Variation in alfalfa (Medicago sativa L.) for germination and seedling vigor at suboptimal temperatures: and laboratory and field response to selection within six alfalfa populations

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Variation in alfalfa (*Medicago sativa* L.) for germination and seedling vigor at suboptimal temperatures; and laboratory and field response to selection within six alfalfa populations

by

Kathy Lynn Esvelt Klos

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Plant Breeding
Major Professor: E. Charles Brummer

Iowa State University
Ames, Iowa
1999
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has met the dissertation requirements of Iowa State University

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Alfalfa germination rate and seedling vigor at cool temperatures are of interest to both growers and breeders. The most common season for planting alfalfa in the Midwestern United States is the spring (Vough et al., 1995). Spring planting takes advantage of existing soil moisture and allows harvest in the seeding year, but alfalfa stand establishment may suffer if soils are cool (Barnes and Scheaffer, 1995, Vough et al., 1995). Planting a forage variety with optimum adaptation to conditions anticipated during growth can improve forage performance (Volenc and Nelson, 1995). Pasture renovation by no-till planting of alfalfa into existing grass stands may also benefit from early spring planting (Tesar and Marble, 1988, Wolf et al., 1989). Planting as early as soil temperatures allow may enable alfalfa to become established before perennial grasses begin their spring growth, thus reducing competition (Miller and Stritzke, 1995). In Iowa, spring seeded alfalfa may encounter suboptimal temperatures (below 10 C) during germination and early seedling growth (Brar et al., 1991, McElgunn, 1973). Good stand establishment is due to good germination, emergence and seedling growth, and thick vigorous stands are essential to high yields (Vough et al., 1995, Miller and Stritzke, 1995). Selection of alfalfa varieties with rapid germination and vigorous growth at temperatures anticipated in early spring plantings may help maximize forage performance during the seeding year.

The objectives of this research were (i) to evaluate variation in germination time and radicle growth among recently developed alfalfa cultivars, one red clover cultivar, and a birdsfoot trefoil cultivar under laboratory conditions at temperatures suboptimal for germination and growth; to evaluate variation in germination rate among seed lots within alfalfa cultivars; and to examine correlations of germination rate and seedling growth at suboptimal
temperatures in the laboratory with seed lot quality, 100-seed mass, and field emergence; (ii) to assess progress realized from phenotypic recurrent selection for germination rate and seedling growth at low temperatures under laboratory conditions in six alfalfa populations; and (iii) to assess progress in the field from phenotypic recurrent selection for cold temperature germination and seedling vigor under laboratory conditions performed in six alfalfa populations.

Dissertation Organization

This dissertation has been organized into four parts. The first part, this chapter, is a review of literature pertinent to the overall subject of study. The remaining chapters are organized as manuscripts to be submitted to refereed publications. Each chapter includes an abstract, introduction, materials and methods, and results and discussion section. References are listed for each part.

Literature Review

Origin and Adaptation of Cultivated Alfalfa

Alfalfa (*Medicago sativa* L.) originated near Iran, with related species located throughout central Asia and into Siberia (Barnes and Sheaffer, 1995; Hill, 1987). Cultivated alfalfa may have been in use as early as 7000 B.C., but the earliest known reference to alfalfa was from Turkey in 1300 B.C. (Hill, 1987). The use of alfalfa as animal feed was documented in the Roman Empire as early as c. 490 B.C. (Barnes and Sheaffer, 1995). Alfalfa, introduced to North America in 1736, is now worldwide in distribution (Barnes and Sheaffer, 1995). About 57% of U.S. production is in the north central states (esp. Wisconsin, Iowa, South Dakota, Minnesota), where it is primarily harvested and stored as hay or silage for on-farm use (Barnes and Sheaffer, 1995).
Alfalfa is adapted to growth in cool or warm, dry climates (Hill, 1987), and is tolerant of temperature extremes (below -25°C and above 50°C) (Barnes and Sheaffer, 1995). The number of harvests per year varies from one in northern and arid regions to ten under irrigation in the southwest (Barnes and Sheaffer, 1995). Production is generally defined as the amount of forage dry matter (DM) per hectare (Sollenberger and Cherney, 1995).

Cultivated alfalfa is naturally outcrossing (Hill, 1987). Most alfalfa cultivars are synthetic populations, produced by intercrossing individual plants selected from a breeding program (Hill, 1987). Because most recently developed synthetics are broad-based populations, formed from more than 40 parents, they can be considered highly heterogenous populations of heterozygous individuals (Barnes and Sheaffer, 1995, Hill, 1987). Breeding of alfalfa cultivars in the last 25 years has focused on increases in yield, especially by increased adaptation (such as winter hardiness) and by incorporation of multiple pest resistances (Barnes and Sheaffer, 1995, Hill, 1987). Some breeding effort has also been applied to improvements in forage quality, and stand establishment (Hill, 1987).

Most alfalfa seedings in the U.S. occur in the early spring or in late summer and fall (Miller and Stritzke, 1995, Vough et al., 1995). Spring seeding takes best advantage of existing moisture and allows harvest during the seeding year, but weed control is usually required (Vough et al., 1995). Seeding early into cold, wet soils can result in poor germination and seedling loss due to fungal disease, but delaying planting until the soil is warm may cause stand failure due to weed pressure and limited moisture (Miller and Stritzke, 1995, Vough et al., 1995). Late summer seeding avoids weed competition, but plantings may encounter stresses due to high temperature, low moisture, and limited time for adequate growth prior to frost (Miller and Stritzke, 1995, Vough et al., 1995).
**Use of Alfalfa in Pasture Production**

Alfalfa makes a high quality pasture for livestock, with the highest feeding value of all commonly grown forage crops (Marten et al., 1988), and produces more protein per hectare than grain or oil crops (Barnes and Sheaffer, 1995). Pastures with alfalfa-grass mixtures are generally more difficult to manage than pastures with alfalfa only, but may offer advantages over pure stands (Vough et al., 1995). Mixtures of alfalfa with grasses reduce the risk of bloat for grazing ruminants, while the alfalfa in the mixture reduces the risk of grass tetany and nitrate poisoning (Barnes and Sheaffer, 1995; Vough et al., 1995). The nitrogen fixing ability of alfalfa reduces the need for nitrogen fertilization of mixed pastures, and increases the overall protein content (Vough et al., 1995). Mixed pastures may be better able to compete with weeds, are usually higher yielding, have a better season-long yield distribution, and produce a better stand than pastures of either all grass or all legumes (Vough et al., 1995). Productivity of permanent pastures can be enhanced by no-till drilling alfalfa into the sward, particularly after herbicide application or overgrazing has suppressed existing vegetation (Tesar and Marble, 1988, Vough et al., 1995). No-till seeding reduces soil erosion and conserves soil moisture for germination and new seedling growth compared to preparing a tilled seedbed (Miller and Stritzke, 1995, Vough et al., 1995). Grass and perennial broadleaf weed competition must be controlled for successful no-till establishment (Barnes and Sheaffer, 1995, Miller and Stritzke, 1995, Vough et al., 1995).

**Alfalfa Seed Size and Seedling Vigor**

Alfalfa seed size is determined by both genetic and environmental factors (Carleton and Cooper, 1972). Seed size is a quantitatively inherited character, with expression controlled by the genotype of the seed parent (Gjuric and Smith, 1997). Genetic variance for seed size within a population is influenced by both additive and non-additive components (Gjuric and
Smith, 1997; Peterson and Barnes, 1982). Heritability of seed size in alfalfa has been estimated as 41.3% on a full-sib family mean basis (Gjuric and Smith, 1997).

Seed size in alfalfa has been weakly (Nel and Burgers, 1968) and strongly (Beveridge and Wilsie, 1959) correlated with seedling vigor. In alfalfa seed stratified by weight, mean seed weight was correlated with total seedling emergence: heavier seed classes had higher emergence irrespective of planting depth or date (Townsend, 1992). Brar et al. (1990) found 100-seed mass to be predictive of main axis root growth within the first 10 d after planting at temperatures between 15 and 25 C. Early growth in alfalfa is associated with larger seed size and proportional to cotyledon size and photosynthetic area (Black, 1959). Carleton and Cooper (1972) observed a correlation between seed size and cotyledon size. Mean mass of seed produced by reciprocal crosses among alfalfa clones was found to be correlated with unifoliate leaf area by Carnahan (1962). However seed mass and seedling height were not correlated (Carnahan, 1962). Carleton and Cooper (1972) compared the effect of genetically and environmentally caused seed size differences upon seedling performance of three legume species and concluded that environmental factors may be the more important in alfalfa. Seeds of alfalfa clones, separated into categories by weight, showed high correlations between seedling height and seed mass, but across all clones, the correlation between seed weight and seedling height was non-significant (Carleton and Cooper, 1972). Environmental factors which can affect alfalfa seed mass and size include soil moisture and plant spacing (Tysdal, 1946).

Germination and Seedling Growth as Affected by Temperature

The germination rate and final germination percentage of legumes decline as temperatures fall below 20 - 25 C (Bewley and Black, 1982, Brar et al., 1991). The percent germination of alfalfa and red clover (Trifolium pratense L.) were affected less than vetch (Vicia) and other clover (Trifolium) species as temperatures decreased from 30 to 10 C (Brar
et al., 1991). The total germination percentage of alfalfa, sainfoin (*Onobrychis viciaefolia* Scop.), sweet clover (*Melilotus officinalis* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) was decreased most by temperatures similar to those encountered in the field under early spring conditions (McElgunn, 1973).

Germination rate is optimum for alfalfa between 15 and 25 C (Brar et al., 1991), but alfalfa is among the earliest forage legumes to germinate at low temperatures (Arakeri and Schmid, 1949). Alfalfa has been shown to germinate at temperatures as low as 0 to 1 C (Coffman, 1923). Alfalfa germination rates increase over the temperature range of 10 to 30 C, but germination response curves differ among cultivars (Brar et al., 1991).

Cultivar differences in germinability at low temperatures have been observed in chickpea (*Cicer arietinum* L.), soybean (*Glycine max* L.), corn (*Zea mays* L.), muskmelon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.), but the genetic control of low temperature germination differs among crop species (Auld, et al., 1988; Hutton and Loy, 1992; Herber, 1986; Hennell, 1949). In alfalfa, Chloupek (1982) found significant genetic variation due to general combining ability, specific combining ability, and reciprocal effects under optimal conditions. In cucumber, additive and dominance variance are equal for days to 50% germination, but additive variance is greater for days to germination (Wehner, 1984). A reciprocal maternal effect, indicating the presence of a cytoplasmic gene for cold germinability, was observed in muskmelon, along with as many as three recessive nuclear genes (Hutton and Loy, 1992). Significant maternal effects, though no reciprocal effect, were also observed in corn along with effects due to embryo genotype (Pinnell, 1949). Environmental effects on cold germinability in corn are large (Pinnell, 1949).

The rate and uniformity of field emergence can affect stand establishment and consequently forage yield (TeKrony and Egli, 1991). Rapid seedling emergence after planting allows plants to avoid problems due to soil crusting, limits the effects of disease while seed is germinating, and reduces non-uniform emergence (Hernn, 1986). Relationships between
laboratory germination tests and field emergence vary. Auld, et al. (1988) compared laboratory tests of germination and radicle elongation of 10 chickpea lines at 5, 13, and 20 C, with field emergence and seedling growth. Cultivars did not differ assessed in the laboratory, but did for field emergence and seedling growth (Auld, et al., 1988). Radicle emergence of bean (*Phaseolus vulgaris L.*) in the laboratory at 8, 10 or 12 C generally does not correlate with field emergence at low temperatures, but radicle emergence under alternating temperatures was predictive of field vigor (Dickson and Boettger, 1984). Saminy et al. (1987) found that cold tests using alternating temperature regimes provided better correlations between laboratory assessments of germination and field emergence than tests using constant temperatures. Laboratory cold tests of soybean correlate well with field emergence (Szyrmer and Szczepanska, 1982).

At early stages of growth, alfalfa and red clover have greater survival rates when non-hardened plants are placed in freezing temperatures than the *Lespedeza* and sweet clover (Arakeri and Schmid, 1949; Tysdal and Pieters, 1931). Early root growth in forage legumes is significantly affected by temperature (Cohen and Tailor, 1969; Brar et al., 1990). Brar, et al. (1990) measured the length of the main root axis and lengths of lateral roots ten d after planting under six temperatures and observed significant cultivar and cultivar*temperature effects. Different legume species had different temperature response curves within the 10 to 25 C range (Brar, et al., 1990).

**Recurrent Selection in Alfalfa**

Recurrent selection is a cyclical breeding method used to increase the mean performance of a population while maintaining genetic variation for future selection (Hallauer, 1985). Methods of recurrent selection differ in their criteria for selection, time of selection, and the generation to be intercrossed to produce the next cycle (Falconer and Mackay, 1996). Included in the phenotypic measurements used for selection are variance due to genotype,
environment, and the interaction between genotype and environment (Hallauer, 1985). Selection may be based on the phenotype of the individual or may rely on information from progeny test means (Falconer and Mackay, 1996). Selection may be made before or after plants are intercrossed, depending on the expression of the trait under selection. Selection before intercrossing will have a greater rate of genetic gain than selection after intercrossing because in the former case, both parents under selection (i.e. there is greater parental control) (Fehr, 1991).

Alfalfa cultivars are heterogenous and are described according their mean response (e.g. avg. yield, percentage resistance) (Barnes and Sheaffer, 1995). Response to recurrent selection in alfalfa for quantitatively inherited traits has been reported for disease resistance, seed size, root characteristics, floral characteristics, and nutritive value (e.g. Gjuric and Smith, 1997, Halimi et al, 1994, Knapp and Teuber, 1994, Pederson et al., 1984, Shenk and Elliott, 1970, Wang et al., 1991). Response to selection is a product of the heritability of the trait, the intensity of selection, and the phenotypic variation observed in the trait (Falconer and Mackay, 1996). Using this relationship, and variables such as time to complete each cycle and level of parental control, genetic gain per cycle may be predicted for a given method of selection (Falconer and Mackay, 1996). Recurrent selection for ease of floret tripping was performed by Knapp et al. (1993). A factorial mating design after one cycle of selection was used to estimate genetic variances and to predict expected genetic gain for several breeding methods. Phenotypic mass selection was the most efficient method of selection for this trait, which had both additive and non-additive sources of genetic variance (Knapp et al., 1993). Three cycles of phenotypic mass selection for ease of floret tripping were performed with observed genetic gains closely mirroring predicted gains (Knapp and Teuber, 1994).

Within a given population, genetic gain from selection may be optimized by adjusting the intensity of selection, and the level of parental control. Gjuric and Smith (1997) conducted bidirectional selection for seed size within a broad-based alfalfa population using three methods
that differed in the level of parental control and the intensity of selection. The greatest increase in seed size was realized with selection in both the seed and pollen parent (full parental control). The greatest decrease in seed size was seen with mass selection and no parental control, but with greater intensity of selection (Gjuric and Smith, 1997).

Bidirectional selection is often used in short-term recurrent selection experiments in alfalfa because there is increased likelihood of detecting statistically significant differences between the positively and negatively selected populations than between a unidirectionally selected population and an unselected control (Falconer and Mackay, 1996). Bidirectional selection often results in different responses for each direction in the first cycles of selection (Falconer and Mackay, 1996). Asymmetry in response to selection may be attributed to several factors, including random drift, differences in selection differential, scalar asymmetry, or the presence of genes of large effect (Falconer and Mackay, 1996). In alfalfa, asymmetrical responses to selection have been observed in bidirectional selection for root bark area (Wang et al., 1991), ease of floret tripping (Knapp and Teuber, 1994), and seed size (Gjuric and Smith, 1997). Conversely, bidirectional selection for Sclerotina crown and stem rot was symmetrical for resistance and susceptibility (Halimi et al., 1994). This trait is likely controlled polygenically, with few effects from major genes (Halimi et al., 1994).

Recurrent selection for the same trait within different alfalfa populations have also resulted in different levels of response. Selection for high and low fresh root weight was performed in six alfalfa cultivars differing in winter hardiness (Pederson et al., 1984). Greatest differences between progeny of high and low root weight selections were seen in cultivars classed as non-winter hardy, but no consistent relationship with winter hardiness was observed in the other cultivars used in the study (Pederson et al., 1984). Hansen and Viands (1989) subjected five alfalfa populations to recurrent phenotypic selection for root regeneration capacity. Selection was successful in four of the five populations, expressed as an increase in secondary roots per plant and an increase in taproot weight. Recurrent selection for increased
seed size was performed in two birdsfoot trefoil (*Lotus corniculatus* L.) cultivars (Draper and Wilsie, 1965). Increase in seed size with three cycles of selection was seen in both populations, but not equally. Gain in seed size was 20% per cycle in ‘Viking’, and 6.25% per cycle in ‘Empire’ birdsfoot trefoil (Draper and Wilsie, 1965). Differences in response to selection among populations may be due to differences in gene frequency between the base populations, to the effects of genetic drift, and to sampling error in estimating the generation means (Falconer and Mackay, 1996).

Inbreeding depression may affect performance of alfalfa populations resulting from recurrent selection. Inbred parents of autotetraploids, such as alfalfa, transmit some of their inbreeding depression to their offspring through the diploid gamete (Hill, 1983, 1987, Busbice et al., 1972). Alfalfa is highly susceptible to inbreeding depression (Busbice et al., 1972). Rapid loss of vigor in alfalfa subjected to eight generations of inbreeding was reported by Tysdal et al. (1942). Busbice et al. (1966), examining this data, concluded that tetragenic and trigenic interactions are important to inbreeding depression in alfalfa. The amount of inbreeding depression in alfalfa is affected by the rate of decrease in heterozygosity produced by different methods of inbreeding (Bartlett and Haldane, 1934; Hill, 1987). Heterozygosis may be halved in fewer than four generations by self-fertilization, and in less than nine generations where sib-mating is performed (Bartlett and Haldane, 1934). Hill (1983) observed heterosis in cultivar crosses between two unrelated non-inbred parents, and between a non-inbred parent and an unrelated inbred parent. A positive correlation between genetic distance as measured by molecular markers and forage yield among tetraploid alfalfa genotypes was observed by Kidwell et al. (1994).

**Recurrent Selection for Germination Rate and Seedling Vigor**

Recurrent selection for germination rate and seedling vigor has been reported in crops other than alfalfa. McConnell and Gardner (1979) selected for cold germinability in two corn
populations by using recurrent mass selection under laboratory conditions. Mean percentage
laboratory germination at 7.2°C was increased 8.8 and 9.9% per cycle (McConnell and
Gardner, 1979). No change in field emergence was observed with selection; however, the
weather conditions at planting may not have been severe enough to cause differences in
performance among lines (McConnell and Gardner, 1979). Laboratory selection to improve
cold germination in cucumber resulted in enhanced field emergence 7 d after planting, but
seedling vigor and yield were unchanged (Staub et al., 1988). Bacon et al. (1986) used mass
selection to select for early field emergence in grain sorghum. Three cycles of selection
increased field emergence 10.6%, with a correlated decrease in days to half-bloom, increases
in plant height and grain yield (Bacon et al., 1986). Selection for seedling vigor in red clover
was evaluated for potential to improve plant growth at later stages of maturity. Seedling traits
were only weakly correlated with mature plant morphology, but selection in red clover for
traits associated with seedling vigor increased individual plant dry weight, and first harvest
yield in the seeding year (Xie and Mosjidis, 1995).

Estimating Heritability of a Quantitative Trait Using Response to Selection

Response to selection may be calculated using estimates of the generation means of
each cycle in the selection experiment. Response to unidirectional selection can be calculated
as the difference between the mean of an unselected control population and the mean of the
selected population. When no control population is available, response to unidirectional
selection can be calculated as the difference between the mean of the source population and the
mean of the selected population. Response to bidirectional selection can be calculated as the
difference between the means of the positively and negatively selected populations (Falconer

The heritability of a quantitative trait is the proportion of the phenotypic variance that is
attributable to additive genetic effects. It is useful in estimating the reliability of the phenotype
in predicting breeding value (Falconer and Mackay, 1996). The results of a recurrent selection experiment may be used to estimate the realized heritability of a quantitative trait by relating response to the selection differential (Falconer and Mackay, 1996). There are several ways of calculating realized heritability, which differ in the expected variance of the estimate (Hill, 1972b). In one method, realized heritability is calculated as the regression of cumulative response on cumulative selection differential:

$$b_C = \frac{1}{\sum_{i=0}^{n}(S_i - \bar{S})(X_i - \bar{X})^2/\sum_{i=0}^{n}(S_i - \bar{S})^2}$$

where $b_C$ is the unbiased estimator of realized heritability, $S_i$ and $X_i$ are the cumulative selection differential and the sample mean of individuals from generation i, respectively, (Hill, 1972b). The variance of $b_C$ is estimated by:

$$V(b_C) = \frac{1}{\sum_{i=0}^{n}(S_i - \bar{S})(S_i - \bar{S})\text{cov}(X_i, X_i)/[\sum_{i=0}^{n}(S_i - \bar{S})^2]^2}$$

where $V(b_C)$ is the sampling variance of the estimator (Hill, 1972b).

The response of a trait to selection and the correlated response of a different trait, determined experimentally, may be used to estimate the realized genetic correlation between them by:

$$r^2_A = (CR_x/R_x)(CR_y/R_y)$$

where R is the direct response to selection and CR is the correlated response to selection (Falconer and Mackay, 1996).

**Summary**

Early spring alfalfa seedlings routinely encounter temperatures suboptimal for germination and growth, often resulting in poor germination and weak stands (Vough et al., 1995). Alfalfa seedlings exhibiting early germination and rapid growth in cold (above
freezing) soils may establish more successfully in the early spring, reducing problems with disease and weed competition. Planting of alfalfa cultivars selected for optimum performance under the conditions anticipated during establishment and growth can improve performance (Volenec and Nelson, 1995).

Selection experiments in corn (McConnell and Gardner, 1979) and cucumber (Staub et al., 1988) suggest that laboratory selection for germination rate may be successful at changing germination rate under laboratory conditions. Changes in seedling emergence with selection in sorghum (Bacon et al., 1986) indicate that traits associated with seedling vigor in the field may also be affected by selection. Selection for seedling vigor in alfalfa may improve plant growth at later stages of maturity. In red clover, selection for traits associated with seedling vigor increased individual plant dry weight, and first harvest yield in the seeding year (Xie and Mosjidis, 1995).

References


CHAPTER 2. CULTIVAR AND SEED LOT VARIATION FOR GERMINATION AND RADICLE GROWTH AT SUBOPTIMAL TEMPERATURES IN ALFALFA

A paper to be submitted to the Agronomy Journal

K.L.E. Klos and E.C. Brummer

Abstract

Successful establishment of alfalfa (*Medicago sativa* L.) in the early spring, particularly with no-till seeding, requires rapid germination and vigorous growth at cool temperatures. This study was conducted (i) to evaluate the extent of variation among ten alfalfa cultivars, ‘Marathon’ red clover (*Trifolium pratense* L.), and ‘Norcen’ birdsfoot trefoil (*Lotus corniculatus* L.) for germination time (GT) and initial radicle growth rate (IRGR) at three temperatures considered suboptimal for germination; (ii) to evaluate variation among seed lots within seven alfalfa cultivars for GT at 5 and 10 °C; and (iii) to determine if field emergence was correlated with traits measured in the laboratory. The IRGR of cultivars increased with temperature and the GT of cultivars decreased, as did the GT of seed lots within cultivars. When seed lots within cultivars were evaluated, differences among cultivars for GT were seen only at 5 °C. Both GT and IRGR measured in the laboratory were correlated with field emergence 8 d after planting at one of two locations tested. Cultivars with superior laboratory GT and IRGR may perform better under cool field conditions.

Abbreviations: GT = Germination Time; IRGR = Initial Radical Growth Rate; FD = Fall Dormancy.
**Introduction**

Forage legumes planted in the early spring routinely encounter temperatures suboptimal for germination and growth, which may result in weak stands (Vough et al., 1995). Seedlings that exhibit rapid germination and growth in cold, but above freezing, soils may establish more successfully. The germination rate of forage legumes decreases rapidly at temperatures below the optimum of between 10 and 25°C, depending on species (Brar et al., 1991; McElgunn, 1973). Brar et al. (1991) reported that the optimum temperature for germination of ‘Maxidor’ and ‘Cimarron’ alfalfa was 15 to 25°C. Seedling root growth of alfalfa and red clover is decreased by temperatures below 15°C (Cohen and Tadmor, 1969; Brar, et al., 1990).

McElgunn (1973) studied the germination responses of alfalfa, birdsfoot trefoil, and other forage legumes at a range of constant and alternating temperatures, finding that alternating cold temperature conditions typical of those in the field reduced both rate and total germination. Because germination rate across a range of temperatures varied among alfalfa cultivars, it may be a useful predictor of field germination and emergence at low temperatures (Brar et al., 1991). Soil temperatures in the upper Midwest during the early spring are below 10°C suggesting that germination responses may be a useful criterion for cultivar selection.

Laboratory screening for germinability at suboptimal temperatures correlated well with field performance in soybean, *Glycine max* (L) Merr. (Szrymer and Szczepąnska, 1982), and in common bean under some temperature regimes (*Phaseolus vulgaris* L.) (Dickson and Boettger, 1984). This relationship has not been studied in alfalfa or commonly used pasture legumes such as birdsfoot trefoil and red clover.

The objectives of this study were (i) to evaluate variation in germination time (GT) and initial radicle growth rate (IRGR) among recently developed alfalfa cultivars, one red clover
cultivar, and a birdsfoot trefoil cultivar under laboratory conditions at temperatures suboptimal for germination and growth; (ii) to evaluate variation in germination rate among seed lots within alfalfa cultivars; and (iii) to estimate correlations of germination rate and seedling growth at suboptimal temperatures in the laboratory with seed lot quality, 100-seed mass, and field emergence.

**Materials and Methods**

**Experiment 1**

To determine variation among cultivars for GT and IRGR, 10 alfalfa cultivars from a range of fall dormancy (FD) classifications, 'Norcen', birdsfoot trefoil, and 'Marathon', red clover were evaluated in a laboratory experiment (Table 1). One lot of seed for each entry was obtained either from commercial sources or from Dr. T. A. Campbell, USDA-ARS.

Germination time (d after planting) and IRGR (mm d\(^{-1}\)) were evaluated in a randomized complete block design with three replications. Treatments were arranged in a split plot design, with temperatures as whole plots and cultivars as sub-plots within temperatures. Replications were necessarily nested within temperatures. Temperature and cultivar effects were considered fixed.

An experimental unit consisted of 100 scarified seeds of a cultivar that were placed on moistened blotter paper in a hinged plastic box. The box was wrapped in black plastic to exclude light and placed in one of three germination chambers set at 5, 10, or 15 C. The boxes were opened daily for 38 d after planting to record the day of germination for each seed. A seed was considered to have germinated if the radicle protruded ≥1 mm from the seed coat. The IRGR was estimated based on the radicle length of each seedling 5 d after germination. Seeds that had not germinated by 38 d were excluded from the analysis. Analyses of variance
Table 1: Mean germination time and initial radicle growth rate (IRGR) of ten alfalfa cultivars, Marathon red clover, and Norcen birdsfoot trefoil at 5, 10, and 15°C.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seedlot</th>
<th>FD †</th>
<th>5°C</th>
<th>10°C</th>
<th>15°C</th>
<th>Mean Germination time</th>
<th>5°C</th>
<th>10°C</th>
<th>15°C</th>
<th>Mean IRGR</th>
</tr>
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<tbody>
<tr>
<td>Norseman</td>
<td>MN9609</td>
<td>1</td>
<td>18.7</td>
<td>4.4</td>
<td>3.2</td>
<td>1.9</td>
<td>5.1</td>
<td>7.9</td>
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<td></td>
</tr>
<tr>
<td>Alfagraze</td>
<td>1996</td>
<td>2</td>
<td>12.4</td>
<td>3.0</td>
<td>2.3</td>
<td>2.0</td>
<td>4.4</td>
<td>7.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vernal</td>
<td>A42834</td>
<td>2</td>
<td>10.6</td>
<td>2.9</td>
<td>2.1</td>
<td>1.9</td>
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<td>7.2</td>
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<td>WL252HQ</td>
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<td>2</td>
<td>12.1</td>
<td>3.0</td>
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<td>4.5</td>
<td>7.4</td>
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<td></td>
</tr>
<tr>
<td>Innovator +Z</td>
<td>1996</td>
<td>3</td>
<td>9.3</td>
<td>2.9</td>
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<td>4</td>
<td>10.9</td>
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<td>Magnum IV</td>
<td>1995</td>
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<td>11.1</td>
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<td>2.0</td>
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<tr>
<td>Lahontan</td>
<td>1996</td>
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<td>3.0</td>
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<td>1.8</td>
<td>4.4</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CUF 101</td>
<td>1996</td>
<td>9</td>
<td>7.9</td>
<td>2.9</td>
<td>2.2</td>
<td>1.9</td>
<td>4.8</td>
<td>7.6</td>
<td></td>
<td></td>
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</tbody>
</table>
Table 1 (continued): Mean germination time and mean initial radicle growth rate for ten alfalfa cultivars, Marathon red clover, and Norcen birdsfoot trefoil at 5, 10, and 15 C.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seedlot</th>
<th>FD †</th>
<th>Germination time</th>
<th>IRGR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 C</td>
<td>10 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(d)</td>
<td>(d)</td>
</tr>
<tr>
<td>Marathon</td>
<td>1995</td>
<td></td>
<td>10.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Norcen</td>
<td>1995</td>
<td></td>
<td>18.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>11.8</td>
<td>3.3</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td></td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Marathon vs. Vernal</td>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Norcen vs. Vernal</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>D vs. ND alfalfa †</td>
<td></td>
<td>**</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>D vs. SD alfalfa †</td>
<td></td>
<td>**</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>SD vs. ND alfalfa †</td>
<td></td>
<td>**</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

† FD = Fall Dormancy; D, 2,3 = dormant; SD, 4-6 = semi-dormant; ND, 9 = non-dormant
were calculated using the GLM procedure of the SAS statistical software package (SAS Institute, 1996). Mean separations were determined using Fisher's protected LSD (P<0.05) (Steel and Torrie, 1980).

Experiment 2

To determine if variation for GT could be caused by variation in seed production practices, seed size, or seed quality, two to four seed lots of seven alfalfa cultivars were evaluated in randomized complete block designs with three replications. After scarification, seed lots were evaluated for hard and dead seed and normal and abnormal germination at 20 C using standard seed quality testing procedures (AOSA, 1991), and for 100-seed mass of lots not treated with fungicide. Mean GT was evaluated with treatments arranged as a split plot design, with temperatures (5 and 10 C) as whole plots and cultivars as sub-plots within temperatures. Seed lots were nested within cultivars and replications were nested within temperatures. Temperature and cultivar effects were considered fixed. Statistical analysis was performed using the GLM and CORR procedures of the SAS statistical software package (SAS Institute, 1996). Mean separations were determined using Fisher's protected LSD (P<0.05) (Steel and Torrie, 1980).

Experiment 3

To determine if the cultivar differences in GT and IRGR correlated with field results, one seed lot of all cultivars that were evaluated in Exp. 1 except 'Lahontan' were planted in a field study in 1998 and evaluated for seedling emergence. Plots were established at the Iowa State Univ. Agron. and Agric. Eng. Research Farm near Ames, IA on 4 April on a Nicollet loam soil (fine-loamy mixed mesic Aquic Hapludoll), and at the Northeast Research Farm near Nashua, IA on 14 April on a Readlyn loam soil (fine-loamy mixed mesic Aquic Hapludoll). The cultivars of interest were part of a larger experiment designed as a rectangular triple lattice
with 70 entries. Fifty seed of each cultivar were planted approximately 1 cm deep in 1 m long rows spaced 75 cm apart. Seedling emergence was recorded 8, 17, and 28 d after planting. Locations and cultivars were considered fixed effects. Statistical analysis was conducted using the MIXED, CORR, and GLM procedures of the SAS statistical software package (Littell et al., 1996; SAS Institute, 1996).

**Results and Discussion**

**Experiment 1**

*Germination time*

Germination time differed among temperatures and cultivars (Table 2). Contrasts between temperatures indicated that GT decreased as temperatures rose from 5 to 15 C (Tables 1 and 2). Cultivar differences were most marked at 5 C, suggesting it is the best temperature of those we tested at which to evaluate GT. Contrasts among cultivars grouped according to FD (dormant, semi-dormant, and non-dormant) showed that more dormant germplasm was generally slower to germinate (Table 1). However, ‘Innovator+Z’, Lahontan, and ‘CUF101’, representing FD 3, 6, and 9, respectively, had equally early GT (Table 1), indicating that FD and GT at low temperatures are not completely associated. Marathon red clover did not germinate earlier at 5 C than Innovator+Z and was not different from ‘Vernal’ alfalfa at any temperature (Table 1). Norcen birdsfoot trefoil had the slowest germination of all entries at all temperatures, except ‘Norseman’ at 5 C (Table 1). A temperature by cultivar interaction was observed, but it was primarily due to a decrease in cultivar differences as temperature increased.

McElgunn (1973) proposed planting a cultivar with optimum germination in the temperature range expected at seeding to improve alfalfa stand establishment. Our results indicated that Innovator+Z may be good candidate for use in early seeding.
Table 2: Analysis of variance for mean days to germination and mean initial radicle growth rate (IRGR) of ten alfalfa cultivars, one red clover cultivar, and one birdsfoot trefoil cultivar.

<table>
<thead>
<tr>
<th>Source</th>
<th>GT</th>
<th>IRGR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Square</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>957.31**</td>
<td>255.49**</td>
</tr>
<tr>
<td>5 vs. 10 C</td>
<td>1303.90**</td>
<td>142.81**</td>
</tr>
<tr>
<td>10 vs. 15 C</td>
<td>11.20*</td>
<td>113.25**</td>
</tr>
<tr>
<td>Replication(Temperature)</td>
<td>0.95*</td>
<td>1.11**</td>
</tr>
<tr>
<td>Cultivar</td>
<td>21.15**</td>
<td>0.84**</td>
</tr>
<tr>
<td>Dormant vs. nondormant alfalfa</td>
<td>24.12**</td>
<td>0.028 ns</td>
</tr>
<tr>
<td>Dormant vs. semidormant alfalfa</td>
<td>15.67**</td>
<td>0.28 ns</td>
</tr>
<tr>
<td>Semidormant vs. nondormant alfalfa</td>
<td>5.94**</td>
<td>0.23 ns</td>
</tr>
<tr>
<td>Temperature*Cultivar</td>
<td>9.3**</td>
<td>0.13 ns</td>
</tr>
<tr>
<td>Error (b)</td>
<td>0.36</td>
<td>0.087</td>
</tr>
</tbody>
</table>

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

ns Not significant (p<0.05).
Initial radicle growth rate

Initial radicle growth rate increased with temperature and differed among cultivars (Tables 1 and 2). Differences among cultivars were not related to FD class, as Norseman, Innovator +Z, '5454', and CUF101 had the highest IRGR (Table 1). Vernal had a faster IRGR than either Marathon or Norcen at all temperatures (except Marathon at 15 C). No temperature by cultivar interaction was observed for IRGR, suggesting that similar cultivar rankings might be obtained at higher temperatures. The lack of correlation between GT and IRGR at 5 C (p<0.05) indicates that the two traits are probably not under the same genetic control. Though evaluating IRGR is feasible, the measurement is time consuming and technically difficult, making it unsuitable for routine use in a breeding program.

Red clover has the ability to establish well in early spring seedings (Taylor and Smith, 1995), but birdsfoot trefoil establishment is difficult (Beuselinck and Grant, 1995). Our data show that alfalfa cultivars appear to have better potential than red clover for GT and IRGR at suboptimal temperatures. Any establishment superiority of red clover must, therefore, be due to other factors. The slow GT and IRGR of birdsfoot trefoil at temperatures prevalent in the upper Midwest may be one cause of the difficulty often seen with its establishment.

Experiment 2

Cultivars and seed lots within cultivars varied for most seed quality parameters (Table 3). With the exception of three seed lots (Vernal 1953, Lahontan 1996, and Lahontan 1970) that had very poor quality, most seed lots were similar and had normal germination greater than 75% (Table 3). Mean 100-seed mass, an estimate of seed size, differed among seed lots within cultivars (Table 3). Three Vernal seed lots and Lahontan 1970 had the lowest seed mass.

The GT at 10 C was significantly (p<0.05) lower than at 5 C, paralleling differences seen in Exp. 1. Likewise, a temperature by cultivar interaction was observed, again due
Table 3: Germination time, percent normally and abnormally germinating seed, percent dead seed and hard seed, and 100-seed mass of seed lots within seven alfalfa cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed Lot</th>
<th>5 C</th>
<th>10 C</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>5454</td>
<td>1995</td>
<td>12.6</td>
<td>3.5</td>
<td>88.3</td>
<td>5.3</td>
<td>4.7</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>5454</td>
<td>1996</td>
<td>12.1</td>
<td>3.9</td>
<td>77.0</td>
<td>7.0</td>
<td>16.0</td>
<td>0.0</td>
<td>2.35</td>
</tr>
<tr>
<td>5454</td>
<td>1997</td>
<td>13.7</td>
<td>3.6</td>
<td>90.3</td>
<td>5.0</td>
<td>3.3</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Alfagraze</td>
<td>1995</td>
<td>14.9</td>
<td>3.9</td>
<td>84.3</td>
<td>5.0</td>
<td>7.3</td>
<td>3.3</td>
<td>2.03</td>
</tr>
<tr>
<td>Alfagraze</td>
<td>1996</td>
<td>12.9</td>
<td>3.9</td>
<td>88.0</td>
<td>5.0</td>
<td>4.7</td>
<td>2.0</td>
<td>2.07</td>
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<tr>
<td>Amerigraze 401+Z</td>
<td>1996</td>
<td>12.9</td>
<td>4.0</td>
<td>77.0</td>
<td>6.0</td>
<td>3.3</td>
<td>13.7</td>
<td>2.27</td>
</tr>
<tr>
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<td>1997</td>
<td>11.4</td>
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<td>82.7</td>
<td>7.7</td>
<td>1.3</td>
<td>8.3</td>
<td></td>
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<tr>
<td>CUF 101</td>
<td>El Centro</td>
<td>8.6</td>
<td>3.5</td>
<td>87.3</td>
<td>8.3</td>
<td>3.3</td>
<td>0.7</td>
<td>2.38</td>
</tr>
<tr>
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<td>San Juaquin</td>
<td>8.2</td>
<td>3.4</td>
<td>91.3</td>
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<td>2.7</td>
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<td>1996</td>
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<td>2.3</td>
<td>11.7</td>
<td>2.43</td>
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</table>
Table 3 (continued): Germination time, percent normally and abnormally germinating seed, percent dead seed and hard seed, and hundred seed weight of seed lots within seven alfalfa cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed Lot</th>
<th>Germination time</th>
<th>normal</th>
<th>abnormal</th>
<th>dead</th>
<th>hard</th>
<th>100-seed mass</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>50C</td>
<td>100C</td>
<td>seed</td>
<td>seed</td>
<td>seed</td>
<td>seed</td>
</tr>
<tr>
<td>Innovator +Z 1996</td>
<td>10.8</td>
<td>3.6</td>
<td>79.7</td>
<td>3.0</td>
<td>11.0</td>
<td>3.0</td>
<td>2.25</td>
</tr>
<tr>
<td>Innovator +Z 1997</td>
<td>10.8</td>
<td>3.5</td>
<td>92.7</td>
<td>3.7</td>
<td>2.0</td>
<td>1.7</td>
<td>treated</td>
</tr>
<tr>
<td>Lahontan 1970</td>
<td>13.8</td>
<td>5.3</td>
<td>25.7</td>
<td>5.7</td>
<td>68.7</td>
<td>0.0</td>
<td>2.24</td>
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<td>3.6</td>
<td>57.0</td>
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<td>38.7</td>
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<td>3.7</td>
<td>39.7</td>
<td>0.0</td>
<td>1.98</td>
</tr>
<tr>
<td>Vernal 1995</td>
<td>10.3</td>
<td>3.3</td>
<td>84.0</td>
<td>6.0</td>
<td>8.7</td>
<td>1.3</td>
<td>1.97</td>
</tr>
<tr>
<td>Vernal 1997</td>
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<td>4.0</td>
<td>85.0</td>
<td>3.7</td>
<td>7.0</td>
<td>4.3</td>
<td>1.65</td>
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<td>Vernal A42834</td>
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<td>3.9</td>
<td>87.7</td>
<td>6.3</td>
<td>2.3</td>
<td>4.0</td>
<td>2.35</td>
</tr>
<tr>
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<td>3.8</td>
<td>78.6</td>
<td>5.6</td>
<td>12.7</td>
<td>3.2</td>
<td>2.19</td>
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<tr>
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<td>1.8</td>
<td>0.3</td>
<td>19.2</td>
<td>3.8</td>
<td>4.6</td>
<td>2.0</td>
<td>0.36</td>
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<tr>
<td>Cultivar main effect</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Seed lot (cultivar) effect</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>*</td>
</tr>
</tbody>
</table>

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.
mainly to a decrease in magnitude among cultivars at 10 C. Seed lot variability for GT was observed within cultivars (Table 3), with differences observed among lots of 'Alfagraze', Innovator +Z, Lahontan, and Vernal. No differences were observed among lots of 5454, 'Amerigraze 401+Z', and CUF 101. The two seed lots of Lahontan differed widely in their GT, a result possibly related to their quality. The variation among seed lots obscured cultivar differences at 10 C, but cultivars could be differentiated at 5 C. Based on this result, evaluation of cultivar differences for GT at suboptimal temperatures should be conducted at 5 C to avoid confounding with seed lot effects. The ranking of cultivars for GT at 5 c was unchanged from Exp. 1, though in this case CUF 101 was significantly (p<0.05) earlier to germinate than Innovator+Z. Germination time at 5 C was weakly correlated with seed mass (r = -0.31, p<0.05), but cultivars did not differ for seed mass (Table 3).

Experiment 3

After planting, soil temperatures at 10 cm were below 10 C for 14 d at Ames and 7 d at Nashua. Average daily air temperatures ranged from 6.4 to 18.6 C at Ames, and from 5.8 to 23.3 C at Nashua during the month after planting (Fig. 1).

Germination time in the field

Emergence 8 d after planting was recorded for both Ames and Nashua. After 8 d, plots at Nashua were affected by insect damage and emergence data for 17 and 27 d after planting was only recorded at Ames.

The location by entry interaction was significant, due largely to differences in ranking among cultivars; therefore, analyses of the individual locations will be discussed. The different rankings at each location may have been due to differences in soil and air temperatures after planting (Fig. 1). Cultivars differed for emergence 8 d after planting at both Ames and Nashua (Table 4). CUF101 had the greatest number of seedlings emerged 8 d after planting at
Table 4: Field emergence of nine alfalfa cultivars, a red clover cultivar, and a birdsfoot trefoil cultivar 8, 17, and 27 days after planting at Ames, IA; and 8 d after planting at Nashua, IA.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed Lot</th>
<th>8 d field emergence</th>
<th>17 d</th>
<th>27 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
</tr>
<tr>
<td>5454</td>
<td>1996</td>
<td>15.5</td>
<td>21.7</td>
<td>22.1</td>
</tr>
<tr>
<td>Alfagraze</td>
<td>1996</td>
<td>7.1</td>
<td>5.7</td>
<td>20.4</td>
</tr>
<tr>
<td>Amerigraze 401+Z</td>
<td>1996</td>
<td>13.5</td>
<td>18.3</td>
<td>28.3</td>
</tr>
<tr>
<td>CUF 101</td>
<td>1996 EC</td>
<td>24.4</td>
<td>13.3</td>
<td>32.5</td>
</tr>
<tr>
<td>Innovator +Z</td>
<td>1996</td>
<td>8.3</td>
<td>19.3</td>
<td>21.7</td>
</tr>
<tr>
<td>Magnum IV</td>
<td>1995</td>
<td>16.3</td>
<td>15.7</td>
<td>32.2</td>
</tr>
<tr>
<td>Norseman</td>
<td>MN9609</td>
<td>15.2</td>
<td>8.3</td>
<td>32.1</td>
</tr>
<tr>
<td>Vernal</td>
<td>1997</td>
<td>14.6</td>
<td>19.7</td>
<td>38.4</td>
</tr>
<tr>
<td>WL252HQ</td>
<td>1996</td>
<td>15.9</td>
<td>17.3</td>
<td>25.1</td>
</tr>
<tr>
<td>Marathon red clover</td>
<td>1995</td>
<td>2.1</td>
<td>18.0</td>
<td>41.4</td>
</tr>
<tr>
<td>Norcenc birdsfoot trefoil</td>
<td>1995</td>
<td>1.4</td>
<td>2.7</td>
<td>34.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>12.2</td>
<td>14.5</td>
<td>29.9</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td></td>
<td>7.4</td>
<td>6.8</td>
<td>7.3</td>
</tr>
</tbody>
</table>
Figure 1: Average daily air temperatures and soil temperatures at a depth of 10 cm for Ames, IA; and Nashua, IA from 1 April, 1998 through 15 May, 1998.
Ames; 5454 emerged fastest at Nashua (Table 4). Of the alfalfa cultivars, Alfagraze had the lowest emergence 8 d after planting at both locations. Norcen birdsfoot trefoil had the slowest emergence of all entries; Marathon red clover was the next slowest entry (Table 4). Differences among cultivars were also observed 17 and 27 d after planting at Ames (Table 4). Marathon red clover had among the greatest emergence counts at 17 and 27 d after planting; Alfagraze was consistently among the poorest (Table 4).

Correlation of field and laboratory results

The germination rate at 5 C in the laboratory was negatively correlated with seedling emergence 8 d after planting at Nashua (r = -0.72, p<0.05), but not at Ames. Norcen birdsfoot trefoil had among the slowest GT at 5 C in the laboratory and among the poorest emergence 8 d after planting at both locations. Innovator +Z was among the earliest to germinate at 5 C, but was among the slowest to emerge at Ames. A positive correlation was observed between IRGR at 10 C in the laboratory and seedling emergence 8 d after planting at Ames (r = 0.76, p<0.05) and for the combined locations (r = 0.79, p<0.05), but not at Nashua.

One hundred-seed mass was measured on the five untreated cultivars tested in the field and was not correlated with emergence 8 d after planting at Ames or Nashua (p<0.05). Carleton and Cooper (1972) found that seed mass was not correlated with seedling vigor in alfalfa, but Townsend (1992), in a similar experiment, found a positive correlation between mean seed mass of yellow flowered alfalfa and total seedling emergence. No correlations between 17 d or 27 d emergence in the field and any laboratory traits were observed, perhaps because temperatures were too warm at these dates to differentiate among genotypes.
Conclusions

We conclude that laboratory evaluation of GT at suboptimal temperature should be conducted at 5 C when utilizing the methods of this study. Differences among cultivars are greater at 5 C than at 10 C, and we were able to differentiate among cultivars at 5 C in the presence of seed lot variation. Because GT at 5 C was correlated with field emergence 8 d after planting only at Nashua, measuring it in the laboratory may be of only limited use in predicting seedling emergence in the field. Initial radicle growth rate at 10 C under laboratory conditions was predicative of seedling emergence of alfalfa cultivars eight days after planting at Ames. Therefore, laboratory evaluation of IRGR may be useful as an indicator of alfalfa seedling emergence rate in the field. Evaluation of IRGR in the laboratory by the method used in this study is time consuming and technically difficult and may not be suitable for large-scale evaluation of forage legume genotypes.

References


CHAPTER 3. RESPONSE OF SIX ALFALFA POPULATIONS TO 
SELECTION UNDER LABORATORY CONDITIONS FOR GERMINATION 
AND SEEDLING VIGOR AT LOW TEMPERATURES

A paper to be submitted to Crop Science

K.L.E. Klos and E.C. Brummer

Abstract

Alfalfa (Medicago sativa L.) seeded in the early spring often encounters temperatures suboptimal for germination and seedling growth. Four selection schemes designed to alter the ability of alfalfa to germinate and grow at suboptimal temperatures were conducted within six commercial alfalfa cultivars: 5454, Alfagraze, Amerigraze 401+Z, Innovator +Z, Magnum IV, and WL252HQ. Two cycles of laboratory selection for early germination at 5°C decreased germination time (GT) by 28.9% (2.8 d) on average. The mean realized heritability of GT was 0.49 averaged over all populations, with a range of 0.38 to 0.67. Response to selection was greatest in the first cycle, suggesting that further decreases in GT may be limited. Two cycles of selection for high seedling vigor increased seedling height (SH) after 45 d at 10°C by 14.9% (7.2 mm). The average realized heritability of SH was 0.18 and ranged from 0.046 to 0.40 for individual populations. Estimates of the realized genetic correlation between GT and SH ranged from 0.02 to 0.60. In some populations, selection for high seedling vigor was effective at increasing SH in the laboratory and continued response to selection may be expected. An increase in GT with selection for high seedling vigor indicated an association between these traits under laboratory conditions. Two cycles of combined selection for both early germination and high seedling vigor resulted in a 28.9% (2.8 d) decrease in GT on
average, without affecting SH. Combined selection against both traits increased GT by 161.9% (15.7 d) and decreased SH by 17.6% (8.5 mm) after two cycles. Selecting for decreased GT and increased SH under low temperatures in the laboratory can be accomplished, but the amount of improvement is population dependant.

**Abbreviations:** EG = Early Germination; HSV = High Seedling Vigor; LG = Late Germination; LSV = Low Seedling Vigor; GT = Germination Time; SH = Seedling Height; C0, C1, C2 = Cycle Zero, Cycle One, and Cycle 2, respectively.

**Introduction**

Spring is the most common season for seeding alfalfa in the Midwestern U.S. because moisture is abundant and forage can be harvested during the establishment year (Vough et al., 1995; Barnes and Sheaffer, 1995). However, soil temperatures during spring are commonly below 10 C and seeding into cold, wet soils can result in poor germination and weak stands (Vough et al., 1995). Thus, alfalfa populations tolerant of cold temperatures during germination and the early stages of growth may be useful for establishing acceptable stands (Volene and Nelson, 1995).

Germination of alfalfa, one of the most cold resistant of the forage legume species, can occur at 0 to 1 C (Arakeri and Schmid, 1949; Tysdal and Pieters, 1931; Coffman, 1923), but is optimum between 15 and 20 C, based on an evaluation of a limited number of alfalfa cultivars (Brar et al., 1991). Variation among cultivars for germination time (GT) and root growth response has been reported across a temperature range of 5 to 30 C (Brar et al., 1990, 1991; Klos, 1999).

Correlations of field and laboratory measurements of germination responses at cold temperatures have been examined in a number of leguminous crop species. Alfalfa field
emergence was positively correlated with initial radicle growth rate measured in the laboratory and negatively correlated with GT measured in the laboratory at one of two locations evaluated (Klos, 1999). In soybean (Glycine max (L.) Merr.) and common bean (Phaseolus vulgaris L.), laboratory germination rate under low temperatures provided a good test for tolerance to cool field conditions (Dickson and Boettger, 1984; Saminy et al., 1987; Szyrmer and Szczepanska, 1981). Progress using recurrent selection for cold temperature germination has been reported in maize, Zea mays L., (McConnell and Gardner, 1979) and cucumber, Cucumis sativus L. (Staub et al. 1988). Narrow sense heritability of days to germination in cucumber was estimated to be between 0.15 and 0.20 when measured at 15 C using parent-progeny regression and 0.61 at 17 C using half-sib family means (Wehner, 1982, 1984). The difference in the estimates was attributed to the evaluation temperature (Wehner, 1984).

The objective of this study was to assess progress realized from recurrent phenotypic selection in six alfalfa populations for GT and seedling growth at low temperatures under laboratory conditions.

**Materials and Methods**

**Selection Methods**

The populations used for recurrent selection in this study represented commonly available cultivars in fall dormancy groups 2 through 4 (Table 1). The developers of the cultivars in this study are listed in Table 1. Seed was obtained either from commercial sources or from Dr. T. A. Campbell, USDA-ARS. Two to three cycles of selection using the following four selection methods were applied to each population.

*Early Germination (EG).*

Germination time (d to germination) was evaluated at 5 C. Three hundred scarified seeds were placed in plastic germination boxes on damp blotter paper, 100 seeds per box.
Table 1: Alfalfa cultivars used in recurrent selection programs for germination time and seedling vigor at low temperatures, and their fall dormancy classification, developer, cultivar parentage, and three traits measured in the laboratory: 100-seed mass, germination time, and seedling height.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>FD†</th>
<th>Developer</th>
<th>No. Parents</th>
<th>Source of Parents</th>
<th>100-Seed Mass</th>
<th>Germination Time</th>
<th>Seedling Ht</th>
</tr>
</thead>
<tbody>
<tr>
<td>5454</td>
<td>4</td>
<td>Pioneer Hi-Bred Int'l.</td>
<td>12</td>
<td>18+ populations</td>
<td>‡</td>
<td>9.2</td>
<td>52.1</td>
</tr>
<tr>
<td>Alfagraze</td>
<td>2</td>
<td>University of Georgia</td>
<td>30</td>
<td>22 cultivars and 1070 PI</td>
<td>2.14</td>
<td>14.9</td>
<td>49.6</td>
</tr>
<tr>
<td>Amerigraze 401+Z</td>
<td>4</td>
<td>ABI Alfalfa</td>
<td>396</td>
<td>'Allegro'</td>
<td>2.44</td>
<td>8.2</td>
<td>47.5</td>
</tr>
<tr>
<td>Innovator +Z</td>
<td>3</td>
<td>ABI Alfalfa</td>
<td>112</td>
<td>27 cultivars and germplasms</td>
<td>2.34</td>
<td>8.5</td>
<td>48.9</td>
</tr>
<tr>
<td>Magnum IV</td>
<td>4</td>
<td>Dairyland Seeds, Inc.</td>
<td>22</td>
<td>8 cultivars and experimental lines</td>
<td>‡</td>
<td>8.5</td>
<td>47.9</td>
</tr>
<tr>
<td>WL252HQ</td>
<td>2</td>
<td>W-L Research</td>
<td>111</td>
<td>4 populations</td>
<td>2.34</td>
<td>8.8</td>
<td>44.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.32</td>
<td>9.7</td>
<td>48.4</td>
</tr>
<tr>
<td>LSD(5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>3.5</td>
<td>ns</td>
</tr>
</tbody>
</table>

† FD = Fall Dormancy; 2 = dormant; 4 = semi-dormant.

‡ Seed mass not determined because treated seed was received.
Boxes were protected from light, and humidity was maintained at 100%. Boxes were opened daily for 15 days and then every second day until 50 d after germination or until all seeds had germinated; the day each seed germinated was recorded. Seeds that did not imbibe water after 5 d were removed from the box and were not included in the analysis. Seeds were considered to have germinated when the radicle protruded ≥ 1 mm from the seed coat. The first 5% of seeds to germinate were selected.

*High Seedling Vigor (HSV).*

Seedling vigor was evaluated on the basis of seedling height (SH) after growth at 10 C. Six hundred scarified seeds were germinated in the greenhouse in 196-cell flats, in equal parts vermiculite and topsoil, two seeds per cell. Three to four d after planting, seedlings were thinned to one plant per cell, and flats were transferred to a growth chamber at 10 C with 20 hr light. Seedling height was recorded 45 d after planting, and the tallest 5% of the seedlings were selected. Selection between seedlings of equal height was made visually for greater leaf number and thicker stem diameter.

*Early Germination and High Seedling Vigor (EG+HSV).*

Three hundred scarified seeds were germinated at 5 C, as described for EG above. The earliest 30 seeds to germinate were transplanted into 196-cell flats with one seedling per cell. Flats were placed in a growth chamber at 10 C with 20 hr light for 45 d when the tallest 15 plants were selected.

*Late Germination and Low Seedling Vigor (LG+LSV).*

Procedures were identical to EG+HSV except that the last 30 seeds to germinate prior to 50 days after planting were transplanted to the growth chamber where the shortest 15 plants were selected after 45 d. Selection between seedlings of equal height was done visually for fewer leaves and thinner stem diameter.

*Control Populations*
Control (unselected) populations of Innovator+Z and 5454 were developed by intercrossing 15 randomly chosen plants of each cultivar in each cycle. No selection was practiced.

**Intercrossing**

Selected plants were transplanted into 15-cm diameter pots in the greenhouse. Plants within each selected population were intercrossed by hand tripping without emasculation. Equal amounts of seed were bulked from each plant in the selected populations for use in the next cycle of selection. Sixty-one populations were created after 2-3 cycles of selection.

**Evaluation**

One hundred seed mass (mg) was measured on all populations as the average of three samples. Laboratory and growth chamber evaluations were performed separately for SH and GT. Evaluation experiments included all CO and selected populations as entries. Experiments were arranged as randomized complete block designs with three replications and individual populations as the only treatment. Population effects were considered fixed.

Germination time was evaluated by placing 100 scarified seeds of each population in a hinged plastic box on moistened blotter paper. Boxes (experimental units) were placed in a germination chamber at 5 C and protected from light. After planting, boxes were opened daily for 15 d and then every second day up to 50 d; the day of germination for each seed was recorded. The mean GT per box was calculated as the average number of days from planting to germination for all viable seeds.

Seedling height was evaluated by planting 96 scarified seeds of each population into three row plots, with 4 cm between rows, in 1500 cm² flats filled with equal parts vermiculite and topsoil. Four populations were planted in each flat; border rows of ‘Vernal’ were placed at
each end. Flats were kept in the greenhouse for 3 d after planting to ensure even germination and then moved to a growth chamber at 10 °C with 20 hr light for 45 d. The first and last seedlings of each row were discarded and seedling height was recorded for all remaining seedlings, excluding the border rows.

Statistical analysis was conducted using the GLM and CORR procedures in the SAS statistical software package (SAS Institute, Inc., 1996). Mean separations were determined using Fisher's protected LSD (p<0.05) (Steel and Torrie, 1980).

**Realized Heritabilities**

Realized heritabilities \( h^2_{realized} \) were calculated as the regression of cumulative response on cumulative selection differential:

\[
 b_C = \frac{\sum_{i=0}^{n} (S_i - \bar{S})(X_i - \bar{X})}{\sum_{i=0}^{n} (S_i - \bar{S})^2}
\]

where \( b_C \) is the unbiased estimator of realized heritability, \( S_i \) and \( X_i \) are the cumulative selection differential and the sample mean of individuals from generation \( i \), respectively, (Hill, 1972b). Estimates of the CO, C1 and C2 parental means used to calculate the selection differentials were obtained at different times in the same controlled environmental chambers. The variance of \( b_C \) was estimated by:

\[
V(b_C) = \frac{\sum_{i=0}^{n} \sum_{j=0}^{n} (S_i - \bar{S})(S_j - \bar{S})\text{cov}(X_i, X_j)}{[\sum_{i=0}^{n} (S_i - \bar{S})^2]^2}
\]

where \( V(b_C) \) is the sampling variance of the estimator (Hill, 1972b).

Estimates of realized genetic correlation between GT and SH were calculated as:

\[
r^2_A = \frac{\text{CR}_X/R_X \cdot \text{CR}_Y/R_Y}{}
\]
where \( R \) is the direct response to selection and \( CR \) is the correlated response to selection based on the total response to two cycles of selection (Falconer and Mackay, 1996).

**Results and Discussion**

Cultivars differed for 100-seed mass and germination time at 5°C, but not for seedling height after 45 d at 10°C (Table 1). One hundred-seed mass of treated seed lots was not evaluated. Alfagraze had the lowest 100-seed mass and the longest mean germination time at 5°C (Table 1).

**Control (unselected) populations**

No differences in GT or SH were observed between CO populations and unselected control populations (Table 2). Therefore, the changes in GT and SH that we observed in some selected populations were probably not caused by random genetic drift or inbreeding depression.

**EG Selection**

Averaged over populations, EG selection decreased GT by 28.9% after two cycles; however, the majority of change occurred in the first cycle (Table 3). Response to selection for EG differed among populations (Table 2). Innovator +Z and 5454 EG cycle 2 (C2) populations were 31.8% and 35.9% (\( p<0.10 \)) earlier to germinate than their respective control-C2 populations but neither were different from their respective EG-C1 populations (Table 2). Selection response in the other cultivars was compared with the CO because no control populations were available. Two cycles of EG selection in Alfagraze, the latest cultivar to germinate at 5°C, resulted in the greatest decrease in GT (52.3%) of any cultivar, but the C1 and C2 populations were not different (Table 2). Decreases in GT with selection for EG were
Table 2: Cycle 0 (C0) means and cycle 1 and 2 deviations from C0 for mean germination time at 5°C and mean seedling height after 45 d at 10°C for four selection methods in six alfalfa cultivars; and control populations in two cultivars.

<table>
<thead>
<tr>
<th>Method/Cycle</th>
<th>5454</th>
<th>Alfagraze</th>
<th>Amerigraze 401+Z</th>
<th>Innovator +Z</th>
<th>Magnum IV</th>
<th>WL252HQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GT §</td>
<td>SH</td>
<td>GT</td>
<td>SH</td>
<td>GT</td>
<td>SH</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>mm</td>
<td>d</td>
<td>mm</td>
<td>d</td>
<td>mm</td>
</tr>
<tr>
<td>C0 Means</td>
<td>9.2</td>
<td>52.1</td>
<td>14.9</td>
<td>49.6</td>
<td>8.2</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>48.9</td>
<td>8.5</td>
<td>47.9</td>
<td>8.5</td>
<td>44.2</td>
</tr>
<tr>
<td>Deviations from C0 mean</td>
<td>-2.7†</td>
<td>-5.2</td>
<td>-7.8</td>
<td>4.0</td>
<td>1.2</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>-1.2</td>
<td>-1.6</td>
<td>-1.2</td>
<td>-5.9</td>
<td>-1.9</td>
<td>3.0</td>
</tr>
<tr>
<td>EG-C1‡</td>
<td>3.5</td>
<td>5.1</td>
<td>-1.0</td>
<td>7.1</td>
<td>-1.1</td>
<td>14.7</td>
</tr>
<tr>
<td>HSV-C1</td>
<td>6.6</td>
<td>2.1</td>
<td>4.5</td>
<td>5.5</td>
<td>0.7</td>
<td>11.6</td>
</tr>
<tr>
<td>HSV-C2</td>
<td>2.2</td>
<td>0.1</td>
<td>6.2</td>
<td>4.5</td>
<td>10.1</td>
<td>-0.5</td>
</tr>
<tr>
<td>EG+HSV-C1</td>
<td>-2.8</td>
<td>0.0</td>
<td>-8.7</td>
<td>1.8</td>
<td>-1.5</td>
<td>6.5</td>
</tr>
<tr>
<td>EG+HSV-C2</td>
<td>-2.8</td>
<td>0.0</td>
<td>-8.7</td>
<td>1.8</td>
<td>-1.5</td>
<td>6.5</td>
</tr>
<tr>
<td>LG+LSV-C1</td>
<td>2.2</td>
<td>0.1</td>
<td>6.2</td>
<td>4.5</td>
<td>10.1</td>
<td>-0.5</td>
</tr>
<tr>
<td>LG+LSV-C2</td>
<td>15.9</td>
<td>-13.8</td>
<td>17.6</td>
<td>-7.5</td>
<td>19.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Control-C1</td>
<td>-0.8</td>
<td>-2.4</td>
<td>-2.2</td>
<td>-4.8</td>
<td>-1.3</td>
<td>-8.0</td>
</tr>
<tr>
<td>Control-C2</td>
<td>0.4</td>
<td>-5.0</td>
<td>1.3</td>
<td>-8.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Germination time LSD (0.05) = 3.5; LSD (0.10) = 2.9; Seedling height LSD (0.05) = 13.7; LSD (0.10) = 11.5.
‡ EG = Early Germination, HSV = High Seedling Vigor, LG = Late Germination, LSV = Low Seedling Vigor, C0 = Cycle 0, C1 = Cycle 1, C2 = Cycle 2.
§ GT = Germination Time; SH = Seedling Height.
Table 3: Cycle 0 (C0) means and cycle 1 and 2 deviations from C0 for 100-seed mass, germination time at 5 C, and seedling height after 45 d at 10 C for four selection methods.

<table>
<thead>
<tr>
<th>Method/Cycle</th>
<th>100-Seed Mass</th>
<th>Germination Time</th>
<th>Seedling Height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>d</td>
<td>mm</td>
</tr>
<tr>
<td>C0 Mean</td>
<td>2.32</td>
<td>9.7</td>
<td>48.4</td>
</tr>
<tr>
<td>EG-C1†</td>
<td>0.22</td>
<td>-2.6</td>
<td>0.4</td>
</tr>
<tr>
<td>EG-C2</td>
<td>0.06</td>
<td>-2.8</td>
<td>-1.9</td>
</tr>
<tr>
<td>HSV-C1</td>
<td>0.40</td>
<td>0.8</td>
<td>5.7</td>
</tr>
<tr>
<td>HSV-C2</td>
<td>0.16</td>
<td>2.8</td>
<td>7.2</td>
</tr>
<tr>
<td>EG+HSV-C1</td>
<td>0.22</td>
<td>-1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>EG+HSV-C2</td>
<td>0.08</td>
<td>-2.8</td>
<td>0.6</td>
</tr>
<tr>
<td>LG+LSV-C1</td>
<td>0.29</td>
<td>6.4</td>
<td>-4.1</td>
</tr>
<tr>
<td>LG+LSV-C2</td>
<td>0.05</td>
<td>15.7</td>
<td>-5.8</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.01</td>
<td>1.4</td>
<td>1.8</td>
</tr>
</tbody>
</table>

† EG = Early Germination, HSV = High Seedling Vigor, LG = Late Germination, LSV = Low Seedling Vigor, C0 = Cycle 0, C1 = Cycle 1, C2 = Cycle 2.
not significant (p<0.10) in the remaining source cultivars (Table 2). A third cycle of selection for early germination was performed in Innovator +Z and Magnum IV with no significant change in GT from cycle 2 (data not shown). More cycles of selection may improve GT within some alfalfa populations, but further substantive decreases appear unlikely. Germination time at low temperature may be near a physiological limit due to limited genetic variability for this trait in these alfalfa cultivars.

The average realized heritability of GT, calculated based on response to EG selection, was 0.49 in these populations (Table 4). This is between the two narrow sense heritability estimates of GT at low temperatures in cucumber of 0.15 to 0.20 and 0.61 (Wehner, 1982, 1984). The average selection differential was similar for all cultivars except Alfagraze, but realized heritability varied from a high of 0.67 in Alfagraze to a low of 0.38 in Magnum IV (Table 4). Because the cultivars used in this study are broad-based synthetic populations (with the possible exception of 5454), they should contain high amounts of genetic variation (Table 1). Interestingly, Amerigraze 401+Z, Magnum IV, and WL252HQ had among the smallest realized heritabilities for GT and the narrowest germplasm bases. The most extreme case was Amerigraze 401+Z, whose parents were selected from only one cultivar. In contrast, Alfagraze may have had the broadest germplasm base, with parents selected from populations tracing to 22 cultivars and 1070 USDA plant introductions. This genetic diversity likely contributed to its significant selection response.

EG selection had no effect on SH (Tables 2 and 3). The average 100-seed mass of EG-C1 and EG-C2 populations were 0.22 mg and 0.06 mg greater, respectively, than C0 but similar responses were observed with all other selection methods (Table 3). The C1 and C2 unselected control populations developed from Innovator +Z were 0.17 and 0.29 mg heavier than C0, respectively. A similar comparison could not be made in 5454 which had been treated with fungicide before we received the seed. Environmental factors are known to affect alfalfa seed size (Carleton and Cooper, 1972). The changes in 100-seed mass we observed
Table 4: Cumulative selection differential, realized heritability, and variance of the realized heritability estimate for germination time and seedling height and the realized genetic correlations between them in six alfalfa populations.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cumulative Selection Differential</th>
<th>Germination Time (Based on EG Selection)</th>
<th>Cumulative Selection Differential</th>
<th>Seedling Height (Based on HSV Selection)</th>
<th>Realized Genetic Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d</td>
<td>h²_{realized}</td>
<td>V(h²)</td>
<td>h²_{realized}</td>
<td>V(h²) (x 10⁻⁴)</td>
</tr>
<tr>
<td>5454</td>
<td>5.3</td>
<td>0.59</td>
<td>0.013</td>
<td>55.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Alfagraze</td>
<td>10.7</td>
<td>0.67</td>
<td>0.074</td>
<td>26.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Amerigraze 401+Z</td>
<td>4.2</td>
<td>0.42</td>
<td>0.050</td>
<td>43.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Innovator +Z</td>
<td>5.0</td>
<td>0.43</td>
<td>0.025</td>
<td>50.1</td>
<td>0.40</td>
</tr>
<tr>
<td>Magnum IV</td>
<td>5.3</td>
<td>0.38</td>
<td>0.049</td>
<td>37.9</td>
<td>0.14</td>
</tr>
<tr>
<td>WL252HQ</td>
<td>4.5</td>
<td>0.47</td>
<td>0.030</td>
<td>32.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean</td>
<td>5.8</td>
<td>0.49</td>
<td>0.040</td>
<td>40.8</td>
<td>0.18</td>
</tr>
</tbody>
</table>
over cycles are likely due to differences in the seed production environments experienced by the selected and source populations. One hundred-seed mass of all populations was not correlated with GT in the laboratory at 5 C (p<0.05).

**HSV Selection**

Two cycles of HSV selection increased SH by 14.9%, averaged over populations (Table 3). HSV-C2 was not significantly different from HSV-C1 indicating that, as with EG selection, most response occurred in the first cycle. The lack of response to selection past the C1 may imply a limit, in some alfalfa populations, to the amount of increase in SH which may be expected with this method or may indicate a complicating factor, such as seed quality, that affected our ability to select superior plants. The response to HSV selection differed among source populations. In Innovator +Z, two cycles of HSV selection increased SH by 68.2% compared to its control-C2 population; in this case the response was greater in the second cycle (Table 2). Linear contrasts among generations of HSV selection in Innovator +Z were significant (p<0.05), indicating the potential for further SH increases. Amerigraze 401+Z HSV-C2 was 24.4% (p<0.10) taller than the C0 (Table 2). Although all other populations tended to have higher SH after selection, the increases were not significant (Table 2). Despite microclimate variation in the greenhouse and growth chamber during evaluation and traits such as seed size that may have hampered our ability to differentiate among populations, selection for increased SH is clearly effective in certain populations.

The average realized heritability of SH, based on response to HSV selection, was 0.18 averaged across these populations and ranged from 0.046 in 5454 to 0.40 in Innovator+Z (Table 4).

Two cycles of HSV selection increased GT by an average of 28.9% in spite of the fact that seeds were germinated in the greenhouse prior to placement in a 10 C growth chamber for selection (Table 3). Cultivars differed in their GT response to selection for HSV, with
significant increases observed in the HSV-C2 of 5454 (68.0%), Alfagraze (46.4%), and Magnum IV (43.3%) (Table 2). In these populations, a significant response occurred past C1 (Table 2) and significant (p<0.05) linear contrasts among generations indicated that GT would continue to increase with HSV selection. In Amerigraze 401+Z, Innovator +Z, and WL252HQ, however, changes in GT with HSV selection were not significant and followed no clear trend (Table 2).

The one hundred-seed mass of C1 and C2 populations selected for HSV was greater than C0 (Table 3), probably due to the seed production environment. The average 100-seed mass of HSV-C2 was lower than that of HSV-C1, indicating that the increases in SH over cycles were not due to seed mass (Table 3). The greatest increase in SH with selection for HSV was observed in Amerigraze 401+Z, which also had the greatest 100-seed mass of the cultivars measured (Table 1). However, one hundred-seed mass was not correlated (p=0.05) with SH in this study. Brar et al. (1990) found 100-seed weight to be predictive of growth of the main axis root at temperatures between 15 and 25°C. Carleton and Cooper (1972), comparing the effects of environmentally and genetically caused seed size differences, reported that environmental effects may be the more important source of seedling performance differences in alfalfa. Selection for seedling vigor may not increase 100-seed mass, but selection based on uniformly sized seed may yield more consistent selection responses.

**Realized genetic correlation**

The realized genetic correlation (\(r^2_\lambda\)) between GT and SH, based on the total response after two cycles of selection, differed among populations and ranged from 0.02 in Innovator +Z to 0.60 in Magnum IV (Table 4). A high genetic correlation indicates that selection for early germination results in decreased SH or that selection for increased SH results in delayed GT. These results were not observed in cultivars with low \(r^2_\lambda\), such as Amerigraze 401 +Z.
and Innovator +Z, populations which are better suited to this selection program. Inconsistency among populations for realized genetic correlation estimates may be due to sampling error, to changes in gene frequency during selection, or may reflect differences in gene frequencies among the CO populations (Falconer and Mackay, 1996).

**Bidirectional selection for germination time and seedling vigor**

Averaged over populations, two cycles of EG+HSV selection decreased GT by 28.9% from CO; most response was observed in CI (Table 3). Significant selection responses were only observed in one cultivar: Alfagraze EG+HSV-C2 had an 89.7% earlier GT than its CO, but CI and C2 did not differ (Table 2). Non-significant (p<0.05) decreases in GT were observed in all other cultivars (Table 2). These results support the conclusion that a decrease in GT with selection for early germination under laboratory conditions is likely to be limited. On average, the SH of EG+HSV-C1 was taller than CO, but this response was lost in C2 (Table 3). The 5454 EG+HSV-C1 was 34.8% taller than the unselected control, but no other SH differences were observed among populations of the other cultivars (Table 2). Generally, the selection intensity with the EG+HSV method may not have been great enough to produce significant changes in SH after only two cycles. Alternatively, selection for GT and SH may be counteracting each other during EG+HSV selection, leading to little improvement for either trait.

In contrast to EG+HSV, selection for LG+LSV was much more successful, resulting in an average increase in GT of 161.9% after two cycles (Table 3). The germination time increased in all cultivars under selection; the smallest increase after two cycles was 144.7% in Innovator +Z and the largest was 234.1% in Amerigraze 401+Z (Table 2). In all cases, LG+LSV-C2 was later to germinate than C1 (Tables 2 and 3), and linear contrasts among generations (p<0.05) indicate the potential for further increases in GT. Seedling height decreased by 12.0% on average after two cycles of LG+LSV selection (Table 3), but selection
was not effective in all cultivars (Table 2). LG+LSV-C2 was 26.5% shorter than the C0 but was not different (p<0.05) from the control-C2 population (Table 2). WL252HQ LG+LSV-C2 was 28.1% shorter (p<0.10) than the C0. Differences in other source populations were not significant (p<0.05).

The germination time and seedling height responses were greater with LG+LSV selection than with EG+HSV selection (Table 3). Selection against these traits may have been more successful because cultivars have already been indirectly selected for high seedling vigor, limiting the potential for further gain, while substantial genetic variation may exist for "unimproving" these traits. An asymmetrical response is commonly observed with divergent selection and may be due to random genetic drift, differences in selection differential, asymmetry in the effects of genetic and environmental variation, the presence of genes of large effect, or to the effects of indirect selection (Falconer and Mackay, 1996).

Conclusions

Both the EG and EG+HSV selection methods were successful at decreasing GT at 5 C in the laboratory. Most of the response to selection occurred in the first cycle and significant change was not observed in all populations. The use of greenhouse-produced seed for the second cycle of selection may have effected the lack of gain. Some populations may have had insufficient genetic variation for early germination at suboptimal temperature and consequently reached a physiological limit to their GT at 5 C. A greater change in GT was seen with LG-LSV selection, which supports the idea of an early germination selection limit. Increases in GT were observed with HSV selection in all populations, indicating an association between seedling vigor and GT under laboratory conditions. GT would likely continue to increase with HSV selection, but the impact of this on field performance has not been investigated. Selection for seedling vigor under laboratory conditions was effective at increasing SH in two
populations. Continued gain may be expected in these germplasms. Clearly, HSV selection is not effective in all alfalfa populations. A consistent environment at germination and the use of seed with uniform mass and quality could improve the heritability of SH under the HSV selection method.

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populations. Crop Sci. 19:765-768.


SAS Institute Inc., Cary, NC.


CHAPTER 4. FIELD RESPONSE IN SIX ALFALFA POPULATIONS SELECTED FOR GERMINATION RATE AND SEEDLING VIGOR

A paper to be submitted to *Crop Science*

K.L.E. Klos and E.C. Brummer

Abstract

Successful establishment of alfalfa (*Medicago sativa* L.) stands in the early spring requires good emergence and vigorous seedling growth at soil temperatures below 10 C. Populations developed from six cultivars by four methods of phenotypic recurrent selection in the laboratory for germination time and seedling height at low temperatures were planted in the field at Ames and Nashua, IA in early spring 1998 and evaluated for emergence, seedling growth, forage yield, and other agronomic traits. On average, seedling height 27 d after planting increased by 20.7% after two cycles of selection for seedling vigor. Two cycles of laboratory selection for early germination increased seedling height 27 d after planting by 9.0%, but the response among cultivars was highly variable. Weak positive correlations were observed between seedling height and first year dry matter yield, total yield, and fall regrowth (p<0.01). Seedling emergence 8 d after planting and other agronomic traits were unaffected by any method of selection. Laboratory selection for seedling vigor under suboptimal temperatures is useful for improving seedling vigor of some alfalfa populations in the field under early spring conditions.
Abbreviations: EG = Early Germination; HSV = High Seedling Vigor; LG = Late Germination; LSV = Low Seedling Vigor; GT = Germination Time; SH = Seedling Height; C0, C1, and C2 = Cycle 0, Cycle 1 and Cycle 2, respectively.

Introduction

Most alfalfa is planted in the upper Midwest during the spring to take advantage of available moisture and to allow harvest in the seeding year (Barnes and Sheaffer, 1995; Vough et al., 1995). Alfalfa seed planted under optimum conditions emerges rapidly, usually within 17 d after planting, but spring soil temperatures in the upper Midwest are often suboptimal for alfalfa germination and growth (Brar et al., 1991; McElgunn, 1973; Vough et al., 1995; Weihing, 1941). At suboptimal temperatures, alfalfa cultivars differ in germination and root growth rates, and some cultivars may have better potential for good stand establishment than others (Brar et al., 1990, 1991; Klos, 1999).

Field emergence of alfalfa 8 d after planting has been positively correlated with laboratory radicle growth and negatively correlated with germination time (Klos, 1999). Laboratory germination rate at low temperatures is a good test for field tolerance to suboptimal temperatures in soybean, Glycine max (L.) Merr., and common bean, Phaseolus vulgaris L. (Dickson and Boettger, 1984; Littlejohns and Tanner, 1976; Szyrmer and Szczepanska, 1981).

Selection under laboratory conditions for germination time (GT) and seedling height (SH) under low temperatures was conducted in six alfalfa cultivars (Klos, 1999). Two cycles of selection for early germination at 5 C decreased the average GT in the laboratory by 28.9%. Laboratory SH was increased by 14.8% after two cycles of selection for seedling vigor at 10 C. McConnell and Gardner (1979) used recurrent phenotypic selection on two maize populations to increase germination under laboratory conditions at 7.2 C by 8.8% and 9.9%.
per cycle. Field emergence and seedling vigor of these populations were unchanged, possibly because of warm weather during testing.

The objective of this study was to assess emergence and seedling vigor in the field after phenotypic recurrent selection in six alfalfa populations for cold temperature germination and seedling vigor under laboratory conditions.

**Materials and Methods**

The six cultivars chosen for recurrent selection represent commonly available cultivars in the Midwestern U.S. in fall dormancy groups 2 through 4 (Table 1). Seed was obtained either from commercial sources or from Dr. T.A. Campbell, USDA-ARS.

**Selection Methods**

Recurrent phenotypic selection was conducted within each cultivar using four methods as described in Klos (1999): (i) early germination (EG) at 5 C, (ii) high seedling vigor (HSV) based on SH after growth at 10 C, (iii) a combination of EG and HSV, and (iv) a combination of late germination (LG) and low seedling vigor (LSV). Additionally, unselected control populations of 'Innovator+Z' and '5454' were developed from 15 randomly chosen plants per cycle.

**Evaluation**

The experiment was planted at the Iowa State Univ. Agronomy and Agric. Eng. Research Farm west of Ames, IA on 4 April, 1998 on a Nicollet loam soil (fine-loamy mixed mesic Aquic Hapludoll) and at the Northwest Research Farm near Nashua, IA on 14 April, 1998 on a Readlyn loam soil (fine-loamy mixed mesic Aquic Hapludoll). The 70 entries, including Cycle 0 (C0), C1, C2 and some C3 populations resulting from all methods of
Table 1: Fall dormancy classifications of alfalfa cultivars (C0) for germination time and seedling vigor at low temperatures and mean of three traits measured at two locations.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>FD†</th>
<th>Ames 8d emergence</th>
<th>Nash. 8d emergence</th>
<th>Ames 27 d height</th>
<th>Nash. 27 d height</th>
<th>Ames Total yield</th>
<th>Nash Total yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>5454</td>
<td>4</td>
<td>15.5</td>
<td>21.7</td>
<td>24.6</td>
<td>41.5</td>
<td>145.9</td>
<td>329.1</td>
</tr>
<tr>
<td>Alfagraze</td>
<td>2</td>
<td>7.1</td>
<td>5.7</td>
<td>23.2</td>
<td>27.0</td>
<td>164.5</td>
<td>213.0</td>
</tr>
<tr>
<td>Amerigraze401+Z</td>
<td>4</td>
<td>13.5</td>
<td>18.3</td>
<td>19.0</td>
<td>40.1</td>
<td>175.8</td>
<td>352.4</td>
</tr>
<tr>
<td>Innovator+Z</td>
<td>3</td>
<td>8.2</td>
<td>19.3</td>
<td>23.4</td>
<td>34.4</td>
<td>182.9</td>
<td>325.6</td>
</tr>
<tr>
<td>Magnum IV</td>
<td>4</td>
<td>16.3</td>
<td>15.7</td>
<td>28.9</td>
<td>41.4</td>
<td>174.6</td>
<td>372.3</td>
</tr>
<tr>
<td>WL252HQ</td>
<td>2</td>
<td>15.9</td>
<td>17.3</td>
<td>23.0</td>
<td>34.8</td>
<td>197.3</td>
<td>334.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>12.8</td>
<td>16.3</td>
<td>23.7</td>
<td>36.5</td>
<td>173.5</td>
<td>321.2</td>
</tr>
<tr>
<td>LSD(5%)</td>
<td></td>
<td>7.4</td>
<td>6.8</td>
<td>4.3</td>
<td>8.7</td>
<td>46.2</td>
<td>72.0</td>
</tr>
</tbody>
</table>

† FD = Fall Dormancy; 2,3 = dormant; 4 = semi-dormant.
selection and 5 check cultivars, were planted in a rectangular triple lattice design. An experimental unit consisted of 50 seeds of each population planted 1 cm deep in rows 1 m long and 75 cm apart. Seedling emergence was recorded 8 d and 27 d after planting. At 27 d after planting, SH was recorded and plots were thinned to 10 uniformly spaced plants. Maturity at first harvest was estimated as the mean developmental stage of the stand (Nelson and Moser, 1995). The average height of the tallest upright stem, dry matter yield, and average regrowth height 7 d after harvest were recorded on all plots for three harvests at each location.

For the analysis of variance locations and entries were considered fixed effects. The response to selection was calculated as the difference between the C2 and CO or Control-C2 populations. Statistical analyses were conducted using the MIXED, CORR, and GLM procedures of the SAS statistical software package (Littell et al., 1996; SAS Institute, Inc., 1996). Mean separations were determined using Fisher’s protected LSD (P<0.05) (Steel and Torrie, 1980).

**Results and Discussion**

Averaged across populations, seedling height 27 d after planting, first harvest plant height, first harvest yield, and total dry matter yield were significantly greater at Nashua than at Ames (Table 2). Insect damage prevented an accurate estimate of final emergence at Nashua. The location by population interaction was significant for all traits, except maturity, plant height at first harvest, and total dry matter yield.

Significant location by population interaction in SH was due to differences in both population rank and the magnitude of separation between populations. CO populations differed at both locations for emergence 8 d after planting, 27 d SH, and total dry matter yield. ‘Alfagraze’, ‘Amerigraze 401+Z’ and Innovator +Z had the lowest 8 d emergence at Ames,
Table 2. Cycle 0 (C0) means and C1 and C2 deviations from C0 for traits measured at two locations for four selection methods.

<table>
<thead>
<tr>
<th>Method and Cycle</th>
<th>100-Seed Mass</th>
<th>8 d Emergence</th>
<th>Seedling Height</th>
<th>1st Harvest Height</th>
<th>1st Harvest Yield</th>
<th>Total Yield</th>
<th>Fall Regrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
</tr>
<tr>
<td>C0 Mean</td>
<td>2.32</td>
<td>12.7</td>
<td>16.3</td>
<td>23.7</td>
<td>36.3</td>
<td>45.4</td>
<td>15.4</td>
</tr>
<tr>
<td>EG-C1 †</td>
<td>0.22</td>
<td>5.8</td>
<td>-2.4</td>
<td>3.4</td>
<td>5.0</td>
<td>0.6</td>
<td>-1.0</td>
</tr>
<tr>
<td>EG-C2</td>
<td>0.06</td>
<td>5.2</td>
<td>-2.2</td>
<td>2.6</td>
<td>2.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>HSV-C1</td>
<td>0.40</td>
<td>10.0</td>
<td>-1.4</td>
<td>6.0</td>
<td>6.8</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>HSV-C2</td>
<td>0.16</td>
<td>7.6</td>
<td>0.5</td>
<td>5.4</td>
<td>6.7</td>
<td>0.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>EG+HSV-C1</td>
<td>0.22</td>
<td>7.0</td>
<td>-1.8</td>
<td>3.6</td>
<td>1.9</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>EG+HSV-C2</td>
<td>0.08</td>
<td>7.7</td>
<td>-0.1</td>
<td>1.6</td>
<td>4.3</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>LG+LSV-C1</td>
<td>0.29</td>
<td>5.1</td>
<td>0.3</td>
<td>0.7</td>
<td>2.6</td>
<td>-1.7</td>
<td>-1.6</td>
</tr>
<tr>
<td>LG+LSV-C2</td>
<td>0.05</td>
<td>7.1</td>
<td>-2.0</td>
<td>1.2</td>
<td>-0.2</td>
<td>-0.7</td>
<td>-3.5</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>0.01</td>
<td>3.0</td>
<td>2.8</td>
<td>1.7</td>
<td>3.6</td>
<td>1.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Ames vs. Nashua ‡

* Significant at the 0.05 probability level.
† EG = Early Germination, HSV = High Seedling Vigor, LG = Late Germination, LSV = Low Seedling Vigor, C0 = Cycle 0, C1 = Cycle 1, C2 = Cycle 2.
‡ Contrast of locations averaged across all C0 populations.
while Alfagraze was lowest at Nashua (Table 1). 'Magnum IV' and 5454 had the tallest seedlings 27 d after planting at Ames, but at Nashua they were only taller than Alfagraze (Table 1). Alfagraze had the lowest yield at Nashua; 5454 was the lowest yielding cultivar at Ames, but only significantly lower than 'WL252HQ'.

EG Selection

On the average, the EG selected populations had improved emergence 8 d after planting at Ames but not at Nashua (Table 2). This was also true of the other three selection methods, indicating that the effect was not due to EG selection per se. The average 100-seed mass of C0 was significantly lower than that of most selected populations. Emergence at Ames was correlated with 100-seed mass ($r = 0.31$, $p<0.01$), but no correlation was observed at Nashua, where conditions were closer to the optimum for alfalfa establishment and growth (Fig. 1). At Nashua, improved 8 d emergence after EG selection was only observed in Alfagraze (C1 and C2) ($p<0.05$, data not shown). Final emergence, evaluated only at Ames, was not significantly different for selected and C0 populations, which indicated that selection in the laboratory for EG at low temperature was ineffective at influencing field emergence. Temperatures in the field at Ames may have been too warm to effectively observe any improvement, a problem encountered by McConnell and Gardner (1979) in their evaluation of early germination in maize.

After the first cycle of selection for EG, 27 d SH increased by 14.3% at Ames and 13.8% at Nashua (Table 2). C2 populations did not show continued improvement for SH at either location and were similar to C0 at Nashua. The individual cultivars responded similarly to EG selection. Seedling height was improved in the C1 at one or both locations for all cultivars except Magnum IV (Table 3). In the C2, SH of EG selected populations remained the same or trended downward from the C1 for all cultivars. Total yield at Nashua was reduced in
Figure 1: Average daily air temperatures and soil temperatures at a depth of 10 cm for Ames, IA; and Nashua, IA from 1 April, 1998 through 15 May, 1998.
the EG-C2 populations after two cycles of selection, but no other traits were affected by EG selection (Table 2).

**HSV Selection**

After two cycles of HSV selection, the average 27 d SH was increased 22.8% at Ames and 18.5% at Nashua (Table 2). At both locations, the second cycle of selection was ineffective at increasing SH beyond the C1 population. All cultivars, except Magnum IV, had a SH increase in at least one location. Alfagraze and Amerigraze 401+Z had the most consistent responses for SH (Table 3). The C1 population of Innovator +Z was significantly improved compared with the C0, but the C2 population was inferior to the C1. Only Amerigraze 401+Z had a positive increase in SH from C1 to C2, which was observed only at Nashua. On the average, emergence was improved with HSV selection (Table 2) as noted above. Selection for HSV did not significantly affect any of the other traits studied (Table 2).

**Bidirectional Selection**

On average, EG+HSV selection increased 27 d SH in both locations (Table 2). At Ames, the C1 populations of EG+HSV, but not the C2, were taller than the C0. At Nashua, C2 populations of EG+HSV, but not the C1, were taller than C0 (Table 2). The location by population interaction for SH was significant. Response to EG+HSV selection among the six cultivars was inconsistent, with significant increases in 27 d SH observed at only one location in Alfagraze, Amerigraze 401+Z, and WL252HQ, none of which had corresponding increases in laboratory SH (Table 3) (Kloos, 1999). The selection intensity used in the EG+HSV method may not have been great enough to produce a consistent change in field performance. Alternatively, improving the two traits simultaneously may be difficult.

Plant height at the first harvest for the C1 populations of LG+LSV decreased by 7.6% from C0 at Ames, but C2 was not different from C0 (Table 2). At Nashua, the plant height at
Table 3: Cycle 0 (C0) means and C1 and C2 deviations from C0 for seedling height 27 d after planting at two locations for four selection methods in six alfalfa cultivars, and unselected controls in two cultivars.

<table>
<thead>
<tr>
<th>Method/Cycle</th>
<th>5454</th>
<th>Alfagraze</th>
<th>Amerigraze 401+Z</th>
<th>Innovator +Z</th>
<th>Magnum IV</th>
<th>WI 252HO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
<td>Nashua</td>
</tr>
<tr>
<td>C0 Mean</td>
<td>24.6</td>
<td>41.5</td>
<td>23.2</td>
<td>27.0</td>
<td>19.0</td>
<td>40.1</td>
</tr>
<tr>
<td>EG-C1 ‡</td>
<td>4.5†</td>
<td>3.8</td>
<td>4.1</td>
<td>16.0</td>
<td>7.2</td>
<td>-4.2</td>
</tr>
<tr>
<td>EG-C2</td>
<td>3.9</td>
<td>5.4</td>
<td>1.1</td>
<td>10.8</td>
<td>7.6</td>
<td>-1.5</td>
</tr>
<tr>
<td>HSV-C1</td>
<td>6.2</td>
<td>-0.4</td>
<td>4.4</td>
<td>18.9</td>
<td>10.6</td>
<td>3.4</td>
</tr>
<tr>
<td>HSV-C2</td>
<td>5.6</td>
<td>6.4</td>
<td>4.2</td>
<td>12.6</td>
<td>12.8</td>
<td>11.7</td>
</tr>
<tr>
<td>EG+HSV-C1</td>
<td>2.7</td>
<td>-6.5</td>
<td>3.3</td>
<td>11.8</td>
<td>9.7</td>
<td>1.9</td>
</tr>
<tr>
<td>EG+HSV-C2</td>
<td>1.0</td>
<td>1.2</td>
<td>2.0</td>
<td>14.1</td>
<td>7.8</td>
<td>7.0</td>
</tr>
<tr>
<td>LG+LSV-C1</td>
<td>2.4</td>
<td>-2.2</td>
<td>-2.6</td>
<td>5.6</td>
<td>6.4</td>
<td>4.9</td>
</tr>
<tr>
<td>LG+LSV-C2</td>
<td>0.6</td>
<td>-4.6</td>
<td>0.2</td>
<td>1.3</td>
<td>7.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Control-C1</td>
<td>0.9</td>
<td>1.3</td>
<td></td>
<td></td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Control-C2</td>
<td>2.6</td>
<td>2.1</td>
<td></td>
<td></td>
<td>2.3</td>
<td>3.9</td>
</tr>
</tbody>
</table>

† Ames LSD (0.05) = 4.2, LSD (0.10) = 3.5; Nashua LSD (0.05) = 8.7, LSD (0.10) = 7.3.

‡ EG = Early Germination, HSV = High Seedling Vigor, LG = Late Germination, LSV = Low Seedling Vigor, C0 = Cycle 0, C1 = Cycle 1, C2 = Cycle 2.
first harvest of the LG+LSV C2 populations, but not C1, was shorter than in the C0 (Table 2).

Correlations among traits

Regrowth of alfalfa following the final cutting of the season was used to evaluate alfalfa populations for fall dormancy. None of the four methods of laboratory selection changed fall regrowth (Table 2). Fall regrowth correlated more strongly with 27 d SH at Ames than at Nashua (Table 4). Seedling height 27 d after planting was correlated at both locations with first harvest plant height and dry matter yield, and total dry matter yield (Table 4). The correlations suggest that increased seedling vigor can improve plant growth at later stages of maturity.

Correlations with laboratory results

Changes in GT and SH under laboratory conditions were reported for these populations (Klos, 1999). Averaged over the six cultivars, EG selection did not affect SH in the laboratory, but increased it in the field (Table 5). No correlation between laboratory GT and field SH was observed (p<0.05), in part due to the increase in both traits with HSV selection (Table 5). In 5454 and Innovator +Z, EG selection decreased GT and increased SH in the field, but field SH increases were also observed in cultivars that were not changed for GT (Amerigraze 401+Z, Innovator +Z, and WL252HQ, data not shown). Selection for EG in the laboratory was effective at increasing SH in some populations, but the procedure is of dubious utility given the inconsistency of the response over cycles and in different germplasms. On average, the pattern of SH increase with cycle of HSV selection was similar in laboratory and field environments (Table 5). HSV selection in Amerigraze 401+Z and Innovator +Z was effective at increasing SH in both the laboratory and the field. In Innovator +Z, an increase in laboratory SH from HSV-C1 to C2 (data not shown) was not observed in the field where C1, but not C2, was taller than CO (Table 3). In the other cultivars, except
Table 4: Correlations among traits measured at two locations.

<table>
<thead>
<tr>
<th></th>
<th>Seedling Ht.</th>
<th>1st Harvest Height</th>
<th>1st Harvest Yield</th>
<th>Total Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ames</td>
<td>Nashua</td>
<td>Ames</td>
<td>Nashua</td>
</tr>
<tr>
<td>1st Harvest Height</td>
<td>0.35**</td>
<td>0.21**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Harvest Yield</td>
<td>0.38**</td>
<td>0.39**</td>
<td>0.42**</td>
<td>0.22**</td>
</tr>
<tr>
<td>Total Yield</td>
<td>0.29**</td>
<td>0.25**</td>
<td>0.21**</td>
<td>0.01 ns</td>
</tr>
<tr>
<td>Fall Regrowth</td>
<td>0.42**</td>
<td>0.26**</td>
<td>0.19**</td>
<td>0.12 ns</td>
</tr>
</tbody>
</table>

* ** Significant at the 0.05, and 0.01 probability levels, respectively.
Table 5: Percentage response of traits measured at two locations to selection averaged over six cultivars and four selection methods.

<table>
<thead>
<tr>
<th>Method/Cycle</th>
<th>Laboratory Germination % change from CO</th>
<th>Laboratory Seedling Height</th>
<th>Field Seedling Height</th>
<th>1st Harvest Height</th>
<th>Total Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG-C1†</td>
<td>-26.8</td>
<td>0.8</td>
<td>14.4</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>EG-C2</td>
<td>-28.9</td>
<td>-3.9</td>
<td>9.0</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>HSV-C1</td>
<td>8.2</td>
<td>11.8</td>
<td>22.1</td>
<td>5.3</td>
<td>11.9</td>
</tr>
<tr>
<td>HSV-C2</td>
<td>28.9</td>
<td>14.9</td>
<td>20.7</td>
<td>-0.3</td>
<td>4.5</td>
</tr>
<tr>
<td>EG+HSV-C1</td>
<td>-17.5</td>
<td>2.7</td>
<td>10.4</td>
<td>2.1</td>
<td>7.4</td>
</tr>
<tr>
<td>EG+HSV-C2</td>
<td>-28.9</td>
<td>1.2</td>
<td>10.4</td>
<td>0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>LG+LSV-C1</td>
<td>66.0</td>
<td>-8.5</td>
<td>5.0</td>
<td>-4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>LG+LSV-C2</td>
<td>161.9</td>
<td>-12.0</td>
<td>2.0</td>
<td>-5.9</td>
<td>0.3</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>14.4</td>
<td>3.7</td>
<td>7.0</td>
<td>4.7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

† EG = Early Germination, HSV = High Seedling Vigor, LG = Late Germination, LSV = Low Seedling Vigor, C0 = Cycle 0, C1 = Cycle 1, C2 = Cycle 2.
Magnum IV, HSV selection only increased SH in the field (data not shown). No change in field SH was observed with selection for LG+LSV (Table 2), despite significant decreases in laboratory SH (Table 5). Based on these results, SH evaluation in the laboratory may not predict field performance for all populations, in spite of positive correlations of $r = 0.50$ at Ames and $r = 0.42$ at Nashua ($p < 0.01$).

Prospects for use in breeding programs

Although the first cycle of selection in the various methods was generally successful in altering the desired traits, the second cycle often contributed no further gain and in some cases reversed the gain observed in cycle 1. The seed used to initiate the selection program was grown in commercial seed production fields. The C2 parents were selected from greenhouse-produced seed, which was larger in mass than C0 and C2 seed, and may have had different seed quality parameters. This may have hindered our ability to accurately make selections for C2.

The location by population interaction was significant for SH due both to rank and magnitude differences among locations. Future evaluation of response to selection for this trait should be performed at multiple locations. Selection by any of these methods had no affect on the first harvest yield, total yield, maturity, or fall dormancy of the cultivars. Improvement in field SH with the various methods of selection was highly variable among cultivars, but was clearly observed in some.

References


Klos, K.L.E. 1999. Variation in alfalfa (Medicago sativa L.) for germination and seedling vigor at suboptimal temperatures; and laboratory and field responses to selection within six alfalfa populations. Ph.D. diss. Iowa State Univ., Ames, IA.


CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

Variation in germination time at suboptimal temperatures was observed among alfalfa cultivars, but was significant in the presence of seed lots only at 5°C. Evaluation of GT in alfalfa should be conducted at 5°C when utilizing the methods of this study. Because GT at 5°C was correlated with field emergence 8 d after planting only at Nashua, measuring it in the laboratory may be of limited use in predicting seedling emergence in the field. Initial radicle growth in the laboratory was correlated with field emergence 8 d after planting at Ames. Therefore, laboratory evaluation of IRGR may be useful as an indicator of alfalfa seedling emergence in the field. However, evaluation of IRGR in the laboratory by the method used in this study is time consuming and technically difficult and may not be suitable for large-scale evaluation of legume genotypes.

Recurrent mass selection in six alfalfa cultivars using selection for early germination at 5°C was successful at decreasing the germination time at 5°C by 28.9% on average, with a realized heritability of 0.49. We conclude that response to selection for germination time at suboptimal temperatures in the laboratory can be expected, but not to the same extent in all populations. Some populations may have had insufficient genetic variation for early germination at suboptimal temperatures and consequently reached a physiological limit to their GT at 5°C. Selection for early germination at low temperatures under laboratory conditions was not effective at influencing field emergence 8 d after planting of alfalfa populations.

Recurrent selection for vigorous seedling growth at 10°C was effective at increasing SH under growth chamber conditions in two populations. A trend towards increased seedling height with selection for seedling vigor was observed in all alfalfa populations. Realized heritability of seedling vigor under growth chamber conditions ranged from 0.013 to 0.13, but
these low estimates may have resulted from environmental variation during evaluation. A consistent environment at germination and the use of seed with uniform mass and quality could improve the heritability of SH under the HSV selection method. Increases in GT were observed with HSV selection in all populations, indicating an association between seedling vigor and GT under laboratory conditions supported by observed genetic correlations as high as $r^2 = 0.60$. GT would likely continue to increase with HSV selection. HSV selection was effective at increasing seedling height in the field at both locations, as was selection for HSV combined with selection for GT. Significant location by population interaction was observed for SH due to differences in both population rank and the magnitude of separation between populations. Fall regrowth was unaffected by any of the four methods of selection.

Although the first cycle of selection in the various methods was generally successful at altering the desired traits, the second cycle often contributed no further gain. The seed used to initiate the selection program was grown in commercial seed production field while parent of subsequent cycles were grown from greenhouse produced seed. The greenhouse produced C1 seed was larger in mass than the C0 or C2 seed, and may have had different seed quality parameters. This may have hindered our ability to accurately make selections for C2.

**Recommendations for future Research**

Selection for vigorous seedling growth may be useful in improving alfalfa breeding populations for seedling growth at suboptimal temperatures in the field. An experimental protocol should be developed for incorporation of seedling growth measurements into existing selection schemes. Use of an alternating temperature regime in future methods would more closely mirror early spring conditions in the field. Genetic components of variation in seedling growth at suboptimal temperatures could be estimated experimentally in populations of interest.
by a diallel cross between individuals. Progeny performance may be used to estimate additive and nonadditive components of genetic variance (Wricke and Weber, 1986). These would be useful in predicting genetic gain from selection for seedling vigor by different methods of recurrent selection.

**References**
