



The effects of soil salinity on growth of and dinitrogen fixation in *Phaseolus vulgaris* L. and *Vicia faba* L.

by Theodore James Kisha

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy

Montana State University

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Abstract:

Faba bean (*Vicia faba* L.) and dry bean (*Phaseolus vulgaris* L.) were evaluated to determine the effects of soil salinity (NaCl and CaCl₂) and fertilizer nitrogen on growth, dinitrogen fixation, and nitrogen accumulation. Plants were evaluated in the field and greenhouse, with and without fertilizer nitrogen, at six salinity levels of 2,4,5,11,15, and 22; and 1.4,3,5,7,9, and 11 mmhos cm⁻¹ ECe, respectively. Data were analyzed using multiple regression. Growth parameters exhibiting sigmoid responses were regressed using an e^{-K} (sigmoid) transform.

Faba bean dry weight and seed yield were reduced 50% at approximately 9 and 10.5 mmhos, respectively. Acetylene reduction activity was maximized at 6 mmhos.

Dry bean growth increased with increased soil salinity, up to 10-15 mmhos. Increased growth may have resulted from improved soil aggregation with the addition of Ca⁺⁺. Seed yield expressed on an area basis reached a maximum at 6 mmhos and declined at the higher salinity levels. Lower yield per area than per plant, at salinity levels greater than 5 mmhos was a result of poor germination. Acetylene reduction decreased as salinity increased.

Nitrogen percentage in seed of both faba bean and dry bean was suppressed at moderate salinity levels in plots receiving fertilizer nitrogen. Nitrogen percentage in seed from unfertilized plots was constant. Plants growing in unfertilized plots (relying primarily on symbiotic reduction of atmospheric nitrogen) were able to supply nitrogen to the seed in adequate amounts at all salinity levels.

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A thesis submitted in partial fulfillment
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in

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Chairperson, Graduate Committee

Approved for the Major Department

9/2/83
Date

Dwane A Miller
Head, Major Department

Approved for the College of Graduate Studies

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Michael Malone
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ABSTRACT

Faba bean (*Vicia faba* L.) and dry bean (*Phaseolus vulgaris* L.) were evaluated to determine the effects of soil salinity (NaCl and CaCl₂) and fertilizer nitrogen on growth, dinitrogen fixation, and nitrogen accumulation. Plants were evaluated in the field and greenhouse, with and without fertilizer nitrogen, at six salinity levels of 2,4,5,11,15, and 22; and 1.4,3,5,7,9, and 11 mmhos cm⁻¹ EC_e, respectively. Data were analyzed using multiple regression. Growth parameters exhibiting sigmoid responses were regressed using an e^{-k} (sigmoid) transform.

Faba bean dry weight and seed yield were reduced 50% at approximately 9 and 10.5 mmhos, respectively. Acetylene reduction activity was maximized at 6 mmhos.

Dry bean growth increased with increased soil salinity, up to 10-15 mmhos. Increased growth may have resulted from improved soil aggregation with the addition of Ca⁺⁺. Seed yield expressed on an area basis reached a maximum at 6 mmhos and declined at the higher salinity levels. Lower yield per area than per plant, at salinity levels greater than 5 mmhos was a result of poor germination. Acetylene reduction decreased as salinity increased.

Nitrogen percentage in seed of both faba bean and dry bean was suppressed at moderate salinity levels in plots receiving fertilizer nitrogen. Nitrogen percentage in seed from unfertilized plots was constant. Plants growing in unfertilized plots (relying primarily on symbiotic reduction of atmospheric nitrogen) were able to supply nitrogen to the seed in adequate amounts at all salinity levels.

CHAPTER I

INTRODUCTION

Soil salinity affects crop production in many areas of the world, including the Northern Great Plains, North Africa, and the Middle East (Chapman, 1975). Saline soils not previously used in agriculture are becoming important as new lands are brought into production to either meet the demand of an increasing population, or to replace land lost to urban development. Additionally, cropping systems in semi-arid regions may lead to an increase in lands affected by excess salinity.

Edible and forage legumes are often significant soil nitrogen contributors and important sources of protein throughout the world. The increasing cost of nitrogen fertilizer increases the importance of plant species capable of biological dinitrogen fixation. Most plant response studies to saline conditions have been in a greenhouse environment. Few experiments have evaluated the effects of salinity on symbiotic dinitrogen fixation in the field.

Faba bean (*V. faba* L.) is a major food legume in the Middle East and is used as a forage in cereal-legume rotations throughout much of the Canadian, Northern Great Plains. It has potential for use in cereal-legume rotations in Montana. Faba bean salinity tolerance would broaden its potential to include use on marginal land where extensive use of nitrogen fertilizer may not be economically practical.

Common bean or dry bean (*P. vulgaris* L.) is one of the most important food legumes in the world. It is grown extensively throughout the United States and is an important crop in Montana. Furrow irrigation practices in the semi-arid regions of Montana increase the chance of dry bean exposure to a saline environment.

The purpose of this research was to define the effects of soil salinity on growth and symbiotic dinitrogen fixation of *V. faba* L. and *P. vulgaris* L. Of particular interest were the effects on these parameters of the soil salinity x fertilizer nitrogen interaction. A sigmoid transformation developed by Jensen and Homeyer (1970) was used in regression analysis of data exhibiting sigmoid characteristics. The use of this sigmoid model allowed direct comparison of growth with and without fertilizer nitrogen. Use of a polynomial for such comparisons was avoided as too cumbersome, as it necessarily would have included a number of interaction factors to express nitrogen effects on plant growth. Research was supported by the Montana Agricultural Experiment Station and the United States Department of Agriculture-Agency for International Development (U.S.A.I.D. No. AG/TAB 610-9-76 and U.S.D.A. No. 801-15-66).

CHAPTER II

LITERATURE REVIEW

Edible legumes are important food sources throughout the world. Approximately 7 million hectares of faba bean (Vicia faba L.) and 26 million ha of dry bean (Phaseolus vulgaris L.) are produced annually worldwide (FAO, 1980). Six-hundred thousand ha of dry bean are grown annually in the USA, with approximately 4,000 ha in Montana (USDA, 1980). Faba bean is being evaluated in Montana as an alternative crop and in long-term rotations with cereals. Salt tolerance is a critical selection criterion for alternative or rotational crops in semi-arid areas. Research involving salt tolerance of food-legume crops is limited, especially regarding salinity effects on symbiotic dinitrogen fixation.

Approximately 25% of the earth's surface is arid or semi-arid with insufficient rainfall to remove salts from the plant root zone (Thorne and Peterson, 1954). Miller and Bahls (1976) estimated that more than 57,000 ha in Montana were non-productive in 1974 because of soil salinity. Additionally, saline areas have been increasing approximately 10% annually in Montana. Increased salinity on non-irrigated agricultural land in Montana has often been attributed to fallowing of saline seep recharge areas. Hydraulic pressures in the fallowed areas may increase movement of ground water and salt to lower areas with inadequate drainage (Thacker, 1976). Soil salinity may be

increased by irrigation and excessive evaporation in areas of inadequate natural drainage (U.S. Salinity Lab, 1954). Soil salinity affects crop production on approximately half of the irrigated land in the western United States (Wadleigh, 1968). Irrigated acreage increased approximately 162,000 ha annually from 1964 to 1978 (U.S. Bureau of Census, 1978).

Cations and anions commonly found in saline soils are Ca^{++} , Mg^{++} , Na^+ , K^+ , CO_3^{--} , HCO_3^- , SO_4^{--} , and Cl^- (U.S. Salinity Lab, 1954). Parent material and location directly affect ion types present in a specific area. Plant response to different ionic species varies (Greenway, 1973; Eaton et al., 1971; Kahane and Poljakoff-Mayber, 1968). Predominant salts in Montana soils are CaSO_4 , MgSO_4 , and Na_2SO_4 (Thompson and Custer, 1976).

Successful farming in saline seep areas incorporates careful management of both recharge and seep areas. Continuous cropping of the recharge area may be used to lower the water table and reduce hydraulic pressure in some areas (Halvorson and Reule, 1976). Proper management should result in a decrease in the lateral flow of ground water and dissolved salts to an accumulation zone. Objections to continuous cropping usually involve economics (Burt and Stauber, 1976). Fallowing is used to stabilize yields on some soils by increasing soil moisture accumulation and nitrogen mineralization.

Legume rotation with cereals in some recharge areas has potential to maintain economic stability and reduce expansion and/or occurrence of saline seeps. Additionally, cereal-legume rotation may provide a continuous income while replenishing soil nitrogen through symbiotic

fixation. Legumes may also have potential use in intermediate zones between recharge and saline seep areas to intercept saline water and decrease salt concentration in the accumulation zone. A salt tolerant legume capable of providing an economic yield offers many other benefits to arid and semi-arid cropping systems. In addition to replenishing soil nitrogen, cereal-legume rotations may be used to control disease, weed, and insect pests.

The use of nitrogen fertilizer has increased more than 350% in the last two decades (U.S. Bureau of Census, 1980). Additionally, nitrogen costs have increased markedly in the last 10 years. Crop management systems using legumes capable of fixing part or all of their nitrogen requirements offer many benefits. Commercial nitrogen fertilizer cost approximately \$108 (kg X 100)⁻¹N in 1982. Comparatively, the cost of inoculating large-seed legumes with commercial Rhizobium inoculum ranges from \$1-4 ha⁻¹.

The effect of soil salinity on yield and dinitrogen fixation in legumes is not well defined. The effect of salinity on plants has been considered a function of the resulting decrease in soil water potential (Schimper, 1903; Kramer, 1959). Schimper described 'physiological dryness' as a result of moisture being "more attracted to the soil than to the root". Wilting of a healthy plant subjected to saline irrigation was presumed to result totally as the inability to withdraw moisture from the soil against an increased potential gradient. However, 'physiological dryness' is not the complete solution to the understanding of salt effects on plant growth. A plant that is gradually subjected to saline irrigations of increasing

salt concentrations may not wilt (Nieman, 1965; Bernstein and Hayward, 1958). Apparently, these plants can partially adjust to increased soil salinity.

Halophytic plants may grow in media having a potential of almost -50 bars by adjusting the potential within the protoplasm in compensation for potential in the root environment (Waisel and Pollak, 1969). It is now well established that glycophytic plants are also capable of osmotic adjustment in salinized media (Bernstein and Hayward, 1958; Waisel, 1972; Stewart and Lee, 1974). Specific effects of osmotic adjustment must be studied further to determine how salinity decreases plant growth.

Waisel (1972) reported that, in general, salinity caused the accumulation of osmotica, which increased protoplasm viscosity and decreased streaming velocity. Other physiological effects, such as a decrease in the number and size of vascular channels in tomato (Lycopersicon esculentum Mill.) and cotton (Gossypium hirsutum L.) (Strogonov, 1962), decrease in the number of stomata in bean (P. vulgaris L.) and cowpea [Vigna unguiculata (L.) Walp.] (Ahmed et al., 1980), and changes in organelle morphology in wheat (Triticum aestivum L.) (Udevenko et al, 1970) have also been observed. Concentration increases of specific ions in a plant as a result of ion accumulation in the soil have been noted in bean and cowpea (Ahmed et al., 1980) and in wheat, barley (Hordeum vulgare L.), oat (Avena sativa L.), pea (Pisum sativum L.), chickpea (Cicer arietinum L.), lentil (Lens culinaris Medic.), cotton, sorghum (Sorghum bicolor L.), corn (Zea mays L.), and rice (Oryza sativa L.) (Das and Mehrota, 1971).

Specific ions have been shown to inhibit *in vitro* enzyme activity (Eaton et al., 1971; Osmond, 1976), modify photosynthesis (Nieman, 1962), decrease incorporation of amino acids into protein (Kahane and Poljakoff-Mayber, 1968), and shift metabolic pathways (Porath and Poljakoff-Mayber, 1968).

Plants growing in NaCl or Na₂SO₄ solutions of one atmosphere potential exhibited a shift in glucose metabolism toward the pentose-phosphate pathway (Porath and Poljakoff-Mayber, 1968). The activity of most glycolytic enzymes was depressed. Only phosphogluconate dehydrogenase remained unaffected by applied salts. The activity of glucophosphate isomerase was inhibited by NaCl but not by Na₂SO₄. This behavior is usually indicative of mature plant tissue. Growing tissues depend on the ATP and carbon frame intermediates provided by glycolysis and the Krebs cycle for synthesis of new tissue. Stress conditions are known to modify the rate of plant senescence under field conditions (Woolhouse, 1978). Sodium and calcium are strong inhibitors of enzyme activity, while Mg⁺⁺ and K⁺ have few adverse effects (Dixon and Webb, 1964). Generally, glycophytes are more sensitive to Na⁺ and halophytes are more sensitive to Ca⁺⁺ (Bernstein and Hayward, 1958).

Ion ratio in a medium is important since certain ions may counteract the deleterious effects of others. La Haye and Epstein (1969) reported that size reduction in *P. vulgaris* L. grown in nutrient solution containing 50 mM NaCl did not occur when the solution contained at least 1 mM CaSO₄. Nieman and Willis (1971) hypothesized that divalent cations stabilize, while monovalent cations

disrupt linkages between the outer cell and proteins required for active solute uptake. Sodium chloride was more effective than Na_2SO_4 in the release or absorption of ions. Calcium, Mg^{++} , K^+ , and proteins were released, Na^+ was absorbed, and glucose and inorganic P uptake was inhibited when either NaCl or Na_2SO_4 was applied to viable carrot root cells. Kahane and Poljakoff-Mayber (1968) also reported differential plant response to NaCl and Na_2SO_4 at the same osmotic concentration. Sodium sulfate inhibited L-leucine uptake and incorporation into protein more than NaCl.

Eaton et al. (1971) reported no reduction in plant growth when SO_4^{--} and Cl^- were applied alone. Sulfate and chloride increased in tomato and cotton plant tissues exposed to SO_2 and HCl. Additionally, K^+ increased with no deleterious effects.

Salts may affect fertility through interference with uptake and/or metabolism of other nutrients. Nieman and Clark (1976) reported an inorganic phosphorous deficit at phosphorylation sites in mature (photosynthesizing) corn leaves when grown in a salinized medium. This resulted in a reduction of ATP and adenylate energy. Additionally, an increase in phosphorous to 2 mM resulted in a toxic inorganic concentration. Bernstein et al. (1974) reported that high phosphorous levels increased salt injury to corn, cabbage (Brassica oleracea L. cv capitata) and broccoli (Brassica oleracea L. cv botrytis). Ferguson and Hedlin (1963) reported greater phosphorous response in barley with increasing soil salinity. The quadratic absorption response of P reached a maximum at approximately 6 mmhos (EC). Wilson (1970) found that a higher proportion of absorbed P

remained in soybean [Glycine max (L.) Merrill] roots subjected to salt, especially when inoculated in the presence of applied nitrogen. Khalil et al. (1967) reported P uptake in corn and cotton to be proportional to the root surface, which decreased with salt in the medium. Additionally, K^+ concentration decreased in cotton as salinity increased. Maas et al. (1972) reported no evidence that soil salinity affects Fe, Mn, or Zn availability in tomato, squash (Curcubita pepo L.), soybean, and snap bean.

Saline soils reduce yields because of poor germination, dry matter production, and seed yield (Das and Mehrota, 1971; Abel and McKenzie, 1964; El Karouri, 1979; Ayoub, 1977). Additionally, salinity may influence legume symbiosis. Salinity may affect Rhizobia survival in the soil, infectivity, nodule development, and dinitrogen reduction in the nodule. Unfortunately, most research results have been based on experiments that initiated saline treatments after germination, or after the plant had fixed nitrogen (Ayers and Eberhard, 1960; Balasubramanian and Sinha, 1976; Bernstein et al., 1974; Weimberg, 1970; Shannon, 1978; Ayers et al., 1952; Patel et al., 1975).

Lakshmi-Kumari et al. (1974) reported that Rhizobia growth in test tubes containing soil extract was unaffected by 0.0-0.6% NaCl. However, alfalfa (Medicago sativa L) symbiosis was indirectly suppressed as a result of decreased root hair number, infection thread number, and size of mucilaginous layer.

Respiration has a direct effect on dinitrogen reduction in plants. Moustafa and Mortenson (1967) reported that ATP/ADP ratios of

0.5 or less completely blocked nitrogenase activity. Sprent (1972) suggested that decreased nitrogen fixation and respiration of soybean nodules under stress resulted from alterations in nodule cortical cell metabolism.

Nitrogenase enzyme inhibition under saline conditions may be a specific ion effect. The active enzyme complex of nitrogenase consists of two metallo-protein subunits (Fe-protein and FeMo-protein), the latter being salt labile. Nitrogen fixation exhibited sigmoid kinetics when plotted versus concentration of Fe-protein with the FeMo-protein held constant (Dalton and Mortenson, 1972). Sodium chloride addition to an *in vitro* reaction mixture resulted in increased sigmoidicity of the response curve (decreased activity). This indicates that some of the Fe protein may be rendered ineffective with the addition of salt (Burns and Hardy, 1975).

Saline irrigation has been reported to suppress symbiotic dinitrogen fixation. Cowpea and mung bean [*Vigna radiata* (L.) R. Wilcz.] grown in a medium containing NaCl exhibited a reduction in dinitrogen fixation proportional to the salt concentration (Balasubramanian and Sinha, 1976). Fixation was suppressed as a result of reduced nodule formation.

Ayers and Eberhard (1960) reported that greenhouse experiments using equal equivalents of NaCl and CaCl₂ at 6 mmhos (EC_e) reduced faba bean dry matter by 50%. Unfortunately, no measurement of seed yield was obtained. El Karouri (1979) reported that faba bean, grown in the Sudan, where the predominant salt was Na₂SO₄, is more salt tolerant. Maximum dry matter and seed yield were reduced 50% at 10.5

and 9.0 mmhos (EC_e), respectively. Neither of these studies evaluated dinitrogen fixation.

Helal and Mengal (1981) grew *V. faba* L. 'Ackerperle' in the greenhouse with 50 mM NaCl under two light regimes (55 and 105 $w\ m^{-2}$). They reported the uptake of $^{14}CO_2$ g^{-1} dry weight was greatly suppressed at low light intensity and slightly suppressed at high light intensity. Additionally, assimilation of photosynthate into cell components, especially into the lipid fraction, was reduced. Their data suggested that high light intensity provided more energy for regulation of internal ionic conditions. Plants grown under the higher light intensity were more capable of accumulating Ca^{++} , Mg^{++} , and K^+ and excluding Na^+ and Cl^- . Helal and Mengal also reported that the concentration of amino acid nitrogen remained the same as salinity increased. Conversely, inorganic nitrogen increased, and protein nitrogen decreased.

Dry bean is one of the most salt sensitive crops (Bernstein, 1964; U.S. Salinity Lab, 1954). Bernstein (1964) reported that dry matter and seed production were reduced 50% at 3.0 and 3.2 mmhos, respectively. Field research involving the effect of soil salinity on dry bean is limited. Sameni et al. (1980) evaluated the interactions of several fertility levels with NaCl salinity on growth of *P. vulgaris* L. in the greenhouse. Shoot dry weight decreased 50% between 1.5 and 2.5 mmhos (EC_e). Yield, percent nitrogen, and total nitrogen decreased as salinity increased. Nitrogen fertilized plants had higher yield and contained more nitrogen than those not fertilized. Sodium and chloride uptake increased (Wignarajah et al, 1975b) and

leaf development was reduced (Nieman, 1965) when *P. vulgaris* L. was grown in NaCl media. Leaf size reduction resulted from decreased cell number (Wignarajah et al, 1975a; Nieman 1965). Cell size was identical for plants grown with or without salt.

Nieman and Poulsen (1971) reported that autotrophic structures (leaves and stems) of dry bean were more sensitive to excess salinity than heterotrophic structures (root). Additionally, Bernstein and Hayward (1958) reported that salt suppressed shoot growth more than root growth.

Strogonov (1962) and Waisel (1972) reported that, in general, salinity suppressed leaf size, reduced stomata number, increased succulence, impaired vascular tissue development, advanced root lignification, inhibited phloem transport, and thickened the cuticle and surface wax layer in plants. Increased salinity decreased transpiration per unit leaf area and increased resistance to water flow in dry bean (Hoffman and Phene, 1971).

Some plants have differential varietal response to soil salinity (Abel and McKenzie, 1964; Rush and Epstein, 1976; Shannon, 1978). Generally, wheat is less salt tolerant than barley (U.S. Salinity Lab, 1954). However, some wheat varieties outyield barley in a saline environment (U.S. Salinity Lab, 1954). Successful plant breeding for salt tolerance may increase substantially, when specific reactions of plants to saline conditions and mechanisms of plant tolerance to salinity are understood.

CHAPTER III

MODEL FOR REGRESSION ANALYSIS

A sigmoid transformation (Jensen and Homeyer, 1970) was used in regression analysis of data exhibiting sigmoid characteristics. This transformation allowed a direct, linear comparison of nitrogen effects. The sigmoid model eliminated the need for numerous interaction factors which often accompany the more common, polynomial regression model.

The ultimate goal of regression analysis is the evaluation of the expected spatial relationship between continuous variables. This implies that a suitable model, arrived at independently of the data being tested, be developed prior to analysis.

Because research may be exploratory, this approach is not always feasible. If the exact function of one variable upon another was known, the research would not have been necessary. However, this should not preclude the use of existing information indirectly associated with the specific effects of the variable(s). For instance, the exact nature of the effects of soil salinity on V. faba L. throughout the growing season is unknown. Previous experiments have shown the yield relationships with salinity to be inverse and linear (El Karouri, 1979; Ayers and Eberhard, 1960). Additionally, plant growth has been shown to be sigmoid (Emmerling, 1880; Winsor, 1932; Tisdale and Nelson, 1975; De Sapia, 1978). Stress may result

in early senescence (Itai and Vaadia, 1965; Wright and Hiron, 1969). Existing information should be used to formulate a model for regression analysis.

The regression coefficient is subject to bias, and variability is increased from lack of fit, if a proposed model is not an accurate description of the true relationship between continuous variables (Draper and Smith, 1966; Snedecor and Cochran, 1980). Therefore, both accuracy and precision are reduced. The closer a proposed model is to the true functional relationship, the more powerful the statistical analysis.

Models may be functional, controlled, or predictive in nature (Draper and Smith, 1966). Functional models describe the actual relationship between response and independent variables. They may be complicated, difficult to interpret and use, and may be intrinsically non-linear for statistical analysis.

Controlled models are similar to functional models, but describe the response only as a function of controlled variables. They are accurate over the parameters measured, easy to interpret, and intrinsically linear for multiple linear regression, with correct data transformation.

A predictive model, while not describing the true functional relationship, is close enough to predict the value of the response in question with reasonable accuracy, although perhaps over a limited range of the independent variable. Precision may become unreasonable when lack-of-fit is extreme.

The most common predictive model used in multiple linear regression to describe curvilinear relationships is the polynomial (Mead and Pike, 1975). Mead and Pike noted that "polynomials seem to be used as the simplest, readily available smoothing curve, without any appeal to their theoretical properties as approximations to the true response functions."

Biological growth curves are commonly expressed using a polynomial predictive model, or simply graphed without mathematical description, because of the difficulty in fitting these curves to a true functional model (Wisehart, 1938; Westermann et al., 1981; Kleinkopf et al., 1981). "Matchacurve" techniques developed by Jensen and Homeyer (1970) and Jensen (1979) provide a means of transforming sigmoid data to an intrinsically linear form for multiple linear regression analysis.

Problems may result when using polynomials as models for plant growth analysis, because treatment effects over time are usually proportional, rather than additive. For example, a treatment expected to increase yield, such as application of fertilizer nitrogen, may increase dry weight from 1.0 to 1.2 g at the seedling stage; while the overall increase at maturity is from 10 to 12 g. The difference in seedling growth is .2 g, and the difference at maturity is 2 g; however both differences represent a 20% proportional increase in yield. To represent this proportional treatment effect over time in an additive, polynomial model requires variables for nitrogen (N), time (T), and the linear by linear variable of nitrogen with time (N x T). Where the relationship is curvilinear, other factors may need to

be included, such as the interaction of nitrogen with the quadratic time factor ($N \times T^2$). With the addition of another main factor, such as salinity (S), the model may have to include the interaction factors: $N \times S$, $N \times S^2$, $S \times T$, and $S \times T^2$. The result is a complicated model in which nitrogen effects are diluted over several interaction factors. Snedecor and Cochran (1980) suggest that the accumulation of interaction factors in a regression model warns that the model may be inadequate. Perhaps a multiplicative model, where $Y=B_0+B_1X_1+B_2X_2$ becomes $Y=B_0+B_1(f(X_1,X_2))$ would be more appropriate.

A predictive model, closely approximating the true functional response of plant growth to time and soil salinity, is $Y=A(S)\exp(-k(T,S))$, where $A(S)$ is a function of salinity (S) and $k(T,S)$ is a function of both time (T) and salinity. The removal of bias resulting from lack-of-fit improves accuracy in measuring and expressing the effects of salinity on sigmoid plant growth. The removal of variation from lack-of-fit improves precision in the analysis of treatment effects.

Jensen (1979) states that the degrees of freedom sacrificed through graphic model development are unknown. It is submitted here that, as in development of a non-linear, multiplicative model using Taylor's Theorem (Snedecor and Cochran, 1980), one degree of freedom should be removed for each parameter in the model estimated graphically. In cases where the parameter is a function, degrees of freedom removed should correspond to the type of function. For instance, if in $A(S)\exp(-k(S,T))$, $A(S)$ is a linear function, two degrees of freedom are removed for that parameter. It must be

emphasized that use of the transformation should be based on theory appropriate to the particular problem at hand. Otherwise, bias may be entered, rather than removed.

The choice of the $A(S)\exp(-k(S,T))$ function was selected because of the high degree of confidence in that function as the true response determined by previous experience in plant growth relationships (Emmerling, 1880; Winsor, 1932; Tisdale and Nelson, 1975; De Sapiro, 1978). The justification for its use is reflected in the high R^2 values found when that transformation was employed. The R^2 values were at least as good as those obtained using a polynomial model for the variables and their interactions. The precision and utility gained by the models produced using "Matchacurve" (Jensen and Homeyer, 1970; Jensen, 1979) demonstrates the value of these techniques when applied to regression analysis of biological growth curves.

The following 2-way functional model (time x salinity) characterizes the process developed by Jensen and Homeyer (1970). A sigmoid curve giving least deviations is hand fit through the data plots (Karst, 1958). Noting the peak value (Y_p) of the response variable and the corresponding time at which this value is reached (X_p), a rescaled curve (Y/Y_p vs X/X_p) is drawn, conforming to the dimensions of 19.05 cm (7.5 inches) in the X direction and 12.7 cm (5.0 inches) in the Y direction. This curve is compared to the standard curves provided in the Matchacurve booklets (Jensen and Homeyer, 1970; Jensen, 1979) and interpolated to provide the best transformation available to describe the data. The scaled function is described by $\exp(-k(T,S))$, where $\exp(-k(T,S))$ is a value ranging

between zero and one, and $k(T,S)$ is a function of time and salinity.

The actual function is (where brackets $[\]$ represent absolute value):

$$Y/Y_p = \frac{\exp(-[(X/X_p-1)/(X_i/X_p-1)]^n) - \exp(-[(X_0/X_p-1)/(X_i/X_p-1)]^n)}{1 - \exp(-[(X_0/X_p-1)/(X_i/X_p-1)]^n)}$$

where X is time, X_p is the time at which maximum response (Y_p) is obtained, X_0 is zero (X_0/X_p is consequently zero), X_i/X_p is the inflection point of the sigmoid curve, \exp is the base of the natural logarithm, and n is a constant. The values of n and X_i/X_p are determined using the standard graphs provided in the matchacurve booklet. The right side of both the numerator and the denominator;

$$\exp(-[(X_0/X_p-1)/(X_i/X_p-1)]^n)$$

is usually a value very close to zero and can be dropped in most cases, simplifying the equation to:

$$Y/Y_p = \exp(-[(X/X_p-1)/(X_i/X_p-1)]^n)$$

In some cases, values for X_p , X_i/X_p , and n may prove to be a function of the independent variable(s), and these functions may be substituted into the equation accordingly.

To complete the model ($A_S \exp(-k(T,S))$) for any particular salinity treatment, the value for A_S must be determined. Regression analysis using the $\exp(-k(T,S))$ transform as the independent variable results

in A_S as the regression coefficient. The equation $Y/Y_p = \exp(-k(T,S))$ becomes $Y = A_S \exp(-k(T,S))$, where $A_S = Y_p$. When the value of A_S for each salinity level (EC_e) has been determined, the function $A(S)$ of A_S vs EC_e can be substituted for A_S , resulting in the equation $Y = A(S) \exp(-k(T,S))$, where $A(S)$ is now a function of salinity. The equation now represents a 3-dimensional response surface.

Goodness of fit may be estimated by least squares analysis over the entire surface, where the sigmoid transformation $A(S) \exp(-k(T,S))$ is the independent variable, and the raw data is the dependent variable. The resulting regression coefficient should be very close to one, and the analysis of residuals should not reveal a pattern which would suggest lack-of-fit of the proposed model.

The following example is provided to illustrate the varying degrees of precision associated with available regression models, when analyzing sigmoid growth.

Hypothetical data representing sigmoid growth of plants grown both with and without fertilizer nitrogen are given in table 1. Coded levels for with nitrogen and without nitrogen are 1 and -1, respectively. Yield on any particular day is approximately 10% greater for plants grown with fertilizer nitrogen. This ultimately results in a final yield of 105 g for plants grown with fertilizer nitrogen and 95 g for plants grown without.

Table 2. gives regression coefficients and their standard errors for each of the models employed: linear, polynomial with all possible variables, polynomial obtained using a backward elimination procedure (Draper and Smith, 1966), and the $\exp(-k(T))$ (sigmoid) transformation.

Table 1. Dependent and independent variables of a hypothetical growth analysis of plants grown with coded levels of fertilizer nitrogen.

<u>N</u>	<u>TIME</u>	<u>DRY WEIGHT</u>
1	20	1.93
-1	20	1.71
1	40	11.07
-1	40	10.23
1	60	38.63
-1	60	34.90
1	80	81.77
-1	80	74.01
1	100	105.11
-1	100	94.89

The standard error of the estimate at 100 days for each of the models is also included.

The fit of each regression model to the actual data points (figs. 1a,1b,1c,1d) illustrates how lack-of-fit decreases as the model closely approaches the true functional relationship. The standard error is greatest for the linear model (fig. 1a), decreases in the polynomial models (figs. 1b and 1c), and is smallest when the sigmoid transformation is used.

The polynomial model using all possible variables (fig. 1b) is a very good fit, but the significance of the nitrogen effects is not readily apparent. Nitrogen effects are diluted over several interaction components. Partial correlation coefficients for the interaction variables are not statistically significant. In fact, when this model is used, nitrogen effects of 40 g in peak yield are not statistically significant!

The backward elimination model, in this case, did show a

significant nitrogen interaction variable ($N \times T^2$). However, this model loses precision because of lack-of-fit. It loses the ability to detect nitrogen differences as the treatment effect gets smaller. The sigmoid model is able to detect nitrogen treatment differences less than 1 g, given the variability defined in this case.

To demonstrate the model development process for the data in this thesis, an example is provided using data for total nitrogen accumulation in faba bean (field experiment). The plot (fig. 2) of the mean values for each salinity level at four harvest dates suggests sigmoid growth with a reduction in time to maturity, proportional to applied salinity stress.

Hand-fit, sigmoid curves giving least deviations (through the means in this case) are given in figure 3. Curves are extrapolated when necessary only for model development. Statistical inferences are reported for data only throughout the period included in the analysis. The time required to attain maximum yield (X_p) is found graphically for each salinity level. Each curve is then divided into ten equal segments, from zero to X_p . As an example, the values for the sigmoid curve representing growth at 21.8 mmhos are given in table 3. Maximum nitrogen content was interpolated to be at 95 days after planting.

