

THE EFFECT OF SEED STORAGE CONDITIONS ON STAND  
ESTABLISHMENT, FORAGE AND SEED PRODUCTION IN  
CRIMSON CLOVER (Trifolium incarnatum L) AND  
PERENNIAL RYEGRASS (Lolium perenne L)

by

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INTRODUCTION

It is a well known fact that generally good seeds produce better stands and increased crop yields when proper cultural practices are followed. Good seeds are usually mature, have high germinability and produce vigorous seedling. High vigor could be conserved by optimum storage condition.

Storage of seed to preserve its quality has been a matter of great interest since the beginning of agricultural history. Preservation of seed viability between harvesting and seeding is a necessary step in agriculture. Furthermore, seeds are often transported long distances from place of production to place of use and it is frequently necessary to store the seeds from one season to the next, or longer. Some countries are partly or entirely dependent for their supplies of certain seeds upon production from other parts of the world. It is, therefore, desirable to retain viability during shipment so that the seeds reach the consuming countries in good condition.

A considerable amount of research has been conducted on optimum storage conditions for seeds of some

crops, but very little has been done with seeds of forage crops. The importance of forage seeds is increasing rapidly as grassland farming increases. Hence, the grassland seeds are vital to the economy of the nation and the world.

The generally accepted measurement for quality of seed is the germination test conducted in the laboratory. The influence of seed storage conditions on production of forage and seed has not been clearly defined. The purpose of this research was to observe how storage conditions influenced quality of seed as measured by stand establishment in the field, plant growth and development, and production of forage and seed. In addition, an attempt was made to correlate germination in the laboratory with emergence under field conditions and determine if a valid estimate of emergence in the field could be made from the results of germination tests in the laboratory.

## LITERATURE REVIEW

There are several environmental, cultural, and internal factors which affect field emergence, stand establishment, growth of forage, and seed production.

A. External factors

Among the environmental factors, soil and air temperature, moisture and light are most important.

1. Temperature: The effects of temperature on the growth and development of grasses have been extensively investigated by Michell (51, p. 22-24), Peterson et al. (61, p. 31-41) and Sullivan et al. (72, p. 708-717). Sprague (69, p. 287-294) studied seedling emergence of eight pasture species under several conditions of controlled environment. He found that the species varied widely in their ability to emerge at different temperatures. At the highest temperature level employed, 100° F. for four hours daily, he noted reduced emergence compared to that at lower temperatures in all species. Laude (45, p. 112), in his studies of six species of perennial grasses, including Lolium perenne, found that this grass was the most

sensitive to high soil temperature. A reduction in percentage of emergence was associated with a delay in emergence of seedlings surviving the heat treatment. Gist and Mott (26, p. 36) reported that increasing temperatures from 60° F. to 90° F. reduced seedling growth of alfalfa, timothy and red clover. Chippendale (14, p. 57-58) noted that timothy germinated poorly at low (5-10° C.) temperatures while orchardgrass did well at this temperature. Timothy germinated quickly, but seedlings were small and grew slowly. Black (6, p. 209) found that hypocotyl extension per unit of cotyledonary reserves increased from 7° C. to 14° C. to 21° C., which was optimum. Bouyocos (10, p. 9-120), Hide (34, p. 31-35), and Smith (68, p. 110) studied soil temperatures in relation to air temperatures and reported that soil temperature at 1/4 to 1/2 inch depth may reach maximums of 30° F. to 40° F. above air temperature. Heldseth et al. (32, p. 294) reported that there are three cardinal growth temperatures for each species and variety of plant. These are the minimum growth temperature below which growth does not take place, the maximum growth temperature above which growth ceases, and the optimum growth temperature at which growth is most

vigorous.

Although there are species differences in general, growth rate of grass declines at constant temperatures higher than  $65^{\circ}$  F. to  $75^{\circ}$  F. The detrimental effect of high temperature may be due to depletion of carbohydrates within the plant. Lethal temperatures for most of the grass species are in the range of from  $120^{\circ}$  F. to  $140^{\circ}$  F.

2. Light: The supply of light energy could be a factor limiting the rate of photosynthesis and indirectly the growth of plants. Gist and Mott (27, p. 585) found that red clover seedlings produced more top growth than alfalfa at 14,400 foot candle hours of light per day. At 2400 foot candle hours of light per day, alfalfa top growth exceeded red clover the first 10 to 40 days after emergence, but red clover was better from 41 to 56 days. Generally, growth response of alfalfa, red clover and birds-foot trefoil were linear for varying light intensities.

Knight (43, p. 556-558) investigated photoperiod response and found that best seed production of dallisgrass resulted when day lengths of 14 and 16 hours were used in combination with high night

temperatures. Night temperatures below 55° F. inhibited seed formation. Dallisgrass failed to produce seed heads when grown under a photoperiod of 8 or 9 hours. Lovvorn (48, p. 575) reported that under short days dallisgrass produced more top growth at 80° F. to 90° F. than at 60° F. to 70° F.

Generally light does not limit stand establishment in the field in adapted areas suitable to crops. Intensity variation may sometimes limit plant growth under unusual conditions or in unadapted areas.

3. Moisture: Moisture is the most important factor influencing distribution of plants within areas of similar temperatures. Placement of seed in a dry soil is generally reported by many workers (2, p. 82-84; 18, p. 119-124; 39, p. 108-109; 19, p. 524-529) to delay emergence and reduce total emergence. Gist and Mott (26, p. 35-36) reported that decreasing soil moisture resulted in reduced seedling growth. Davies (16, p. 11-24), working with ryegrass, found that relative drought, especially when accompanied by high temperatures, and



high rainfall when accompanied by long periods of low temperatures, result in low germination and high seedling mortality. Low temperature, accompanied by but slight rainfall, causes slow germination which need not necessarily result in poor establishment, provided more favorable conditions follow. Tysdal (76, p. 526-529) found that high soil moisture resulted in considerably increased forage yield of alfalfa and did not inhibit seed production, but lowest seed yields and highest amounts of shriveled seeds were produced by thick stands of frequently irrigated, lodged plants. Hollowell (35, p. 232-245) reported that atmospheric humidity did not affect seed setting of red clover. More seeds, more heads per plant, and greater vegetative growth were produced on soil of medium moisture content than soil either high or low in moisture.

From the above review, it is obvious that soil moisture, when either excessive or deficient, is a limiting factor in stand establishment and production for most grasses and legumes.

4. Soil: The kind of soil, its fertility and pH, have a great bearing on the establishment of stand and production of forage and seed. Soil serves as a medium in which plants live, as a source of plant nutrients and as a source of water. As early as 1927 Davies (17, p. 46-53) noted that heavier soils tend to give poorer stands of forage species than lighter soils, and fertilizers may improve seedling vigor under many circumstances.

B. Internal factors

1. Seed size and weight: Among the internal factors affecting establishment of seedlings and subsequent production of forage and seed are, genetic characteristics, seed size and weight, seed viability and vigor, maturity of seed, past history and age of seed.

Effect of seed size on establishment has been studied by many workers. Kidd and West (41, p. 138) stated that the balance of evidence favors the conclusion that larger seeds give rise to more vigorous plants and better yields. Davies (17, p. 47), referring to the most commonly used British grasses, commented that under normal field conditions, the size of endosperm is an

important factor in determining the potential ability of a species to establish itself. Milton (52, p. 174-181) found that strains of perennial ryegrass and timothy with heavy seeds became better established than light seeded strains, even though the same weight of seed was planted giving more seed per acre for the light seed. Plummer (62, p. 23-31) noted in 18 forage species, that heavier seeded grasses emerged faster and better than light seeded grasses when planted deep. In 1927, Davies (17, p. 46-49) reported correlation coefficients ranging from 0.698 to 0.795, between low seed weight and per cent stand establishment for a number of forage species under investigation.

Erickson (22, p. 969-971) found a direct association of germination and seedling vigor with seed size, though effect on seedling vigor decreased with the age of the seedling. Hunt (37, p. 86-95), working on a number of grasses, including perennial ryegrass, showed a correlation of 0.798 between seed weight and number of established seedlings per 100 seeds planted. A correlation of 0.90 between seed weight and seedling vigor was obtained by Hawk and Welch (31,

p. 811-815). Peace (60, p. 36-38) obtained significant correlations, both phenotypic and genotypic, between seed weight and seedling vigor with 20 strains of smooth brome grass.

From the above review, it is clear that most workers agree that larger seeds produce seedlings that emerge faster, and grow at faster rates. However, there are differences in average seedling vigor between species and among strains in the species.

2. Seed viability: Most investigators agree that the most important factors affecting viability of seeds in storage are moisture and temperature. Excellent reviews on this subject may be found in Blackman (5, p. 31-36), Crocker (15, p. 235-274), Duvel (21, p. 9-89), Barton (3, p. 379-403) and Toole (75, p. 3-8).

The effects of temperature and moisture on seed viability counteract each other when one is low and the other is high. Their effects multiply when both are high. Ching et al. (13, p. 681-683) reported that crimson clover containing 6 per cent moisture remained viable after three years storage in hermetically sealed cans at 3° C., 22° C. and

38° C. Perennial ryegrass containing 8 per cent moisture preserved well at 3° C. and 22° C. Foy (24, p. 11-14), Kearns and Toole (40, p. 24) and Gane (25, p. 90-91) studied the influence of storage temperatures and relative humidity upon the viability of chewings fescue seed. Their respective experiments demonstrated that seed maintained at a given moisture level the greatest loss of viability was associated with high temperatures. Barton (4, p. 217-220) found in tomato seed that though germination after six years in storage was still high, vigor had fallen off markedly and the percentage of seedlings capable of emerging from the soil was very low. A similar result was noted by Simpson (67, p. 416), who reported that weakened but still germinable seeds are difficult to detect by laboratory methods. Cotton seed stored at high temperature with high moisture for two and one-half years, failed to emerge from the soil in the field and the germination per cent obtained in the field were considerably below that obtained in the laboratory.

From the above review of seed viability and seedling vigor, it can be concluded that excessive

moisture in the seed speeds metabolic changes that result in loss of viability. High temperatures further increase the rate of change. Exposure to high temperature and high moisture reduces the potential life span of seed, even before the percentage of germination is reduced. Weakening of seed may result from unfavorable conditions of storage and/or long periods of storage.

3. Seed maturity: Seed maturity plays an important role in seed weight, seed germination, seedling vigor, and stand establishment. Grabe (29, p. 253-256) conducted a study of bromegrass on the effect of stage of maturity on seed size, viability, and seedling vigor. Maximum dry weight of seed and fall germination potential were reached by 17 to 18 days after anthesis. It was found that ability to germinate and seedling vigor were directly proportional to seed weight, which reflects the amount of food reserves in the seed. McAlister (49, p. 442-453) reported that seeds harvested at the pre-milk stage and milk stage were inferior in most instances both in viability and longevity, compared with seeds harvested when at the dough stage or mature.

Immature seeds did not emerge and did not retain germinability in storage. Herman (33, p. 876-885), working with crested wheatgrass (Agropyron cristatum), found that seed weight, emergence from different seeding depths, and height of seedlings all increased as seed was harvested closer to maturity. Viable seeds were obtained by harvesting earlier than hard dough stage, but such seeds did not produce vigorous seedlings.

Thus, from the above review, it is clear that vigor and germination percentage of the seed increase with maturity.

4. Genetic factors: It has been observed that not only seed size but also genetic factors are related to seedling vigor (30, p. 19). McDoland et al. (50, p. 22-24) studied plant to plant variation in bromegrass and found significant differences in yield, spreading habit, and plant height, both among families and between inbred and open pollinated progenies. Kneebone and Cremer (42, p. 473-476), working with several range grasses, Lawrence (46, p. 215-219) with intermediate wheatgrass, and Peace (61, p. 36-38) reported similar results. Newell and Keim (60, p. 422-433) found that northern

strains of brome grass, though excellent in quality, often produced weak seedling growth in the fall (seedlings in the field), while southern strains from Nebraska and Kansas were very good in seedling vigor. Black (7, p. 3-10) noted that the relative growth rates of three strains of brome grass were identical, though these strains differed in seedling weight at any one time, due to initial seed weight differences. There is a wide difference among grass species in root and shoot development in early stages of growth. Plummer (62, p. 24-32) reported that brome grass was one of the best in root growth during the first 28 days in the greenhouse, while crested wheatgrass was outstanding in root development in the field and the first few months after seeding.

In summary, one may conclude that genetic factors are known to influence seedling vigor. However, cultural practices, e.g. seeding time, environmental influences, and seeding method, may alter the plant performance and yield.

#### C. Cultural practices

Cultural practices, such as seeding depth, time of



seeding, seed treatment, companion crops, irrigation, pollination, fertilizer, plant population and control of weeds, diseases and insects, have a marked influence on stand establishment and production of forage and seed.

Love and Hanson (47, p. 373-382) were among the first to study the effect of different depths of seeding on stands. They found that crested wheatgrass and bromegrass could emerge from depths up to 3 inches, but best emergence occurred at  $1/4$  to  $1/2$  inch for the former, and  $1/4$  to 1 inch with the latter. Murphy and Arny (58, p. 19-26), Ahlgren (1, p. 137-144), and Robertson (64, p. 5-11) reported that grass and legume seeds, unless sown very shallow, did not produce a high percentage of emerged seedlings. Approximately  $1/2$  inch was a satisfactory depth for seeding perennial ryegrass and other species included in the experiment, although seeding on the surface can produce good stands under ideal conditions. Moore (53, p. 374-378) found that emergence of seedlings from extra large or extremely small crimson clover seeds was reduced to a greater extent by deeper seeding than from a medium depth. Seedlings that emerged from deeper seeding not only were slower in emergence, but were much weaker

than those that emerged from the optimum depth.

Thatcher et al. (74, p. 9-40) did an extensive study of different methods of sowing meadows and found that deep seeding is recommended for early sowing and shallow for late sowing. Blaser (9, p. 119-121) reported that, in a mixture, species best adapted to current environments become aggressive and dominant while the plants less adapted are suppressed. In general, an increase in stand density produces an increase in total forage yield, but seed heads are produced fewer in close spacing as found by Knight and Hollowell (44, p. 74-76). Hawk and Welch (31, p. 411-417), working with brome grass, found a varietal difference in seedling vigor. Certain inter-relationships between variety, depth of sowing, and seed size in forage establishment have been reported by Black (7, p. 3-13).

Weeds are recognized to be of considerable economic importance in crop production. Several investigations (11, p. 68; 12, p. 193; 28, p. 165-168) have demonstrated that weed infestation may bring reduction in yield by competition with crops for moisture, nutrients, and light. Competition begins early and often persists over a major part of the growing season.

Forage species are attacked by large numbers of pathogens. The importance of disease varies according to the grass species and the environment in which it is grown. Bird (9, p. 554-558) reported that rust can have a marked effect on both yield and vigor of individual plants and strains of timothy. Sampson et al. (66, p. 253-263) found that several leaf spotting and defoliative diseases weaken clovers and other plants.

Management methods and environment have an important effect on the development of many foliar diseases. Sometimes susceptibility to insects restricts the production of forage and seed. Rhodesgrass scale is a limiting factor in southeastern Texas as reported by Richerd et al. (63, p. 1-5).

D. Correlation of laboratory germination with field emergence

Generally, seedling vigor and seed germination tests conducted in the laboratory reflect seed quality. In the laboratory, there are optimum conditions close to the ideal environment. However, when seeds are planted in the field, the environment is seldom optimum. Many seeds that could germinate under optimum conditions are not vigorous enough to survive in unfavorable environment. Everson (54, p. 14-16) and

Munn (57, p. 285-286) reported that seeds generally germinate higher in the laboratory than in the field, but the laboratory test gives an idea what we can expect in the field. Samples that are good in the laboratory most likely will be good in the field. Rogler (65, p. 217-229) obtained a correlation of 0.9 between field and greenhouse emergence ratings for a number of crested wheatgrass seed lots at six depths.

## MATERIALS AND METHODS

The seeds of perennial ryegrass (Lolium perenne L) and crimson clover (Trifolium incarnatum L) with 6, 8, 12, 16 and 20 per cent moisture content stored for 48 months at 3° C., 22° C., 38° C. and at variable warehouse temperature in hermetically sealed cans, were used for this experiment. Seeds with 12 per cent moisture stored at 22° C. and 38° C. with 16 and 20 per cent moisture stored at 22° C., 38° C. and variable warehouse were not included in this experiment because they were non-viable. A more detailed description of these seeds was published by Ching et al. (13, p. 680-684). Germination percentages of both kinds of seeds were checked in the laboratory before seeding in the field.

The soil for the field experiment was Amity silty clay loam. Ammonium phosphate (16-20-0) fertilizer was added at the rate of 200 pounds per acre during preparation of the seedbed. The experimental design was a randomized block with 4 replications. Each plot contained five rows 10 feet long and 3 feet apart. Seeding was done during the first week in October 1959. Two hundred seeds were sown in each row. Crimson clover seed was inoculated with "Nitragin" (a commercial culture of rhizobia) to promote nodulation.

Emergence counts of crimson clover were made 10-12 days after planting. The average percentage of emergence was taken on the three center rows of each plot and the results were used to correlate field emergence with laboratory germinations. No counts were made in ryegrass because of difficulty in distinguishing between seedlings of volunteer annual ryegrass and perennial ryegrass.

Weeding was done three times during the growing period by hand hoeing in December 1959, February and April 1960. In addition, a spray treatment of dinitro amine at 3 pounds per acre was applied in December 1959 to control broadleaved weeds.

Plant height of both ryegrass and crimson clover was measured in centimeters in the last week of February 1960. The counting of tillers was difficult in the field because of inability to distinguish between individual plants of either crimson clover or perennial ryegrass. Consequently, the plants in one of the border rows in each plot of crimson clover were dug the second week of May 1960. Total number of surviving plants, number of tillers per plant, length of roots and tillers, and number of heads per plant were recorded in the laboratory. With ryegrass, even in the laboratory, it was difficult to separate individual plants.

Dry weight and moisture content of forage from

the material described above in both crimson clover and perennial ryegrass were determined by taking a weighed green sample of approximately 100 grams from each plot and drying in a forced air oven at  $85^{\circ}$  C. for 24 hours.

To study the effect of seed storage condition on forage production, total forage yield was taken before blooming. In crimson clover, the last row was dug and roots were removed before weighing to determine the total forage yield. The ryegrass was cut 2 inches above the ground with hand sickles and then weighed. At the time of digging and harvesting, care was taken to prevent loss of moisture by keeping the forage in plastic bags and weighing immediately after harvest.

For more information regarding the effect of storage condition on the quality of forage, the protein content of the forage from all the treatments was determined by macro-Kjeldahl method in the plant nutrition laboratory of the Horticulture Department. The following procedure was used.

A 500 gram sample of fresh forage of both ryegrass and crimson clover was dried at  $60^{\circ}$  C., then ground and the material passed through a screen of 20-40 mesh. Duplicate samples of 1 gram of each treatment were analyzed.

The protein content was calculated by multiplying nitrogen percentage by 6.25.

Seeds of crimson clover and ryegrass were harvested by hand in the month of July 1960 when they were fully mature and just before shattering. Only the second, third and fourth rows of each plot were harvested for yield of seed and threshed by machine. The perennial ryegrass threshed easily and almost all seeds were recovered, but threshing of crimson clover was far from satisfactory. Further cleaning was accomplished with a hand clipper and a seed blower.

In the case of crimson clover, only two replications were harvested because the other two replications were accidentally mowed. All four replications of ryegrass were harvested. The seed yields were recorded in grams per plot.



## EXPERIMENTAL RESULTS

Crimson CloverCorrelation of germination between laboratory and field

The emergence of seedlings in the field was lower than the percentage germination in respective laboratory tests. The speed of emergence of ryegrass was slower than crimson clover. In crimson clover, the seedlings started to emerge five days after seeding, but in ryegrass, emergence of seedlings was first noted nine days after seeding. The results of laboratory germination and field emergence for various seed lots stored under different conditions for three and four years are shown in Table 1 for 1958 and in Table 2 for 1959.

There was a high correlation between laboratory germinations and field emergence, as shown by the correlation coefficients  $r = 0.940^*$  in 1958<sup>#</sup> and  $0.981^{**}$  in 1959. Both of them are significantly correlated at 5 and 1 per cent level.

<sup>#</sup>These data have been taken from the experiment of 1958 conducted by Ching and Overmiller. The seed was sown in the field after 36 months storage under different conditions.

\*N = 12

\*\*N = 13

Table 1. Comparison of percentage laboratory germination with field emergence of crimson clover stored for 36 months under different storage conditions in 1958.

Sample	Treatment	Condition	Replications			
			1	2	3	4
			%	%	%	%
B	*6-3° C.	Lab	92	86	94	94
		Field	61	64	68	69
C	6-22° C.	Lab	84	88	92	92
		Field	60	72	59	55
D	6-38° C.	Lab	86	90	86	82
		Field	69	55	58	63
E	6-V.	Lab	94	88	94	86
		Field	72	68	64	72
F	8-3° C.	Lab	88	88	84	80
		Field	77	63	70	61
G	8-22° C.	Lab	84	88	78	82
		Field	66	55	64	54
H	8-38° C.	Lab	76	84	82	70
		Field	55	36	42	38
I	8-V.	Lab	90	90	92	82
		Field	66	71	64	72
J	12-3° C.	Lab	90	92	88	88
		Field	64	55	70	56
K	12-V.	Lab	70	82	64	70
		Field	32	30	36	32
L	16-3° C.	Lab	92	90	82	96
		Field	47	62	48	66
M	20-3° C.	Lab	16	26	42	40
		Field	11	4	4	12

\*First number = percentage moisture in seed, the second is the storage temperature.

V = variable warehouse conditions.

Table 1a. Correlation between percentage laboratory germination with field emergence of crimson clover stored for 36 months under different storage conditions in 1958.

Sample	Treatment	Germination percentage	
		Laboratory	Field
B	6-3° C.*	91 <sup>1/</sup>	65 <sup>1/</sup>
C	6-22° C.	89	61
D	6-38° C.	86	61
E	6-V.	90	69
F	8-3° C.	85	67
G	8-22° C.	83	59
H	8-38° C.	78	42
I	8-V.	88	68
J	12-3° C.	89	61
K	12-V.	71	35
L	16-3° C.	90	55
M	20-3° C.	31	7

$r = 0.940$  D.F. = 10

\*First number = percentage moisture in seed, the second is the storage temperature.

V = variable warehouse conditions.

<sup>1/</sup> Mean of four replications.

Table 2. Comparison of percentage laboratory germination with field emergence of crimson clover stored for 48 months at different storage conditions in 1959.

Sample	Treatment	Condition	Replications			
			1 %	2 %	3 %	4 %
A	*Control	Lab	84	83	84	83
		Field	59	58	58	59
B	**6-3° C.	Lab	79	74	84	87
		Field	45	61	49	40
C	6-22° C.	Lab	79	74	80	69
		Field	46	59	57	52
D	6-38° C.	Lab	74	78	81	76
		Field	58	58	60	40
E	6-V.	Lab	88	91	83	82
		Field	59	62	65	54
F	8-3° C.	Lab	85	84	85	89
		Field	56	60	60	46
G	8-22° C.	Lab	84	81	82	79
		Field	59	57	52	57
H	8-38° C.	Lab	23	28	19	25
		Field	24	21	28	20
I	8-V.	Lab	86	84	87	90
		Field	52	64	53	53
J	12-3° C.	Lab	92	89	86	90
		Field	55	47	60	57
K	12-V.	Lab	23	32	27	30
		Field	10	25	22	17
L	16-3° C.	Lab	87	83	89	83
		Field	48	62	51	56
M	20-3° C.	Lab	4	3	3	6
		Field	2	4	4	2

\*Freshly harvested

\*\*First number = percentage moisture in seed, the second is the storage temperature.

V = variable warehouse conditions.

Table 2a. Correlation between percentage laboratory germination with field emergence of crimson clover stored for 48 months at different storage conditions in 1959.

Sample	Treatment	Germination percentage	
		Laboratory*	Field*
A	Control	83	58
B	6-3° C.**	80	49
C	6-22° C.	75	53
D	6-38° C.	77	54
E	6-V.	85	60
F	8-3° C.	85	55
G	8-22° C.	81	56
H	8-38° C.	24	21
I	8-V.	86	53
J	12-3° C.	89	52
K	12-V.	27	18
L	16-3° C.	85	54
M	20-3° C.	4	3

$r = 0.981$  D.F. = 11

\*\*First number = percentage moisture in seed, the second is the storage temperature.

V = variable warehouse conditions.

\*Mean of four replications.

Effect of seed storage conditions on field emergence

There was a significant variation in total emergence among the treatments. Sample H (8-38° C.), K (12-V.) and M (20-3° C.) gave very poor germination in the laboratory, as well as poor emergence in the field. These three samples were significantly different from the other ten samples.

Comparatively, sample H (8-38° C.) and K (12-V.) were better than sample M (20-3° C.). There was no significant difference between control and the other ten treatments when compared by L.S.D. Field emergence results are summarized in Table 3 and the analysis of variance is shown in Table 4.

Table 3. Summary of the experimental results of the effect of seed storage conditions on stand establishment and forage production in crimson clover (*Trifolium incarnatum*) in 1959.

Sample	Treatment	No. of seedlings emerged 12-15 days after sowing 200 seeds	No. of plants survived per 200 seeds	Forage Yield in Grams			Plant ht. in cm.	No. of tillers per plant	No. of flower heads per plant	Root length in cm.
				Fresh wt. per plant	Fresh wt. per 200 seeds	Dry wt. per 200 seeds				
A	Control +	112	61	20.8	1251	198.2	47.1	3.0 +	8	11.8
B	6-3° C #	98	71	21.3	1536	246.5	50.4	3.0	9	13.6*
C	6-22° C	108	67	25.6	1646	247.7	48.3	3.0	8	8.9**
D	6-38° C	108	68	19.6	1359	220.7	52.7	3.0	8	12.6
E	6-V	120	70	18.1	1286	200.7	49.5	3.0	7	12.1
F	8-3° C	112	54	17.5	976	161.2	46.2	3.0	9	13.0
G	8-22° C	112	82	20.6	1736	281.5*	50.7	3.0	9	13.8**
H	8-38° C	40**	12	12.2**	97**	17.5**	31.9**	1.0**	3**	8.4**
I	8-V	112	55	22.4	1241	203.7	47.0	3.0	7	12.9
J	12-3° C	110	73	18.8	1386	218.2	48.8	3.0	6	10.9
K	12-V	38**	14	10.6**	155**	28.0**	33.8**	2.0**	5**	10.3*
L	16-3° C	108	65	19.3	1224	194.2	50.0	3.0	8	12.2
M	20-3° C	6**	2	15.7**	31**	4.0**	35.7**	---	---	---
L.S.D.	.05	14.40		6.16	698.40	76.70	6.60	0.60	2.03	1.48
L.S.D.	.01	19.20		8.24	934.50	102.60	8.84	0.80	2.72	1.98

\* Significant at 5 per cent level

\*\* Significant at 1 per cent level

V Variable warehouse temperature

--- Not enough plants

+ Fresh seed harvested in 1959

# The first no. designates the seed moisture content, the second is the storage temperature

Table 4. Analysis of variance for the emergence count, total forage yield, forage yield per plant, total dry weight of forage of crimson clover.

Source of variation	D.F.	MS			
		Emergence count	Total forage yield per 200 seeds	Forage yield per plant	Total dry weight per 200 seeds
Treatment	12	5302.46**	1388773.52**	65.40**	34545.49**
Replication	3	563.74**	721793.52*	199.78**	13071.25**
Error	36	191.701	238883.16		2882.89
Total	51				

\*\*Significant at 1 per cent level.

\*Significant at 5 per cent level.



### Effect of seed storage conditions on forage yield

There was a significant variation among the treatments in fresh weight and dry weight or forage production per plant. Sample H, K and M gave lower yields of fresh material as well as of dry forage than the control. The forage yield per plant was lowest in sample H, K and M. Sample G (8-22° C.) gave a higher yield of dry material than the control and it was significantly different from the control.

The effect of seed storage conditions on seedling emergence survival and plant development of crimson clover is summarized in Table 3 and the analysis of variance for total forage production, forage yield per plant, and total dry weight is shown in Table 4.

### Effect of seed storage conditions on plant growth and development

There was a significant difference in plant height among the treatments. Sample H (8-38° C.), K (12-V.) and M (20-3° C.) were statistically different from the control and from other treatments. These three samples were comparatively shorter in height than the others. The analysis of variance for plant height

and number of tillers per plant is given in Table 5 and 6 respectively. In sample M there were not sufficient plants to make observations regarding number of tillers per plant. Sample G (8-22° C.), H (8-38° C.) and K (12-V.) were significantly different from the control when compared by L.S.D. (Table 3). Sample G had more tillers than the control and other treatments, but samples H and K had fewer tillers than the control and other treatments. The analysis of variance for number of flower heads per plant is given in Table 6. The results obtained show that there were significant differences between treatments, and samples H and K were significantly lower in number of heads per plant than the others.

There were highly significant differences among the treatments in root length. Sample B (6-3° C.), C (6-22° C.), G (8-22° C.), H (8-38° C.) and K (12-V.) were significantly different than the control. Samples H and C produced shorter roots than the other treatments. There was greater variation among the treatments in root length than in plant height, number of tillers, or flower heads per plant. The analysis of variance for root length is given in Table 6.

Table 5. Analysis of variance for the height of plant in cm. of crimson clover.

Source of variation	DF	MS	F
Treat	12	194.07	9.245**
Rep	3	29.79	1.419
Error	36	20.99	
Total	51		

\*\*Significant at 1 per cent level.

Table 6. Analysis of variance for number of tillers per plant, number of heads per plant, plant root length for crimson clover.

Source of variation	DF	MS		
		Number of tillers per plant	Number of heads per plant	Plant root length
Treatments	11	1.589**	12.64**	12.18**
Replication	3	1.816**	7.21*	2.26
Error	33	0.176	1.99	1.06
Total	47			

\*Significant at 5 per cent level.

\*\*Significant at 1 per cent level.

Effect of seed storage conditions on seed production

The results obtained indicated that there were significant differences between treatments. Sample H (8-38° C.), K (12-V.) and M (20-3° C.) produced lower total seed yields than other treatments. But the yield of sample K per plant was not significantly different from the control, whereas sample M produced a highest yield per plant and it differs significantly from other treatments. The effect of seed storage on seed production is summarized in Table 7 and analysis of variance for total seed production and seed per plant is shown in Table 9.

Table 7. The effect of seed storage conditions on seed production of crimson clover (T. incarnatum)

Sample	Treatment	Total seed yield per plot in gms* per 600 seeds	Seed yield per plant in gms
A	Control	272	1.88
B	6-3° C. <u>1</u> /	426	1.85
C	6-22° C.	360	2.19
D	6-38° C.	371	1.66
E	6-V.	382	1.86
F	8-3° C.	270	1.46
G	8-22° C.	451	1.43
H	8-38° C.	52**	0.34**
I	8-V.	375	1.86
J	12-3° C.	311	1.51
K	12-V.	64**	1.71
L	16-3° C.	338	1.93
M	20-3° C.	31**	4.27***
L.S.D.	.05	190.4	1.15
L.S.D.	.01	267.0	1.62

\*Only 2 replications harvested

\*\*Significant at 5 per cent level

\*\*\*Significant at 1 per cent level

1/First number = percentage moisture in seed, second is the storage temperature.

V = variable warehouse temperature.

Effect of seed storage conditions on protein content in forage

A highly significant difference between treatments was found in the protein content of crimson clover forage. Sample C (6-22° C.) had a significantly higher protein content than the control and other treatments, whereas sample G (8-22° C.) had lowest content of protein which differed significantly from the control.

Samples F (8-3° C.), H (8-38° C.), L (16-3° C.) were not different from the control, but all others were significantly different from the control. The experimental results are given in Table 8, and the analysis of variance in Table 9.

Table 8. Effect of seed storage conditions on protein content of crimson clover (T. incarnatum)

Sample	Treatment	Protein content
A	Control	16.2
B	6-3° C. <sup>1/</sup>	17.8**
C	6-22° C.	18.9**
D	6-38° C.	17.6**
E	6-V.	16.7**
F	8-3° C.	16.4
G	8-22° C.	15.7**
H	8-38° C.	16.2
I	8-V.	16.7**
J	12-3° C.	17.7**
K	12-V.	16.7**
L	16-3° C.	16.4
M	20-3° C.	18.1*
L.S.D.	.05	0.35
L.S.D.	.01	0.49

\*Significant at 5 per cent level

\*\*Significant at 1 per cent level

<sup>1/</sup>First number = percentage moisture in seed, second is the storage temperature.

V = variable warehouse temperature.

Table 9. Analysis of variance for seed yield per 600 seeds, seed yield per plant and protein content of forage of crimson clover.

Source of variation	DF	MS		
		Seed yield per 600 seeds	Seed yield per plant	Forage protein content
Treatment	12	41712.95**	1.596**	1.610**
Replication	1	4551.37	1.980*	0.119
Error	12	7647.30	.281	0.026
Total	25			

\*Significant at 5 per cent level

\*\*Significant at 1 per cent level

### Perennial Ryegrass

#### Effect of seed storage conditions on forage production

In Sample H (8-38° C.) and M (20-3° C.) there were no plants to make observations. The results show a significant difference among the treatments. Sample K (12-V.), C (6-22° C.) and D (6-38° C.) were significantly different from the control and produced lower yields of forage. Among these three samples, sample K gave lowest yield. The experimental results of forage production from various samples is summarized in Table 10, and analysis of variance for fresh and dry forage yields is given in Table 11.



Table 10. Effect of seed storage conditions on forage production of perennial ryegrass.

Sample	Treatments	Plant ht. in cm.	Forage yield per 200 seeds in grams	
			Fresh	Dry
A	Control	8.8	2009	518.0
B	6-3° C. <u>1</u>	8.9	2409	621.0*
C	6-22° C.	7.8*	1355**	366.7**
D	6-38° C.	7.4**	1000**	272.5**
E	6-V.	7.6*	1787	480.7
F	8-3° C.	9.0	1969	526.0
G	8-22° C.	7.8*	1656	440.0
H	8-38° C.	--	--	--
I	8-V.	8.4	2161	566.0
J	12-3° C.	8.1	1970	513.2
K	12-V.	6.2**	76**	18.7**
L	16-3° C.	7.9*	1945	521.0
M	20-3° C.	--	--	--
L.S.D.	.05		462.3	99.9
L.S.D.	.01		623.7	134.6

\*Significant at 5 per cent level

\*\*Significant at 1 per cent level

-- No plants

1/First number = percentage moisture in seed, second is the storage temperature.

V = variable warehouse temperature.

Table 11. Analysis of variance for fresh forage, dry forage yield per 200 seeds and plant height of perennial ryegrass.

Source of variation	DF	MS		
		Fresh forage yield	Dry forage yield	plant height
Treatment	10	1609245.12**	114702.86**	2.52**
Replication	3	1110286.38**	54518.68**	1.37*
Error	30	102520.11	4792.19	0.29
Total	43			

\*\*Significant at 1 per cent level

\*Significant at 5 per cent level

The effect of storage condition of seed on total dry weight of forage is similar to the fresh weight except sample B, which produced more dry weight than the control. Samples C (6-22° C.), D (6-38° C.) and K (12-V.) gave lower dry weights than the other treatments, whereas sample B (6-3° C.) produced significantly higher total dry weight than all the other treatments. Seed stored at higher temperatures yielded less fresh and dry forage than the control.

The height of plants of various treatments was significant different (Tables 10 and 11). Samples C (6-22° C.), D (6-38° C.), E (6-V.), G (8-22° C.), K (12-V.), and L (16-3° C.) were significantly lower in height than the control and other treatments (Table 10).

This shows that when seed is stored at high temperatures at and above  $22^{\circ}$  C. and with high seed moisture content, even at low temperatures, e.g.  $3^{\circ}$  C., the plant height is reduced.

#### Effect of seed storage conditions on seed production

The treatments were significantly different among themselves. Sample D ( $6-38^{\circ}$  C.) and K (12-V.) gave lower yields than the control and other treatments. There were no plants in samples H ( $20-3^{\circ}$  C.) and M ( $20-3^{\circ}$  C.). Seed stored at high temperatures, e.g.  $38^{\circ}$  C., and at warehouse temperatures with high moisture content (12 per cent) produced less seed than the control, both being affected by either high moisture content or high temperature in storage. The effects of seed storage on seed production are summarized in Table 12 and analysis of variance in Table 13.

Table 12. Effect of seed storage conditions on seed production of perennial ryegrass

Sample	Treatments	Seed yield per plot in gms
A	Control	639.7
B	6-3° C.*	604.5
C	6-22° C.	570.7
D	6-38° C.	382.5**
E	6-V.	580.5
F	8-3° C.	639.5
G	8-22° C.	590.0
H	8-38° C.	--
I	8-V.	615.0
J	12-3° C.	651.0
K	12-V.	39.5**
L	16-3° C.	642.7
M	20-3° C.	--
L.S.D. .05		123.1
L.S.D. .01		165.8

\*\*Significant at 1 per cent level

\*First number = percentage moisture content in seed,  
second is the storage temperature.

V = variable warehouse temperature.

Table 13. Analysis of variance for seed yield of perennial ryegrass per plot.

Source of variation	DF	MS	F
Rep	3	41092.27	5.65*
Treat	10	135179.37	18.58**
Error	30	7271.74	
Total	43		

\*Significant at 5 per cent level

\*\* Significant at 1 per cent level

Effect of seed storage conditions on protein content of forage

The treatments are significantly different among themselves. Samples B (6-3° C.), C (6-22° C.), G (8-22° C.) and I (8-V.) had lower protein content than the control, and samples F (8-3° C.) and K (12-V.) had higher protein content than the control. Sample B had the lowest protein content. The result does not show any definite trend. The protein content of forage from various treatments is given in Table 14 and the analysis of variance in Table 15.

Table 14. Effect of seed storage conditions on protein content of forage of perennial ryegrass.

Sample	Treatments	Protein content
A	Control	7.65
B	6-3° C. <u>1</u> /	6.47**
C	6-22° C.	6.84**
D	6-38° C.	7.34
E	6-V.	7.65
F	8-3° C.	8.09*
G	8-22° C.	7.09**
H	8-38° C.	--
I	8-V.	7.15*
J	12-3° C.	7.94
K	12-V.	9.56**
L	16-3° C.	7.87
M	20-3° C.	--
L.S.D. .05		0.38
L.S.D. .01		0.54

\*Significant at 5 per cent level

\*\*Significant at 1 per cent level

1/First number = percentage moisture content in seed,  
second is the storage temperature.

V = variable warehouse temperature.

Table 15. Analysis of variance for protein content  
in forage of perennial ryegrass

Source of variation	Df	MS	F
Treat	10	1.335	45**
Rep	1	0.070	2.36
Error	10	0.0296	
Total	21		

\*\*Significant at 1 per cent level

## DISCUSSION

There are a number of limiting factors in the establishment of forage crop seedlings as discussed by Willard (78, p. 431-447). Essentials to proper germination of live seeds are sufficient air and moisture and favorable temperature. Other limiting factors are soil pH, kind of soil, depth of seeding, availability of oxygen, crusted soil surface, seed size, age of seed, vitality of seed, permeability of seed coat, absence or presence of soil micro-organisms, and genetic factors. Failure of seedlings after germination could be due to lack of sufficient plant nutrients, poor drainage, drought, competition from weeds, insects and diseases.

The results for both 1958 and 1959 showed that the percentage emergence of seedlings in the field was less than the corresponding laboratory germinations. This agrees with the findings of early workers (Munn 35, p. 55; 56, p. 87), Whitecomb (79, p. 60-62) and Stahl (73, p. 42-56). All samples of crimson clover included in the experiment showed a marked decline of emergence in the field. Samples H (8-38° C.), K (12-V.) and M (20-3° C.) had very poor germination in the field. Only 3 per cent of the seed which had been



stored with high moisture (20%) was able to emerge from the soil.

In the laboratory the germination test is conducted under optimum conditions, thus it reflects the ability of the seed to grow under ideal conditions. The field test shows the number of seedlings that may be produced from a given number of seed under less optimum conditions.

Reduced emergence of seed in the field may be caused by the factors mentioned above. If field conditions are as favorable as in the laboratory, the laboratory germination and field emergence may be expected to be essentially alike. The seed lots used in this experiment all originated at the same source, seeded in an adapted area and given identical culture and management. No disease or insect pest was there. Environmental, cultural and genetic factors, therefore, may not be accounted for in the differences in emergence among the treatments.

Here, vitality may be considered the important factor causing reduction of emergence in the field. Certain lots of seeds which show indication of weakened vitality in the laboratory test give very poor results in the field or may fail entirely. Seed stored for a

long time or stored at high temperature and moisture suffers reduction in vigor, even before the percentage of germination is reduced. Generally, weakening of seed results from unfavorable storage conditions and long duration of storage. Seed stored under unfavorable conditions loses vigor and ability to emerge after seeding. Similar results have been reported by Barton (4, p. 217-220) and Simpson (67, p. 416).

In this experiment, a high correlation between laboratory test and field emergence was found, both in 1958 ( $r = 0.940$ ) and in 1959 ( $r = 0.981$ ). Larger seeds, such as peas and corn, which have more reserve material are better able to cope with unfavorable conditions in the field, such as being covered too deeply, drying out, or other conditions not conducive to emergence. The percentage emergence of larger seeds is closer to the percentage germination in the laboratory. Conversely, the field emergence of small seeded grasses and legumes is generally much below the laboratory germination. In this experiment, a close relationship between laboratory germination and field emergence has been found in perennial ryegrass and crimson clover. The laboratory test points to what one can expect in the field. Samples that are good in the laboratory are most likely

to be good in the field. By determining the correction factor for different kinds of seeds or a regression equation on the results of laboratory germinations, one may predict the emergence of seed in the field. The laboratory test, along with a correction factor or regression equation, is a good criterion of the number of seeds to sow per acre to secure a stand of a given number of plants per acre under adapted conditions. By knowing laboratory germination and a correction factor or regression equation, waste of seed in sowing can be avoided. The laboratory test is the only test by which the quality of seed can be predicted at present.

The effect of seed storage conditions on forage and seed production in both crimson clover and perennial ryegrass showed a significant difference among the treatments. Samples H (8-38° C.), K (12-V.) and M (20-3° C.) in the case of crimson clover gave significantly lower yields of forage as well as of seed. Ryegrass samples H (8-38° C.) and M (20-3° C.) did not produce any plants in the field. Samples C (6-22° C.), D (6-38° C.) and K (12-V.) gave significantly lower yields than other treatments. This indicates that perennial ryegrass seed is more sensitive to unfavorable storage conditions than crimson clover seed because

samples C (6-22° C.) and D (6-38° C.) in crimson clover were not significantly different from other treatments. The fresh and dry forage yields of crimson clover were also significantly lower in samples H (8-38° C.), K (12-V.) and M (20-3° C.) than other treatments. Sample G (8-22° C.) of crimson clover did not differ significantly from the control in fresh forage yield, but gave significantly higher dry yield.

The forage and seed yield per plant were also statistically lower in samples H (8-38° C.), K (12-V.) and M (20-3° C.) of crimson clover. Only in sample M (20-3° C.) was the seed yield per plant higher than other treatments. Probably this was due to less competition among the plants, because there were very few plants in the plots. The reduction in forage and seed yield was the result of thin stands as well as poor plant development. The seeds stored at high temperature with high moisture gave significantly lower total production, as well as low production per plant. Storage conditions not only affect the stand of the plant in the field, but reduce the growth and development of an individual plant. The height of the plant, number of tillers per plant and number of flower heads

per plant were less in plants produced by seed stored under unfavorable conditions. There was much variation in the root length of crimson clover. Samples C (6-20° C.), H (8-38° C.) and K (12-V.) had shorter roots than all other treatments and were significantly different from the control. These variations did not appear to be correlated with storage conditions. Less root variation might have been obtained by taking into consideration the total root length or comparative total root weight.

From the results of this experiment, it can be concluded that seed storage conditions do affect height of plant, number of tillers, number of flower heads, root length, forage yield, and seed production of crimson clover. For perennial ryegrass also, it can be said that unfavorable seed storage conditions, e.g. high moisture content and high temperatures, affect the height of the plant, forage yield, and seed production. The total weight of sample B (6-3° C.) of ryegrass was not significantly different than the control but total dry weight was significantly higher than the control. It might be implied from this result that seed could be stored under favorable conditions for a long time and still retain the reproductive value.

In future studies, improved techniques such as space plantings, thinning out the stand to equal numbers of plants for equal interplant competition in the field, or starting the plants in the greenhouse in bands and transplanting the seedlings in the field at equal spacing or density may be used to overcome some of the variability encountered in the experiment and increase precision. If the effect of stand variability can be eliminated, results will be more accurate.

Protein content is one of the criteria for judging the quality of forage. Variation in protein content in forage is generally attributed to climatic conditions, soil fertility, seasonal factors and stage of maturity and plant competition (71, p. 2-46; 20, p. 563-565; 23, p. 371-374; 36, p. 565-568; 38, p. 68).

The results of this experiment do not show any trend in protein content of forage as related to storage condition. Although the protein values in various treatments are significantly different among themselves, no definite conclusion of effect of storage condition of the seed on protein content of forage can be drawn. Climatic and fertility factors need to be studied singly and in known combinations as to their effect on composition of forage. This has

not yet proved possible under field conditions.

Sullivan (70, p. 934-936) found in more than 100 plants of Poa pratensis a range of nitrogen content from 2.56 to 3.72 per cent, with differences being highly significant. Wieland et al. (77, p. 219-276) found in a study of 28 plants of Poa pratensis, a variation in crude protein content of the dry matter from 8.2 to 13.6 per cent. Variation of from 9.1 to 17.5 per cent crude protein was observed among 35 plants of Lolium perenne.

The method of analyzing protein content used in this experiment is not sensitive enough for accurate results. A better method probably should be employed in this kind of study.

## SUMMARY AND CONCLUSIONS

A study was conducted in 1959 to observe the effect of storage condition of seeds of crimson clover (Trifolium incarnatum) and perennial ryegrass (Lolium perenne) on stand establishment and production of forage and seed. Also, protein content of forage was determined by the macro-Kjeldahl method to study effect of storage condition on quality of forage. The seed used in this experiment had been stored at 3° C., 22° C., 38° C., and warehouse temperature with 6, 8, 12, 16 and 20 per cent moisture content, in hermetically sealed cans for 48 months. Germination percent of the seed was checked in the laboratory before seeding in the field. Four replications of completely randomized plots were planted in October 1959. Emergence counts of crimson clover seedlings were made 10 to 12 days after seeding. Perennial ryegrass seedlings were not counted. Other observations on crimson clover were height of plant, number of tillers per plant, number of flower heads per plant, root length of dug plants. In perennial ryegrass, only plant height was measured. For determining forage yield one row per plot for each of crimson clover and perennial ryegrass was harvested. Three rows of



crimson clover and perennial ryegrass per plot was harvested for seed yields in June.

The results of the experiment show:

1. That per cent of field emergence is lower than laboratory germination.
2. There is a close relationship between laboratory germination and field emergence as shown by correlation coefficients  $r = 0.940$  in 1958 and  $r = 0.981$  in 1959.
3. The seed with high moisture stored at high temperature gave poor germination in the field as well as in the laboratory.
4. Perennial ryegrass was found to be more sensitive to high temperature than crimson clover.
5. The fresh forage yield, dry forage yield and seed yield in both crimson clover and ryegrass was lower in samples H (8-38° C.), K (12-V.) and M (20-3° C.) than the control. Seed with high moisture content stored at high temperature and warehouse condition produced less forage and seed yield than the control.
6. Storage condition affected not only total forage and seed production, but also the growth and development of the individual

plant. Seed with high moisture stored at high temperature produced shorter plants, fewer tillers, and fewer flower heads.

There was much variation in root length between the treatments.

7. Variations among the treatments in protein content were significant, but were apparently not related to storage conditions of seed.

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