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GERMINATION AND VEGETATIVE PROPAGATION OF NATIVE PRAIRIE FORBS

BY

JAMES T. SORENSEN

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Botany, South Dakota
State University

1972

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GERMINATION AND VEGETATIVE PROPAGATION

OF NATIVE PRAIRIE FORBS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Botany-Biology Department Date

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JTS

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INTRODUCTION

Public Awareness

In the late 1960's and early 1970's there arose an increased awareness of the ecological relationships of nature to man. Although this relationship has been known for some time and numerous books have been written on the subject, public awareness has been slow in coming. But for some unknown reason man has taken a turn to nature. He is becoming more involved with conservation of nature and appreciative of its aesthetic beauty.

With this new awareness there has come a new era of camping, hiking, and riding, to mention only a few activities which man needs or wants to enable him to enjoy his new relationship with nature. As strange as it may seem, one of these new interests is the prairie. There have been many demands placed on the prairie by man but never one so unique. In his awareness, man now has become concerned over his management of the prairie and now is looking at its aesthetic beauty, historical dialog, and restoration.

Prairie Decline

Prairies once occupied an estimated billion acres or nearly one-fifth of the North American continent, but this community, like no other natural community has been vastly altered by European settlement. Of the once vast expanse of central grasslands, only scattered remnants remain and even these are being rapidly destroyed by intent or neglect (Jenkins 1971).

Investigation by the International Union for the Conservation of Nature so far indicates that about 10 percent of our global flora must now be considered threatened with extinction. An estimated 20,000 plant species may soon disappear. So far, these studies show that many species are endemic to ocean islands having never been subjected to herbivores until man introduced them with his colonization (Tinker 1971).

Areas comprising fairly pure prairie flora today are limited chiefly to railroad right-of-ways, neglected cemeteries and certain islands. Thus, due to man's activities, the prairie community has nearly disappeared (Bland 1970). Kilburn (1970) cited that bluffs and banks of rivers, such as the Mississippi, Illinois, Missouri and Ohio Rivers, form the bulk of relict native prairie stands. With the disappearance of most of the original tall grass prairie from the midwest, there has been, in recent years, an increased interest in the possibility of replanting and restoring a part of this diverse and beautiful grassland community (Schramm 1970).

Prairie Restoration and Uses

In 1963 Morton Arboretum personnel started prairie restoration on 25 acres by transplantings, taken from a seventy-five mile radius, and seedlings grown in a greenhouse. Broadcast seeding was also used. Arboretum personnel did not realize how difficult prairie re-establishment would prove to be, nor how much interest would develop in such a project. By 1968, 120 prairie plant species, covering about six acres, had been planted (Schulenberg 1970). Bland (1970) cited that Michigan

Botanical Garden personnel sum up their work by saying, "Although forty-four prairie species have become established much is left to be found out about prairie establishment." It is important to point out that a restored prairie is a far cry from the original, greatly lacking in diversity and proper composition (Schramm 1970).

With the advent of awareness have come new demands on the prairie but of the nature of conservation instead of destruction. Yet another demand is one of landscaping and designing, where the unique beauty of each plant can be expressed.

Wilson (1971) stated that thousands of years ago Nature designed for the American plains and prairies four grasses that work as well for "people pasture" on city lots as for livestock pasture on the range . . . along with native wildflowers. Landscape architects are depending more and more on what nature originally designed for a region, in addition to, or instead of temperamental exotics that have to be babied and sometimes look out of place.

Mayer (1971) stated that prairie wild flowers have become very popular for aesthetic appeal. They are being used today on the grounds of museums, campuses, arboretums, industrial and residential sites, and highway right-of-ways. There is key interest in these plants because of their aesthetic pleasures; but, more important, once established, they are hardy and require a minimum of maintenance such as watering and fertilizing. These plants have also been found to be highly effective in holding soil particles together and retarding erosion.

Objectives

If prairies are to be restored or prairie plants used for landscaping and other commercial uses, there will be a large demand for seed from forbs which does not exist in sufficient quantity at the present time. This seed source must come from the small relict areas which still possess a gene pool representative of the native prairie. Jenkins (1971) points out that in any natural community there are a few dominant species which are very abundant and a large number of species which are more or less rare. In a prairie in Wisconsin, with 240 species present, 12 species were represented by as many individual plants as all the other 228 species combined. It is these 228 species, the majority of which are forbs, that this thesis is concerned about. Extensive research has been done on the grasses of the prairie, and these grasses can be established with relative ease compared to the forbs. Essentially no research has been done with forbs in comparison to prairie grasses. At present the capability to provide commercial sources of native prairie seeds only exists with some of the grasses and relatively none of the forbs. The objectives of this thesis are two fold: (1) to determine the germination potential of several native prairie forbs, and (2) to determine the vegetative propagation potential of several native prairie forbs.

Germination

Before consistent artificial establishment of a species can be accomplished, its particular germination requirements must be known

(Mayer and Polijakoff-Mayber 1963). In addition to germination requirements, year to year differences in seed quality and quantity affect establishment. Little information has been available on the influence of environmental condition during flowering and seed set on the subsequent germination of a seed crop (Amen 1963).

The importance of environmental influences on seed germination was investigated by Nichols (1934). The effects of winter temperature on more than 200 species, almost all from the northern part of the United States, were studied. Subjection to cold temperature was necessary for germination of 16.3 percent, beneficial for 31.9 percent, without effect on 38.7 percent and detrimental to the germination of 12.1 percent of the species tested. In some instances failure to germinate was due to immaturity at the time of collection; in others, to the short viability of the seed or to its inability to survive desiccation; others, to the greater length of time required after ripening. Nichols not only determined that refrigeration increased germination but also shortened the germination period.

Other conditions, including cold treatment, were studied by Blake (1935). Generally, seeds showed a seasonal response in the amount of dormancy, with greater germination in spring and fall. Dry-frozen seeds generally germinated better than those unfrozen, and stratification increased germination even more, especially for forbs. Blake also found that weather conditions during flowering and seed set appeared to affect subsequent germination, which accounts for differences in germination between annual harvests of seeds. Below normal

temperature and above normal rainfall seemed to increase subsequent germination, while drought lowered germination.

Greene and Curtis (1950) did stratification on 51 species and scarification on 12 species. Stratification benefited 73 percent and harmed 14 percent, while scarification benefited 83 percent and harmed 8 percent. Germination values for the same species under comparable conditions fluctuated greatly for seeds collected in different years. In some cases this fluctuation appeared to be related to the same factors as are affected by stratification, since the variation was greater in the stratified seeds than in the non-stratified.

Comparison of germination results of prairie species from various reports emphasized that there was annual difference in germination and that stratification was beneficial (Christiansen 1967, Tolstead 1941).

In summary, flowering and seed set are greatly influenced by weather conditions accounting for the annual differences in seed germination. Stratification was found to either break dormancy, increase germination or shorten the period for germination. In some instances stratification was found to be detrimental. Scarification was found to be effective on hard seed coats but was detrimental to some seeds.

Vegetative Propagation

Although there are reams of literature on vegetative propagation, there is very little which is applicable to prairie forbs. Most of the literature deals with horticultural varieties.

Chase and Strain (1966) found that stem cuttings of many woody desert perennials could be induced to form roots, contrary to earlier reports. For most of the shrubs which rooted, a compound called ROOTONE was used. Application of the hormone was by the dip method. These cuttings were placed in a rooting box under continuous light with moderate bottom heat. A dip treatment with 200 ppm IAA may have enhanced rooting but was found to give a poorer response than Rootone.

Taylor and Hamblin (1963) used Hormodin or Rootone and found them to stimulate rooting on a large number of prairie plants. Audus (1959) reported a number of compounds, IAA, NAA, and IBA for root stimulation. The majority of plants researched were woody plants or shrubs, not all being native to the prairie.

In summary, there was very little information on vegetative propagation of native prairie forbs. Contrary to some earlier reports, vegetative propagation can be accomplished by use of hormones.

METHODS AND MATERIALS

The methods and materials of this paper are divided into two parts. The first part deals with the germination of native prairie forb seeds while the second part deals with the vegatative propagation of native forbs.

Lawrence et al. (1947) listed essential data that is desirable for completeness of studies on native plants. The data which he recommended as being essential has been incorporated into the methods of this paper.

GERMINATION

Collection

Two prairies were used for collection of seeds; the Sioux Prairie, twenty miles south of Brookings on Highway 77 (T 107N R 50W), and Hide-A-Woods, twenty miles east by northeast of Brookings (T 111N R 48W). These two prairies are similar to the Cayler Prairie in northwest Iowa (Aikman and Thorne 1956).

Seeds of nineteen different native prairie forbs were collected from September 17, 1971, to November 1, 1971. Four different species of seeds were collected from June 6, 1972, to June 21, 1972. Several collections were conducted so that spring, summer, and fall flowering forbs could be tested for germination. All collection was done by hand; and seeds were collected when they were mature, which was at time of drying or at time of dehiscence.

Seeds which required thrashing were thrashed by rubbing on hard corrugated rubber (Hartmann and Kester 1968) and then screened, the screen having 256 holes per square inch. Not all chaff was removed; therefore, seeds were then hand picked. Seeds which were too delicate to be thrashed mechanically were thrashed by hand.

All seeds were stored dry at room temperature, 70-75° F, and in the dark. If seeds are to be stored for more than six months, a low temperature, low humidity, and a sealed container is required to preserve the viability of the seed (Hartmann and Kester 1968, Mayer and Poljakoff-Mayber 1963).

Seed Development and Viability

As seeds set not all seeds fill; consequently, there can be a high proportion of empty seed coats. It becomes important to determine filled seed versus nonfilled seed prior to subjecting the seed to germination tests (Lawrence et al. 1947). This determination was made on ten forbs through physical examination by pinching the seed with forceps.

All filled seeds were tested for percent viability by soaking for 24 hours in 0.1 percent tetrazolium (Hartmann and Kester 1968, Machlis and Torrey 1956). Seeds that have a hard coat were cut in half with a razor blade prior to treatment.

Germination Tests

Normal Germination. Seeds of twenty-three native prairie forbs underwent normal germination. The seeds were treated with ARASAN, a

fungicide, and placed in a petri dish with moist filter paper. Nichols (1934) also used the petri dish method. A growth chamber was used to maintain a constant temperature of 70° F (Greene and Curtis 1959, Tolstead 1941) and complete darkness, except when the seeds were removed for counting germination. This treatment was conducted for a thirty day period with daily recordings as to the number of seeds which had germinated. Once a seed had germinated it was removed from the petri dish. Seeds were considered germinated once the radicle protruded through the seed coat. One hundred seeds were germinated per species, except for those seeds which were limited in number. A germination trial consisted of four replications, twenty-five seeds per replication, totaling one hundred seeds tested. Additional seeds from those species which failed to germinate were then subject to other methods of treatment to determine what method would break seed dormancy.

Scarification. Seeds of seven forbs were scarificated. Scarification was done by placing the seeds between two sheets of sand paper (coarseness number, SP-150) and by lightly rubbing the seeds (Hartmann and Kester 1968). Because of the limited number of seeds, this method was used to minimize seed destruction. Once scarified, the seeds were germinated under the procedure described in normal germination.

Moist Cold Treatment (Stratification). Seeds of thirteen forbs underwent moist cold treatment. Seeds were placed in a petri dish

with a moist filter paper and were chilled in a refrigerator at 38° F (Bland 1970, Christiansen 1967, Hartmann and Kester 1968) for a period of one, two, and three months. At each monthly interval, one hundred seeds were removed and germinated as described in normal germination.

Puncture Treatment. Two forbs, Ratibida[1] and Lilium, were tested for an impermeable membrane. Each forb was subjected to two treatments of 0.1 percent tetrazolium. In one treatment, the seed membrane was punctured with a probe to allow the tetrazolium to enter the seed embryo to determine seed viability. In the second treatment, the seed membrane was not punctured, thus determining by the use of tetrazolium as an indicator whether or not the seed membrane was permeable or impermeable.

Chemical Treatment. Gibberellic acid was used to induce germination of two forbs, Cicuta and Gentiana (Mayer and Polijakoff-Mayber 1963). The seeds were soaked in 100 ppm gibberellic acid for a two-week period and a one-month period and then germinated as described in normal germination.

VEGETATIVE PROPAGATION

Collection

During the months of June and July, vegetative material for the rooting experiment was collected from the Sioux Prairie. Young plants were selected, cut with a knife, and immediately placed in a plastic

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[1]Scientific and colloquial nomenclature is listed on page 43.

bag containing a moist paper towel to prevent wilting. Once the plant had been collected, it was immediately transported to the laboratory and placed in pans of distilled water. The plants were rinsed in distilled water, then placed in a 1 percent solution of calcium hypochlorite for five minutes and then rinsed again in distilled water. Calcium hypochlorite acts as a disinfectant.

All buds, flowers, and lower leaves were removed to reduce transporation and nutritional requirements. A basal diagonal cut of the stem was made with a razor blade, about one inch below a node. The plants were then placed in a rooting jar and on a rooting bench with a sixteen-hour light period.

Rooting Jars

One-half pint jars, with five holes per lid, were used as rooting containers (Machlis and Torrey 1956). Aluminum foil was wrapped around the jar to prevent light inhibition of roots.

An upright wooden stake was secured to the side of the jar to support a one-gallon transparent plastic polyethylene bag. This bag was slipped over the stake and secured by a rubber band around the lid of the jar (Figure 1) to reduce transporation. Coggeshall (1953) stated that polyethylene plastic film is an air permeable, water impermeable plastic that allows for an exchange of oxygen and carbon dioxide, while at the same time retaining the moisture inside the plastic, thereby keeping the humidity very high. Taylor and Hamblin (1963) stated that the success in handling cuttings depends almost entirely on how well

Fig. 1. This shows the apparatus used for rooting. Also illustrated is the use of the polyethylene bag.

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the cuttings are covered. They continued by saying that if the atmosphere becomes dry, failure will result. Taylor and Hamblin recommended the use of polyethylene film for covering of cuttings. Leiss (1970) stated that the most important factor in vegetative propagation is to prevent wilting of the cuttings.

Rooting Media

Three treatments were conducted on each species: a control, consisting of half strength Hoaglands; IBA at 2 ppm with 1/2 strength Hoaglands; and Kinetin at 5 ppm with 1/2 strength Hoaglands. It was later determined that chlorine needed to be added to reduce contamination. To each treatment was added 0.2 mg/l of calcium hypochlorite which resulted in 0.1 ppm chlorine. Each treatment contained two replications with five plants per replication.

After seven days, the old solution was poured out and a new solution added. This was done to retard bacterial and fungal growth and to maintain proper osmotic pressure.

RESULTS

The results of this paper are divided into two parts. The first part deals with the results from the different germination trials and the second part deals with the results from the vegetative propagation experiments.

GERMINATION

Seed viability (Table I) was analyzed with tetrazolium to determine live seeds versus dead seeds, thus giving an indication as to the total potential in the seed's ability to germinate if given all the correct environmental factors. This table shows that the majority of seeds possessed a high potential to germinate. Percent seed viability ranged from 100 percent to 92 percent except for one species, Pedicularis, which was 58 percent.

All species which were subjected to germination trials are in Table I except for the two Liatris species which were infested by weevils and Erigeron because of a shortage of seed.

Percent seed development (Table II) was determined by mechanically pinching the seed coat with a forceps. Ten out of the twenty-three species which underwent germination were subjected to the seed development test. As illustrated in Table II, not all hulls are filled, for seed development ranged from 93.3 percent to 24.5 percent. Determination of seed development was done on only those seeds which were enclosed in a hull.

TABLE I. Seed Viability. This table shows the seed viability of the twenty-three seeds which were subjected to germination trials. Seed viability determination was by means of tetrazolium (Hartmann and Kester 1968, Machlis and Torrey 1956).

Species	Percentage
Achillea millefolium	100
Allium spp.	100
Amorpha canescens	92
Anemone cylindrica	98
Anemone patens	98
Antennaria spp.	96.5
Aster sericeus	100
Astragalus canadensis	96
Astragalus crassicarpus	100
Cicuta maculata	100
Echinacea angustifolia	100
Erigeron strigosus	[a]
Gentiana puberula	92.3
Geum triflorum	100
Liatris ligulistylis	[b]
Liatris punctata	[b]
Lilium philadelphicum	98[a]
Pedicularis candensis	58
Petalostemum spp.	100
Potentilla arguta	100
Ratibida columnifera	100[c]
Vernonia fasciculata	100
Zizia spp.	100

[a] ran out of seed
[b] weevils in seed
[c] after puncture treatment

TABLE II. Seed Development. This table illustrates that not all seeds have matured into a fully developed seed. Those seeds in this table all have a hull which masks whether or not the seed has developed. Determination of seed development was done by pinching the seed with forceps.

Species	Percentage
Amenome patens	91.2
Amorpha canescens	81.0
Aster sericeus	42.0
Echinacea angustifolia	76.5
Geum triflorum	93.3
Liatris ligulistylis	66.5
Liatris punctata	76.5
Petalostemum spp.	24.5
Ratibida columnifera	47.5
Vernonia fasciculata	54.5

The findings from normal germination, i.e., a petri dish, with a moist filter paper, placed in a growth chamber at 70° F, are illustrated in Table III. In this table is found percent germination, number of days required from beginning to end of the germination period, and days required to reach 50 percent and 75 percent germination. Thus, this table not only shows percent germination but rate of germination.

To illustrate the data in this table, Anemone patens will be discussed. Germination (78 percent) began on the eleventh day after the seeds were placed in the growth chamber and stopped on the twenty-fourth day--a total of thirteen days germinating period. On the seventeenth day, six days from start of germination, 50 percent of the seeds had germinated and by the nineteenth day, eight days from beginning germination, 75 percent germination had been attained.

Only one species in normal germination displayed any unusual characteristics. Echinacea was found to have a corky seed covering. When this was removed germination was 92 percent, but when not removed germination was 13 percent.

Table IV illustrates and compares the findings of the one-month moist cold treatment, two-month moist cold treatment, and normal germination. In this table one can compare the beginning time of germination, days duration of germination, and days required to reach 50 percent and 75 percent germination, along with the effect that moist cold treatment has had on the percent and rate of germination. It was found that this treatment had four responses, which are as follows:

TABLE III. Normal Germination. This table shows the results from normal germination, a process where seeds are germinated in a petri dish under controlled conditions. Each germination trial consists of four replications, twenty-five seeds per replication, totaling one hundred seeds per trial.

Species	Percent Germination	Days to Germinate	Days required for 50 Percent Germination	Days required for 75 Percent Germination
Achillea millefolium	87	2-8	3	5
Allium spp.	36.7[a]	16-23	-	-
Amorpha canescens	63	2-18	8	-
Anemone cylindrica	96	13-23	13	14
Anemone patens	78	11-24	17	19
Antennaria spp.	2	7-11	-	-
Aster sericeus	71	2-24	6	-
Astragalus canadensis	8	4-9	-	-
Astragalus crassicarpus	0	-	-	-
Cicuta maculata	0	-	-	-
Echinacea angustifolia	92[b]	2-9	4	6
Echinacea angustifolia	13[c]	5-11	-	-
Erigeron strigosus	70	3-18	6	-
Gentiana puberula	0	-	-	-
Geum triflorum	90	7-16	10	12
Liatris ligulistylis	41	4-26	-	-
Liatris punctata	47	8-22	-	-
Lilium philadelphicum	30[d]	9-17	-	-
Pedicularis candensis	0	-	-	-
Petalostemum spp.	2	3-4	-	-
Potentilla arguta	0	-	-	-
Ratibida columnifera	0	-	-	-
Vernonia fasciculata	0	-	-	-
Zizia spp.	4	10-18	-	-

[a] based on 30 seeds
[b] hull removed
[c] hull not removed
[d] based on 50 seeds

TABLE IV. One and two month moist cold treatment (MCT). This table illustrates the effect of one and two month moist cold treatment. Seeds were refrigerated at 38° F for one and two months and then germinated under normal germinating conditions. Also, normal germination can be compared to one month and two month moist cold treatments.

Species		Percent Germination	Days to Germinate	Days required for 50 Percent Germination	Days required for 75 Percent Germination
Achillea millefolium	NG*	87	2-8	3	5
	1 mo. MCT	79	1-15	3	7
	2 mo. MCT	-[a]	-	-	-
Anemone cylindrica	NG	96	13-23	13	14
	1 mo. MCT	96	5-8	7	7
	2 mo. MCT	98	4-7	3	3
Anemone patens	NG	78	11-24	17	19
	1 mo. MCT	75	7-27	14	27
	2 mo. MCT	82	4-15	9	12
Antennaria spp.	NG	2	7-11	-	-
	1 mo. MCT	20	2-10	-	-
	2 mo. MCT	45	1-6	-	-
Aster sericeus	NG	71	2-24	6	-
	1 mo. MCT	63	2-18	2	-
	2 mo. MCT	-[b]	-	-	-
Erigeron strigosus	NG	70	3-18	6	-
	1 mo. MCT	16	2-8	-	-
	2 mo. MCT	0	-	-	-
Geum triflorum	NG	90	7-16	10	12
	1 mo. MCT	91	3-7	4	4
	2 mo. MCT	80	1-4	3	3
Pedicularis candensis	NG	0	-	-	-
	1 mo. MCT	0	-	-	-
	2 mo. MCT	0	-	-	-
Potentilla arguta	NG	0	-	-	-
	1 mo. MCT	58	3-8	5	-
	2 mo. MCT	49	1-2	-	-
Zizia spp.	NG	4	10-18	-	-
	1 mo. MCT	46	5-12	-	-
	2 mo. MCT	55	1-12	4	-

[a] seeds had heavy mold
[b] seeds starting to germinate in moist cold treatment
*Normal Germination

1. broke seed dormancy
2. increased percent germination
3. decreased percent germination
4. had little or no effect on germination

Potentilla is an example of the moist cold treatment breaking the seed dormancy with one month cold treatment. Both Antennaria and Zizia show how cold treatment has increased percent germination with increasing cold period and how it has also increased the rate of germination. Erigeron is an example of how cold treatment decreased the percent germination by starting with 70 percent under normal germination, 16 percent after one month cold treatment, and 0 percent after two months treatment.

Pedicularis could be used as an example of cold treatment having no effect, however the seeds may be morphologically or physiologically deficient. Anemone cylindrica illustrates how the cold treatment did not affect percent germination but that it did increase the rate of germination. In fact, cold treatment increased the rate of germination of all species except Achillea which decreased in percent germination as well as rate of germination.

Table V illustrates the effect of the three-month moist cold treatment on three of the twenty-three species tested. In this table a comparison can be made with percent germination to length of cold treatment and days duration for germination.

Cicuta and Gentiana both show an increase in percent germination with an increase in cold treatment. However, Vernonia does not display the same trend. At some time during the two-month cold treatment,

TABLE V. Three Month Moist Cold Treatment (MCT). This table shows the effect of three month moist cold treatment. These seeds were refrigerated at 38° F and germinated under normal conditions.

Species		Percent Germination	Days to Germinate
Cicuta maculata	NG*	0	-
	1 mo. MCT	0	-
	2 mo. MCT	5	4-5
	3 mo. MCT	37	1-9
Gentiana puberula	NG	0	-
	1 mo. MCT	0	-
	2 mo. MCT	32	3-13
	3 mo. MCT	55	1-8
Vernonia fasciculata	NG	0	-
	1 mo. MCT	38	3
	2 mo. MCT	0	-
	3 mo. MCT	24	2-5

*Normal Germination

Vernonia apparently went into a secondary dormancy; the percent germination was zero for this period. However, by the end of the third month cold treatment, Vernonia had started to germinate again. This will be more completely considered in the discussion.

The effectiveness and harmfulness of scarification to seven species is shown in Table VI. The effectiveness of scarification in increasing germination can be compared to before and after treatments. The days of duration for germination and days required for 50 percent and 75 percent germination show the rate of change in germination.

The first four species in this table have hard seed coats, and one can see that scarification has been beneficial in breaking seed dormancy or in increasing percent germination.

For example, Amorpha's germination increased from 63 percent to 93 percent. Before scarification, it took eighteen days to germinate and eight days to reach 50 percent germination; whereas, afterwards, it took eight days to complete germination, two days to reach 50 percent, and four days to reach 75 percent germination.

Allium appeared to have a hard seed coat when observed under 20 power magnification, but scarification was found to be detrimental. Zizia and Cicuta both possess a corky seed covering and a low percent germination. This could indicate that the corky seed covering was inhibiting germination, but scarification was found to be detrimental to Zizia and of no benefit to Cicuta.

Table VII illustrates whether or not the puncture treatment was beneficial in increasing percent germination. Both Ratibida and Lilium

TABLE VI. Scarification. This table illustrates the effect of scarification. Seeds were scarified by rubbing the seeds between two sheets of sand paper (Hartmann and Kester 1968).

Species	Percent	Days	Germination 50 Percent	Germination 75 Percent
Amorpha canescens	63*	2-18	8	-
	92	1-8	2	4
Astragalus canadensis	8*	4-9	-	-
	89	1-12	1	2
Astragalus crassicarpus	0*	-	-	-
	96	1-23	3	4
Petalostemum spp.	2	3-4	-	-
	98	1-2	1	1
Allium spp.	26.7*[a]	16-23	-	-
	0			
Zizia spp.	4*	10-18	-	-
	0	-	-	-
Cicuta maculata	0*	-	-	-
	0	-	-	-

*results from normal germination

[a]based on 40 seeds

TABLE VII. Puncture Treatment. This table shows two species which have an impermeable membrane to water. Tetrazolium was used as an indicator to determine if puncture, or acetone, or ether treatments were beneficial in breaking the seed membrane.

Species	NG* no Puncture	NG Puncture	Tetrazolium no Puncture	Tetrazolium Puncture	Acetone and Tetrazolium	Ether and Tetrazolium	MCT**
Ratibida columnifera	0%	95%	0	100%	0	0	11%
Lilium philadelphicum	30% [a]	79% [c]	0	98%	0	0	66% [b]

* normal germination
** moist cold treatment
[a] based on 50 seeds
[b] based on 30 seeds
[c] based on 24 seeds

were benefited by puncture, indicating an impermeable membrane or wax coating. Tests with tetrazolium, without puncture, indicated non-viable seeds; but after puncture Ratibida had 100 percent and Lilium 98 percent seed viability which indicated the barrier is to water. Both species were soaked in acetone and ether for five minutes and then tested with tetrazolium with negative results. Therefore, the barrier is probably not a fat or wax layer but an impermeable membrane. The membrane coating of Lilium is less impermeable than Ratibida, for under normal germination Lilium germinated 30 percent and Ratibida 0 percent.

It should also be noted that when subjected to one month cold treatment, Ratibida germinated better than normal germination but not as well as with the puncture treatment. For Lilium, the puncture method was better than normal germination, but the cold treatment was best.

Chemical treatment with gibberellic acid was conducted on the two species requiring the longest after-ripening time (Table VIII). This treatment had no effect on Cicuta but it did shorten the length of time for Gentiana to start germination.

VEGETATIVE PROPAGATION

The results of the vegetative propagation experiment were inconclusive, for when field material was used no roots developed. The main difficulty encountered was bacterial or fungal growth in the liquid rooting medium which resulted in decomposition of the cuttings.

TABLE VIII. Chemical Treatment. This table shows the effect gibberellic acid has on breaking long after ripening periods.

Species		Percent Germination	Days to Germinate
Cicuta maculata	NG*	0	-
	2 week	0	-
	1 month	0	-
Gentiana	NG	0	-
	2 week	30	7-25
	1 month	36	4-22

*Normal Germination

However, when these same forbs were grown from seed in laboratory conditions and subjected to the same method of rooting, some species rooted, as is illustrated in Table IX. Phaseolus vulgaris was used as a control species because of its rootability and because it would also serve as a means of evaluating the rooting method employed.

TABLE IX. Preliminary Vegetative Propagation Trials. This table shows the results of preliminary vegetative propagation trials of laboratory grown forbs.

Species	Control	IBA	Kinetin
Phaseolus vulgaris*	+	+	+
Achillea millifolium	0	+	0
Amorpha canescens	0	+	0
Astragalus canadensis	0	+	0
Astragalus crassicarpus	0	+	+
Petalostemum spp.	0	0	0

+ developed roots
0 failed to root
* variety-bountiful

DISCUSSION

As mentioned earlier, there is neither a sufficient quantity nor quality of native prairie forb seeds available at present to be utilized on a commercial basis. If this seed is to be made available for public use, the seeds must first be collected and then studied.

At present, collection of seed is a laborious process, all of it being done by hand. One can see from Table I that once the seed is collected, seed viability is high. In other words, when the seed is subjected to the right environmental conditions, one is insured of a successful germination. But, on the other hand, Table II illustrates that not all seeds which were collected developed seeds; thus, leaving the researcher or commercial producer the problem of determining what conditions favored good seed set. Amen (1963) cited that there is little information available on the influence of environmental conditions during flowering and seed set on the subsequent germination of a seed crop. Blake (1935) stated that weather conditions during flowering and seed set appear to affect subsequent germination and to account for differences in germination between annual harvests of seeds. Blake continued by saying that below-normal temperature and above-normal rainfall appeared to increase subsequent germination while drought lowered germination.

Not only was seed development a problem in collection, but little is known about the best time for collection. If seeds were left to mature on the plant, the researcher found that these seeds were

sometimes quick to disperse once they reached maturity. The composites were especially bad, for there would be seed one day and most of it could be gone the next day. One solution to this problem would be to collect the seed in the dough stage as is done in agronomic crops. This method of collection would probably work on some and not on others.

Prior to commercial use of native prairie forb seeds, the environmental characteristics must be known so that the seed can be successfully germinated on a large scale. In this paper, the environmental characteristics of twenty-three native prairie forbs were studied. Of these forbs, sixteen species or 69.5 percent germinated under normal conditions--normal conditions described under normal germination in the methods and materials. Those seeds which germinated under normal conditions present no real problem for commercial use. On the other hand, those seeds which did not germinate do present a problem, and it will be those seeds which are discussed in the rest of this paper.

Seeds have been genetically adapted to the environment through natural selection. It has been this process of selection which has developed a number of mechanisms which result in seed dormancy. Seed dormancy can result from physiological or morphological characteristics, such as temperature or light requirements, permeability of seed coat, or inhibitors. In this study, light requirement for seed dormancy was not dealt with.

The moist cold treatment was utilized in breaking temperature dormancy. During this treatment, after-ripening took place, resulting in responses such as embryonic growth or metabolic change in the embryo

(Hartmann and Kester 1968, Mayer and Poljakaff-Mayber 1963). Under this mode of treatment four responses were noted: the breaking of the dormancy of those species which did not germinate under normal conditions, increased germination and rate of germination, decreased germination, and no effect on germination. These same results were also noted by Nichols (1934) where he stated that stratification was necessary for 16.3 percent, beneficial for 31.9 percent, detrimental to 12.1 percent and without effect on 39.7 percent.

Greene and Curtis (1950) found that stratification benefited 73 percent and was harmful to 14 percent. Tolstead (1941), Christiansen (1967), and Blake (1935) found the same basic response to stratification or moist cold treatment.

It was found that different lengths of time were required to break dormancy. Potentilla required only one month whereas Gentiana required two months. Cicuta required two months, but germination was very low and by three months germination had increased considerably.

Vernonia presented an interesting aspect of secondary dormancy. This species germinated after one month of cold treatment and failed to germinate after two months. However, after three months, Vernonia started to germinate again. Germination generally resulted when all correct stimulants were present, resulting in growth. When a stimulus was removed, the seed then went into this secondary dormancy.

It was noted while Vernonia was undergoing moist cold treatment that the petri dishes became somewhat dry. Hartmann and Kester (1968) stated that during the drying of seeds in moist cold treatment, the seeds would go into secondary dormancy.

A very widespread cause of seed dormancy is the presence of a seed coat. Mayer and Poljakoff-Mayber (1963) stated that the seed coat is usually a multi-layered membrane containing a number of layers or cells. The impermeability of the seed coat, or its selective permeability, is frequently the cause of dormancy. They continued by saying that a hard seed coat is resistant to abrasion and may be covered with a wax-like layer resulting in impermeability to water, impermeability to gases, or mechanically constraining the embryo.

The method most commonly used to break hard seed coat dormancy is scarification. The results of this study show that four species benefited from scarification, all of the family Leguminosae, this family having a hard seed coat which is impermeable to water. Not only did scarification break seed dormancy, but it increased the percent germination and rate of germination of those species which germinated under normal conditions. It should also be noted that scarification was harmful to three species. Greene and Curtis (1950) also found scarification both beneficial and harmful. Of the species tested, 83 percent benefited and 8 percent were harmed.

Not only does seed dormancy result from a hard seed coat but also from an impermeable membrane or paricarp which is not hard but impervious to water or gases. Both Ratibida and Lilium have this characteristic of an impermeable membrane. From the results, one can conclude that the dormancy of these two species is due to an impermeable membrane. Also, one-month cold treatment has broken this dormancy by apparently reducing impermeability of the membrane.

Often in the seed coat are chemical inhibitors which will act as a dormancy mechanism until they are removed through leaching, digestion, or removal of the seed coat. In this study, three species, Echinacea, Zizia, and Cicuta, were investigated for inhibitors.

Echinacea was found to have a higher germination when the seed coat was removed (Table III). Water extracts of the seed coats were made at levels of one, two, and three times as concentrated as in the normal seed coat. Seeds without seed coats were germinated with these extracts. The extracts appeared to have some effect but no conclusive results were obtained.

The seed coats of Zizia and Cicuta were removed by scarification, thus removing any inhibitor which might exist (Table VI). The results were nonconclusive for neither species germinated. It is felt that failure to germinate is a result of scarification being damaging to the seed, since Zizia germinated under normal conditions. Greene and Curtis (1950) also scarified Zizia and found this treatment to be detrimental. It was found that both Zizia and Cicuta increased in germination in proportion to length of time in cold treatment. This increase could be a result of the leaching of inhibitors or just metabolic change within the embryo.

Seeds which fail to germinate or require extra long treatments to break dormancy can be subjected to stimulants, such as hormones, which may shorten the time needed to germinate or break dormancy. Both Cicuta and Gentiana germinated when subjected to the moist cold treatment, but the length of time was the longest required by any of the

species and germination was low. Both of the species were treated with gibberellic acid. This treatment shortened the length of time required for germination of Gentiana but had no effect on Cicuta (Table VIII). The actual effect gibberellic acid has on the seed is difficult to determine. Mayer and Poljakoff-Mayber (1963) stated that gibberellic acid has the ability to reverse the inhibition of germination caused by high osmotic pressure. Cleland (1969) stated gibberellic acid can activate amylase in the endosperm. Amylase is a hydrolytic enzyme which converts the starch in the endosperm to glucose, which then can be utilized by the embryo as energy for growth. More research with hormones is needed before their actual effect will be known.

Pedicularis was the only species out of twenty-three which failed to germinate. It is difficult to way why. Its failure to germinate could be attributed to the fact that the seed had not matured, for seed viability was only 58 percent. Those which indicated viability may not have been physiologically or morphologically developed enough to germinate, thus requiring a longer time after ripening.

From this study several comparisons can be drawn from other studies which have done germination tests on native prairie plants. Table X shows a comparison between methods used in normal germination. In this study and Nichols (1934) the petri dish method was used, while Greene and Curtis (1950) and Blake (1935) used soil flats. One can see that the petri dish method had a much higher overall percent germination. It should be noted that 50 percent and 75 percent germination levels were also higher in the petri dish method.

TABLE X. Results of Normal Germination from Four Studies. This table shows a comparison between the petri dish and soil flat methods of germination.

	Author			
Response	Sorensen[x] 1972	Green and Curtis[xx] 1950	Blake[xx] 1935	Nichols[x] 1934
Average number of species which germinated over 50 percent	30.7%	9.8%	7.4%	29.5%
Average number of species which germinated over 75 percent	17.3%	3.9%	0	10.2%
Average of those that did not germinate	30.4%	39.0%	11.1%	-
Overall average germination	35.6%	13.7%	11.2%	38.7%
Number of species	23	51	27	88

[x] germination in petri dish
[xx] germination in soil flats

The difference between the results of these two methods can be attributed to the fact that soil flat germination represents seedling survival whereas the petri dish method represents the actual germination.

Although all five studies of moist cold treatment varied in methods of treatment, four basic responses were found (Table XI). Of these studies 40.9 percent germinated better after cold treatment, while 26.2 percent germinated only after treatment, 18.7 percent showed no effect from treatment and 17.1 percent were harmed by cold treatment. The most striking thing about cold treatment is that these same four responses have been noted even though method of treatments has varied.

The objectives in studying vegetative propagation of native prairie forbs are not to substitute this method over seeding in establishing prairie species but to use this method as a tool to aid in obtaining seed or in propagation of unusual varieties. For example, on the Sioux Prairie there has been found a wide genetic variation in Phlox pilosa, Echinacea angustifolia, and Rudbeckia hirta, to mention only a few. By seed collection, if the seed is available, or by vegetative propagation, these genetic variations could be propagated under conditions which would insure seed collection. Vegetative propagation is of particular value to species such as Psoralea esculenta, for it is very difficult to obtain the seed of this plant. Thus, vegetative propagation can be used to secure this seed under laboratory conditions.

TABLE XI. Results of cold treatment from five studies. This table shows the cumulative results of five studies on cold treatment as a means of breaking or enhancing germination.

Response	Author					Average
	Sorensen 1972	Greene and Curtis 1950	Tolstead 1941	Blake 1935	Nichols 1934	
Germination only after treatment	30.7%	39.2%	28.6%	16.2%	16.3%	26.2%
Germination better after cold treatment	23.1%	33.3%	57.2%	51.2%	38.7%	40.9%
No effect from cold treatment	15.3%	13.9%	-	18.9%	30.5%	18.7%
Cold treatment harmful	30.7%	13.7%	14.2%	13.5%	13.5%	17.1%
Number of species	13	51	28	37	141	

Vegetative propagation consists of a number of methods, one of these being used in this study. Others are the dip method, root cuttings, or division, each method having its advantages and disadvantages. Taylor and Hamblin (1963) listed a large number of wild flowers which can be vegetatively propagated. The method most often used by Taylor and Hamblin was division; the use of hormones was seldom listed by them.

Probably the most universal way to propagate vegetatively is by division and the least used is the one studied here. The reason for the selection of the method used here was two fold. First, the collection of a portion of the plant could be used for propagation with no threat to the survival of that plant. This is especially important to rare or endangered species. The method of division or root cutting leaves no alternative but to take the parent plant from the field. If the plant does not survive, the plant or species may become extinct.

Secondly, the liquid culture rooting method could be controlled. Often, hormones are applied by the dip method and then the cuttings are placed in a porous rooting media, such as sand. This was the method used by Chase and Strain (1966) and the method suggested by Taylor and Hamblin (1963) which has been found to be successful in vegetatively propagating prairie plants. The major disadvantage of this method is that the concentration of the hormones is never known. Therefore, the liquid media for rooting was selected because the concentration can be known and it is not detrimental to the parent plant.

Hormones selected were indolebutyric acid (IBA) and kinetin. IBA was selected because of its universal ability to form roots and its stability over indoleacetic acid. Kinetin was selected because of its ability to form callus. Those plants which would not root with IBA might form callus with the aid of kinetin, which then could be subjected to IBA to stimulate root development.

Preliminary trials of the liquid rooting method proved to be feasible, as shown in Table IX. IBA worked well on all species but one, while only one species responded to kinetin. However, when this method was used on field material, contamination became a major problem even though the cuttings had been disinfected with 1 percent calcium hypochlorite solution. Contamination was almost eliminated when 0.1 ppm chlorine was put into the rooting medium, thus nearly eliminating the contamination. Leiss (1970) cited the use of chlorine to kill fungi and bacteria in water which was being used for rootings.

Other than contamination, one of the possible reasons for lack of rooting was the time the cuttings were taken. Hitchcock and Zimmerman (1946) found that the change in structure or protoplasm of the plant with age was a major controlling factor on the mechanism in plants through which chemicals acted and roots were formed. They continued by saying the apparent age factor could be generally overcome with the aid of hormone-like substances which served as chemical growth regulators. Hitchcock and Zimmerman (1932) stated that when maximum rooting was obtained at a particular time of the year, it was difficult to tell whether such results were due entirely to age of shoot

or to some difinite portion of the cutting. Hess (1951) suggested that there are inhibitors or lack of certain cofactors which make some plants difficult to root.

Roberts (1969) found that buds on Douglas fir cuttings had a great deal to do with their rootability. These buds appeared at various times to be a source of inhibition, promotion, and competition.

Leiss (1970) summed up vegetative propagation by saying the propagator himself and his experience in propagation--when and how to take cuttings--by feel, so to speak, is very important; this is where science has failed us so far. This feel cannot be described. It is intuition which you either have or you do not.

There are many factors which affect the rootability of plants and there has been little work done with the native prairie forbs, but there is no reason why these plants should behave in any different way than agronomic plants do. As research proceeds in this area some plants will be easy to root and others difficult. The biggest problem is to determine the correct time to take cuttings and the best method for rooting.

SUMMARY

As yet there is not a large quantity of native prairie forb seed available; this study as well as other studies indicate that native forb seed can be successfully germinated. Seed viability poses no real problem, but seed set does present some difficulties. However, once this seed is raised agronomically, environmental conditions can be controlled to some extent and seed set will probably no longer be a problem. At present, seed collection is a laborious process; but once these forbs are raised as one-stand crops, mechanization will then solve the problem in collection.

Of the twenty-three species studied it was found that 69.5 percent (16 species) germinated under normal germination, 21.7 percent (5 species) required moist cold treatment before the seeds would germinate, 4.4 percent (1 species) required scarification before germination and 4.4 percent (1 species) never germinated.

Of the thirteen species subjected to moist cold treatment it was found that there were four responses: 30.7 percent germinated only after cold treatment, 23.1 percent increased in germination percentage, 15.3 percent had no effect and moist cold treatment was harmful to 30.7 percent. These same four responses have also been found in other studies of cold treatment.

Scarification was found to be beneficial to 57.1 percent and harmful to 42.9 percent. It should also be noted that scarification not only broke seed dormancy; but on those seeds which germinated normally,

scarification increased the rate of germination. Other studies have also found scarification to have this same effect.

Chemical induction of germination with gibberellic acid was found to be beneficial to only one of the two species tested. Gibberellic acid did increase the rate of germination and percentage of germination.

Below is a consolidated listing of all forbs studied giving scientific name, colloquial name and the best method for germination:

Scientific Name	Colloquial Name	Best Method For Germination
Achillea millefolium L.	Common yarrow	Normal germination
Allium spp. L.	Wild onion	Normal germination
Amorpha canescens Pursh	Lead plant	Scarification
Anemone cylindrica Gray	Meadow anemone	Moist cold treatment
Anemone patens L.	Pasque flower	Moist cold treatment
Antennaria spp. Gaertn.	Pussy toes	Moist cold treatment
Aster sericeus Vent.	Silky aster	Normal germination
Astragalus canadensis L.	Milk vetch	Scarification
Astragalus crassicarpus Nutt.	Buffalo bean	Scarification
Cicuta maculata L.	Water hemlock	Moist cold treatment
Echinacea angustifolia DC.	Purple coneflower	Normal germination
Erigeron strigosus Muhl.	Daisy fleabane	Normal germination
Gentiana puberula Michx.	Downy gentiana	Chemical treatment
Geum triflorum Pursh	Prairie smoke	Normal germination
Liatris ligulistylis (Nels.) K. Scham.	Rocky mountain gay feather	Normal germination
Liatris punctata Hook.	Dotted gay feather	Normal germination
Lilium philadelphicum L.	Wood lily	Puncture treatment
Pedicularis candensis L.	Common lousewort	Unknown
Petalostemum spp. Michx.	Prairie clover	Scarification
Potentilla arguta Pursh	Tall cinquefoil	Moist cold treatment
Ratibida columnifers (Nutt.) Wooton and Standl.	Prairie coneflower	Puncture treatment
Vernonia fasciculata Michx.	Western ironweed	Moist cold treatment
Zizia spp. W. D. J. Koch	Alexanders	Moist cold treatment

In other studies, vegetative propagation has been successful with native plants. However, in this study it was found that the liquid rooting method became contaminated easily when field material was used; but when laboratory grown forbs were used contamination was not a problem. However, when 0.1 ppm calcium hypochlorite was added to the liquid medium the contamination was reduced considerably. It is felt that the morphological and physiological age of the plant has profound effect in determining whether or not a plant will root. This study indicates that vegetation propagation is important in propagating genetic variations and in securing rare or endangered plants under laboratory conditions.

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