

AN ABSTRACT OF THE THESIS OF

Judy Ka	aye Schnell	for the <u></u>	of Science Degree
in	Biology	presented on	<u>October 3, 1990</u>
Title:	Seed Ecology	y of Illinois Bundl	eflower
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Desmanthus illinoensis

Abstract approved: James M Mayo

Seed ecology of Illinois bundleflower (<u>Desmanthus</u> <u>illinoensis</u>) was studied by looking at several aspects of ecology. Germination at varying temperatures with six different scarifying techniques was assessed. Scarification, which included mechanical and chemical, was observed at 10C, 13C, 17C, and 24C. Germination rates varied from 2% to 99%, depending on treatment. Germination increased with all scarification treatments and suggests that germination is inhibited by an extremely hard seed coat.

Heat treatments simulating fire were applied for eight seconds and thirty seconds at temperatures ranging from 100C to 500C at 50C intervals. Germination percentage of all heat-treated seeds was greater than the control. This indicates that Illinois bundleflower could be considered a fire species.

The effect of moisture stress on Illinois bundleflower seed germination was determined using mannitol as the osmoticum. Mannitol solutions varying from -0.033 MPa to -2.5 MPa were used to germinate Illinois bundleflower seeds after a 10 minute scarification in concentrated sulfuric acid. Illinois bundleflower seeds germinated at water potentials as low as -1.0 MPa. This suggests that Illinois bundleflower seeds can germinate at relatively low soil water potentials.

Xylem pressure potentials of Illinois bundleflower seedlings were measured to determine lethal water potentials reached before death. Scarified seeds (10 minutes in concentrated sulfuric acid) were germinated, then planted 50 to a pot and placed in the greenhouse. Plants were watered every other day until they were 6" high. Two pots were kept as watered controls and water was withheld from all other plants. Xylem pressure potentials of the plants were checked with a pressure chamber. After obtaining xylem pressure potentials, the plants were watered and observed to see if plants recovered or died. Illinois bundleflower seedlings withstood 15 days of drought conditions and a xylem pressure potential of -3.5 MPa. Any xylem pressure potential below -3.5 MPa resulted in death for the seedlings.

The possibility for ley farming techniques was applied using Illinois bundleflower in an established wheat field. Plots were established and seeded at a rate of 10 lbs/a, or 171 seeds in a 9 ft² plot. Seeds were scarified in concentrated sulfuric acid for 10 minutes before planting. Rows of Illinois bundleflower were planted between wheat rows and the plots were checked periodically for establishment. Plants were counted by row and plot. Biomass will be checked after this year's wheat harvest (i.e. year 2).

SEED ECOLOGY OF

ILLINOIS BUNDLEFLOWER

(Desmanthus illinoensis)

A Research Thesis Submitted to The Division of Biological Sciences Emporia State University

In Partial Fulfillment of the Requirements for the Degree Master of Science

by

Judy Kaye Schnell October 4, 1990

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ACKNOWLEDGEMENT

Many thanks are extended to the faculty at Emporia State University who have given me moral support throughout my thesis work. Extra special thanks are given to Drs. Tom Eddy and Richard Schrock for their help and for serving as committee members during my thesis work. Special thanks also belong to Dr. David Edds for his encouragement and his willingness to help when Dr. Mayo was not available. His dedication and new ideas have been of great help. Thanks also to Dr. Rod Sobieski for his help and support. His work with the graduate students should be commended.

Special thanks belong to Dr. James Mayo. His outstanding knowledge in his field of biology (ecophysiology) and his willingness to convey his knowledge to his students have made me realize the importance of plants and soils in our society today. His understanding and support have been unending. He has not only been an excellent instructor, but a good friend as well. Without his support and ideas, this thesis would not have been successful.

Special thanks to my children, Amber and Thorin Schnell, for their support and endurance of the many hours spent at work on this thesis. Also many special thanks to Warren Voorhees for his help with the field work and his moral support and love to my children and me. He has made my work much easier and more meaningful. Special thanks also belong to my dear friends, Bob Prather, Tanya Tims, Greg Taylor, Craig Romery, and Mark LaBarge. Without their support and their "two cents", my college career would not have been the same.

Thanks also belong to my father, W.R. Moore and my sister, Dorothy Hollabaugh. Without their moral support and of course my dad's financial backing, I never would have been able to finish.

Many thanks belong to Dr. Richard Keeling for his cartoon drawing in the back of this thesis and to Gary Anstey for his pencil drawing in the front of this thesis.

Special thanks also to Thea Anstey for her assistance in the greenhouse, and her moral support throughout this entire research effort.

INTRODUCTION

Illinois bundleflower (Desmanthus illinoensis) is a warm season, perennial herbaceous legume (Fig. 1). Illinois bundleflower, according to Jones and Luchsinger (1986), belong in the Order Fabales; Family Mimosaceae. However, Radford, Dickison, Massey, and Bell (1974) place Illinois bundleflower in the Order Rosales; Family Leguminosae (segregate order Fabales). The genus was derived from the Greek words (desme) "bundle" and (anthos) "flower" - because the inflorescence is a head composed of many small flowers. The name illinoensis was derived from (ensis) "from" and by the specimens of Michaux, a french botanist, who established the species in the Illinois territory in 1803 (Stevens, 1943). It is a prolific seed producer which produces seed heads in late June-August. The immature fruits occur in loose pods, which contort, mature and form a bundle (Fig. 2). According to The Flora of the Great Plains, Illinois bundleflower can be found readily on rocky, open wooded slopes, prairies, ravines, stream banks, roadsides or waste places (McGregor et al, 1986). McGregor et al (1977) and Gates (1940) have noted that Illinois bundleflower occurs extensively throughout the state of Kansas, whereas Weaver and Fitzpatrick (1980) make no mention of Illinois bundleflower in their monograph on the Kansas prairie.

Illinois bundleflower is listed as a plant normally found in climax prairie communities. Illinois bundleflower

Figure 1. Photograph of <u>Desmanthus illinoensis</u>.



Figure 2. Seed pods of Illinois bundleflower.



is reported to be readily eaten by all classes of livestock and is rated by some authorities as the most important native prairie legume (McGregor et al, 1986). Illinois bundleflower is commonly used in range revegetation programs by the Soil Conservation Service for wildlife purposes (Pritchard, 1989). It is used for revegetation of disturbed and deteriorated rangelands in Texas and Oklahoma (Call, 1985).

Since Illinois bundleflower has not been found in a climax community (Weaver et al, 1980), questions have been raised as to what classification it deserves. With this study, I will try to clarify the successional status of Illinois bundleflower.

The objectives of this study are to evaluate; 1) the germinability of Illinois bundleflower, and what techniques promote germination; 2) the effect of temperature upon germination; 3) the effect of water potential upon germination; 4) does heat from native prairie fires play a role in germination; 5) the effect of drought (xylem pressure potential) upon seedling growth and development; and 6) since Illinois bundleflower is a legume, can it be used in place of red clover in ley farming techniques? Each of these aspects of Illinois bundleflower ecology is discussed in detail in the following pages.

Germination

Germination is a subtle measure of seed quality. Total germination and the rate at which it is achieved represent accurate measures of seed quality (Dunlap and Barnett, 1984). Germination and methods used will show us the quality of native seed and what the germination rate might be in the natural environment.

Illinois bundleflower has an extremely hard seed coat, which requires scarification or stratification in order to germinate. Scarification techniques have been tried before by Everitt & Gausman (1984) and Call (1985). Everitt & Gausman (1984), soaked Illinois bundleflower seed from 3-5 minutes in concentrated sulfuric acid (H_2SO_4) which increased their germination. Call (1985) tried a chemical scarification treatment of soaking in concentrated H_2SO_4 for 15 minutes and mechanical scarification by cutting the seed coat at the end of the seed opposite the micropyle. Various germination techniques for native plants have been reviewed by Young & Young (1986). Techniques recommended include chemical scarification, mechanical scarification, stratification and a chemical (usually NO_3) treatment.

Scarification is the partial removal of the outer seed coat by chemical or mechanical means. Chemical scarification of Illinois bundleflower with concentrated (H_2SO_4) is recommended by Young & Young (1986). Since 3-5 minute and 15 minute soaking intervals had previously been tried, a shorter and a longer time interval were used. Soaking times of 1 minute, 10 minutes, and 30 minutes were adopted.

Mechanical scarification has previously been tried by Call (1985). The seed coat was removed by cutting the seed at the end of the micropyle.

Stratification is another common seed treatment used to promote germination. Stratification inhibits germination in order to promote germination at a later time. Stratification techniques recommended by Salisbury and Ross (1985), consist of placing seeds in a moist environment, cold or warm, for varying time periods. Both stratification techniques were tried.

Chemical treatments are often used to increase germination. Even though no references were found concerning the use of chemical treatment of Illinois bundleflower, potassium nitrate solutions (usually 2%) were reported to improve germination of other species (Copeland and McDonald, 1985).

The techniques described in the previous pages suggest the best means of increasing Illinois bundleflower germination. The important question is what time of the year does Illinois bundleflower germinate (i.e. what is the optimum temperature for germination?). Is seed germination most likely to occur in winter, spring, or summer? Since we know very little about the plant, four temperatures were chosen in which three of the four germination techniques would be performed. Temperatures chosen were 10C, 13C, 17C, and 24C.

Fire Scarification

A fire species is one which must have heat from a native prairie fire, forest fire, etc., in order for germination or for seed release to occur. An excellent example of a fire species is Jack and Lodgepole Pine. Seeds of these pines are released only after the pine cones have been exposed to heat (U.S.D.A). Illinois bundleflower having such a hard seed coat, needs some type of scarification in order to increase germination. There are no studies of the direct effect of fire on Illinois bundleflower germination. Temperatures during native prairie fires have been measured (Wright and Bailey, 1982). Depending upon where seeds are located (on the plant or on the ground) the temperature can be quite different. Using this information, a range of temperatures from 100C to 500C was administered at time intervals of 1 min and 30 sec. Some seeds were whisked through fire three or four times. The whisking effect attempted to simulate conditions of seeds that were in soil or covered by damp litter. Results of these tests determined the role fire plays in germination (i.e., if Illinois bundleflower can be considered a fire species).

Effect of Moisture Stress on Germination

Germination can also be effected by exposing seed to different levels of moisture stress (i.e. water potential). Biochemical processes, as well as physical process, cannot take place unless tissue hydration occurs. (Hegarty and Ross, 1978). Moisture available to the seed governs the amount of water a seed can imbibe. Moisture is available only over a limited range of soil water potentials. Field capacity (= -0.033 MPa) is reached when soil contains all the water it can hold against gravity. This is optimum for germination of most species. However, different species may germinate better at a lower soil moisture content (i.e. water potential). Some seeds may even be able to germinate near the permanent wilting point. The permanent wilting point is reached when a test species, even if placed in 100% relative humidity overnight, cannot obtain water to overcome wilting (Salisbury and Ross, 1985). The level of drought stress that Illinois bundleflower can withstand and still germinate will indicate if it is of potential use in Kansas in reseeding old fields.

Effects of Water Stress on Seedlings

Another important step in reseeding is to determine how much stress seedlings can withstand. The moisture stress point in seedlings can vary from moisture needed for seed germination. Moisture stress in plants occurs whenever the

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transpiration rate exceeds the water absorption rate, so plant cells are less than fully turgid (Mastalerz, 1977). As moisture stress continues, stomata will close and photosynthesis is reduced. Further stress can impede protein synthesis, leading ultimately to death. The amount of stress a seedling can withstand will determine the extent of drought Illinois bundleflower can withstand.

Potential Use of Illinois Bundleflower in a Crop Rotation

A major concern to many people is protecting the environment. A common method of dealing with weeds in farming is spraying with pesticides. Even though 430 million pounds of pesticides are applied yearly to United States crops, crop damage appears to have increased (Lipske, 1990). One way to reduce the massive use of pesticides is by adopting natural farming techniques. Some farmers in Eastern Kansas have adopted a natural alternative to indiscriminately using chemicals for crop dusting. Red clover is established in wheat fields not only to control weeds in winter wheat, but to nourish the soil with nitrogen. Sweetclover is also used in a similar manner. It is one of the most drought-resistant legumes. It grows on heavy clay soils and rocky terrain, and is tolerant to poorly drained soils. During a two year period of growth, sweetclover adds more nitrogen to the soil than any other legume. Approximately 100-120 pounds of nitrogen is made

available to the following crop (Reinhardt et al, 1978). Sweetclover, which is currently being used in Eastern Kansas, cannot be used in Western Kansas due to drought sensitivity. If clover is harvested along with wheat in Eastern Kansas, the green clover can ruin the entire crop of wheat or straw. Yellow sweetclover also represents the same type of problems. What type of legume remains short, blooms later in the summer or after wheat harvest, and is a drought hardy plant? Illinois bundleflower was tested for use as a substitute for red clover, by planting it between rows of It is known from past studies by Linda Strange (ESU wheat. special project) that Illinois bundleflower is capable of nitrogen fixation and should provide nitrogen to the soil. The degree of drought hardiness is tested by the seedling stress tests.

Another aspect of Illinois bundleflower is its use as a component in ley farming. Ley farming is the growing of grass or legumes in rotation with grain or tilled crops as a soil conservation measure. Ley farming has become exceedingly popular in Australia and has been carried over to remote areas of Africa. In the early phase of Southern Australian agriculture, scrub lands were cleared to grow wheat. Because of the low initial fertility of the soils, yields of wheat soon declined because of the depletion of nitrogen and phosphorus in the soil. Soon after large increases in the price of wool, farmers reduced acreage of farming to a minimum and made sheep production their major source of income. Annual legume pastures were widely sown, and became integrated with the areas cropped. The major part of the improvement came in rotation of pastures sown in rotation with wheat. Production of wheat in some areas increased as much as 68% and protein content of wheat grain rose approximately 2% (Puckridge and French, 1983). Since this period, the integration of growing an annual legume pasture in rotation with a cereal crop has been maintained.

Some techniques similar to ley farming, can be seen in the United States today. Many farmers double crop their lands, and many will double with a legume cereal crop. Although double cropping with a legume crop is similar to ley farming you are not actually achieving the essence of the term ley farming. The important aspect of ley farming is to establish a legume seedbank so that reseeding is not required each year. The closest aspect we have in the United States today is the rotation program known as the Perpetuating Alternative Legume System (PALS). The program was studied in the Palouse area of eastern Washington by Goldstein and Young (Pesek, 1989). The rotation included the biennial legume black medic, which was observed to reseed itself for as many as 30 years following establishment. The PALS rotation period was three years and consisted of spring peas plus black medic in year one, black medic in year two, and winter wheat in year three.

In three of four tests performed, the PALS rotation was equally or more profitable on a per acre basis than the conventional rotation used in this area. With tests performed at Dr. Mayo's wheat field, can Illinois bundleflower be established in these forms for use in the PALS rotation program or as a substitute for sweetclover in wheat fields for dryer areas that cannot use red clover?

MATERIALS AND METHODS

Germination Techniques

Five (5) different methods of germination methods at varying temperatures were tested. These consisted of stratification, chemical scarification, mechanical scarification, chemical enhancement, and a control group. Germination experiments were compared at four (4) different temperatures in a Precision Scientific incubator Model 805. Light was not provided inside the incubator for germination Temperatures used were 27C, 17C, 13C, and 10C. One process. hundred seeds were used in each treatment. Each treatment was replicated three (3) times and ran for a two week period or until 99% germination had been achieved. Seeds were collected from plant seed heads found in a disturbed field northeast of Emporia. The seed heads were ground, and seeds were separated by blowing debris away from seeds with an air hose. Some seeds used in this study were obtained from USDA Plant Materials Center.

Stratification

Warm moist and cold moist stratification were studied February 1988 to April 1988 according to the techniques from Young and Young (1986). Seeds were placed in moist sand in a sealed but not airtight jar. One jar of seeds in a refrigerator were kept at 3C and a second jar of seeds was kept in a science lab room at room temperature which was approximately 24C. Germination was then tested by spreading seeds in a germination tray lined and covered with filter paper to retain moisture. Distilled water was used to moisten filter paper. Germination trays were incubated for 10 days until germination results were obtained.

Mechanical Scarification

Mechanical scarification was followed as described by Young and Young (1986). Mechanical scarification was achieved by the removal of a portion of the outer seed coat. 1,100 seeds were nicked to allow imbibition of water. Seeds were nicked individually with a razor blade using caution to avoid removing a portion of the embryo. These seeds were placed in germination trays containing thin Kotex maxi pads as the absorbent material. The pads were soaked with distilled water and placed in an incubator for germination. Germination was measured for 27C, 17C, 13C, and 10C after 12 days.

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Chemical Scarification

Chemical scarification was achieved by soaking seeds in an 18 M concentrated sulfuric acid (Everitt and Gausman, 1984). Seeds were placed in a plastic beaker with 25 small holes bored in bottom. Concentrated (H_2SO_4) was poured into a larger plastic beaker and the beaker containing the seeds was submerged into the acid beaker. Twelve hundred (1200) seeds were soaked for periods of one, ten, and 30 minutes. After soaking, the seeds were thoroughly rinsed with tap water. Seeds were then germinated at 27C, 17C, 13C, or 10C for a two-week period as described above.

Chemical Enhancement

Chemical enhancement, using potassium nitrate in a 0.2% solution was studied as described by Copeland and McDonald (1985). Seeds were placed in germination tray containing absorbent pads. Potassium nitrate at a concentration of 0.2% was used in place of distilled water to moisten pads. These seeds were then germinated at 27C, 17C, 13C, or 10C as described above.

Control

The control consisted of germinating seeds without any treatment. These seeds were germinated at 27C, 17C, 13C, or 10C as described above.

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Fire Scarification

Illinois bundleflower seeds were treated with fire at temperatures approximating those in prairie grass fires (Wright and Bailey, 1982). A Bunsen burner was used to produce appropriate temperatures. Temperatures were monitored with an Omega CL6053 Precision Calibrator and an iron-constantan thermocouple. Temperatures ranged in 50 degree increments from 500 to 100 degrees Fahrenheit. Two sets of seeds were tested at each temperature. One seed set was placed directly into flame for 30 seconds (Figure 3), while another seed set was whisked back and forth over the flame for 30 seconds or until the first seed popped. Seeds treated at 150 and 100 degrees Fahrenheit were left in flame for one minute during continuous flame tests and whisked tests.

After heat treatment, 25 seeds of each treatment were placed in germination trays on absorbent material (Figure 4). Distilled water was used to moisten absorbent pads and trays were placed in a Precision Scientific Model 805 incubator at 24 degrees Celsius for 7 days.

Moisture Stress on Germination

Moisture stress upon germination was conducted using mannitol as the osmoticum (Uhvites, 1946). Mannitol solutions were made to provide osmotic potentials ranging from -0.033 MPa to -2.5 MPa (Table 1). One hundred seeds, Figure 3. Seeds placed directly into flame for 30 seconds.



Figure 4. Heat treated seeds shown in germination trays.

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Table 1. Mannitol/100g H₂0 used to simulate water stress in MPa.

MPa	g/100g of H ₂ 0
-0.033	0.23
-0.1	0.7
-0.3	2.1
-0.5	3.5
-0.7	4.9
-1.0	7.0
-1.5	10.5
-2.0	14.0
-2.5	17.5

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scarified for 10 minutes in 18 M H₂SO₄ were placed in each germination tray and wetted with appropriate mannitol solutions. Trays were then placed in a Precision Scientific Model 805 incubator at 24C for four days. Mannitol solutions were prepared fresh for each trial to reduce mold growth. Tests were replicated three times at each osmotic potential.

Effects of Water Stress of Seedlings

Water potential determination on Illinois bundleflower seedlings were started in the greenhouse on September 1, 1989. Seeds were treated for ten (10) minutes in concentrated (H_2SO_4) and dried in preparation for water potential testing. Twenty-seven (27) pots were planted with 50 seeds placed in each pot. Seedlings were grown in the ESU greenhouse under an approximately 12 hour photoperiod and temperature regime. On September 18, 1989, two (2) pots of plants, 18 days from germination, were set aside as controls. All other plants were set aside so that water could be withheld until recovery no longer occurred. Water was withheld from seedlings for periods ranging from one to twenty days.

Starting on September 18, 1989, seedlings were taken from the ESU greenhouse at dawn and brought into the lab. One reading was taken daily from the control group and one reading was taken from a plant that had water withheld for a specified period of time. Readings were obtained by use of the pressure chamber according to Scholander et al (1965). After pressure chamber readings were obtained, the plants were watered to check for recovery. This proceeded up to day 20 of withholding water and plants showed no sign of recovery after water was reestablished.

Potential Use of Illinois bundleflower in Crop Rotation

Scarified seeds (10 minutes in 18 M concentrated (H_2SO_4) were planted in a wheatfield located in Chase County, KS (Soil series, Reading silt loam, 0 to 1 percent slopes). Ten plots located 50 feet apart were placed in a random fashion. Each plot was 3 ft x 3 ft or a 9 square foot area and was seeded at a rate of 10 lbs/acre. 171 seeds were planted in each plot in rows between rows of previously sown wheat, much in the manner that yellow sweet clover is interplanted (Reinhardt et al, 1978). The number of seeds per row varied depending upon the number of rows per plot of wheat. Plants were counted periodically, by plot, until the wheat was harvested.

Wheat was harvested from plots three days before total field harvest was performed. Paired plots were harvested (experimental plots plus plots located beside experimental plots) by hand. Wheat was taken into Room 169 and allowed to dry for two weeks before removal of seed from the seed heads. Removal of the seed from the wheat was also

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performed by hand. Yield was obtained for each plot by weighing seeds on a Mettler PM 460 DeltaRange balance. Weights were obtained for controls and experimental plots.

RESULTS AND DISCUSSION

Germination

Results of germination techniques performed at 24C can be seen in Figure 5. The lowest germination rate found was the control group which only showed a 4.5% germination rate. This is a large variation from results found by Call (1985) who reported 43% germination of first year seeds. Latting (1961) also reported control groups with an 18% germination rate. The difference between my results and those of Latting can be explained by the difference in seed viability due to the differences in ecotone between an area in Oklahoma versus Kansas. The difference between the control reported by Call (1985) may be due to a difference in the variety found in Texas versus that found in Kansas.

The addition of potassium nitrate to the germination test resulted in a germination rate of 5.66%. This was not a significant difference (P>0.19) from the control and indicates no increase in germination.

Stratification tests performed resulted in a 7% germination rate in warm-moist treatment, and a 14% germination rate in cold-moist treatments. When comparing Figure 5. Germination rates obtained at 24C. Treatments included stratification with potassium nitrate (P.N.), chemic scarifications at 1 min, 10 min, and 30 min in H_2SO_4 , and mechanical scarification (M.S.).

these germination results to that of the control (4.5%), a slight increase in germination can be seen by both treatments. Stratification does not increase germination as does scarification, and suggests that overwintering has no effect on germination.

Results of chemical scarification at various time intervals are also shown in Figure 5. Soaking seeds for 1 minute in H₂SO₄ resulted in a 11.67% germination. This is not significantly different from the control at the P>0.05 level. Results from the 30 minute treatment showed an increase in germination to 78.67%, while the 10 minute H₂SO₄ treatment resulted in a 93.33% germination rate. These are significantly different (P>0.005 and P>0.0002 respectively) from the control. Results from other studies (Table 2), show results to be lower than acid scarification results found during this study. Differences in results could be explained by the age classes of the seeds or that the area around Emporia may be the center of the range given for Illinois bundleflower (McGregor, et al, 1977). If Emporia Kansas is the center of its range, the difference may be more the viability of the seed that the age class itself. However, Latting (1961) reported that after maturity there is a rapid development of an impermeable seed coat. The ages of seeds could explain the differences in chemical scarification results. All studies show a dramatic increase in germination over the control, but that there was not a

Table 2. Comparison of different germination trials.

Treatment	Schnell	Latting+	Call [~]
Control	5%	18%	43%
Sulfuric Acid*	93%	76%	82%
Scarification	98%	97%	968

+ Latting (1961)
 Call (1985)
* Sulfuric Acid treatments by Schnell & Latting
 were soaked for 10 min. versus Call's 15 min.

significant difference between the 10 minute and the 30 minute scarification treatments.

Mechanical scarification results are similar to those reported by Call (1985) and Latting (1961). Results from this study of 98.5% prove mechanical scarification to be the best method of scarifying Illinois bundleflower seeds. This information is of major importance because most scarification in nature may be achieved in this manner. Mechanical scarification, which is not a natural occurrence, could occur by mowing roadsides, or by haying in native prairies or meadows. This might account for the abundance of Illinois bundleflower found on roadsides.

With the finding of the optimum germination treatment, the next question to answer was at what temperature did Illinois bundleflower germinate best. Three additional temperatures were tried using scarification methods previously performed at 24C. Temperatures of 17C, 13C, and 10C were used for germination. The results of germination treatments at 17C are shown in Figure 6. The control and the potassium nitrate treatments show an extremely low germination rate, as was found previously in tests performed at 24C. All other treatments resulted in an increase in germination. Germination results at 13C are shown in Figure 7. All germination rates decreased drastically and were zero in the control, potassium nitrate, and 1 minute in H_2SO_4 . Germination trials at 10C showed no germination in

Figure 6. Results of germination at 17C. Treatments included stratification with potassium nitrate (P.N.), chemical scarifications at 1 min, 10 min, and 30 min in H_2SO_4 , and mechanical scarification (M.S.).



Figure 7. Results of germination at 13C. Treatments included straatification with potassium nitrate (P.N.), chemical scarifications at 1 min, 10 min, and 30 min in H_2SO_4 , and mechanical scarification (M.S.).



any treatment. This indicates that soil temperatures of 10C $(50^{0}F)$ or lower are too low for germination to occur.

The overall effects of temperature on germination can be seen in Figure 8. Germination at 13C is not affected by treatment. This implies that 13C is too cool for Illinois bundleflower to germinate and is one reason to classify Illinois bundleflower as a warm season plant. Germination at 17C and 24C shows the same trend except for the treatment at 17C and 1 minute in H_2SO_4 . There is no explanation for this increase. Results shown in figure 8 suggest that a temperature between 17C and 24C can be considered optimum for germination.

Fire Scarification

The effect of fire scarification was brought to my attention during a field trip to Ross Natural History Reservation in Lyon County, Kansas. I noticed that the amount of Illinois bundleflower had increased dramatically in the burned areas of Ross Reservation. Tom Zogelman, in a special project for Plant Ecology, germinated seeds that had been collected from the burned area the previous year. The increased germination that he found led to the experiments performed for this thesis.

Temperatures ranging from 100C to 500C were replicated as described in the methods and materials section and results are shown in Table 3. Although, some temperatures Figure 8. Effect of temperature on germination.



Temperature	<pre>% Germination</pre>	
500	0	
450	12	
400	32	
350	12	
300	16	
250	28	
200	32	
150	36	
100	28	

Table 3. Effects of varying temperature on germination.

show no germination, this would be expected in nature. Some seeds may not be scarified at all during a native prairie fire, with some being completely destroyed. This however, does not occur to all seeds and will scarify some seeds at each of the varying temperatures tried. Germination did increase and this indicates that fire scarification can play a role in germination. Whether this indicates that Illinois bundleflower could be considered a fire species is still of some doubt, but demonstrates that fire scarification is important in Illinois bundleflower seed ecology.

The importance of fire in grass ecology has been seen in the grass <u>Rottboellia</u> <u>exaltata</u> as reported by Rodriquez and Redundo (Harlan, 1982). This grass also seems to be dependent upon fire as a means of increasing stands of <u>Rottboellis exaltata</u>. Stands of Illinois bundleflower also show an increase after a fire. This supports the importance of fire in the ecology of Illinois bundleflower.

Effects of Moisture Stress on Germination

Several studies of this nature have been performed on plants such as alfalfa (<u>Medicago</u> sp.), calabrese (<u>Brassica</u> <u>oleracea</u> var. <u>italica</u> Plenck), cress (<u>Lepidium sativum</u> L.), and loblolly pine (<u>Pinus taeda</u>). Studies performed on calabrese and cress (Hegarty and Ross, 1978) used a solution of polyethylene glycol (PEG) 6000, which is often used as an osmoticum. Studies performed by Dunlap and Barnett

(1984) and Unvites (1946) used mannitol solutions to adjust water potential. The study by Dunlap and Barnett on the manipulation of loblolly pine seed germination with simulated moisture stress showed low water potentials had a large influence. Their study showed that germination rate of 90% on loblolly was unaffected until a water potential reached -1.0 MPa or lower which dropped germination rates down to between 10 - 15%. In studies performed by Hegarty and Ross (1978) on calabrese and cress, different techniques were used and compared. Seeds were kept in a -1.0 MPa PEG solution for 10 days. At the end of the 10 day period, 1/3 of the seeds were left in the -1.0 MPa solution for germination. Another 1/3 of the seeds were placed into water for germination. The remaining seeds were placed into water for 6 1/2 hours to allow imbibition, then were placed back into the -1.0 MPa PEG solution for germination. Results from this study showed that the percent germination was lowest for seeds kept at -1.0 MPa continuously. Germination results rose to around 75% for those seeds that were removed after 10 days at -1.0 MPa in PEG solution, introduced to water, and reintroduced to PEG solution. The highest germination results achieved were the seeds that were soaked at -1.0 MPa for 10 days and then introduced to water for germination. This shows that although seeds have been kept at a low water potential level, when placed back at a higher water potential they are still capable of

germination.

The study on the effect of osmotic pressure on water absorption and germination of alfalfa seeds by Uhvites (1946) shows that germination remained at around 58% in tap water, but dropped to 4% when introduced to a solution of mannitol at -0.7 MPa. This information concurs with results seen in calabrese and cress. If this is possible for mustard, pepper grass, pine, and alfalfa, is it possible for a legume?

Different levels of water stress were used in germination trials and results of these studies on Illinois bundleflower can be seen in Figure 9. Germination on Illinois bundleflower seeds remained at 93% or above until the solution water potential (mannitol as the osmoticum) reached -1.0 MPa. At levels of -1.5 MPa a mean germination of 1.66% was achieved, and at -2.0 MPa, germination was zero. This follows the study by Dunlap and Barnett and indicates that Illinois bundleflower can germinate during periods of modest soil drought. This makes Illinois bundleflower a more tolerable species over plants such as alfalfa to be used in reseeding old fields and in ley farming techniques.

Effects of Water Stress on Seedlings

The effect of water stress on seedlings plays a major role in how well a stand of Illinois bundleflower will be Figure 9. Effect of mannitol induced moisture stress on seed germination. Units are megapascals (MPa).



established. The effect of drought in seed germination has been established by the work performed earlier. Now the question of how water stress affects seedlings must be known before the introduction of Illinois bundleflower into reseeding mixtures.

The procedure of George Davis (pers. comm.) from the University of Alberta at Edmonton was followed.

Seedlings were placed in the greenhouse as described in the methods and materials section of this thesis. Control plants were watered daily from day 1 to day 20 near end of photoperiod. Experimental groups were labeled as to how long water had been withheld (i.e. day 10, day 11, etc.) and XPP was determined daily at dawn. Dawn readings were taken in order to compare the XPP with soil water potential since at dawn they are close to being the same. Figure 10 shows seedlings prior to water stress tests. All plants were checked to make sure they were healthy and comparable to those used as the controls. Figure 11 shows seedlings after 20 days of water stress. Figure 12 shows the results from the Xylem Pressure Potential (XPP) tests. Plants were carried through to day 20 but XPP could not be measured on the experimental group past -6.4 MPa which was initially reached on day 16, so the graph illustrates a two day period after measurements could not be made. The control plants (XPP) varied between -9 MPa and -13 MPa for the entire 20 day period. Starting at day 10 XPP started to drop at a

Figure 10. Seedlings prior to water stress.



Figure 11. Seedlings after water stress.



Figure 12. Effects of drought on seedlings. Xylem pressure potential (XPP) expressed as megapascals (MPa).



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slow pace at an average of about -0.5 MPa daily until day 15. From day 15 to day 16 XPP dropped from -3.5 MPa to -6.4 MPa. All readings from this point were read at -6.4 MPa or lower since the pressure chamber does not extend beyond -6.4 MPa. At this point in time (day 20), the experiment was terminated. Observations were made concerning:

1. When wilting occurred.

2. Recovery from wilting.

3. Recovered from wilting but later died.

4. Plants that never recovered.

These results are shown in Figure 13. Illinois bundleflower seedlings showed no signs of wilting until day 11. At day 11 through day 15 plants wilted but were able to recover after watering. On days 16 and 17, Illinois bundleflower seedlings showed signs of recovery after water was restored but soon showed signs of wilting again and eventually died. Plants from day 18 on never recovered. Figure 14 shows plants from days 11 - 13 which recovered after water was supplied. Figure 15 shows plants from day 16, 17 and 18. Plant 17 shows sign of recovery but as can be seen, day 16 has already died along with the day 18 plants. Seedlings from day 17 soon died.

Results from this study show that seedlings can withstand periods of drought of up to about a 2 week period or until XPP reached a -3.5 MPa. The drastic change from day 15 to day 16 did not allow us to monitor the gradual Figure 13. Seedling response to moisture stress. Xylem pressure potential (XPP) expressed as megapascals (MPa).



Figure 13. Seedling response to moisture stress. Xylem pressure potential (XPP) expressed as megapascals (MPa).



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Figure 14. Seedlings after recovery from XPP test.



Figure 15. Seedlings after non-recovery from XPP test.



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change or at what point XPP the plant could have reached and recovered. This does show that Illinois bundleflower is capable of removing water from the soil at very low water potentials which makes the seedlings drought resistance.

Potential Use of Illinois Bundleflower in a Crop Rotation

The first aspect of concern in crop rotation was to establish plots in an existing wheat field. Results showing percent establishment in a wheatfield in Chase County, Kansas can be seen in Table 4. A mean establishment of 28% was found. These results show that Illinois bundleflower can be broadcast into an already existing wheatfield and a stand established: to the point that seeds are produced the first year. Figure 16 shows Illinois bundleflower plants established, while figure 17 shows seed heads produced the year of planting (plot 9).

With the establishment of Illinois bundleflower into the wheatfield, the next question was to see if wheat yield would be increased the next year. Results of interplanting Illinois bundleflower with wheat can be seen in Table 5. Plot 7 was accidentally destroyed by disking. However, results from all other plots are shown.

A t-test was performed on the results, and it was found that wheat yield was significantly lower (0.01) in the plots containing Illinois bundleflower. Based upon these results, I can not recommend substituting Illinois bundleflower for

Plot	Plants Established	Percent
1	46	
2	29	17
3	35	20
4	74	43
5	32	19
6	49	29
7	46	27
8	68	40
9	48	28
10	48	28

Table 4. Percent establishment in wheatfield.
Figure 16. Illinois bundleflower in wheat field.



Figure 17. Illinois bundleflower seed heads in wheat field.



Plot	Control	Experimental	Difference
1	64.72	53.705	+11.015
2	174.21	124.37	+49.84
3	172.65	66.10	+106.55
4	135.86	150.64	-14.78
5	179.65	105.53	+74.12
6	177.77	94.00	+83.77
7	*	*	*
8	195.69	131.96	+63.73
9	311.65	188.86	+122.79
10	273.98	116.26	+157.72

Table 5. Results of interplanting with wheat.

* Plot 7 inadvertently destroyed.

sweetclover in ley farming. The reason for decreased yield is not clear. Perhaps it is due to competition or an allelopathic event.

Competition between wheat and Illinois bundleflower would most likely occur over available water. Since rainfall was more than adequate during this study, and the study was conducted on a Reading silt loam, I cannot demonstrate that competition for water was the limiting factor. At this time I would suggest that the yield reduction was caused by allelopathy. Further studies would need to be performed to determine whether or not allelopathy was the cause of the lower yield.

Another factor to take into consideration was the amount of weeds (mainly Johnson grass) that was found in the plots with the Illinois bundleflower. Wheat is usually competitive with weeds, including Johnson grass, but the Illinois bundleflower plots were very weedy. Was this from the lack of disking in the plots before planting (weeds were removed by hand three times during summer months) or because of the Illinois bundleflower? If Illinois bundleflower affected wheat by allelopathy, why was it not having a similar effect on the weeds (i.e. Johnson grass). Without further work, I recommend that Illinois bundleflower not be used in rotation with wheat.

CONCLUSIONS

From this study, several conclusions can be made about various aspects of the ecology of Illinois bundleflower. First, we know how to increase germination in the lab for broadcasting into previously reseeded fields, and how to treat seeds for establishment in with other native grass seeds for the initial reseeding of abandoned cultivated lands, (go-back).

Secondly, we know that fire will increase Illinois bundleflower stands which are pre-existing. This helps increase stands of Illinois bundleflower to increase nitrification.

Thirdly, we also know that Illinois bundleflower is a drought-hardy plant, that can establish itself in times of low water availability. This is important for both establishment and growth of the plant.

The use of Illinois bundleflower in crop rotations is doubtful. This does not indicate that the plant is not of major importance, but as a substitute for sweetclover in ley farming, it does not appear to be successful and may reduce crop yields. This does not suggest that Illinois bundleflower should not be incorporated into seed mixtures which will be reseeding old cultivated fields. Its ability to fix nitrogen and its ability to withstand periods of drought make it a well-adapted legume for native prairie restoration. The importance of Illinois bundleflower in a prairie community has previously been established in this thesis. We know how to increase germination in the lab as well as in the field, and we know how drought resistant the seeds and seedlings are. However, how Illinois bundleflower is classified has not been satisfactorily discussed.

During this entire research project and the gathering of many seeds and plants, Illinois bundleflower was never found in a native prairie that was considered to be in excellent condition. This is not only true in Lyon, Coffey, and Chase counties, but in Cherokee, Crawford, Bourbon, Miami, and Anderson counties as well. This suggests that Illinois bundleflower is not a climax species. A climax species is classified as one that will continually reproduce when a community has reached its climax. This does not seem to be the case with Illinois bundleflower. A seral species is considered to be a plant that has an important position in the seral stages leading to climax. As was proven before in a class project by Linda Strange, Illinois bundleflower was found to contain nodules which proves that it is capable of nitrogen fixation. The theory I am proposing is that since Illinois bundleflower is a legume capable of fixing nitrogen, and is mainly seen in areas that have been disturbed rather than areas considered in excellent condition, that Illinois bundleflower is a seral species and should not be classified as a climax species.

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My proof of this statement can be seen from my work at Dr. Mayo's farm (Mayo Manor). Dr. Mayo has a perfect study area: a hay meadow, (which was calculated by a step loop method to be in excellent condition); and adjacent to it an area that at one time was farmed and left to go-back to native prairie. In an intense investigation of both areas, not only by myself, but by Bernard Sietman, Illinois bundleflower was not found in the hay meadow but was found extensively in the go-back. This information along with findings in most areas of Kansas, leads me to believe that Illinois bundleflower will flourish in an area that lacks an adequate nitrogen pool. Once a nitrogen pool has been established, and the climax grasses which are also capable of fixing nitrogen have been established, Illinois bundleflower may slowly die out. This would explain why Illinois bundleflower is only found in disturbed areas.

Another interesting factor encountered during this research was the use of Illinois bundleflower as a wildlife food source. Many people have stated that Illinois bundleflower is a palatable species, and I have taken them at their word. No data supporting this issue has been found. Studies at Emporia State University have been performed by individuals such as Mark LaBarge and Kevin May on the use of Illinois bundleflower seeds as a food for wildlife. Mark LaBarge (in a class project) found that Illinois bundleflower is comparable to soybean in nutritional values. This suggests that Illinois bundleflower should be an excellent food source. Mark LaBarge fed seeds to prairie chickens and discovered that the prairie chicken was not able to digest the seed. The same results were seen in a study by Tom Zogelman in which he fed Illinois bundleflower to quail and domestic chickens. Apparently, the seed coat is too hard for the digestive tracts of birds to dissolve.

Another study performed in the summer of 1990 by Kevin May dealt with the feeding of Illinois bundleflower seeds to Norway rats. The purpose of this study was to see if the ability to crack the seed with animals' teeth would release the nutrients available. The results of this study showed that the Norway rats also lost weight when exclusively feed Illinois bundleflower seeds. Whether the fact that it is the hard seed coat or the lack of palatability was not answered.

The palatability of the plant has not been directly studied. Whether it is considered an "ice cream" plant to cattle would be an interesting follow up study. At this time, Illinois bundleflower is considered a good forage for cattle.

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