikelets.

Table 1. Effect of salinity on the numbers of leaves and spikelets produced by the mainstems of two wheat genotypes.

Salinity Level	Leaf	Spikelet
	no.	
	Anza: Greenhouse	
control	10.00 a†	22.27 a
medium	9.27 a,b	19.40 b
high	8.93 b	18.23 b
	Yecora Rojo: Greenhouse	
control	8.33 a	20.10 a
medium	7.47 b	17.20 b
high	7.10 b	16.50 b
	Anza: Field Plots	
control	10.97 a	22.47 a
medium	9.90 b	16.17 b
high	9.90 b	14.80 b
	Yecora Rojo: Field Plots	
control	8.21 a	19.30 a
medium	8.07 b	16.20 b
high	8.03 b	13.13 c

† Leaf or spikelet means followed by a different letter within a given variety and location were found to be statistically different from one another at the 0.05 level using Tukey's Studentized Range test.

Appropriate Parameter Restrictions for the Three-Piece Linear-Spline Model

From the results shown in Table 1, it appeared reasonable to assume that both the average leaf and spikelet primordia counts should decrease with increasing salinity. This assumption in turn influenced the choice of

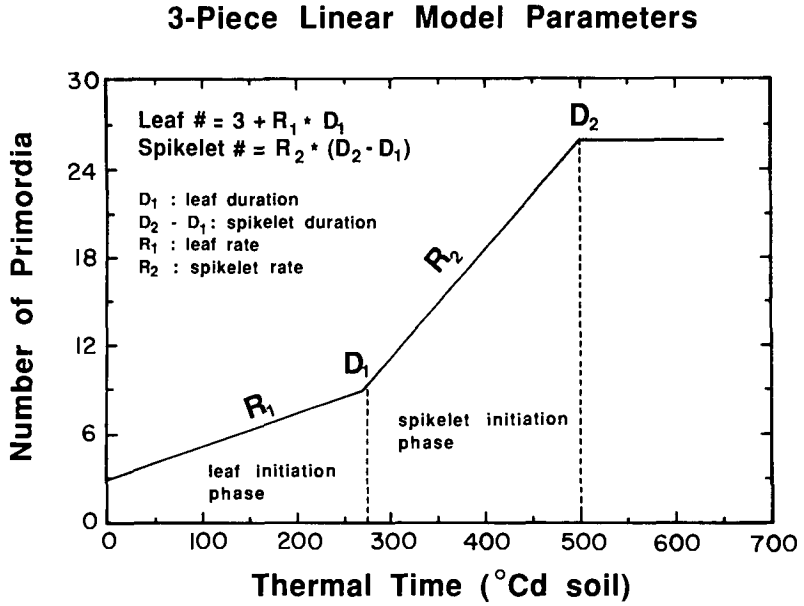

Fig. 1. Schematic of the three-piece linear approximation of the organogenesis process.

Table 2. Summary statistics for all potential linear spline models fit to greenhouse data.

Model	SSE	df	MSE	σ^2_L	σ^2_S	σ^2_A	Order of predicted leaf count†
			Anza				
full	534.10	348	1.535	0.42	2.79	1.61	C > M > H
lr	537.22	350	1.535	0.87	3.67	2.27	H > M > C
ld	544.48	350	1.556	0.12	1.64	0.88	C > M > H
sr	547.37	350	1.564	0.26	1.57	0.92	C > H > M
td	671.42	350	1.918	0.30	5.22	2.76	C > H > M
ldsr	549.05	352	1.560	0.18	1.44	0.81	C > M > H
lrtd	673.36	352	1.913	0.47	4.94	2.71	M > C > H
duration	671.66	352	1.908	0.28	5.22	2.75	C > M > H
rate	549.76	352	1.562	0.56	2.11	1.34	H > M > C
srtd	694.56	352	1.973	0.46	7.13	3.80	C > H > M
			Yecora Rojo				
full	375.50	306	1.227	3.40	0.92	2.16	C > M > H
lr	378.16	308	1.228	3.57	0.78	2.18	H > C > M
ld	382.54	308	1.242	3.51	0.74	2.13	M > C > H
sr	382.80	308	1.243	3.48	0.86	2.17	C > M > H
td	402.66	308	1.307	3.21	1.05	2.13	C > M > H
ldsr	383.87	310	1.238	3.52	0.79	2.16	C > M > H
lrtd	405.57	310	1.308	3.42	0.86	2.14	C > H > M
duration	412.58	310	1.331	3.54	0.82	2.18	M > C > H
rate	385.79	310	1.245	3.71	0.75	2.23	H > M > C
srtd	447.26	310	1.443	3.84	1.64	2.74	C > H > M

† Order of treatments producing a decreasing number of mainstem leaves. (C)ontrol, (M)edium, and (H)igh salinity treatments are as defined in Materials and Methods.

parameter restrictions that could be made within the three-piece linear-spline model (see Eq. [2] and [3]). For example, both the leaf initiation rate (β_1) and the leaf duration (δ_1) cannot be assumed to be constant across salinity levels. To do so would imply that the expected average leaf counts are constant across salinity stress levels, which is counter to our basic assumption made above.

In light of Eq. [2] and [3], we can invoke the following argument. The three-piece linear-spline model contains four independent parameters, any one of which can be restricted to be constant across all three salinity levels without theoretically violating the requirement that the average leaf and spikelet counts decrease with increasing salinity. Hence, we can construct four separate parameter restrictions; one which restricts only the leaf initiation rate (β_1), the spikelet initiation rate (β_2), the duration of leaf initiation (δ_1), or the total duration of primordia initiation (δ_2). For the remainder of this paper we will refer to these four models as *single parameter restricted models* and abbreviate them as *lr*, *sr*, *ld*, and *td*, respectively.

There are a total of six ways to restrict any two parameters in the linear-spline model. However, as already discussed, restricting both the leaf rate and leaf duration implies that the average leaf counts remain constant across the salinity levels and hence this combination of parameter restrictions can be removed from further consideration. The remaining five combinations of two parameter restrictions are all theoretically valid; a leaf rate—spikelet rate restriction (β_1, β_2), a leaf duration—total duration restriction (δ_1, δ_2), leaf rate—total duration restriction (β_1, δ_2), a leaf duration—spikelet rate restriction (δ_1, β_2), and a spikelet rate—total duration restriction (β_2, δ_2). We shall refer to these five models as *double parameter restricted models* and abbreviate them as *rate*, *duration*, *lrtd*, *ldsr*, and *srtd*, respectively. Note that the first model implies that the primordia initiation rates remain constant across the salinity levels; only the durations change. The second model implies just the opposite; the primordia durations remain constant while the rates change. The next two models represent mixture models, and the final model implies that all the changes from increasing salinity stress occur during the leaf initiation phase.

Finally, there is another model to consider that does not force either the leaf or spikelet counts to remain constant across increasing salinity levels. This is simply a model with no parameter restrictions; e.g., all four parameters are allowed to vary across the salinity levels. This represents an *unrestricted model* and is abbreviated in this paper as the *full* model.

Final Model Selection

In all, there were 10 models under consideration: the full, or unrestricted model, four models with one restricted parameter, and five models with two parameter restrictions. Each of these models was in turn fit to all of the apical morphogenesis data and used to generate the summary statistics discussed in the Methods section. The summary statistics for all the models fit to the greenhouse data are shown in Table 2. The first column identifies the model, the second through fourth columns contain the sum of squares error (SSE), degrees of freedom (df), and mean squared error (MSE) statistics, the fifth and sixth columns contain the prediction variance estimates for the leaf (σ^2_L) and spikelet (σ^2_S) counts, the seventh column lists the average prediction variance (σ^2_A, an average of columns five and six), and the eighth column lists the order of the predicted leaf number. All the models for both varieties of wheat grown in the greenhouse predicted decreasing spikelet counts as the salinity levels increased.

The MSE for the unrestricted (full) model fit the Anza greenhouse data was 1.535. Three of the single parameter restriction models had MSE estimates that were within 2% of the unrestricted MSE estimate; the restricted leaf rate, leaf duration, and spikelet rate models, respectively. Additionally, two of the five double parameter restriction models had MSE estimates within 2%; viz., the restricted leaf rate—spikelet rate model and the restricted leaf duration—spikelet rate model. Among the single parameter models, both the restricted leaf duration and spikelet rate models produced low prediction variance estimates for both the leaf and spikelet counts. However, the spikelet rate model produced a biased leaf ordering (estimating the average total leaf number under the high salinity treatment to be greater than the average total leaf number under the medium treatment). Among the double parameter models the restricted leaf duration—spikelet rate model produced the smallest prediction variance estimates and a correct (unbiased) leaf ordering. Overall, the restricted leaf duration—spikelet rate model produced the lowest spikelet and average prediction variance estimates.

The MSE for the unrestricted model fit to the Yecora Rojo greenhouse data was 1.227. Again, three of the four single parameter and two of the five double parameter restricted models produced MSE estimates within 2% of the unrestricted estimate. All of these five re-

Table 3. Summary statistics for all potential linear spline models fit to field data.

Model	SSE	df	MSE	σ^2_L	σ^2_S	σ^2_A	Order of predicted leaf count†
			Anza				
full	734.28	306	2.400	3.22	8.88	6.05	M > H > C
lr	750.68	308	2.437	1.26	7.21	4.24	H > M > C
ld	768.94	308	2.497	0.09	3.26	1.68	C > M > H
sr	735.37	308	2.388	3.51	9.18	6.35	M > H > C
td	819.73	308	2.662	4.15	9.45	6.80	M > H > C
ldsr	790.10	310	2.549	0.29	3.29	1.79	C > M > H
lrtd	841.83	310	2.716	0.20	3.51	1.86	C > M > H
duration	840.16	310	2.710	0.14	3.22	1.68	C > M > H
rate	751.77	310	2.425	1.43	7.95	4.69	H > M > C
srtd	975.10	310	3.145	3.99	5.85	4.92	M > H > C
			Yecora Rojo				
full	462.05	251	1.841	0.30	0.59	0.45	C > H > M
lr	463.75	253	1.833	0.30	0.50	0.40	H > C > M
ld	463.32	253	1.831	0.29	0.65	0.47	C > M > H
sr	471.02	253	1.862	0.50	0.35	0.43	H > C > M
td	479.93	253	1.897	0.34	1.10	0.72	C > H > M
ldsr	481.04	255	1.886	0.49	1.13	0.81	C > M > H
lrtd	481.66	255	1.889	0.29	0.84	0.57	C > H > M
duration	483.68	255	1.897	0.31	0.93	0.62	C > M > H
rate	471.28	255	1.841	0.59	0.26	0.43	H > M > C
srtd	536.43	255	2.104	0.67	0.63	0.65	H > C > M

† Order of treatments producing a decreasing number of mainstem leaves. (C)ontrol, (M)edium, and (H)igh salinity treatments are as defined in Materials and Methods.

Table 4. Cumulative summary statistics for all potential linear spline models.

Prediction Model	SSE	df	MSE	σ^2_L	σ^2_S	σ^2_A	No. of incorrect leaf prediction orderings†
			Greenhouse				
full	909.60	654	1.391	1.91	1.85	1.88	0
lr	915.38	658	1.391	2.22	2.22	2.22	2
ld	927.02	658	1.409	1.82	1.19	1.51	1
sr	930.17	658	1.414	1.87	1.22	1.55	1
td	1074.1	658	1.632	1.75	3.14	2.45	1
ldsr	932.92	662	1.409	1.85	1.12	1.49	0
lrtd	1078.9	662	1.630	1.95	2.90	2.43	2
duration	1084.2	662	1.638	1.91	3.02	2.47	1
rate	935.55	662	1.413	2.13	1.43	1.78	2
srtd	1141.8	662	1.725	2.15	4.38	3.27	2
			Field Plots				
full	1196.3	557	2.148	1.76	4.74	3.25	2
lr	1214.4	561	2.165	0.78	3.86	2.32	2
ld	1232.3	561	2.197	0.19	1.95	1.07	0
sr	1206.4	561	2.150	2.01	4.77	3.39	2
td	1299.7	561	2.317	2.24	5.28	3.76	2
ldsr	1271.1	565	2.250	0.39	2.21	1.30	0
lrtd	1323.5	565	2.343	0.25	2.18	1.22	1
duration	1323.8	565	2.343	0.22	2.07	1.15	0
rate	1223.1	565	2.165	1.01	4.10	2.56	2
srtd	1511.5	565	2.675	2.33	3.24	2.79	2
			All Data (ld and ldsr models only)				
ld	2159.3	1219	1.771	1.00	1.57	1.29	1
ldsr	2204.1	1227	1.796	1.12	1.66	1.39	0

† Number of times the proposed model departs from the expected sequence, i.e. C > M > H.

stricted models produced virtually equivalent leaf and spikelet prediction error estimates. However, only the restricted spikelet rate, restricted total duration, and the restricted leaf duration—spikelet rate models produced decreasing average total leaf numbers with increasing salinity.

The summary statistics for these same 10 models fit to the field data are shown in Table 3. As with the greenhouse data, all the models for both varieties of wheat grown in the field predicted decreasing spikelet counts as the salinity levels increased. For the Anza field data, it is clear from the prediction variance estimates and the ordering of the predicted leaf counts that the restricted leaf duration model is the only acceptable single parameter restricted model.

Three models with two parameter restrictions predicted decreasing leaf counts and roughly equivalent average prediction variance estimates. Of these three, the restricted leaf duration—spikelet rate model had the lowest MSE (2.549). Note that this MSE estimate was about 2% higher than the observed 2.497 MSE estimate found with the restricted leaf duration model.

The MSE for the unrestricted model fit to the Yecora Rojo field data was 1.841. Two of the models with single parameter restrictions actually produced MSE estimates that were lower than this; the other two models had MSE estimates that were within 3% of the unrestricted estimate. With the exception of the restricted total duration model, all of the prediction variance error estimates were roughly equivalent. Again, however, only the restricted leaf duration model predicted decreasing leaf counts as the salinity level increased. Four of the five models with two parameter restrictions had MSE estimates that were within 3% of the unrestricted MSE estimate. Of these four, two predicted decreasing leaf counts; the restricted leaf duration—spikelet rate model and the restricted leaf duration—total duration model. Between these two, the latter model produced marginally lower prediction error variances.

Two facts stand out from the summary statistics shown in Tables 2 and 3. The first is that the linear-spline model with no parameter restrictions generally appeared inferior to one or more of the models with one or two parameter restrictions. For example, the restricted leaf duration model always had a MSE within 3% of the full model, was more likely to correctly predict decreasing average leaf counts, and usually produced lower prediction error estimates. These results implied that the unrestricted model was over-parameterized. In other words, the data did not support the hypothesis that all four of the crop attributes were changing within these two wheat varieties under this range of salinity stress.

The second fact apparent from Tables 2 and 3 was that no one alternative model (containing either one or two parameter restrictions) universally performed the best under all the statistical criteria all of the time. However, there were two restricted parameter models that seemed to perform well consistently; the restricted leaf duration model and the restricted leaf duration—spikelet rate model. The evidence for this is shown in Table 4, where the summary statistics for each location have been combined across both wheat varieties. (Note that in Table 4, the final column now gives the number of incorrect orderings, rather than the order of predicted leaf counts.) For the greenhouse data, the "best" model appeared to be the restricted leaf duration—spikelet rate model. This

Table 5. Final parameter estimates and standard deviations for the restricted leaf duration and spikelet rate model.

Salinity Level	Greenhouse Anza	Greenhouse Yecora Rojo	Field Plots Anza	Field Plots Yecora Rojo
	β_1 — Leaf rate, leaves (°Cd)$^{-1}$			
control	0.0200	0.0199	0.0231	0.0191
medium	0.0188	0.0195	0.0192	0.0169
high	0.0178	0.0182	0.0177	0.0152
Std. Dev.	(0.0015)	(0.0010)	(0.0017)	(0.0020)
	β_1 — Leaf duration, °Cd			
all	318.7	337.3	380.7	331.1
Std. Dev.	(4.3)	(3.5)	(14.7)	(21.4)
	β_2 — Spikelet rate, spikelets (°Cd)$^{-1}$			
all	0.0741	0.1096	0.0841	0.0915
Std. Dev.	(0.0024)	(0.0041)	(0.0071)	(0.0166)
	δ_2-δ_1 — Spikelet duration, °Cd			
control	322.6	171.3	271.5	207.9
medium	273.9	152.6	228.0	180.6
high	259.1	144.9	185.9	163.1
Std. Dev.	(11.9)	(5.7)	(25.9)	(30.2)

Table 6. Final leaf and spikelet primordia number estimates and standard deviations found with the restricted leaf duration and spikelet rate model.

Salinity Level	Leaf		Spikelet	
	no.			
	Anza: Greenhouse			
control	9.39	(0.48)	23.89	(0.70)
medium	8.98	(0.43)	20.28	(0.46)
high	8.66	(0.49)	19.19	(1.04)
	Yecora Rojo: Greenhouse			
control	9.73	(0.46)	18.78	(0.62)
medium	9.57	(0.22)	16.69	(0.48)
high	9.15	(0.37)	15.89	(0.46)
	Anza: Field Plots			
control	11.78	(0.37)	22.84	(0.30)
medium	10.32	(1.17)	19.18	(1.71)
high	9.72	(0.63)	15.64	(0.83)
	Yecora Rojo: Field Plots			
control	9.31	(0.83)	19.02	(0.87)
medium	8.59	(0.91)	16.52	(0.84)
high	8.04	(0.82)	14.92	(1.01)

model's average MSE is within 1.3% of the full model's MSE estimate, it had the absolute lowest spikelet prediction error variance and lowest average prediction error variance, and was the only model with parameter restrictions that correctly predicted decreasing average leaf counts within both wheat varieties. The only two other reasonable models were the restricted leaf duration and the restricted spikelet rate models, which appeared to perform well in all but the leaf ordering categories.

For the field plot data, the best model appeared to be the restricted leaf duration model. Its MSE was within 2.3% of the full model, it produced the absolute lowest leaf, spikelet, and average prediction error variance estimates, and it correctly predicted decreasing leaf counts within both varieties. Three double parameter restricted models also appeared viable; the restricted leaf duration—spikelet rate model; the restricted leaf rate—total duration model, and the restricted leaf duration—total duration model. However, neither the restricted leaf rate—total duration model nor the restricted leaf duration—total duration model fit the greenhouse data very well.

At the bottom of Table 4 the overall average summary statistics for the restricted leaf duration model and the restricted leaf duration—spikelet rate model are shown. These results suggest that both models fit the apical morphogenesis data quite well; the restricted leaf duration model appeared to be somewhat more biased, but it did have slightly lower overall prediction error variances.

In light of all the criteria, the results of the data analysis strongly suggested that there was no evidence of a change in the leaf primordia durations within the range of the applied salinity stress levels. For the greenhouse data, there also appeared to be no conclusive evidence suggesting that a change in the spikelet primordia initiation rates was occurring. The fact that the greenhouse data were collected under more carefully controlled environmental conditions combined with the viability of the restricted leaf duration—spikelet rate model with respect to the field plot data suggested that the two crop attributes primarily affected by the increasing salinity stress levels were the leaf primordia initiation rate and the duration of spikelet primordia initiation. The finding that salinity has a greater effect on the duration of spikelet initiation than on the rate is consistent with the results of Oosterhuis and Cartwright (1983) who reported that the spikelet initiation phase of the spring wheat cultivar, Devuli, was shortened by water deficit, while the rate was unaffected. In barley, the duration of spikelet initiation was strongly influenced by planting density, and the rate was unchanged (Kirby and Faris, 1970). Evidence from studies of the developmental patterns of diverse genotypes also indicates that the duration of spikelet initiation, rather than the rate, is more closely related to spikelet number in wheat (Rawson, 1970; Rashid and Halloran, 1984) and barley (Kitchen and Rasmusson, 1983).

Linear-Spline Parameter Estimates

The final parameter estimates associated with the restricted leaf duration—spikelet rate model for both wheat varieties within both locations are shown in Table 5. The leaf rate, spikelet rate, and leaf duration attributes correspond directly to the parameters, β_1, β_2, and δ_1, respectively. The spikelet duration was found by subtracting the leaf duration parameter estimate from the total duration parameter estimate; e.g., $\delta_2 - \delta_1$. A comparison between the parameter estimates of two wheat varieties revealed that, compared to Anza, Yecora Rojo exhibited a more rapid spikelet initiation rate and shorter durations of spikelet initiation. The same could not be said about either the leaf rate or duration estimates; trends in these parameters between the two varieties were inconsistent across the two locations.

The standard deviations shown in Table 5 confirmed that the greenhouse data were less variable than data from the lysimeters. These standard deviations were computed from "jack-knifed" variance estimates derived in the following manner. The apical morphogenesis data were first split into three distinct sets corresponding to either the greenhouse sand tank or field lysimeter replications. Every pair of observations within each of these replication subsets was then randomly split into two dis-

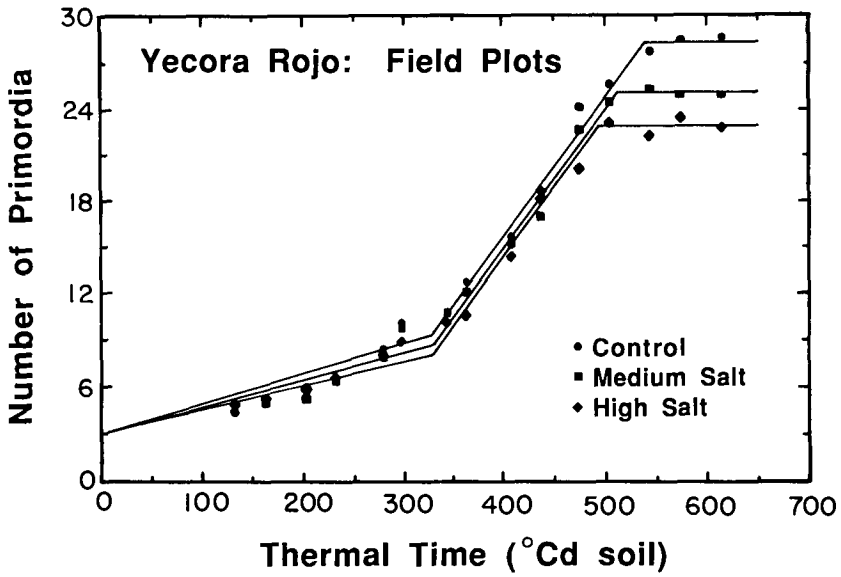

Fig. 2. Primordia production of field-grown Yecora Rojo as a function of °C d_{soil}. During phase 1 the electrical conductivities of the soil water = 4.1 (solid circle), 15.8 (solid square), and 23.1 (solid diamond) dS m^{-1}. During phase 2, these values were 3.6 (solid circle), 17.3 (solid square), and 24.4 (solid diamond) dS m^{-1}. Each data point is the mean of six measurements. Fitted lines were generated by the restricted leaf duration—spikelet rate model.

tinct subsets, yielding six distinct data sets altogether. The three-piece linear-spline model was then sequentially fit to each of these six data sets, and the resulting parameter estimates were then used to compute variance estimates. Note that the leaf rate and spikelet duration variance estimates were found by pooling the individual variance estimates associated with each salinity level together.

A plot of the restricted leaf duration—spikelet rate model fit to the Yecora Rojo field lysimeter data is shown in Fig. 2. In this plot, each data point represents the average of six apical morphogenesis observations for a given salinity level. The model appears to fit the data well, with the exception of the slight nonlinearity apparent at the beginning of the leaf initiation phase. This nonlinearity was probably the result of the constraint placed on the intercept, whereby α_0 is set equal to 3, the number of leaf primordia that are already differentiated in wheat seeds at planting, (t_0). Without constraint, intercept estimates for both cultivars and for all salinity levels were highly dependent upon the experimental location. Values from the greenhouse were consistent with the number of primordia (three–four) presumably present in the seed. That values from the lysimeter experiment ranged between 0 and 2, however, suggests that initiation of the first few primordia proceeded at a slower-than-average rate. Differences in substrate as well as in irrigation frequency in the two locations may have created different moisture conditions for the restoration of the water status of the seed and for subsequent metabolic reactivation and the reorganization of cell membranes and macromolecules. The apparent delay in leaf primordia initiation by wheat grown under field conditions has been noted by other investigators (Kirby et al., 1987; Delecolle et al., 1989).

Final Primordia Estimates

Table 6 contains the final leaf and spikelet primordia estimates for both wheat varieties within each location. The standard errors associated with these estimates were, as before, computed from jack-knifed variance estimates; e.g., by estimating the total leaf and spikelet counts from the parameter estimates associated with each of the six distinct apical morphogenesis subsets. The results in Table 6 support the hypothesis of an inverse primordia count, salinity stress relationship. The agreement between the predicted primordia counts found with the restricted leaf duration—spikelet rate model and the observed leaf and spikelet counts was encouraging. Eight out of 12 of the predicted leaf and spikelet counts were within one unit of the observed counts. In general the model tended to overestimate the number of primordia formed at the lower rate during phase 1. This finding implies that all of the foliar structures, i.e., the full complement of leaf primordia along with the primordium destined to become the spike collar, are initiated immediately prior to the abrupt change in initiation rate that has been associated with the transition from vegetative to reproductive development (Kirby, 1974). The only serious inconsistency occurred within the Yecora Rojo leaf counts associated with the greenhouse data, where the predicted leaf primordia estimates tended to be about two units higher than the observed counts. However, this was an artifact of the observed apical morphogenesis data, rather than the specific parameter restrictions contained within the final model. The fact that all ten of the linear-spline models examined during the data analysis overpredicted these leaf counts indicates that the spike collar along with one spikelet primordium may have been initiated during phase 1.

CONCLUSION

Numerous researchers have found that salinity reduces the growth rate of the whole plant as well as that of specific shoot organs (e.g., Aspinall, 1986). The duration of plant development is also affected by salinity and the time from planting to maturity for many cereal crops typically decreases with increasing salinity stress (Maas

and Poss, 1989). The three-piece linear-spline model describes the time-course of primordia production in terms of well-defined and significant stages of morphological development of the wheat apex. Salinity affected the two phases of primordium initiation differentially. In the first phase the leaf initiation rate decreased while the duration was unaffected by salinity. Thus, salinity had no influence on the timing of the transition from vegetative to reproductive development. Thereafter, spikelet primordia were initiated at an increased rate that was not further influenced by salinity treatment. Reduction in spikelet number in response to salinity was due to decreased duration of the spikelet primordium initiation phase. Salinity-induced changes in this important stage of wheat development may also be reflected in the timing of subsequent life-cycle phases that lead to differences in time to maturity.

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