



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 CaCl<sub>2</sub>) 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<sup>-1</sup> ECe, respectively. Data were analyzed using multiple regression. Growth parameters exhibiting sigmoid responses were regressed using an e<sup>-K</sup> (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<sup>++</sup>. 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

Agronomy

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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 CaCl<sub>2</sub>) 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<sup>-1</sup> EC<sub>e</sub>, respectively. Data were analyzed using multiple regression. Growth parameters exhibiting sigmoid responses were regressed using an e<sup>-k</sup> (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<sup>++</sup>. 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  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{CO}_3^{--}$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{--}$ , and  $\text{Cl}^-$  (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  $\text{CaSO}_4$ ,  $\text{MgSO}_4$ , and  $\text{Na}_2\text{SO}_4$  (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)<sup>-1</sup>N in 1982. Comparatively, the cost of inoculating large-seed legumes with commercial Rhizobium inoculum ranges from \$1-4 ha<sup>-1</sup>.

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 Na<sub>2</sub>SO<sub>4</sub> 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 Na<sub>2</sub>SO<sub>4</sub>. 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<sup>++</sup> and K<sup>+</sup> have few adverse effects (Dixon and Webb, 1964). Generally, glycophytes are more sensitive to Na<sup>+</sup> and halophytes are more sensitive to Ca<sup>++</sup> (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 CaSO<sub>4</sub>. 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  $\text{Na}_2\text{SO}_4$  in the release or absorption of ions. Calcium,  $\text{Mg}^{++}$ ,  $\text{K}^+$ , and proteins were released,  $\text{Na}^+$  was absorbed, and glucose and inorganic P uptake was inhibited when either NaCl or  $\text{Na}_2\text{SO}_4$  was applied to viable carrot root cells. Kahane and Poljakoff-Mayber (1968) also reported differential plant response to NaCl and  $\text{Na}_2\text{SO}_4$  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  $\text{SO}_4^{--}$  and  $\text{Cl}^-$  were applied alone. Sulfate and chloride increased in tomato and cotton plant tissues exposed to  $\text{SO}_2$  and HCl. Additionally,  $\text{K}^+$  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 CaCl<sub>2</sub> at 6 mmhos (EC<sub>e</sub>) 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 Na<sub>2</sub>SO<sub>4</sub>, 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.

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<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.

Table 2. Variables and regression coefficients with their standard errors and P-values for comparison of regression analyses using linear, polynomial and sigmoid models.

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<u>VARIABLE</u>	<u>B</u>	<u>SE(B)</u>	<u>P-VALUE</u>
LINEAR			
N	-1.76	7.041	0.811
T	1.32	.106	0.001
N x T	.07	.106	0.594
INTERCEPT= -33.65 $R^2=.963$ SE(EST) AT MAX. YIELD = 7.35			
POLYNOMIAL (ALL POSSIBLE VAR)			
N	2.125	13.49	0.899
T	-3.142	.882	0.070
N x T	-.1727	.882	0.863
T <sup>2</sup>	.0755	.0164	0.044
N x T <sup>2</sup>	.0040	.0164	0.829
T <sup>3</sup>	-.0004	.0001	0.053
N x T <sup>3</sup>	.00002	.0001	0.846
INTERCEPT= 37.96 $R^2=.998$ SE(EST) AT MAX. YIELD = 3.94			
POLYNOMIAL (BACKWARD ELIM.)			
T	-3.14	.565	0.003
T <sup>2</sup>	.07	.010	0.001
T <sup>3</sup>	.0003	.00006	0.001
N x T <sup>2</sup>	.0005	.00014	0.013
INTERCEPT= 37.96 $R^2=.998$ SE(EST) AT MAX. YIELD = 2.24			
SIGMOID TRANSFORM			
EXP(-k)	99.96	.077	0.001
N x EXP(-k)	5.06	.049	0.001
INTERCEPT=.03 $R^2=.999$ SE(EST) AT MAX. YIELD = 0.07			

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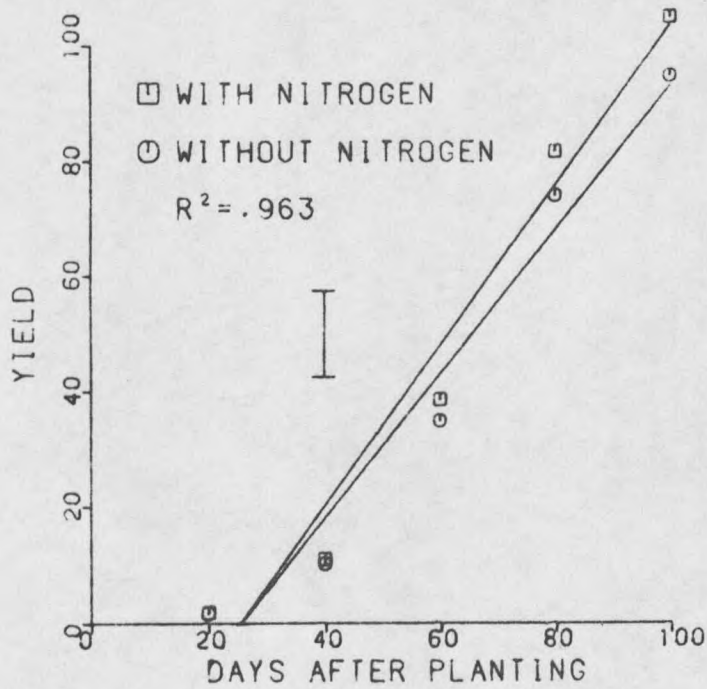


Figure 1a. Fit of linear model to data points representing sigmoid growth pattern. Hash mark represents standard error of the estimate at 100 days.

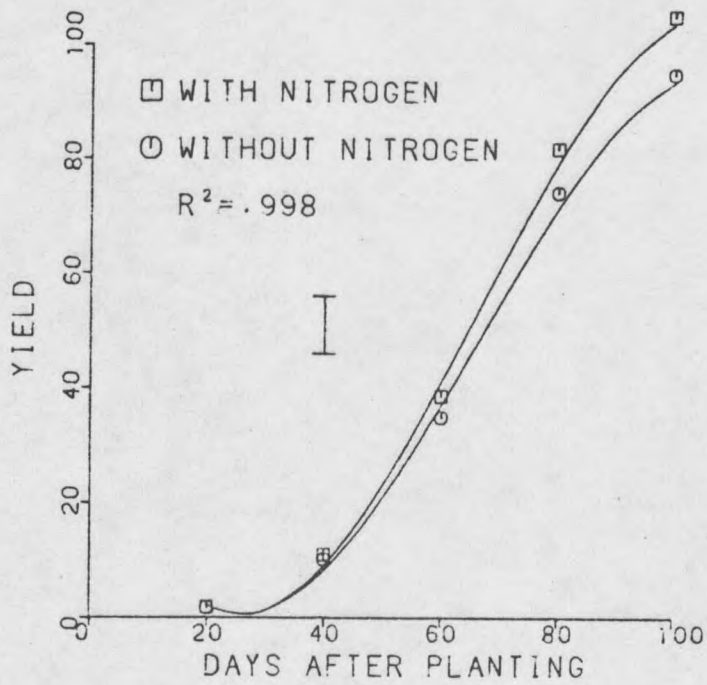


Figure 1b. Fit of polynomial model using all possible variables to data representing sigmoid growth pattern. Hash mark represents standard error of the estimate at 100 days.

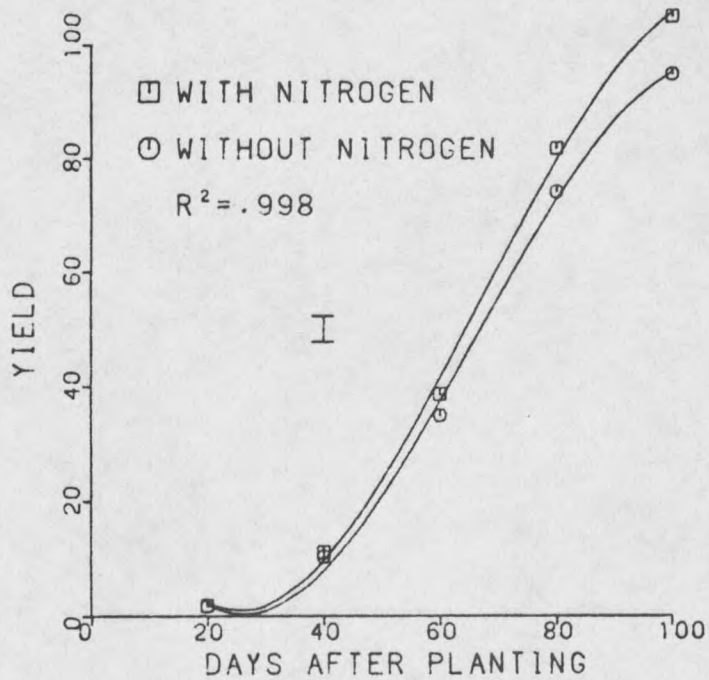


Figure 1c. Fit of polynomial developed using backward elimination to data representing sigmoid growth pattern. Hash mark represents standard error of the estimate at 100 days.

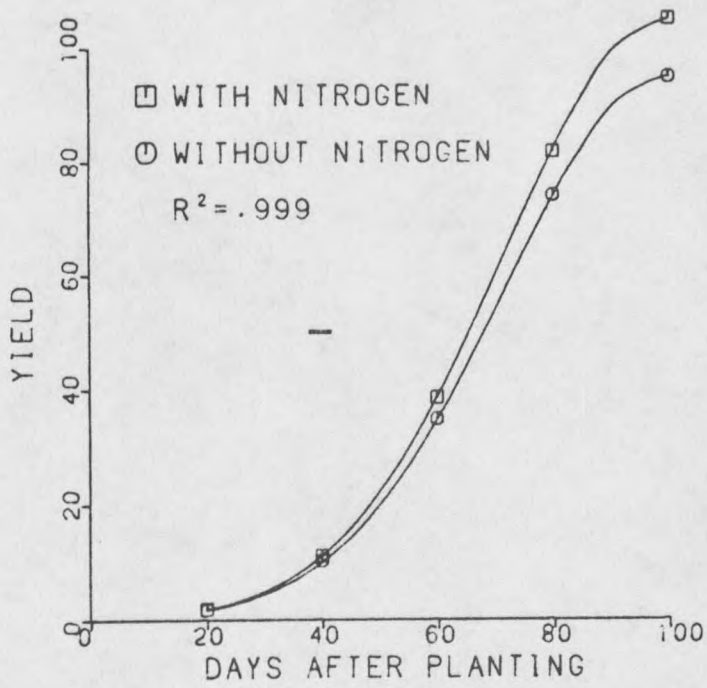


Figure 1d. Fit of sigmoid model to data representing sigmoid growth pattern. Hash mark represents standard error of the estimate at 100 days.

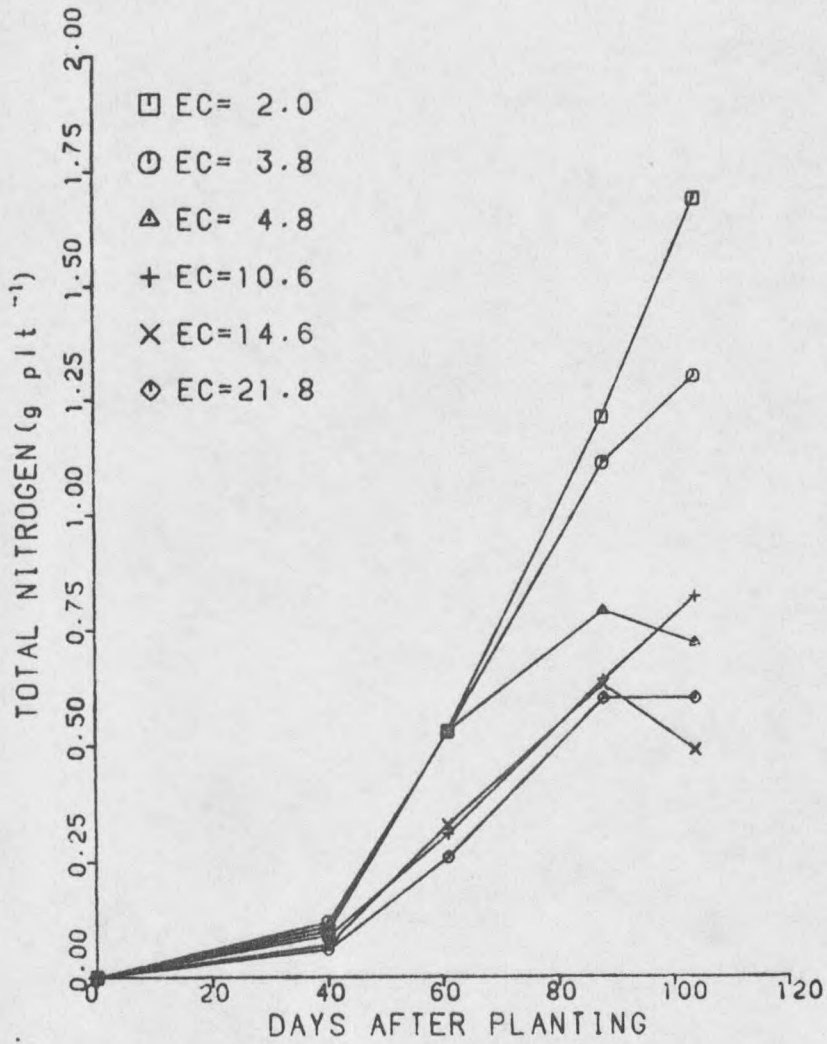


Figure 2. Mean values for total nitrogen for *V. faba* L. for each salinity value at four harvest dates at the SARC Huntley, MT. Plots are taken through the origin to demonstrate sigmoidicity.

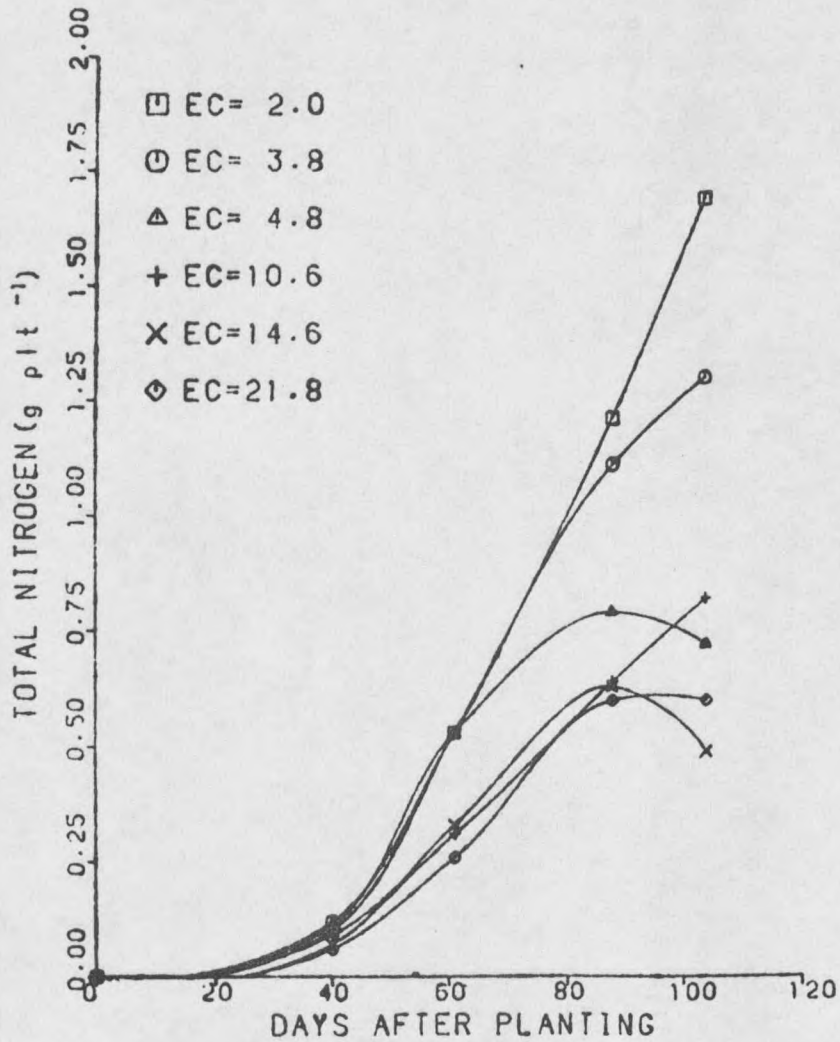


Figure 3. Hand-fit, sigmoid curves through the means of total nitrogen for *V. faba* L. measured at six salinity levels and four harvest dates at the SARC Huntley, MT. Plots are taken through the origin and extrapolated to  $X_p$  where necessary for model development.

To scale the sigmoid model for comparison to the standard curves given in the matchacurve booklet (Jensen and Homeyer, 1970), values for X and Y are converted to  $X/X_p$  and  $Y/Y_p$ , respectively (table 3.). The scaled sigmoid curve of  $X/X_p$  vs  $Y/Y_p$  is then graphed with an X axis of 19.05 cm (7.5 inches) and a Y axis of 12.7 cm (5.0 inches), and superimposed on the standards (fig 4.) until the proper family of curves is found. The scaled inflection point ( $X_i/X_p$ ) is found graphically, by interpolation if necessary.

Table 3. Scaled values of total nitrogen (Y) vs time (X) at 21.8 mmhos for faba bean at the SARC, Huntley, MT.

---

X	0.0	9.5	19.0	28.5	38.0	47.5	57.0	66.5	76.0	85.5	95.0
Y	0.0	0.0	.01	.02	.04	.10	.20	.33	.49	.61	.66
$X/X_p$	0.0	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.0
$Y/Y_p$	0.0	.00	.02	.03	.06	.15	.30	.50	.74	.92	1.0

---

When the values for  $n$ ,  $X_i/X_p$ , and  $X_p$  have been determined for each salinity value, they are plotted to determine their relationships with salinity. For total nitrogen, the exponent ( $n$ ) is constant (2.0),  $X_i/X_p$  (fig.5) is a function  $f(S)$  where:

$$f(S) = .59 + .0034S$$

and  $X_p$  (fig. 6) is a function  $g(S)$  where:

$$g(S) = 90.71 + 64.4S^{-1.1}$$

When these functions are substituted into the scaled curve form, the model becomes:

$$\exp(-k(S,T)) = \exp(-[(T/g(S)-1)/(f(S)-1)]^{2.0})$$

The only step left is to find a scalar function,  $A(S)$  (see page 24) which, when multiplied times the standardized curve form ( $\exp(-k(S,T))$ ), will describe the response surface as a function of salinity. To describe  $A(S)$ , values of  $Y_p$  are plotted vs salinity (fig. 7). This completes the model development process.

A plot of the standard error vs mean yield at each harvest date (fig. 8) for total nitrogen reveals that standard error is proportional to the square of the mean yield. Furthermore, an F-test of the null hypothesis that the sigmoid model goes through the origin is not significant. Accounting for these facts, a weighted least squares analysis was carried out according to Snedecor and Cochran (1975). The original analysis tested a response surface of 24 data points (4 harvest dates x 6 salinity Treatments). The analysis of variance used 6 degrees of freedom for regression and 18 degrees of freedom for lack-of-fit. Adequacy of the model was then tested using  $S_d^2/S^2$ , where  $S_d^2$  is the mean square for lack-of-fit (the mean square of the deviations of the 24 data points from the response surface), and  $S^2$  is the pooled, within treatments mean square. Since the lack-of-fit test was not significant, the regression model was tested again using the pooled mean square of  $S_d^2$  and  $S^2$  (Snedecor and Cochran, 1980). This allowed greater precision when testing for nitrogen effects.

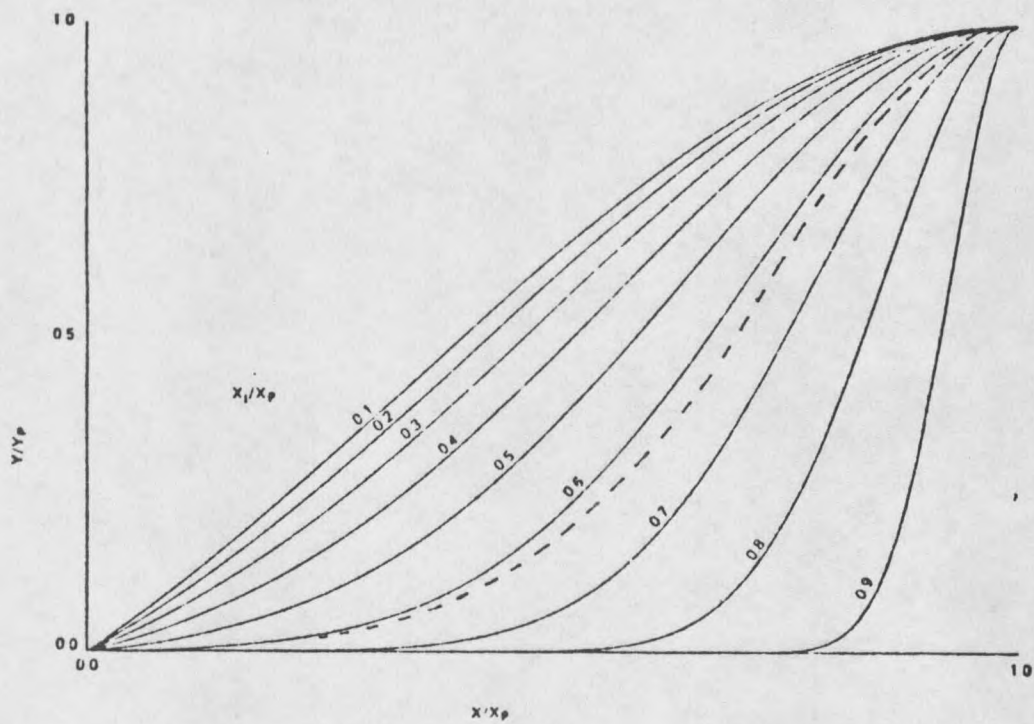


Figure 4. Set of standard curves of the family  $n=2.0$  (from Jensen and Homeyer, 1970). Dotted line represents the scaled curve of total nitrogen for *V. faba* L. at the SARC Huntley, MT.

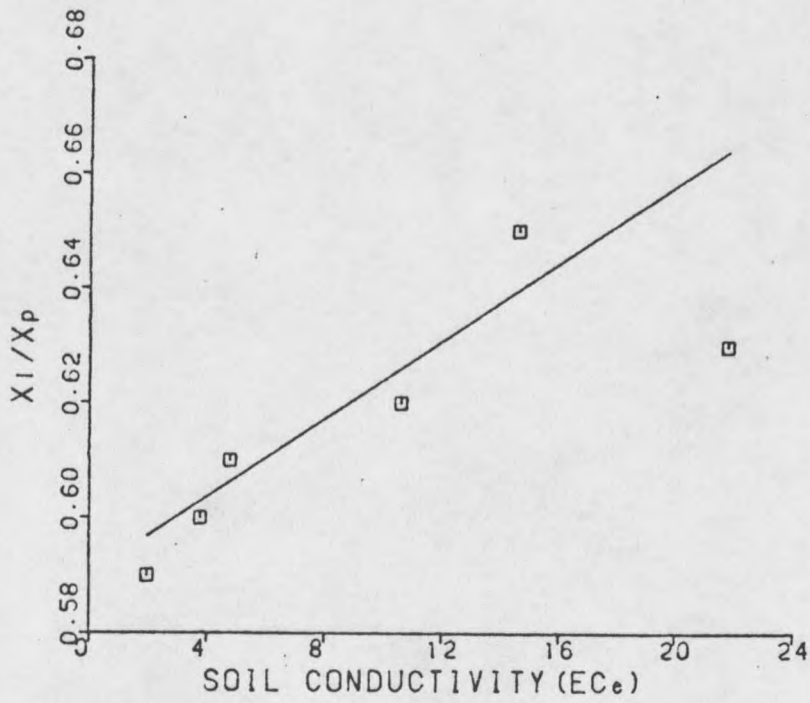


Figure 5. Inflection points ( $X_i/X_p$ ) of the family of six curves drawn through the means of total nitrogen for *V. faba* L. at the SARC Huntley, MT.

Least squares analysis of the raw data is accomplished over the entire response surface using total nitrogen measured at (S,T) vs the value of  $\exp(-k(S,T))$ . Since this is a comparison of a number against its estimated value, the resulting regression coefficient assigned by least squares analysis should be very close to one. The regression coefficient assigned in this case (table 4) is .98 with a standard error of  $\pm .05$ . The  $R^2$  of .63 is significant at  $P < .001$ . The model is a very good fit.

Table 4. Least squares fit of the sigmoid regression model.

<u>VARIABLE</u>	<u>B</u>	<u>SE(B)</u>	<u>P-VALUE</u>
A(S) $\exp(-k(S,T))$	.98	.06	0.001
NA(S) $\exp(-k(S,T))$	.13	.08	0.095
N(A(S) $\exp(-k(S,T))$ ) <sup>2</sup>	.14	.07	0.050

$$R^2 = .63$$

To determine the effects of fertilizer nitrogen on the average response surface, two other additional variables,  $(NA(S)\exp(-k(S,T)))$  and  $(N(A(S)\exp(-k(S,T)))^2$ , were introduced. The addition of these variables reduces the evaluation of nitrogen effects to a comparison of the curvilinear relationships of the response surfaces with  $(N = 1)$  and without  $(N = -1)$ . (An example in which nitrogen effects are linear in the comparison between response surfaces is given in figure 9.) The regression coefficients assigned to these factors (table 4) indicate the fractional increase or decrease of nitrogen accumulation

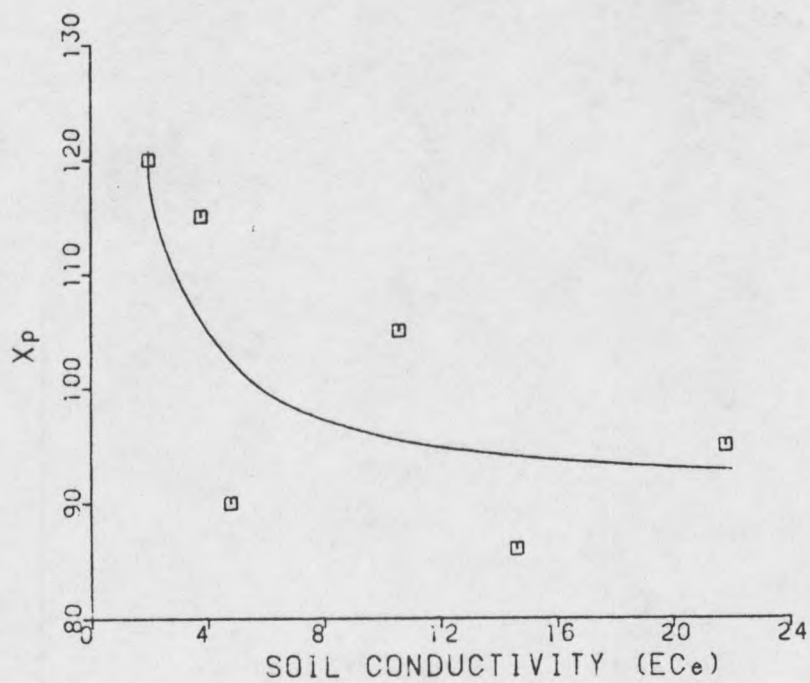


Figure 6. Time ( $X_p$ ) to accumulation of peak total nitrogen content for the family of six curves drawn through the means of total nitrogen for *V. faba* L. at the SARC Huntley, MT.

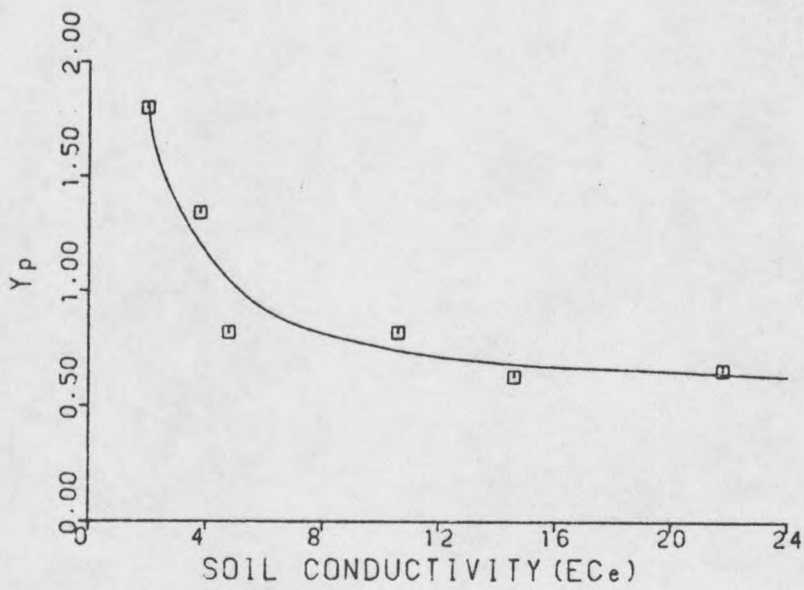


Figure 7. Peak yields ( $Y_p$ ) of the family of six sigmoid curves drawn through the means of total nitrogen for V. faba L. at the SARC Huntley, MT.

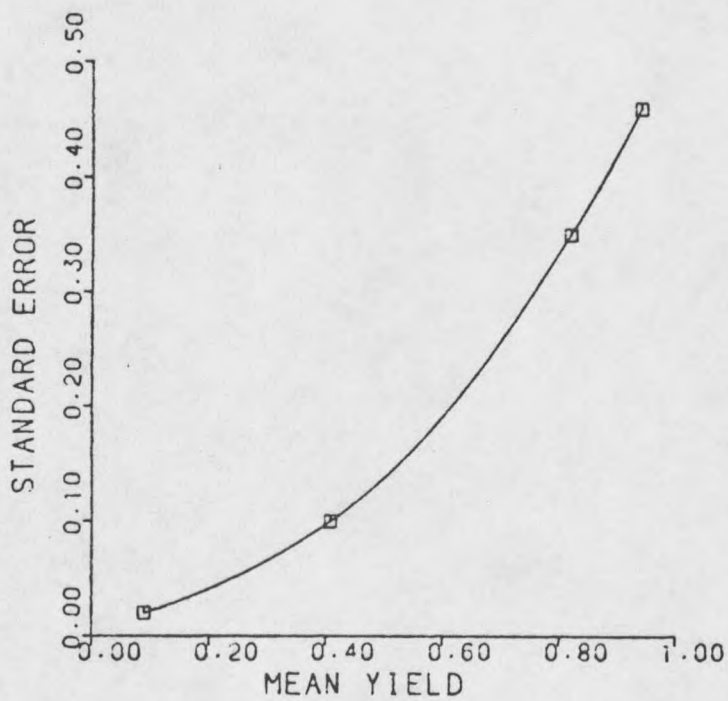


Figure 8. Standard error of total nitrogen measurements for *V. faba* L. at the SARC Huntley, MT. Standard error is proportional to the square of the mean yield.

over the entire response surface. The completed regression model is now:

$$Y = B_1A(S)\exp(-k(S,T)) + B_2NA(S)\exp(-k(S,T)) + B_3N(A(S))^2\exp(-k(S,T))$$

The standard error of the regression coefficients given by least squares analysis (table 4) are variables analogous to the increase or decrease in the response surface with fertilizer N. The standard error (.05) of the regression coefficient (.98) for the response surface  $A(S)\exp(-k(S,T))$  corresponds to approximately  $\pm 5\%$  of the value of the response surface. In other words, the standard error is equal to  $.05 \times A(S)\exp(-k(S,T))$  (fig.10).

It must be conceded that a model of this complexity fit through four data points (not including the origin) is tenuous, and inferences made through the interpolation between harvest dates must be considered as estimates. The comparison between nitrogen treatments is valid, however; and the regression model is valid if discussion is limited to inferences made at the harvest dates. Although interpolation to peak values may be of concern, the same must be said of the polynomial model. New data is not presently available to further evaluate the hypothesis, but intuitive confidence in the response surface is great enough to warrant adoption of the model as the best means of analysis available in the interim. The precision gained in analyzing for nitrogen effects strengthens this confidence.

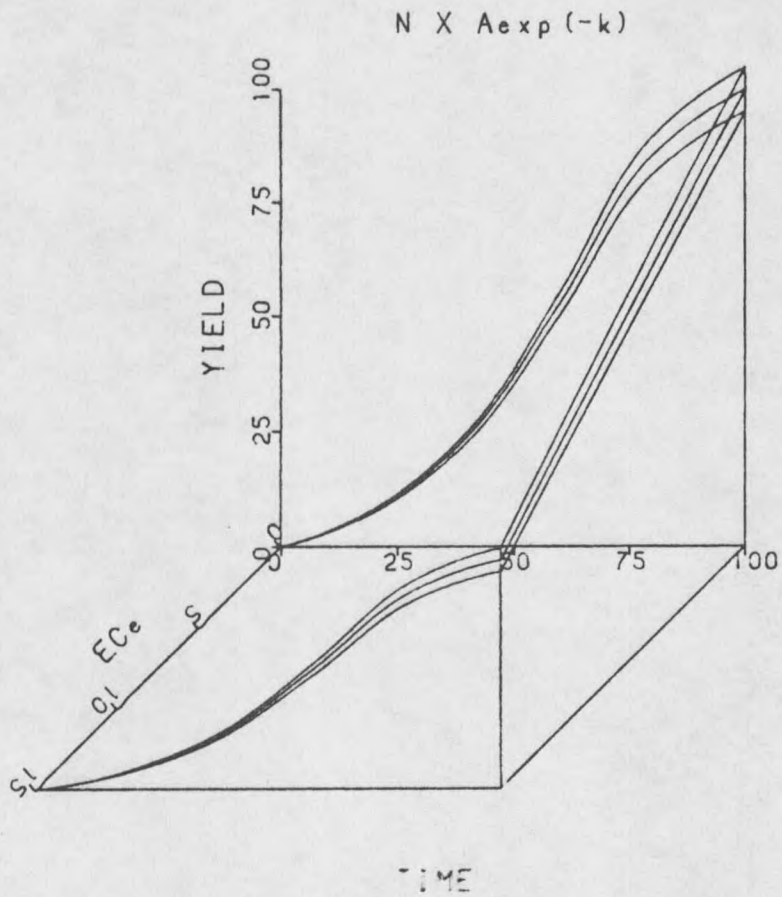


Figure 9. Model of nitrogen effects on a multiplicative, sigmoid response surface. The increase or decrease in yield is proportional to the average surface.

A prime consideration for future experiments is the inclusion of more data points on the time axis.

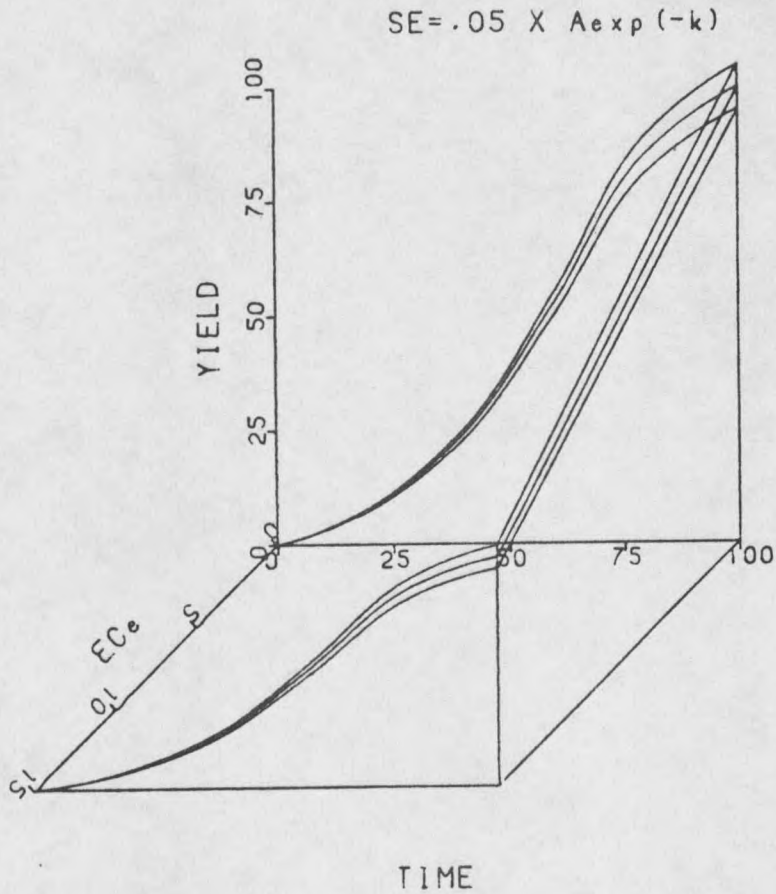


Figure 10. Model of standard error on a multiplicative, sigmoid response surface. The standard error is proportional to the yield.

## CHAPTER IV

EFFECTS OF SALINITY ON VICIA FABIA L.

Faba bean is moderately tolerant to saline conditions. Fifty percent yield reduction has been reported at 6 mmhos in equal equivalents of NaCl and CaCl<sub>2</sub> (Ayers and Eberhard, 1960) and at 9 mmhos when the predominant salt was Na<sub>2</sub>SO<sub>4</sub> (El Karouri, 1979). Many of the other large-seeded legumes that may be used in a cereal-legume rotation are highly sensitive to soil salinity. Properly inoculated faba bean plants can supply all the nitrogen required for optimum growth through symbiotic dinitrogen fixation and soil residual nitrogen in low nitrate soils (Richards and Soper, 1979). Faba bean requires a cool season for optimum development and is well adapted to the cereal-growing areas of western Canada (Duke, 1981). Therefore, faba bean has potential for use in cereal-legume rotations in cool, semi-arid regions of the United States.

Limited information is available regarding the effects of soil salinity on plant and seed yield (U.S. Salinity Lab, 1954; Ayers and Eberhard, 1960; El Karouri, 1979). Effects of soil salinity on symbiotic dinitrogen fixation and the interaction with fertilizer nitrogen have not been determined.

## MATERIALS AND METHODS

Field experiment. The effects of soil salinity on nodulation, dinitrogen fixation, growth and development of faba bean (*V. faba* L. 'Ackerperle') were evaluated in 1980 at the Southern Agricultural Research Station, Huntley, MT.

The soil was a Ryorp silt clay (Typic Cryocrepts) with 2% O.M., pH 8, and N, P, and K in the low, high, and high sufficiency range. The experimental area had been fallowed the previous year. Chopped wheat straw (12,000 kg ha<sup>-1</sup>) was soil incorporated 14 months prior to planting to reduce indigenous soil nitrogen.

A randomized complete block, split-block design with four replications was utilized. Six main plots (6 m<sup>2</sup>) received equal equivalents of NaCl and CaCl<sub>2</sub> resulting in approximate soil conductivity levels of 2, 4, 5, 11, 15, and 22 mmhos (EC<sub>e</sub>) at planting. Soil conductivity was monitored before and during the growing season at four week intervals (fig. 11). One part soil was mixed with two parts water (w/w) and allowed to settle before measuring conductivity. Solution conductivity was related to the saturation extract (fig. 12). Salt treatments were split into subplots containing 0 and 200 kg ha<sup>-1</sup> nitrogen applied as NH<sub>4</sub>NO<sub>3</sub>. Indigenous nitrate nitrogen in subplots not receiving fertilizer nitrogen was approximately 40 kg ha<sup>-1</sup> at planting.

Seed were planted 1 May 1980, 2.54 cm deep, in eight-row plots. Rows were 30 cm apart with 11 seed m<sup>-1</sup> (330,000 seed ha<sup>-1</sup>). Seed were sized to control variation in seedling vigor and inoculated with

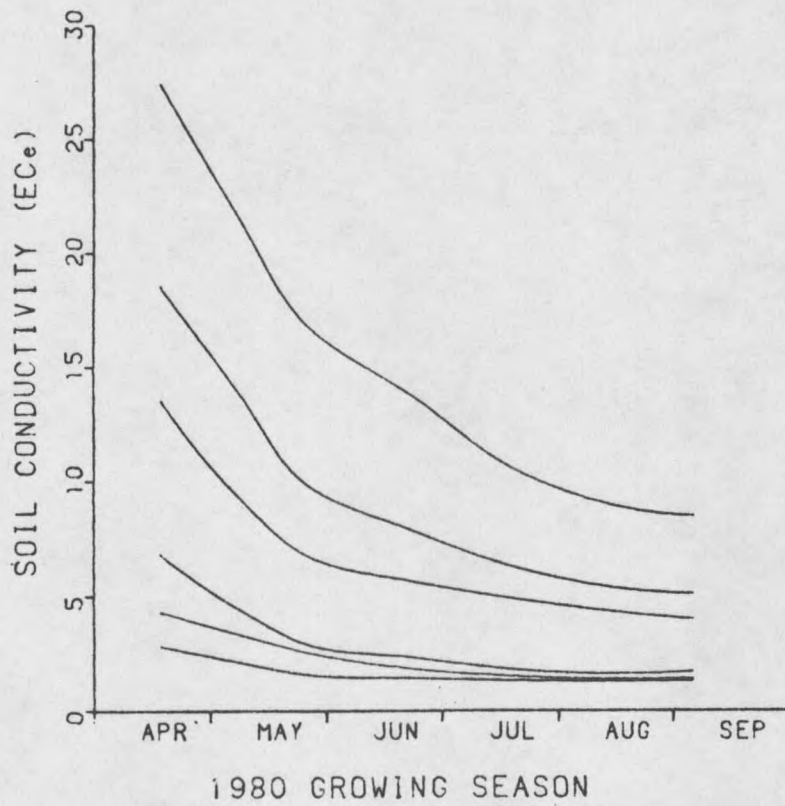


Figure 11. Soil conductivity ( $EC_e$ ) ranges for the six salt treatments at the SARC Huntley, MT.

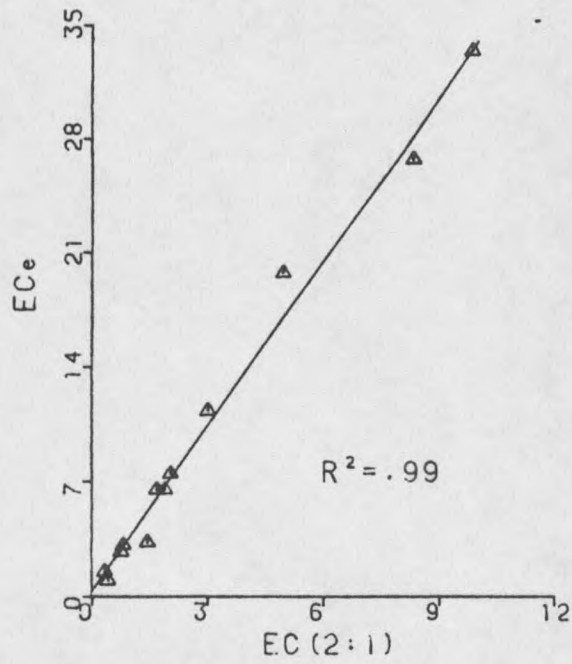


Figure 12.  $EC_e$  and  $EC_{2:1}$  comparison from the SARC Huntley, MT.

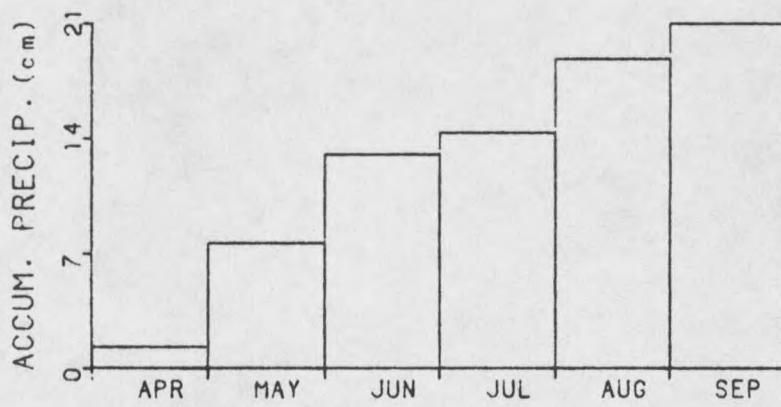
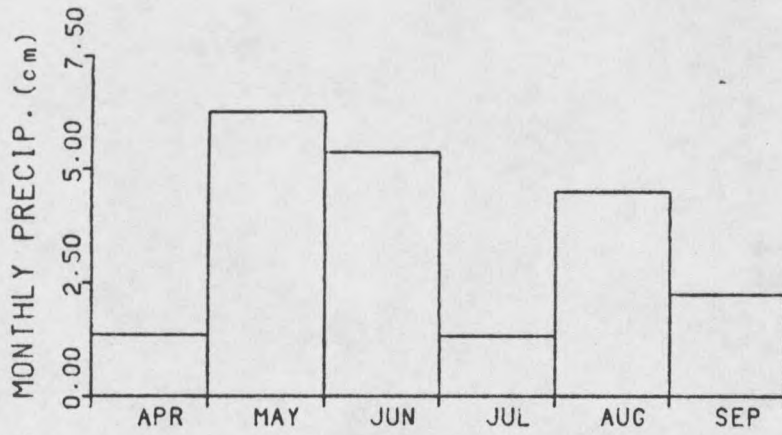
commercial, peat base Rhizobium (strain Q, Nitragin Co. Milwaukee, WI).

Weekly irrigations of 1.8 cm were applied with an overhead sprinkler system. Growing season precipitation is given in figure 13. Plots were hand-weeded weekly.

Dinitrogen fixation, nodule number, root volume, leaf area, shoot dry weight, root dry weight, shoot nitrogen (%w/w), and root nitrogen (%w/w), were measured 40, 61, 88, and 104 days after planting. Seed yield was measured at harvest and was determined on a per plant and a per area basis.

Dinitrogen fixation was measured by Kjeldahl and acetylene reduction as described by Burris (1972), with the following modifications. Root systems of two plants from each treatment from each of four replications were extracted with a 15 X 15 cm cutting cylinder. Plants were taken from the middle four rows to exclude border effects. Root samples were washed, blotted dry, and incubated for one hour immediately after extraction in 500 ml, sealed assay chambers containing 10% purified acetylene. Fifty milliliters of air was withdrawn and replaced with 50 ml of acetylene.

Gas samples were extracted with a syringe and stored in 7 ml evacuated blood sampling tubes (Vacutainer<sup>®</sup>). A Tracor Model 550 gas chromatograph with dual H<sub>2</sub> flame ionization detector and 30 m X .32 cm column of Porapak T<sup>®</sup> was used for determination of acetylene reduction. Twenty-five microliter gas samples were injected into the gas chromatograph with a Hamilton gas syringe with Chaney adapter.



1980 GROWING SEASON

Figure 13. Growing season precipitation at the SARC Huntley, MT.

A linear relationship between sample concentration in parts per million (ppm) and peak height was obtained utilizing helium as a carrier gas at 30 ml min<sup>-1</sup> flow rate. Column temperature was set at 100°C. Air and hydrogen flow rates to the flame ionization detector were 472 and 50 ml min<sup>-1</sup>, respectively. Ethylene production was calculated in micromoles by relating ppm (obtained by peak height) to the volume of the incubation chamber minus the root volume. Root volume was determined by water displacement in a graduated cylinder.

Plant shoots and roots were bagged in the field and refrigerated during transport to the laboratory. Turgid leaf area was measured with an Hayashi Denko automatic area meter (Model AAM-5). Nodules were counted, removed from the roots, dried for 24 hours at 67°C, and weighed. Plant and seed samples were dried at 67°C for 24 hours, weighed and ground in a Wiley mill. Nitrogen content was determined in the Soil and Plant Tissue Analysis Laboratory at Bozeman, MT using a modified macro-Kjeldahl technique described by Gavlak (1981). Seed yield was estimated from plants harvested from the center of the plots in a 1 m<sup>2</sup> area. Sigmoid curves for growth parameters were fitted from techniques developed by Jensen and Homeyer (1970) and subjected to regression analysis (chapter III).

Greenhouse experiments. A series of controlled environment experiments were used to verify the results obtained in the field. Broadbean (*Vicia faba* L. 'Ackerperle') seed were inoculated (same as field experiment) and planted in 4 X 25 cm 'conetainers'® (Ray Leach Co., Canby, OR) filled with a silt-loam soil (Pachic Cryoborolls) (74 ppm NO<sub>3</sub><sup>-</sup>-N; 74 ppm P; 152 ppm K; 3% O.M.; pH 7.5; and 1.6 mmho EC<sub>e</sub>).

A randomized complete block design was used with four replications. Plants were grown under metal halide lamps at 2500 einsteins with a 16/8 hour light/dark regime, 25/16°C photo/nyctotemperature, and 60% relative humidity. Modified half-Hoagland's solution (Hoagland and Arnon, 1938) was adjusted to six EC<sub>25</sub> levels of 1.4, 3, 5, 7, 9, and 11 mmhos with equal equivalents of NaCl and CaCl<sub>2</sub> (Table 4).

Eight plants from each treatment were harvested at 14, 21, 28, 35, and 42 days. Containers were not large enough to mature broadbean plants. Analyses were similar to those reported for the field experiment with the exception of the acetylene reduction assay. A container with an intact plant was placed in a 600 ml test tube capped with a rubber stopper fitted with a septum. Fifty cubic centimeters of chamber gas was drawn from each test tube and replaced with 50 cc purified acetylene. Plants were incubated for 1 hour and 7 ml gas samples were extracted for quantitative gas chromatography utilizing procedures employed in the field experiment.

#### RESULTS AND DISCUSSION

Field experiment. Increased soil salinity resulted in a corresponding decrease in emergence (fig. 14). Emergence was less than 40% of the control at 22 mmhos. Compared to other crops, faba bean is moderately tolerant to soil salinity during early seedling growth (Abel and McKenzie, 1964; Bernstein and Hayward, 1958; U.S. Salinity Lab, 1954). Because of the reduction in emergence in a saline environment, the effects of salinity on other growth parameters estimated from the

Table 5. Nutrient solutions applied in greenhouse studies <sup>1</sup>

Chemical	Stock Solution (g/L)	Nutrient Solution (ml stock /L solution)	
		with nitrogen	without nitrogen
<u>macronutrients</u>			
Fe(10% chelate)	19.92	1.25	1.25
KH <sub>2</sub> PO <sub>4</sub>	27.20	1.25	1.25
MgSO <sub>4</sub> ·7H <sub>2</sub> O	98.60	1.25	1.25
K <sub>2</sub> SO <sub>4</sub>	87.00	.50	.50
KNO <sub>3</sub>	101.11	2.50	--
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	236.16	2.50	--
CaCl <sub>2</sub> ·2H <sub>2</sub> O	147.00	.75	3.25
KCl	74.56	--	2.5
<u>micronutrients</u> (combined stock solution)			
Zn SO <sub>4</sub> ·7H <sub>2</sub> O	.088	.625	.625
H <sub>3</sub> BO <sub>3</sub>	1.144		
Mn Cl <sub>2</sub> ·4H <sub>2</sub> O	.724		
Cu SO <sub>4</sub> ·5H <sub>2</sub> O	.032		
H <sub>2</sub> MO <sub>4</sub> ·H <sub>2</sub> O	.008		

<sup>1</sup>/ Modified from Hoagland and Arnon (1938)

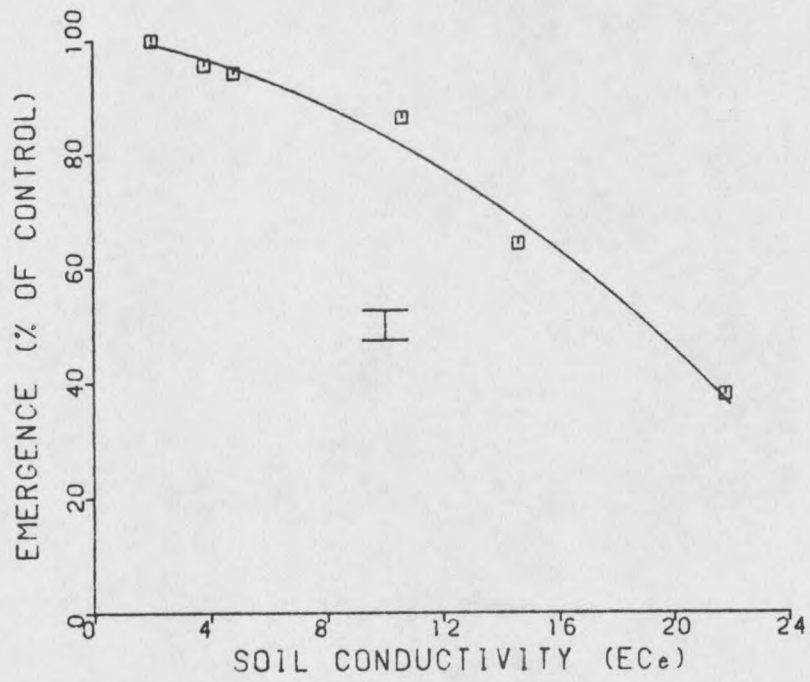


Figure 14. Emergence of *V. faba* L. at the SARC Huntley, MT.

effect on individual plant data may be inaccurate. Yields may be considerably reduced on an area basis.

Total dry matter accumulation decreased with increased soil salinity (fig. 15). This decrease was also evident in shoot, root, and seed yields (fig. 16, 17, 18). Increased soil salinity decreased the growing time required to obtain the highest dry matter yield. It is not unusual for stress conditions to effect early senescence (Itai and Vaadia, 1965; Wright and Hiron, 1969). Reduction in dry matter production by 50% occurred at approximately 9 mmhos (Maximum yield is taken as the yield of the control at day 104. Fifty percent reduction in yield is then calculated using the regression of peak yield on  $EC_e$ ). These data show faba bean slightly less salt tolerant than previous results (El Karouri, 1979). El Karouri reported a 50% reduction in dry matter at 10.5 mmhos. El Karouri found shoot dry weight and seed yield both to be linearly and inversely related to soil conductivity. In this field experiment dry matter yields were curvilinear. This may have been the result of the decreasing soil salinity (fig. 11). Quantitative values inferred by regression analysis using the values of  $EC_e$  at planting must be considered approximate.

Fertilizer nitrogen significantly increased shoot dry weight (fig. 16). Shoot dry weight was 13% greater in plants receiving fertilizer nitrogen. Fertilizer nitrogen had no significant effect on total plant dry weight (shoot, root, and seed). Faba bean has been reported to be nitrogen self-sufficient from symbiotic fixation in low nitrate soils (Dean and Clark, 1977; Richards and Soper, 1979).

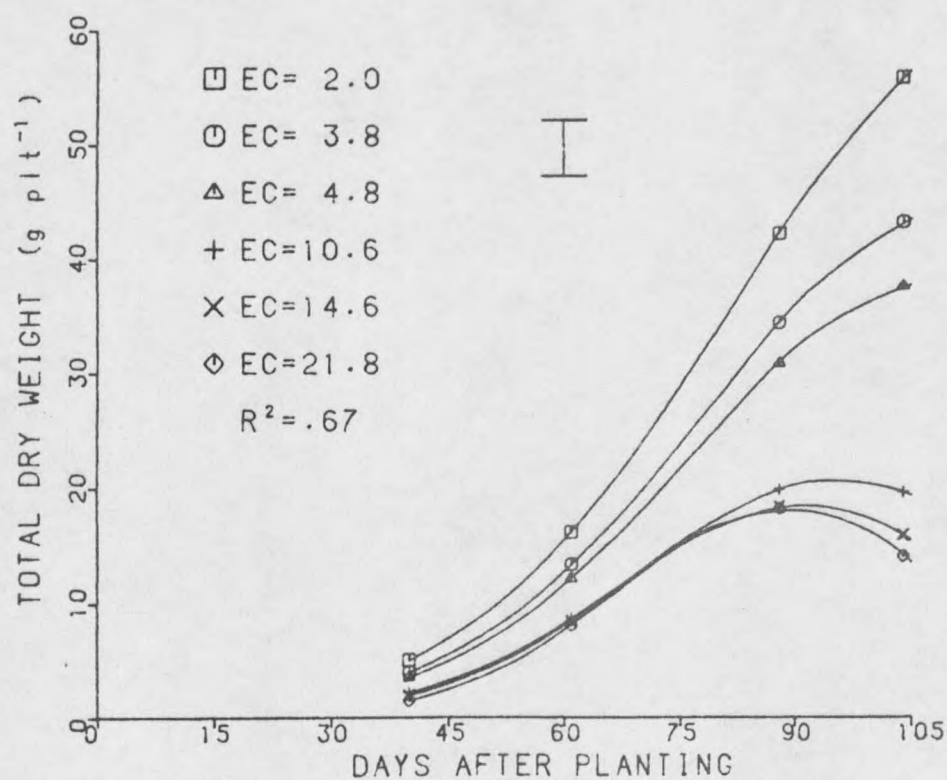


Figure 15. Regression model of total dry matter accumulation in *V. Faba* L. at the SARC Huntley, MT. The response surface is the result of 24 data points (4 harvests x 6 salinity treatments), each the mean of sixteen plants. The standard error of the response surface is 5% and is represented on the graph as 2.2 g at the highest yield.  $R^2$  for the response surface is significant to .001.

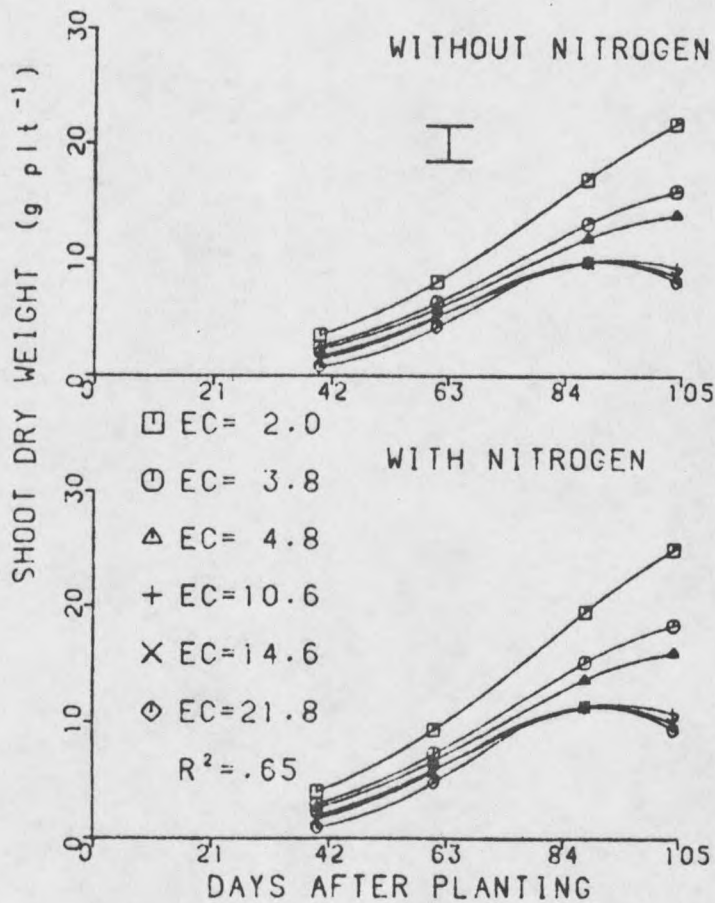


Figure 16. Regression model of shoot dry weight accumulation in *V. faba* L. at the SARC Huntley, MT. The 4-dimensional response surface is the result of 48 data points (4 harvests x 6 salinity treatments x 2 nitrogen treatments), each the mean of eight plants. The standard error of the response surface is 5%, represented at maximum yield by 1.08 g.  $R^2$  is significant to .001.

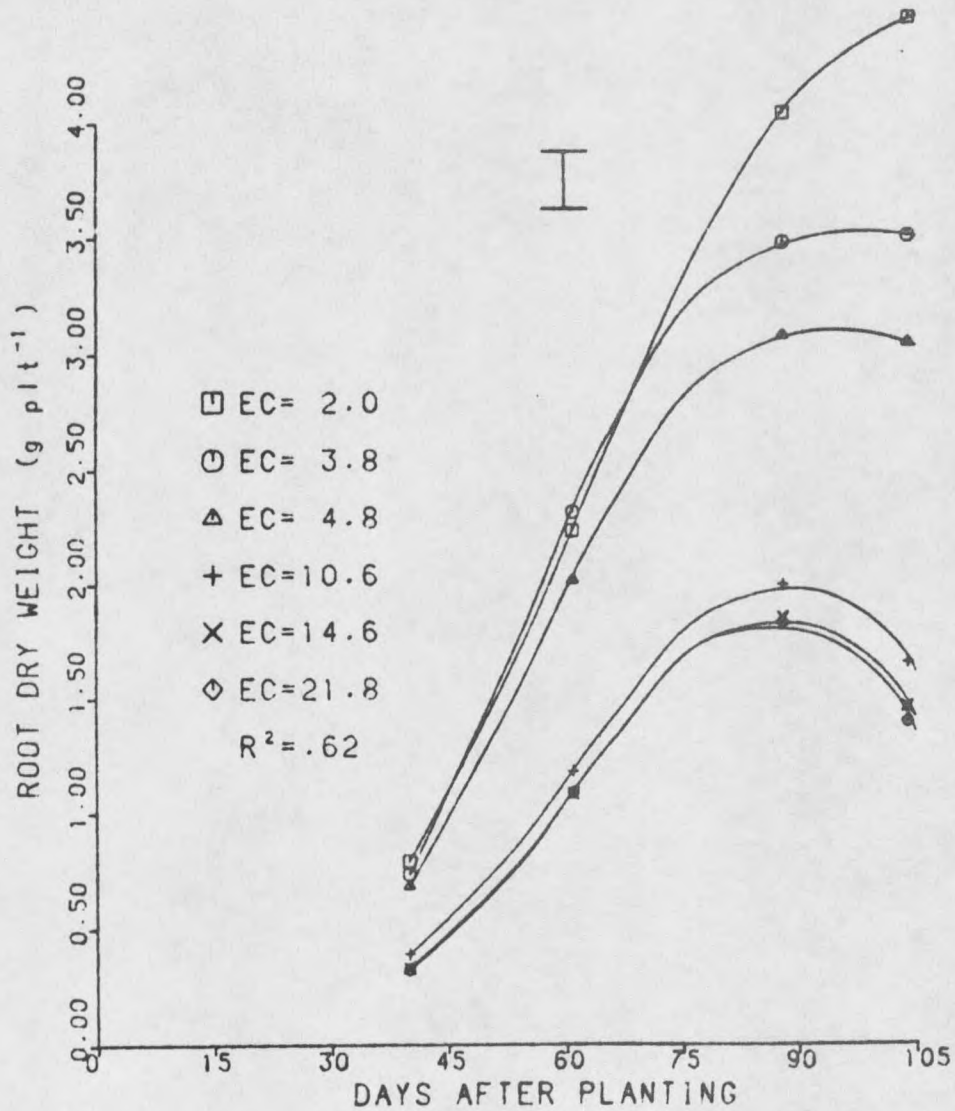


Figure 17. Regression model of root dry weight in *V. faba* L. at the SARC Huntley, MT. The response surface is the result of 24 data points (4 harvests x 6 salinity treatments), each the mean of 16 plants. The standard error of the response surface is 3%, represented at maximum yield by .12 g.  $R^2$  is significant to .001.

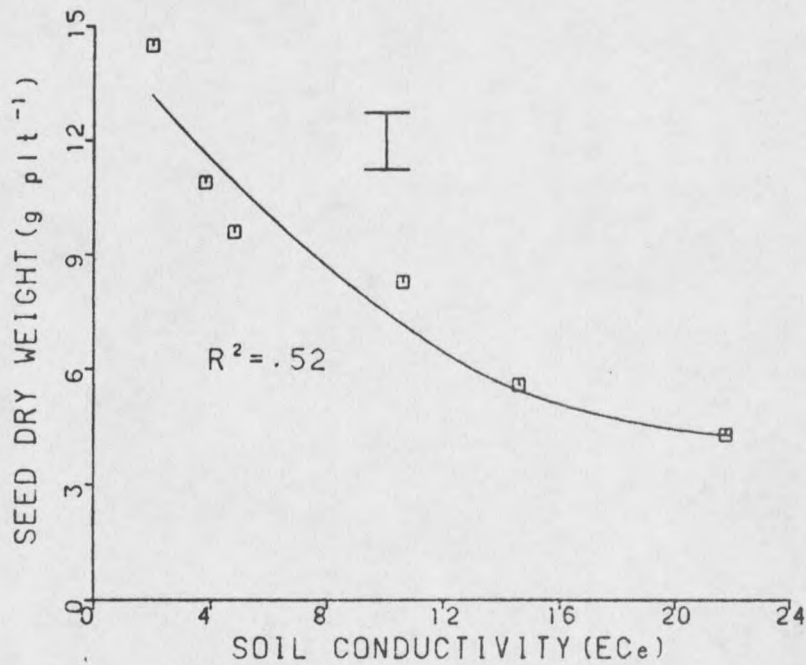


Figure 18. Average seed yield for *V. faba* L. at the SARC Huntley, MT. Symbols represent the means of four replications of plants harvested within one square meter. The standard error of the estimate at 10 mmhos is given.  $R^2$  is significant to .001.

Although there was no significant difference in root dry weight with added fertilizer nitrogen, it must be recalled that since roots were removed with a soil core of specified dimensions, extent of root penetration was not measured. Although the bulk of the root system was removed with the core, the measurement was more of root density than total dry weight.

When the means of leaf area measurements at the six salinity treatments were plotted over time (fig. 19), the pattern obtained is not that of a symmetrical, bell-shaped curve. All available information is used when forming the regression model, but analysis includes only those data points within the range of sigmoidicity (88 days). Because peak leaf area is found through interpolation of the best sigmoid fit through the data points, quantitative interpretation of the results is approximate. However, because the model is a good approximation at the means, it remains useful for orthogonal comparison of nitrogen treatment effects. This comparison results in no significant difference in leaf area as a result of nitrogen treatment. An interesting feature of the model (fig 20.) is that maximum leaf area is obtained at 75 days, approximately coincidental with maximum growth rate (inflection point of fig. 15). This is theoretically sound and increases confidence in the model as representative of leaf area vs time.

A polynomial model for leaf area is given in figure 21. This model provides estimates of  $X_p$  and  $Y_p$  very close to those obtained using the sigmoid model.

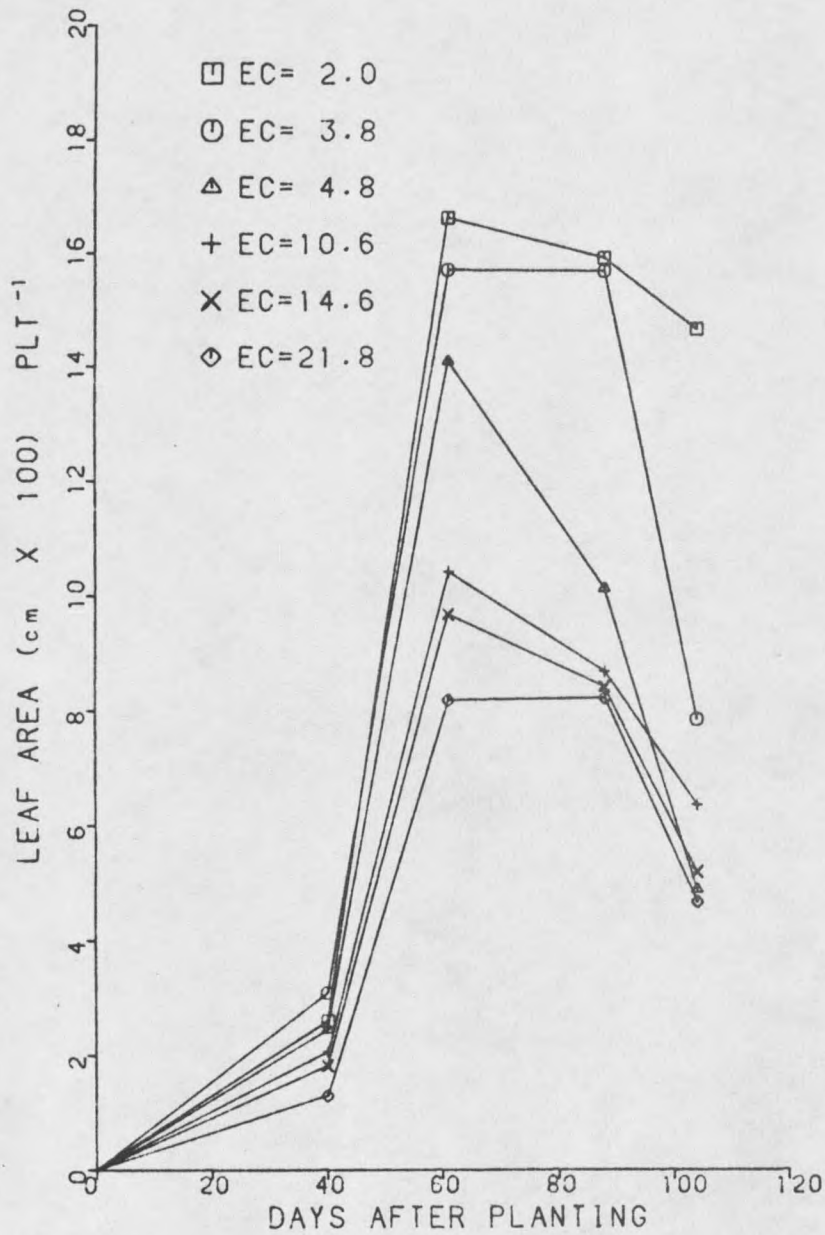


Figure 19. Mean values for leaf area in *V. faba* L. for each salinity value at four harvest dates at the SARC Huntley, MT.

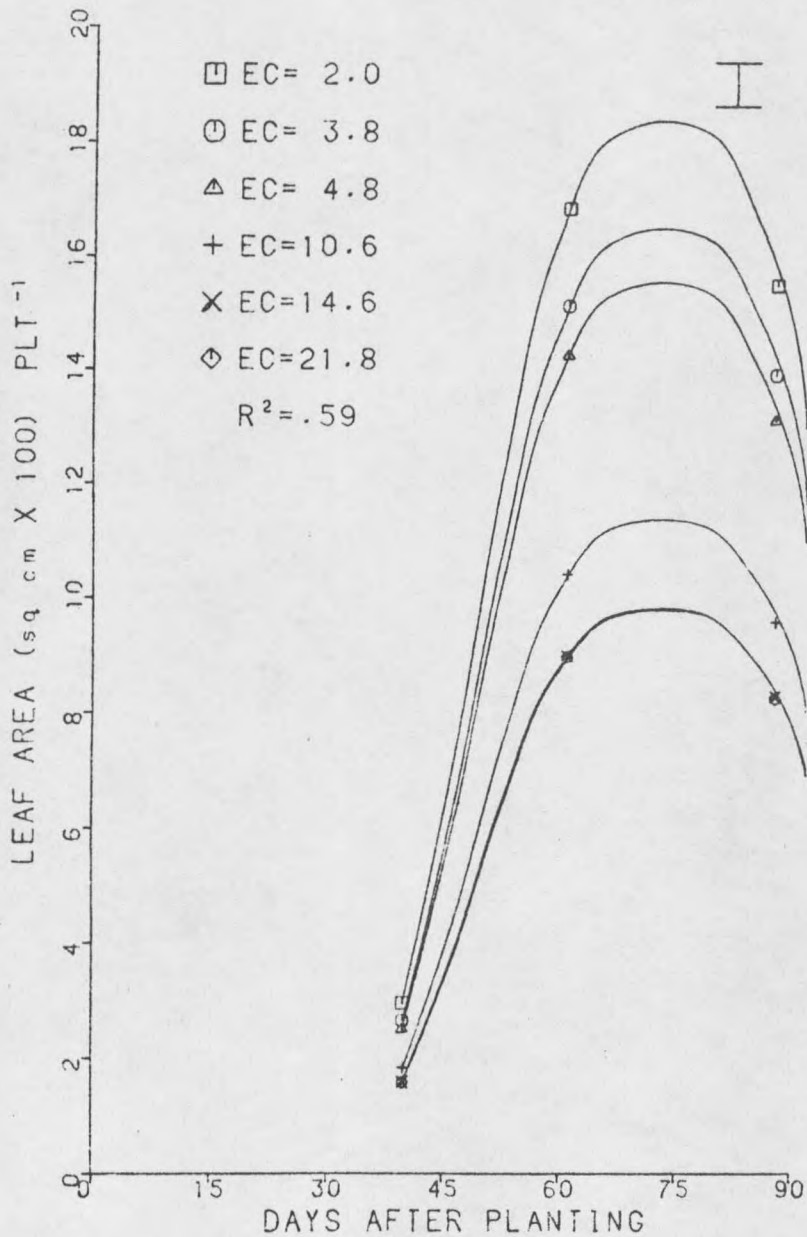


Figure 20. Regression model of leaf area in *V. faba* L. at the SARC Huntley, MT. The response surface is the result of 18 data points (3 harvests x 6 salinity treatments) each the mean of 16 plants. The standard error of the response surface is 2%, represented at maximum leaf area by 38 cm<sup>2</sup>.

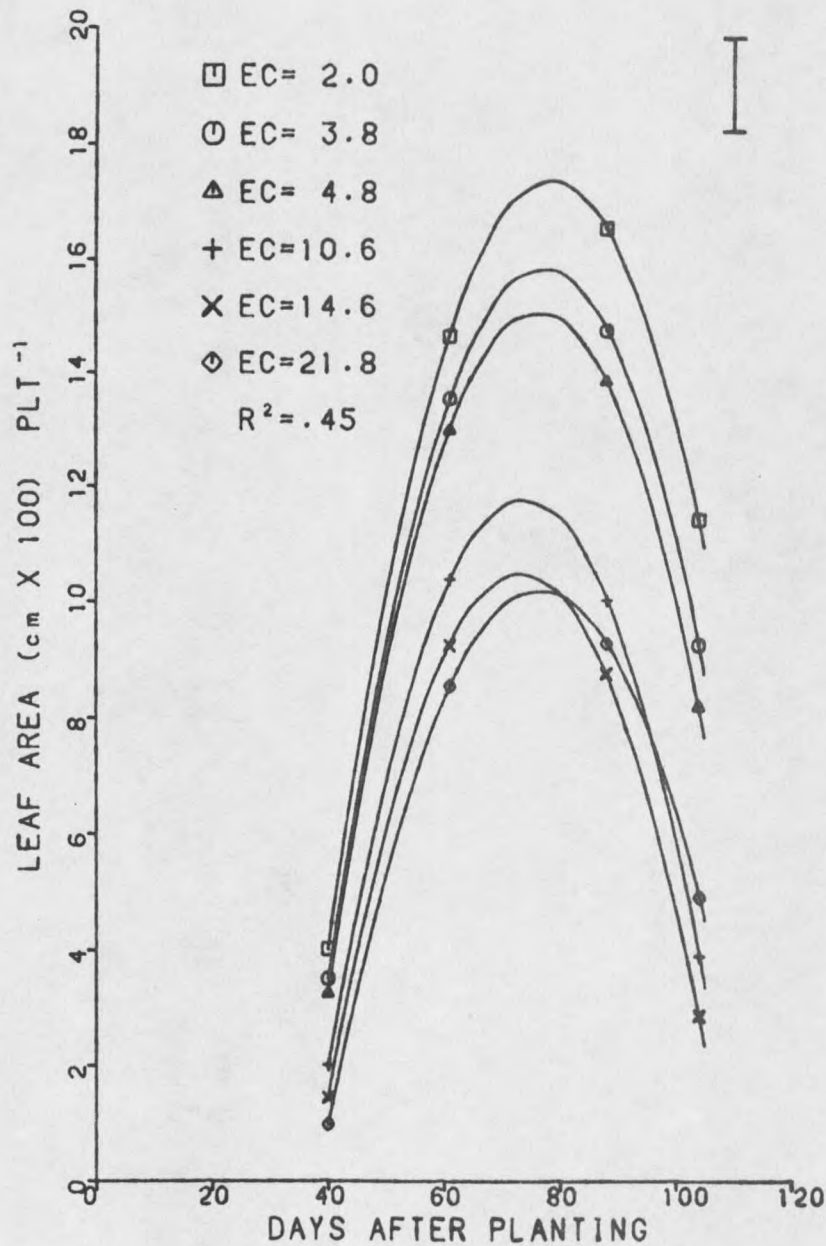


Figure 21. Polynomial regression model of leaf area in *V. faba* L. at the SARC Huntley, MT. The response surface is the result of 24 data points (4 harvest x 6 salinity treatments) each the mean of 16 plants.

Acetylene reduction (AR) increased markedly at the low to moderate soil salinity levels (fig. 22). Improved soil aggregation from the addition of calcium was evident when soil samples were taken. The improved aeration in the rhizosphere may have accounted for the increased nodulation and subsequent increase in AR activity. Dinitrogen fixation is closely linked to respiration (Dalton and Mortenson, 1972). Minchin and Pate (1975) found dinitrogen fixation inhibited by inadequate gas exchange.

Regression analysis indicates maximum AR at 6 mmhos. Soil salinity above 10 mmhos resulted in a proportional decline in AR activity. Dinitrogen fixation in saline environments should be considered a function of a salinity X soil type (%clay) interaction. Specific ion effects (monovalent vs divalent) should be markedly evident in soils with a high clay content.

Total nitrogen accumulation (fig. 23) was 26% higher in plants receiving fertilizer nitrogen, only when no excess soil salinity was present. In treatments in which salt was applied, the converse was true. Total nitrogen accumulation was 26% greater in plants relying primarily on symbiotic dinitrogen fixation.

Shoot nitrogen content presented a situation similar to that encountered in forming the leaf area model. A plot of the means (fig. 24) shows the data are not represented by a bell-shaped curve. Furthermore the response surfaces obtained with nitrogen treatments (figs. 25a,25b) are not symmetrical about an average surface, so an orthogonal comparison is not possible.

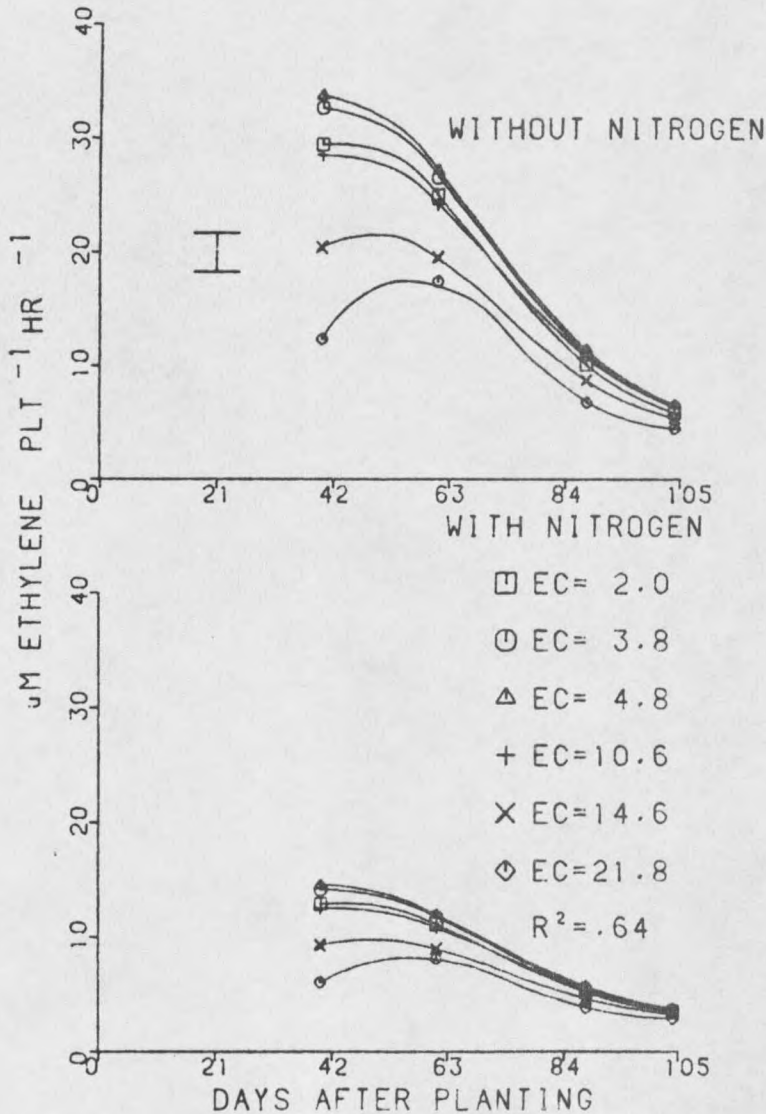


Figure 22. Regression model of acetylene reduction (AR) activity in *V. faba* L. at the SARC Huntley, MT. The 4-dimensional response surface is the result of 48 data points (4 harvests x 6 salinity treatments x 2 nitrogen treatments) each the mean of eight plants. The standard error of the response surface is 5%, represented at maximum AR by 1.65  $\mu\text{M}$ .  $R^2$  for the response surface is significant to .001.

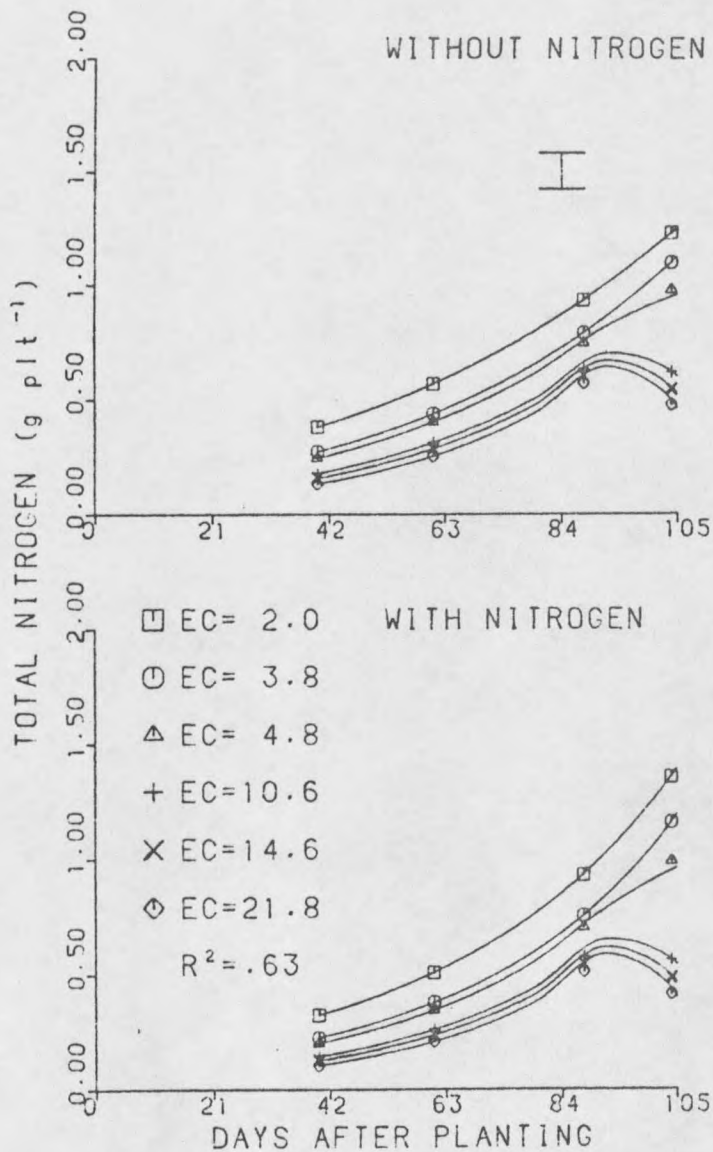


Figure 23. Regression model of total nitrogen accumulation in *V. faba* L. at the SARC Huntley, MT. The 4-dimensional response surface is the result of 48 data points (4 harvests x 6 salinity treatments x 2 Nitrogen treatments) each the mean of eight plants. The standard error of the response surface is 6% of the yield, represented at maximum nitrogen content by .08 g.  $R^2$  for the response surface is significant to .001.

The most interesting feature of the response surfaces obtained (figs. 25a,25b) is their agreement with the results obtained in AR analysis (fig. 22) and seed nitrogen analysis (fig. 26). When values of  $Y_p$  for shoot nitrogen are plotted against soil conductivity (fig. 25b),  $Y_p$  for plants relying primarily on symbiotic dinitrogen fixation was curvilinear with a maximum at six mmhos; approximately the salinity value at which maximum AR activity was obtained (fig. 22). This coincides with the plant's ability to provide adequate nitrogen to the developing seed (top line, fig. 26).

In contrast, the relationship found between shoot nitrogen and soil conductivity in plants receiving heavy nitrogen fertilization was inverse (fig. 25a,25b). As salinity increased, shoot nitrogen decreased. The result was a decrease in nitrogen to the seed (bottom line, fig. 26).

Shoot nitrogen reached its peak coincidental with maximum acetylene reduction activity, maximum leaf area, and maximum growth rate. Shoot nitrogen declined, presumably as a result of protein hydrolysis in the senescing leaves and translocation to the developing seeds (Beever, 1976).

A polynomial model for shoot nitrogen is also given (fig. 27). This model found significant differences between nitrogen treatments, but did not represent the subtle differences indicated by the sigmoid model and concurred in by AR (fig. 22) and seed nitrogen (fig. 26).

Shoot nitrogen percentage increased with increased salinity and with application of fertilizer nitrogen (fig. 28). Nitrate accumulation has been demonstrated in various crops under heavy

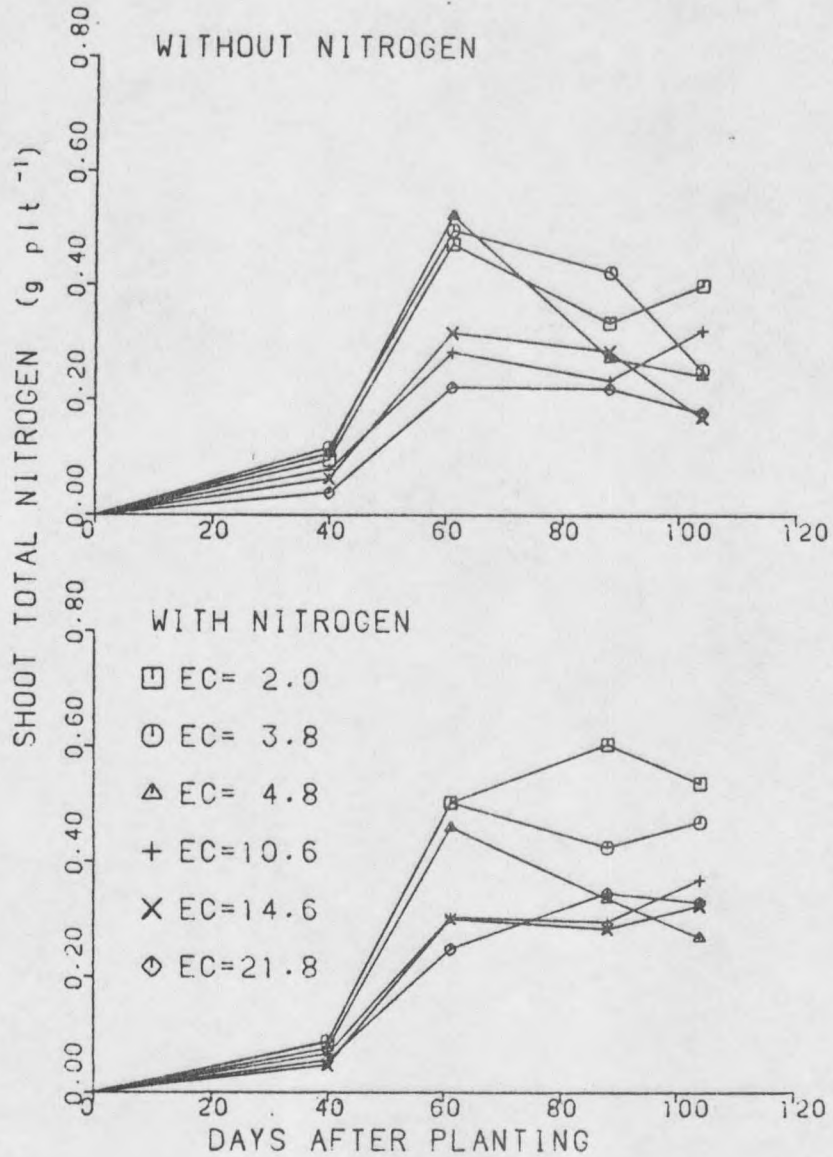


Figure 24. Shoot nitrogen accumulation in *V. faba* L. at the SARC Huntley, MT. Each symbol represents the mean of measurements taken on eight plants.

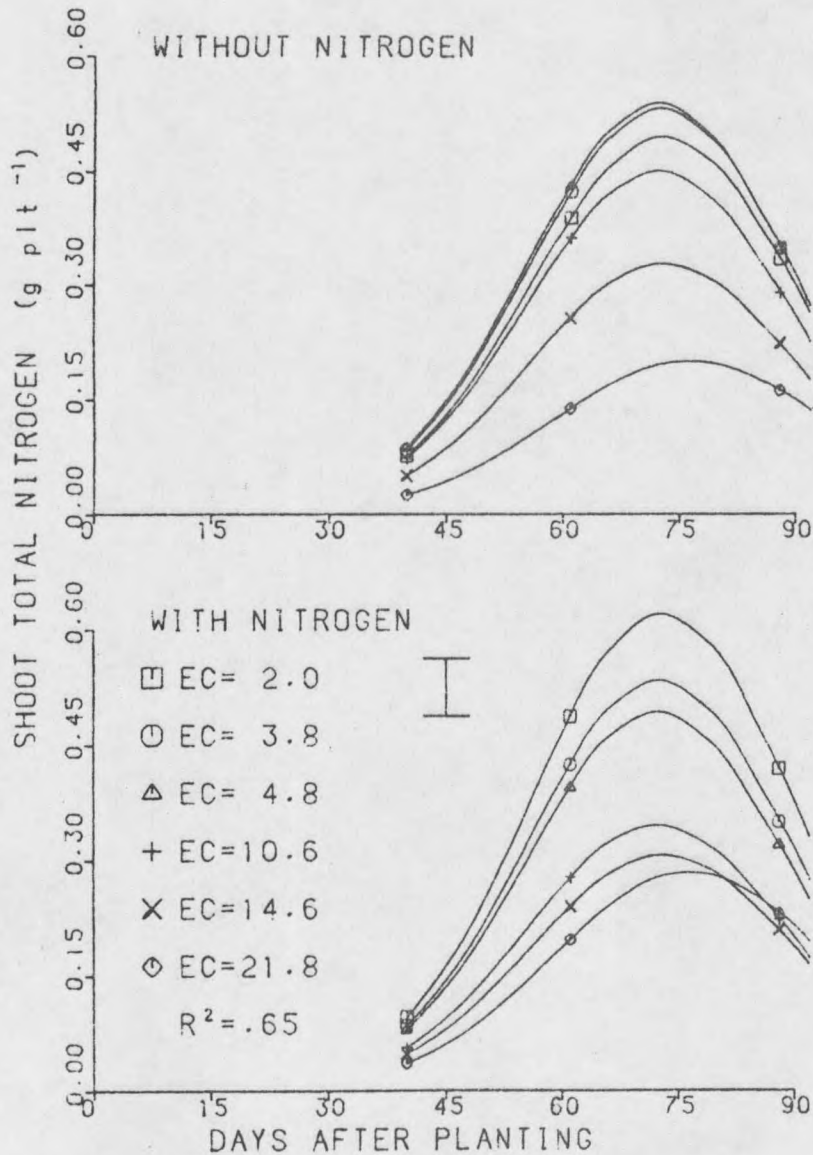


Figure 25a. Regression model of shoot nitrogen accumulation in *V. faba* L. at the SARC Huntley, MT. The 4-dimensional response surface is the result of 36 data points (3 harvests x 6 salinity treatments x 2 nitrogen treatments, each the mean of eight plants). The standard error of the response surface is 3%, represented at maximum shoot nitrogen content by .07 g. R<sup>2</sup> for the response surface is significant to .001.

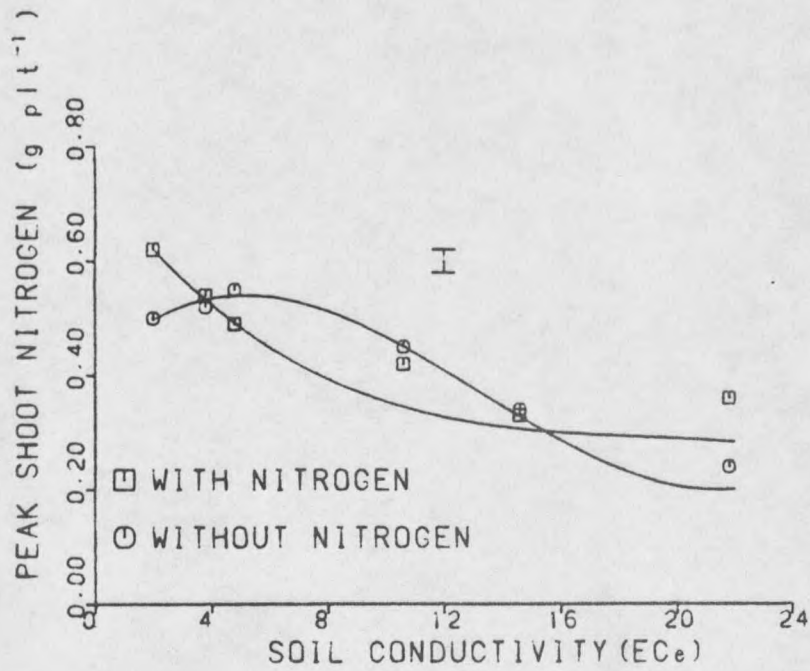


Figure 25b. Peak shoot nitrogen content in *V. faba* L. at the SARC Huntley, MT. Symbols represent the peaks of the sigmoid curves given in figure 25a.

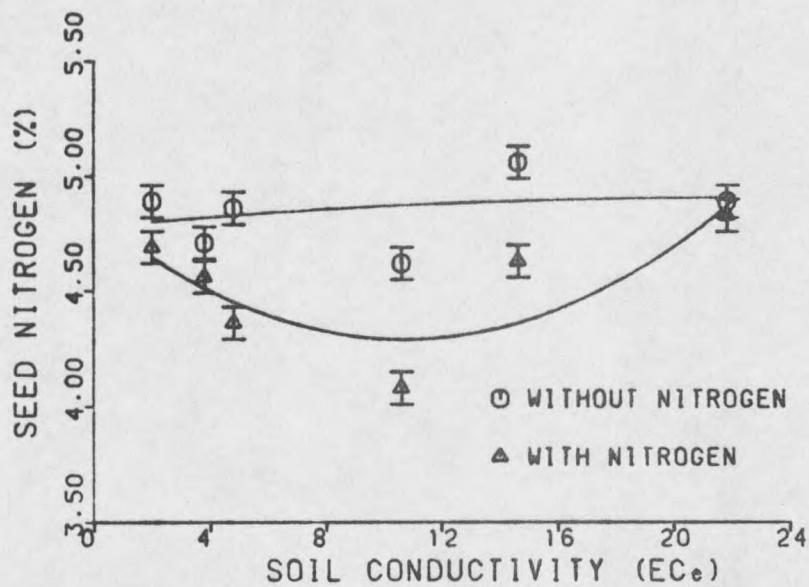


Figure 26. Seed nitrogen percentage of *V. faba* L. at the SARC Huntley, MT. Symbols represent means from four replications of random samples from seed harvested within one square meter. Hashmarks represent the standard error of the nitrogen treatment difference.

fertilization (Boatwright and Haas, 1961). Available nitrate may have exceeded the amount necessary to meet the needs of the reduced plant metabolism under saline conditions. Another explanation may be the accumulation of free amino acids as a means of osmotic adjustment to the absolute increase in soil potential with increasing salinity (Stewart and Lee, 1974). Helal and Mengel (1981) reported that inorganic nitrogen increased while protein nitrogen decreased in V. faba L. under saline stress.

The proportion of nitrogen to plant dry weight has been shown to decrease following initial development (Beevers, 1976). This was reflected in the decline of shoot nitrogen percentage and root nitrogen percentage (fig. 28 and 29). Root percent nitrogen increased slightly at the end of the growing season. This may have resulted from translocation termination of fixed or absorbed nitrogen from the root to the above ground portion of the plant.

Seed yield was determined on a per plant and a per area basis (fig. 18 and 30). Fifty percent reduction in seed yield occurred at 12 and 10.5 mmhos, respectively. This illustrates the danger in assessing the salinity tolerance of agronomic crops by measuring yield on a per plant basis. Fertilizer nitrogen had no effect on seed yield.

Percent nitrogen in the seed is given in figure 26. A salinity x fertilizer nitrogen interaction is evident, especially at the moderate salinity levels. With excess soil salinity present, soil nitrogen apparently was unable to provide adequate nitrogen to the relatively large number of developing seeds. This reduction in available

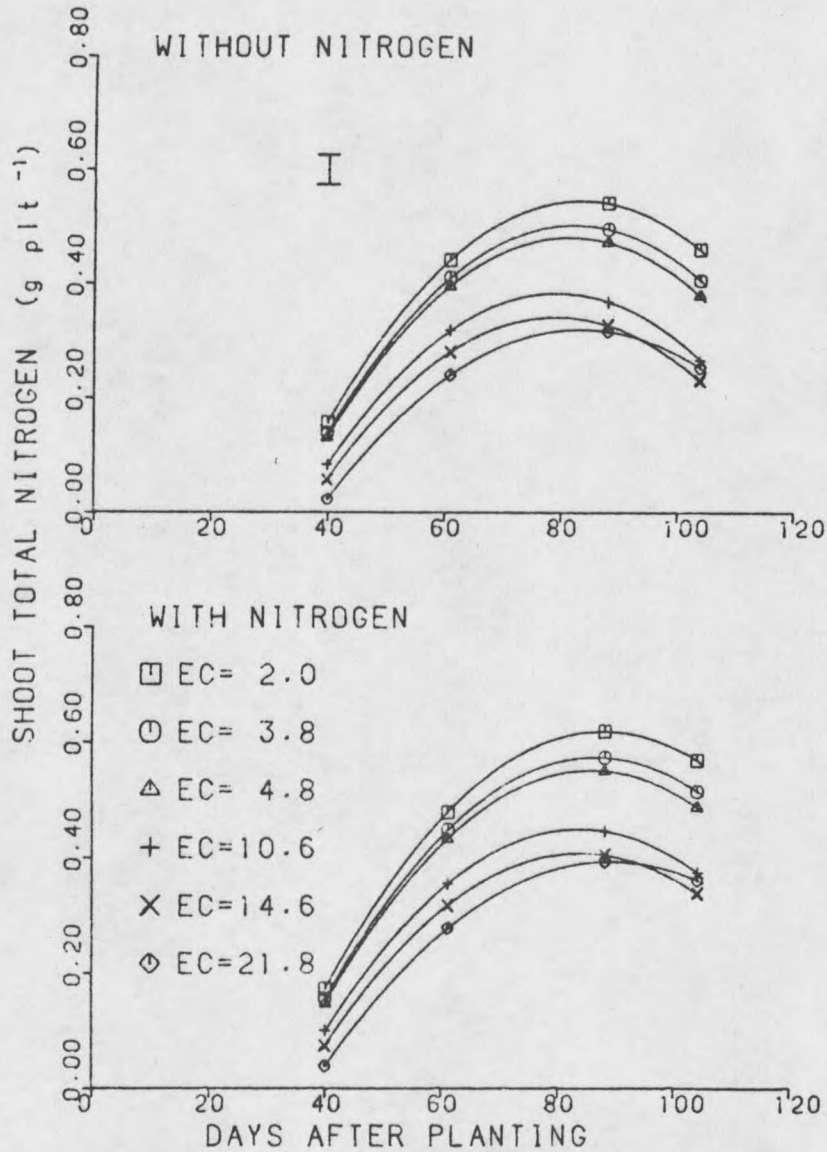


Figure 27. Polynomial regression model for shoot nitrogen accumulation in *V. faba* L. at the SARC Huntley, MT. The 4-dimensional response surface is the result of 48 data points (4 harvests x 6 salinity treatments x 2 nitrogen treatments), each the mean of eight plants.  $R^2$  for the response surface is significant to .001.

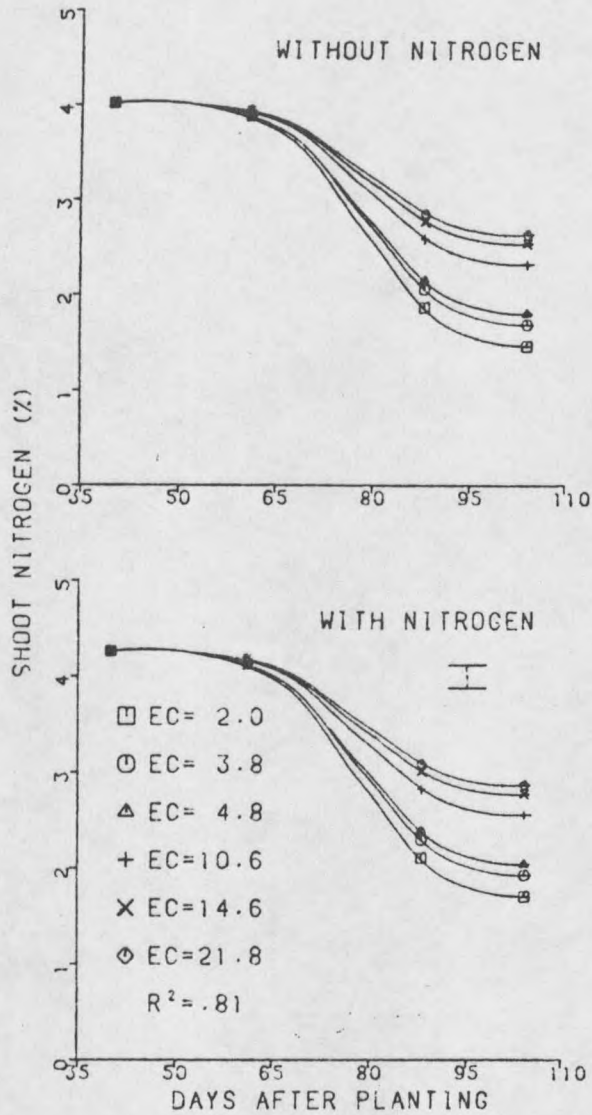


Figure 28. Regression model of shoot nitrogen percentage in *V. faba* L. at the SARC Huntley, MT. The 4-dimensional response surface is the result of 48 data points (4 harvests x 6 salinity treatments x 2 nitrogen treatments), each the mean of eight plants. The standard error of the response surface is 3%, represented at 104 days by .12% nitrogen.  $R^2$  for the response surface is significant to .001.

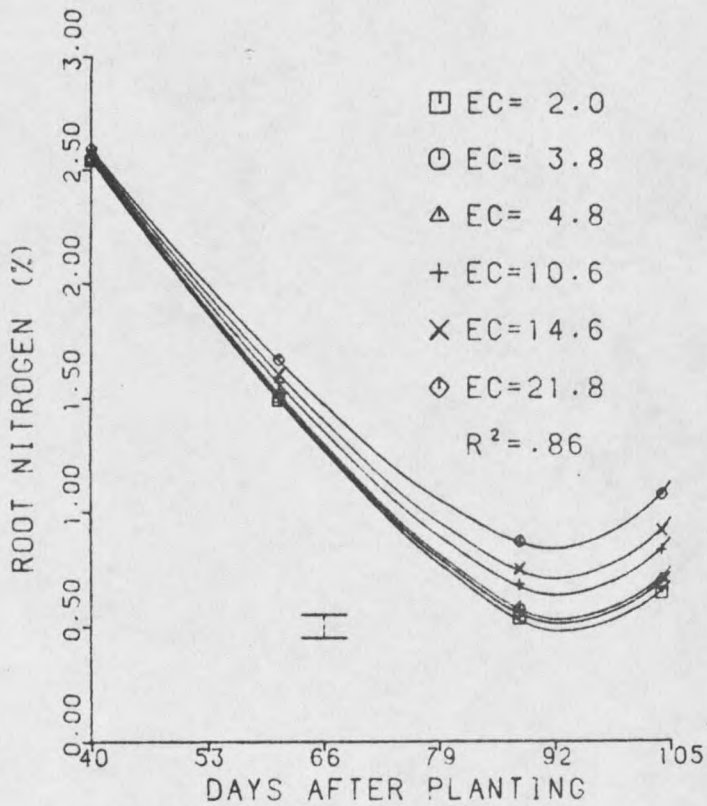


Figure 29. Regression model of root nitrogen percentage in *V. faba* L. at the SARC, Huntley, MT. The response surface is the result of 24 data points (4 harvests x 6 salinity treatments), each the mean of 16 plants. The standard error of the estimate at 10 mmhos, 92 days after planting, is given.  $R^2$  for the response surface is significant to .001.

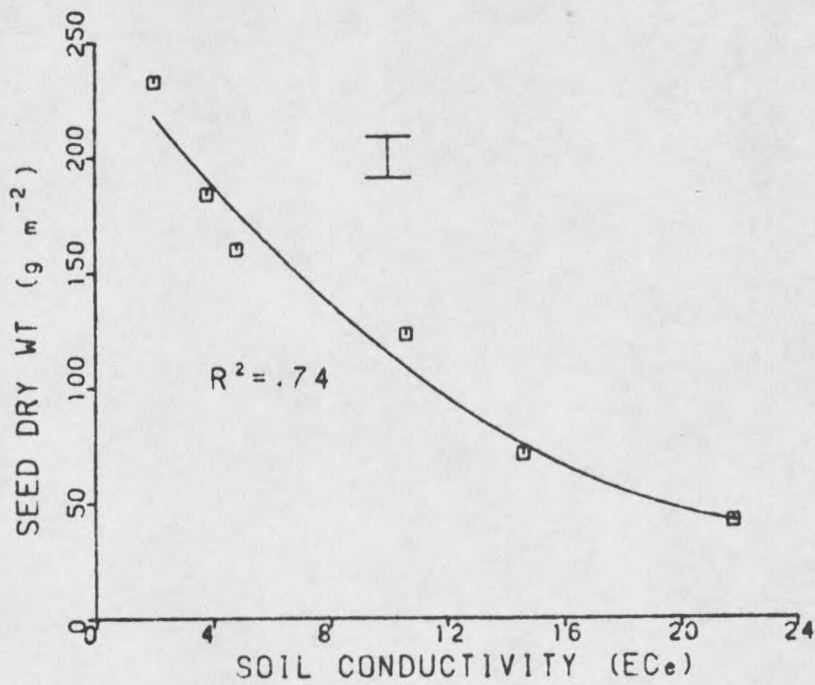


Figure 30. Seed dry weight for *V. faba* L. at the SARC Huntley, MT. Symbols represent means from four replications of plants harvested within one square meter. The standard error of the estimate at 10 mmhos is given.  $R^2$  is significant to .001.

nitrogen is less important at higher salinity levels, where the decreased seed number was more adequately supplied. Conversely, symbiotic fixed nitrogen was adequate to supply developing seeds throughout the entire soil salinity range.

Greenhouse experiments. Greenhouse data basically agree with field results. However, unlike the curvilinear salinity effect on yield under field conditions, yield reduction with salinity in the greenhouse was linear. No seed was formed in the limited time in which the plants were grown. Growth in containers restricted root and subsequent shoot growth.

Leaf area (fig. 31) was unaffected by fertilizer nitrogen and decreased with salinity. A 50% reduction in maximum leaf area was found at 9.4 mmhos. Maximum leaf area was obtained at 35 days compared to the 75 days required in the field. The regression models used were proportioned using maximum leaf area as a convenient reference point, because maximum leaf area roughly coincided with the inflection points in the growth curves (maximum growth rate). It was also shown that the plants were approaching maximum height at the end of 42 days (fig. 32).

Total dry weight (fig. 33) decreased in proportion to salinity treatment. A fifty percent reduction in maximum yield occurred at 8.7 mmhos. Plants grown with nitrogen exhibited a 6% increase in total dry weight over greenhouse plants grown without nitrogen.

Shoot dry weight (fig. 34) decreased to 50% of maximum yield at 8.9 mmhos. Shoots of plants grown with nitrogen accumulated 8% more dry weight. This is less tolerance for shoot dry matter accumulation

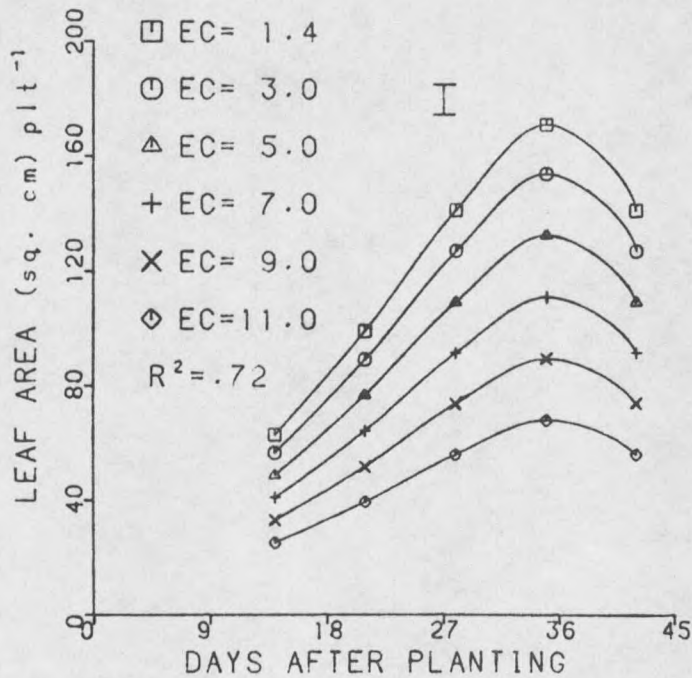


Figure 31. Regression model of leaf area of *V. faba* L. grown in containers. The response surface is the result of 30 data points (5 harvests x 6 salinity treatments), each the mean of 16 plants. The standard error of the response surface is 3%, represented at maximum leaf area by 5.1 cm<sup>2</sup>.  $R^2$  for the response surface is significant to .001.

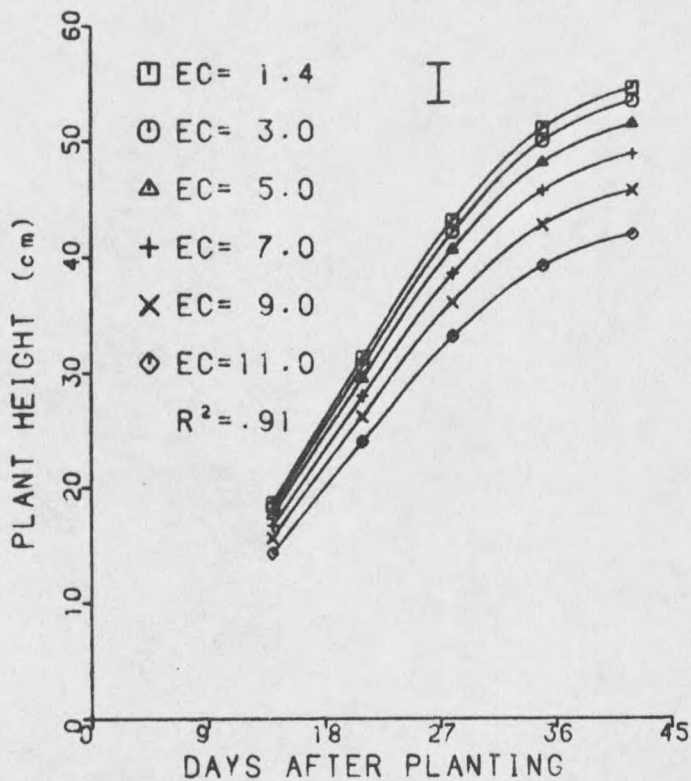


Figure 32. Regression model of height of *V. faba* L. grown in containers. The response surface is the result of 30 data points (5 harvests x 6 salinity treatments), each the mean of 16 plants. The standard error of the response surface is 3%, represented at maximum height by 1.7cm.  $R^2$  for the response surface is significant to .001.

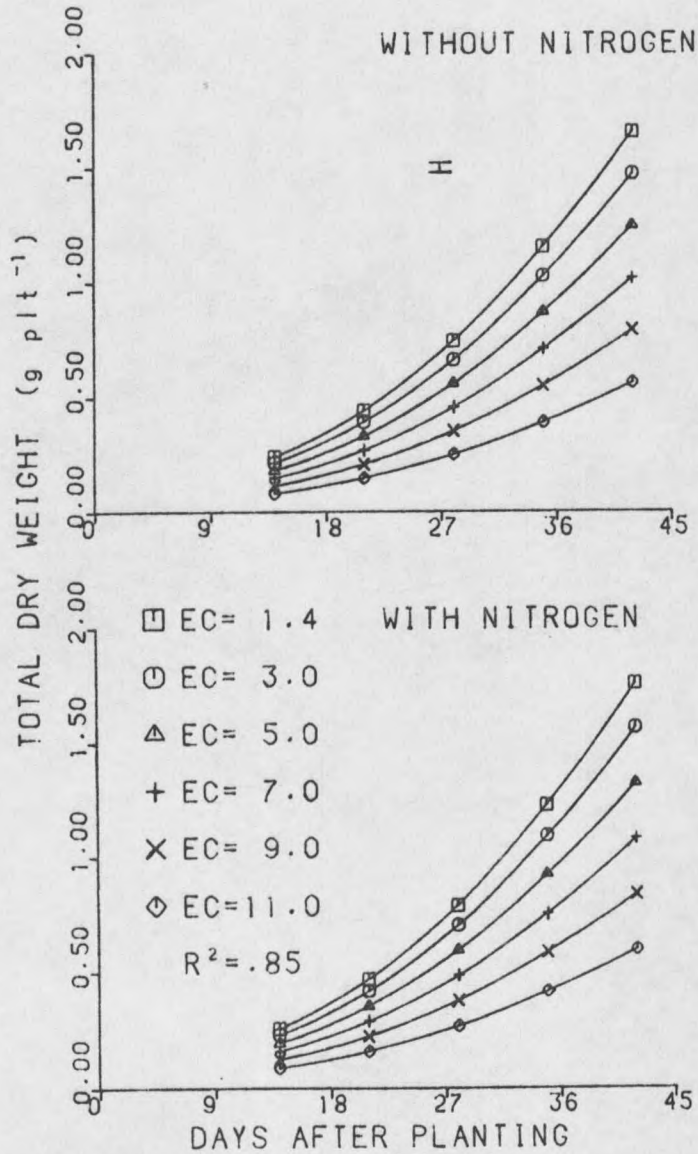


Figure 33. Regression model of total dry weight for *V. faba* L. grown in containers. The four dimensional response surface is the result of 60 data points (5 harvests x 6 salinity treatments x 2 nitrogen treatments), each the mean of eight plants. Standard error for the response surface is 2%, represented at maximum weight by .04 g. R<sup>2</sup> of the response surface is significant to .001.

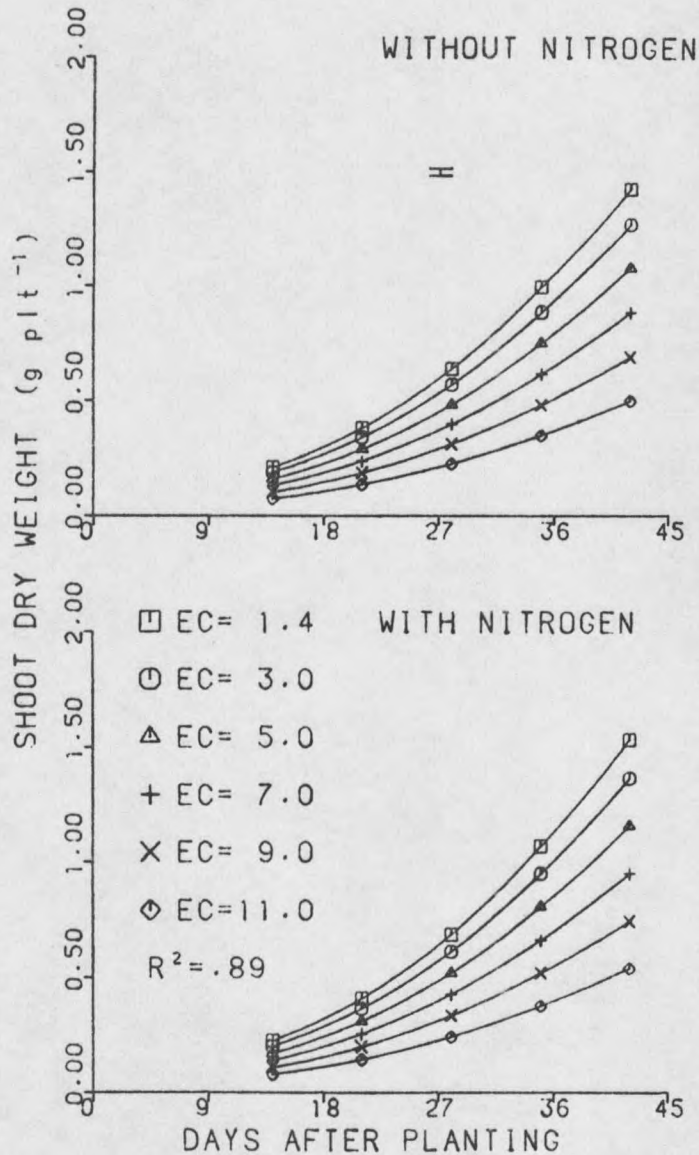


Figure 34. Regression model of shoot dry weight in *V. faba* L. grown in containers. The response surface is the result of 60 data points (5 harvests x 6 salinity treatments x 2 nitrogen treatments), each the mean of eight plants. The standard error of the response surface is 2%, represented at maximum weight by .03 g.  $R^2$  for the response surface is significant to .001.

by vegetative growth than that reported by El Karouri (1979) but more than that reported by Ayers and Eberhard (1960). They had found a 50% reduction in vegetative dry weight at 10.5 and 6 mmhos respectively. The predominant salt in the field experiment by El Karouri was  $\text{Na}_2\text{SO}_4$  while Ayers and Eberhard used equal equivalents of  $\text{NaCl}$  and  $\text{CaCl}_2$ .

Root dry weight (fig. 35) decreased to 50% of maximum yield at 8.4 mmhos. Nitrogen treatment had no effect on root dry weight.

Acetylene reduction is given in figure 36. Activity during the first 35 days followed a pattern similar to the sigmoid growth, development, and decay exhibited by the field-grown plants. Acetylene reduction was inversely proportional to salinity. This pattern changed on the final harvest as AR activity at the three lowest salinity treatments experienced a second rapid rise. Acetylene reduction was highest at 5 mmhos. The cause of this increase is unknown.

Percent nitrogen in both the shoot (fig. 37) and the root (fig. 38) was significantly greater in plants receiving nitrate nitrogen. Percent nitrogen increased with salinity but decreased overall with time. This agrees with results obtained in the field.

Total nitrogen decreased with salinity (fig. 39). Plants receiving nitrate nitrogen accumulated 50% more nitrogen than those grown with no nitrate. Shoot total nitrogen (fig. 40) also decreased with salinity and increased with fertilizer nitrogen. Shoots contained 50% more nitrogen when plants were grown with nitrate in the nutrient solution.

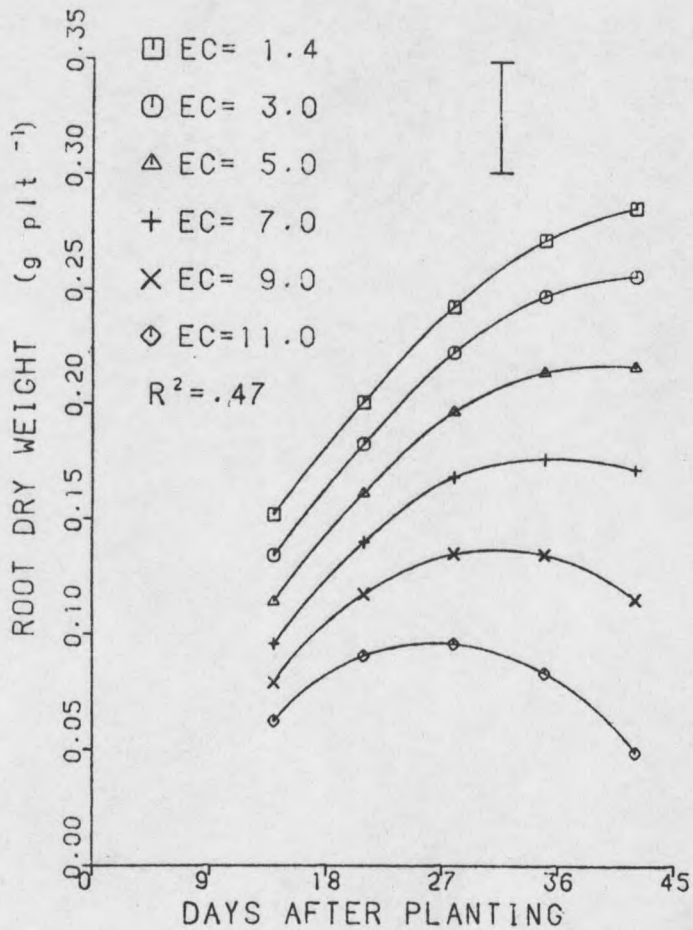


Figure 35. Regression model of root dry weight for *V. faba* L. grown in containers. The response surface is the result of 30 data points (5 harvests x 6 salinity treatments) each the mean of 16 plants. The standard error of the response surface is 8%, represented at maximum root dry weight by .02 g.  $R^2$  for the response surface is significant to .001.

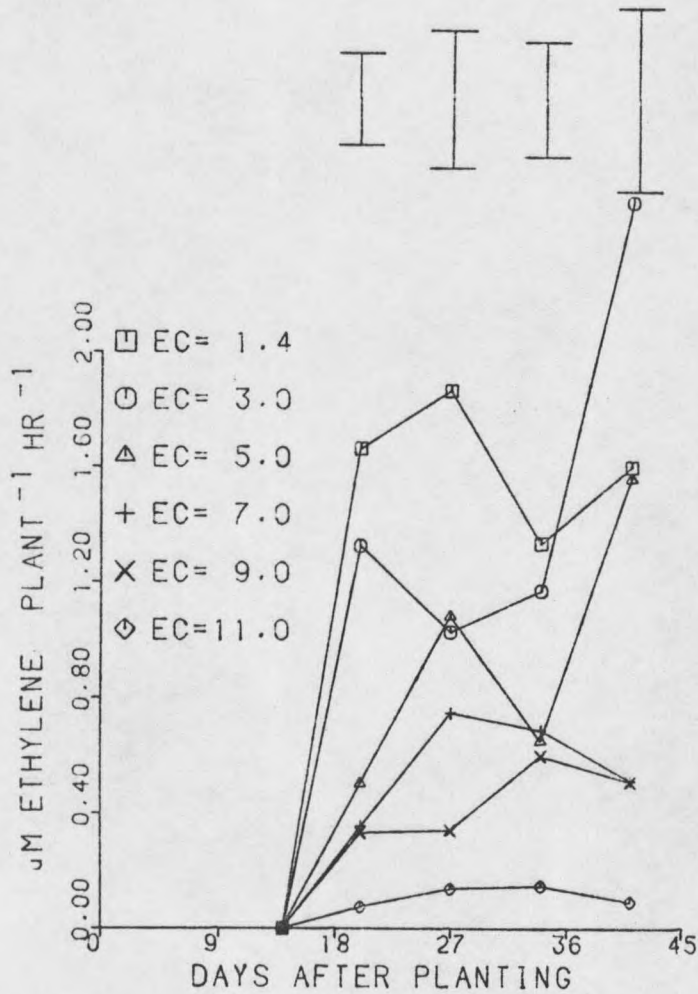


Figure 36. Acetylene reduction activity of *V. faba* L. grown in containers. Each symbol represents the mean of eight plants grown without fertilizer nitrogen. Activity in plants grown without fertilizer nitrogen was very low.



















































































































































































