MONTANA STATE UNIVERSITY

Evaluation of germination procedure, mechanical scarification, and environmental variation of germination of cicer milkvetch (Astragalus cicer L.) seed
by Richard David Austin

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University
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Abstract:
A study was conducted to determine methods of raising the low levels of germination in cicer milkvetch to acceptable standards.

Two studies were carried out to determine the optimum conditions for germination. Four ages of seed, two germination solutions and five temperature regimes were used. It was found that H2O was the best germination solution, and either 15-25 or 25C the best temperature. The first year of storage of seed gave the greatest increase in germination percentage.

Laboratory, small commercial, and large commercial scarifiers were used to mechanically scarify cicer milkvetch seed. Two principles of scarification, rubbing, and rubbing plus throwing the seed against a cylinder were employed. All scarification experiments demonstrated the practicality of scarification for raising the levels of germination. Overscarification occurred in some cases. Percentage losses of seed through scarification were calculated for one trial.

Two quick tests for detection of levels of scarification were used. They were the Chromic-Nitric 1-hour test which was not successful and a 24-hour swell test which was successful.

The effects of stage of maturity of racemes at harvest and time of harvest during the summer were studied in relation to germination percentage, hard seed, individual seed weight and seed number. Attempts to manipulate these two conditions proved unsuccessful in regulating the above four factors.

EVALUATIONS ON GERMINATION PROCEDURE, MECHANICAL SCARIFICATION, AND ENVIRONMENTAL VARIATION ON GERMINATION OF CICER MILKVETCH (*Astragalus cicer* L.) SEED

by

RICHARD DAVID AUSTIN

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

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INTRODUCTION

Cicer milkvetch (*Astragalus cicer* L.) was introduced into the United States from the Stockholm Botanical Gardens, Stockholm, Sweden, in the early 1930's. Cicer milkvetch has many desirable characteristics; however, full evaluation of its potential as a forage crop has been hampered by its low germination, 1-20%. Initial work through chipping studies indicated this was due to a hard seed coat.

Research to overcome low germination was needed so a comprehensive field evaluation could be made of cicer milkvetch. The purpose of this investigation was to establish a standard for germination, raise the germination percentage through mechanical scarification, and evaluate the effects of environment on germination percentage, hard seed coat, individual seed weight and seed number per raceme.

REVIEW OF LITERATURE

Cicer milkvetch (Astragalus cicer L.) is a little known legume in the United States and has received limited attention (17, 39, 46). As a forage crop it has many favorable characteristics. It has been under observation at plant materials centers in the West since 1934. Cicer milkvetch is a perennial herbaceous legume. Its growth habit varies from prostrate to upright with decumbent being the more common form. Stems are succulent and leaves are pinnately compound with numerous leaflets. Flowers are white to pale yellow borne on racemes. Bladder-like seed pods contain the seed and are green to greenish red in color when immature. They turn black as the seed matures, and essentially, no shattering of mature seed occurs. Seed is produced over a long period of time with some plants being in continuous bloom from June to the first frost. The seeds are of medium size, bright yellow to pale green in color with extremely hard shiny coats. Laboratory germination varies from 1-20% depending on the lot of seed. Reproduction is by seed and rhizomes. Rapid rhizomatous spread and formation of a dense mat often occur after two or more years of growth.

Cicer milkvetch is adapted to a range of moderate to coarse textured soils. In comparison to alfalfa it will tolerate a higher pH than alfalfa. A stand has been established on a soil having a pH of 9.8. It equals alfalfa in low pH tolerance. Cicer will grow exceedingly well on sites too wet for establishment of alfalfa. Cicer milkvetch has been adapted to dryland conditions where the annual precipitation is 14 inches or greater. It is adapted to high elevations because of its frost tolerance.

Production is equal to alfalfa when properly managed. Cicer milkvetch has no toxic alkaloids and no cases of bloat have been reported, although Cooper (11) showed it to an extremely high foam producer. It appears to be relatively free from diseases or insect damage. Seed production is high with average seed yields of 756 lbs./A. being reported by the SCS Plant Materials Center in Bridger, Montana. The percentage of leaves by weight is higher than that of alfalfa in the hay stage and crude protein is slightly lower than that of alfalfa at the hay stage (20). Once established, cicer milkvetch is a good competitor with grasses in a mixed stand. When planted with Russian wildrye it had a tendency to crowd out Russian wildrye (35). It is slow to establish. This is attributed to low seedling vigor and slow seedling emergence. Low germination has been shown by Little (24) to be caused by an impermeable seed coat.

Hard seeds are mechanically dormant seeds which do not germinate. For germination to take place the impermeable seed coat must become permeable. Hard seeds occur universally among the family Leguminosae and have been found in members of other families. Gramineae, Malvacea, Chenopodiacea and Solanacea are examples as reported by Barton (4).

Early work by White (43) proposed that impermeability of hard seeds was caused by a thicker cuticle layer over the palisade cells. Coe and Martin (10) did an extensive study on the structure and chemical nature of the seed coat and its relation to impermeable seeds of sweetclover. They found no difference in the chemical structure between the coats of permeable and impermeable seeds. The principle difference was in the character and amount of thickening of the walls of the malpighian cells. The outer membrane is instrumental in conferring impermeability on the seed. Quinlivan (30), in his work with hard seeds of sand-plain lupine, found that hard seeds with a moisture content above 10% are conditionally hard and those with a moisture content below 8.5% are absolutely hard and will not soften when exposed to moisture unless daily temperature fluctuations of 15 up to 65C are present. He also found moisture penetration occurring primarily at ramdom sites over the testa but not normally at the hilum or strophiole.

Environment and genetics have been studied to determine their effects on impermeable seed. Lute (27), in her study on impermeable seed of alfalfa, stated that there appeared to be no relationship between altitude and production of impermeable seed. In contrast, Dexter (13) tested a large number of samples of alfalfa seed collected from several states to include a wide range of climatic conditions. These samples were tested for germination and hard seed content. He found that seed from lower altitudes tended to be lower in hard seed content than seed from higher altitudes. Also, second or later cuttings seemed somewhat higher in hard seed than first cutting or earlier harvest. Seed from irrigated fields seemed much the same in hard seed content as seed from dryland fields. Varietal differences in hard seed content were small and unreliable.

According to Stewart and Carlson (37), brightly colored seed in alfalfa was indicative of the following quality characteristics: large seed size, high hard seed content, and optimum maturity.

Whitcomb (42) found a high degree of variation occurring in the hard seed content of different kinds of small seeded legumes. He also found differences in lots of the same kind of seed. In checking on the time of harvest he found the hard seed content of immature seeds was lower than that of ripe seeds but germination of immature seeds was lower than that of mature seeds.

Williams and Elliott (44), in their investigation of the ecological significance of seed coat impermeability to moisture in crimson, subterranean and rose clover in a Mediterranean climate, found that crimson and subterranean followed a general pattern of decline of impermeability during summer months after seed maturity. Rose clover, in contrast, maintained a very high percentage of impermeable seed throughout the summer, autumn and winter months. They concluded that the ecological significance of seed coat impermeability, relative to survival of annual leguminous species, involved four major characteristics. These were: production of impermeable seed, rate at which seeds become permeable, degree of influence of environmental factors favorable to germination, and breakdown of impermeability and the survival and production of plants developing from impermeable seed after delayed germination.

Fayemi (15), studying the effects of temperature on the rate of swelling and germination of legume seeds, found that the time from exposure to the initial absorption of water by the seeds became shorter as the temperature increased for alfalfa, red clover, alsike clover, and ladino clover. The exception was crimson clover.

Hoverland and Elkins (21) found that crimson clover varieties originating in northern locations were more tolerant of low temperatures and less tolerant of high temperatures during germination than were varieties from southern locations.

No inheritance of impermeability in seeds of crimson clover was found by James (22). He selfed parents with a difference of about 60% in seed coat permeability and found a difference of less than 1% in the first generation. He concluded from his data that it appears doubtful that impermeability is inherited unless the possible inheritable factors are masked by the environmental factors to the extent that the different effects cannot be separated.

Analysis of F_1 and F_2 data in a diverse sample of wild blue lupine from Portugal by Forbs (16) showed that hard-seededness was controlled by a single pair of dominant genes, SS, whereas soft-seededness in two cultivated varieties was controlled by the allelic recessive pair, ss.

Storage of seed has been shown to have a definite effect on impermeability and viability. Hafenrichter _et al._ (18) found that cicer milkvetch improved in germination when stored at cool dry conditions at two locations. Germination increased and hard seed content decreased with time. Lute (26) found that many hard seeds became permeable in storage at the end of 13 years and impermeable seeds stored for the same length of time did not differ in death rate. Whitcomb (41) found that all impermeable seeds of alfalfa in storage became permeable in 11 years. Hanson and Moore (19) stored alfalfa, white and crimson clover, and lespedeza under subfreezing conditions for periods up to 48 months and under standard laboratory conditions. The lot stored under subfreezing conditions maintained higher viability and had higher germinations.

Many different processes have been applied to impermeable seeds to increase their permeability to water and oxygen. Some of the most common processes used have been heat, aging, mechanical abrasion, and chemical degradation. Time, cost, and unreliable results have been limiting factors in commercial application of these.

Acid scarification has successfully been applied to hard-seeded legumes, but results are extremely variable and the treatment is hazardous (7, 10, 41).

Various means of scarification from chipping and sandpapering to large commercial scarification have been used. Results have been variable depending on the method, lot of seed, and kind of seed. Whitcomb (41) found the following degrees of injury in seeds that were mechanically scarified: no visible injury, seed coats cracked, embryos partly exposed but not broken, small portions of the embryo broken away, and one third or more of the seed broken away. Alfalfa seeds mechanically and acid scarified have been shown to deteriorate rapidly under storage (5). Little (24) was able to successfully demonstrate chipping as an effective means of increasing laboratory germination with cicer milkvetch.

Heat has also been used successfully to increase permeability in hard seeds. Aasheim (1), in checking the thermal death point of certain crops, increased germination in alfalfa from 52 to 90%. He used 100C for 15 minutes.

He had a similar response for ruby milkvetch, sweetclover, and yellow trefoil. Swobada (48), using a temperature of 105C for periods of 1, 2, and 3 minutes, found that cicer milkvetch failed to respond to heat treatment and showed only a slight increase in germination. Whitcomb (41) found a temperature of 75C for 3 hours increased germination in alfalfa by 23%. Rincker (33) used a dry heat of 220F for 4 minutes. He was able to show an 81 and 69% reduction of hard seed in alfalfa and red clover, respectively, with a corresponding increase in germination. Hard-seeded sweetclover didn't respond the same as alfalfa to an identical heat treatment. He found that overheating posed a real threat to the viability of specific lots of seed, and samples of seed from the same crop varied from each other in response to the same heat treatment.

Kendall (23) tested the effect of amino acids on germination of red clover seed. He found significant differences in germinations but the differences were not sufficiently consistent to be useful for identification purposes.

Works and Erickson (45) used infrared radiation treatments on hard seeds of alfalfa, red and alsike clover, and sweetclover. Treatments increased germination of all species but sweetclover seed lots responded with only a slight increase in germination.

STANDARDIZATION OF A PROCEDURE FOR GERMINATION OF CICER MILKVETCH

Materials and Methods

Two experiments were designed to determine a standard procedure to be used for germination of cicer milkvetch seed.

The procedure for germination consisted of placing 4 replications of 100 seeds each in 4" x 4" plastic trays on double layers of blotter paper saturated with water. This was done for each treatment. A dark germinator was used and seeds were counted at intervals of 7 and 14 days. All cicer milkvetch seed used in this study was harvested and provided by the SCS Bridger Plant Materials Center, Bridger, Montana.

In the first experiment seed of four ages representing the 1964, 1965, 1966, and 1967 years were germinated. Germination temperatures were 15, 20, 25, 30, and an alternating 15-25C.

In the second experiment the same general procedure and seed were used with the exception that 1964 seed was not included. Germination temperatures were 15-25 and 25C. Two solutions, .2% KNO_3 and water, were used for germination.

Results and Discussion

In the first experiment on germination, temperature significantly influenced germination of cicer milkvetch (Table I). Germination at temperatures of 15-25 and 25C was similar and when averaged across age of seed gave the highest % germination. Mean germinations of 20.9 and 19.8% were obtained for 15-25 and 25C respectively. These low germination percentages are typical of unscarified cicer milkvetch.

Table I. The effect of temperature and age of seed on % germination of cicer milkvetch seed.1/

Temperature in C.	Years seed produced				
	1964	1965	1966	1967	Avg.
	%	%	%	%	%
15C	1.8	1.8	2.5	1.5	1.9 d
20C	11.3	16.8	13.3	6.8	12.0 c
25C	18.3	25.8	24.5	11.0	19.9 a
15-25C	18.5	23.3	30.8	11.3	20.9 a
30C	16.0	15.5	24.8	10.8	16.8 b
Avg.	16.4 c	20.8 b	23.9 a	10.3 d	

1/ Averages followed by the same letter are not significantly different at the .05 level of probability. (Duncans Multiple Range Test).

The mean germination was lowest in the year of harvest. Seed which had been stored for one year had the highest mean germination. This indicated that cicer milkvetch seed gains its greatest increase in germination during the first year of storage. A study by Hafenrichter (18) on storage of cicer milkvetch seed gave similar results. After storage of one year germination began to decline.

In the second experiment, as in the first, no significant differences were found between temperatures of 15-25 and 25C (Table II). A significant difference was again recorded for the ages of seed for the years 1965, 1966, and 1967. The pattern of germination for ages of seed was quite similar to that in the first experiment. Germination temperatures and age of seed had a significant interaction. The 1965 and 1967 seed showed no significant differences between the two temperatures. The 1966 seed had a significantly higher germination at 15-25C than at a constant 25C. One possible explanation is that those seeds which had begun the breakdown of the hard seed coat under storage were less vigorous and the constant 25C temperature was less favorable for their germination.

Significant differences in germination were found for the two solutions when averaged across temperatures and age of seed. KNO_3 depressed germination of 1965 and 1966 seed at both temperatures. Water and KNO_3 gave equal results for 1967 seed at 15-25C, but at 25C, KNO_3 caused a slight increase in germination over water. Age of seed affected germination in solutions. The 1965 and 1966 seed germinated better in water than in KNO_3. Only a slight difference existed between the two solutions for

Table II. Mean germination for 3 ages of cicer milkvetch seed at 2 temperatures in 2 different solutions.1/

	Solution				
	H_2O		KNO_3		
	Temperature		Temperature		
	15-25C	25C	15-25C	25C	Avg.
	%	%	%	%	%
1965	18.3	13.5	18.5	14.0	16.1 a
1966	23.5	16.5	16.8	14.5	17.8 a
1967	7.5	7.5	7.5	8.0	7.6 b

	H_2O	KNO_3
Average Solutions	15.3 a	12.3 b
	15-25C	25C
Average Temperature	14.5 a	13.2 a

1/ Averages followed by the same letter are not significantly different at the .05 level of probability. (Duncans Multiple Range Test).

1967 seed while the 1966 and 1965 seed differed by 17%. KNO_3 did not depress the germination of the 1967 seed. In the 1966 seed which had aged for one year the KNO_3 may have affected those seeds which were marginal in germination strength. This was also true for 1965 seed which also germinated less in KNO_3.

A possible explanation for the depressing effect of KNO_3 might be from increased osmotic concentration of the solution. This could possibly affect the uptake of water by the less vigorous but permeable seed and prevent germination (29).

From these two experiments a standard procedure for use in germination of cicer milkvetch seed was established. Cicer milkvetch seed should be germinated in 4" x 4" trays on blotter paper saturated with water at temperatures of 15-25C for 14 days in the dark. This procedure was used for all subsequent experiments.

EXPERIMENTAL SCARIFICATION OF CICER MILKVETCH SEED USING A VANE-TYPE SCARIFIER

Materials and Methods

A small vane-type scarifier was used for experimental scarification. It operated on the principle of throwing the seed against an abrasive surface causing a fracturing of the seed coat. The two sources of variation were: (1) control of rpm through a rheostat, and (2) number of times (X) the seed was put through the scarifier. One lot of cicer milkvetch seed was passed through the scarifier 1 through 4 times. Four replications of 100 seeds each were germinated, counted, and averaged for each treatment.

Results and Discussion

As the rheostat setting and number of times through the scarifier increased, germination percentage increased (Table III). The exception to this occurred when the seed was run through 4 times at rheostat settings of 55 and 60. This resulted in lower germination and higher death losses due to overscarification. Average death losses for these two treatments were 33 and 36% respectively, which were higher than those for any other treatments. Scarification increased germination from 13 to 56%.

Table III. Effects of degree of seed scarification on germination of cicer milkvetch seed.

Rheostat setting	Number of times through scarifier				Avg.
	1	2	3	4	
	%	%	%	%	%
40	13	26	35	--	24.7
45	13	37	41	39	32.5
50	22	42	46	59	42.3
55	23	36	50	38	36.8
60	28	42	56	38	41.0
Avg.	19.8	36.6	45.6	43.5	

SCARIFICATION OF CICER MILKVETCH SEED IN A SMALL COMMERCIAL SCARIFIER

Materials and Methods

Two scarification trials were carried out for testing commercial scarification. A small Model No. 2 Foresberg Huller was obtained from the Northrup King Seed Company. This machine combined a throwing and rubbing action as a means of scarification. The scarifier consisted of a solid tapered cylinder with cup-shaped teeth that forced the seed against a metal screen which lined the drum. The cylinder traveled at a fixed speed of approximately 1400 rpms. The two variables controlling amount of scarification were cylinder-to-drum clearance and number of times (X) seed was put through the scarifier. Seed from 1966 and 1967 was scarified.

Treatments of 1X through 4X at a single clearance were used in the first trial. In the second trial seed was put through the scarifier from 4X to 10X with the same clearance setting. Mean germination was based on 4 replications of 100 seeds each for each treatment. In the second trial a clean-out percentage was calculated for each treatment to determine the percentage lost through scarification. A 100-gram sample was cleaned and the % loss determined for each treatment.

Results and Discussion

Germination increased with number of times through the scarifier up to 3X in the first trial (Table IV). Scarification was most effective on 1966 seed. Alfalfa put through the scarifier 4X was reduced to flour whereas most of the cicer milkvetch seed remained in a whole state and germination was equal to that at 3X.

Table IV. Average germination of two lots of cicer milkvetch seed when passed through a scarifier from 1X to 4X in a commercial scarifier.1/

Treatment	Age of seed years 1966	1967	Avg.
	%	%	%
Check	22.0	19.5	20.8 d
1X	74.8	45.0	59.9 c
2X	81.5	56.8	69.1 b
3X	75.5	70.8	73.1 a
4X	80.5	75.0	77.8 a
Avg.	66.9 a	53.4 b	

1/ Averages followed by the same letter are not significantly different at the .05 level of probability. (Duncans Multiple Range Test).

In general for the second trial, germination percentage and percentage loss increased with number of times the seed was put through the scarifier (Table V). Normal unscarified seed lost less than 1%, whereas seed scarified 10X lost 30.8%. Germination based on cleaned seed with no allowance for loss was 25% for the unscarified check and 94.8% for 10X. Germination percentage was similar at 4-6X; at 7-8X; and 9-10X. Average germination for this range of settings was 80.41, 86.64, and 93.75%, respectively.

These data show that scarification of cicer milkvetch seed is feasible on a commercial basis. However, setting used for scarification of alfalfa cannot be used as a guide for scarification of cicer milkvetch seed.

Table V. Average germination percentages and percent losses of cicer milkvetch seed scarified from 4X to 10X in a commercial scarifier.1/

Treatment	% germination of cleaned seed	% loss on seed lot
Check	25.0 d	0.4
4X	78.8 c	8.8
5X	81.0 c	9.2
6X	81.5 c	8.9
7X	85.3 b	14.1
8X	88.0 b	28.4
9X	92.8 a	28.1
10X	94.8 a	30.8

1/ Averages followed by the same letter are not significantly different at the .05 level of probability. (Duncans Multiple Range Test).

SCARIFICATION OF CICER MILKVETCH SEED IN LARGE COMMERCIAL MACHINES AND TECHNIQUES FOR RAPID DETERMINATION OF DEGREE OF SCARIFICATION

Materials and Methods

Two experiments were conducted using large commercial scarifiers and two techniques were tried for determination of levels of scarification. In the first study, Crippen Manufacturing Company scarified a lot of cicer milkvetch seed with a Model S Scarifier & Huller. This machine draws the seed into a revolving, circular, abrasive-lined drum by air suction. The seed coat is rubbed as the seed is circulated. The degree of scarification is regulated by control of rpm of the main shaft. Seed was scarified at 4 different rpm settings (Table VI). Four replications of 100 seeds each were germinated for each rpm setting.

In the second experiment a Wes Gro Process Brand Polisher furnished by the Northrup King Seed Co. was used. The principle of scarification by this machine is a rubbing action on the seed coat produced by brushes carrying the seed around a large vertical perforated steel drum. Seed is fed by gravity down through the top of the machine. Treatments consisted of (1) varying tension on the brushes, (2) regulation of feed rate, and (3) number of times (X) the seed was put through the scarifier. Ten treatments including the unscarified check were used. One lot of seed was used. Mean germinations were calculated from 4 replications of 100 seeds for each treatment.

A staining technique developed by Simmons (36) of the University of Wyoming was used for determination of amount of scarification which had taken place. This technique was used on seed processed through the

Table VI. Scarification results from cicer milkvetch seed scarified in a Crippen Large Scarifier.1/

Setting	Avg.
	%
Check	25.3 d
700 rpm	29.5 c
900 rpm	41.8 b
1300 rpm	49.3 a
1600 rpm	51.5 a

1/ Averages followed by the same letter are not significantly different at the .05 level of probability. (Duncans Multiple Range Test).

Wes Gro Process Brand Polisher. The stain was Chromic-Nitric Acid. Seed was soaked in stain for 1 hour and then checked for the percentage of seeds which were stained.

An additional experiment was conducted to determine the potential of using the number of swelled seeds in a 24-hour period as a means of detecting germination potential of scarified seed. Seed from 1966 and 1967 was used. Four treatments and a check were used for each year. Treatments for 1966 and 1967 consisted of putting the seed through from 1 through 4 times (X). All seed was scarified in a Model No. 2 Foresberg Huller. Four replications of 100 seeds each were averaged to obtain the means for treatments. A correlation was run between percent germination and percent swell. The standard germination procedure was used for germination.

Results and Discussion

In the first experiment involving seed scarified by the Crippen Manufacturing Company, germination increased from 25.3% with no scarification to 49.2% with an rpm setting of 1300. A higher setting of 1600 rpms gave no significant increase. This machine which employs a rubbing action was as effective as the machine which employed a rubbing plus fracturing action.

In the second experiment with seed from Northrup King Seed Company's scarifier, untreated seed germination was 28.0% (Table VII). The first setting used was that commonly used for scarification of alfalfa seed. This only raised germination to 36.2% which was significantly different from the check. The highest germination percentage obtained, 84.0%,

Table VII. Results from scarification trials and staining trials.1/

Treatment	Avg. Germination	Avg. Stain
	%	%
Alfalfa setting	36.2 f	8.5 e
3x's tighter	36.5 f	7.3 e
5x's tighter	45.0 e	9.8 de
5x's tighter (1 run)	43.0 e	8.8 e
6x's tighter (1500 lbs./hr.)	46.0 e	13.0 d
6x's tighter (180 lbs./hr.)	56.0 d	17.5 c
5x's tighter (2 runs)	68.0 c	12.5 d
5x's tighter (3 runs)	78.0 b	22.5 b
5x's tighter (4 runs)	84.0 a	35.8 a
Check	28.0 g	8.2 e

1/ Averages followed by the same letter are not significantly different at the .05 level of probability. (Duncans Multiple Range Test).