Improvement of chickpea stand establishment in cool soils
by Shirley Ann Bollinger

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University
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Abstract:
Chickpea germinates poorly in cool soil and seedling establishment is highly variable. The objective of studies undertaken was to find out why chickpea performs poorly in pool soil and to evaluate storage conditions, seed moisture content, seed priming, location grown and seed vigor as ways to improve chickpea stand establishment.

Field and laboratory trials indicated cool storage increases germination and lessens hard seed expression when planted in cool soil. Further experiments controlling storage temperature and relative humidity indicated elevated seed moisture content also reduced hard seed. Seed priming with polyethylene glycol increased percentage germination, reduced hard seed and increased the speed of germination. Chickpea grown at three locations, Pullman and Lind, Washington and Manhattan, Montana, had significantly different amounts of hard seed, with the Manhattan location having the least. A greenhouse study and several seed vigor tests, including; cool germination, seedling growth rate, electrical conductivity, and accelerating aging were conducted on eight chickpea seedlots to determine which tests would give the most accurate estimate of chickpea seed vigor. Accelerating aging proved to be the most reliable test in predicting seed vigor.

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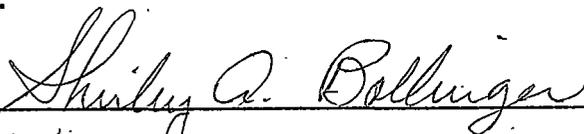Approved for the College of Graduate Studies

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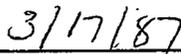## STATEMENT OF PERMISSION TO USE

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ABSTRACT

Chickpea germinates poorly in cool soil and seedling establishment is highly variable. The objective of studies undertaken was to find out why chickpea performs poorly in cool soil and to evaluate storage conditions, seed moisture content, seed priming, location grown and seed vigor as ways to improve chickpea stand establishment.

Field and laboratory trials indicated cool storage increases germination and lessens hard seed expression when planted in cool soil. Further experiments controlling storage temperature and relative humidity indicated elevated seed moisture content also reduced hard seed. Seed priming with polyethylene glycol increased percentage germination, reduced hard seed and increased the speed of germination. Chickpea grown at three locations, Pullman and Lind, Washington and Manhattan, Montana, had significantly different amounts of hard seed, with the Manhattan location having the least. A greenhouse study and several seed vigor tests, including; cool germination, seedling growth rate, electrical conductivity, and accelerating aging were conducted on eight chickpea seedlots to determine which tests would give the most accurate estimate of chickpea seed vigor. Accelerating aging proved to be the most reliable test in predicting seed vigor.

CHAPTER I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool-season crop with alternative crop potential for Montana. Variable market conditions, increased disease problems in specific United States production areas, increased market value, and crop rotation potential with small grains has increased interest in chickpea production in the northwestern United States. Montana has the cool, dry climate required for growing chickpea. However, because of the short growing season, seeds must be planted in cold soils, causing establishment problems. Preliminary studies indicate that cold germination temperatures increase hard seed. High vigor seed reduces the probability of stand failure. Higher vigor seed enhances stand establishment under unfavorable conditions.

High vigor chickpea seeds, with proper treatment, will germinate better in cool soils. Also, better yields are obtained with early planting.

The main objectives of this study were to find a treatment to reduce hard seed under cool conditions and to determine which seed vigor tests would be most accurate in determining high vigor seed.

CHAPTER II

LITERATURE REVIEW

Crop

Chickpea (*Cicer arietinum* L.), also called garbanzo bean, is a cool-season legume which originated in Asia Minor. The earliest record of its use is 5450 B.C. in Turkey. Its cultivation spread eastward to India and westward to the Mediterranean (Simmonds, 1976).

Chickpea, and other grain legumes, provide the major protein source for people in Asia, Africa and Latin America. Chickpea is a good dietary complement to cereals because of the high lysine content and 20-22% crude protein which is 80% digestable. Proper chickpea and cereal mixtures can provide all the essential amino acids for humans. The carbohydrate (50-60%) and oil (5%) content of chickpea, contribute to energy requirements (Simmonds, 1976). Chickpea utilizes atmospheric nitrogen and has maintained soil fertility in some areas of India for centuries (Van Der Maesen, 1972). As compared to other grain legumes, chickpea is second in area planted (exceeding ten million hectares) and third in quantity produced (6.3 million metric tonnes) in the world.

There are two types of chickpea. The large-seeded kabuli type is grown primarily as a summer crop in the

Mediterranean. The small-seeded desi type is grown during the winter in Asia. Eighty-five percent of the world's production of chickpea is the desi type which is grown in India, where they are processed into dhal (split pea without seed coat) for human consumption. Seeds are eaten fresh, parched, boiled, fried and sprouted for salads. They are also dried and ground into flour for snacks and sweetmeats. Young plants and green pods are eaten like spinach. A favorite hors d'oeuvres in the Mediterranean area is hummus, which is mashed kabuli chickpea mixed with oils and spices (Duke, 1981). Other uses include production of starch for textile sizing, adhesive for plywood and an indigo like dye from the leaves. Seed, husks, leaves and stems are used to feed horses and cattle. Granular secretions of malic and oxalic acids from leaves, stems and pods are used both medicinally and as a vinegar. A cooked chickpea and milk mixture can be fed to infants to control diarrhea (Duke, 1981).

Chickpea is also grown in South America, Mexico and the United States. The United States produces approximately 3500 metric tonnes. California produces approximately 3240 hectares and the Palouse region of eastern Washington and western Idaho produce approximately 2000 hectares of chickpeas (Welty, et al, 1982). Disease problems in other areas, market value ($25-50/cwt), nitrogen fixing ability, and rotation benefits with small grains make chickpea a

promising crop for some areas of Montana. Montana's cool, dry summers should provide the right environment for production of chickpea (Welty, et al, 1982).

Botanical Description

Chickpea (*Cicer arietinum* L.) belongs to the Papilionacaea subfamily of Fabaceae. Chickpea is a self-pollinated, branched, annual that reachs a height of 60 cm with a tap root ranging from 0.6 to 1.8 meters long. The leaves are odd-pinnately compound with three to eight leaflet pairs. The flowers (0.6 to 1.25 cm) are borne on inflorences that grow from the stem axes. The kabuli type has large leaflets and white flowers, while the desi type has small leaflets and flowers that are either white, pink, purplish or blue. The seeds are borne in pubescent pods (2 to 5 cm long) and have an anterior "beak and groove" about two-thirds of the distance around the seed (Muehlbauer, et al, 1982).

The chickpea seed coat is identified by two microscopic characteristics, the external palisade and "hour glass" cells (Corner, 1951). The seed coat has a blistered, undulating surface. Seed coats of desi type chickpea are three times as thick as the kabuli type. Desi seeds have several layers of palisade cells, while the kabuli has only one. (Singh, et al., 1984). The hilum is located in a sunken pouch below the seed surface with a second layer of palisade cells on top of the first layer. A vascular bundle

(tracheid bar) is located at the bottom of the pouch extending into the mesophyll. (Patel, et al, 1976). Under the seed coat surface is a layer of palisade cells, also called macrosclereids, which are typically very long with wavy, thickened cell walls (Chowdhury, 1970). There is a bulbous lumen with a curved outer end at the base of the cells. A 'mucilage stratum', found only in chickpea, is located below the palisade cells (Chowdhury, 1970). Osteosclereids, also called "hour glass" cells, are located between the mesophyll and the palisade cells. The osteosclereid ends are broad and come in contact with the adjoining osteosclereids only at the broad ends which providesa very large intersclereid space. The lateral walls adjacent to the intersclereid space are uniformally thick (Patel, 1976).

Growing Conditions

Optimum growing conditions for chickpea are: full sun on a south or east facing slope; a cool dry climate and a well drained soil with a pH of 5.5 to 8.6. They tolerate poor soils, but do best on heavy clay. Daily temperature fluctuations of 10-30 C with cold nights (18-21 C) and heavy dews are best for growth. Some cultivars can withstand -3 C in the early growth stage or under snow cover. Optimum annual rainfall is approximately 63-76 cm. *Rhizobium* inoculation on first-planted soil will increase chickpea yields 10-60% (Duke, 1981).

Welty, et al. (1982) have studied varietal responses, plant population for maximum yield, planting date, fertility, weed control and irrigation for production in Montana. They recommend a seeding rate of one to two seeds per linear foot in 15 cm rows (170-225 kg/ha) with an optimum planting date of May 7. Nitrogen fertilizer did not increase yields when seeds were inoculated with *Rhizobium*. Herbicides such as profuluralin, ethalfluralin, trifluralin and dinoseb provided excellent control of broadleaf weeds and metolachlor controlled green foxtail. Some irrigation may be necessary for maximum yields in Montana.

Hard Seed

Montana has a good late season for growing chickpea. However, because of the short growing season, seeds must be planted in cold soils which may cause establishment problems. Germination at 5 C caused an increase in hardseededness and reduced germination rates when seed was stored in warm conditions (23 C). Further experiments indicated that cool storage reduced hard seed content (Frisbee, et al., 1987). These data suggest that there is a relationship between seed coat permeability and storage temperature and/or relative humidity.

Hard seeds have been defined as seeds unable to imbibe water (Rolston, 1978). These seeds are common in many species of Fabaceae (legumes). The presence of a palisade

layer of macrosclerid cells is typical in water impermeable seed. The hardness and impermeability of the seed coat is caused by the contraction of the walls of the palisade cells as the seed ripens (Corner, 1951).

Another phenomenon of impermeability in the palisade cells is the light line (Corner, 1951). The cell wall in this region appears to be compact. Phenolic compounds are also thought to contribute to water impermeability (Esau, 1977). Riggio-Bevilacqua, et al. (1985) indicated that the cuticle is the impermeable layer in legume seeds. The hilum, which acts as a valve to prevent the entry of moisture into the seed, while permitting loss of moisture, may also contribute to hardseededness. This phenomenon is reported to cause a high degree of desiccation (Esau, 1977). The impermeability of the seed coat of *Cercis siliquastrum* (Judas tree) is affected by a combination of water failing to pass through the hilum and an inner non-cellular lipidic layer at the edge of the hypodermis (Riggio-Bevilacqua, 1985).

Hardseededness could be the result of low seed moisture. Soybean (*Glysine max* L.) studies conducted by Harrington (1949) showed that 15 days storage at 10 % relative humidity, resulted in a seed moisture of 7 % with 76.5 % hard seed. Fifteen days storage at 66.5 % relative humidity, resulted in a seed moisture of 11.3 % and 5.2 % hard seed. Seed stored for 60 days at 75 % relative humidity, had a resultant seed moisture of 12.5 % and no hard seed.

In another study done with white beans (*Phaseolus vulgaris* L.) by Lebedeff (1947), seeds were dried to ten different moisture contents ranging from 14.11 % to 5.39 %. There were no hard seeds in the control which contained 15.14 % moisture, but seed with 14.11 % moisture had 1 % hard seed. The percentage of hard seeds increased with each reduction in seed moisture content, when moisture content was reduced to 5.59 % almost 90 % of the seeds were hard. Permeability of West Austrailian blue lupine (*Lupinus digitatus* L.) directly related to the amount of seed moisture, with storage temperature not affecting hardseededness (Gladstones, 1958).

Environmental conditions during the growing season have been shown to affect the proportion of hard seeds produced by some annual legumes. According to Bewley and Black (1985), photoperiods can affect seed coat thickness and color. Phenolic substances produced during air drying are oxidized to dark-colored compounds that may contribute to hardseededness. Drying of the seed in some leguminous species is controlled by the hilum, which in turn is regulated by environmental conditions. When the relative humidity is low, the cells around the hilar fissure shrink, thus opening the tissue and allowing more moisture to escape. A cultivar of alfalfa (*Medicago sativa* L.) grown in different areas of the United States (USA) exhibited wide variations in the amount of hard seed (Gunn, 1972). Northwestern

USA grown alfalfa usually has 40 to 50 % hard seed, while alfalfa grown in southwestern USA seldom has more than 20 to 30 % hard seed. The effects of temperature during and immediately following maturation is believed to play a major role in determining the amount of hard seed. When temperatures are low, hard seed content is highest. Argel and Humphreys (1983) reported that temperature during seed formation of carribean stylo (*Stylosanthes hamata* (L.) Taub. cv. Verano) is an important factor modifying the development of hardseededness. More soft seed may be produced in cool areas, when flowering and maturation occurred during cold periods and in seasons which are abnormally cool. It is possible that temperature acts as a modifying factor on the development of hardseededness exerted by atmospheric humidity (Argel and Humphreys, 1983). Hardseededness after 120 days storage at 77 and 32 % relative humidty was positively correlated to temperatures of 21, 24 and 27 C. Correlation coefficients between seed pod moisture content and percentage hard seed were greater as temperature decreased. Argels and Humphreys, 1983) gave no evidence that variations in soil moisture supply or levels of illuminance influenced hardseededness.

The length of the growing period in the spring months may be a critical factor in the development of hardseededness in subteranean clover (*Trifolium subterraneum* L.) Environments with relatively long spring growing periods

caused a higher proportion of hard seed and plants maturing under soil moisture stress produced fewer hard seed. High soil moisture availability during seedfill of soybeans reduces hard seed by disrupting seed-coat integrity (Hill, et al, 1986).

Argels and Humphreys, 1983, also identified some morphological characteristics of the seed which are changed by temperature which influence seed moisture content and the development of hardseededness. Carribean stylo seed formed under high temperature had more lignin and hemicellulose, less cellulose, and shorter palisade cells than seed formed under cooler temperature. The testa of hard seed had a more regular, even reticulate surface than that of soft seed.

Seed Priming

The partial imbibition of seeds in polyethylene glycol (PEG) is called osmoconditioning or seed priming. This technique has induced early and uniform germination of vegetables, soybean, cereals and forage grasses (Bodsworth and Bewley, 1981). PEG in aqueous solutions creates a negative water potential so seed will imbibe enough moisture to start the physiological changes necessary for germination, but will not actually germinate. Physiological repair processes such as restoration of membranes and mobilization of storage reserves are stimulated by priming (Knypl and Khan, 1981). Khan, et al., (1978) studied lettuce seed osmoconditioning

and found that it caused the activation and/or synthesis of enzymes used in the breakdown of proteins, lipids, and phosphate esters used in glycolysis.

There are several factors to consider when priming seed. Each species and possibly each seedlot within that species requires a different concentration of osmoticum, priming duration and priming temperature. Heydecker, et al.,(1973), found that with onion seed, a temperature of 10 C, a priming duration of 23 days and a potential of -10 bars gave the best results. Studies by Knypl and Khan (1981) showed 15 C, 4 to 8 days and -8.6 to -11.9 bars to be the optimum priming treatments for soybean. Bodsworth and Bewley (1981) reported optimum priming treatments of 10 C at -10 bars for six days; for maize, 10 C at -10 bars for 2 days; for wheat and barley, 10 C at -10 bars for 1 day; for sorghum, and 10 C at -5 bars for 6 days; for soybean. Abernathy (1986) reported variability among seedlots of Cicer milkvetch (*Astragalus cicer* L.)indicating the optimum osmoconditioning may need to be determined for each seed lot.

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Seed Vigor Tests
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Differences in "germination energy" or seed vigor was first observed by Nobbe (1876). The definition of seed vigor adopted by the Association of Official Seed Analysts (AOSA , 1983) is: "Seed vigor comprises those properties which determine the potential for rapid, uniform emergence

and development of normal seedlings under a wide range of field conditions".

The AOSA (1983) classifies seed vigor tests three ways: (1) seedling growth and evaluation tests, (2) stress tests, and (3) biochemical tests. Seedling growth and evaluation tests include seedling vigor classification and seedling growth rate tests. Accelerating aging test, cold test and cool germination tests are stress tests. Biochemical tests are tetrazolium and conductivity tests. There is no one test that is accepted as a standard for seed vigor. Each seed laboratory standardizes its own test procedures, but standardization among laboratories has been difficult.

The seedling vigor classification test divides normal seedling into strong and weak categories. The test was proposed for vigor assessment of soybean, cotton, peanut and green bean (Woodstock 1976). This test provides a means of separating seedlings free of deficiencies from those with deficiencies symptomatic of either low vigor or reduced quality. The seedling growth rate test was developed to obtain reproducibility in strong and weak classifications. Biochemical measurements and vegetative growth in the field have been correlated with seedling growth rate (AOSA, 1983).

The accelerated aging test stresses seed prior to germination. The basis for this test is that high vigor seed tolerates high temperature and humidity during storage better than low vigor seed. Delouche (1965) first developed

this test for predicting the relative storability of seeds, but now it is also used as a vigor test (AOSA, 1983).

Cool germination is a stress test to evaluate field condition effects. High vigor seed will perform better under adverse conditions than low vigor seeds (AOSA, 1983).

The tetrazolium test is essentially a measurement of dehydrogenase enzyme activity. Lakon (1942) first developed this technique for seed viability testing and later the staining patterns of the tissues were used to assess vigor (Moore and Goodsell 1965, Moore 1972). Living cells of a seed soaked in tetrazolium solution will stain red while dead cells remain colorless. Evaluation of the amount and placement of red color within the seed and turgidity of tissues are used to distinguish high vigor seed from low vigor seed (AOSA, 1983).

Measurement of electrolytes leaking from seed is the basis for the conductivity test. Dry seed loses membrane integrity, but, upon imbibition membranes are restored. However, before membranes are restored there is leakage of electrolytes. The vigorous seed more rapidly restores membrane integrity and exhibits less leakage of electrolytes. Conductivity tests have shown positive correlation with field emergence for field corn and soybean (Tao 1980).

CHAPTER III

STORAGE CONDITIONS

Introduction

Hardseededness in some legumes has long been recognized as being related to storage conditions with high temperature and low humidity favoring its development. Studies conducted by Frisbee, et al. (1987), indicated that hardseededness was significantly reduced with cool storage, when germinated at 5 C.

Chickpea seed exhibits very little hardseededness when germinated at optimum temperature (27 C), but when germinated at 5 C hardseededness increases. The 5 C germination temperature is similar to the field conditions of early planting of chickpea in Montana.

Studies were undertaken in 1985 and 1986 to determine if cool storage of chickpea improved field emergence and reduced hardseededness under cool soil conditions. The relationship between cool storage, seed moisture content, and hardseededness was evaluated.

Materials and Methods

A field study was conducted in 1985 using two desi seedlots of 'Garnet" chickpea, harvested in 1981 and 1983 at Pullman, Washington. Two field plantings were made, April 23

and May 21, to evaluate the effects of soil temperature on emergence. Soil temperature April 23 was 4 C, and 20 C on May 21.

Six storage conditions were used for the April planting and nine storage conditions were used for the May planting. Storage conditions used for the April planting were: 1) 1981 seed stored 36 months warm (room temperature), 2) 1981 seed stored warm for 35 months, then 1 month in cool (10 C, 50 % relative humidity) storage, 3) 1981 seed stored cool for 6 months, then warm for 1 month, 4) 1981 seed stored for 7 months cool, 5) 1983 seed stored for 17 months cool, then 1 month warm, and 6) 1983 seed stored for 18 months cool (Table 1).

The May planting had the following storage conditions: 1) 1981 seed stored for 36 months warm. 2) 1981 seed stored for 35 months warm, then 1 month cool, 3) 1981 seed stored for 34 months warm, then 2 months cool, 4) 1981 seed stored for 6 months cool, then 2 months warm, 5) 1981 seed stored for 7 months cool, then 1 month warm, 6) 1981 seed stored for 8 months cool. 7) 1983 seed stored for 16 months cool, then 2 months warm. 8) 1983 seed stored for 17 months cool, then 1 month warm. 9) 1983 seed stored for 18 months cool (Table 1).

Three weeks before planting, seed was treated with methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benlate) at the rate of 3 gms ai per kilogram of seed to control

fungi (*Ascochyta*).   One day before planting, approximately one gram of chickpea *Rhizobium* spp. innoculum was added to each packet of seed.

The field study was established near Manhattan, Montana on a Manhattan very fine, sandy-loam soil (coarse-loamy, mixed, Typic Calciborolls).   This site received 14 cm of precipitation during April through August.   A randomized block design with four replications was used with four rows, six meters long, spaced 0.3 meters apart.   Three seeds were planted per linear foot at a depth of five cm.

Two center rows, one-half meter long, were used for emergence counts.   Counts were taken daily for eight days beginning 12 days after planting, for the April planting. Daily emergence counts for the May planting were initiated seven days after planting and continued for 10 days, with a final count on the 15th day.   One-half meter of the center two rows of each plot was hand harvested.   The April planting was harvested on August 1 and the May planting was harvested on September 5.   Harvested samples were cleaned using a belt thrasher and an aspirator. Samples were weighed to evaluate yield.   Five hundred seeds were counted and weighed to determine seed size.

Germination tests were conducted to evaluate hard seed content.   Four replications of fifty seeds each were placed in plastic germination boxes (4x14x13 cm) with two H_2O

saturated blotters. Boxes were placed in 5 and 27 C incubators. Germination counts were taken after one week for seeds in the 27 C germinator and after two weeks for those in the 5 C incubator.

Seed moisture content was measured by drying two replicates of 25 gm of seed at 100 C for 24 hours. Percentage moisture was determined on a wet-weight basis. Data were analyzed with the Plant and Soil Science Discovery computer system using "MSUSTAT" (Lund, 1983).

The 1986 field study was conducted similarily to the 1985 study with the following exceptions; seedlots were 1981 and 1985 'Garnet' chickpea from Pullman, Washington. The 1981 seedlot was stored for four years cool or for four years warm. The 1985 seedlot was stored for one year cool or warm. Field planting was made on May 1 at the Arthur H. Post field research laboratory near Bozeman, Montana. The soil at the field research laboratory is classified as an Amsterdam variant of silt loam (fine-silty, mixed family of Typic Haploborolls). Rainfall for the growing season was 25 cm. Daily emergence counts were taken 20 days after planting for 13 days, with a final count taken on the 19th day after emergence. Plots were infested with *Ascochyta* fungi in mid-season so seed harvest was not possible. Germination tests of the seed were conducted using six replications with 50 seeds per replication in a 5 C germinator. Germination

counts were made daily for three weeks and percentage germination and speed of germination were calculated.

Results and Discussion

The April, 1985 planting had significant differences in total emergence, but no differences in speed of emergence, yield and seed size. The best treatment was the 1981 seedlot, which was stored for seven months cool with a total seedling emergence of seven (Table 1). These results suggest that the 1981 seedlot is more responsive to warm or cool storage when planted under cool soil conditions.

The May, 1985 planting had significant differences in total emergence and seed size, with no differences in speed of emergence and yield. The highest total emergence resulted from the 1983 seedlot stored 18 months cool; 17 months cool, then 1 month warm; 16 months cool, then 2 months warm and the 1981 seedlot stored eight months cool. These seedlots had total emergences of 12, 11 and 9 respectively (Table 1). The 1981 seedlot stored for 36 months warm produced the smallest seeds (Table 1).

Four similar treatments from the early and late plantings were compared to determine the effects of soil temperature on emergence and yield. There were significant differences in total emergence for planting time and storage conditions (Table 2). The early planting had significantly

Table 1: Total emergence (TE), speed of emergence (EI), seed yield and seed size for early and late plantings of chickpea.

Early Planting (April 23)

Months Storage warm or cool	Seed-lot	TE (per meter row)	EI	Yield (Mg ha^{-1})	Seed Size (500 wt)
36 wm 0 cl	1981	3	.98	3.60	76.64
35 wm 1 cl	1981	4	1.23	3.91	77.09
6 cl 1 wm	1981	3	1.08	3.52	72.96
7 cl 0 wm	1981	7	2.25	4.13	69.85
17 cl 1 wm	1983	4	1.12	3.77	71.12
18 cl 0 wm	1983	4	1.25	3.91	71.65
LSD .05		2.5	NS	NS	NS

Late Planting (May 21)

Months Storage warm or cool	Seed-lot	TE (per meter row)	EI	Yield (Mg ha^{-1})	Seed Size (500 wt)
36 wm 0 cl	1981	8	2.79	2.80	73.20
35 wm 1 cl	1981	8	2.60	3.19	77.09
34 wm 2 cl	1981	6	2.06	2.94	77.37
6 cl 2 wm	1981	8	2.99	3.11	76.84
7 cl 1 wm	1981	8	2.30	3.07	76.90
8 cl 0 wm	1981	9	3.13	2.75	78.81
16 cl 2 wm	1983	11	2.81	3.32	79.53
17 cl 1 wm	1983	9	2.52	3.01	77.38
18 cl 0 wm	1983	12	4.29	3.11	78.56
LSD .05		3.3	NS	NS	2.96

fewer seedlings emerge than the late planting. Cool storage allowed more seedlings to emerge in the early planting than did warm storage. The storage conditions did not affect emergence at the late planting. The yield was significantly greater for the early planting even though more seedlings had emerged for the late planting (Table 2). Early plantings are necessary in Montana since chickpea needs a long growing season to produce maximum yield. Seed