

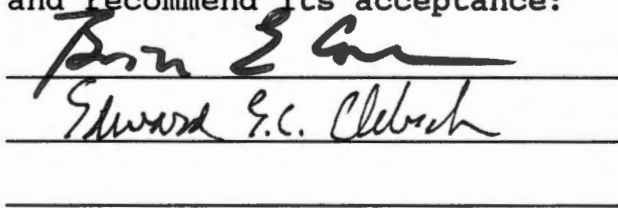
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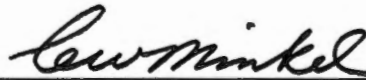
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**THE POSSIBLE ALLELOPATHIC EFFECT OF
SCHIZACHYRIUM SCOPARIUM (MICHX.) NASH
ON GERMINATION OF SELECTED
HERBACEOUS SPECIES**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Albert C. Hill, Jr.

May, 1992

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THESIS

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*To my wonderful wife Jennifer,
for all her love and support.
To my precious daughter Katie,
for all the joy she gives me.*

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ABSTRACT

The principal objective of this research was to determine if *Schizachyrium scoparium* (Michx.) Nash (little bluestem) exhibited any allelopathic influence on the germination of selected weed and wildflower seed. Experiments were designed to test the effects of leachates, mulches, and teas, collected or made from plant parts of *S. scoparium*. Germination tests were conducted using petri dishes for leachates and teas. Some tests involved germinating seed on sand covered with different mulches of *S. scoparium* clippings. Another test involved sowing seed in pots which had *S. scoparium* plants growing in them. All results were analyzed using ANOVA and LSD.

Plants of *Schizachyrium scoparium* were grown in glazed crockery pots that were fitted so leachates from the plant roots could be collected. The leachates were used to moisten filter paper disks upon which test seed were placed. There was no significant difference in germination due to treatment.

Little bluestem plants were barerooted, cutup and sorted as inflorescences, leaves, and roots, placed in paper bags and air-dried. Samples of each tissue type were weighed (12 g) and then soaked in 900 ml of distilled water for twenty-four hours. The resulting teas were used in petri dish germination tests. The tests involved four species, none of which were significantly affected by the little bluestem teas.

Mulches made from the clippings of *S. scoparium* were placed over the top of seed which had been sown in crocks

filled with sand. Both dried and fresh little bluestem material was used for mulch. Significant inhibition was observed with all test species and bluestem mulches. The fresh clippings were more inhibitory than dried mulches in three out of four species tested.

Although the majority of experiments did not support the hypothesis that *Schizachyrium scoparium* was allelopathic the results of the mulch test could not be ignored. Further experimentation needs to be conducted in order to verify the results obtained.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
1. Origin and Meaning of Allelopathy	3
2. Studies in the Literature	6
3. The Role of Allelopathy in Plant Succession	14
4. Little Bluestem	16
III. MATERIALS AND METHODS	19
Experiment 1	20
Experiment 2	23
Experiment 3	27
Experiment 4	29
IV. RESULTS AND DISCUSSION	34
Experiment 1	34
Experiment 2	36
Experiment 3	38
Experiment 4	40
V. SUMMARY AND CONCLUSIONS	42
LIST OF REFERENCES	46
VITA	49

CHAPTER I

INTRODUCTION

The planting of roadside wildflowers has been gaining in popularity over the past several years. State highway departments have invested much time and effort into attempts at establishing these plantings without much lasting success. It has been proposed that the planting of wildflowers along highways would ultimately reduce roadside maintenance costs, but current establishment practices are much more expensive than mowing. This research was undertaken with the purpose of gaining further insight into the successful long term establishment of roadside wildflowers. It is thought that when successful establishment of wildflowers is reliable there would be a savings in maintenance cost as well as the added aesthetic value over conventional roadside treatments.

It has been observed that certain species of plants influence the composition of the plant communities in which they exist by emitting leachable chemical compounds into the environment (Aamissepp and Osvald, 1962). This interference of one species on another can promote a very stable, long enduring community (Muller, 1966; Rice, 1984). The contrived reproduction of this naturally occurring phenomenon could possibly aid in extended establishment of roadside wildflower plantings. If allelopathic inhibition by a species, suitable for inclusion in wildflower seed mixtures, could be exploited

the resulting roadside plantings might persist with less required maintenance.

Many wildflower authorities recommend the inclusion of native grasses in wildflower blends to improve establishment (Martin, 1986; Paulson, 1989). *Schizachyrium scoparium* (Michx.) Nash (little bluestem) is a widely recommended grass species for this purpose. Since much of the research done on wildflower plantings has been done regarding prairie reclamation it is understandable that the grassland climax would be used as an ideal model for this type of planting. Little bluestem is one of the predominant grass species occurring in the different types of climax grassland and it is very widely distributed in the United States (Hitchcock, 1950).

Andropogon virginicus (broomsedge) a close relative of little bluestem, has been shown to exhibit allelopathic properties (Priester and Pennington, 1978; Rice, 1972). Little bluestem was once classified under the genus *Andropogon* (Gould and Shaw, 1983). Both little bluestem and broomsedge occupy similar ecological niches and often are found together in the same community. Since they share such close association, it is reasonable to suspect that little bluestem might also demonstrate allelopathic properties. The objective of this research was to determine if *Schizachyrium scoparium* exhibited allelopathic influences on the germination of selected herbaceous species.

CHAPTER II

REVIEW OF LITERATURE

1. Origin and Meaning of Allelopathy

Aamissepp and Osvald (1962) stated "In studying natural plant communities and crops of different kinds it soon becomes evident that higher plants, trees and shrubs, herbs and grasses, exercise a considerable influence upon each other". They were referring to an interaction between plants known as **allelopathy**. Rice cites (1984) Molisch, who in 1937, first used the term allelopathy to describe the "biochemical interactions between all types of plants including microorganisms".

A confusion often arises in differentiating between the terms competition and allelopathy. Competition is a situation where different organisms vie for limited resources often to the disadvantage of one or the other (Barbour et al., 1987). A key point which contrasts allelopathy from competition is that allelopathy involves the release of a chemical compound into the environment causing some response by sensitive species (Rice, 1984).

The phenomenon of allelopathic interference between plants has been the focus of much speculation, interest, and confusion in the many disciplines involved in the study of plants. There have been numerous references of suspected

plant interactions since the time of Theophrastus in circa 300 B.C. when he observed "... that the chick pea (*Cicer arietinum*) does not reinvigorate the ground as other related plants (legumes) do but 'exhaust' it instead" (Rice, 1984). The general acknowledgement of the occurrence of "soil sickness" or "soil fatigue" has been documented since early times. Most of these early observations were made regarding interactions between agricultural crops and associated weed species. The possible role of allelopathy in vegetational composition was also suspected by some. In 1832, DeCandolle proposed that the spatial grouping of plants in natural plant communities is due to the fact that certain plants are capable of influencing other plants by the production of inhibitory by-products from their roots (Aamissepp and Osvald, 1962).

The types of biochemical interactions caused by allelopathic species usually results in adverse affects on seed germination, seedling development, stunting of root elongation, or cellular disorganization in the roots (Anderson, 1983). The initial stages of testing for allelopathic influence usually begins with germination experiments and evaluations of seedling development. Chemical inhibition is more operative during these critical developmental stages (Muller, 1966). Allelopathy is generally thought of in the context of having a negative influence on sensitive species but in some situations it may be stimulatory in effect. It has been reported that sub-lethal

concentrations of phytotoxic compounds can actually stimulate growth (Weidenhamer, et al. 1989).

Researchers most often look for the inhibitory influence of a species. In the chaparral communities of California, Muller observed that the same suppression affecting annual species also seemed to contribute to the decline of shrubs emitting the inhibitory compounds (Muller, 1966). This autotoxic effect probably allows inroads to be gained by other species which are not susceptible to the toxins and thereby play an important role in succession (Abdul-Wahab and Rice, 1967).

An understanding of the different ways that allelopathic chemicals enter the environment is fundamental to the study of biochemical interactions between plants. Allelopathic compounds may enter the environment by means of volitization, root secretions, leachings from foliage, or leachates from the decomposition of plant residues (Anderson, 1983; Rice, 1984). In his studies of the California chaparral Muller demonstrated that volatile phytotoxic terpenes are given off by *Salvia leucophylla* (Muller, 1966). It has been repeatedly demonstrated that roots are responsible for some allelopathic compounds being added to the environment (Aamissepp and Osvald, 1962). Some early tests involved the taking of soil samples in close proximity to inhibitory species and then attempting to germinate seeds in these samples. In 1921, the pioneering work of Lyons and Wilson demonstrated a variety of compounds

are exuded by living roots of several crop species (Rice, 1984). The leaching of chemicals from above ground plant parts by the action of rain has been demonstrated to be an effective allelopathic mechanism (Muller, 1966). It has been demonstrated that aqueous extracts of various organs of *Helianthus annuus* significantly reduced growth of eight test species based on oven-dry weights (Wilson and Rice, 1968).

The inhibitory effects of leaf litter and plant residues have received much attention especially regarding the toxicity of compounds released by the decay of plant parts. In tests studying decomposition of residues under aerobic and anaerobic conditions, Patrick (1971) determined that phytotoxic compounds resulted under both circumstances.

2. Studies in the Literature

Rice (1972) tried to determine if *Andropogon virginicus* (Broomsedge) was allelopathic to other species common in Eastern Oklahoma old-field succession. Broomsedge has been observed to invade old-fields at about three to five years and become dominant for periods of eight or more years before being replaced by *Schizachyrium scoparium* (Little Bluestem) in later stages of succession. The study also examined four species of nitrogen fixers to determine if the *Andropogon* had an inhibitory effect on them. Some researchers theorize inhibition of soil bacteria by allelopathic species could

affect nitrogen availability and be a contributing factor to the vegetation composition. It has been shown that broomsedge competes well and proliferates on soils of low fertility (Rice, 1972).

In a preliminary series of tests aqueous extracts of fresh roots and shoots of broomsedge were used to grow seedlings of four old-field species. They were grown in hydroponic culture for 10 days and then oven dried and weighed. It was found that both root and shoot extracts significantly inhibited growth of all test seedlings. The shoot extracts were considerably more inhibitory to all species than root extracts (Rice, 1972).

In a second test, sterilized root and shoot extracts were used in Petri dish culture comparing two species of symbiotic nitrogen fixers and two species of free living nitrogen fixers. The soil bacteria were allowed to develop for two days and then the spread of each culture was measured. It was observed that extracts of both roots and shoots were inhibitory to both free-living and symbiotic nitrogen fixers. Again the shoot extracts were more inhibitory than root extracts.

In a third test, either 1g of clipped tops of fresh broomsedge or 1g of last year's old dry tops were incorporated into a mixture of sand and soil using 1g of clippings to 454g of soil. Seeds of five old-field species were sown and the germination recorded and then thinned to two plants per pot.

They were grown for 17 days and then harvested, oven dried and weighed. The decaying old material inhibited seed germination of four test species while the fresh material only inhibited three. The germination of test species which are associated with the first successional stage was the most severely inhibited. The decaying fresh material was significantly more inhibitory to seedling growth.

In a final test, fresh clippings of broomsedge were incorporated in a sand/soil mixture and seeds of *Lespedeza* and *Trifolium* were inoculated and sown. They were grown for six weeks, harvested and fresh weights taken. The roots were inspected for possible adverse effects on nodulation. It was found that 1g of decaying broomsedge in 454g of soil significantly inhibited the growth and nodulation of both legumes.

The results of all four studies demonstrated *Andropogon virginicus* is allelopathic to species commonly found in early successional old-fields. It was determined that old dried tops of broomsedge were as inhibitory as fresh green clippings. This suggests that the allelochemicals causing the inhibition were either not water soluble or they are released by decomposition in the soil. The observed inhibition of selected legumes of old-field succession and adverse effects on soil nitrogen fixers makes it reasonable to suggest that broomsedge might lower available nitrogen levels in the field. The influence of broomsedge on species associated with old-

fields demonstrates the possible significance in succession and vegetation patterning caused by allelopathy (Rice, 1972).

A study by Tinnin and Muller (1972) was conducted to determine if the straw of *Avena fatua* contained any allelopathic compounds which might explain its dominance in annual grasslands (Tinnin and Muller, 1972). *Avena fatua* was introduced from Europe prior to 1662, and is now dominant in some annual grasslands in many parts of the country. It is closely related to *A. sativa* which has been shown to have allelopathic influences on some species.

A series of tests including two types of bioassays and a variety of soil trials was conducted. In a sponge bioassay, extracts were made using finely milled straw of *A. fatua* and allowing it to soak in distilled water for a period of time. The resulting extract was used to moisten a piece of cellulose sponge in a petri dish onto which a piece of filter paper was placed. Each dish had 10 seeds of *Bromus rigidus* spaced evenly on the paper and the dishes were then sealed with Parafilm. The petri dishes were placed in a dark growth chamber. The success of germination and the length of the radicles were noted after a growth period of 48 hours and 72 hours. The straw of *Avena fatua* apparently contained water soluble compounds which significantly inhibited radicle elongation of *Bromus rigidus* seedlings.

An agar bioassay was conducted to determine if compounds in the straw of *A. fatua* could diffuse through a barrier. The

extract treatment consisted of ground straw placed in 500ml storage dishes and covered with adequate distilled water to moisten the straw. This extract was covered with 15ml of a two percent sterile agar and allowed to solidify. Another 10ml layer of agar was added on top of the first creating an agar barrier devoid of straw. A sheet of filter paper was placed on top of the solidified agar on which seeds of four species were tested. Ten seeds of each species were used except for the *Centaurea* in which 20 seeds were used per dish. The same steps as for the previous assay were followed. The ground straw of *A. fatua* beneath the agar barrier inhibited germination of *Centaurea melitensis* but no other species. However the radicle growth of all four species was inhibited (Tinnin and Muller, 1972).

Two types of soil trials were done by assessing the effects of water extracts on seedlings and the addition of straw on seedlings grown in pots. An unsterilized clay soil was used as potting media in which seedlings of three species were grown. These are species associated in the grasslands where *A. fatua* dominates. Plants of each species were potted in 10 x 10 x 15cm plastic containers and watered with an aqueous extract of ground straw. The extract was made by soaking 20g of straw in two liters of tap water for an hour. The amount of seedling growth, expressed as dry weight of the tops, was determined at the end of eight weeks in the greenhouse. The addition of aqueous extracts of *Avena fatua*

straw added to clay soil caused significant reduction in germination of only *C. melitensis* one of three species used (Tinnin and Muller, 1972).

The second soil test used either washed sand or clay in the plastic containers previously mentioned. The same three species were used but in this case the treatment was four or five grams of ground *Avena fatua* straw incorporated into the top two centimeters of soil. Seeds of the plants were sown and watered with tap water and grown in the greenhouse. Germination success was recorded and the resulting seedlings thinned to equal numbers. The tops were harvested at the end of eight weeks and growth determined by dry weight. *A. fatua* and *C. melitensis* were reduced by the addition of ground straw in both sand and clay medias. The germination of *C. melitensis* was inhibited significantly as well (Tinnin and Muller, 1972).

The authors demonstrated that the straw of *Avena fatua* contains water soluble compounds which are differentially toxic to the germination and growth of certain species of the annual grasslands. They were able to demonstrate the potential for *A. fatua* to influence the vegetative composition of the grasslands where it often becomes dominant (Tinnin and Muller, 1972).

Wilson and Rice (1968) conducted a wide range of tests to determine the allelopathic influence of *Helianthus annuus* (sunflower) and its role in old-field succession in central

Oklahoma and southeastern Kansas. Their theory was that the presence of *Helianthus* in the initial pioneer weed stage of succession in old-fields shortened the duration of this stage and moved it towards the annual grassland phase. Field observations suggested that competition was not the major factor for replacement of the weed stage by *Aristida oligantha*, the dominant species of these annual grasslands. Field surveys were conducted prior to any laboratory studies in order to verify vegetation patterns and growth inhibition reported to be associated with sunflower. Quadrat frames of 0.25, 0.50, and 1.0m² were laid around the base of sunflowers and clippings were taken and separated by species. The comparisons of oven dried weights and species density figures supported earlier field observations of possible allelopathic influence of sunflower (Wilson and Rice, 1968).

One test compared the effects of extracts of various *Helianthus* organs to seedling growth. The seedlings of eight species associated with the initial stage of succession were tested. They were grown in plastic vials in solutions composed of one part extract to five parts complete nutrient solution for 12 days (hydroponic culture). The oven dried weights were compared to determine effects. All extracts significantly reduced the oven-dry weights of the eight test species. The extracts made from leaves and inflorescences appeared to be more inhibitory to growth than the extracts of stems and roots (Wilson and Rice, 1968).

A series of seed germination trials was conducted employing the petri dish method and testing extracts of various sunflower parts. The treatment solutions were the same as used in the previous experiment. Seeds were allowed to germinate in the dark for eight days. The petri dish series indicated that all extracts reduced germination of *Haplopappus ciliatus* and *Rudbeckia hirta*. Solutions containing extracts of inflorescences caused a reduction in all species except *Digitaria sanguinalis* with only a slight effect on *Amaranthus retroflexus*. The germination of *Aristida oligantha* was not significantly altered by any of the extracts tested (Wilson and Rice, 1968).

The effects of dried sunflower leaves on germination and seedling growth were tested. The powdered leaves of dried *Helianthus* were incorporated into a soil and sand medium in which seeds of eight species were sown. When germination was completed the plants were thinned to the five largest seedlings, grown for an additional 14 days then harvested and oven-dry weights recorded. The dried, decaying leaves of sunflower incorporated into soil had an inhibitory effect on *Erigeron*, *Amaranthus*, *Haplopappus* and *Helianthus*. The growth of *Aristida* seedlings was actually stimulated while growth of all other species was significantly reduced (Wilson and Rice, 1968).

These findings by Wilson and Rice seem to support the theory of the important role of *Helianthus* in the early stages

of old-field succession. The inhibition of several species associated in the pioneer weed stage of old-fields verifies the suspected allelopathic properties of sunflower. They demonstrated that allelopathy does play a significant role in succession of old-fields in parts of Oklahoma and Kansas (Wilson and Rice, 1968).

3. The Role of Allelopathy in Plant Succession

Braun defines **succession** as ... "vegetational change, the replacement of one community by another as a result of natural and progressive (rarely regressive) development" (Braun, 1974). The factors affecting the advancement of a plant community through the stages of succession have usually been attributed to abiotic factors and the resulting competition. The many phenomena which can be observed affecting plant community development cannot be attributed solely to competition and the interactions involving abiotic factors (Aamissepp and Osvald, 1962). There have been many studies supporting the implied role of allelopathy in plant succession and its impact on those communities. The work of Rice, Muller and others makes a strong case for the significance of allelopathy in plant community interactions.

A critical phase in succession involves the migration of propagules into a community and their subsequent germination and development (Clements, 1916). These opportunities for

shifts in population composition hinge on the success or failure of seed germination and seedling survival. Bell and Muller observed that the patterning effect within a community occurred at the time of germination which is before factors of competition are involved (Rice, 1984). The presence of allelopathic species would have a profound affect on the composition of a community by having an inhibitory influence on the germination or subsequent growth and development of certain species.

A considerable portion of the research studying the influence of allelopathy is regarding old-field succession. Secondary succession is caused by severe disturbance to a climax community (Braun, 1974). The disturbance may be due to natural causes or the activities of man. The characteristic phases of old-field succession in the east include pioneer weeds, perennial bunchgrasses, pine and juniper invaders and ultimately hardwood climax species (Braun, 1974). In Oklahoma old-field succession follows a slightly different development since the climax is prairie. The four stages of old-field succession in Oklahoma are pioneer weed, annual grass, perennial bunchgrass dominated by *Andropogon scoparius* and the climax prairie (Wilson and Rice, 1968). The perennial bunchgrass stage of succession remains for extended periods. Quarterman (1957) observed that *Andropogon virginicus* is present from the first stage of succession but that it may dominate for eight years or more once established. It seems

that east of the Mississippi river the prairie formation is a local subclimax of the deciduous forest (Clements, 1916).

A subclimax community is a premature stabilization of any intermediate community which may last for prolonged periods or could be replaced by climax vegetation (Braun, 1974). It is possible that allelopathic interference may contribute in some instances to a community reaching a subclimax state.

4. Little Bluestem

Little bluestem is a native grass that has had a confused identity in botanical literature. DeSelm (1953) observed that "Little bluestem has been placed in three genera other than *Andropogon*, has had seven specific epithets and eleven subspecific, varietal or subvarietal descriptions". The currently accepted nomenclature is *Schizachyrium scoparium* (Michx.) Nash although many authors still refer to it by a previous label, *Andropogon scoparius* Michx. (Brown, 1979; Gould and Shaw, 1983; Oakes, 1990). Little bluestem exhibits much phenotypic variation over its range with three varietal forms presently acknowledged. This variety of forms is probably why it is so often confused taxonomically. It is most widely represented in the western U.S. by *Schizachyrium scoparium* var. *frequens*, and in the east by *S. scoparium* var. *divergens*, or along the Atlantic coast by *S. scoparium* var. *littoralis* (Gould and Shaw, 1983).

Little bluestem is a widely adapted grass species found throughout North America. It thrives on poor, infertile soils that will not support many other species (Oakes, 1990). In the United States it is listed as native to 44 of the lower 48 states being absent from the Pacific coastal states and Nevada (Brown, 1979; Gould and Shaw, 1983; Hitchcock, 1950).

Schizachyrium scoparium is most frequently associated with the prairies of the mid-west and great plains regions. It is considered one of the most important of the tall-grass prairie species along with *Andropogon gerardi* (Big Bluestem) and *Sorghastrum nutans* (Indian Grass) (Gould and Shaw, 1983). Although it is associated with prairies, little bluestem is commonly seen invading old-fields (Brown, 1979). In the communities where little bluestem occurs it often becomes dominant. Little bluestem may make up 55 to 90 percent of the vegetational composition in prairie grassland communities (Gould and Shaw, 1983). It is usually a dominant or codominant species in the various types of communities in which it occurs. In the sand plains of New England where pitch pine is the dominant species, herbaceous communities of primarily little bluestem occupy the gaps between the pines (Braun, 1974).

The association of little bluestem (*Schizachyrium*) with the broomsedges (*Andropogon*) is easy. They both seem to occupy similar ecological niches often occurring together in the same community. The broomsedges are characteristic

species associated with old-fields. In old-fields *Andropogon virginicus* is a major dominant in the herbaceous stages of succession (Quarterman, 1957). It has been proposed that *Andropogon virginicus* exhibits some allelopathic properties. Priester and Pennington (1978) studied broomsedge and found that extracts were inhibitory to young loblolly pine seedlings. This influence might explain why *Andropogon* is able to remain in almost pure stands for extended periods before giving way to woody invaders. Broomsedge plays an important role in the development of Oklahoma old-field communities possibly due to allelopathic properties it exhibits (Rice, 1972). It is reasonable to suggest that little bluestem might also exhibit some allelopathic influence which contributes to the dominance it achieves in some communities.

CHAPTER III

MATERIALS AND METHODS

The experimentation was carried out in the compounds, greenhouses, and laboratories on the Agriculture campus of the University of Tennessee, Knoxville. All of the experiments were run between June and October of 1991. The experiments were conducted in randomized complete block design. The effects of various leachates and mulches were observed to determine the influence on seed germination of selected herbaceous species. Effort was made to obtain leachable compounds of little bluestem to evaluate the possible allelopathic properties of this native grass. Leachates were made from various parts of little bluestem plants to isolate the origin of any allelochemical. Evaluations were based on germination response only and not seedling development.

Seeds were obtained from commercial sources and from seed available within the department. Eight species of wildflower seed were purchased from Wildseed, Inc., of Eagle Lake, Texas. The little bluestem seed was purchased from the Bamert Seed Company, of Muleshoe, Texas. Weed seeds were available from previous researchers in the department of Ornamental Horticulture and Landscape Design. All of the seed were tested to determine germination rates under experimental conditions.

Sand was used instead of organic media to minimize any possible influence of leachable compounds on the little bluestem leachates. The inert sand would leach well by not binding any compounds produced by the little bluestem plants.

The controls consisted of distilled water for the leachate experiments and vermiculite for the mulch experiments.

Experiment 1

Introduction

The purpose of this experiment was to test for leachable germination inhibitors which might be exuded from the roots of little bluestem. Root exudation is an important mechanism by which allelopathic compounds are released into the environment (Rice, 1984). Seeds of wildflowers and weeds can be germinated using the petri dish method. A filter paper disk is placed in a petri dish then the seeds are evenly arranged and the disk is then moistened with the leachate to be tested (Evenari, 1949).

Materials and Methods

This experiment required two phases, one involved the method for collecting the leachate and the other was the technique for testing germination using the leachates collected.

Three 7.2l glazed crockery pots were used to contain the little bluestem plants. The bottom of each pot was fitted with a #6 rubber stopper which had a 7.5cm long section of 5ml

serological pipet pushed through until it was flush with the tapered end of the stopper. A 21.5cm long section of 10mm rubber tubing was forced over the protruding end of the pipet until it was flush with the broad end of the stopper. Large hemostat type forceps were used to clamp off the ends of the tubing when the pots were not allowed to drain.

The rubber stoppers extended into the interior of the pots thus interfering with drainage. Paraffin was melted and poured into the bottom of each pot until a level was reached that allowed positive drainage into the tubing. Prior to filling the pots with sand, 5cm x 5cm squares of fine screen were placed over the exit hole to prevent it from clogging with sand.

The pots were moved into the greenhouse and each was placed on top of two stacks of bricks on the greenhouse bench which allowed the tubing to hang beneath the pots with the hemostats in place. Each stack of bricks was carefully leveled before the pots were put in place.

The treatment pots were filled with silica sand and individual little bluestem plants were planted in each and grown until they were well established. A control pot was filled with silica sand only. Distilled water was used for all irrigation in the experiment. The plants were fertilized with Peter's Peat-Lite Special 15-16-17¹ at 200ppm once weekly for three weeks. The fertilization was discontinued after

¹ W. R. Grace and Co., Fogelsville, PA

three weeks because of concern regarding the soluble salts and their possible effects on the leachates.

The leachates were collected every seven days for a period of eight weeks. Through the week the plants were watered sparingly to avoid waterlogging the roots. Collections were made by adding one to one and one half liters of distilled water to each pot and opening the hemostat and catching the run-off in a pitcher. Leachates from all three pots were mixed together and one liter of the resulting leachate was conserved in a poly bottle. Solubridge tests were run with a Hach² conductivity meter on both the treatment and control leachates for a period of three weeks after fertilization had been discontinued. The bottles were labeled and stored at room temperature in the dark until needed.

The experiment was a randomized complete block design using four replications, two treatments, and seeds of six species of plants. The following species were used:

Cichorium intybus

Chrysanthemum leucanthemum

Dracopis amplexicaulis (syn. *Rudbeckia amplexicaulis*)

Gaillardia pulchella

Lepidium virginicum

Plantago lanceolata

² Hach Chemical Co., Loveland, CO

Petri dishes (100mm x 15mm) were lined with #4 Whatman³ filter paper disks and ten seeds of each species evenly arranged on the paper. Another piece of filter paper was placed on top of the seed and moistened with five milliliters of leachate. The lids were placed over the dishes and sealed with Parafilm⁴ to conserve moisture. Each replication was randomized, put in flats and placed on a table in the laboratory protected from direct sunlight.

The tests were run for twelve days with observations being made every other day. Each petri dish was held up to an overhead light source in order to silhouette any emerging radicles. When the experiment was terminated the final counts were made by opening each petri dish and separating the filter papers so that exact totals could be confirmed.

Experiment 2

Introduction

Some allelopathic compounds are water soluble and are leached from the leaves of inhibitory species which are responsible for vegetational patterning (Rice, 1984).

Materials and Methods

This experiment was designed to simulate field conditions while still controlling as many variables as possible. Little bluestem plants were grown outside in pots filled with sand and sunk in a raised bed also filled with sand. Seeds of

³ Whatman International Ltd., Maidstone, England

⁴ American National Can Co., Greenwich, CT

eight herbaceous species were tested to see if germination was affected when they are planted in the same container with little bluestem plants. The experiment was a completely randomized block design using four replications, two treatments, and the seeds of eight herbaceous species. The following species were used in this experiment:

*Cichorium intybus**

*Chrysanthemum leucanthemum**

Dracopis amplexicaulis(syn.Rudbeckia amplexicaulis)*

*Gaillardia pulchella**

Lepidium virginicum

Plantago lanceolata

Sonchus oleraceus

Oxalis stricta

[* Indicates wildflower species commercially available]

There were seven seeds of each species per treatment. Washed silica sand was used for potting medium to avoid any possibility of influencing results with the presence of organic matter other than that of little bluestem. White, glazed crockery pots with a volume of 7.2l (21cm dia.) were filled with silica sand to within approximately four centimeters of the rim and sunk in a raised bed of silica sand to within five centimeters of the rim. The raised bed was 27.5cm deep and constructed of pressure treated boards. The

bed was lined with Weed-X⁵ fabric, a porous landscape weed barrier material, to keep out organic contaminants. The crockery pots were arranged in a grid of three rows and four columns spaced 15cm apart.

Plants of *Schizachryium scoparium* grown in 15cm pots were bare-rooted to remove the organic media from the root mass. The cleaned plants were then potted in the crocks of sand one plant per pot. The control treatments did not contain little bluestem plants.

A wooden block (3.1cm x 2.3cm x 8cm) was made into a seeding spacer by drilling 0.4cm diameter holes through the edge at one centimeter intervals making seven evenly spaced holes. The idea of the spacer block was to facilitate the spacing of seed out from the crown of the plant in a radial pattern at one centimeter intervals towards the outside rim of the crock.

There were three pots per replication. The control pots had seven seeds of each of the eight test species arranged in a radial distribution every 45 degrees of the compass. The treatments consisted of four species of wildflower seed with one little bluestem plant in one crock and four species of weed seed with one little bluestem plant in another. In the pots containing little bluestem seven seed of each species were sown in a single row aligned with one directional axis of the compass. This part of the experiment was not randomized

⁵ Dalen Products, Inc., Knoxville, TN

to ensure that each species of seed occurred along each orientation of the compass at least once.

Shade cloth was erected over the top of the beds until the plants overcame transplanting shock and were well established. The plants were watered with tap water through a water breaker held 1.8M above the bed to simulate rainfall. Initially, watering was performed twice daily while the little bluestem transplants were establishing. After the shade cloth was removed a single daily watering was done. After another two weeks watering was reduced to every other day. The little bluestem was fertilized once weekly from transplanting until one week prior to sowing the test seed. Peter's Peat-Lite Special 15-16-17⁶ was mixed with distilled water to a concentration of 200ppm nitrogen.

The test seed was counted and sown August 22, 1991 according to the treatment design. The bed was watered to keep the seed moist and provide the best possible environment for germination. Effort was made to simulate natural rainfall when irrigating the seed.

Daily observations of germination were made. Germination was first detected on August 27th. Records were kept on emergence observed in each pot indicating the species, orientation to the compass, and how many centimeters it was away from the little bluestem plant. The seeds were originally spaced one centimeter apart for eight centimeters

⁶ W.R. Grace and Co., Fogelsville, PA

radiating out from the little bluestem plant. Due to the dislodging of some seed by watering no conclusions regarding zonal influence could be made. The experiment was terminated after 10 days.

Experiment 3

Introduction

Allelopathic compounds may egress into the environment from particular organs of inhibitory species. Extracts of various organs of an allelopathic species had differing effects on germination and seedling growth (Wilson and Rice, 1968). This experiment was designed to assay the effects of extracts of different organs of little bluestem on seed germination.

Materials and Methods

The experiment was designed as a randomized complete block design with five replications, four treatments and four test species. The following herbaceous species were used in the germination trials: *Cichorium intybus*, *Chrysanthemum leucanthemum*, *Dracopis amplexicaulis*, and *Gaillardia pulchella*. Treatments consisted of dried extracts of leaves, inflorescences, and roots of little bluestem with distilled water as the control.

Three little bluestem plants that were grown in sand were barerooted, clipped into 8-10cm sections and separated into organ categories. The plant parts were placed in large paper grocery bags and allowed to air dry at room temperature for

three weeks. All the plants collected were going to seed. Treatment #1 consisted of leaf blades taken only from the plant base. Treatment #2 consisted of the seedheads and upright stems of the plant. Treatment #3 was roots that were cut just below the original soil line and washed to remove sand. Distilled water was the control or treatment #4.

A 7.2l glazed pot filled with sand was used to establish the approximate amount of little bluestem litter that was required to simulate 5mm depth in the field. It was determined that 12.0g of material was required to achieve the desired depth of litter.

Extracts were prepared by soaking 12g (dry weight) of material in 900ml of distilled water. Parafilm⁷ was placed over the top to prevent contamination. The beakers were placed in the dark and allowed to soak for twenty-four hours. The resulting liquids were filtered through Baxter⁸ qualitative filter paper and collected in clean 1000ml bottles.

The petri dish method of germination testing was followed using the extracts which had been prepared. A circle of filter paper was placed in each petri dish then ten seed per dish were evenly arranged. Another piece of filter paper was placed on top of the seeds and moistened with 5ml of the

⁷ American National Can Co., Greenwich, CT

⁸ Baxter Healthcare Corporation, McGaw Park, IL

appropriate extract. The lids were put in place and sealed with Parafilm to conserve moisture.

The trial was conducted over a period of twelve days. Observations were made by holding the dishes up to an overhead light source to silhouette any emerging radicles. Counts were made every other day and recorded. When the experiment was terminated each petri dish was opened and counts verified to ensure accuracy.

Experiment 4

Introduction

One of the acknowledged means of allelopathic compounds entering the environment is by the decomposition of plant residues. The purpose of this experiment was to determine if fresh or dried residues of little bluestem exhibited any allelopathic influence on the germination of wildflower seed. Sand was selected as the medium so that any results might be based solely on residues from little bluestem and not any other organic source.

Materials and Methods

The experiment was set up in the randomized complete block design using four replications, three treatments, and four species. The test was run indoors under greenhouse conditions. In a rectangular raised bed filled with silica sand 12 glazed 7.2l (21cm inside dia.) crockery pots were sunk in sand to a depth of 17.5cm. Then each pot was filled with silica sand to within three to four centimeters of their rims.

The pots were spaced with approximately 10cm between the rims of the crocks with four rows and three columns.

The treatments consisted of air dried clippings of little bluestem leaves, fresh green clippings of little bluestem leaves and horticultural grade, coarse vermiculite as the control. These were to be used as mulches placed over the top of seeds of the test species.

The clippings were collected from little bluestem plants that had been grown in sand media outdoors through the summer. The material collected for the dried grass treatment was taken from plants at a stage of growth when their floral stalks were initiated but not yet fully mature. Samples were made primarily of basal leaves and cut into eight to ten centimeter long sections to approximate the size of clippings left by a tractor driven mower. Clippings were then placed in brown paper grocery bags and allowed to air dry in the lab at room temperature for two weeks. The fresh clippings were taken just prior to the initiation of the experiment.

Vermiculite was selected for the control treatment assuming it would function as an inert mulch. It would also serve as a top dressing over the test seed serving to conserve moisture and offering optimal conditions for germination.

Samples of the dry leaves were placed in similarly prepared crocks to simulate leaf litter depth of approximately one and one quarter centimeter and then weighed to establish the amounts of mulching material to use in each treatment. It

was found that 12.0g of dry leaf material closely approximated the desired mulching depth.

The dried little bluestem leaves were then weighed and placed in the test crocks according to the treatment randomization order. Small frames with screen wire mesh were placed over the top of the pots. The mesh frames would allow air, water, and light penetration to react with the leaf material. The crocks were watered every other day, except when it rained, to help accelerate the process of decomposition so that perhaps the resulting leachable compounds might simulate those found under field conditions. This treatment was allowed to leach into the sand over a period of four weeks prior to sowing test seed.

The four test species were *Cichorium intybus*, *Chrysanthemum leucanthemum*, *Dracopis amplexicaulis*, and *Gaillardia pulchella*. The first two were chosen because they were introduced species that have escaped and adapted across much of the country. The other two are native to the U.S. and are also very well adapted to a wide geographic area. In each crock 25 seeds were sown for each of the four species. All four test species germinated extremely well in preliminary trials.

It was determined that due to the cooler temperatures of October the actual experiment would be run in the greenhouse. The 12 pots were moved to a greenhouse bench and reset in the same order and orientation as before. The screened frames

were removed from on top of the dried little bluestem leaves as they were no longer necessary.

Fresh green clippings were collected, weighed and placed in plastic sandwich bags in 12.0g allotments. The vermiculite was also weighed and prepackaged in 12.0g amounts.

A compass was used to indicate the magnetic north point on the inside rim of each pot. Plastic labels were inserted at this point flush with the inside wall of the pot. They were labeled with the replicate number and treatment code that each pot was to receive.

A simple seeding device was made to facilitate the sowing of seed in a uniform reproducible manner. It was fashioned from a clear, square piece of Lucite⁹ measuring 6cm x 6cm x 0.5cm through which 25 three millimeter holes were drilled. The holes were arranged in a grid configuration of five rows and five columns spaced one centimeter on center in both directions. When the device was placed on top of the media the seed could be put in each hole achieving uniform distribution. Since the Lucite is clear each cell could be seen so duplication of seeding was avoided.

A 20cm diameter circular frame and some string were used to make crosshairs which would nest just inside the pots. It was oriented so that the crosshair axis aligned running southwest to northeast and southeast to northwest. Beginning

⁹ Dupont Co., Willmington, DE

with the north quadrant, the seed spacing device was then placed on top of the sand and one seed dropped into each hole. The device was lifted off and moved to the next quadrant progressing in a clockwise rotation. The seeding order was based on alphabetical sequence of the genus of each seed and was rotated with each pot so that all the seeds would be in each compass orientation at least once.

The sand medium was tamped lightly to level the surface prior to seeding and as each pot was seeded it was lightly firmed to insure good contact between seed and sand for optimum germination. The dry bluestem straw was lifted off of each pot before tamping and seeding. The mulches were applied as evenly as possible. The pots were misted with tap water using a nozzle attachment on a garden hose held approximately 45cm above the pots. Mist was applied three to four times a day to prevent the seed from drying out. It was also desired to encourage leaching by both of the bluestem straws onto the seeds below.

The seeds were sown October 20th, 1991 and germination counts were made every five days. The experiment was terminated after 15 days on November 4th. Mulches were removed on the last count so that they would not conceal any germinated seed from detection. When each germinated seed was recorded it was then removed from the pot to avoid counting any seedling more than once.

CHAPTER IV

RESULTS AND DISCUSSION

All the resulting germination data were analyzed by ANOVA and the means were separated by Least Significant Difference method at the 95 percent level of probability. In the experiments utilizing the petri dish method, germination was acknowledged upon the emergence of the radicle. In experiments where seeds were sown in sand, the emergence of cotyledons above the sand was counted as germination. The final counts were confirmed and totals adjusted to reflect actual radicle or cotyledon emergence.

Some of the test seed was chosen to evaluate possible interaction between little bluestem and species commonly found in wildflower mixtures. Little bluestem is the most widely recommended native grass for combining with wildflower plantings. The other seed used in the tests were weed species that are commonly found in this area. All of the test species exhibited high germination percentages in preliminary testing.

Experiment 1

There was no statistically significant effect of the leachate overall in the experiment according to the ANOVA. However, there was a significant difference between species. There was a significant inhibition of *Chrysanthemum* and *Gaillardia* according to LSD but only marginally (Table 1).

TABLE 1. Effects of little bluestem leachate on the germination of six herbaceous species.

Species	Treatment	Mean ^a
<i>Cichorium intybus</i>	control	7.8
	leachate	8.0
<i>Chrysanthemum leucanthemum</i>	control	8.8 ^b
	leachate	6.3
<i>Dracopis amplexicaulis</i>	control	8.5
	leachate	9.8
<i>Gaillardia pulchella</i>	control	7.5 ^b
	leachate	5.0
<i>Lepidium virginicum</i>	control	8.3
	leachate	9.0
<i>Plantago lanceolata</i>	control	6.3
	leachate	5.5

Data summarized from four replications.

a Mean number germinated out of 10 seed after 12 days.

b Significantly different from the calculated $LSD_{05}=2.4$.

Due to the collection method it was difficult to ascertain whether concentrations of the leachate approached levels found under field conditions. It was stated by many researchers that too high a concentration of allelochemicals can be as ineffective as too low a concentration. Thus it would be important to accurately reproduce concentration levels of compounds found in the field in order to support any arguments for allelopathic interaction in a community. The concentration may have been diluted too much by the volume of distilled water added. It is also possible that an adequate root density to soil volume ratio was not achieved. In such case the planting of more seedlings per container might have more correctly simulated field concentrations.

Experiment 2

The overall germination for this experiment fell below expectations regardless of any treatment influence. The only difference in germination was observed between the various species. Some germination variability between species was expected. The treatment did not produce any effect according to LSD comparison (Table 2).

If the number of seeds per species had been increased it is conceivable that small allelopathic effects might have been recognized. It is more difficult working with such small numbers to be confident in any conclusions the data might indicate.

TABLE 2. Effect of the presence of little bluestem plants on the germination of eight herbaceous species.

Species	Treatment	Mean ^a
<i>Cichorium intybus</i>	Grass	2.3
	Control	2.3
<i>Chrysanthemum leucanthemum</i>	Grass	3.0
	Control	2.5
<i>Dracopis amplexicaulis</i>	Grass	3.3
	Control	2.8
<i>Gaillardia pulchella</i>	Grass	3.0
	Control	1.8
<i>Lepidium virginicum</i>	Grass	4.5
	Control	5.8
<i>Plantago lanceolata</i>	Grass	2.3
	Control	2.3
<i>Sonchus oleraceus</i>	Grass	2.0
	Control	1.5
<i>Oxalis stricta</i>	Grass	0.3
	Control	0.5

Data summary of four replications.

Control treatment was sand only.

LSD_{.05} = 2.0

^a Mean number germinated out of 7 seed after 10 days.

The use of clay based media may have had different results than was experienced with sand. Some researchers found that allelochemicals could be accumulated on soil colloids and held there until rain released the compounds into the soil solution. If any compounds were released into the sand or washed from the leaves it is very likely that they did not remain in contact with the seeds long enough to produce an effect. Any compounds that may have been produced were probably leached away.

Experiment 3

There was no significant effect observed on germination due to any of the leachate treatments according to ANOVA. The LSD method indicated that *Gaillardia* tended to be inhibited by the leachate made from floral parts (Table 3). The germination for *Gaillardia* was extremely low in this experiment compared with the other species.

The age and condition of the collected materials could be the critical factor in this type of test. Fresh green clippings may have been required to produce any allelopathic effect. Material collected at a certain stage of development may exhibit more allelopathic influence.

It is possible that the plant parts were allowed to leach too long in the distilled water. The active compounds may be released soon after being submerged and start to break down under anaerobic conditions. Shorter soaking times

TABLE 3. Effect of leachates made from different organs of little bluestem on the germination of four herbaceous species.

Species	Treatment ^b	Mean ^a
<i>Cichorium intybus</i>	T-1	7.2
	T-2	8.0
	T-3	8.4
	T-4	7.2
<i>Chrysanthemum leucanthemum</i>	T-1	6.8
	T-2	6.2
	T-3	7.4
	T-4	6.2
<i>Dracopis amplexicaulis</i>	T-1	7.8
	T-2	7.8
	T-3	7.6
	T-4	8.8
<i>Gaillardia pulchella</i>	T-1	3.6
	T-2	1.6
	T-3	2.8
	T-4	4.4

Data summary of four replications.

LSD_{.05} = 1.9

a Mean number germinated out of 10 seed after 12 days

b T-1 Leachate made from leaves

T-2 Leachate made from inflorescences

T-3 Leachate made from roots

T-4 Control of distilled water

may have resulted in higher concentrations of active allelochemicals. The concentration level of the compounds may not have been adequate to produce any significant effect.

Experiment 4

There was a significant effect of both little bluestem mulches on the germination of all four species tested (Table 4). However there was not any effect attributed to species or treatment and species interaction according to the ANOVA. It appears that little bluestem did exhibit some allelopathic potential.

It would be desirable to test mulches of different little bluestem organs to determine if one exhibited more influence than another. The results may vary depending on the developmental stage at which the clippings were collected.

TABLE 4. Effects of dried and fresh straw of little bluestem on germination of four herbaceous species.

Species	Treatment ^b	Mean ^a
<i>Cichorium intybus</i>	Control	20.0
	Dried	14.0
	Fresh	12.5
<i>Chrysanthemum leucanthemum</i>	Control	20.0
	Dried	9.0
	Fresh	7.0
<i>Dracopis amplexicaulis</i>	Control	20.3
	Dried	11.0
	Fresh	13.3
<i>Gaillardia pulchella</i>	Control	21.5
	Dried	12.0
	Fresh	10.3

Data summary of four replications.

Mulches placed over sand media

LSD_{.05} = 6.4

a Mean number of seed germinated out of 25 after 15 days

b Dried Little Bluestem 12.0g

Fresh Little Bluestem 12.0g

Control was 12.0g of coarse vermiculite

CHAPTER IV

SUMMARY AND CONCLUSIONS

The objectives of this research were two-fold. The primary goal was to determine whether *Schizachyrium scoparium* exhibited any allelopathic properties regarding the germination of certain herbaceous species. The secondary purpose was to determine if little bluestem might chemically influence community composition in such a way that this ability could be exploited in roadside wildflower establishment. Allelopathy has been proposed as an important factor in affecting the species composition within some plant communities (Muller, 1966; Rice, 1984). Little bluestem has been recommended as a primary component in wildflower seed mixtures by many authorities. The prairies of the western United States are ideal models on which to base the composition of wildflower seed mixtures for roadside planting. Little bluestem is one of the predominant species of the climax prairie as well as being widely distributed throughout the United States. The stability of these western plant communities, if reproducible, could be a significant development in the establishment of wildflower plantings.

A series of experiments was run with the purpose of simulating conditions that might act to release allelopathic compounds into the environment. Primarily the tests consisted of collecting or making leachates from various parts of the

little bluestem plant and subjecting certain species of seed to the resulting leachates. Mulches consisting of clippings of little bluestem were also tested to evaluate their allelopathic potential. Observations were made to determine if the germination of the various test species were affected by any of the compounds produced by little bluestem. Every attempt was made to eliminate other possible sources of leachable compounds such as organic potting media.

The overall results did not strongly support the theorized existence of allelopathic properties in *Schizachyrium scoparium* but one of the tests did indicate some inhibition of germination by little bluestem.

In Experiment 1 seeds of six species were subjected to leachate that was collected from the root zone of little bluestem using the petri dish method of germination testing. Comparing the differences between treatment and control with the calculated LSD (2.4) two species were affected. *Chrysanthemum leucanthemum* and *Gaillardia pulchella* were slightly inhibited by the leachate treatment with differences of 2.5 for each species.

Experiment 2 was to determine if seed sown in close proximity to the base of little bluestem plants would be inhibited in germination. The germination in this experiment was lower than expected and thus probably not suitable for basing any conclusions. It is likely the test seed was washed deeper into the sand medium than optimal for good germination.

Perhaps the use of vermiculite as a mulching material would have prevented the dislodging problem and offered reliable results.

Experiment 3 was designed to determine if leachates made from different parts of little bluestem had varying effects on germination. There was no significant inhibition by any of the three leachate treatments. It is possible that the concentrations of the leachates were not sufficient to cause any response. Further experimentation should involve soaking the little bluestem clippings for varying periods of time before collecting and testing.

Experiment 4 was to determine if fresh or dried clippings of little bluestem used as a mulch exhibited any influence on seed germination. There was a significant difference when comparisons were made with the calculated LSD value (6.4) and the differences between the treatments and control germination means. The fresh little bluestem mulch was slightly more inhibitory to germination than the dried mulch in this experiment. The results of this test were encouraging but far from conclusive. Further testing should involve repetition of this test both in the greenhouse and in the field.

The overall results of the experiments discussed herein did not strongly support any hypothetical allelopathic properties of little bluestem. This researcher believes that with more refinement of technique and better simulation of field conditions findings might be more conclusive. There is

a need for study and experimentation in the field with indigenous populations of *Schizachyrium scoparium* to substantiate suspected allelopathic properties.

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VITA

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