

AN ABSTRACT OF THE THESIS OF

Mohamed Jabbes for the degree of Master of Science
in Rangeland Resources presented on January 18, 1991
Title: Evaluation of Germination Response and Early
Seedling development of Selected Medicago and Hedysarum
species.

Abstract Approved:

Redacted for Privacy

Douglas E. Johnson

Germination strategies and early seedling development of selected Medicago and Hedysarum species were evaluated to identify plants with high potential for range rehabilitation in central Tunisia. Temperature (5°C to 25°C) and water stress (0 MPa to -0.8 MPa) affected the germination percentage of all Medicago and Hedysarum species. Water stress had a greater effect on percent germination than did temperature, however, the nature of the effect of water potential depended on temperature. Australian medics were more depressed by temperature extremes and low water potential than were Tunisian accessions. Optimum germination of Medicago polymorpha var. Circle Valley and Medicago trusatula var. Jemalong was at 15 °C and 0 MPa.

Germination was highly reduced at higher temperatures and water stress. Tunisian Medicago truncatula germinated better at lower temperatures (5 °C to 15 °C). Hedysarum carnosum germinated more completely at high temperatures (15°C to 20°C). Medicago laciniata germinated well across a wide range of temperature but germination decreased as water stress increased.

Rapid rates of root elongation are beneficial to plants in semiarid environments. High temperature accelerated rates of root elongation and low temperature retarded the rates. The degree of retardation varied with the species and the temperature range. Maximum root elongation occurred at 15°C and minimum root elongation occurred at 5°C. Medicago laciniata had the fastest root elongation rate at 5°C and 15°C. Medicago truncatula was equal to Medicago laciniata at 5 °C. Hedysarum carnosum had rapid root elongation at 10°C and 15°C compared to 5°C. A Tunisian accession Medicago polymorpha had the slowest root elongation at all temperatures.

A quantitative growth analysis was used to assess the effect of environmental conditions on the species performance over a period of 49 days. Mean relative growth rate (mRGR) varied among species. This variation suggested size hierarchies in relative performance among species. The largest plant, Tunisian Medicago truncatula had the largest mRGR and the smallest plant, The Tunisian Medicago polymorpha had the lowest mRGR. The derived parameters,

leaf area ratio (LAR) and unit leaf ratio (ULR), were not consistent with the size hierarchies obtained by mRGR. The Root to shoot ratio (R/S) varied among the species. The Tunisian Medicago truncatula had high root to shoot ratios at low temperature and its R/S ratio decreased at higher temperature. Slow growing species had high R/S ratio. Medicago laciniata was an exception, it produced low R/S ratio because of its long, and thin root system.

Evaluation of Germination Response and Early Seedling
Development of Selected Medicago and Hedysarum Species.

By

Mohamed Jabbes

a THESIS

submitted to

Oregon State University

In Partial Fulfillment of
the Requirements for the
Degree of

Master of Science

Completed January 18, 1991

Commencement June 1991

APPROVED:

Redacted for Privacy

Assistant Professor of Range Resources in Charge of major

Redacted for Privacy

Head of Department of Rangeland Resources

Redacted for Privacy

Dean of Graduate School

Date thesis is presented January 18, 1991

Typed by Mohamed Jabbes

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**Evaluation of Germination Response and Early Seedling
Development of Selected Medicago and Hedysarum Species**

INTRODUCTION

Tunisia is a North African country characterized by a Mediterranean climate. Winters are cool to cold and relatively wet; summers are hot and dry. In the central portion of Tunisia rainfall is irregular and sparse, ranging from 100 mm to 400 mm.

Most of the central and southern region is rangeland. The people that live there and the livestock they raise are heavily dependent upon these rangelands. In addition to range vegetation, the principle forages for livestock are weeds from fallow croplands and crop residues (Johnson, 1987). Traditionally, these rangelands have supplied the bulk of forage upon which livestock depend. In recent times livestock number have increased dramatically. In central Tunisia, where slightly less than half of the country's sheep population exists, farmer's revenues and their well-being are heavily dependent on the returns from sheep flocks (Johnson, 1987).

Management and planning of rangelands have been given little attention. Marginal lands often belong to the government and everyone is free to graze animals, but none is willing to improve the vegetation. Intensive overgraz-

ing of these state owned (or collective lands) depress range production and the ability of the remaining plants to recover. Overgrazing has resulted in reduced yield of beneficial plants and a shift in plant species composition to unpalatable plants. In addition, there is a loss of vegetative cover. Soil erosion has been accelerated which contributes to ecological retrogression. Precipitation that should be held by vegetation so that it can percolate into the soil, under current conditions runs off very quickly carrying with it topsoil and causing gullies and washouts. Gintzberger (1984) reported that rangeland improvement and regeneration is a matter of great concern in North Africa and the Middle East. Socioeconomic and demographic changes have completely altered the fragile balance of traditional grazing patterns, which are based on conventional wisdom of the regional ecology (Telehique 1981). Johnson (1987) reported that an increasing population rate (2.4/annum) coupled with a desire for a higher standard of living, has increased the demands placed upon rangelands. Without improved scientifically sound management, rangeland production will continue to be erratic and the general productivity of the flocks will be low.

In addition to the abuse of rangelands and the increased number of livestock, there is the problem of diminishing rangeland areas. Land that was historically range is being converted to croplands and olive groves. Between

1920 - 1981 cereals and fruit production increased at the expense of pasture and rangeland. For instance, in 1943 there were 0.86 ha of range available for each domestic animal fed continuously on natural pasture; by 1986 there was only 0.38 ha per animal (Dahman, 1987).

The Ministry of Agriculture of Tunisia has recognized the need to improve these areas and has established several projects. The Central Tunisian Range Improvement Project began in 1982. Its goal was to improve rangelands of this area and to provide livestock with adequate feed supplies. Other projects and programs have encouraged sedentarization of nomads and provision of health and educational programs to them.

Several promising ways have been implemented to improve range areas. One initial activity was the introduction of imported perennial grass species such as crested wheatgrass (Agropyron desertorum var. Nordan) and orchardgrass (Dactylis glomerata var. Palestine) for land rehabilitation. Unfortunately, stands of both of these species have failed to persist. Plants had good growth following adequate precipitation during the establishment year but plants lost vigor the second year and by the third year stands were weak and sparse. In spite of the fact that both these varieties are cool season and summer dormant, crested wheatgrass and Palestine orchardgrass cannot tolerate prolonged summer drought. High summer temperature may

also preclude their use and root growth may have been affected. It is likely that these conditions decreased the plant's ability to extract water and nutrients from the soil profile. This would affect their competitiveness, allowing weeds to dominate the site. Although crested wheatgrass and Palestine orchardgrass did not persist, the search for adapted perennial grasses continues.

Another promising technique is the use of forage or fodder reserve shrubs. Attempts are being made to revegetate range areas of central Tunisia with perennial shrubs, such as spineless cactus (Opuntia ficus-indica var. inermis) and salt bushes (Atriplex species). Limitations to this program are: the cost of establishment is high, mortality of transplants is high, and (in the case of cactus) the contribution to the livestock diet is small. Availability of seeds and adapted genetic material is poor and cost is high. Shrub transplanting is labor intensive and difficult to organize. As Hadri (1980) proposed:

"Range rehabilitation based upon the "all perennial" and fodder shrubs policy faces many major problems, including establishment failure, high cost, and conflict with the uses the local population wishes to employ on the range."

In addition, grazing management systems for perennial shrubs have not been developed for (or adapted to) North African ecological and social conditions which increases

the risk of overgrazing and the destruction of plantations. Le Houarou (1980) reported that rangeland legislation which could control abuse is generally absent in North Africa.

Commercially available annual legumes, especially medics, have also been suggested as a method of improving rangelands. Low yield and lack of competitiveness against weeds growing on the sites has been observed in Central Tunisia. I hypothesize that unsuccessful medics have a slower rate of growth at cool temperatures than weedy species common on these sites. Introduction of the Australian ley farming system (based on annual medics) appears to lack success because of frost intolerance (Bailey, 1967). Medics may lose up to 30% of their plant population and thus much of their production potential because of winter kill (Ben Ali 1986).

Annual medics (Medicago species) have been successfully used in areas with a similar Mediterranean climate but without occurrence of prolonged frost. Cocks and Ebrahim (1987) reported that livestock production on fallow lands in the semi-arid area of Syria can be increased by using the otherwise unproductive fallow for self-regenerating annual legumes such as Medicago. Furthermore, Dahman (1987) reported that annual medics have shown great potential for improving the productivity of both cereals and livestock in the semi-arid areas of Tunisia. Annual medics can help to satisfy the immediate feed requirements of

rangeland stock in Tunisia. Annual medics are nutritious and highly palatable to all classes of livestock. Since, medics have their origin in North Africa and the Middle East (Heyn 1963) they are already part of the stock diet. An increase of the medic component in the natural vegetation could be achieved by reintroducing more productive and persistent local ecotypes among other plants (Gintzberger et al 1983).

It is important to focus on the search for ecotypes of annual medics or those that can establish and persist in central Tunisia. The examination of the indigenous species has not been carried out in great detail until recently (Papastyliyanou 1987). Chatterton (1984) added that little has been done in encouraging production of indigenous species that have regenerated naturally in North Africa and the Middle East. Farming system using locally adapted annual Medicago species have obvious practical potential.

The major objective of the use of indigenous Medicago species in central Tunisia is to produce species with significantly greater winter above and belowground growth. Germination strategies (rate of germination at low temperature and water stress) and early seedling growth and development at low temperature of a number of Medicago and Hedysarum species from Tunisia were evaluated. These parameters will be contrasted to the commercially available

strains. From this I hope to identify commercial varieties that closely approximate the local species, or determine which characteristics are different and should be selected for in plant breeding programs.

This study was undertaken to accomplish the following objectives:

1. Determine the germination performances of five Medicago and two Hedysarum species under various temperatures and water stresses.
2. Quantify the rates of root elongation of these species and accessions under controlled temperatures. This information can suggest the effect of such rates on competition for moisture later in the season and plant survival. Faster growing roots should be able to preempt resources from slower growing species.
3. Evaluate growth parameters of Medicago and Hedysarum species in relation to temperature.

LITERATURE REVIEW

Although there has not been a great deal of work done with annual medics from this region, the literature does reveal some helpful points. The Minister of Agriculture has mentioned that medics are well known as valuable forages to Tunisian farmers. Previously they were abundant in Tunisian rangelands and appear to be declining because of overgrazing. Identification and collection of local medic species suitable for rangeland conditions is therefore a major phase of this project. Gintzenberger and Rahman (1981) reported that collecting native forage species already adapted to an area provides highly valuable material for immediate use in land rehabilitation projects.

Taylor and Hughes (1979) pointed out that many annual legume species occur naturally in the Mediterranean region. These annual legumes grow during the cool winter, set seeds in spring, then senesce and die before summer. In artificial pastures, seed produced after initial sowing provide a reliable seed bank in the soil. Medics have a hard seed coat, which is an ecological adaptation that allows only part of the seed to germinate the following autumn (Quinlyvan 1966). Medics therefore can survive drought years by avoidance because seeds may remain dormant for several years. After a prolonged drought seeds gradually break dormancy, germinate and grow. Because of these adaptations, medic species have proved to be a useful component

in permanent pastures and on rangelands of New Zealand, Australia and California where dry summers are frequent (Levy 1937 and Martin et al. 1967).

In spite of the fact that these regions are hot and dry, plant response to winter conditions (cool to cold and moist) may be more important in determining the vegetative community. Many investigators have pointed out that growth during periods with cold temperatures limit the areas where medics can be used (Doolette 1977).

Taylor and Hughes (1976) suggested that it might be possible to use Mediterranean legumes as the "cool season" component of a double cropping system. In their 1979 study, they found that a growth reduction of 30% to 70% of some legume crops is clearly seen at cooler sites. They added that the establishment of legumes and their winter and spring growth was slow at certain sites in New Zealand and that most of the forage harvested was produced in mid spring. Muhmes (1985) said that cultivars used in the ley farming system do not tolerate extreme cold temperature. Consequently, the system presently is restricted to areas with mild winters. He added that under these conditions the annual legume varieties display vigorous winter-spring growth and produces large quantities of seed.

Van Heerden (1984) suggested that temperature as well as day length should be incorporated in any model used for the description of the relationship between climatic fac-

tors and length of the period from seedling emergence to flowering in the species concerned. The exact relationship between temperature and day length in medics has not been examined.

Cold hardiness and winter growth are traits that one would like to have in annual medics improve establishment and forage production (Ben Ali 1986). However, these traits appear to be independent and antagonistic. At Canberra, Australia, Daday (1964) has shown that Medicago sativa's cold hardiness and winter growth are inherited separately, but both characters can be brought together through genetic combination. It is generally found that if plants are growing quickly they cannot be frost hardened (Riviera and Corneli 1931), whereas treatment that retards growth increases hardiness. Germination cold tolerance and emergence cold tolerance are likely to be closely related in medic similarly to the association found in other plants (Dickson and Boettger 1984).

Larson (1964) reported that cold sensitive varieties of forage plants may initiate growth quickly during periods of warm weather in late winter, whereas cold tolerant varieties require much longer warm periods before they initiate growth. He added that plant species that cannot adjust carbohydrate concentrations (as solutes) in the cytoplasm may not be able to develop cold tolerance regardless of temperature exposure. This is particularly true

when carbohydrate reserves are reduced to critical needs.

Gintzberger and Rahman (1984) have grown numerous ecotypes of medics collected in Libya at two Australian rainfed sites. They obtained an extreme earliness of flowering and outstanding winter vigor from the Lybian accessions, especially in Medicago littoralis, as compared to the two earliest and drought resistant Australian cultivars, var. "Harbinger" and "Cyprus."

It should be possible to obtain ecotypes with the ability to withstand cold temperatures from regions where winter frost is common. These ecotypes could be evaluated or be used in breeding programs. This technique was used by Scully (1987) in his studies on Phaseolus acutifolius A. Gray.

Taylor and Hughes (1979) have tried to introduce cool season legumes for forage in New Zealand, they found two species of Medicago which show promise as annual cool-season forage crops. They added that Medicago seedlings generally established rapidly, presumably because of their large seeds, and that Medicago species yielded consistently well, about 9.0 to 11 tons DM/ha. In Central Tunisia yields have been comparable to this in the Dorsale in high rainfall years but are typically much lower in the semi-arid regions (400-1200 KgDM/ha).

ECOLOGICAL IMPORTANCE OF ROOTS

Range plants compete with each other for limited resources such as: light, water, nutrients, soil space, and air (Harris 1967). In arid and semi-arid systems the primary limiting factor is soil water. Plant species that are able to adjust the growth pattern of their root system to establish in a new zone of absorption may have competitive advantage.

In arid environments such as central Tunisia, soil surface layers dry out very quickly requiring rapid seedling root elongation for successful establishment. Therefore, maximum root elongation in the preemergent and early post-emergent stages is necessary to ensure that the seedling root reaches the relatively moist subsurface layers before the emerging plant becomes subjected to atmospheric moisture stress (Moore 1943, Whally et al. 1966).

The major benefit of using indigenous Medicago species in Central Tunisia is to produce plants with significantly greater winter above and belowground growth. However selecting for this trait is especially difficult because growth at low temperature may be genetically complex with characteristics influenced by numerous environmental factors and their interactions. However, indigenous species may have developed cold tolerance through natural selection pressure in the field. Through natural pressure, plants without cold resistance are eliminated from natural popula-

tions, and the remaining plants are cold resistant.

Timing of phenological development in roots has important survival value for the plant. In Tunisia most of the precipitation occurs in the winter period, so water generally has enough time to percolate to deeper strata. Unfortunately, during this period of adequate soil moisture plants have greatly reduced or ceased their growth because of temperature. Therefore, seedlings that are able to slowly continue root growth at this season will have roots that occupy a greater volume of soil and be poised to capture more resources.

Harris (1967) studied the seedling root systems of Bromus tectorum L. (an annual grass) and Agropyron spicatum (a bunchgrass) to determine plant attributes that resulted in competitive advantage and dominance. He also assessed the ability of B. tectorum to maintain dominance on the sites indefinitely. He determined that B. tectorum root systems grew at a faster rate during the early seedling stage than root systems of A. spicatum. Growth continued throughout the winter. Thus B. tectorum exhausted available moisture supplies in advance of the competing A. spicatum roots. As a result A. spicatum seedlings had less resources, available which caused their demise. Bromus tectorum roots grew mainly in the winter when shoot growth is much reduced. Available carbohydrates are partitioned for root growth. In this experiment Bromus tectorum

competition, reduced the elongation rate of A. spicatum seedling roots. Harris (1967) concluded that the greater root elongation rate of Bromus tectorum and its ability to grow at lower temperatures was the key characteristic which account for its dominance over A. spicatum.

Root Length

Range seedlings need a continuous supply of water and nutrients for their growth and establishment. Root development obviously controls the volume of soil from which these materials can be drawn. Any study of the efficiency of root systems to capture belowground resources should take into account root length and its association with surface area. Studies based only on root weight are incomplete. Root length and surface area are important characteristics of a plant's competitive ability. They are also indicative of the capability to take up water and nutrients from the soil. The development of long roots permits the plant species to cover greater soil volume and allow to extract soil moisture at a greater distances in the soil profile. Horst et al. (1967) emphasised that root weight is less suitable index than root length. He added that length is better correlated with surface development, a most important factor affecting physiological activity and exchange of substances. Also Newman (1965) revealed that most quantitative studies of roots have used weight as the

of assessing the amount of roots; however it is generally accepted that the capacity to take up water and salts is usually more closely related to the surface area or total length of the root system than to weight.

Caldwell and Richards (1986) used sensitivity analysis to illustrate root and soil characteristics that were important in nutrient absorption and thus competitive effectiveness. They cited the example of Silberbush and Barbar (1983) who used sensitivity analysis for examining potassium uptake by soybeans. The analysis showed that the most sensitive parameters are those related to root morphology: root growth and radius. They added that growth in root length was the most sensitive parameter because both root length and the soil volume explored increase with growth.

Caldwell and Richards (1986) studied competitive abilities in the bunchgrass species Agropyron spicatum and Agropyron desertorum growing in an Artemisia tridentata mixture. They found that A. desertorum had deeper roots than A. spicatum. In addition average crown volume of Artemisia grown with A. spicatum was more than twice the mean volume of the Artemisia interplanted with A. desertorum. They determined that A. desertorum invests less energy per unit root area which allows the plant to produce more root surface area that can occupy a greater portion of the soil profile. They concluded that thinner roots of A.

desertorum seem to provide greater rooting density and allow this species to dominate belowground space. It is therefore advantageous to possess a root system that is sufficient for nutrient uptake and water supply but simultaneously has low biomass maintenance costs.

Fertility

The effect of temperature on root growth is complicated by interrelations with other factors such as soil fertility and soil conditions. The soil environment (soil type, texture, structure, temperature, moisture and nutrients availability) influences the severity of competition between roots and governs the pattern of the root system. Although the general form of root is governed by heredity, it is very responsive to environmental conditions (Weaver 1926). Deficiency of water and nutrients leads to long root growth and restriction of side root formation. This favors an accelerated root penetration of the soil (Horst 1967). Asher (1966) added that on soils which are deficient in plant nutrients or which dry out rapidly after rain, the rate at which root penetrate the soil may be important for seedling survival.

In soils poor in nutrients roots also tend to branch less than in richer soils so roots grow deeper (Troughton 1968). Root system can grow toward areas rich in soil nutrients and then branch. Water and nutrients are impor-

tant ecological factors influencing the zone where root biomass is concentrated (Kramer 1989).

Contribution of Legumes to Soil Fertility

Legume species can contribute to soil fertility in central Tunisia especially on sites with depleted soils. Adapted legume species, such as Medicago will add nitrogen to the soil and increase subsequent forage production. Legumes are very important nitrogen sources. Through their symbiotic association with rhizobia in their root system, legume species are able to fix elemental nitrogen (N_2) from air and transform it to a form used by plants. The input of nitrogen by leguminous pastures is considered to be the most important factor for improving cereal production in the ley farming system (Papastylyano 1987). Andrew (1965) and Stoneman (1973) added that legumes contribute to soil productivity by improving soil structure.

Dahlman (1965) evaluated root system dynamics in stable prairies in Missouri. He found that humus accumulation was greater than 25 Metric tons/ha in the top fifteen centimeters. Thorpe (1948) reported 40-100 Metric tons/ha in North Dakota and Canadian prairies and 10-20 Metric tons/ha in warmer regions. Dahlman (1965) estimated annual nitrogen production of 2500 kg/ha (assuming 5% nitrogen in organic matter).

Soil erosion is a serious problem to exposed range-

lands. In addition to soil fertility root systems have an obvious role in protecting soils from erosion. Weaver (1926) said:

"roots exert an important effect in connecting together soil particles and thus stabilizing the soil."

Crawford et al. (1989) found that cereal-fallow systems in southern Australia caused significant degradation in soil structure while erosion by water and wind became common. In contrast growth of a pasture-ley (medics) between crops improved soil structure.

Temperature

Temperature is a major influencing factor on shoot and root growth. Even though there is extensive literature on root development, temperature effects on elongation have received less attention. Plant activities are significantly affected by temperature and ceased at or below critical levels. In Tunisia, low winter temperature limits the development of both roots and shoots of even winter annual species and may kill or injure other plants. Root absorption of water and nutrients, as well as, physical and chemical processes are greatly reduced by low temperature. Because root growth, as well as mineral salt and water uptake, are dependent on metabolic processes, it is to be expected that soil temperature will influence root growth

and activity and indirectly the whole plant growth (Horst 1967). Atkin (1973) found that depression of shoot growth of corn grown at low temperature was caused by altered production of growth substances in the root systems. He added that reduction of top growth could be caused by lowered translocation of nutrient elements through root systems. Low soil temperature affects germination of seeds and seedling development. Troughton (1957) found that unfavorable soil temperature reduces the rate of root initiation and causes a depressed lateral root growth and reduced length. Cohen and Tadmor (1969) studied seedling root penetration as a function of temperature of the small seeded range plants, Agropyron desertorum Shult, A. elongatum Hort, Medicago polymorpha, and Medicago truncatula. They compared them to the large seeded species, Avena sterilis, wheat and barley. They found that the rate of seedling root elongation of the range species varies between .5cm and 2cm day⁻¹ compared to 2cm to 7cm day⁻¹ for wheat and barley. Higher temperatures accelerated root elongation while lower temperatures retarded it. A pronounced critical effect of low temperature and small seed weight on establishment of the small seeded range plants was also observed. They suggested that severe detrimental effects on establishment occur, especially in winter rainfall areas where the growth period coincides with low temperature. Breeding range plants with larger seeds and

faster root elongation seems to be essential for better establishment, especially if establishment under suboptimal conditions is needed (Cohen and Tadmor 1969).

Asher (1966) studied the rate of root elongation of twelve annual pasture species in a glasshouse. He found that roots of three hardy Mediterranean weed species (filaree, ripgut brome grass, and capeweed) penetrated the soil faster than those of the sown pasture species. The rate of root penetration of most species also changed with time.

IMPORTANCE OF ROOT STUDIES

In spite of the important role that roots play in a plant's survival, little is known about root properties in the soil and growth behavior. This is because roots are very difficult to observe and are often considered to be secondary organs. Caldwell (1987) said:

"The significance of some phenomenon (ie interference between root systems) should not be overlooked but on the other hand demonstration of these phenomenon in the field remains elusive".

He added that competition for soil resources is also difficult to prove unambiguously in a field setting.

A comprehensive picture of root system characteristics under field conditions is seldom available. Little is known about the attributes of root systems which enable some species to acquire resources more efficiently than

others in competitive situations. Whereas detailed information, and in some instances reproducible results have been obtained, on shoot growth, reports on root growth are in disagreement or are contradictory (Horst 1967). Knowledge of root growth, and the factors influencing it, should play a more important role than it has in the past (Chouki 1986). Knowledge of root growth and behavior can be used to select plants that enhance forage production with increased capture of resources.

GROWTH ANALYSIS

The establishment of a satisfactory stand and high dry matter yield is very important in forage production; however, it is important to know more than the final yield or the end result. Events along the way may have had a marked influence on the final outcome (Seddiick, 1980). Beginning with the early works of Blackman (1919) and Kidd and West (1919), a discipline of study has developed that attempts to integrate the effects of environment, development, and size on plant growth. These techniques are collectively called growth analysis (Radosavich, 1984).

Growth analysis is used to quantify plant growth from a series of mathematical equations. Growth analysis is usually developed from individual plant growth, generally at the early stages. The growth analysis approach involves measurements made at frequent intervals throughout the life

cycle of the plant. Consequently, investigators can separate the components of plant growth and compare them under a range of environmental conditions. Such experiments provide valuable information for understanding the basis of differential success when plants are growing in mixed stands. By comparing the growth parameters of various plant species, we may better understand their competitive nature (Radosavich 1984).

Investigators have looked at growth analysis when species are grown separately and in mixture. Williams (1963) found that the relative growth rate (R) of three clover species was similar. However, in mixed stands R of subterranean clover was increased at the expense of the other two in the mix. Such differences in growth rates are of great ecological importance, because the maximum growth rates of plants are a major determinant of their short term dynamic responses to any changes in their environment (Tillman 1982).

Studying resource availability, assimilation, and allocation can help understand how plants react, uptake, use, and distributes resources. It has been suggested that plants grow with a certain allometry, meaning that the size and function of one organ is related and dependent of upon the size and function of another associated organ (Radosavich 1984, Tillman 1982). The root plays an important role in nutrient and water uptake and distribution, while the

shoot provides carbohydrate and certain growth substances to the root. Environment can change the relationship of one organ to another, usually as a result of limitation in supply of an essential factor leading to a competitive relationship and thus change in distribution of dry matter.

It is obvious that environment will have an impact on the way the plant grows and develops and distributes its dry matter. These effects are very complex where interaction among environmental factors are occurring.

Recently, growth analysis has been widely used by many investigators in the USA and around the world.

Relative growth rate (RGR) expresses the dry weight increase in a time interval in relation to the initial weight (Evans 1972). The formula for calculating RGR is:

$$RGR = 1/W * \Delta W / \Delta T$$

where W is weight and T is time.

Relative growth rate adjusts for initial plant size and provides a comparison of the plants relative performances. When a species has established a growth advantage, it would have a great result for competition during the whole season. Radosavich (1984) stated

"It appears that a rapid growth, that is, large value for RGR, is an important characteristic of weeds common to arable land and other productive sites."

He added:

"Perhaps the RGR can serve as an indicator of potential competitive ability among crop and weed species."

A subdivision of parameters of relative growth rate could be a more meaningful index of growth. These parameters are unit leaf ratio (ULR) and leaf area ratio (LAR).

ULR of a plant is the gain of plant material per unit of leaf area per unit of time:

$$ULR = 1/LA * \Delta W/\Delta T$$

W refers to plant dry weight, LA refers to leaf area and T is time. LAR of a plant is the ratio of total leaf area per total plant mass:

$$LAR = LA / W$$

where LA refers to leaf area, and W refers to plant dry weight. The product of both ULR and LAR equals relative growth rate (RGR).

ECOLOGICAL AND PHYSIOLOGICAL FACTORS OF THE SPECIES USED FOR THE STUDY

The Fabaceae is one of the largest families of flowering plants after the Asteraceae and the Orchidaceae. It is represented by 16,000 to 19,000 species in 750 genera (Allen 1981). The distribution is worldwide. Economically, it is a very important family, second to the grasses in providing a variety of food sources to humans and animals. This family is very diverse. It plays an important role in plant ecosystems around the world.

Legumes, through their symbiotic association with the bacteria, Rhizobium, fix elemental nitrogen (N_2) from the atmosphere and transform it into a plant usable form. Nitrogen fixation enables the plant to meet its requirements and gain ecological advantage over other species. As pioneer plants, legumes form the hub of an efficient nitrogen source for the entire ecosystem (Allen et al 1964). Allen (1981) added that biological nitrogen fixation, particularly of the symbiotic type, plays a crucial ecological role in maintaining adequate nitrogen resources in the plant world.

Today, the most important forage crop is alfalfa which belongs to the genus Medicago (Lesin et al 1979). Its agronomic significance such as, forage yield and quality, and the improvement of soil properties has expanded and intensified its use over a wide geographical areas of the

world.

The annual Medicago species originated in the Mediterranean basin. They have shown potential for growth and improvement in less desirable soils and on rangelands in areas with Mediterranean climates. Some of these "problem" soil types in Australia support excellent stands of naturalized medics, mainly burr medic or goldfields medic (Quinlivan 1965). Medicago species, facilitated by their spiny burrs, have been distributed to many areas around the continents. The extensive trade in unscoured wool, stock, and other commodities from Mediterranean countries over hundreds and perhaps thousands of years, has undoubtedly been the mean by which these spiny burred species spread from their original homeland (Quinlivan 1965).

The annual Medicago species constitute an important component of Mediterranean natural pastures by producing high quality feed readily available to grazing animals. They improve soil properties and increase nitrogen availability in the soil. The quantity of nitrogen fixed by legumes varies depending on biotic factors (stand age, density, vigor, etc.), efficiency of the symbiotic association with the bacteria, and abiotic factors (soil type, temperature, etc.). Substantial nitrogen can be fixed by these plants. Several investigations in wheat growing areas have show that Medicago can increase soil nitrogen by 60 to 70 kg per hectare in one growing season. Even though

they play an important role, little is known about physiological and ecological adaptations of medics. This is particularly true for the annual medics where we don't know their potential growth and development or even their distribution. More research needs to be carried out before such species come into widespread agricultural use, but the potential is there (Le Houarou 1979).

Medicago truncatula Gaertner: barrel medic

Medicago truncatula is an omni-mediterranean species (Lesin and Lesin 1979) and valuable pasture plant (Tumble and Donald 1938). It has been grown extensively in Mediterranean areas and regions of similar climate. It occurs naturally in the mountainous areas of Mogods, the coastal range, the dorsale, and central and south Tunisia. In Australia, where Medicago truncatula has become naturalized, it grows well on fine textured soils with high cation and phosphorus status. These soils are black earths, or grey and brown soils of heavy texture (Robson 1969). It grew poorly on coarse textured acid soils of granitic or quartzic origin (Robson 1969). In Tunisia, its natural habitat is fine textured, alluvial soils. It is found in outwash areas from oueds, meadows, and hillsides (Pottier 1981).

The plant grows between 15-80cm high, procumbent to ascending (Lesin and Lesin 1979). It has hairy stems and

leaflets. The leaflets are wedge shaped, and coarse toothed toward the outer extremes (Quinlivan 1965). This species has a cluster of one to three small yellow flowers sitting on a peduncle. The pod is barrel-like in shape, spiny, and coiled with 3.6-6 turns in a clockwise direction. The seed pod contains 6-10 kidney shaped seeds.

Medicago truncatula is primarily represented by three cultivars in Australia: commercial barrel medic, Jemalong barrel medic, and Cyprus barrel medic. Cyprus barrel medic is more widely used in Australia and has been replacing other commercial strains. Cyprus barrel medic matures 3 to 4 weeks earlier and has proven to be generally superior.

Medicago polymorpha L : burr medic

Even though Medicago polymorpha was originally confined to the Mediterranean basin, it has spread all over the world except in areas of cold winter temperatures. Low temperature has been found to destroy imbibed seeds and spring frost can kill seedlings (Lesin and Lesin 1979).

Medicago polymorpha is grown in the cereal producing areas of Tunisia where environmental conditions (soil, temperature, precipitation etc.) are favorable. Even though barrel medic use is increasing, this species still constitutes an important pasture plant. The natural habitat of burr medic is fine textured soils, although it has been found growing well on alkaline and poorly drained

soils (Andrew and Hely 1960).

The plant is about 20-70cm high, with decumbent to ascending growth and glabrous leaflets. The flowers are small, yellow, and occurs in clusters of 2-8 (Lesin and Lesin 1979). Seed pods are rather polymorphic and spiny, with coils (1.5 to 7) turning clockwise. The burr usually contains from 3 to 6 seeds. Slender, hooked spines on the burr are effective dispersal mechanisms which enabled the seeds to be distributed widely. The spiny burr can decrease the market value of sheep wool. Medicago polymorpha is represented by three varieties: Medicago polymorpha L var *brevispina*, Medicago polymorpha L var *polymorpha* and Medicago polymorpha L var *vulgaris*.

Medicago laciniata (L.) Mill., leafcut medic

Leafcut medic is an annual legume that prefers to grow in dry light soils of steppes and Hemadas (Jordan 1981). In Tunisia, it is found growing in dry sandy soils and alluvium or in stony desert-like environments. It is frequently the only Medicago species that can survive these harsh southern Tunisian conditions (Pottier 1981). Heyn (1963) has identified two varieties of Medicago laciniata: Medicago laciniata var *laciniata* and Medicago laciniata var *brachyantha* Boiss. The first variety (var. *laciniata*) has lacinate leaves, larger seed pods, and long spines. It is adapted to a wetter habitats where it is commonly found on

northern slopes and wadi beds dominated by associations of Artemisia herba-alba (Friedman and Elberse 1976). The second variety (var brachyantha) has smaller seed pods, coils 2-4, and serrate leaves. The var brachyantha is adapted to more xeric habitats and commonly found in the southern slopes and hill tops dominated by the association of Zygophyllum dumosi (Friedman and Elberse 1976). A hard seed coat allows seeds to remain dormant and viable for several years. Friedman (1974) has tested 20 year old seeds and found that 85% germinated after 48 hours with favorable moisture. Seeds are dispersed by rodents and livestock.

Hedysarum carnosum Desf.

The genus Hedysarum is well distributed in temperate Europe, Mediterranean North Africa, Asia Minor, Siberia, and North America (Allen 1981). Hedysarum carnosum is a biennial legume native to the arid lands of North Africa. It is commonly found in the central part of the country growing on saline soils. The natural habitats of the species is fine textured, clay loam, saline and alkaline soils (Le Houarou 1969), and in natural pastures of calcareous and gypseferous soils (Pottier 1981). The foliage of this species is palatable and relished by livestock. Native populations of this plant produce a high proportion of hard seeds which result in poor germination under field

conditions during the first year. However higher germination can be obtained when seeds are scarified to allow water imbibition. Haddar (1965) has tested the species in controlled environments and found germination can approach 90%. Under field conditions hard seeds require high humidity and soil moisture for germination, emergence and seedling root establishment. The species is grazed by horses, cattle, and sheep when green but livestock avoid it when it is dry.

The plant is glabrous and fleshy. Stems are erect with spreading lower branches. Leaves are 5-7, foliate and oblong (Pottier 1981). Flowers are large 10-12mm, showy pink, in a dense clusters (axillary erect raceme).

GERMINATION EXPERIMENT

Materials and Methods

Thirteen species of medics were given by Dr. Walter L. Graves, Farm Advisor, Cooperative Agricultural Extension, University of California. Dr. Daalool Abderazak, Professor, Institut Nationale Agronomique de Tunisie, Tunis, Tunisia, has provided 15 species of medics from the gene pool bank in Tunisia. Other plant material was obtained from the forage laboratory, Institute Nationale Agronomique Tunis (INAT), and the Central Tunisian Rangeland Development Project in Kairouan, Tunisia.

From the accumulated seeds, the following were chosen for the experiment:

1. Medicago truncatula, (W. Graves) Tunisian accessions
2. Medicago truncatula var. Jemalong
3. Medicago polymorpha, (W. Graves) Tunisian accession
4. Medicago polymorpha Circle Valley
5. Medicago laciniata, (W. Graves) Tunisian accession
6. Hedysarum carnosum, (INAT) Kairouan, Tunisia
7. Hedysarum carnosum, (INAT) Tunisian

The imported Medicago truncatula var. Jemalong and Medicago polymorpha var. Circle Valley were selected because they are widely used in Tunisia in the ley farming system and have failed in some areas.

Seeds of medics and Hedysarum species were germinated in growth chambers under all combinations of temperature (5, 10, 15, 20, 25°C) and water stress (-0, -0.2, -0.4, -0.6, and -0.8 MPa). Alternating periods of 16 hours light and 8 hours dark were maintained. The temperature was controlled to within $\pm 1^\circ\text{C}$.

The osmotic solutions were obtained by using polyethylene glycol (8000) and distilled water following procedures outlined by Michel (1983). Solutions were tested with a vapor pressure osmometer (model 5100cc Wescor Inc., Logan, Utah) and adjustments were made until the values gave the desired water potential.

Germination took place in 16 x 16cm germination boxes (plastic) with tightly fitting lids. The substrata consisted of one germination blotter. Each box was enclosed in a plastic bag (ziploc) to maintain high humidity and prevent desiccation (Doeshner 1985). The PEG (8000) solution or distilled water was added to the substrata until saturation and some puddling on the bottom of the container occurred. Three observations of 25 seeds were used for each treatment. Daily germination counts were made for 28 days. Seeds were counted as germinated when the radicle was twice the length of the seed.

To evaluate the percent germination of each species, mean percent germination was adjusted based on the percent germination of the treatment control. Optimum temperature

of 15°C and water potentials of zero MPa was used as a control treatment. The adjusted mean percent germination was the ratio of the percent germination of each species over the percent germination of the treatment control. Germination rate (Mean Germination Time) was calculated (Scott 1984) for each treatment using the following formula:

$$\text{Mean Germination Time} = \frac{\sum T_i N_i}{S}$$

Where T_i is the number of days after sowing, N_i is the number of seeds that germinated on day i , and S is the number of seeds that germinated.

The Mean Germination Time represents the average time in days until a seed (of each species) germinates.

Data analysis

The experimental design was a factorial set of treatments (5 temperatures x 5 water potentials x 7 species) in a split plot design with 2 blocks (time) and three observations per treatment. The experiment was replicated twice over time and time was used as a block. Percent germination and Mean Germination Time (MGT) were analysed using Analysis of Variance. Fishers protected LSD was used to separate the mean percent germination of the species tested at a probability level of $P=.05$ (Milliken and Johnson 1984).

Mathematical contrasts were used to partition treatment sum of squares into a number of components that illustrated the nature of interactions. For percent germination species and temperature, species and water potential, and temperature and water potential interactions were examined. Temperature and water potential interactions were analyzed to determine their effects upon Mean Germination Time (MGT).

GERMINATION EXPERIMENT RESULTS

Percent Germination

The results of the experiment conducted to assess the effect of temperature and water stress on the germination percentages and Mean Germination Time of five Medicago and two Hedysarum species are shown in Figures 1-3 and tables 1-3. The analysis of variance revealed no significant three-way interactions between species, temperature, and water potential for percent germination (Appendix Table A). However, significant interaction were found between species and temperature ($P = .0001$), and temperature and water potential ($P = .0002$).

Percent germination of the local accessions Medicago truncatula TA was higher than other species at 5°C (Figure 1). Percent germination of the local Medicago truncatula TA was 82% at 5°C (Figure 1). However the percent germination of the imported medic species, Medicago truncatula Jemalong and Medicago polymorpha Circle Valley was 63%, 67% at 5°C respectively. These results suggested that there was some difference in germination ability at low temperature between the local species Medicago truncatula and imported species (Table 1). At higher temperature of 25°C Hedysarum carnosum TA, Hedysarum carnosum KA, and Medicago laciniata TA had higher percent germination than the other medic species (Table 1). These results suggested that Medicago species (except Medicago laciniata) had an optimum

temperature for germination of 15°C and no water stress while Hedysarum accessions and Medicago laciniata TA optimum temperature for germination was 20°C (Table 1). The data indicated that the percent germination declined as water potential decreased below 0 MPa for all the species tested. Percent germination was the greatest at water potentials of 0 MPa but decreased significantly at water potentials of -0.8 MPa for all species (Table 1).

At temperature extremes (5°C, 20°C and 25°C) and at low water potentials (-0.6 and -0.8 MPa) imported varieties were more affected than local species. At 5°C, germination percentage ranged from a low of 38% for Hedysarum carnosum TA to a high of 63% for Medicago truncatula Tunisian accession (Figure 1). At 25°C, Medicago truncatula JA germinated 28% of its seeds while Medicago laciniata Kairouan accession germinated 62% (Figure 1).

The rate of decline in germination percent as temperature was increased from 15°C through 25°C was slower for all local Medicago and Hedysarum accessions than imported medics ($P = .0001$). Medicago laciniata and Hedysarum carnosum Kairouan accession response was not affected by temperature increase until it reached a temperature greater than 20°C (Figure 1). However Medicago truncatula, Medicago truncatula Jemalong, and Medicago polymorpha Circle Valley declined as soon as the temperature rose above 15°C (Figure 1).

The interaction of species X temperature indicated that some species (such as Medicago polymorpha CV, and Medicago truncatula JA were affected more than others by temperature (Figures 1 and 2).

The interaction of temperature and water stress is non linear. Both cubic and quadratic models of water stress coupled with quadratic temperature effects were significant ($P = .0001$ and $P = .0134$ respectively). Water stress had a greater effect on species germination response than temperature, however the nature of the effect of water stress depended on temperature (Table 2).

Mean Germination Time

Analysis of variance showed no significant three way interaction between species, temperature, and water potential for germination time (Appendix Table B). A significant interaction was detected between temperature and water stress ($P = 0.0019$). The lack of interaction between species X temperature, and species X water stress indicated that the response of each species germination rate was similarly affected by either temperature and water stress. Increasing water stress increased the time to germination (Figure 4). At high moisture stress, a small number of seeds germinated late and at lower moisture stress germination was relatively fast.

Examination of these data suggested that germination

time is a nonlinear function of temperature and water stress (Figure 4 and Table 2). A quadratic model of water stress and temperature was significant at $P = .001$. The interaction of the linear component of temperature and quadratic component of water stress was also significant ($P = .0225$).

As temperature increased germination time decreased. For example, at 5 °C seeds required more than 12 days to germinate while at 25°C it decreased to only 8 days. Conversely, as water stress increased time to germination tended to increase. At 5°C and -0.8 Mpa seeds of Medicago and Hedysarum required more than 17 days to germinate. This trend breaks down at high water stress coupled with high temperatures. I hypothesize that at 20°C and higher, the respiration rate of seeds is greater and only those seeds that germinate quickly can survive long enough to be counted as germinated. In addition at high temperature and high water stress only a few seeds germinated. This resulted in a curvilinear relationship as shown in Figure 4. The germination time (MGT) at 20 and 25°C at -0.6 and -0.8Mpa was decreased to 11 and 8 days, respectively.

Table 1. Three way table of adjusted mean percent germination of *Medicago* and *Hedysarum* species as influenced by temperatures and water stress.

WS,MPa	Temperatures								
	5 °C			15 °C			25 °C		
	0	-.4	-.8	0	-.4	-.8	0	-.4	-.8
Species									
HCTA	53 c	51 b	8 d	75 c	62 ab	12 c	83 a	56 a	13 b
HCKA	68 b	57 ab	29 b	88 b	71 a	37 ab	92 a	59 a	22 ab
MLTA	70 ab	61 a	42 a	85 bc	64 ab	34 ab	93 a	65 a	27 a
MPTA	58 bc	46 b	23 c	72 c	64 ab	45 a	67 b	31 b	16 b
MPCV	64 bc	58 ab	29 b	91 ab	57 bc	33 ab	56 bc	22 c	9 c
MTTA	82 a	61 a	37 ab	100 a	69 ab	14 c	47 c	32 c	7 c
MTJA	63 bc	64 a	28 b	98 a	56 c	27 b	46 c	29 c	6 c

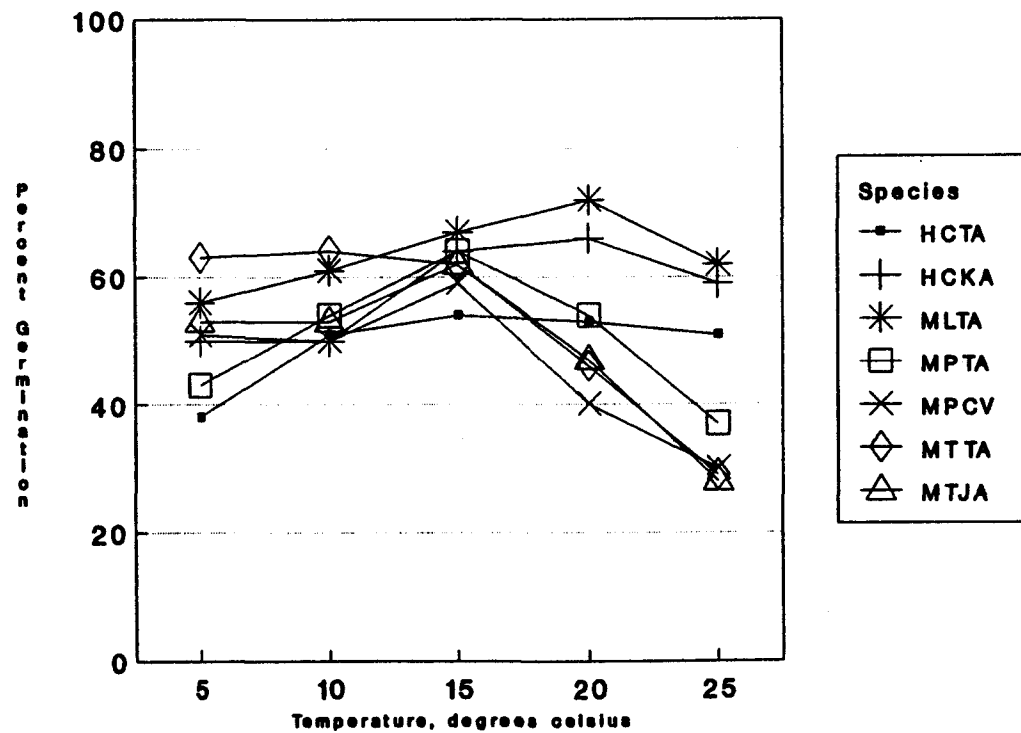


Figure 1. Effect of temperature on germination percent of local and imported Medicago, and Hedysarum species. Germination percent was averaged over water potentials.

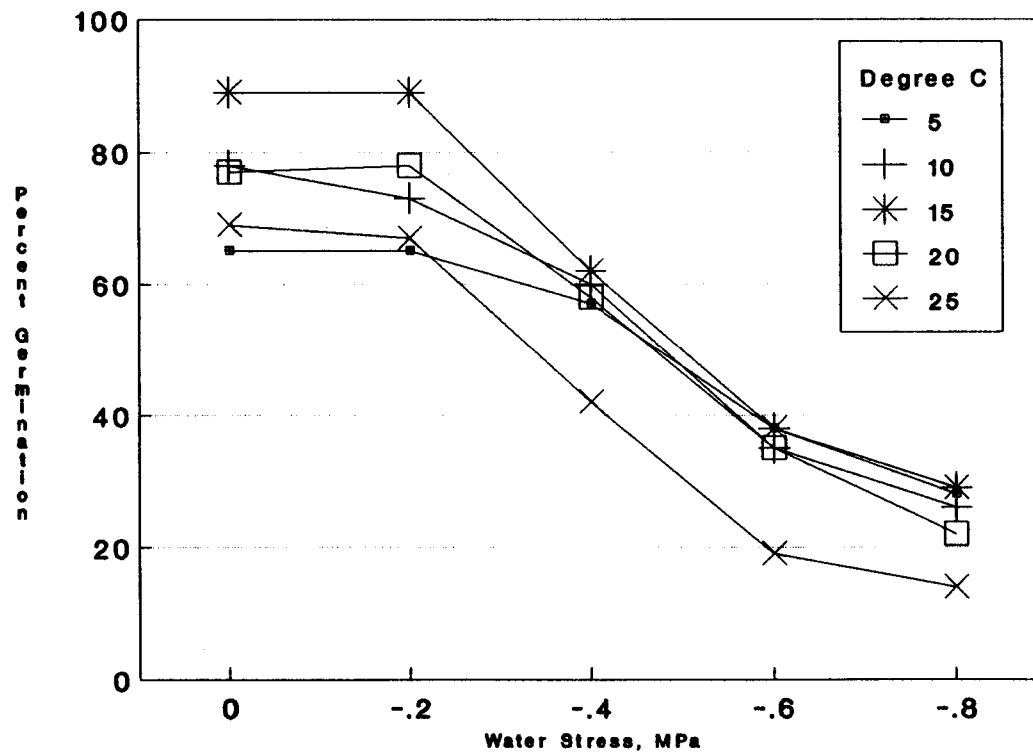


Figure 2. Interaction effect of temperature and water stress on germination percent of Medicago, and Hedysarum species. germination percent was averaged over all species.

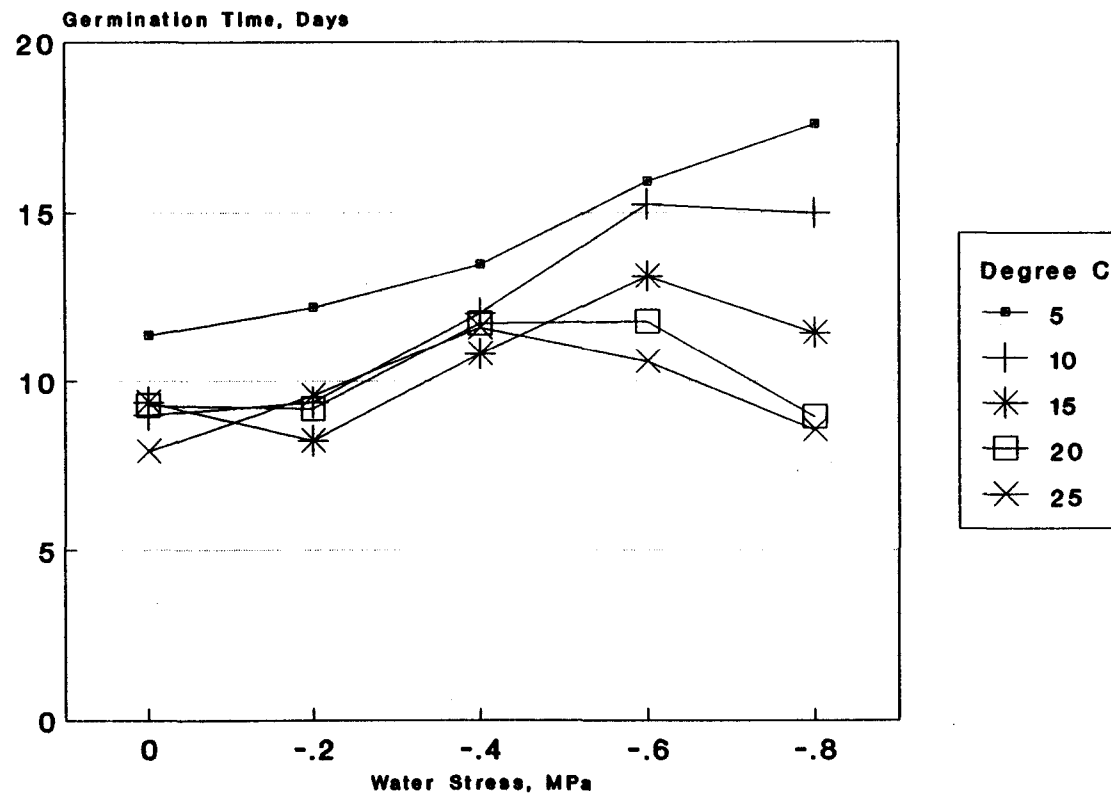


Figure 3. Interaction effect of temperature, and water stress on germination rates of Medicago, and Hedysarum species. Data was averaged over all species.

Table 2. P-values for source of variation of contrasts of percent germination, Interaction between Sp*temp, and Temp*WS.

contrast	P-Values
<u>Species * Temperature</u>	
Local species vs imported species	.0001
Hedysarum Carnosum KA + Medicago laciniata vs imported medics	.0001
Local Medicago vs imported Medicago species	.0242
Hedysarum species vs Medicago species	.0001
<u>Temperature (Temp) * Water stress (WS)</u>	
Temp. L * WS L	.3478
Temp. L * WS Q	.7586
Temp. L * WS C	.8390
Temp. Q * WS L	.1053
Temp. Q * WS Q	.0134
Temp. Q * WS C	.0001

L = Linear Q = Quadratic C = cubic

Table 3. P-values for source of variation of contrast of germination time, interaction between Temperature * Water Stress.

Contrast	P-values
Temperature * Water stress	
Temp. L * WS L	.4356
Temp. L * WS Q	.0225
Temp. L * WS C	.7738
Temp. Q * WS L	.9703
Temp. Q * WS Q	.0001
Temp. Q * WS C	.6239

L = Linear Q = Quadratic C = cubic

Root Growth Experiment

Materials and Methods

Seeds of the same five Medicago, and two Hedysarum species that were used in the germination experiment were examined to determine root dynamics.

Racks were made to set the tubes at an angle of 17° from the vertical plane. Each rack has 36 squares of 7.5cm on each side and 45cm long legs.

The experiment was conducted during the spring of 1990 in controlled environment, growth chambers. Plants were grown at three constant temperatures: 5°C, 10°C, and 15°C. A period of 16 hours day with constant daily irradiance of 1000 ft-c illumination at plant level was followed by 8 hours darkness (night). Plants were grown in glass tubes (51mm outer diameter and 60cm long for the 5°C treatment, and 51mm outer diameter and 90cm long for the 10°C and 15°C treatments). The tubes were sealed at the bottom with a rubber stopper that contained a small opening to allow excess water drainage yet prevent leakage of soil. Tubes were filled with washed river sand.

To prevent the exposure of the roots to light the glass tube was slipped into an ABS black pipe of 5cm diameter. Tubes were placed in a wooden racks at an angle of 17° from vertical to ensure that roots grew against the glass. Each rack had 36 squares of 7.5cm on each side and

45cm long legs. Tubes were watered to field capacity with nutrient solution which was mixed according to a soil analysis completed two days prior to the beginning of the experiment. Nutrient solution was added as needed and at all times there was an ample supply of water and minerals. Five seeds of each species were sown per glass tube at a depth of 1cm. Plants were allowed to grow in the glass house for seven days at 18°C, thinned to one plant per tube then moved to growth chambers for temperature treatments. Total root length was measured and recorded every week for six weeks.

Data Analysis

The study was conducted as a completely randomized design. Root elongation was evaluated by Analysis of Variance performed with the Statistical Analysis Systems (SAS Institute Inc. 1987).

The plot of root length vs time for each species at each temperature was tested for linearity. A regression line was generated for each experimental unit (individual plant) at each temperature. Each temperature was analysed separately using thirty five regression lines (7 species * 5 observations). Analysis of variance was performed on the slopes (Δ length in cm per week) of these regressions which were considered as a treatment responses (Appendix Tables C-E). Contrasts were performed to identify differences in root elongation rates.

ROOT GROWTH EXPERIMENT RESULTS

Analysis of variance revealed significant differences among the species tested (Figures 4-7, Appendix Tables C-E). Root elongation rate was slowest at 5°C (Figure 4), intermediate at 10°C (Figure 5), and highest for all species at 15°C (Figure 6). Elongation varied with species and temperature. The average root elongation rates of species tested at 5°C, 10°C, and 15°C is shown in figure 7.

A sharp decrease in rate of root elongation occurred as temperature declined for Hedysarum carnosum Kairouan accession and Hedysarum carnosum Tunisian accession. The rate of elongation was decreased from 11.7 and 9.8 cm/week at 15°C to 3.6 and 3.5 cm/week respectively at 5°C.

Roots of Medicago laciniata and Medicago truncatula penetrated faster than other species at 5°C ($p = .0001$), however at higher temperatures (15°C) Hedysarum carnosum KA and Medicago polymorpha var. Circle Valley had similar growth rates (Figure 7 and Appendix Table G).

Contrasts were used to test whether there was a difference in root growth among groups of these species. There was no significant difference in response between local and imported species tested at all temperatures (Table 4). Medicago laciniata had the highest growth rate at 5°C, 15°C and slightly lower at 10°C ($P = .0001$ at 5 °C, $p = .1779$ at 10°C, and $P = .0001$ at 15°C). This species had slower elongation at 10°C because one of the plants was

stunted which reduced mean root growth for the entire treatment. Medicago truncatula TA elongation was not different than other species at 10°C ($P = .1825$) and 15°C ($p = .6158$).

Medicago species had greater rates of root elongation than the Hedysarum carnosum accessions at 5°C but this difference disappeared as temperature increases (Table 4). Hedysarum carnosum accessions showed greater response to increased temperature than Medicago accessions, especially between 5°C and 10°C. Hedysarum species had an increase of 55% in rate of root elongation between 5°C and 10°C compared to about 33% increase of the medic species (Appendix Table F and Figure 7). At higher temperature 15°C there was no difference in speed of elongation between medics and Hedysarum accessions (Table 3). Both Hedysarum accessions have similar rate of root elongation at 5°C, however when temperature was increased Hedysarum carnosum KA had higher rates ($P = .011$).

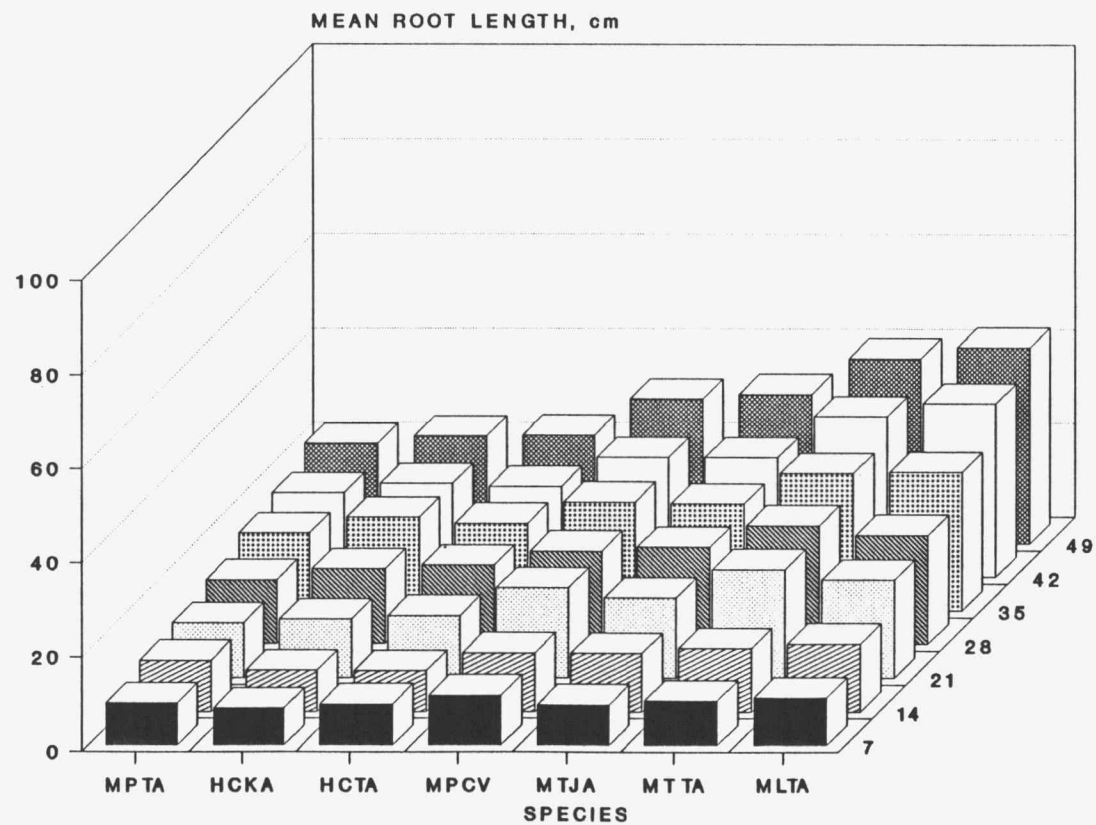


Figure 4. Mean root length of Medicago, and Hedysarum species grown at 5°C.

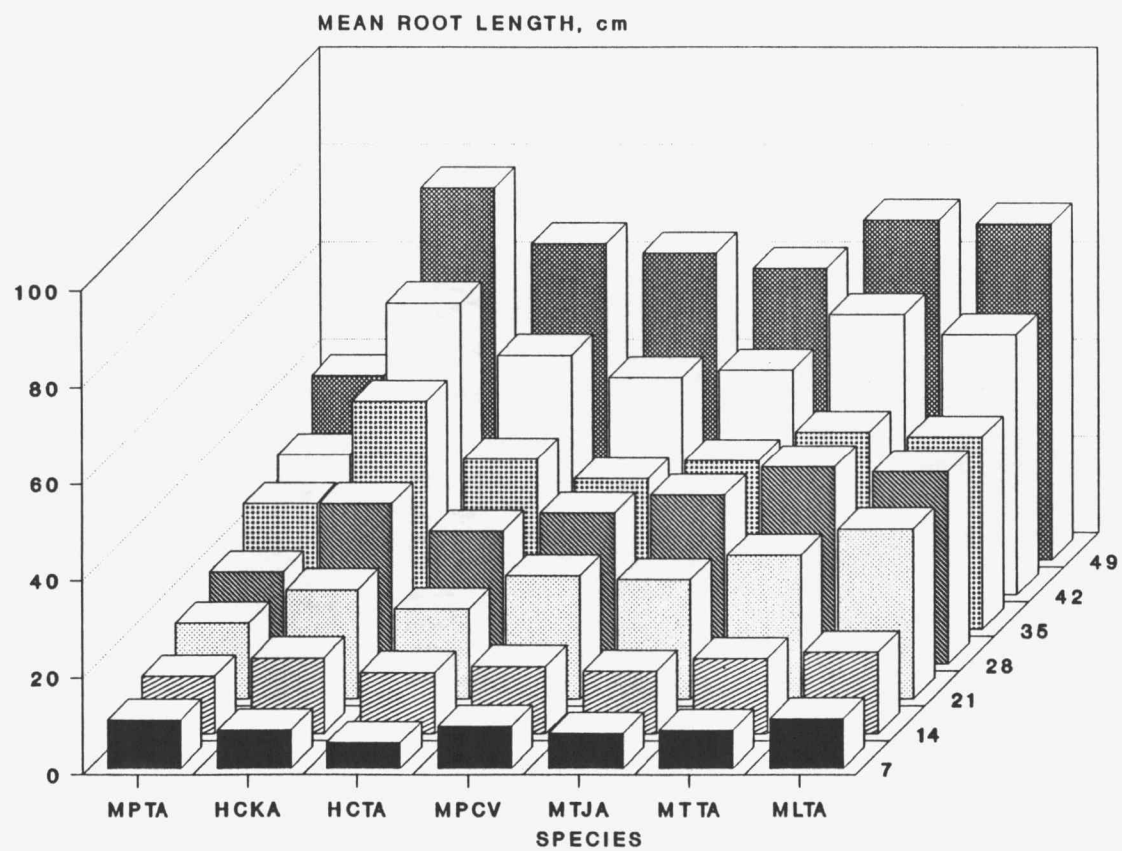


Figure 5. Mean root length of Medicago, and Hedysarum species grown at 10°C.

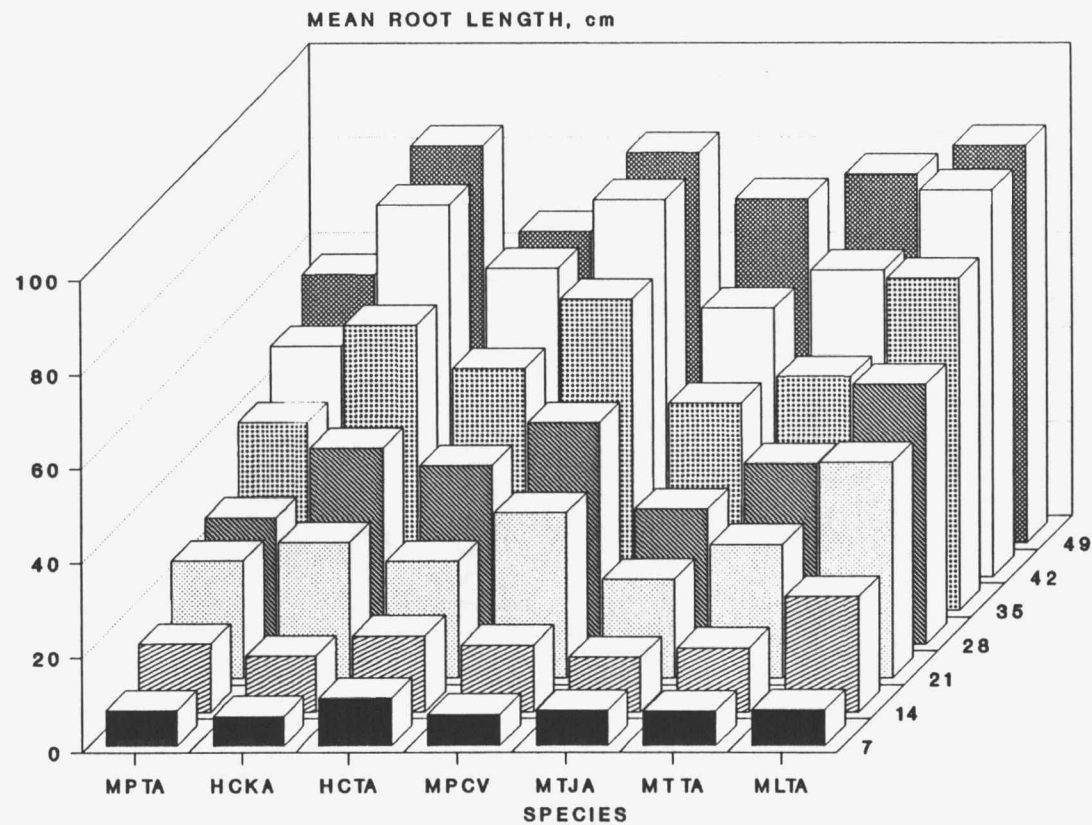


Figure 6. Mean root length of Medicago, and Hedysarum species grown at 15°C.

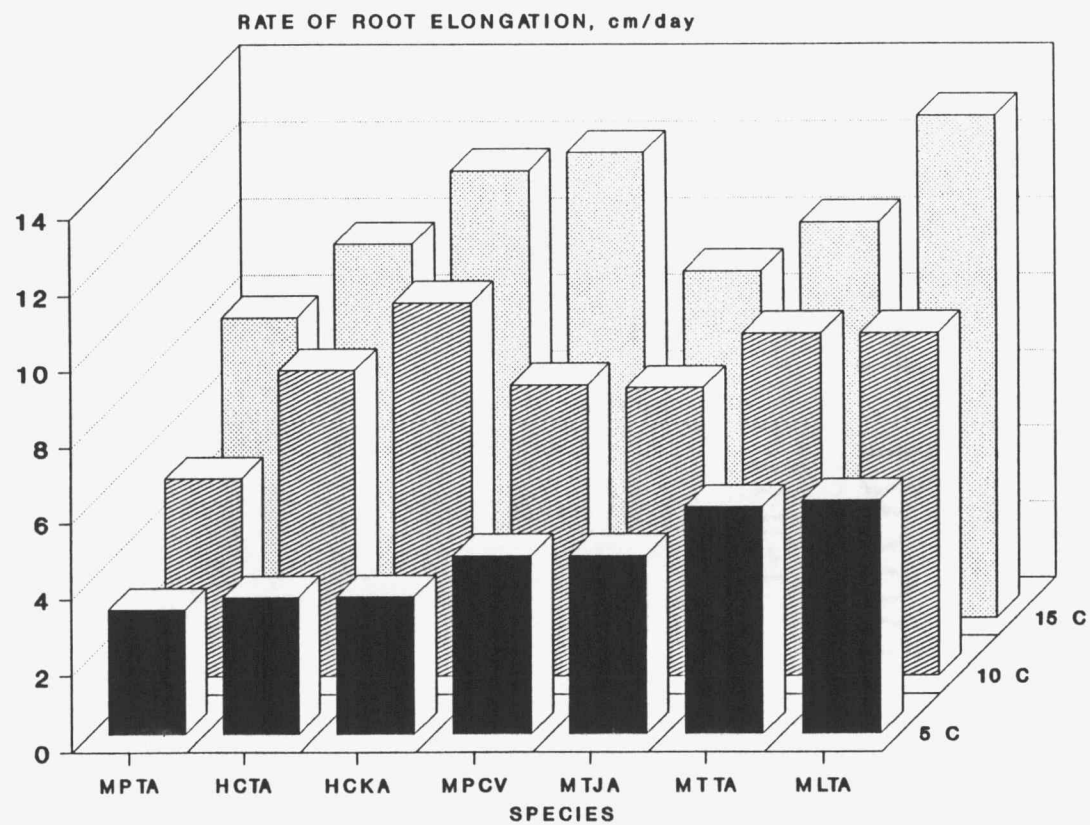


Figure 7. Rate of root elongation of Medicago, and Hedysarum species grown at 5°C, 10°C, and 15°C.

Table 4. P-values for source of variation of contrasts of root length at 5°C, 10°C, and 15°C

Source	Root Length
<u>Temperature 5 °C</u>	
Local species vs imported species	.4598
Local Medics vs imported Medics	.1023
Medics vs Hedysarums	.0001
Medicago Truncatula TA vs Others	.0001
Medicago laciniata vs Others	.0001
Hedysarum carnosum TA vs Hedysarum carnosum KA	.9698
<u>Temperature 10 °C</u>	
Local species vs imported species	.2678
Local Medics vs imported Medics	.6994
Medics vs Hedysarums	.063
Medicago truncatula TA vs Others	.1825
Medicago laciniata vs others	.1779
Hedysarum carnosum TA vs Hedysarum carnosum KA	.0905
<u>Temperature 15 °C</u>	
Local species vs imported species	.8933
Local Medics vs imported Medics	.7022
Medics vs Hedysarums	.5894
Medicago truncatula TA vs Others	.6158
Medicago laciniata vs Others	.0001
Hedysarum carnosum TA vs Hedysarum carnosum KA	.011

Local species = Hedysarum carnosum TA & KA, Medicago laciniata, Medicago polymorpha TA, and Medicago truncatula TA

Imported species = Medicago polymorpha Circle Valley, and Medicago truncatula Jemalong

GROWTH ANALYSIS EXPERIMENT

Materials and Methods

Root and shoot biomass accumulation was investigated (for the seven species previously used) by growing plants in a controlled environment. Light, rooting media, and temperature were controlled in growth chambers. Five seeds of each species were sown at a depth of 1 cm. in a 2 liters plastic pots containing washed river sand. Plants were watered as needed with nutrient solution prepared according to the soil analysis so that there was an ample supply of water and mineral nutrients. Plants were grown for one week in the glass house at a temperature of 18°C, thinned to one plant per pot, and moved to growth chambers. The pots were spaced to avoid interplant shading. Three constant temperature treatments were maintained (5°C, 10°C and 15°C). Plants were monitored for six weeks. A randomly selected plant from each treatment group was harvested every 7 days (Evans 1972, and Kreusler 1875). Seven harvests occurred between April 21 and June 2nd, 1990.

Destructive sampling of one plant of each species provided a measure of fresh and dry weight of total plant, shoots (leaves and stems separately measured then summed), and roots. Leaf area was measured by an automatic area meter (Lincor model 3100). Five measurements were taken and the average was calculated. Above ground tissues were

allowed to dry in an oven at 80°C for about 24 hours (AOAC, 1980). Cool dry samples were weighed. Roots were washed to remove sand, blotted out evenly, and placed in plastic bags to be weighed. The procedures for drying and weighing were the same as that for above ground tissues.

Data analysis

Growth analysis parameters were evaluated by analysis of variance using Statistical Analysis systems (SAS). Species tested showed exponential growth over the period of the experiment. Regression of the log transformed dry matter accumulation ($\ln W_t$) in each species over time represents the Relative Growth Rate. Pairwise t-test was performed by L S-Means using general Linear Model (Statistical Analysis Systems 1989) at $P=.05$).

GROWTH ANALYSIS EXPERIMENTAL RESULTS

Mean relative growth rate (mRGR), leaf area ratio (LAR), unit leaf rate (ULR), and above and below ground dry phytomass were measured. Growth parameters for Medicago and Hedysarum species as measured in this study are shown in Tables 5-8. Plant dry weight gains over 49 days indicated that local Medicago truncatula TA had largest biomass accumulation while local Medicago polymorpha TA had the lowest (Table 5). Significant differences in mean relative growth rate (mRGR) were found among the species (Tables 6-8). The mean relative growth rates of both Medicago truncatula TA (0.0688 day^{-1}) and imported Medicago truncatula JA (0.0665 day^{-1}) species were the highest at 5°C (Table 6). Mean RGR of Medicago polymorpha TA (0.0641 day^{-1}) and imported Medicago polymorpha CV (0.063 day^{-1}) were lowest at 5°C . Derived parameters, leaf area ratio (LAR) and Unit leaf ratio (ULR), also show significant differences among the species. Medicago laciniata and Medicago polymorpha TA had the highest leaf area ratio (LAR) and intermediate unit leaf ratio (ULR) (Table 6). On the other hand, the largest species, Medicago truncatula TA, had low LAR and high ULR (Table 6).

Root to shoot ratios were significantly different between species. Medicago truncatula TA had the greatest root to shoot ratio (1.72), while Medicago polymorpha TA had the lowest ratio (0.793).

Mean growth rate increased with temperature, as would be expected. At 10°C, Medicago truncatula TA and Medicago truncatula JA had the highest mRGR (Table 7). Medicago laciniata had the lowest mRGR (Table 7). Derived growth parameters (LAR and ULR) did not show great variation among the species (Table 7).

Mean relative growth rate varied between species at 15°C which suggested size hierarchies may be important to relative performance. The largest plant Medicago truncatula TA had the largest mRGR (0.1072 day⁻¹) and the smallest plant Medicago polymorpha TA had the lowest (mRGR = 0.789 day⁻¹). At this temperature, Medicago laciniata rapidly produced aboveground biomass (0.3891 g Final DM, Table 8) compared to its performance at lower temperatures. Mean RGR was equal to that of Medicago truncatula TA (Table 8).

Table 5. *Medicago* and *Hedysarum* species starting, and ending weights (g plant⁻¹) and RGRs (g g⁻¹ of initial weight day⁻¹).

Species	Initial DM	RGR	Final DM
<u>Temperature 5 oC</u>			
<i>Hedysarum carnosum</i> TA	.0038	.065	.0562
<i>Hedysarum carnosum</i> KA	.005	.065	.1095
<i>Medicago laciniata</i>	.0034	.065	.0484
<i>Medicago polymorpha</i> TA	.0049	.064	.0595
<i>Medicago polymorpha</i> CV	.0071	.063	.1002
<i>Medicago truncatula</i> TA	.0092	.067	.1522
<i>Medicago truncatula</i> JA	.0059	.069	.0972
<u>Temperature, 10 oC</u>			
<i>Hedysarum carnosum</i> TA	.007	.073	.1696
<i>Hedysarum carnosum</i> KA	.0071	.069	.2059
<i>Medicago laciniata</i>	.0069	.086	.1850
<i>Medicago polymorpha</i> TA	.0045	.078	.1090
<i>Medicago polymorpha</i> CV	.0062	.093	.2591
<i>Medicago truncatula</i> TA	.0112	.093	.5542
<i>Medicago truncatula</i> JA	.0071	.088	.2665
<u>Temperature, 15 oC</u>			
<i>Hedysarum carnosum</i> TA	.009	.093	.3380
<i>Hedysarum carnosum</i> KA	.0062	.084	.2517
<i>Medicago laciniata</i>	.0049	.107	.3891
<i>Medicago polymorpha</i> TA	.0086	.079	.2263
<i>Medicago polymorpha</i> CV	.0109	.104	.5984
<i>Medicago truncatula</i> TA	.0077	.107	.6525
<i>Medicago truncatula</i> JA	.0099	.103	.5635

Table 6. Mean values of growth parameters for Medicago and Hedysarum species grown at a temperature of 5°C.

Species	Parameters			
	RGR (Day ⁻¹)	LAR (cm ² g ⁻¹)	ULR 1(10 ⁻⁴ g cm ⁻² day ⁻¹)	R/S
Hedysarum carnosum TA	.0645 bc	137.9 ab	4.8 c	1.32 b
Hedysarum carnosum KA	.0652 b	116.2 bc	6.0 b	1.01 cd
Medicago laciniata	.0653 b	143.9 ab	5.3 b	1.09 c
Medicago polymorpha TA	.0641 c	157.4 a	5.1 b	.793 d
Medicago polymorpha CV	.063 c	126.8 bc	5.8 b	1.22 bc
Medicago truncatula TA	.0665 ab	75.1 d	9.7 a	1.72 a
Medicago truncatula JA	.0688 a	109.3 c	6.7 b	1.02 c

RGR = $\frac{1}{t} \ln \frac{wt}{w_0}$; ULR = RGR/LAR ; LAR = LA/W

W = Plant weight T = time

Means with the same letter are not significant at
P =0.05

Table 7. Mean values of growth parameters for Medicago and Hedysarum species grown at a temperature of 10°C.

Species	Parameters			
	RGR (Day ⁻¹)	LAR (cm ² g ⁻¹)	ULR (10 ⁻⁴ g cm ⁻² day ⁻¹)	R/S
Hedysarum Carnosum TA	.0725 d	129.27 a	6.06 c	.8228 d
Hedysarum carnoisum KA	.0725 d	132.1 a	6.73 bc	.5914 e
Medicago laciniata	.0685 e	123.09 a	6.28 bc	.9757 cd
Medicago polymorpha TA	.0861 b	122.68 a	7.27 bc	1.061 bc
Medicago polymorpha CV	.0785 c	116.85 ab	7.11 bc	1.199 ab
Medicago trucatula TA	.093 a	96.47 b	12.4 a	1.039 bc
Medicago truncatula JA	.0926 a	121.96 ab	8.49 b	1.284 a

$RGR = \frac{1}{t} \ln \frac{wt}{w}$; $ULR = RGR/LAR$; $LAR = LA/WT$
W = Plant weight T = time
Means with the same letter are not significant at
P = 0.05

Table 8. Mean values of growth parameters for Medicago and Hedysarum species grown at a temperature of 15°C.

Species	Parameters			
	RGR (Day ⁻¹)	LAR (cm ² g ⁻¹)	ULR (10 ⁻⁴ g cm ⁻² day ⁻¹)	R/S
Hedysarum carnosum TA	.0934 c	180.4 a	5.73 d	.5407 d
Hedysarum carnosum KA	.0843 d	174.3 ab	5.34 e	.6828 d
Medicago laciniata	.1071 a	141.9 c	8.02 bc	.6707 d
Medicago polymorpha TA	.0789 e	133.2 c	6.79 cd	1.037 bc
Medicago polymorpha CV	.1041 b	121.9 c	9.26 b	1.229 ab
Medicago truncatula TA	.1072 a	155.0 bc	7.91 bcd	.9536 c
Medicago truncatula JA	.1029 b	92.0 d	12.0 a	1.388 a

RGR = $\frac{1}{T} \ln \frac{W_t}{W_0}$; ULR = RGR/LAR ; LAR = LA/Wt

W = Plant weight T = time

Means with the same letter are not significant at

P =0.05

DISCUSSION AND CONCLUSION

Species used in restoration of depleted rangelands in central Tunisia must be able to germinate and establish under adverse conditions of temperature and water stress. The ability of forage species to grow under these conditions differ markedly. Even within a species, plants at different stages of their life cycle differ in their resilience to environmental stress.

Germination and the initial stages of seedling development are the most important periods for survival and establishment of range plants under natural conditions. Seed germination is affected by numerous factors such as: temperature, moisture, salinity, light and their combined effects. Effects of temperature and water potential on germination of range plants has been studied by several investigators (Doescher 1985, Bahler et al. 1988, Francois and Goodin 1972, Owens and Call 1985, Fullbright 1988, and Eddleman and Romo 1988). They found that decreasing osmotic potential delays germination and/or reduced the rate of germination of the species tested. Even though solutions used in the germination experiment were treated with fungicide, temperature and high humidity inside the boxes were conducive to fungus growth during the experiment. In this experiment, hard seed coat and germination inhibitors may have been the cause of reduced germination in some of the species. Quinlyvan (1968) treated several Medicago species

(barrel medic cv Cyprus, strand medic cv Harbinger, burr medic, and snail medic) after field maturity with fluctuating temperature of 15°C and 75°C. After six months, he found that a high proportion of hard seeds remained among these medics. He also found that the rate of seed coat softening increased for clovers with increasing maximum temperature but this trend was not found in medics. He suggested that hard seededness of medics might be the reason for establishment failures in Western Australia.

Characterization of germination properties over a combination of temperatures (5°C, 10°C, 15°C, 20°C, and 25°C), and osmotic potentials (0, -0.2, -0.4, -0.6, -0.8 MPa) showed differences in the ability to germinate among species. This study showed that local Medicago laciniata and Hedysarum carnosum Kairouan accession have higher performance (greater % germination) over wide range of temperatures and osmotic potentials. These species have evolved under the irregular environmental conditions of central Tunisia where rainfall is scanty and temperature fluctuate widely between seasons (and often within seasons). On the other hand, imported Medicago truncatula var. Jemalong and Medicago polymorpha var. Circle Valley were selected and developed for Australian conditions. Because they are normally grown under moderate temperatures and relatively higher precipitation, they don't germinate under as wide a range of temperature and water stress

conditions. Doesher (1985) studied the effect of moisture and temperature stress on germination of the cool season plant Idaho fescue (Festuca idahoensis Elmer). He found that its germination pattern reflected seasonal and climatic environmental conditions which naturally occur during germination. Medicago truncatula TA germinated better at low temperatures of 5°C, 10°C, and 15°C, but when temperature was increased to 20°C, and 25°C the germination declined. This difference in germination response to different treatments indicates the natural selectivity for those adaptations which aid the adaptations of ecotypes to its environment.

These results agree with field observations, Medicago truncatula TA germinates early when moisture is available and young seedlings appear in the Fall (October-November) often in great numbers, and continue growth throughout the winter. Since the species is native to central Tunisia it is not surprising that germination occurs under cool temperatures. The ability to germinate early and continue growth through the winter may be an important physiological characteristics of survival value to this species. This species germinates early and develops a good root system well in advance of the drying soil profile. This should provide a competitive advantage over other species growing on the sites.

Even though a low percentage germination was obtained

because of several factors, valuable information has been secured from this experiment. Favorable planting dates can be selected on the basis of these temperature and moisture requirements for germination and knowledge of the areas to be revegetated (Ashby and Helmers 1955). In Central Tunisia, late fall (mid-November) temperature drops below 15°C, so plants should be seeded at least two weeks prior to this period. This would allow the seeded plants to germinate, and produce an adequate root system before inhibiting winter temperature begins. Since precipitation is erratic in the fall and often does not occur until winter, species such as Medicago truncatula TA, that are able to germinate under low temperatures will establish on the site.

Plants respond differently to environmental factors, because of their differences in geographical areas of adaptations and in the biological mechanisms that have evolved for coping with unstable environments. Roots of both local ecotypes, Medicago truncatula TA and Medicago laciniata TA penetrated the soil faster than other species tested; however, the rate of root penetration changed as a function of temperature. The distinct relationship between cold tolerance, low temperature growth and survival and climate of origin has been shown for white clover (Ollerenshaw and Baker 1981, 1982, and 1983) for perennial grass (Lorenzetti et al. 1971) and for Medicago species (Cocks

and Ehram 1987). Ollrenshaw (1984) has found that ecotypes of Trifolium repens collected from cold environment are more tolerant to cold temperature and grow better root and shoot materials than species of more temperate regions, and that populations from high altitude are more tolerant than those of low altitude. Lorenzetti et al. (1971) have found the same relationship between low temperature growth and the winter temperature at the place of origin from Lolium perenne. The results of the experiment showed a relationship between rate of root growth under cool temperature and winter temperature of the site where the species were collected. The species tested can be classified into three groups based on their results in this trial. Group A, the local Medicago truncatula and Medicago laciniata, have originated in areas where the winter temperature is low, showed outstanding growth under the cold temperature treatment. Group B, Hedysarum carnosum Tunisian accession and Hedysarum carnosum Kairouan accession collected in warmer sites had slow root growth at low temperature but when temperature was increased their rate of root elongation increased. Group C, the imported commercial strains, Medicago Polymorpha cv circle valley and Medicago truncatula cv jamelong have shown low root elongation rates. The result correlate with field observations that Australian varieties are only adapted to mild areas near the Mediterranean Sea. On cooler sites slow growth and high winter

kills occurs for those species (Ben Ali 1986, Cocks and Abdel-Moneim 1986). From these results it is very important to focus on the study of the species from the sites of origin and screen for the species that show great potential to be used in the range sites of central Tunisia.

Increasing the soil volume penetrated by root systems would increase the available moisture to the plant and reduce or delay water stress. Rates of root elongation have been found to be an important aspect of seedling survival on dry sites. Several investigations on root development of several plant species have demonstrated the importance of the development of root system and that a marked differences in root development between species occur. Plummer (1943) found a marked differences in root elongation between several range species. He found correlations between rate of root development and subsequent establishment. Kasper (1981) found that a soybean genotype with a dominant, rapidly elongating tap root may later have a deeper root system and better water availability than a genotype with a weak, slow growing taproot. Total root development prior to the season of drought appears to be directly associated with initial success or failure (Plummer 1943). Medicago species with slow root development, Medicago polymorpha Tunisian accession, Medicago truncatula var. Jemalong, Hedysarum carnosum Tunisian accession and Hedysarum carnosum Kairouan accession may be difficult to

get established under low winter temperature in the field. However Medicago truncatula Tunisian accession and Medicago laciniata possess high rates of root elongation may be able to establish early in the season and continue to grow successfully in the plant community. The high degree of morphological plasticity of Medicago laciniata root system could be an adaptive mechanism which permits this species to survive harsh climatic conditions. Its high rate of root elongation and wide adaptations to a range of temperature constitute an ecological advantage.

The extent of root system, that is the depth to which it penetrates the soil, spreads laterally is dependent upon several factors, including soil moisture, and composition of the soil (Richard 1986). Although under favorable conditions a plant may develop a more or less characteristic root system, unfavorable conditions in the soil can bring about marked alterations in the form of root system (Wiersum 1957). Soil condition have an important bearing on initial root development. The natural habitat of Medicago laciniata in dry land soils and alluvians of central and southern Tunisia. Because of the irregularity of the rainy period and the rapid drying out of the soil surface, the establishment of the species is heavily dependent on early and rapid root elongation in the soil profile. Wiersum (1957) found that the speed of germination of several range species on sandy soils was greater than on

fine textured soils. Medicago polymorpha is adapted to a finer textured soils with higher water holding capacity and slower drying out of the soil surface. Since more water is available at the soil surface, the species tend to have shorter roots and penetrate at slower rate into the soil profile however they tend to develop lateral branches and occupy a greater soil volume. Even though root tips exert tremendous force to penetrate soil profile, the mechanical resistance of fine and dense soil slows down and restricts root growth. Medicago laciniata invests less energy per unit root area which allow the plant to produce more root surface area that occupy greater portion of the soil profile. The thinner root of Medicago laciniata is an ecological adaptation that allow the species to dominate the below ground area. Harris (1967) suggested that the rapid elongation of Bromus tectorum could be attributed to its cell structure where the roots are both smaller in diameter and the cell wall have a fraction of the thickness observed in Agropyron spicatum. Medicago laciniata appeared to invest more energy into an increased root length than the other species.

The importance of seed size on initial shoot and root growth and development and its effect on the competitive ability of the seedling and final plant development have been studied by several investigators. Great amount of food reserves in the seed can serve as a source of energy

to root growth until the plant develop new leaves and initiate photosynthetic activity. Seed size and rate of root elongation at seedling emergence and development stage have been found to be closely related. The small seeded range species are difficult to get started and establish especially under adverse field conditions of central Tunisia. The species used in this experiment had a rate of root elongation between .5cm and .87cm/day, .7cm and 1.28 cm/day, and 1.12cm and 1.87cm/day at a temperature of 5°C, 10°C, and 15°C respectively. Cohen et Tedmor (1969) have found similar results when they tested Medicago polymorpha and Medicago truncatula among other range species. Investigations on the effect of seed size among the varieties tested have not been performed. However the rates of root elongation obtained was slow compared to the rates of larger seeds of barley and wheat of 2cm to 7cm/day obtained by Cohen and Tedmor (1969). The suggestion of Brouwer (1962) that the supply of resource materials to the root system determines the rate of root elongation support these observations. Cameron et al. (1962), Hove and Kleinendorst (1962), and Crawford et al. (1989) have found a relationship between seedling vigor and seed size, and that seedling vigor increase with seed size. Root growth and development is detrimental for range species establishment in areas where temperature and moisture are limiting. Breeding for larger seed with faster root elongation seems

to be essential for better establishment (Cohen and Tedmor 1969). Since Medicago laciniata and Medicago truncatula have shown good adaptations to grow under the adverse conditions of central Tunisia, plant breeders could use these potential species and develop larger seeds. This relationship of seed size and seedling vigor was used to develop Medicago rugosa cv Paraponta in Australia which have twice the seed weight and 20% better seedling vigor than Medicago rugosa cv paragosa (Mackey 1978).

Species with slower root elongation rates have most of their roots at the top surface soil and less roots at greater depth than species with higher rates. Kasper et al. (1984) found that soybean cultivars with faster rates of root elongation have more roots at depths of 150cm and 200cm than slower cultivars. Soybean cultivars with slower root elongation concentrate their bulk of roots at a depth of 100 cm.

Growth analysis experiment revealed distinct variation in mean relative growth rate (mRGR) among the species tested. This variation suggested a size hierarchies in relative performance (Biomass production) among the species. Grime and Hunt (1975) said that values of relative growth rate are a clue to the relative productivity of species growing under field condition. The size hierarchies suggested is similar to the results obtained by relative biomass production of the species. The largest

plant species, Medicago trusatula (local accession) (0.6525 g) had the highest mean relative growth rate (mRGR). The smallest plant species, Medicago polymorpha (local accession) (0.2263 g) had the lowest mean relative growth rate (mRGR). Such differences in mean relative growth rate suggests that species with high mRGR may be more productive and thus more competitive.

Plant species may differ considerably in relative growth rate (RGR). This may be caused by habitat related variation in abiotic factors, like temperature, water, light, and nutrients, or by biotic factors like competition, disease or grazing pressure (Poorter 1989). The ecological significance of the differences in relative growth rate among species or populations of similar or different habitat were investigated (Grime and Hunt 1975, Rouch and Radosavich 1985, and Poorter 1989). Grime and Hunt (1975) revealed that species with high RGR grow faster and rapidly capture greater space. These characteristics are important in competitive situations. Medicago is an annual species, so high mRGR facilitate the rapid completion of life cycle (Grime and Hunt 1975). Medicago trusatula (local accession) was able to quickly uptake water and nutrient resources available to produce high amounts of biomass. Also complete its life cycle prior to the late spring drought conditions that occurs in central Tunisia. Medicago trusatula cv jamelong showed a high potential in

mRGR, however it produced less plant biomass than the local Medicago truncatula. Slow growing species such as Medicago laciniata may have an ecological advantage. Medicago laciniata is found in areas where no medic species is growing. The species is well adapted to less fertile soils and relatively dry areas. Grime and Hunt (1975) suggested that slow growing species require less resources for their growth and therefore will not use up the available resources very quickly. Also the species growth ability is not affected by adverse conditions as a rapid growing species.

The derived parameters, leaf area ratio (LAR) and Unit leaf ratio (ULR) were not consistent with the results (size hierarchies) obtained by mRGR. Medicago truncatula have shown an impressive adaptation. At cold temperature (5°C and 10°C) Medicago truncatula (local accession) had low LAR and High NAR, however when the temperature was increased to 15°C the inverse was obtained. High NAR indicate that high yielding plants tend to have greater dry matter production per unit leaf area than did low yielding genotypes. Poorter (1989) suggested that lower LAR may be the result of adaptation to frost or chilling. Wilson (1966) has studied the growth parameters of two arctic and temperate species. He found variation in LAR and not in NAR. He postulated that this difference in LAR may be due to differences in SLA. Changes in temperature within the plant is controlled by intrinsic factors such as water, solutes..etc. Moisture

is an important factor because of its high specific heat and thermal conductivity. Medicago truncatula may have a small cell size, low water content, and higher photosynthate concentration. These characteristics are conducive to cold tolerance. On the other hand at optimum temperature Medicago truncatula Tunisian accession has higher LAR and low NAR. The high leaf area produced would result in higher interception and utilization of solar energy. This would result in a greater amount of photosynthate available for biomass production. Variation in root to shoot ratio (R/S) illustrate the ability of the species to uptake water and nutrients. However the variation in R/S ratio was not consistent with the size hierarchies obtained by the relative growth rate. The experiment showed differences in root morphology among the species. Hedysarum species produced thick and short roots with high moisture content. Medicago Laciniata had low R/S ratio, but it produced long and thin roots. The greater root length enabled the species to occupy greater soil volume to extract resources deep in the soil profile.

CONCLUSION

In Tunisia, land use systems designed to increase the production of livestock, and improve soil conditions (texture, structure, and organic matter content) should receive considerable attention. Annual legumes such as Medicago species are able to produce high quality feed, improve soil properties, and increase nitrogen availability. However, the success of these species depends mainly on how well the onset and duration of a plant's stages of development are matched to the constraints of Central Tunisian environmental conditions.

The experiment performed suggested that local ecotypes are better than improved varieties in terms of ecological adaptations to the temperature treatments. I conclude that the local ecotypes are better adapted to the environment of Central Tunisia.

From these results it is very important to focus on the study of indigenous species and screen for the species that show great potential to be used in the range sites of Central Tunisia.

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APPENDICES

Appendix Table A. Analysis of variance for germination percent of Medicago and Hedysarum Species

Source	DF	S. Of Squares	M. Squares	F	P
Time	1	1112	1112	9.69	.0022
Temperature	4	13682	3420	30	.0001
Time * Temp.	4	938	234.5	2.04	.0905
Species	6	10914	1819	15.85	.0001
Water Stress	4	154795	38698	337	.0001
Sp. * Temp	24	16541	689	6.0	.0001
Sp. * WS	24	3337	139	1.21	.238
Temp.* WS	16	5549	347	3.02	.0002
Sp*Temp*Ws	96	7285	76	.66	.990
Error	170	19513	114		
Correc. Tot.	349	233669			

R-Square = 91

CV = 20

Appendix Table B. Analysis of variance for germination time of Medicago and Hedysarum species

Source	DF	S. of Squares	M. Square	F	P
Time	1	515.31	515.31	41.75	.0001
Temperature	4	911.34	227.83	2.48	.2001
Time * Temp.	4	367.29	91.82	7.44	.0001
Species	6	394.24	65.71	5.32	.0001
Water stress	4	816.30	204.07	16.54	.0001
Sp * Temp.	24	392.38	16.35	1.32	.1543
Sp * WS	24	441.23	18.38	1.49	.0763
Temp. * WS	16	491.97	30.75	2.49	.0019
Sp*Temp*Ws	96	992.5	10.34	.84	.892
Error	170	2098	12.34		
Corrected Total	349	7420			

Appendix Table C. Analysis of variance for root growth slopes grown at 5°C

Source	DF	Sum of squares	Mean squares	F	P
Species	6	39.808	39.808	16.634	.0001
Error	28	11.101	11.101	.396	
Corrected Totals	34	50.91	50.910		

Appendix Table D. Analysis of variance for root growth slopes grown at 10°C

Source	DF	Sum of Squares	Mean Squares	F	P
Species	6	56.731	9.455	3.7	.0078
Error	28	71.493	2.553		
Corrected Total	34	128.2243			

Appendix Table E. Analysis of variance for root growth slopes grown at 15°C

Source	DF	Sum of Squares	Mean Squares	F	P
Species	6	102.387	17.064	13.86	.0001
Error	28	34.462	1.230		
Corrected Totals	34	136.850			

Appendix Table F. Change in root growth rate (cm week⁻¹) as a function of temperature.

Speceies	Temperature		
	5 oC	10 oC	15 oC
	Rate	Rate	Rate
<u>Hedysarum carnosum</u> TA	3.58 c	8.05 ab	9.85 c
<u>Hedysarum carnosum</u> KA	3.59 c	9.20 a	11.75 ab
<u>Medicago laciniata</u>	6.12 a	9.02 ab	13.17 a
<u>Medicago polymorpha</u> TA	3.26 c	5.20 c	7.93 d
<u>Medicago polymorpha</u> CV	4.68 b	7.66 bc	12.23 a
<u>Medicago truncatula</u> TA	5.95 a	9.01 ab	10.41 bc
<u>Medicago truncatula</u> JA	4.68 b	7.58 cb	9.14 cd

LSD_(.05) = 0.8157 at 5 °C

LSD_(.05) = 2.07 at 10 °C

LSD_(.05) = 1.437 at 15 oC

Appendix Table G. Analysis of variance for Relative Growth Rate parameter of Medicago and Hedysarum species

Source	DF	F	P
Temperature	2	491	.0001
Species	6	28	.0001
Replication	1	.31	.5758
Days	1	.0001	.9905
Temp * Sp.	12	19.5	.0001
Days * temp.	2	.0001	.9999
Days * Temp.	6	.0001	1.0
Days*Temp*Sp	12	.0001	1.0

Appendix Table H. Analysis of variance for Leaf Area Ratio parameter of Medicago and Hedysarum Species

Source	DF	F	P
Temperature	2	7.17	.0009
Species	6	1.58	.1531
Replication	1	0.20	.6534
Days	1	160.4	.0001
Temp * Sp.	12	1.42	.1556
Days * temp.	2	1.59	.2064
Days * Temp.	6	0.56	.7593
Days*Temp*Sp	12	2.57	.0031

Appendix Table I. Analysis of variance for Unit Leaf Ratio parameter of Medicago and Hedysarum species.

Source	DF	F	P
Temperature	2	5.32	.0055
Species	6	8.14	.0001
Replication	1	1.04	.3088
Days	1	101	.0001
Temp * Sp.	12	2.53	.0037
Days * temp.	2	2.93	.0551
Days * Temp.	6	2.7	.0148
Days*Temp*Sp	12	1.74	.0058

Appendix Table J. Analysis of variance for Root to Shoot Ratios parameter of Medicago and Hedysarum species.

Source	DF	F	P
Temperature	2	32.99	.0001
Species	6	8.39	.0001
Replication	1	2.28	.1324
Days	1	363.85	.0001
Temp * Sp.	12	2.99	.0006
Days * temp.	2	20.19	.0001
Days * Temp.	6	3.23	.0045
Days*Temp*Sp	12	1.33	.2036