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Germination, emergence, and early growth of Spartina pectinata

Wade Herold Williams
University of Northern Iowa

GERMINATION, EMERGENCE, AND EARLY GROWTH OF *SPARTINA PECTINATA*

An Abstract of a Thesis

Submitted

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Wade Herold Williams

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ABSTRACT

Spartina pectinata Link is frequently omitted from lowland prairie restorations because the seed often fails to grow when planted, and inclusion is limited to plugs of young plants. Anecdotal reports indicated improvement of seed germination following stratification. The effects of seed stratification on the germination of *S. pectinata* seed were systematically studied. Seed was obtained from both relict populations of plants and germplasm plants as defined by Englert and White (1977). Seed from 5 different collections were subjected to 4 different seed stratifying treatments: imbibed-chilled (1°C for 15 to 45 days); imbibed-frozen (-12°C for 4 to 7 days); or dried-warmed (23°C for 120 days); or aged (4°C for 1.5 to 2.5 years). Control (unstratified) seed for all trials was fresh, dried-chilled seed of the same collection, stored in a refrigerator at 4°C. In addition, seed from 4 collections planted in soil were subjected to 2 different seed stratifying methods (imbibed-chilled, imbibed-frozen) under greenhouse conditions. Emergence of germinating seedlings was monitored for post-emergence damping-off. Also, seedlings from 2 collections were grown in the greenhouse and growth measurements were made weekly for 6 weeks after emergence from the soil. Control seed ranged in average viability from 66.5% (±7) to 99% (±1), in average germination from 59.5% (±7) to 94% (±2.2), and in average emergence from 38.5% (±3.8) to 84.5% (±6.8). The stratification treatments did not improve these values. Longer stratification periods at 1°C for imbibed-chilled seed increased the germination percentage in the early phase of germination, this early increase was also reflected in coleoptile emergence from the soil. Total germination and emergence at the end of the trials were the same as the control.

Stratifying seed by imbibed-freezing, dried-warming, or aging up to 1.5 years had no effect on its germination, though control seed germinated significantly better than 2.5-year old seed. Germination rates varied in collections from different sites. Post-emergence damping-off was not observed for any seed collection, but mildew became a problem for seed stratified by imbibed-chill beyond 45 days. For both relict and germplasm plants, the total length of leaves and roots steadily increased during the first 6 weeks. The majority of growth was below ground. Average seed depth decreased during early growth, rising several seed thicknesses from an average planting depth range of 0.62 cm (±0.11) to 0.69 cm (±09). Endosperm starch levels steadily decreased through week 4 and were depleted by week 5. Six week-old seedlings had an average total shoot length range of 30.40 cm (±5.89) to 33.32 cm (±4.71) and an average total root length range of 139.40 (±39.40) cm to 169.11 cm (±52.42). It can be concluded from this study that *Spartina pectinata* should be included in prairie restoration projects, as viable seed will germinate readily with or without stratification.

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3/20/01 Date	Dr. Daryl D. Smith, Chair, Thesis Committee
March 20, 2001 Date	Dr. Jean M. Gerrath, Thesis Committee Member
3/28/01 Date	Dr. Kurt W. Pontasch, Thesis Committee Member
March 20, 2001 Date	Dr. Mark D. Ecker. Thesis Committee Member
4/30/01 Date	Dr. John W. Somervill, Dean, Graduate College

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INTRODUCTION AND LITERATURE REVIEW

Introduction

Spartina pectinata Link (prairie cordgrass, or sloughgrass), once a dominant canopy species of the lowland tallgrass prairie, is in need of careful study. Public interest in prairie restoration* projects is steadily increasing, but *S. pectinata* has been left behind. It has proven difficult to grow from seed, and its incorporation into projects is largely restricted to seedling plugs from container-grown live plants. These plants, which can be produced by division of its fast-growing rhizomes (Wynia 1994), are expensive to grow, transport, and plant, but most importantly seedlings can not be included in sufficient quantities to properly represent *S. pectinata* in habitat-appropriate projects. Ironically many associated wet prairie forbs are fairly well known and easily grown from seed. The ability to grow *S. pectinata* from seed would and should be a major step toward achieving appropriate species composition when restoring lowland prairie habitat. Seed sources are readily available as relict populations of *S. pectinata* are common throughout its former range (Mobberley 1956; Kucera 1961).

Literature reports are not optimistic regarding the production of *S. pectinata* from seed. On the subject of seed production, authors and researchers are either silent (Silveus 1933; Kucera 1961; Gould and Shaw 1983; Brown 1979) or pessimistic, describing plants as 'grain-free,' 'empty glumes,' 'good seed sparingly produced,' 'seed with low viability,'or 'seed heavily damaged by insects (Pammel *et al.* 1904; Britton and Brown

* Restoration as defined by Packard and Mutel (1997) includes prairie reconstruction from bare ground, and rehabilitation of degraded prairies.

1913; Pohl 1966; Santos 1968; Runkel and Roosa 1989; Gleason and Cronquist 1991; Shirley 1994; Wynia 1994; Christiansen and Müller 1999). Of the viable seed produced, some indicated that germination was prevented by an unknown dormancy and required stratification prior to germination (Eddleman 1977; Long *et al.* 1990; Shipley and Parent 1991; Shirley 1994), while others reported seed to germinate readily but methods, results, and outcomes were variable (Weaver 1954; Santos 1968; Shipley and Peters 1990; Shipley and Parent 1991; USDA-NRCS 1992-3). Several Midwestern prairie plant merchants have reported success in growing *S. pectinata* from seed, but this information has not found its way into the literature.

There are steadily increasing opportunities for use of *S. pectinata* seed in restorations, if seed were available. For example, after the summer floods of 1993, the United States Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS) acquired floodplain farmland along several Iowa rivers. Working along the Iowa river, the USDA-NRCS, in partnership with the United States Fish and Wildlife Service, sought to reconstruct large tracts of wet prairie. *S. pectinata* was the former dominant species of this area and would have been a natural choice for reintroduction.

Meanwhile along Iowa's state and county roads, roadside managers are planting native prairie species as part of the state's Integrated Roadside Vegetation Management program (Ehley 1989). It could be included with other locally-adapted species to help reduce maintenance costs and soil erosion while improving wildlife habitat.

Finally, across the northern Great Plains and Midwest, the USDA-NRCS detected the emergence of a potential sales market. Commercial supplies of *S. pectinata* seed are

limited, yet at least six potential uses for *S. pectinata* have been identified: prairie restoration, streambank stabilization, windstrip barriers, filter strips, riparian buffers, and prairie landscaping (Tober *et al.* 1998). To answer this need, the Plant Materials Center at Bismarck, North Dakota is developing a cultivated variety from relict plants (USDA-NRCS 1997).

Objectives

The inconsistencies between reports on the production of *S. pectinata* from seed indicated a need to systematically re-evaluate the germination of this species after different seed storage conditions. The objectives of this research were to:

1) Compare the effectiveness of 4 stratifying treatments on relict and germplasm *S. pectinata* seed.

2) Investigate the growth and development of *S. pectinata* from several seedling collections.

Literature Review

"At the Mississippi, the prairie for the most part extends to the water's edge, and renders the scenery truly beautiful . . . Fancy a natural meadow of deep green grass and beautiful flowers, rising with a gentle slope for miles so that, in the vast panorama, thousands of acres are exposed to the eye."

--from a geological reconnaissance by Owen (1852)

A Natural Resource

Historic habitat and cultural use. *Spartina pectinata* is native to North America, and was the most abundant grass of the wet tallgrass prairie. Before Euro-American settlement, *S. pectinata* covered hundreds of square miles of prairie bottomlands, and often grew in almost pure stands. The species was especially prevalent on the flood plains of the great rivers and their tributaries (Mobberley 1956; Weaver 1954, 1960; Pohl 1966).

S. pectinata had several cultural uses. Prairie Indians, such as the Mandan and Hastada, used the leaves to thatch the tops of permanent lodges and cache pits, and line interiors of the underground pits (Wilson 1917; Madson 1982). Euro-American settlers adapted this practice, thatching the tops of haystacks and outbuildings. Settlers also used the sod itself, forming building blocks by skinning it off in courses 1-foot wide, 2 or 3-feet long, and 4 inches thick. With its dense matrix of interlocked roots, *S. pectinata* sod was prized by pioneers, and considered the best for constructing houses (Madson 1982; USDI-NPS-JENA 2000).

Extant distribution. Today *S. pectinata* is most common in the Midwest, Northeast, and southern Canada (Mobberley 1956; Kucera 1961). In addition, the plant has been introduced into Italy (Mobberley 1956). Along the East Coast, *S. pectinata* thrives equally well in salt and fresh water. In New England salt marshes, it grows intermingled with *S. patens* (Ait.) Muhl. (saltmeadow cordgrass). They hybridize to form a third species, *S.* x *caespitosa* (A.A. Eaton) Fern. (Mobberley 1956). Across the western Great Plains, *S. pectinata* is restricted to stream and marsh margins where it shares habitat with *S. gracilis* Trin. (alkali cordgrass) (Mobberley 1956; McGregor and Barkley 1986).

In freshwater environments, *S. pectinata* grows on wet-to-seasonally mesic ground. Its habitat includes marsh margins, sloughs, swales, floodplains, drained fields, wet ditches, and railroad and highway embankments (Britton and Brown 1913; Silveus 1933; Weaver 1954, 1991; Mobberley 1956; Kucera 1961; Pohl 1966; Brown 1979; Gleason and Cronquist 1991; Eilers and Roosa 1994; Ladd and Oberle 1995; Christiansen and Müller 1999). Wynia (1994) noted it tolerated some fairly dry sites. Mobberley

(1956) alone claimed that contrary to most information, primary habitat seemed to be open, dry prairie.

Locally, *S. pectinata* is mostly absent from shoulders and ditches along highways and paved secondary roads, but is common along gravel roads. Quite likely the plant was excluded from modern thoroughfares due to the rhizomes being removed during the intensive earth moving process necessary to construct a paved road. At the same time the seed bank was probably eliminated by being removed or buried.

Weaver (1954, 1991) called an area dominated by *S. pectinata* a 'sloughgrass community,' and enumerated common forbs found in more open areas. Crist and Glenn-Lewin (1978) called a community where *S. pectinata* was prominent a 'pothole border.' Currier *et al.* (1978), identified *S. pectinata* as 1 of 8 dominant vegetation types in a central Iowa marsh. These authors seemed to agree that along a hydric gradient, *S. pectinata* grew near the high water mark. Currier *et al.* (1978) noted that *S. pectinata* expanded laterally along a hydric gradient, and was associated with *Carex* spp. (sedges) at its margins. Crist and Glenn-Lewin (1978) found *S. pectinata* associated with 4 other dominant species: *Polygonum coccineum* Muhl. (water smartweed), *Calamagrostis canadensis* (Michx.) Beauv. (bluejoint), *Poa palustris* L. (fowl meadowgrass), and *Carex stricta* Lam. Weaver (1954) observed that downslope *S. pectinata* was bordered by various tall sedges (*Carex* spp.), rushes (*Juncus* spp.), marsh grasses (*Poa* spp.) and other hydrophytes (Weaver 1954). Upslope it gave way to *Andropogon gerardii* Vitman (big bluestem) through a transitional zone characterized by *Panicum virgatum* L. (switchgrass) and *Elymus canadensis* L. (Canada wild rye) (Weaver 1954).

Taxonomy. Chapman (1996) placed *S. pectinata* in the family Poaceae, subfamily Chloridoideae (a subfamily mostly associated with drier tropics and subtropics), and tribe Chloridae. Variation in both field specimen morphology and DNA analysis has left many authors divided about the plant's taxonomy (Mobberley 1956).

Wild vs. cultivated. This study used both relict and germplasm grass seed. Englert and White (1997) defined 'relict' and 'germplasm' as 2 classes of a 5-class plant materials hierarchy (Appendix 1).

Relict *S. pectinata* seed from across its range has been used in germination experiments by several authors. Santos (1968) worked with seed from Iowa, Illinois, and Missouri, Weaver (1954, 1991) with seed from Iowa and Nebraska, W Lovelace (personal communication, 1998 Nov 5) and J Keiser (personal communication, 1999 Feb 8) with seed from Missouri, Wynia (1994) and E Jacobson (personal communication, 2000 Jul 17) with seed from Kansas, Tober *et al.* (1998) with seed from North Dakota, South Dakota, and Minnesota, Eddleman (1977) and Eddleman and Meinhardt (1981) with seed from Montana and Wyoming, and Shipley and Peters (1990) and Shipley and Parent (1991) with seed from Quebec and Ontario, Canada.

Tober *et al.* (1998) documented construction of the *Red River* 'natural' germplasm by the USDA-NRCS Plant Materials Center in Bismarck, North Dakota, and described it as a composite of 4 accessions from South Dakota, North Dakota, and Minnesota. There were 2 reasons given to justify the germplasm's development and release: limited commercial supplies of northern relict seed; and the lack of a cultivar.

Flowering and Seed Set

Reproductive morphology. *S. pectinata* is an obligate perennial, warm-season grass (Kucera 1961; Shipley and Parent 1991). Its graceful culms grow up to 2.5 m in height (Mobberley 1956) and have scabrous margins (Britton and Brown 1913) with leaves produced from a mat of coarse, thick, many-branched rhizomes (Wynia 1994). The root system is equally coarse, rather poorly branched, and deep, penetrating almost vertically downward to depths of 2.5 to 4 m (Weaver 1954).

In Iowa, it blooms from late June to September or October (Mobberley 1956; Pohl 1966). Flowers are borne on tall panicles (Mobberley 1956). Spikelets are 1-flowered (Gould and Shaw 1983), and the flowers are perfect (Britton and Brown 1913), having 1 ovary, 2 plumose stigmas and filamentous, purple anthers. After flowering, some spikelets produce a single caryopsis (Mobberley 1956).

In local populations, flowering spikes can be consistently identified by 3 characteristics: the purple-colored anthers that dangle from spikelets, the distinct grape-fragrance produced by the tiny flowers, and insects that tend to cover the flowering spikes (especially small bees, flies and beetles). This suggests that insects may play a role in the pollination of these plants, a condition not unheard of in other grasses. A few tropical rain forest grass species have evolved an insect pollination mechanism (Proctor *et al.* 1996), though the main exceptions to wind pollination in Poaceae are those species that are self-pollinated (often cleistogamous) or apomictic (Campbell *et al.* 1983). Insect pollination of *S. pectinata* flowers is an area that needs more study.

Younger plants form clumps and often produce many flowering panicles. Older colonies reproduce mainly from spreading rhizomes, and produce panicles mostly along the colony perimeter (Pohl 1966). In local pastures where it lacks competition from other grasses, colonizing *S. pectinata* tends to form large, symmetrical circles, sometimes many meters in diameter, presumably from the uniform outward growth of rhizomes. Few flowering panicles are produced in the centers of the older colonies.

By late summer, many cordgrass colonies have produced light-colored panicles (Silveus 1933). Each panicle axis is 3-angled, and individual spikes are appressed to somewhat spreading (Mobberley 1956). The spike rachis is 3-angled, and produces spikelets in rows on 2 sides. Spikelets are alternate, appressed, closely imbricate, and have short pedicels that disarticulate below the glumes (Mobberley 1956; Pohl 1966). The first glume is usually shorter than the second, but longer than the floret. Both glumes are awned, with the second glume awn longer than the first, and sometimes almost 1 centimeter in length. The paper-thin palea is slightly longer than the lemma (Mobberley 1956; Kucera 1961).

Seed set. Low seed set is common in Midwest stands of *S. pectinata.* Mobberley (1956) examined plant material collected throughout the plant's extant range, and found seed set to be highly variable from year to year. He offered no explanation for this variation.

Santos (1968) sought to find the cause of low seed set in Midwest populations by studying relict plants from Iowa, Illinois and Missouri. For all plants examined, seed set was relatively low, both in the field (0.9 to 39%) and from transplants raised in

greenhouses (12.6 to 20%). The author suspected the problem was developmental in nature, but after examining flower production, and male and female gametophyte development, conceded that developmental physiology appeared normal. Ultimately a list of other possible causes were suggested: self sterility, insufficient panicles produced to supply pollen for fertilization, insect injury, pollen germination failure, and/or unfavorable weather conditions for reproduction.

Nicholson and Langille (1965) found that seed production was improved by application of nitrogen fertilizer. Smith and Smith (1997) called for nitrogen application on established stands, but the purpose of this fertilizer was not stated.

Seed Handling

Harvest. Timing of harvest has been shown to affect the quality of seed. Budy *et al.* (1986) pointed out that timing of seed collection was important. If seed was collected too early, yields were low, and immature seed failed to germinate. If collection was delayed, seed might shatter and be lost on the ground. Those working with *S. pectinata* seed over the years developed at least 3 different criteria for judging when to harvest: calendar date, seed moisture, and shattering potential. Some studies indicated a 2 to 3 month window of opportunity for seed harvest while others tended to focus more tightly on a period slightly before, to several weeks after, the first frost.

Eddleman (1977) stated that prairie cordgrass could be harvested in southern Montana from late August to early November. The average frost for that area was 6 September (Koss *et al.* 1988). Shipley and Parent (1991) harvested seed in September and October in southeast Ontario and southwest Quebec, an area with a first frost date of

about 6 October (Bingham and Halvorson 2000). Shirley (1994) recommended the month of October as a seed harvest date, but did not specify location. Smith and Smith (1997) harvested seed from 5 to 30 October in Bismarck, North Dakota, an area which experienced its average first frost on 7 September (Koss *et al.* 1988). USDA-NRCS (1997) reported that in New England, seed typically matured within 1 or 2 weeks of frost. This was a region with first frost dates that ranged from 4 September in northern Maine to 22 October in southern Massachusetts (Koss *et al.* 1988).

Hartmann *et al.* (1997) noted that developing seed should be sampled often and harvested once the seed had reached physiological maturity (no further increase in dry weight) and when seed moisture was 12 to 15%.

Wynia (1994) cautioned that shattering during maturation could be a problem, though this was contradicted by Tober *et al.* (1998). Broome *et al.* (1973) worked in a similar situation with *S. alterniflora* along the North Carolina coast, and concluded that seed should be collected as near the shattering stage as possible, since seed performance was affected by maturity. He added that spikes shattered more easily after a month in cold storage at temperatures just above freezing.

Several techniques have been developed to harvest and process *S. pectinata* spikes. Broome *et al.* (1973), Eddleman (1977), and Wynia (1994) agreed that mature spikes from small patches of both species could easily be harvested by hand. Eddleman (1977) suggested clipping spikes with electric shears and drying them in large open paper bags. GA Houseal (personal communication, 1999 Sept 15) also used large open paper bags to air dry seed. Broome *et al.* (1973) noted the difficulty in clipping large numbers

of *S. alterniflora* spikes and described a mechanical spike clipper invented specifically for the study. Wynia (1994), Smith and Smith (1997), and Tober *et al.* (1998) recommend harvesting larger patches of *S. pectinata* mechanically with a combine. Tober *et al.* recommended combining with a straight head, and cautioned that since the leaves had such a high fiber content, combining should be done after the leaves have dried. Kromray (2000) observed that combines were designed to harvest agricultural crops and might do a poor job collecting native seed. He offered an excellent discussion on design modifications that improved combine performance with native grasses and forbs.

Cleaning. Ultimately, effective seed cleaning techniques might be the key to producing high quality, pure, live seed. GA Houseal (personal communication, 2000 May 17) disarticulated spikelets by rubbing spikes across a screen of ½-inch hardware cloth stretched over a frame. Eddleman (1977) achieved nearly 100% pure live seed by running spikes through a head thresher, mechanical flail, a screen separation in a clipper mill, and finally a seed blower. Smith and Smith (1997) used a debearder, hammer mill, and a screen separation in a clipper 4-screen fanning mill.

Storage. *S. pectinata* has been stored under a variety of conditions. Wynia (1994) recommended that for ideal storage, seed should be kept in a cool, dry place. Smith and Smith (1997) specified that a safe storage moisture content for seed was 12%, and 15% in paper bags. GA Houseal (personal communication, 2000 Sept 7) stored seed between 2 and 5°C. Plant Materials Specialists at the USDA-NRCS Plant Material Centers in Bismarck, ND (W Duckwitz, personal communication, 2000 Dec 11) and Elsberry, MO (S Bruckerhoff, personal communication, 2000 Dec 11) spoke of not exceeding a

combined temperature (°F) and relative humidity (%) index of 90. The Bismarck Plant Materials Center stored seed at 4.4°C in humidity of 40-50%; the Elsberry Plant Materials Center stored seed at 15.6°C in humidity of 10-20%. Eddleman (1977) stored seed dry at 20°C up to 12 months without a noticeable decline in viability.

Weight. The caryopses are quite small, and difficult to weigh accurately. VA Berg (personal communication, 1999 Feb 8) recommended weighing *S. pectinata* caryopses in batches of 25, due to small size, limitations of scale sensitivity, and convenience. Shipley and Parent (1991) weighed seed in 4 batches of 25 and obtained an average seed weight value of 71×10^{-5} g, though it was unclear if these were spikelets or caryopses. Eddleman (1977) reported a fairly uniform range of seed weight, 282 to 291 spikelets per gram, over 2 growing seasons.

Germination and Growth

Viability. Several authors characterized *S. pectinata* as producing seed with low viability (Runkle and Roosa 1989; Shirley 1994; Christiansen and Müller 1999). Roberts (1972) defined a viable seed as one which could germinate under favorable conditions, providing dormancy had been removed. Christensen (1972) noted that a common cause of low viability in otherwise healthy looking seed was fungal attack. Ellis *et al.* (1985) stated that the tetrazolium test was developed to measure seed viability, since it was otherwise impossible to distinguish a viable, dormant seed from non-viable seed.

Stratifying nomenclature. Traditionally, stratification meant chilling a seed to remove dormancy (or after-ripening), but the concept has expanded to include different temperatures and imbibed states (Bewley and Black 1994; Hartmann *et al.* 1997).

Stratification should not be confused with vernalization. Treatment is often the same, but the end goal differs. Seeds and growing plants are vernalized to promote flower production; but seeds are stratified to promote after-ripening (Taiz and Zeiger 1991; Hartmann *et al.* 1997).

Breaking dormancy. Seed dormancy in *S. pectinata* has been a contentious issue. Dormancy was defined by Bewley and Black (1994) as the ability of a young plant to suspend its developmental processes until conditions necessary for germination were met. Ellis *et al.* (1985) noted that dormancy was common in the seed of wild plants. Simpson (1990) and Hartmann *et al.* (1997) both called dormancy a polymorphic trait, and Buchanan *et al.* (2000) detailed the biochemistry of breaking dormancy. Authors who have studied *S. pectinata* have a variety of opinions about seed handling strategies needed to overcome seed dormancy. Eddleman (1977) found dormancy to be polymorphic, with 40% of the seed tested having no apparent dormancy. Shirley (1994) claimed double dormancy, and indicated that 2 years was required to germinate seed. The assumption that dormancy was an obstacle to germination was implicit in the experiments of Long *et al.* (1990), Shipley and Parent (1991), W Lovelace (personal communication, 1998 Nov 5), J Keiser (personal communication, 1999 Feb 8). Broome *et al.* (1973) also assumed that dormancy was blocking germination in the closely related species *S. alterniflora.*

Mayer and Poljakoff-Mayber (1982) noted that dry storage of dormant seed often produced after-ripening. Hartmann *et al.* (1997) described after-ripening as the process a seed goes through that results in the removal of primary dormancy. Wynia (1994)

observed after-ripening in *S. pectinata*, noting that no special seed treatment was required for seed propagation.

Mimicking the over-wintering conditions of a seed's natural habitat is a common approach researchers use when trying to break the seed dormancy of a particular species. For example, Budy *et al.* (1986) found that the seed of many wetland plants required storage in a cool, wet environment before they would germinate. It is therefore not surprising that many researchers have found, or assumed that the seed of *S. pectinata* required a period of imbibed-chill to germinate. Eddleman (1977) chilled imbibed *S. pectinata* seed on cellulose pads from 30 to 60 days at 4°C. He found that only imbibed-chill was effective in breaking seed dormancy. Long *et al.* (1990) also noted that a significant amount of chilling stimulated seed to germinate quickly, but gave no details. Shipley and Parent (1991) chilled imbibed seed buried in damp sand for 9 months at 4°C. W Lovelace (personal communication, 1998 Nov 5) imbibed-chilled seed buried in a mixture of composted rice hulls, pine bark and sand for 60 days at -0.5°C. J Keiser (personal communication, 1999 Mar 4) found that the pine bark medium used by Lovelace often harbored gnat larvae, which fed on the seminal roots, killing the seedlings. In two different experiments, Keiser imbibed seed in a damp mixture of sand and vermiculite, and either chilled them in a cooler for 60 days at +0.5°C, or froze them in a food freezer overnight (no temperature cited). Likewise for *S. alterniflora,* Broome *et al.* (1973) found that dry seed stored up to 30 days at temperatures just above freezing increased the germination percentage of the seed.

Germination. Simpson (1990) noted that for grasses, germination was characterized by cell expansion in the coleorhiza, then by cell expansion in the coleoptile and scutellum. Langer (1979) and Ritchie *et al.* (1992) stated that the first evidence of seed germination observable to the human eye was usually the emergence of the seminal root from the seed coat, followed by the coleoptile and lateral roots. Weaver (1954) found that on wet soil in a well-lighted environment, *S. pectinata* seed germinated readily. Shipley and Parent (1991) considered *S. pectinata* seed to have germinated if any part of the embryo had emerged from the seed coat. Similarly Broome *et al.* (1973) considered *S. alterniflora* seed to have germinated when the coleoptile had emerged from the seed coat.

A wide range of average germination rates has been reported for *S. pectinata.* Shipley and Parent (1991) reported 41%, both Eddleman (1977) and Santos (1968) reported rates varying between about 50 to 91 or 100%, respectively, W Lovelace (personal communication, 1998 Nov 5) reported about 60%, and J Keiser (personal communication, 1998 Mar 4) also reported nearly 100%. Broome *et al.* (1973), working on *S. alterniflora* reported average germination rates ranging from 0 to nearly 100%.

Galinato and van der Valk (1986) found that for wetland species in general, maximum germination was achieved if seed was exposed to a temperature cycle ranging from a high at noon of 30°C, to a low at midnight of 20°C. Eddleman (1977) observed a similar effect on *S. pectinata*, noting that seed germinated vigorously under 2 different cycling temperatures: 30 to 20°C, and 20 to 5°C, and a constant temperature of 20°C. He found seed germinated poorly under constant cold (10°C) or warm (30°C) temperatures.

Mayer and Poljakoff-Mayber (1982) indicated that seeds of grasses were generally stimulated to germinate by light, though this was contradicted by Simpson (1990). Weaver (1954) remarked that *S. pectinata* did not renew growth activity until the second week of April, during which time daylength increased rapidly. In Cedar Falls, Iowa seed on the ground during mid-April received 14.2 hours of daylight, by mid-May 15.4 hours, and by mid-June 16.7 hours, a two month increase of 2.5 hours (McCarthy 2000). Since the discovery of the phytochrome mechanism in seeds (Bewley and Black 1994), it is not surprising to find that longer day length (and correspondingly shorter nights) helps warm season grass seed break dormancy. Light and phytochrome were shown to be the dormancy breaking mechanism in at least 2 warm season native grass seeds: *P. virgatum* (Holm and Miller 1972) and *Sorghastrum nutans* Nash (Indian grass) (Emal and Conard 1973), and the combination of after-ripening and light improved *S. nutans* germination. For optimal growth in past *S. pectinata* experiments, Eddleman (1977) illuminated *S. pectinata* seed from 8 to 14 hours per day, and Shipley and Parent (1991) for about 15 hours per day.

The AOSA (1998) did not specify a laboratory method for germinating *S. pectinata* seed, but did specify a method for germinating *A. gerardii,* calling for a 30-20° temperature cycle, and a 16 hours of light on a daily basis. This may be appropriate for *S. pectinata*, because *S. pectinata* and *A. gerardii* often grow intermingled along northeast Iowa roadsides; both are warm season grasses, and both have about the same phenology.

Seedling emergence. Opinions varied regarding depth of planting to study emergence from ½ the seed diameter (DD Smith, personal communication, 1999 Nov 9)

to 4 times the seed diameter (Hartmann *et al.*1997) to 1 to 2 cm (Morgan 1997). Mayer and Poljakoff-Mayber (1982) and Ritchie *et al.* (1992) noted that shallow soil was warm, and favorable to both seed germination and seedling emergence.

Shipley and Parent (1991) observed a lag time of 6 days between planting of *S. pectinata* seed and coleoptile emergence. Langer (1979) and Ritchie *et al.* (1992) described how a seedling's mesocotyl propelled its coleoptile upwards through the soil, then stopped when the coleoptile tip encountered sunlight. The USDA-NRCS (1992-3) studied the emergence of 21 source-identified collections of *S. pectinata* seedlings from soil and reported a range of emergence from 0 to 66%.

S. pectinata seedlings may be susceptible to damping-off, even under optimum conditions. Thomson *et al.* (2000) defined damping-off as seedling rot caused by fungi (*Rhizoctonia*, *Phytophthora*, and *Cylindrocladium* were most common), and remarked that it was a major concern to germinating and emerging seedlings. Weaver (1954) stated that wet soil conditions typically dominated *S. pectinata* habitat during late spring and early summer when seed was germinating. Hartmann *et al.* (1997) noted that this was ideal habitat for the growth of pre- and post-emergence damping-off fungi, and that warm season plants such as *S. pectinata* were particularly susceptible.

Post germination. The first 6 weeks of *S. pectinata* growth generally follow a phenology described by several authors (Weaver 1954, 1991; Pohl 1966; Langer 1979; Ries and Svejcar 1991; Raven *et al.* 1992; Ritchie *et al.* 1992). Ries and Svejcar (1991) outlined a 3 stage development pattern for grass seedlings where the plant was initially

heterotrophic (sustained by endosperm), then transitional (sustained by the seminal root and first leaves), and finally autotrophic (sustained by the canopy and adventitious roots)

Most authors agreed that the seminal root system was fibrous, reticulate, and highly absorptive (Weaver 1954; Pohl 1966; Langer 1979; Ries and Svejcar 1991; Raven *et al.* 1992; Ritchie *et al.* 1992), and many remarked that these roots were thin, shallow, and short-lived, but Weaver and Zink (1945) disagreed. They found that of 14 prairie grasses studied, most had well developed seminal roots that penetrated 2 or 3 feet into the soil, and lived at least a full growing season. Langer (1979) and Ries and Svejcar (1991) stated that adventitious roots were the main absorbing system.

Weaver (1954) described 3 kinds of *S. pectinata* roots: vertical (a uniform thickness of several millimeters, poorly branched), lateral (thread-like, abundant, 2 to 8 centimeters long, poorly branched) and shallow (produced under wet conditions, fine, highly branched).

Langer (1979), Weaver (1991), and Ritchie *et al.* (1992) stated that grass root morphology was initially dependent on 2 factors: seed planting depth (for seminal root initiation) and location of the shoot apex at the time of emergence (for adventitious root initiation). It was the combination of these 2 factors that occasionally produced a several centimeter separation between the 2 young root systems, which were otherwise connected only by a single thread-like mesocotyl.

Ritchie *et al.* (1992) found that for corn plants, the first leaf and adventitious roots are produced simultaneously. Weaver (1954) found a similar pattern for *S. pectinata* tiller development on 4 and 5 week old seedlings. Langer (1979) noted that leaf production by

many grasses was both temperature dependant, and more variable than root production. Growth of the same leaf in summer was often 3 to 4 times faster than in winter. Shipley and Peters (1990) found *S. pectinata* seedlings that emerged in mid July produced a root to shoot growth ratio of nearly 4:1 after 10 and 30 days.

Ries and Svejcar (1991), and Ries (1996, 1999) consistently found that successful establishment of a grass stand after 3 years could be forecast by only 6 weeks after emergence from the soil. Ries and Svejcar (1991) considered plants to be autotrophic once endosperm starch reserves had been depleted and adventitious roots formed. Seedlings in all 3 studies were described in terms of numbers and lengths of leaves, adventitious roots, and tillers, leaf area, and shoots present per square meter. The single best predictive factor favoring establishment was number of adventitious roots. Ries (1999) also found the cool season perennial *Agropyron smithii* Rydb. (Western wheatgrass) was slow to establish. This plant, like *S. pectinata*, readily reproduced vegetatively from rhizomes.

Insect Attack

There have been a number anecdotal observations of insect damage to *S. pectinata* seed, but very little definitive (sp) research is reported in the literature. Wynia (1994), and Christiansen and Müller (1999) characterized the spikes of *S. pectinata* as heavily insect damaged. Christiansen and Müller (1999) noted that larvae tunnel through the spikes, destroying the few seeds that are set. Wynia (1994) identified 2 genera of larval stage insects that affected seed production: *Batra* sp. (Lepidoptera: Olethreutidae) was a seed predator; and *Eucosma* sp. (Lepidoptera: Tortricidae) was a stem borer. E Jacobson

(personal communication, 2000 Jul 17) tentatively identified a spike burrowing insect larva as belonging to the order Diptera.

Insect attack on *S. pectinata* spikes may be more severe in the south. E Jacobson (personal communication, 2000 Jul 17) found 100% of caryopses in spikes of Kansas prairie cordgrass had been destroyed by insects, Santos (1968) reported that floret destruction by insects in Iowa, Illinois, and Missouri ranged from 6 to 77.6%, W Duckwitz (personal communication, 2000 Jul 17) characterized predation on North Dakota cordgrass caryopses as minimal, and Eddleman (1977) implied that insect predation on Montana cordgrass was low (3 to 7%) .

A few authors have studied insect species active on *S. pectinata* stems and leaves. Holder (1990) identified *S. pectinata* as the host plant for a guild of 11 species of sap-feeding insects from 5 families. He concluded that the sap-feeding guild hosted by *S. pectinata* was similar to that hosted by *S. patens*. Among *S. pectinata* clumps, there were greater numbers of insects on larger clumps, but species diversity was not significantly different between large and small clumps. The effect of these insects on the plants was not discussed. Holder and Wilson (1992) found that *Prokelisia crocea* (Van Duzee) (Homoptera: Delphacidae) fed and reproduced on *S. pectinata* stems and leaves. The study focused on the morphology of different instars, and again did not consider the insect's effect on the plants. Johnson and Knapp (1996) estimated the impact of *Ischnodemus falicus* (Say) (Hemiptera: Lygaeidae) on photosynthesis and production of *S. pectinata* growing on Kansas prairies, and found that plants hosting high insect densities experienced significantly reduced photosynthesis and above ground biomass production,

but that the effect was only temporary. When the infestations ended, photosynthesis and growth rebounded to normal rates.

One study indicated insect associations with *S. pectinata.* Wilson *et al.* (1993) examined the frequency and seasonal distribution of planthopper fauna (Homoptera) at a prairie in Missouri, and found a total of 47 species representing 22 genera and 7 families. *S. pectinata* was named as a major floristic component of this site.

Statistical Treatment

Germination studies result in longitudinal data. Cobb (1998) recommended using a block design to analyze longitudinal data. This design diminished the effects of variation among experimental units by maintaining homogeneity among factors. The author also offered extended discussion and examples on experimental design.

The appropriate statistical model to evaluate longitudinal data is a mixed linear regression model, or 'mixed model' for short (McLean *et al.* 1991; Spector 1993; SI 1999; Baker 2000). A rapid growth of computational power has fueled programming advances in mixed models (Spector 1993; Baker 2000), and "how-to" information about these models for researchers working outside the realm of statistics is increasingly available (Spector 1993; Littell *et al.* 1996). On the web, the SAS Institute (1999) has made its version 8 programming manual available, and Baker (2000) presented an integrated primer on mixed model methodology for biologists with longitudinal data.

Nomenclature

Several sources were consulted to name plants. For members of *Spartina*, Mobberley (1956); for Iowa flora other than *Spartina*, Eilers and Roosa (1994); for flora of the Northeastern United States, Gleason and Cronquist (1991); for flora of the Great Plains, McGregor and Barkley (1986). The remaining names were established from their respective literature sources.

MATERIALS AND METHODS

The presentation of Materials and Methods is divided into 5 sections: Preparation, Seed Testing, Growth, Statistical Analysis of Data, and Nomenclature. Preparation describes the tasks involved in obtaining, storing, and handling seed. Seed Testing describes how germination, viability, and emergence tests were conducted. Growth describes how measurements were made on developing seedlings. Statistical Analysis of Data describes how the data was described, with an emphasis on the use of mixed linear regression models. Nomenclature identifies literature sources for scientific names.

Preparation

General

Experiments to measure the germination and growth of *Spartina pectinata* seeds and seedlings were conducted from 1998 to 2000 at the University of Northern Iowa, Cedar Falls, Iowa. Primary emphasis in this study was placed on systematically evaluating the germination of seeds after treatment under different conditions. Seed from 5 *S. pectinata* collections (Table 1) were used to assess 4 seed stratifying techniques with 14-day germination tests. In addition, emergence tests were used to check for damping-off of soil-grown seedlings after seed stratification and germination. Four seed collections were used to assess 2 seed stratifying techniques with 18-day emergence tests. Growth tests were used to insure that soil-grown seedlings produced normal looking plants after germination. Two seed collections were grown into seedlings for 6 weeks to describe their early phenology.

Table 1 Seed collections of *S. pectinata* used to measure germination, emergence and growth trials

Seed Collection	Agency/Origin	Serial Number	Used for Trials*	Abbr.
1997 *Red River* germplasm	USDA-NRCS	SG1-97-W40	g	RR97
1998 *Red River* germplasm	USDA-NRCS	SG1-98-W40	g, e, d	RR98
1999 *Red River* germplasm	USDA-NRCS	SG1-99-E12	g, e	RR99
1998 Story County, Iowa	IA Ecotype	SP2-28	g, e, d	SC98
1999 Butler County, Iowa	hand collected	---	g, e	BC99

*Trials: g = germination, e = emergence, d = growth

Seed Origin and Collection

Relict seed was collected from Story and Butler Counties in Iowa during the fall of 1998 and 1999, respectively. Seed collected in Story County (SC98) was from Doolittle Prairie State Preserve, (LaFayette Township, latitude 42° 08' 55" N, longitude 93° 35' 20" W). Seed collected in Butler County (BC99) was from a wet pasture along the west side of Union road, 1 mile north of US Highway 3 (Jackson Township, latitude 42° 46' 00" N, longitude 92° 45' 00" W).

The Story County relict seed was supplied by the Iowa Ecotype Project, University of Northern Iowa Roadside Program, Cedar Falls, Iowa. Three separate age classes, 1997-99, of the germplasm *Red River* (RR97, RR98, RR99) were supplied by the USDA-NRCS Plant Materials Center, Bismarck, North Dakota, which also developed the germplasm.

Relict seed was harvested by clipping spikes from plants and storing them in small paper bags to dry for several days at room temperature. Spikelets were disarticulated by

rubbing spikes across a screen of ½-inch hardware cloth stretched over a frame. Germplasm seed was disarticulated, cleaned, and shipped to the researcher by the Bismarck Plant Materials Center. After drying, seed was kept in *Ziploc* bags and chilled in a refrigerator to maintain optimal moisture content and viability.

To be assured that only plump, healthy-looking caryopses were used in experiments, caryopses were carefully dissected out of their spikelet coverings using tweezers and a magnifying lamp. The naked caryopses were stored in small paper envelopes. Seeds were weighed in batches of 26 on a digital scale that had a 1 mg sensitivity.

Seed Treatments

Four different seed stratifying treatments were studied (Table 2): imbibed-chilled, imbibed-frozen, aged, and dried-warmed. The imbibed-chilled treatment was done to confirm the anecdotal information on stratifying from native seed merchants; the imbibed-frozen treatment is similar to over-wintering conditions in the seed's natural habitat; the different ages of Red River germplasm readily permitted a comparison of the effect of aging on germination. The dried-warmed stratified seed was a serendipitous late addition. These were left in a paper bag to dry at room temperature, but overlooked when the other seed was packaged and placed in the refrigerator. They were discovered 5 months later. Each treatment consisted of an experimental seed lot of 200 caryopses divided into 4 replicates of 50. Each replicate succeeded the next by 1 week. Controls for all trials were fresh, dried-chilled seed of the same collection, stored in a refrigerator at 4°C. The imbibed-chilled stratifying treatment involved cooling seeds that were imbibing on moist

Table 2 A summary of seed stratification methods used to test germination and emergence

Stratifying Treatment	Temp. (°C)	Time	Trial No.	Seed Type	Remarks
		Germination Trials			
Imbibed-chilled	1	0, 15, 30, or 45 d	1	RR98, SC98	---
			2	RR99, BC99	---
Imbibed-frozen	-18	0, 4 or 7 d	3	RR99, BC99	---
Aged	4	0.5, 1.5 or 2.5 y	4	RR99	Dried-chilled for years in *Ziploc* bags
Dried-warmed	23	0 d	5	BC99	Stored in a paper bag at room temperature
		Emergence Trials			
Imbibed-chilled	1	0, 15, 30, or 45 d	1	RR98, SC98	---
			2	RR99, BC99	---
Imbibed-frozen	-18	0, 4 or 7 d	3	RR99, BC99	—

Legend: SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA, RR = *Red River* germplasm seed; 98 = 1998, 99 = 1999; d = day, y = year; Zero-time controls for all trials were fresh, dried-chilled seed of the same collection, stored at 4°C.

blotter paper to a temperature of 1°C for 15, 30 or 45 days. The imbibed-frozen stratifying treatment consisted of freezing seed that was imbibing on moist blotter paper down to a temperature of -18°C for 4 or 7 days. The age-stratified treatment used older dried-chilled seed, aged 1.5 and 2.5 years, which were kept refrigerated in *Ziploc* bags until needed. All 4 stratifying treatments were used to test germination whereas only the imbibed-chilled and imbibed-frozen treatments were used to test emergence.

Different *S. pectinata* seed was assigned to different trials depending on availability. Four collections of seed were imbibed-chilled: 2 age classes of *Red River* (RR98 and RR99), relict seed from Story County (SC98) and Butler County (BC99).

Two collections of seed were frozen: 1 age class of *Red River* (RR99) and relict seed from Butler County (BC99). Three collections of seed were aged: all were *Red River* (RR97, RR98, RR99). One collection of seed was dried-warmed: relict seed from Butler County (BC99).

Germination Experiment Preparation

Seeds were germinated in semi-transparent, 14.3 x 14.3 x 3.8 centimeter polypropylene boxes containing 12 x 12 centimeter squares of moist, single layer, steel blue germination blotter paper (Table 2). To prepare the boxes, blotter paper was soaked in distilled water, then the water was expressed by pressing the stacked blotters between wood blocks squeezed with hand tightened c-clamps. Once a sheet of moistened blotter paper was fitted into a clean box, 50 caryopses were sprinkled on the paper and counted, any broken caryopses were replaced. The tightly fitting lids of the boxes helped maintain an atmosphere of nearly 100% relative humidity for imbibing seed. Seed to be chilled was covered prior to stratification with a second layer of dampened blotter paper. This second layer helped protect seed from drying while stratifying.

Emergence Experiment Preparation

Seeds were sown in 52.7 x 26.7 x 5.7 centimeter plastic cell-pak sheets cut in rectangular units of 6 cells (6-paks) and filled with steam sterilized soil (Table 2). Fifty caryopses were counted and sprinkled over firmly pressed soil (8 seeds per cell plus 2 seeds randomly assigned), covered with a thin soil layer, and pressed firmly again. Six-paks were well watered, drained, and placed in flats. If chilling was required, 6-paks were placed in quart *Ziploc* plastic bags to control humidity. Then flats were bubble wrapped,

sealed with common packing tape and stored in either the refrigerator or freezer. The wrap was used to help maintain a constant stratifying temperature inside the package.

Imbibition and Chilling

Imbibed seeds damaged easily, and the AOSA (1992) cautioned that damaged seeds often failed to develop properly. Because of this, seeds used for germination and emergence trials were placed in their respective containers before imbibition, and once imbibed, only the containers were handled, not the seeds.

A refrigerator set to 1°C , and freezer set to -18°C were used to chill all imbibed-cold and -frozen stratified seed. The -18°C temperature was arbitrary, since this was the maximum cooling provided by the freezer. The boxes and insulated 6-paks were stacked randomly in the refrigerator or freezer. Tubs of crushed ice were loaded inside the refrigerator to help maintain the desired seed chilling temperature. The tubs were refreshed every several days as necessary.

Seed Testing

Germination Trials

The main emphasis of this study was to systematically evaluate the germination of seeds after treatment in different conditions. To test germination, boxes containing the seeds were placed in a *Percival* I-35LLVL growth chamber (Boone, IA) for 14 days. The boxes were arranged randomly on fiberglass lunch trays and exposed to an alternating light and temperature schedule: 16 hours light at 30°C, then 8 hours dark at 20°C.

A seed was considered to have germinated if any part of the embryo had emerged from the seed coat (Shipley and Parent 1991). Once emerged, the embryo was considered

a seedling. Germinated and mildewed seeds were counted and removed daily from each box, and remaining caryopses misted to dampen the blotters. Abnormal seedling development was ignored (AOSA 1992). Figure 1 presents a photograph of 24 hour-old *S. pectinata* seedlings.

Seed Viability

Any seed that sprouted during the 14-day germination trial was considered viable. Seeds that remained firm, but not germinated (ungerminated) after 14 days were checked for viability. Ungerminated seeds were first razor scored in a cross pattern to expose the endosperm and embryo, then soaked in a 0.8% tetrazolium chloride (TZ) solution with a 0.05 molar phosphate buffer (pH 7.5) for 24 hours (Appendix 2). Viable seeds were

Figure 1 Twenty four hour-old *S. pectinata* RR99 seedlings (note: coleoptile emerges from caryopsis before radicle) (10x)

those whose endosperm and embryo stained uniformly red by the TZ test. Figure 2a diagrams the seed scoring technique used to determine viability. This technique slightly modified AOSA (1998) TZ rules, which called for longitudinal scoring of a grass seed prior to testing. It was observed that for *S. pectinata* seeds, longitudinal scoring alone resulted in only partial staining of what appeared to be otherwise viable seeds because the staining did not reach the green embryo (Figure 2b). A transverse score was found to correct this defect (Figure 2c). Reported seed viability for each trial was determined by the formula:

$$\% \text{ viability} = \frac{\text{germinated seeds} + \text{firm, viable, ungerminated seeds}}{50 \text{ seeds}} \times 100$$

A linear regression was performed on the data collected from each stratified seed collection to assess the effects that stratifying had on seed viability over time.

Emergence Trials

Emergence experiments were conducted to insure that stratified, germinated seeds did not damp-off as seedlings. To test emergence, 6-paks were arranged randomly in flats, and flats were arranged randomly on a greenhouse mist bench for 18 days and kept well watered. Only natural light was used during experiments, and temperature was allowed to fluctuate accordingly. Sides of the black plastic flats were wrapped with aluminum foil to reflect sunlight that would otherwise heat perimeter cells. Seedlings were considered to have emerged if any portion of the green coleoptile tips could be seen protruding above the soil surface. Flats were checked daily for emerged coleoptiles, which were counted and removed.

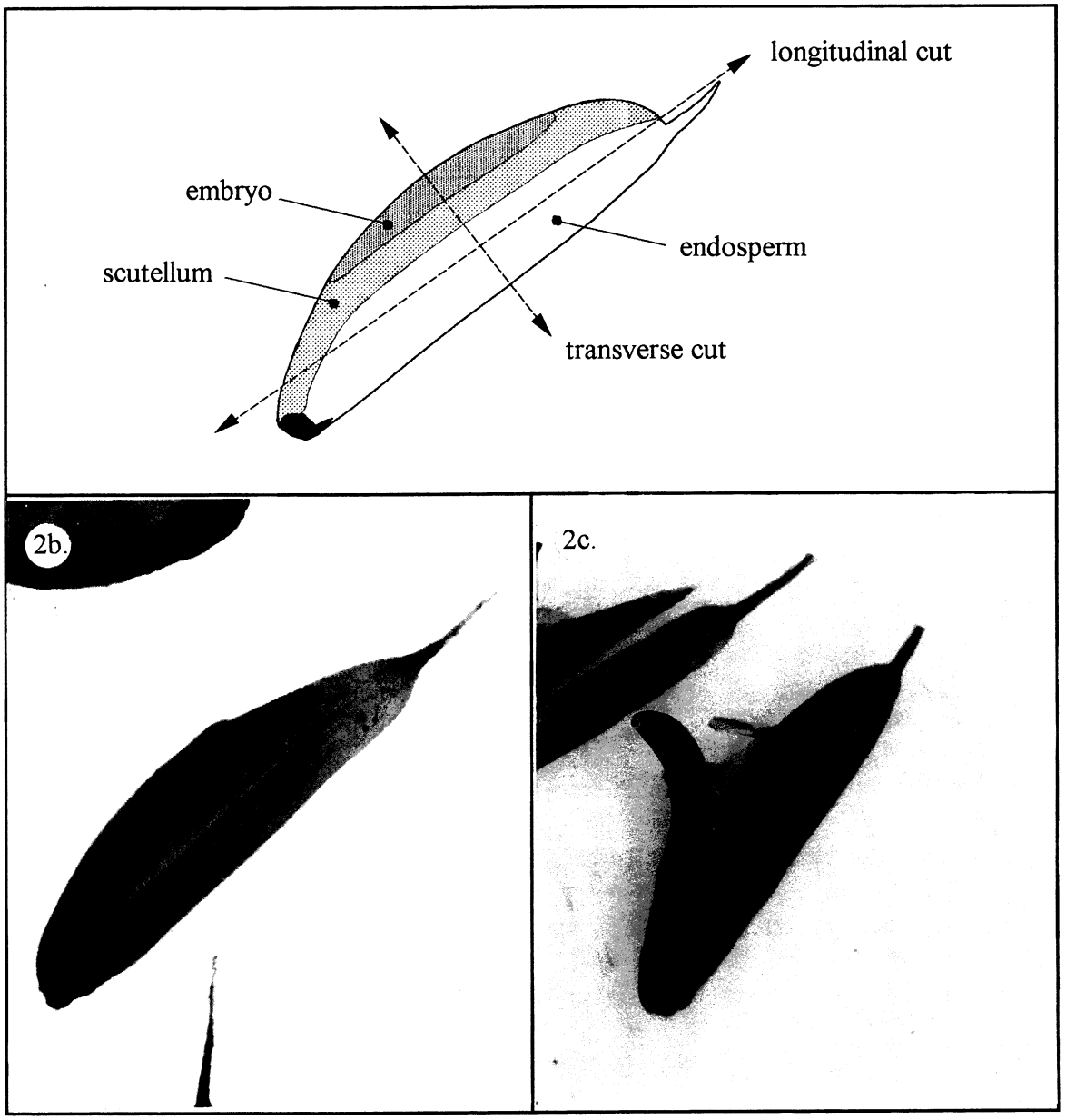


Figure 2a. Illustration of *S. pectinata* caryopsis score pattern to achieve a successful tetrazolium test (TZ) on a viable caryopsis

2b Incomplete TZ staining after only longitudinal scoring of the caryopsis (note unstained embryo) (15x)

2c. Complete TZ staining after longitudinal and transverse score of caryopsis (10x)

Growth

Establishing Age Classes

Growth experiments were conducted to insure that soil-grown seedlings produced normal looking plants after germination. To observe and measure the growth of seedlings, 2 collections of *S. pectinata* seed, *Red River* (RR98) and relict seed from Story County (SC98), were sown into 4 cell-pak flats filled with steam sterilized soil. Seed collections were sown into cells in an alternated checkerboard pattern. Two caryopses per cell were sprinkled over firmly pressed soil, covered with a thin soil layer, and pressed firmly again. Flats were well watered, drained, and wrapped in bubble wrap, sealed with common packing tape, and stored in the refrigerator. The wrap was used to help maintain a constant stratifying temperature inside the package. Flats of seed were imbibed-chilled at 1°C for 20 days (it had been observed during germination experiments that this treatment created an early seed germination effect, which tended to produce even-aged classes of seedlings), then unwrapped and arranged randomly on a greenhouse mist bench. Seeds and seedlings were kept well watered, received natural light, and the temperature was allowed to fluctuate accordingly. Flats were checked daily, new coleoptiles noted, and seedlings were kept thinned to 1 plant per cell. Monitoring the date and place of each emerged coleoptile established uniform seedling age classes.

Sampling

Plants were destructively sampled weekly for 6 weeks starting 1 week after emergence. Each week 10 seedlings of each collection and age class were randomly washed from soil plugs. Seedlings were measured on a *Mylar* sheet ruled in a grid of

⅛-inch squares. Bare roots were kept moist with distilled water. Eight direct measurements were taken: presampling height (longest leaf tip to soil); postsampling height (leaf tip to seed top); leaf count; leaf length; main root count (seed root(s) + adventitious roots); root length; root reticulation (total number of root branches); IKI test result (positive or negative). Three derived measurements were calculated: seed planting depth (postsampling height - presampling height); total shoot length (sum leaf length); and total root length (sum individual roots; a threshold length of 0.635-cm (¼- in) was chosen for convenience, and shorter roots and shoots were ignored). To create a photographic record of growth, each week a few washed, whole seedlings were floated in a rectangular, water-filled glass dish and photographed against a black background.

IKI Test

Starch reserves in the endosperm of each seed were observed by dissection and staining: the pericarp was removed and iodine potassium iodide (IKI) applied. Often, the endosperm had to be gently stirred with forceps to stain the remaining starch.

Growth Observations and Records

Growth measurements were made on low power using an Olympus dissecting microscope with a fiber optic ring stage illumination. An Olympus BX40 compound microscope was used to examine *Dipteran* larvae and nematodes attacking and stunting SC98 seedlings during weeks 2 and 3. Light microscope photographs were taken with an Olympus PMC35DX camera and PM-CB20 Photolink Exposure Control Unit using 200 ASA print and slide film. Floated seedlings were photographed under natural light with a

Nikon FM camera using 64 ASA slide film. Floated seedlings were photographed under natural light with a Nikon FM camera using 64 ASA slide film.

Growth Data Presentation

The emphasis for the presentation of growth data is placed on measuring a seedling's presumed transition between heterotrophy and autotrophy as hypothesized by Ries and Svejcar(1991), and Ries (1996, 1999). Measurements are presented in table and graph form. Data is assessed in cursory fashion with mixed models to check for possible fixed factor interactions. Photographs are presented to highlight aspects of seedling growth.

Explanation of Mixed Models

Mixed models were used to assess germination and emergence data. Data were analyzed by fitting several mixed models using the statistical software package SAS (release 6.09 enhanced). In order to make comparisons amongst the fitted models, it was necessary to: minimize random error; identify the significance of fixed effects, and their interactions; and simplify the models wherever possible.

The use of mixed models required that for each seed stratifying treatment, the sampling unit, or subject, and one or more fixed effects had to be identified. In our experiments, sampling units were the containers holding seeds (either sandwich boxes for germination trials, or 6-paks for emergence trials). Henceforth, we defined the sampling unit as BOX, and response variable as GERM. Fixed effects were factors with specific levels of interest. We identified 3 fixed effects: seed collection, seed stratifying time, and

elapsed time within a trial. Therefore, we defined seed collection as SEED, seed stratifying time as STRAT, and elapsed time within a trial as TIME.

Random effects were those that crept into an experiment but were not the result of deliberate experimental treatments. To make meaningful comparisons of treatment results, unwanted random effects had to be filtered from the data. Examples of unwanted random effects for this project might have included: seed handling damage; microclimate variation; bouncing seeds onto the lid or walls of a sandwich box by jarring; or raindrop splash rearranging seeds. Random effects were filtered by a random complete block design and covariance modeling. A random complete block design was built into experiments by arranging the subjects from complete blocks randomly in their treatment environments (refrigerator, freezer, germination chamber, greenhouse bench). This tended to minimize the magnitude of any single error by spreading it uniformly over an experiment. Figure 3 presents a sample complete block matrix. Fixed effects and the subject were represented by columns, called blocks. Each subsequent block subdivided the previous block, horizontally, into successively smaller sub-blocks. The design acted as a data collection table. Data repeatedly collected from each subject were entered into the appropriate cell within the *time* block.

In addition, the covariance structure within subjects was modeled to insure that inferences about each mean were valid. Each of 5 models presupposed a different covariance structure for the data. Using different mixed models produced a gradient of estimated covariance structures. Appendix 3 presents a sample data set and programs of the 5 mixed models used. A standard general linear regression model, (GLM) was the

Fixed effect *seed*	Fixed effect *strat* (d)	Subject *box*	Fixed effect *time* (d)						
			1	2	3	. . .	12	13	14
Red River	0	1							
		2							
		3							
		4							
	15	1							
		2							
		3							
		4							
	30	1							
		2							
		3							
		4							
	45	1					cell		
		2							
		3							
		4							

Figure 3 A sample complete block design matrix used to collect germination data (adapted from Spector 1993; Neter *et al.* 1996; Cobb 1998)

simplest. It assumed no within-subject correlation and its results, compared to mixed models were poor. The compound symmetry (CS) and autoregressive order (one) (AR(1)) models were chosen as middle-of-the-road mixed models with different covariance structures. Both possessed the same mean structure, fixed effect parameters, and interactions, but CS assumed the same within-subject variability between data points regardless of time separations, while AR(1) had a within-subject variability that declined exponentially as time between data points increased. This difference was due to the

variable nature of the AR(1) covariance structure, which allowed for observations on the same subject that were closer in time to be more highly correlated than measurements at times that were farther apart. Finally, heterogeneous CS (CS_{hetero}) and heterogeneous AR(1) ($AR(1)_{hetero}$) were the most complex models, with CS_{hetero} more complex than CS, and $AR(1)_{hetero}$ more complex than AR(1). Essentially a separate CS or AR(1) model was fit for each level of *strat* complexity. The addition of extra parameters for each level of *strat* sharply increased model detail, but better covariance fit tended to occur at the expense of too many parameters. For example, if all subjects in the CS model had the same correlation structure, the best fit would default to the simpler CS model. The concept of parsimony strikes a balance between numbers of model parameters and the resulting model.

The PROC MIXED procedure from SAS generated 3 indices of covariance fit: Akaike's Information Criterion (AIC); Schwarz's Bayesian Criterion (BIC); and log Likelihood (LnL). Numbers of parameters (P) were calculated by the equation AIC - LnL = P. A "best-fitting" model was selected parsimoniously by choosing the covariance fit index closest to zero and fewest number of parameters. For example:

Model	**LnL**	**AIC**	**BIC**	**P**
GLM	-1578.010	-1579.010	-1581.130	1
CS	-1290.800	-1292.800	-1297.020	2
AR(1)	-1221.650	-1223.650	**-1227.870**	**2**
CS_{hetero}	-1275.960	-1287.960	-1313.300	12
$AR(1)_{hetero}$	**-1206.540**	**-1218.540**	-1243.870	12

Inspection of the covariance fitting indices on the proceeding page showed that the model with the fewest number of parameters and smallest negative index was AR(1). By using parsimony to choose the best fitting covariance, the effects of random error were minimized. Each mixed model also reported the level of significance for fixed effects, both singly and for 2- and 3-way interactions. The highest order interaction was of most interest. To continue the previous example, fixed effects for the AR(1) model reported the following p-values:

Fixed Effects	***Pr > F***
strat	0.0064
time	0.0001
seed	0.0001
*strat*seed*	0.0016
*strat*time*	0.0001
*time*seed*	0.0001
strat*time*seed	**0.0001**

Here the highest order significant interaction observed was between all 3 fixed effects ($P < 0.05$ level of significance). As a consequence, no generalizations could be made for any of the lower order fixed effects, and comparisons had to be made on a cell-by-cell basis. Unfortunately, this example (Trial 1) generated more than 16,000 comparisons and served to illustrate that some of the least squares means (ls-means) difference reports were extremely large.

Selected results are presented with graphs and ls-means difference tests. Graphs are used to qualitatively present average daily seed germination and seedling emergence.

Figure 4 presents a sample graph. Early in trials, some seeds germinated more rapidly than others. (Zone A in the sample graph), but eventually most seeds germinated (Zone B in the sample graph). We used ls-means differences tests to quantify these patterns. The first test compared differences observed on a rapid event day (day 3 for germination trials, day 4 for emergence trials) and differences observed on the last day (day 14 for germination trials, day 18 for emergence trials). The most complex results were generated by trials that had a 3-way fixed factor interaction. Germination Trial 1 serves as an example, where for any day, there were 28 possible combinations of stratifying times and seed collections:

Within RR98	**Within SC98**	**Between RR98 and SC98**	
00RR vs. 15RR	00SC vs. 15SC	00RR vs. 00SC	30RR vs. 45SC
00RR vs. 30RR	00SC vs 30SC	00RR vs. 15SC	45RR vs. 45SC
00RR vs. 45RR	00SC vs. 45SC	00RR vs. 30SC	00SC vs. 15RR
15RR vs. 30RR	15SC vs. 30SC	00RR vs. 45SC	00SC vs. 30RR
15RR vs. 45RR	15SC vs. 45SC	15RR vs. 15SC	00SC vs. 45RR
30RR vs. 45RR	30SC vs. 45SC	15 RR vs. 30SC	15SC vs. 30RR
		15 RR vs. 45SC	15SC vs. 45RR
		30RR vs. 30SC	30SC vs. 45RR

Legend: 00 = seed stratified for 0 days, 15 = seed stratified for 15 days, etc.; RR = *Red River* germplasm seed, SC = relict seed from Story County, IA.

The second ls-means difference test was used to determine how many days elapsed before a particular seed treatment reached its final result. Here comparisons were made between daily results (day 1, 2, 3...) and results observed on the last day for each respective treatment in each respective seed trial. In order to contrast the overall α level

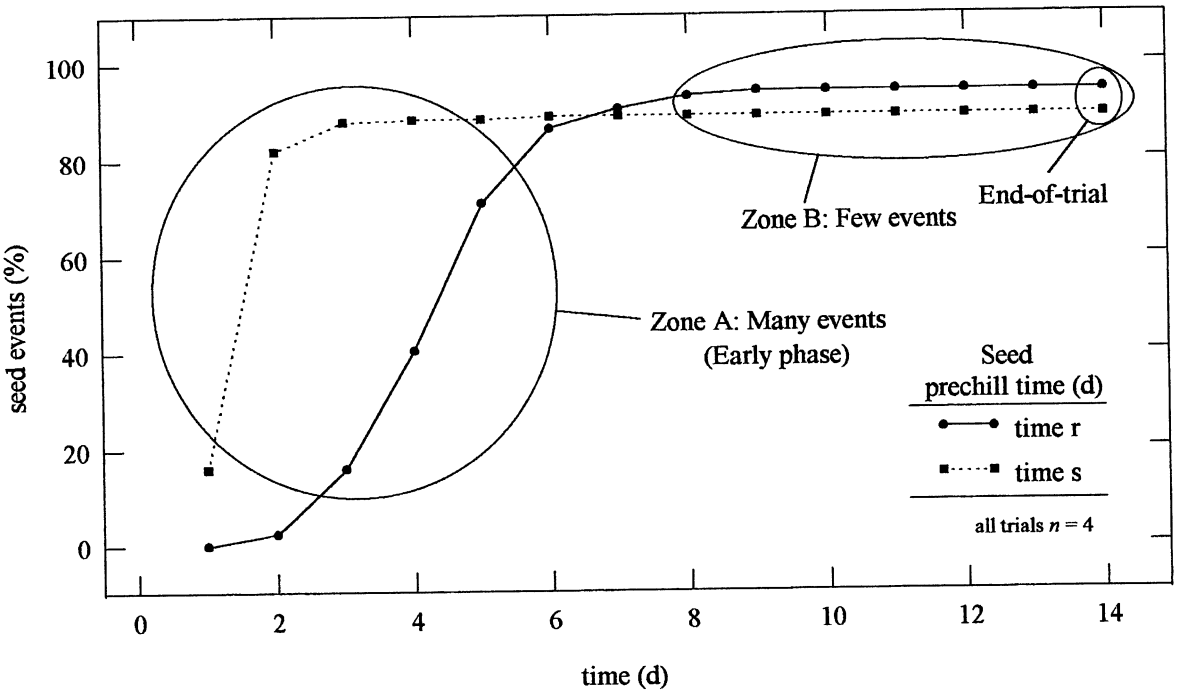


Figure 4 A typical graph of seed germination or emergence for seed stratified for times r and s

for all comparisons, a Bonferroni adjustment required individual significance levels to be 0.05/number of comparisons. To improve clarity, results from this second ls-means comparison were converted to bar graphs.

RESULTS

The presentation of Results is subdivided into 4 sections: Seed Production, Germination, Emergence, and Growth. Seed Production presents data for seed set and seed weight. The Germination section includes 5 trials testing seed viability and germination. The Emergence section consists of 3 trials related to emergence of the seedling from the soil. The Growth section includes 9 sets of plant measurements describing the first 6 weeks of seedling growth.

Seed Production

Seed Weight

Table 3 presents the average weight of *S. pectinata* seed from 1998 and 1999 collections (RR98, SC98, RR99, BC99). Average seed weight (from 20 batches of 26 seeds) ranged from 1.4 mg (±0.03) for RR98, to 2 mg (±0.03) for SC98 with other collections having an intermediate weight. Seed weight for each collection was derived from the average weight of 20 batches of seeds, with each batch numbering 26 seeds.

Table 3 Seed weight of *S. pectinata*

			Weight (mg)			
Collection	Seeds/batch	Batches	Batch $\bar{x}$	($S_{\bar{x}}$)	Seed $\bar{x}$	($S_{\bar{x}}$)
SC98	26	20	52.6	(0.88)	2.0	(0.03)
BC99	26	20	39.7	(0.85)	1.5	(0.03)
RR98	26	20	36.7	(0.80)	1.4	(0.03)
RR99	26	20	44.7	(1.45)	1.7	(0.03)

Legend: SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA, RR = *Red River* germplasm seed; 98 = 1998, 99 = 1999; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error.

Germination

The systematic evaluation of germination data from 5 collections of *S. pectinata* seed (RR97, RR98, RR99, SC98, BC99) after different seed stratifying treatments was the main focus of this study. The duration of seed germination trials was 14 days.

Seed Viability

Table 4 presents the average % viability of seed used in germination trials. The average viability of fresh, untreated seed ranged from 66.5% (±7) for BC99 in Trial 2 to 99% (±1) for RR99 in Trial 3. It was found that the average viability of most stratified seed (Figure 5) declined over time. The sharpest decrease in viability occurred for imbibed-chilled SC98, which declined an average of 20% over 45 days.

Germination Reports

Seed experiments traditionally reported total % germination at the conclusion of a test. To provide greater detail for the mixed model analysis, an early germination phase, day 3 was added to the end-of-trial results, day 14. Percent germination of *S. pectinata* seed is presented: for day 3, early-phase (Table 5); and for day 14, end-of-trial (Table 6). Average total germination after 14 days ranged from 50% (±2.5) for 30 day imbibed-chilled BC99 seed in Trial 2 to 98.5% (±1) for 4 day imbibed-frozen RR99 seed in Trial 3.

Mixed Model Analysis

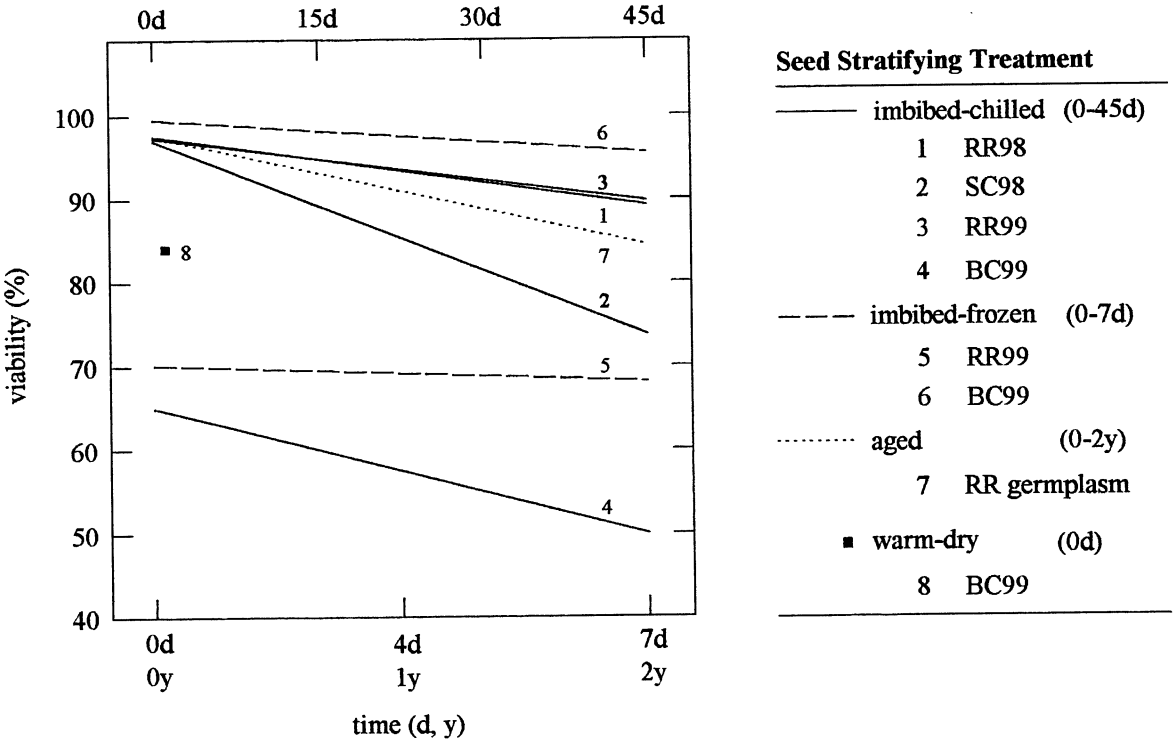
Data sets for germination and emergence were evaluated by 5 mixed models: a general linear regression model (GLM), a homogeneous and a heterogeneous compound symmetry model (CS, CS_{hetero}), and a homogeneous and a heterogeneous autoregressive

Table 4 Percent viability of *S. pectinata* seed used in germination trials

Stratifying Treatment	Time	% Germination RR98 $\bar{x}$	RR98 $(S_{\bar{x}})$	SC98 $\bar{x}$	SC98 $(S_{\bar{x}})$	RR99 $\bar{x}$	RR99 $(S_{\bar{x}})$	BC99 $\bar{x}$	BC99 $(S_{\bar{x}})$
		Trial 1				Trial 2			
Imbibed-chilled	0d	**96.0**	**(1.2)**	**95.5**	**(1.0)**	**93.5**	**(3.9)**	**66.5**	**(7.0)**
	15d	98.5	(1.0)	94.0	(1.5)	98.5	(1.0)	59.0	(2.9)
	30d	89.0	(2.7)	76.5	(6.6)	96.0	(2.5)	52.5	(2.5)
	45d	90.0	(2.2)	75.5	(1.3)	86.0	(5.1)	52.0	(3.9)
						Trial 3			
Imbibed-frozen	0d					**99.0**	**(1.0)**	**68.0**	**(2.5)**
	4d					98.5	(1.0)	73.5	(1.3)
	7d					95.0	(1.7)	66.0	(2.5)
						Trial 4			
Aged	0y					**97.0**	**(1.9)**		
	1y					92.0	(1.4)		
	2y					84.0	(3.2)		
								Trial 5	
Dried-warmed	0d							**84.0**	**(6.2)**

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error; **bold** shows untreated seed; $n = 4$.

order (one) model (AR(1), $AR(1)_{hetero}$). Each model generated 3 indices of covariance fit: Akaike's Information Criterion (AIC); Schwarz's Bayesian Criterion (BIC); and Log Likelihood (LnL). Each 'best-fitting' model was selected parsimoniously by choosing the covariance fit index with the largest value and fewest parameters. Models also generated a list of fixed effects, their interactions, and least squares means (ls-means) comparisons. For each seed trial, the highest order fixed effect interaction was of most interest, since it dictated how the fixed effects could be summarized when making comparisons within



Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; $n = 4$.

Figure 5 Seed viability decline with stratification (from Table 4)

and between seed trials. Results are presented 4 ways and this presentation pattern is repeated for each trial:

1) Total % emergence of seedlings was tracked daily and graphed.

2) Ls-means comparisons for different seed stratifying treatments were made for day 3 (early into trial) and day 14 (end of trial).

3) Ls-means comparisons within each seed stratifying treatment were made to estimate time to complete germination.

4) Results of the second ls-means comparison test were converted to bar graphs for greater clarity.

Table 5 Percent germination of *S. pectinata* seed for germination trials from day 3, early-phase

Stratifying Treatment	Time	% Germination							
		RR98		SC98		RR99		BC99	
		$\bar{x}$	($S_{\bar{x}}$)	$\bar{x}$	($S_{\bar{x}}$)	$\bar{x}$	($S_{\bar{x}}$)	$\bar{x}$	($S_{\bar{x}}$)
		Trial 1				Trial 2			
Imbibed-chilled	0d	**16.0**	**(4.5)**	**50.5**	**(7.3)**	**2.5**	**(1.0)**	**9.5**	**(2.6)**
	15d	58.0	(9.1)	65.5	(8.8)	68.5	(13.0)	15.5	(1.7)
	30d	88.0	(2.9)	69.5	(5.4)	94.5	(2.1)	24.5	(4.1)
	45d	88.0	(2.9)	73.0	(3.0)	84.0	(5.0)	36.5	(4.6)
						Trial 3			
Imbibed-frozen	0d					**11.5**	**(4.9)**	**8.5**	**(2.8)**
	4d					5.0	(1.7)	9.5	(3.3)
	7d					11.0	(5.3)	5.5	(1.5)
						Trial 4			
Aged	0y					**24.0**	**(1.4)**		
	1y					6.5	(3.9)		
	2y					2.5	(1.3)		
								Trial 5	
Dried-warmed	0d							**4.5**	**(1.0)**

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error; **bold** shows untreated seed; $n = 4$.

Table 7 presents fit indices and model parameters for the covariance structure of all 5 mixed models for each seed germination trial. Each respective most parsimonious case is underlined, and the corresponding fixed factor report for each best model is also indicated. Of the 3 model fitting predictors tested, BIC consistently provided the most parsimonious solution. Of the 5 models tested, it was either AR(1) or $AR(1)_{hetero}$ that provided the best fitting covariance structure. Specifically, Germination Trials 1, 3 and 4

Table 6 Percent germination of *S. pectinata* seed for germination trials from day 14, end-of-trial

Stratifying Treatment	Time	% Germination RR98 $\bar{x}$	RR98 ($S_{\bar{x}}$)	SC98 $\bar{x}$	SC98 ($S_{\bar{x}}$)	RR99 $\bar{x}$	RR99 ($S_{\bar{x}}$)	BC99 $\bar{x}$	BC99 ($S_{\bar{x}}$)
		Trial 1				Trial 2			
Imbibed-chilled	0d	**94.0**	**(2.2)**	**87.5**	**(5.1)**	**85.5**	**(3.9)**	**59.5**	**(7.0)**
	15d	96.0	(1.6)	90.5	(3.3)	97.5	(1.0)	52.0	(2.9)
	30d	89.0	(2.7)	76.0	(6.4)	96.0	(2.5)	50.0	(2.5)
	45d	90.0	(2.2)	75.5	(1.3)	85.5	(5.1)	50.5	(3.9)
						Trial 3			
Imbibed-frozen	0d					**89.0**	**(4.7)**	**63.0**	**(3.3)**
	4d					98.5	(1.0)	66.0	(2.6)
	7d					91.5	(2.6)	57.0	(2.1)
						Trial 4			
Aged	0y					**93.5**	**(2.6)**		
	1y					88.5	(2.2)		
	2y					77.5	(3.8)		
								Trial 5	
Dried-warmed	0d							**77.0**	**(7.9)**

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error; **bold** shows untreated seed; $n = 4$.

(imbibed-chilled seed, imbibed-frozen seed, and aged seed) were best modeled with AR(1). Germination Trials 2 and 5 (imbibed-chilled seed, dried-warmed seed) were best modeled with $AR(1)_{hetero}$. The highest-order significant interaction observed (at the $P < 0.05$ level of significance) between the fixed factors *seed* (seed collection), *strat* (seed stratifying time), and *time* (elapsed time within a trial) was:

Table 7 Assessment of germination data covariance structure by 3 fit indices and parameters (each best case underlined), and the fixed effects of each best case

Model	LnL	AIC	BIC	P	Fixed Effects	*Pr > F*
Germination Trial 1: Imbibed-chilled seed					**AR(1)**	
GLM	-1578.010	-1579.010	-1581.130	1	*strat*	0.0064
CS	-1290.800	-1292.800	-1297.020	2	*time*	0.0001
AR(1)	-1221.650	-1223.650	**-1227.870**	**2**	*seed*	0.0001
CS_{hetero}	-1275.960	-1287.960	-1313.300	12	*strat*seed*	0.0016
$AR(1)_{hetero}$	**-1206.540**	**-1218.540**	-1243.870	12	*strat*time*	0.0001
					*time*seed*	0.0001
					*strat*time*seed*	0.0001
Germination Trial 2: Imbibed-chilled seed					**AR(1) heterogeneous**	
GLM	-1120.040	-1121.040	-1122.950	1	*strat*	0.0026
CS	-980.850	-982.850	-986.667	2	*time*	0.0001
AR(1)	-855.111	-857.111	-860.928	2	*seed*	0.0001
CS_{hetero}	-931.369	-939.369	-954.637	8	*strat*seed*	0.0028
$AR(1)_{hetero}$	**-837.458**	**-845.458**	**-860.727**	**8**	*strat*time*	0.0001
					*time*seed*	0.0001
					*strat*time*seed*	0.0001
Germination Trial 3: Imbibed-frozen seed					**AR(1)**	
GLM	-748.577	-749.577	-751.341	1	*strat*	ns
CS	-655.304	-657.304	-660.834	2	*time*	0.0001
AR(1)	-586.901	-588.901	**-592.430**	**2**	*seed*	0.0001
CS_{hetero}	-652.610	-658.610	-669.199	6	*strat*seed*	ns
$AR(1)_{hetero}$	**-581.945**	**-587.945**	-598.533	6	*strat*time*	ns
					*time*seed*	0.0001
					*strat*time*seed*	ns
Germination Trial 4: Aged seed					**AR(1)**	
GLM	-336.010	-337.010	-338.428	1	*strat*	ns
CS	-293.492	-295.492	-298.329	2	*time*	0.0001
AR(1)	-274.827	-276.827	**-279.663**	**2**	*strat*time*	0.0001
CS_{hetero}	-287.357	-293.357	-301.866	6		
$AR(1)_{hetero}$	**-270.346**	**-276.346**	-284.855	6		

Legend: AIC = Akaike's Information Criterion, BIC = Schwarz's Bayesian Criterion, LnL = Log Likelihood, P = parameters; GLM = general linear model, CS = compound symmetry model; AR(1) = autoregressive order (one), hetero = heterogeneous; *strat* = seed stratifying time, *time* = elapsed time within a trial, *seed* = seed collection; $\alpha = 0.05$.

(table continues)

Table 7 Continued

Model	LnL	AIC	BIC	P	Fixed Effects	*Pr > F*
Germination Trial 5: Dried-warmed seed					**AR(1) heterogeneous**	
GLM	-309.681	-310.681	-311.897	1	*strat*	ns
CS	-276.355	-280.355	-285.217	4	*time*	0.0001
AR(1)	-277.483	-279.483	-281.914	2	*strat*time*	ns
CS_{hetero}	-220.412	-222.412	-224.843	2		
$AR(1)_{hetero}$	**-214.476**	**-218.476**	**-223.337**	**4**		

Legend: AIC = Akaike's Information Criterion, BIC = Schwarz's Bayesian Criterion, LnL = Log Likelihood, P = parameters; GLM = general linear model, CS = compound symmetry model; AR(1) = autoregressive order (one), hetero = heterogeneous; *strat* = seed stratifying time, *time* = elapsed time within a trial, *seed* = seed collection; $\alpha = 0.05$.

1) A 3-way interaction for Germination Trial 1 and 2.

2) A 2-way interaction between *strat* and *seed* for Germination Trial 3.

3) A 2-way interaction between *strat* and *time* for Germination Trial 4.

4) No interaction, but the factor *time* was significant for Germination Trial 5.

Germination Trial 1

RR98 and SC98 seed was stratified by the imbibed-chill technique. Figures 6 and 7 present respective graphs of average % germination for each seed collection. The best-fitting mixed model AR(1) was selected to further examine these data. A significant 3-way fixed factor interaction of *strat* by *time* by *seed* (at the $P < 0.05$ level of significance) was observed. Two ls-means comparisons were made.

Because of the relatively large numbers of pairs of means to be compared (28), and the desire to control the overall α level at 0.05, a Bonferroni adjsutment was employed. Significant differences were observed for any mean comparison's P value $< 0.05/28$.

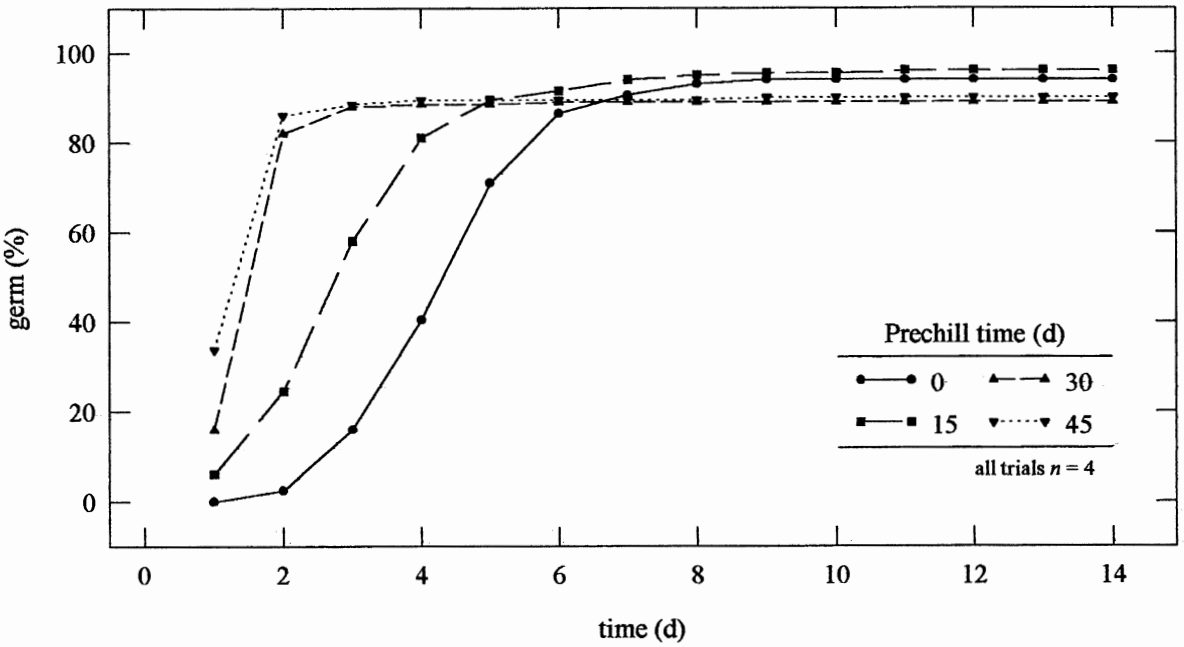


Figure 6 Average % germination of imbibed-chilled RR98 seed from Germination Trial 1

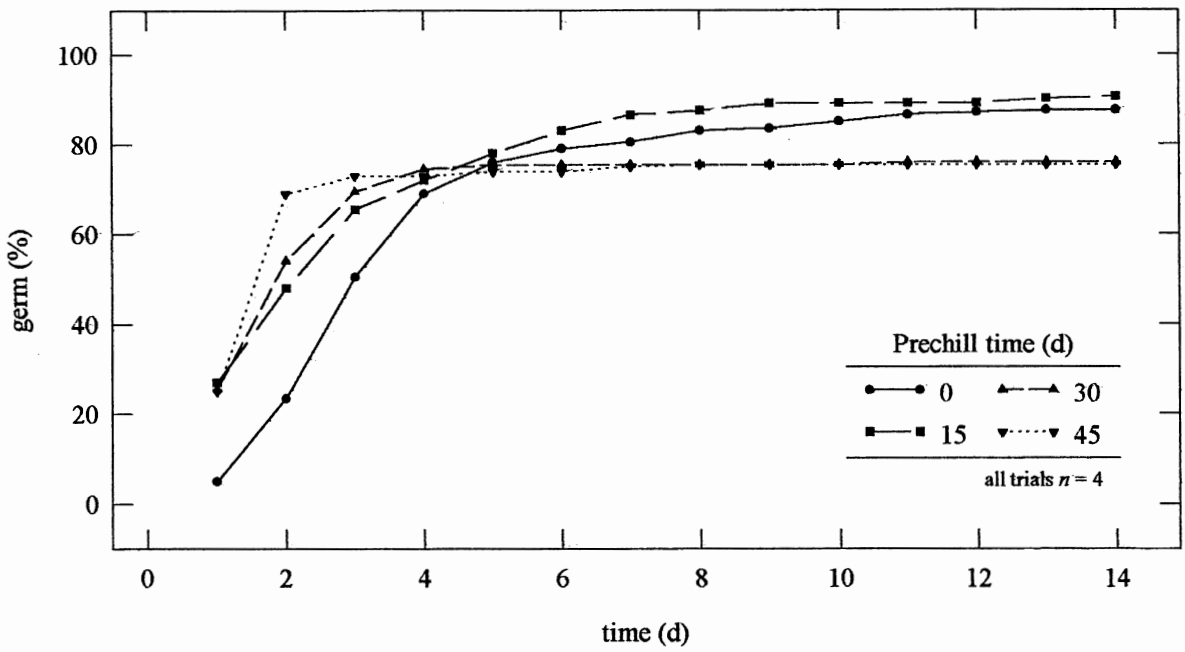


Figure 7 Average % germination of imbibed-chilled SC98 seed from Germination Trial 1

Table 8 shows ls-means comparisons of the stratifying treatments of each seed collection for day 3 and 14 (Bonferroni $\alpha = 0.0018$ (0.05/28) level of significance). Comparisons within RR98 found 5 of 6 average % germination values to be significantly different at day 3 (0RR vs. 15, 30, and 45RR; 15RR vs. 30 and 45RR) but those differences disappeared by day 14. Comparisons within SC98 produced 1 difference at day 3 (0SC vs. 45SC), and no differences at day 14. Comparisons between seed collections stratified for identical times found 1 difference at day 3 (0RR vs. 0SC), and no difference at day 14. Remaining comparisons between seed collections found 9 of 12 average % germination values to be significantly different at day 3 (0RR vs. 15, 30, and 45SC; 0SC vs. 30 and 45RR; 15SC vs. 30 and 45 RR; 30SC vs. 45RR) but those differences disappeared by day 14 while 2 others arose (15RR vs. 30 and 45SC).

Table 9 and Figure 8 show ls-means comparisons within each seed stratifying treatment between each successive day (days 1-13) and the last day (day 14) (Bonferroni $\alpha = 0.0038$ (0.05/13) level of significance) for each collection. This test was used to estimate time to complete germination for seeds of any particular stratifying treatment and was a measure of the change in seed germination potential. In Table 9, the first nonsignificant difference in each column of *seed* by *strat* comparisons is underlined. Complete germination for 0 day imbibed-chilled RR98 and SC98 seed was reached after 7 or 6 days, respectively, and successively longer stratifying times diminished caryopsis dormancy. A 45 day stratifying treatment for either seed collection produced a statistically complete germination in only 2 days. Figure 8 is used to more clearly show the results of Table 9.

Table 8 Least squares means comparisons within and between RR98 and SC98 for imbibed-chilled seed from Germination Trial 1, for days 3 and 14

Least squares means comparison	Day 3	Day 14
within RR98		
0 RR vs. 15 RR	0.0001	ns
0 RR vs. 30 RR	0.0001	ns
0 RR vs. 45 RR	0.0001	ns
15 RR vs. 30 RR	0.0001	ns
15 RR vs. 45 RR	0.0001	ns
30 RR vs. 45 RR	ns	ns
within SC98		
0 SC vs. 15 SC	ns	ns
0 SC vs. 30 SC	ns	ns
0 SC vs. 45 SC	0.0003	ns
15 SC vs. 30 SC	ns	ns
15 SC vs. 45 SC	ns	ns
30 SC vs. 45 SC	ns	ns
between RR99 and SC98		
0 RR vs. 0 SC	0.0001	ns
15 RR vs. 15 SC	ns	ns
30 RR vs. 30 SC	ns	ns
45 RR vs. 45 SC	ns	ns
0 RR vs. 15 SC	0.0001	ns
0 RR vs. 30 SC	0.0001	ns
0 RR vs. 45 SC	0.0001	ns
0 SC vs. 15 RR	ns	ns
0 SC vs. 30 RR	0.0001	ns
0 SC vs. 45 RR	0.0001	ns
15 RR vs. 30 SC	ns	0.0009
15 RR vs. 45 SC	ns	0.0009
15 SC vs. 30 RR	0.0003	ns
15 SC vs. 45 RR	0.0002	ns
30 RR vs. 45 SC	ns	ns
30 SC vs. 45 RR	0.0009	ns

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998; ns = not significant; $\alpha = 0.0018$ (0.05/28); $n = 4$.

Table 9 Using least squares means differences between days 1-13 and 14 to estimate time to complete germination for imbibed-chilled seed from Germination Trial 1

	RR98 *strat*				SC98 *strat*			
Day	0	15	30	45	0	15	30	45
1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	0.0001	0.1737	0.4365	0.0001	0.0001	0.0001	0.2064
3	0.0001	0.0001	0.8421	0.7651	0.0001	0.0001	0.2326	0.6186
4	0.0001	0.0023	0.9185	0.9185	0.0002	0.0002	0.8379	0.6091
5	0.0001	0.1706	0.9159	0.9159	0.0157	0.0087	0.9159	0.7515
6	0.1010	0.3244	1	0.9128	0.0632	0.101	0.9128	0.7424
7	0.4227	0.6468	1	0.9088	0.1094	0.3596	0.9088	0.9088
8	0.8088	0.8088	1	0.9037	0.2766	0.468	0.9037	1
9	1	0.8969	1	1	0.3004	0.6975	0.8969	1
10	1	0.8875	1	1	0.4794	0.6712	0.8875	1
11	1	1	1	1	0.7496	0.6322	1	1
12	1	1	1	1	0.8487	0.5672	1	1
13	1	1	1	1	1	0.7923	1	1

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998 ; *strat* = seed stratifying time; $\alpha = 0.0038$ (0.05/13); $n = 4$.

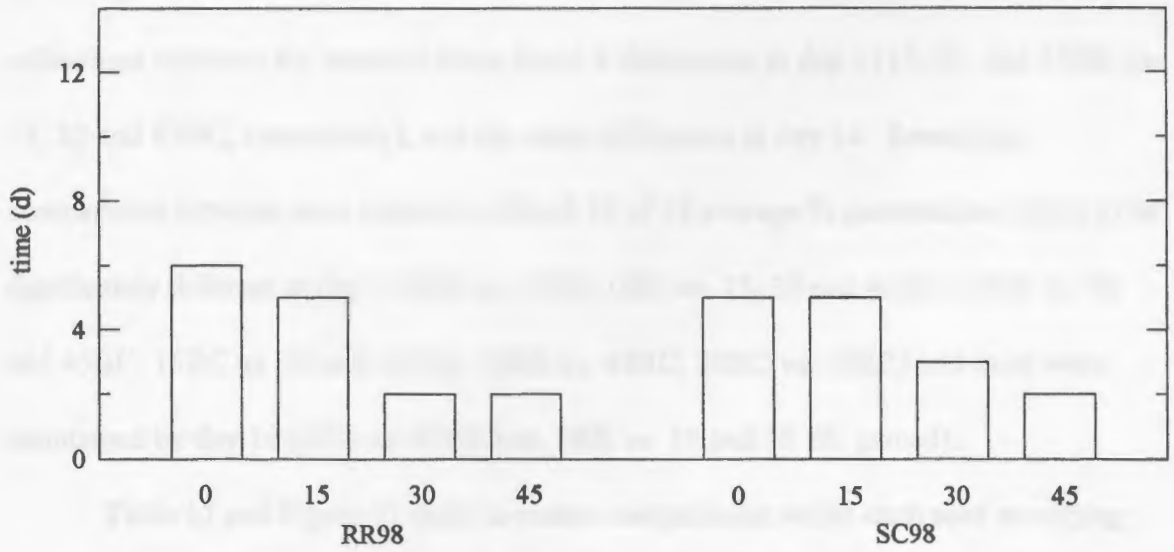


Figure 8 Conversion of Table 9 into a bar graph; Time to complete germination for imbibed-chilled seed from Germination Trial 1

Germination Trial 2

RR99 and BC99 seed was stratified by the imbibed-chill technique. Figures 9 and 10 present respective graphs of average % germination for each seed collection. The best-fitting mixed model heterogeneous AR(1) was chosen to further examine these data. A significant 3-way fixed factor interaction of *strat* by *time* by *seed* (at the $P < 0.05$ level of significance) was observed. Two ls-means comparisons were made.

Table 10 shows ls-means comparisons of the stratifying treatments of each seed collection for day 3 and 14 (Bonferroni $\alpha = 0.0018$ (0.05/28) level of significance). Comparisons within RR99 found 4 of 6 average % germination values to be significantly different at day 3 (0RR vs. 15, 30, and 45RR; 15RR vs. 30RR) but those differences disappeared by day 14. Comparisons within BC99 produced 2 differences at day 3 (0BC vs. 45BC; 15BC vs. 45BC), and no differences at day 14. Comparisons between seed collections stratified for identical times found 3 differences at day 3 (15, 30, and 45RR vs. 15, 30 and 45BC, respectively), and the same differences at day 14. Remaining comparisons between seed collections found 10 of 12 average % germination values to be significantly different at day 3 (0RR vs. 45BC; 0BC vs. 15, 30 and 45RR; 15RR vs. 30 and 45BC; 15BC vs. 30 and 45 RR; 30RR vs. 45BC; 30BC vs. 45RR) and most were maintained by day 14 (0BC vs. 45RR lost, 0RR vs. 15 and 30 BC gained).

Table 11 and Figure 11 show ls-means comparisons within each seed stratifying treatment between each successive day (days 1-13) and the last day (day 14) (Bonferroni $\alpha = 0.0038$ (0.05/13) level of significance) for each seed collection. This test was used to estimate time to complete germination for seeds of any particular stratifying treatment

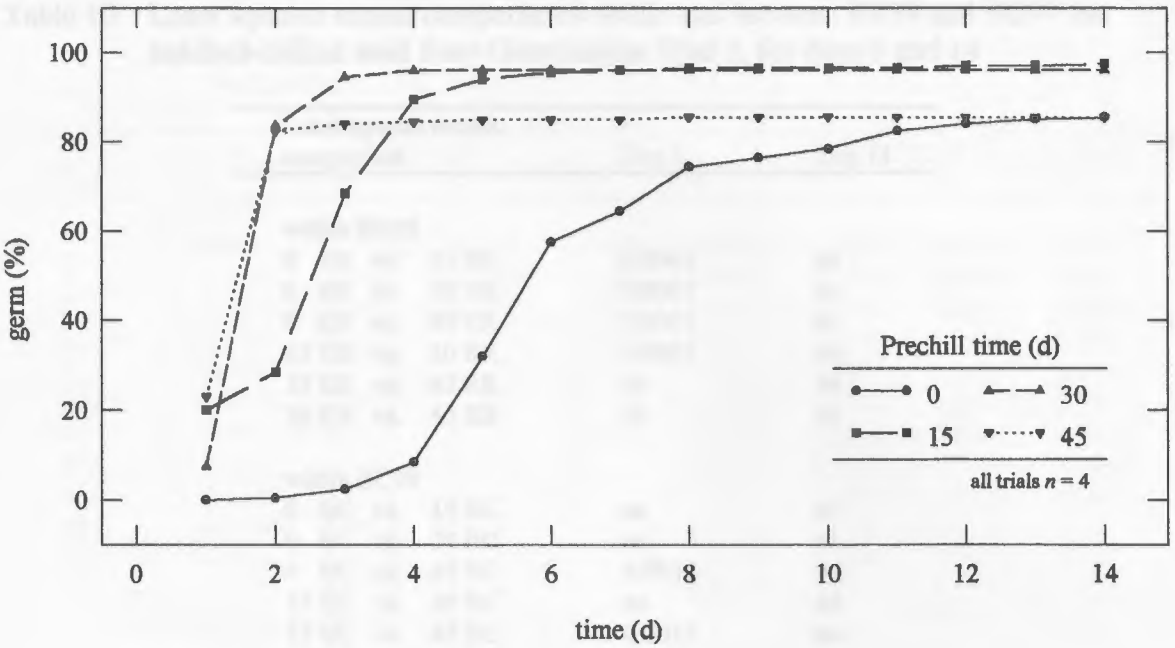


Figure 9 Average % germination of imbibed-chilled RR99 seed from Germination Trial 2

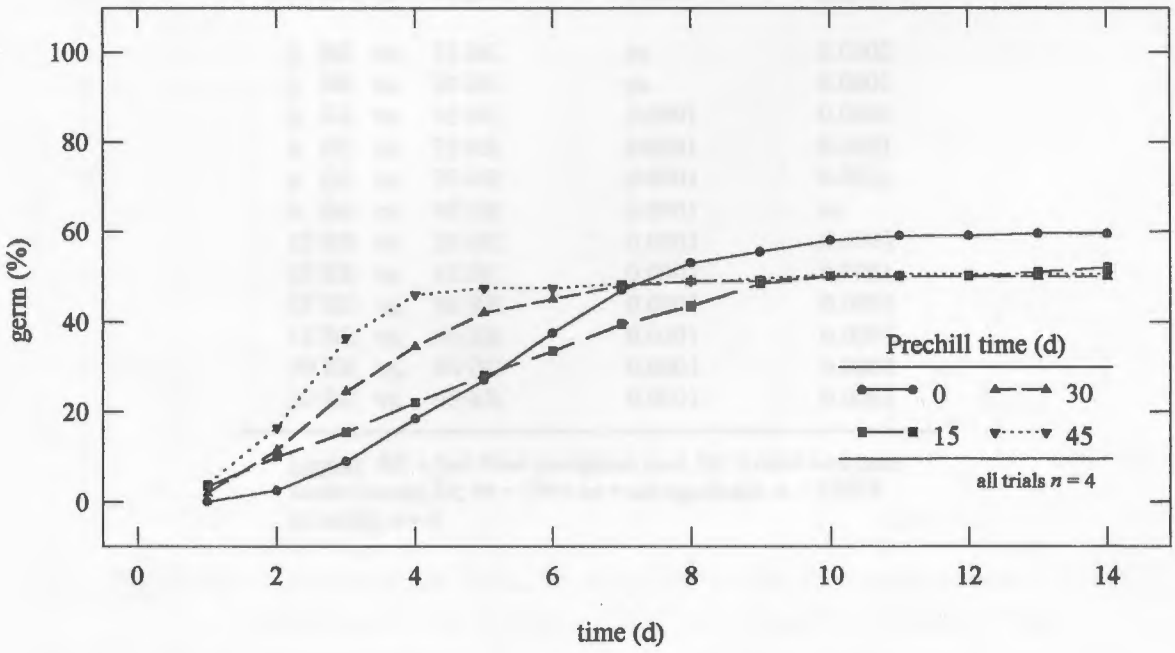


Figure 10 Average % germination of imbibed-chilled BC99 seed from Germination Trial 2

Table 10 Least squares means comparisons within and between RR99 and BC99 for imbibed-chilled seed from Germination Trial 2, for days 3 and 14

Least squares means comparison			Day 3	Day 14
within RR99				
0	RR	vs. 15 RR	0.0001	ns
0	RR	vs. 30 RR	0.0001	ns
0	RR	vs. 45 RR	0.0001	ns
15	RR	vs. 30 RR	0.0001	ns
15	RR	vs. 45 RR	ns	ns
30	RR	vs. 45 RR	ns	ns
within BC99				
0	BC	vs. 15 BC	ns	ns
0	BC	vs. 30 BC	ns	ns
0	BC	vs. 45 BC	0.0012	ns
15	BC	vs. 30 BC	ns	ns
15	BC	vs. 45 BC	0.0013	ns
30	BC	vs. 45 BC	ns	ns
between RR99 and BC99				
0	RR	vs. 0 BC	ns	ns
15	RR	vs. 15 BC	0.0001	0.0001
30	RR	vs. 30 BC	0.0001	0.0001
45	RR	vs. 45 BC	0.0001	0.0001
0	RR	vs. 15 BC	ns	0.0002
0	RR	vs. 30 BC	ns	0.0001
0	RR	vs. 45 BC	0.0001	0.0001
0	BC	vs. 15 RR	0.0001	0.0001
0	BC	vs. 30 RR	0.0001	0.0001
0	BC	vs. 45 RR	0.0001	ns
15	RR	vs. 30 BC	0.0001	0.0001
15	RR	vs. 45 BC	0.0001	0.0001
15	BC	vs. 30 RR	0.0001	0.0001
15	BC	vs. 45 RR	0.0001	0.0001
30	RR	vs. 45 BC	0.0001	0.0001
30	BC	vs. 45 RR	0.0001	0.0001

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; ns = not significant; $\alpha = 0.0018$ (0.05/28); $n = 4$.

Table 11 Using least squares means differences between days 1-13 and 14 to estimate time to complete germination for imbibed-chilled seed from Germination Trial 2

	RR99 *strat*				BC99 *strat*			
Day	0	15	30	45	0	15	30	45
1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	0.0001	0.0010	0.5891	0.0001	0.0001	0.0001	0.0001
3	0.0001	0.0001	0.6873	0.7822	0.0001	0.0001	0.0001	0.0103
4	0.0001	0.1431	1	0.8498	0.0001	0.0001	0.0001	0.3943
5	0.0001	0.5170	1	0.9221	0.0001	0.0001	0.0285	0.5576
6	0.0001	0.7074	1	0.9191	0.0014	0.0002	0.1625	0.5426
7	0.0014	0.7742	1	0.9154	0.0556	0.0133	0.5669	0.6711
8	0.0739	0.8443	1	1	0.2901	0.0959	0.7675	0.7365
9	0.1157	0.8385	1	1	0.4838	0.6836	0.7575	0.8101
10	0.1793	0.8296	1	1	0.7732	0.9143	1	1
11	0.5140	0.8150	1	1	0.9133	0.9069	1	1
12	0.6950	0.8931	1	1	0.8960	0.8931	1	1
13	0.8562	0.8589	1	1	1	1	1	1

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; *strat* = seed stratifying time; $\alpha = 0.0038$ (0.05/13); $n = 4$.

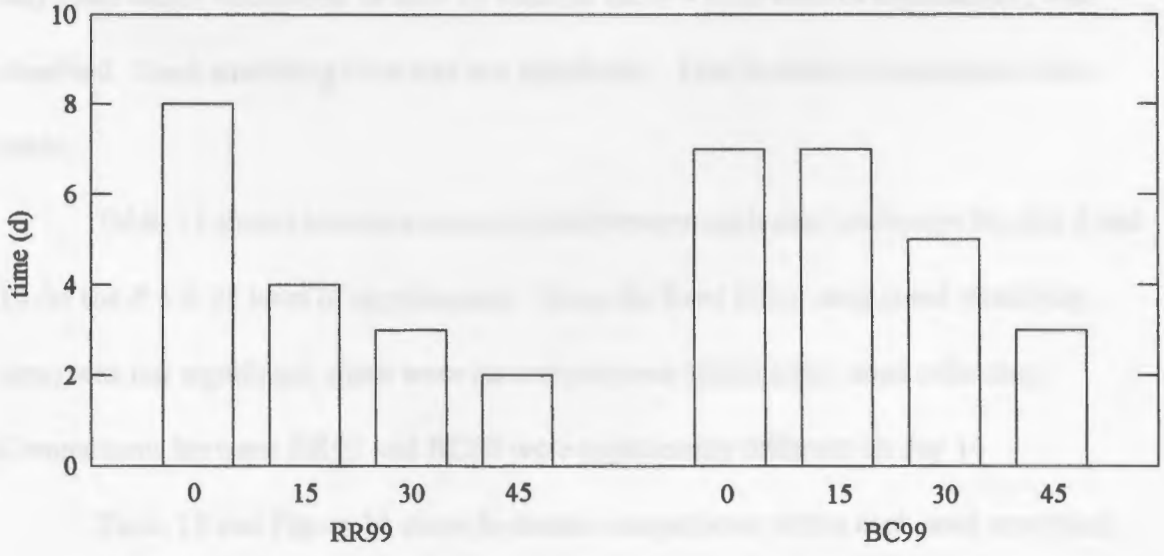


Figure 11 Conversion of Table 10 into a bar graph; Time to complete germination for imbibed-chilled seed from Germination Trial 2

and was a measure of the change in seed germination potential. In Table 11, the first nonsignificant difference in each column of *seed* by *strat* comparisons is underlined. Complete germination for 0 day imbibed-chilled RR99 and BC99 seed was reached after 8 or 7 days, respectively, and successively longer stratifying times diminished caryopsis dormancy. A 45 day stratifying treatment for either seed collection produced a statistically complete germination in 2 or 3 days, respectively. Figure 11 is used to more clearly show the results of Table 11.

Germination Trial 3

RR99 and BC99 seed was stratified by the imbibed-freezing technique. Figures 12 and 13 present respective graphs of average % germination for each seed collection. The best-fitting mixed model AR(1) was chosen to further examine these data. A significant 2-way fixed factor interaction of *time* by *seed* (at the $P < 0.05$ level of significance) was observed. Seed stratifying time was not significant. Two ls-means comparisons were made.

Table 12 shows ls-means comparisons between each seed collection for day 3 and 14 (at the $P < 0.05$ level of significance). Since the fixed effect *strat* (seed stratifying time) was not significant, there were no comparisons within either seed collection. Comparisons between RR99 and BC99 were significantly different on day 14.

Table 13 and Figure 14 show ls-means comparisons within each seed stratifying treatment between each successive day (days 1-13) and the last day (day 14) (Bonferroni $\alpha = 0.0038$ (0.05/13) level of significance) for each collection. This test was used to estimate time to complete germination for seeds of any particular stratifying treatment

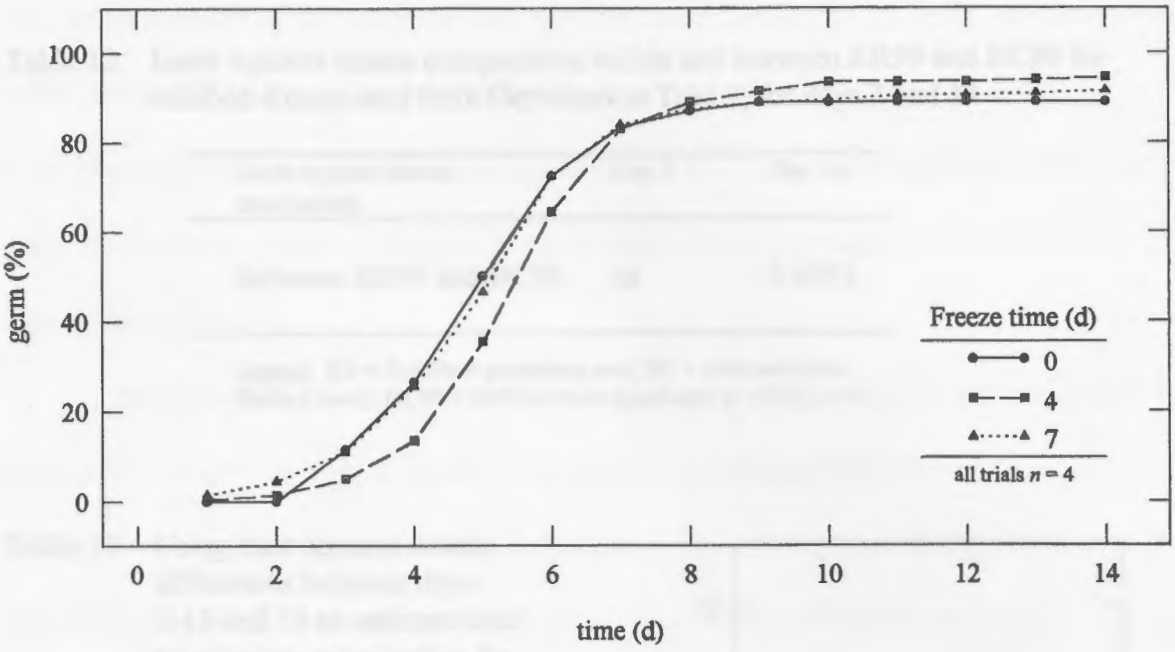


Figure 12 Average % germination of imbibed-frozen RR99 seed from Germination Trial 3

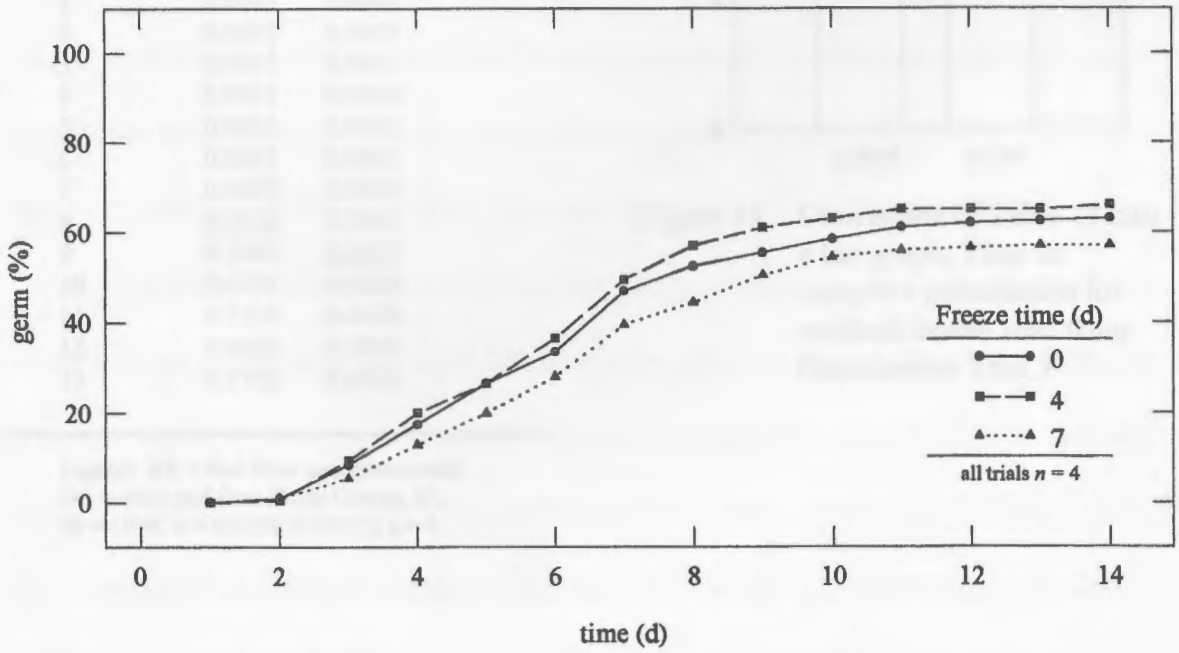


Figure 13 Average % germination of imbibed-frozen BC99 seed from Germination Trial 3

Table 12 Least squares means comparisons within and between RR99 and BC99 for imbibed-frozen seed from Germination Trial 3, for days 3 and 14

Least squares means comparison	Day 3	Day 14
between RR99 and BC99	ns	0.0001

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; ns = not significant; $\alpha = 0.05$, $n = 4$.

Table 13 Using least squares means differences between days 1-13 and 14 to estimate time to complete germination for imbibed-frozen seed from Germination Trial 3

Day	RR99	BC99
1	0.0001	0.0001
2	0.0001	0.0001
3	0.0001	0.0001
4	0.0001	0.0001
5	0.0001	0.0001
6	0.0001	0.0001
7	0.0007	0.0001
8	0.0936	0.0001
9	0.3967	0.0037
10	0.6191	0.0984
11	0.7133	0.4626
12	0.6662	0.5897
13	0.7702	0.6613

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; $\alpha = 0.0038$ (0.05/13); $n = 4$.

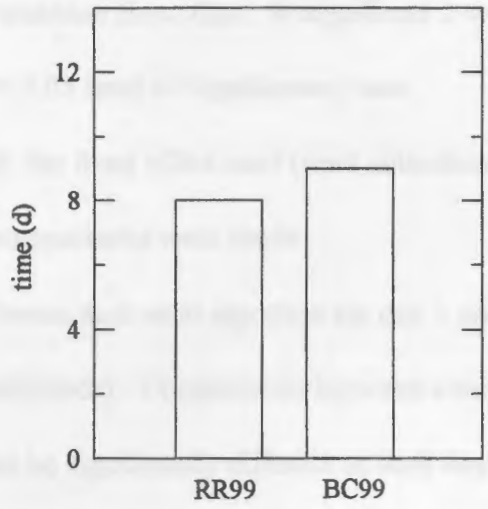


Figure 14 Conversion of Table 13 into a bar graph; Time to complete germination for imbibed-frozen seed from Germination Trial 3

and was a measure of the change in seed germination potential. In Table 13, the first nonsignificant difference in each column of *seed* by *strat* comparisons is underlined. Complete germination for imbibed-frozen RR99 and BC99 seed was reached after 9 or 8 days, respectively. Figure 14 is used to more clearly show the results of Table 13.

Germination Trial 4

Red River germplasm seed was stratified by aging. Figure 15 presents respective graphs of average % germination for 2 year old, 1 year old, and fresh seed. The best-fitting mixed model AR(1) was chosen to further examine these data. A significant 2-way fixed factor interaction of *strat* by *time* (at the $P < 0.05$ level of significance) was observed. Since only 1 seed collection was tested, the fixed effect *seed* (seed collection) did not apply to this experiment. Two ls-means comparisons were made.

Table 14 shows ls-means comparisons between each seed age class for day 3 and 14 (Bonferroni $\alpha = 0.0018$ (0.05/28) level of significance). Comparisons between seed age classes found average % germination values to be significantly different at both day 3 and 14 for RR99 and RR98 vs. RR97, but not between RR99 and RR98.

Table 15 and Figure 16 show ls-means comparisons within each age class of *Red River* germplasm seed between each successive day (days 1-13) and the last day (day 14). This test was used to estimate time to complete germination for seed from each age class, and was a measure of the change in seed germination potential with age. In Table 15, the first nonsignificant difference in each column of *strat* by *time* comparisons is underlined. Complete germination for RR97, 98 and 99 seed was reached after 7 days. Figure 16 is used to more clearly show the results of Table 15.

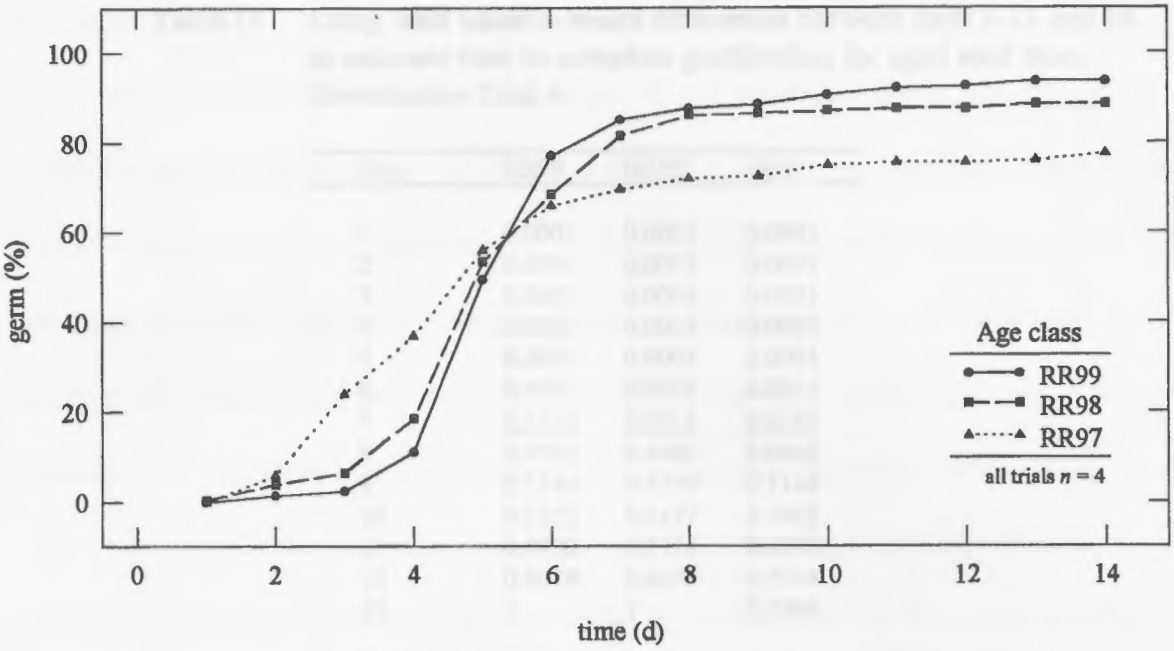


Figure 15 Average % germination of aged *Red River* germplasm seed from Germination Trial 4

Table 14 Least squares means comparisons between RR97, 98, and 99 aged seed from Germination Trial 4, both during early-phase, and at end-of-trial

Least squares means comparison	Day 3	Day 14
RR99 vs. RR98	ns	ns
RR99 vs. RR97	0.0001	0.0001
RR98 vs. RR97	0.0001	0.0040

Legend: RR = *Red River* germplasm seed, 97-99 = 1997-1999; ns = not significant; $\alpha = 0.0167$ (0.05/3); $n = 4$.

Table 15 Using least squares means differences between days 1-13 and 14 to estimate time to complete germination for aged seed from Germination Trial 4

Day	RR99	RR98	RR97
1	0.0001	0.0001	0.0001
2	0.0001	0.0001	0.0001
3	0.0001	0.0001	0.0001
4	0.0001	0.0001	0.0001
5	0.0001	0.0001	0.0001
6	0.0001	0.0001	0.0012
7	0.0135	0.0411	0.0199
8	0.0702	0.4480	0.0966
9	0.1144	0.5259	0.1144
10	0.3121	0.6127	0.3993
11	0.5790	0.7114	0.4597
12	0.6679	0.6679	0.3914
13	1	1	0.3904

Legend: RR = *Red River* germplasm seed; 97-99 = 1997-1999; $\alpha = 0.0038$ (0.05/13); $n = 4$.

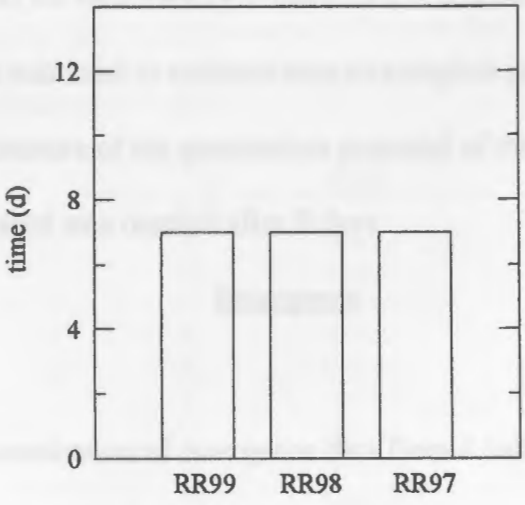


Figure 16 Conversion of Table 15 into a bar graph; Time to complete germination for aged seed from Germination Trial 4

Germination Trial 5

BC99 seed was stratified by the dried-warm and dried-chill techniques. Figure 17 presents average % germination for each seed stratifying treatment. The best-fitting mixed model heterogenous AR(1) was chosen to further examine these data. The fixed effect *time* (elapsed time within a trial) was observed to be significant (at the $P < 0.05$ level of significance). Since only 1 seed collection was tested, the fixed effect *seed* (seed collection) did not apply to this experiment. There were no higher-order interactions.

Since the fixed effect *strat* (seed stratifying time) was not significant, there were no ls-means comparisons for stratifying times within the seed collection. Likewise since there was only 1 seed collection, there were no comparisons between seed collections.

Table 16 presents ls-means comparisons between each successive day (days 1-13) and the last day (day 14) for BC99 seed (Bonferroni $\alpha = 0.0038$ (0.05/13) level of significance). This test was used to estimate time to complete germination for the seed collection, and was a measure of the germination potential of the seed. Complete germination for BC99 seed was reached after 9 days.

Emergence

Emergence Reports

The systematic evaluation of emergence data from 4 collections of *S. pectinata* seed (RR98, SC98, RR99, BC99) was used to provide supplemental information following germination. Emergence tests were conducted to insure that stratified, germinated seeds did not damp-off as seedlings. The duration of seed emergence trials was 18 days. Obviously, seedling emergence from soil was slower than coleoptile

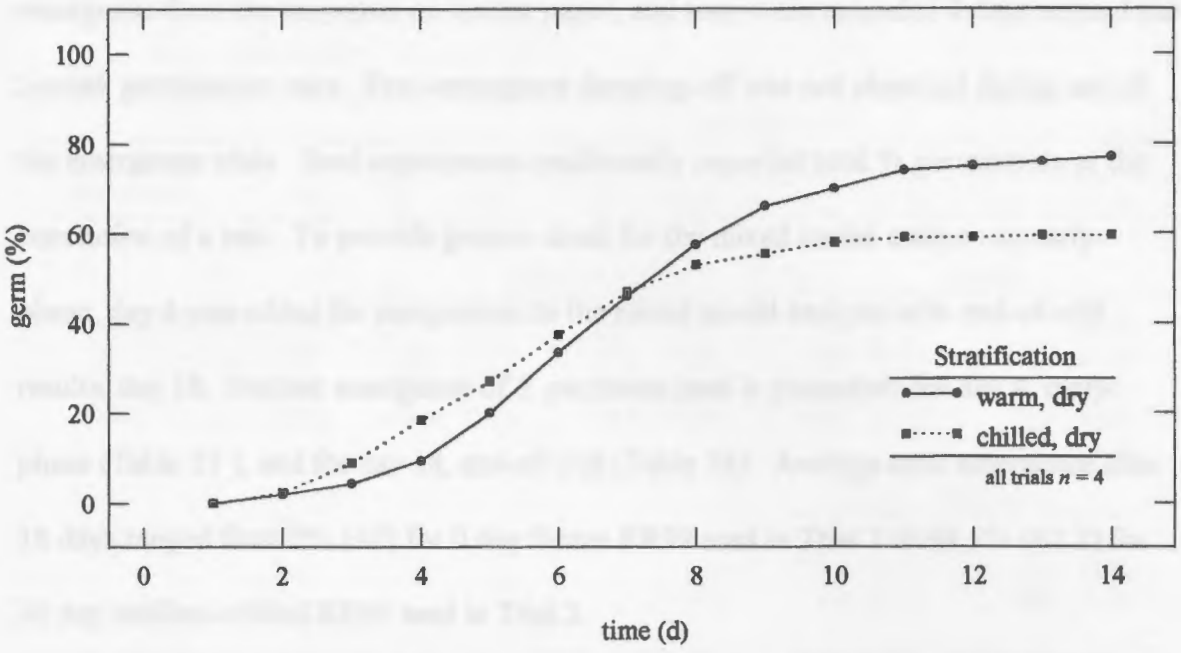


Figure 17 Average % germination of dried-warmed and dried-chilled BC99 seed from Germination Trial 5

Table 16 Using least squares means differences between days 1-13 and 14 to estimate time to complete germination for dried-warmed seed from Germination Trial 5

Day	BC99
1	0.0001
2	0.0001
3	0.0001
4	0.0001
5	0.0001
6	0.0001
7	0.0001
8	0.0015
9	0.0439
10	0.2066
11	0.5542
12	0.6116
13	0.7781

Legend: BC = relict seed from Butler County, IA; 99 = 1999; $\alpha = 0.0038$ (0.05/13); $n = 4$.

emergence from the caryopsis on blotter paper, and tests were extended 4 days beyond the 2-week germination tests. Post-emergence damping-off was not observed during any of the emergence trials. Seed experiments traditionally reported total % germination at the conclusion of a test. To provide greater detail for the mixed model analysis an early-phase, day 4 was added for comparison to the mixed model analysis with end-of-trial results, day 18. Percent emergence of *S. pectinata* seed is presented: for day 4, early-phase (Table 17); and for day 18, end-of-trial (Table 18). Average total emergence after 18 days ranged from 2% (±2) for 0 day frozen RR99 seed in Trial 3 to 94.5% (±2.2) for 30 day imbibed-chilled RR99 seed in Trial 2.

Mixed Model Analysis

The aforementioned mixed models were fit to the data sets using SAS as presented in Germination. Table 19 presents fit indices and model parameters for the covariance structure of all 5 mixed models for each germination trial. Each respective most parsimonious case is underlined, and the corresponding fixed factor report for each best model is also indicated. Of the 3 model fitting predictors tested, BIC consistently provided the most parsimonious solution. Of the 5 models tested, it was either AR(1) or $AR(1)_{hetero}$ that provided the best fitting covariance structure. Specifically, Emergence Trials 1 and 2 (imbibed-chilled seeds) were best modeled with AR(1). Emergence Trial 3 (imbibed-frozen seed) was best modeled with $AR(1)_{hetero}$. The highest-order significant interaction observed (at the $P < 0.05$ level of significance) between the fixed factors *seed* (seed collection), *strat* (seed stratifying time), and *time* (elapsed time within a trial) was:

Table 17 Percent emergence of *S. pectinata* seed for emergence trials from day 4, early-phase

Stratifying Treatment	Time	% Germination RR98 $\bar{x}$	RR98 ($S_{\bar{x}}$)	SC98 $\bar{x}$	SC98 ($S_{\bar{x}}$)	RR99 $\bar{x}$	RR99 ($S_{\bar{x}}$)	BC99 $\bar{x}$	BC99 ($S_{\bar{x}}$)
		Trial 1				Trial 2			
Imbibed-chilled	0d	**0.5**	**(0.5)**	**14.0**	**(2.4)**	**0.0**	**(0.0)**	**0.5**	**(0.5)**
	15d	7.0	(1.3)	20.0	(5.7)	5.0	(1.7)	4.5	(1.9)
	30d	71.5	(5.9)	48.5	(5.2)	55.5	(9.5)	9.5	(3.8)
	45d	72.0	(5.9)	64.0	(2.9)	64.5	(8.3)	2.0	(0.8)
						Trial 3			
Imbibed-frozen	0d					**2.0**	**(2.0)**	**4.5**	**(3.3)**
	4d					3.5	(2.1)	8.0	(4.6)
	7d					2.5	(1.5)	8.0	(2.7)

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error; **bold** shows untreated seed; $n = 4$.

1) A 3-way interaction for Emergence Trial 1 and 2.

2) A 2-way interaction between *strat* and *seed* for Emergence Trial 3.

Emergence Trial 1

RR98 and SC98 seed was stratified by the imbibed-chill technique. Figures 18 and 19 present respective graphs of average % emergence for each seed collection. The best-fitting mixed model AR(1) was chosen to further examine these data. A significant 3-way fixed factor interaction of *strat* by *time* by *seed* (at the $P < 0.05$ level of significance) was observed. Two ls-means comparisons were made.

Table 20 shows ls-means comparisons of the stratifying treatments of each seed collection for day 3 and 14 (Bonferroni $\alpha = 0.0018$ (0.05/28) level of significance).

Table 18 Percent emergence of *S. pectinata* seed for emergence trials from day 18, end-of-trial

Stratifying Treatment	Time	% Germination							
		RR98		SC98		RR99		BC99	
		$\bar{x}$	($S_{\bar{x}}$)	$\bar{x}$	($S_{\bar{x}}$)	$\bar{x}$	($S_{\bar{x}}$)	$\bar{x}$	($S_{\bar{x}}$)
		Trial 1				Trial 2			
Imbibed-chilled	0d	**76.0**	**(7.8)**	**61.5**	**(3.1)**	**84.5**	**(6.8)**	**38.5**	**(3.8)**
	15d	85.5	(1.5)	69.0	(8.9)	82.0	(5.8)	40.5	(4.0)
	30d	84.5	(3.3)	67.0	(1.0)	94.5	(2.2)	45.0	(6.0)
	45d	74.5	(4.6)	69.0	(3.4)	88.5	(3.1)	29.0	(4.1)
						Trial 3			
Imbibed-frozen	0d					**2.0**	**(2.0)**	**10.5**	**(8.5)**
	4d					6.0	(3.6)	12.0	(5.4)
	7d					2.5	(1.5)	10.5	(2.1)

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; x = mean, S_x = standard error; **bold** shows untreated seed; $n = 4$.

Comparisons within both RR98 and SC98 found 4 of 6 average % emergence values to be significantly different at day 3 (0RR vs. 30 & 45RR; 15RR vs. 30 & 45RR) but those differences disappeared by day 14. Comparisons between seed collections stratified for identical times found 1 difference at day 3 (30RR vs. 30SC), but no difference at day 14. Remaining comparisons between seed collections found 9 of 12 average % emergence values to be significantly different at day 3 (0RR vs. 30, 45RR; 0SC vs. 30, 45RR, 15RR vs. 30, 45SC, 15SC vs. 30, 45RR, 30SC vs. 45RR) and all but 1 of those differences (0SC vs. 30RR) disappeared by day 14 while 1 other arose (0SC vs. 30RR).

Table 21 and Figure 20 show ls-means comparisons within each seed stratifying treatment between each successive day (days 1-17) and the last day (day 18) (Bonferroni

Table 19 Assessment of germination data covariance structure by 3 fit indices and parameters (each best case underlined), and the fixed effects of each best case

Model	LnL	AIC	BIC	P	Fixed Effects	*Pr > F*
Germination Trial 1: Imbibed-chilled seed					**AR(1)**	
GLM	-1435.47	-1436.47	-1438.51	1	*strat*	0.0001
CS	-1234.42	-1236.42	-1240.51	2	*time*	0.0001
AR(1)	-1067.77	-1069.77	**-1073.87**	**2**	*seed*	0.0005
CS_{hetero}	-1183.53	-1191.53	-1207.91	8	*strat*seed*	ns
$AR(1)_{hetero}$	**-1054.88**	**-1062.88**	-1079.27	8	*strat*time*	0.0001
					*time*seed*	ns
					*strat*time*seed*	0.0001
Germination Trial 2: Imbibed-chilled seed					**AR(1)**	
GLM	-1381.10	-1382.10	-1384.13	1	*strat*	0.0001
CS	-1239.55	-1241.55	-1245.62	2	*time*	0.0001
AR(1)	-1008.69	**-1010.69**	**-1014.76**	**2**	*seed*	0.0001
CS_{hetero}	-1206.03	-1214.03	-1230.30	8	*strat*seed*	0.0007
$AR(1)_{hetero}$	**-1002.95**	-1010.95	-1027.22	8	*strat*time*	0.0001
					*time*seed*	0.0001
					*strat*time*seed*	0.0001
Germination Trial 3: Imbibed-frozen seed					**AR(1) heterogeneous**	
GLM	-974.99	-975.99	-977.88	1	*strat*	ns
CS	-706.92	-708.92	-712.71	2	*time*	0.0001
AR(1)	-423.71	-425.71	-429.49	2	*seed*	0.0350
CS_{hetero}	-610.70	-616.70	-628.04	6	*strat*seed*	ns
$AR(1)_{hetero}$	**-405.95**	**-411.95**	**-423.29**	**6**	*strat*time*	ns
					*time*seed*	ns
					*strat*time*seed*	ns

Legend: AIC = Akaike's Information Criterion, BIC = Schwarz's Bayesian Criterion, LnL = Log Likelihood, P = parameters; GLM = general linear model, CS = compound symmetry model; AR(1) = autoregressive order (one), hetero = heterogeneous; *strat* = seed stratifying time, *time* = elapsed time within a trial, *seed* = seed collection; $\alpha = 0.05$.

$\alpha = 0.0029$ (0.05/17) level of significance) for each seed collection. This test was used to estimate time to complete germination for seeds of any particular stratifying treatment and was a measure of the change in seed germination potential. In Table 21, the first nonsignificant difference in each column of *seed* by *strat* comparisons is underlined.

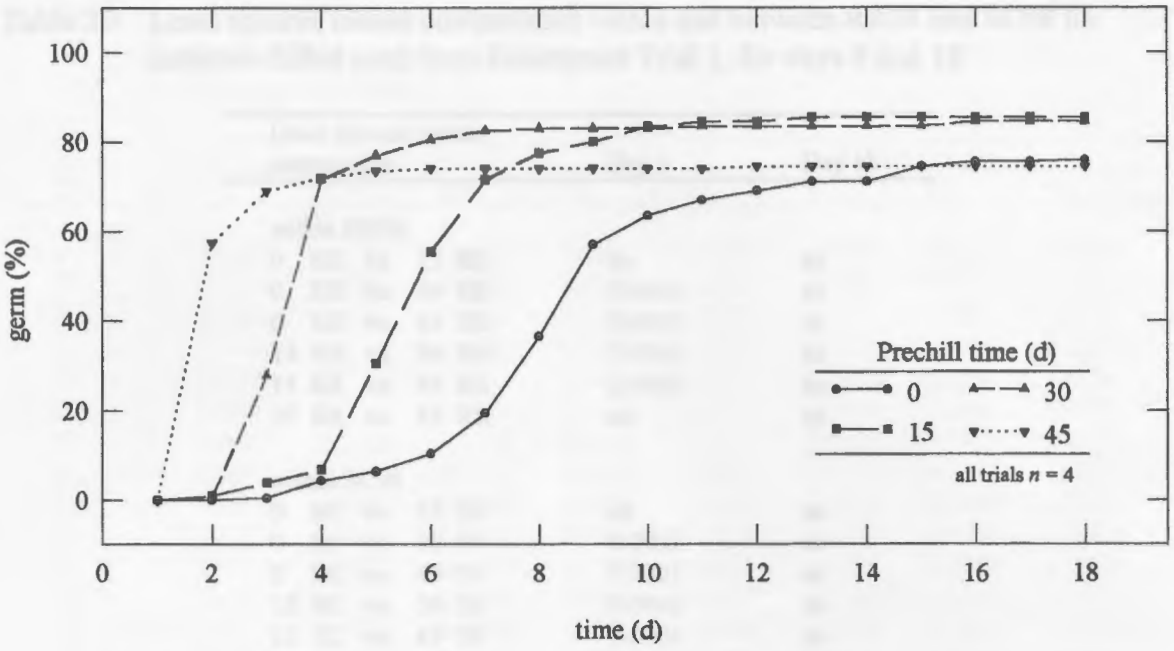


Figure 18 Average % emergence of imbibed-chilled RR98 seed from Emergence Trial 1

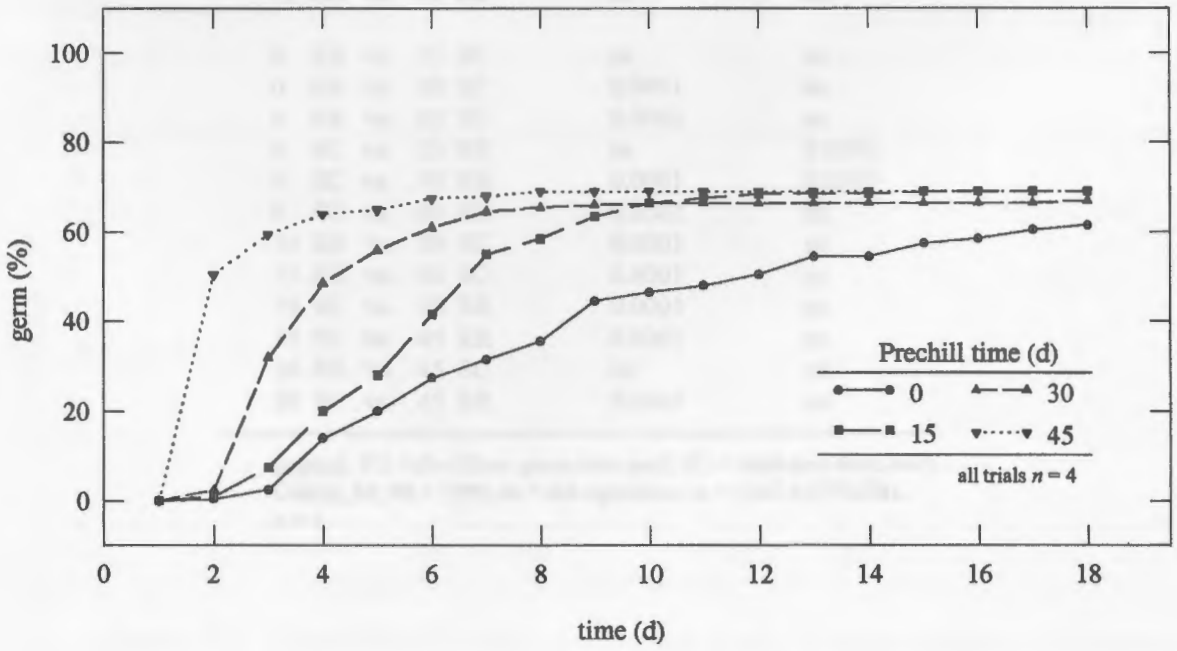


Figure 19 Average % emergence of imbibed-chilled SC98 seed from Emergence Trial 1

Table 20 Least squares means comparisons within and between RR98 and SC98 for imbibed-chilled seed from Emergence Trial 1, for days 4 and 18

Least squares means comparison	Day 4	Day 18
within RR98		
0 RR vs. 15 RR	ns	ns
0 RR vs. 30 RR	0.0001	ns
0 RR vs. 45 RR	0.0001	ns
15 RR vs. 30 RR	0.0001	ns
15 RR vs. 45 RR	0.0001	ns
30 RR vs. 45 RR	ns	ns
within SC98		
0 SC vs. 15 SC	ns	ns
0 SC vs. 30 SC	0.0001	ns
0 SC vs. 45 SC	0.0001	ns
15 SC vs. 30 SC	0.0001	ns
15 SC vs. 45 SC	0.0001	ns
30 SC vs. 45 SC	ns	ns
between RR98 AND SC98		
0 RR vs. 0 SC	ns	ns
15 RR vs. 15 SC	ns	ns
30 RR vs. 30 SC	0.0003	ns
45 RR vs. 45 SC	ns	ns
0 RR vs. 15 SC	ns	ns
0 RR vs. 30 SC	0.0001	ns
0 RR vs. 45 SC	0.0001	ns
0 SC vs. 15 RR	ns	0.0002
0 SC vs. 30 RR	0.0001	0.0003
0 SC vs. 45 RR	0.0001	ns
15 RR vs. 30 SC	0.0001	ns
15 RR vs. 45 SC	0.0001	ns
15 SC vs. 30 RR	0.0001	ns
15 SC vs. 45 RR	0.0001	ns
30 RR vs. 45 SC	ns	ns
30 SC vs. 45 RR	0.0003	ns

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998; ns = not significant; $\alpha = 0.0018$ (0.05/28); $n = 4$.

Table 21 Using least squares means differences between days 1-17 and 18 to estimate time to complete emergence for imbibed-chilled seed from Emergence Trial 1

	RR98 *strat*				SC98 *strat*			
Day	0	15	30	45	0	15	30	45
1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	0.0001	0.0001	0.0038	0.0001	0.0001	0.0001	0.0016
3	0.0001	0.0001	0.0001	0.3412	0.0001	0.0001	0.0001	0.1005
4	0.0001	0.0001	0.0229	0.6609	0.0001	0.0001	0.0013	0.3805
5	0.0001	0.0001	0.1818	0.8585	0.0001	0.0001	0.0504	0.4760
6	0.0001	0.0001	0.4680	0.9277	0.0001	0.0001	0.2765	0.7855
7	0.0001	0.0098	0.7109	0.9262	0.0001	0.0098	0.6432	0.8530
8	0.0001	0.1291	0.7757	0.9243	0.0001	0.0466	0.7757	1.0000
9	0.0002	0.2824	0.7693	0.9221	0.0010	0.2824	0.8449	1.0000
10	0.0117	0.6855	0.7613	0.9193	0.0025	0.6127	0.9193	1.0000
11	0.0578	0.8327	0.8327	0.9159	0.0045	0.7514	0.9159	1.0000
12	0.1199	0.8239	0.8239	1.0000	0.0147	0.9114	0.9114	1.0000
13	0.2353	1.0000	0.8122	1.0000	0.0968	0.9054	0.9054	1.0000
14	0.1962	1.0000	0.7958	1.0000	0.0706	0.8971	0.8971	1.0000
15	0.6626	1.0000	0.7711	1.0000	0.2449	1.0000	0.8843	1.0000
16	0.8624	1.0000	1.0000	1.0000	0.2985	1.0000	0.8624	1.0000
17	0.8115	1.0000	1.0000	1.0000	0.6335	1.0000	0.8115	1.0000

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998 ; *strat* = seed stratifying time; $\alpha = 0.0029$ (0.05/17); $n = 4$.

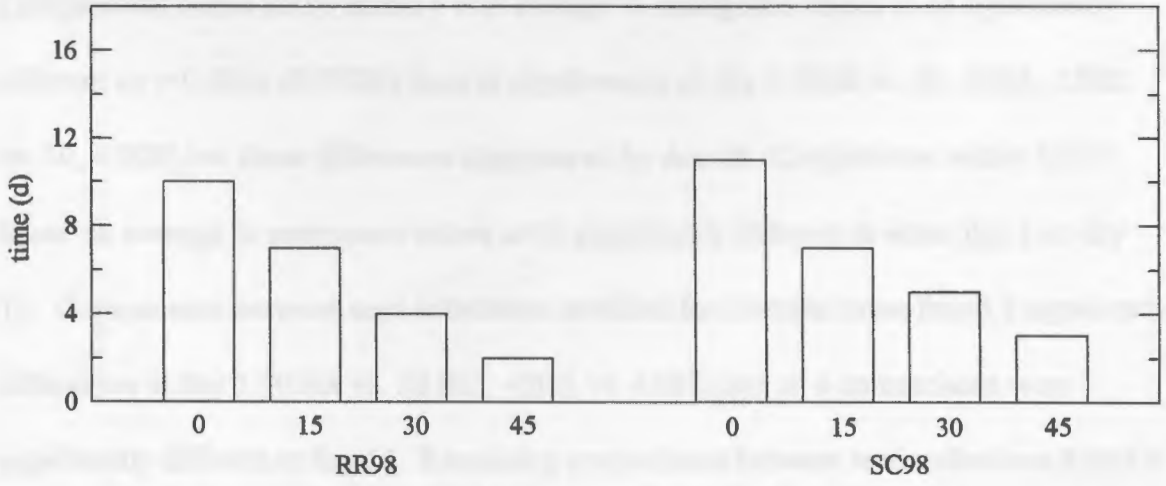


Figure 20 Conversion of Table 21 into a bar graph; Time to complete emergence for imbibed-chilled seed from Emergence Trial 1

Complete emergence for 0 day imbibed-chilled RR98 and SC98 seed was reached after 10 or 11 days, respectively, and successively longer stratifying times diminished caryopsis dormancy. A 45 day stratifying treatment for either seed collection produced a statistically complete emergence in 2 or 3 days. Figure 20 is used to more clearly show the results of Table 21.

Emergence Trial 2

RR99 and BC99 seed was stratified by the imbibed-chill technique. Figures 21 and 22 present respective graphs of average % emergence for each seed collection. The best-fitting mixed model AR(1) was chosen to further examine these data. A significant 3-way fixed factor interaction of *strat* by *time* by *seed* (at the $P < 0.05$ level of significance) was observed. Two ls-means comparisons were made.

Table 22 shows ls-means comparisons of the stratifying treatments of each seed collection for day 3 and 14 (Bonferroni $\alpha = 0.0018$ (0.05/28) level of significance). Comparisons within RR99 found 4 of 6 average % emergence values to be significantly different ($\alpha = 0.0018$ (0.05/28) level of significance) at day 3 (0RR vs. 30, 45RR; 15RR vs. 30, 45RR) but those differences disappeared by day 18. Comparisons within BC99 found no average % emergence values to be significantly different at either day 3 or day 18. Comparisons between seed collections stratified for identical times found 2 significant differences at day 3 (30RR vs. 30 BC; 45RR vs. 45BC) but all 4 comparisons were significantly different at day 18. Remaining comparisons between seed collections found 6 of 12 average % emergence values to be significantly different at day 3 (0BC vs. 30,

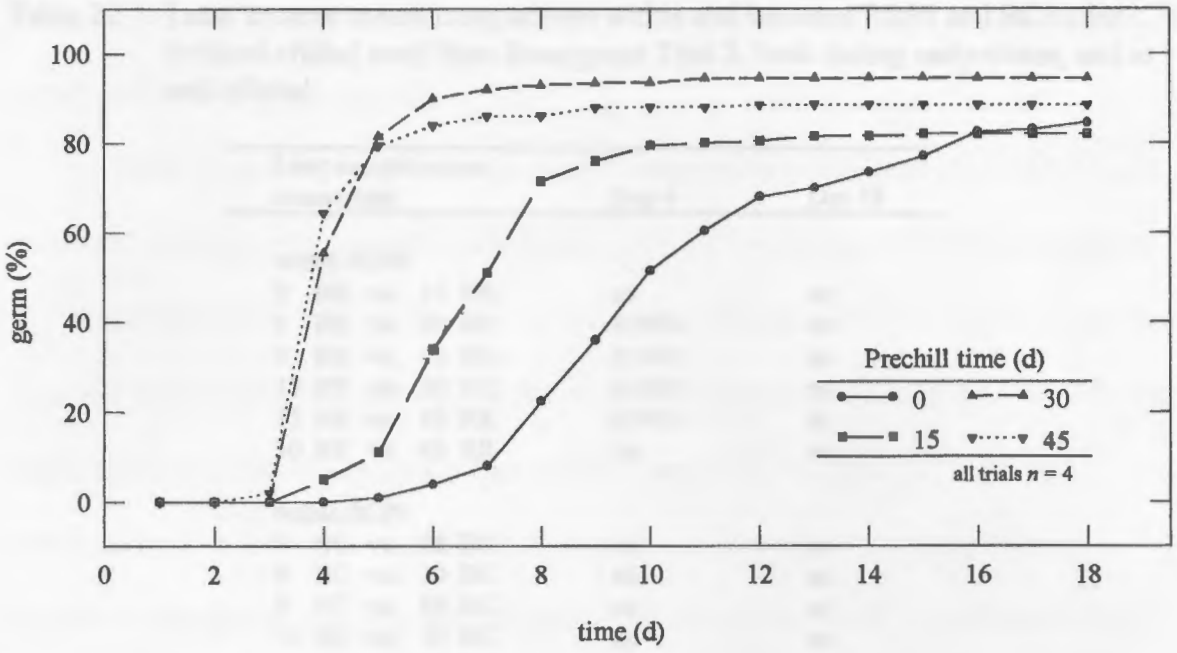


Figure 21 Average % emergence of imbibed-chilled RR99 seed from Emergence Trial 2

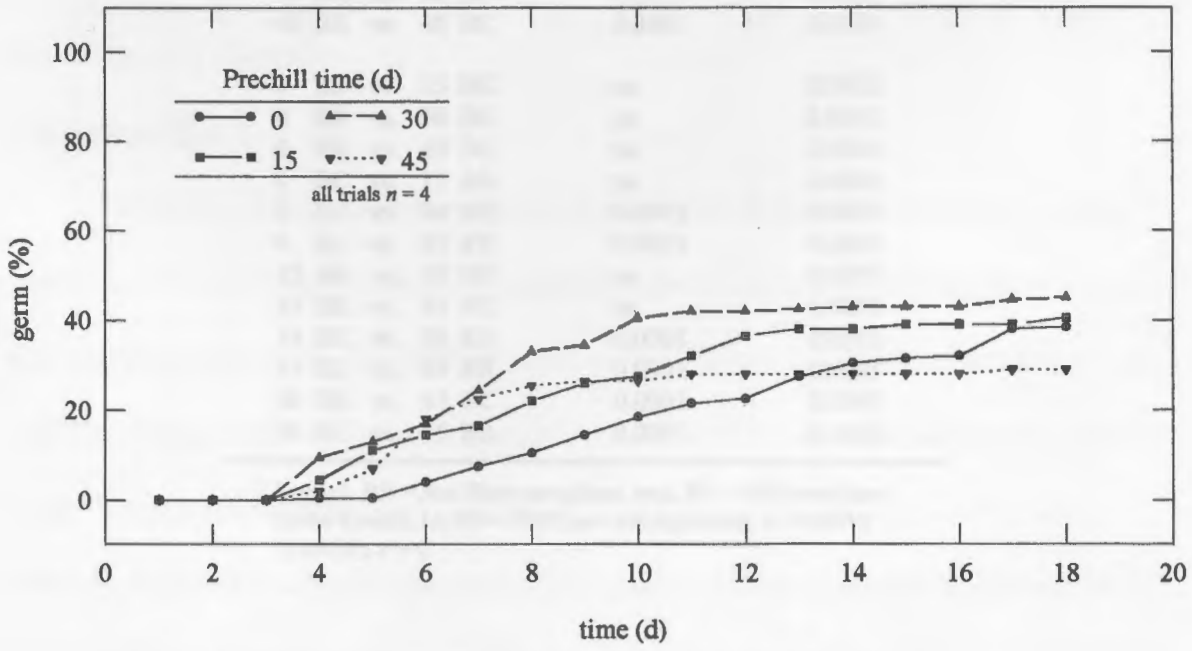


Figure 22 Average % emergence of imbibed-chilled BC99 seed from Emergence Trial 2

Table 22 Least squares means comparisons within and between RR99 and BC99 for imbibed-chilled seed from Emergence Trial 2, both during early-phase, and at end-of-trial

Least squares means comparison	Day 4	Day 18
within RR99		
0 RR vs. 15 RR	ns	ns
0 RR vs. 30 RR	0.0001	ns
0 RR vs. 45 RR	0.0001	ns
15 RR vs. 30 RR	0.0001	ns
15 RR vs. 45 RR	0.0001	ns
30 RR vs. 45 RR	ns	ns
within BC99		
0 BC vs. 15 BC	ns	ns
0 BC vs. 30 BC	ns	ns
0 BC vs. 45 BC	ns	ns
15 BC vs. 30 BC	ns	ns
15 BC vs. 45 BC	ns	ns
30 BC vs. 45 BC	ns	ns
between RR99 AND BC99		
0 RR vs. 0 BC	ns	0.0001
15 RR vs. 15 BC	ns	0.0001
30 RR vs. 30 BC	0.0001	0.0001
45 RR vs. 45 BC	0.0001	0.0001
0 RR vs. 15 BC	ns	0.0001
0 RR vs. 30 BC	ns	0.0001
0 RR vs. 45 BC	ns	0.0001
0 BC vs. 15 RR	ns	0.0001
0 BC vs. 30 RR	0.0001	0.0001
0 BC vs. 45 RR	0.0001	0.0001
15 RR vs. 30 BC	ns	0.0001
15 RR vs. 45 BC	ns	0.0001
15 BC vs. 30 RR	0.0001	0.0001
15 BC vs. 45 RR	0.0001	0.0001
30 RR vs. 45 BC	0.0001	0.0001
30 BC vs. 45 RR	0.0001	0.0001

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; ns = not significant; $\alpha = 0.0018$ (0.05/28); $n = 4$.

45RR; 15BC vs. 30, 45RR; 30RR vs. 45BC, 30BC vs. 45RR) but all 12 comparisons were significantly different at day 18.

Table 23 and Figure 23 show ls-means comparisons within each seed stratifying treatment between each successive day (days 1-17) and the last day (day 18) (Bonferroni $\alpha = 0.0029$ (0.05/17) level of significance) for each seed collection. This test was used to estimate time to complete germination for seeds of any particular stratifying treatment and was a measure of the change in seed germination potential. In Table 23, the first nonsignificant difference in each column of *seed* by *strat* comparisons is underlined. Complete emergence for 0 day imbibed-chilled RR99 and BC99 seed was reached after 15 or 13 days, respectively, and successively longer stratifying times diminished caryopsis dormancy. A 45 day stratifying treatment for either seed collection produced a statistically complete emergence in 5 or 6 days. Figure 23 is used to more clearly show the results of Table 23.

Emergence Trial 3

RR99 and BC99 seed was stratified by the imbibed-freezing technique, but few seedlings emerged. Figures 24 and 25 present respective graphs of average % emergence for each seed collection. The ordinate scale on both figures was adjusted to show detail. The best-fitting mixed model heterogeneous AR(1) was chosen to further examine these data. No interaction was observed between fixed factors, though *time* (elapsed time within a trial) and *seed* (seed collection) were observed to be separately significant (at $P < 0.05$ level of significance). Two ls-means comparisons were made.

Table 23 Using least squares means differences between days 1-17 and 18 to estimate time to complete emergence for imbibed-chilled seed from Emergence Trial 2

	RR99 *strat*				BC99 *strat*			
Day	RR0	RR15	RR30	RR45	BC0	BC15	BC30	BC45
1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
4	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
5	0.0001	0.0001	0.0144	0.0895	0.0001	0.0001	0.0001	0.0001
6	0.0001	0.0001	0.3863	0.3863	0.0001	0.0001	0.0001	0.0346
7	0.0001	0.0001	0.6226	0.6226	0.0001	0.0001	0.0001	0.2010
8	0.0001	0.0344	0.7619	0.6135	0.0001	0.0002	0.0157	0.4796
9	0.0001	0.2119	0.8350	0.9171	0.0001	0.0027	0.0292	0.6027
10	0.0001	0.5894	0.8291	0.9171	0.0001	0.0052	0.3315	0.5894
11	0.0001	0.6520	1.0000	0.9140	0.0001	0.0558	0.4988	0.8216
12	0.0001	0.7213	1.0000	0.9102	0.0002	0.3416	0.4756	0.8120
13	0.0003	0.8988	1.0000	1.0000	0.0054	0.5250	0.5250	0.7992
14	0.0024	0.8897	1.0000	1.0000	0.0269	0.4881	0.5791	0.7815
15	0.0195	1.0000	1.0000	1.0000	0.0292	0.6394	0.5322	0.7548
16	0.4560	1.0000	1.0000	1.0000	0.0157	0.5760	0.4560	0.7093
17	0.4411	1.0000	1.0000	1.0000	0.7973	0.4411	0.7973	1.0000

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; *strat* = seed stratifying time; $\alpha = 0.0029$ (0.05/17); $n = 4$.

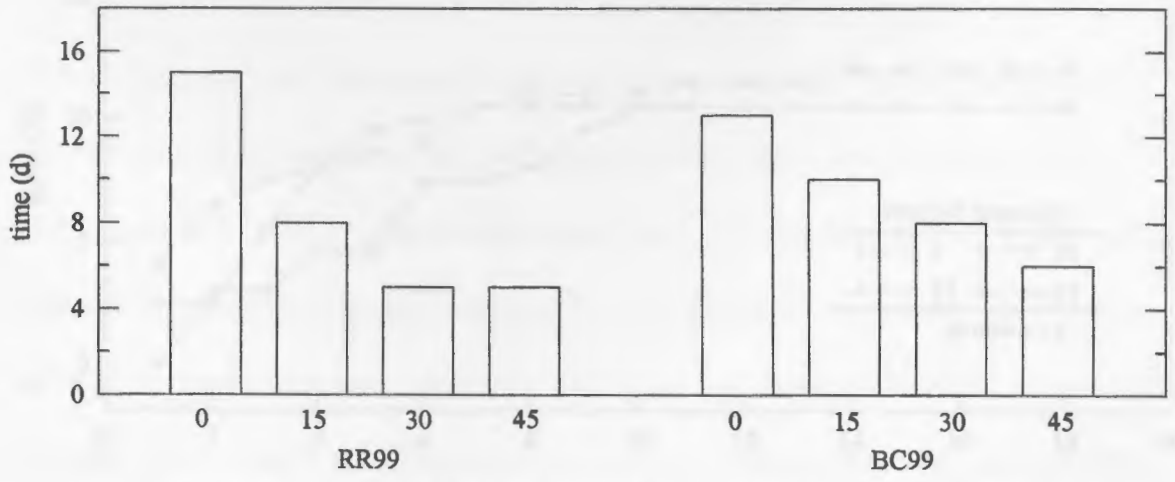


Figure 23 Conversion of Table 23 into a bar graph; Time to complete emergence for imbibed-chilled seed from Emergence Trial 2

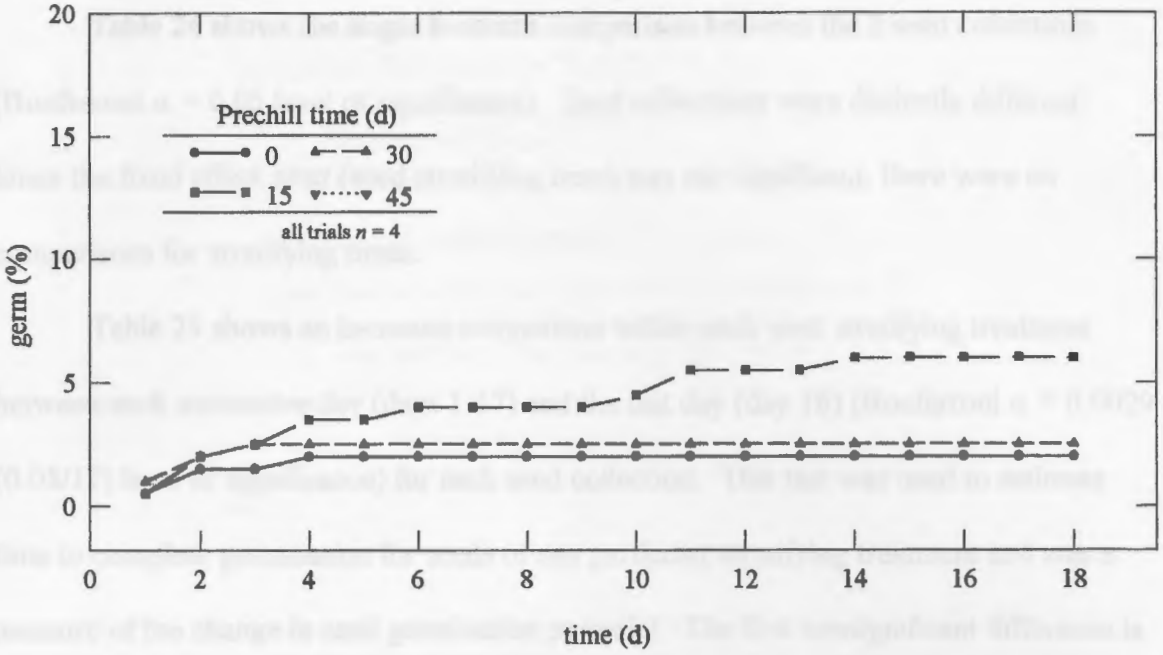


Figure 24 Average % emergence of imbibed-frozen RR99 seed from Emergence Trial 2

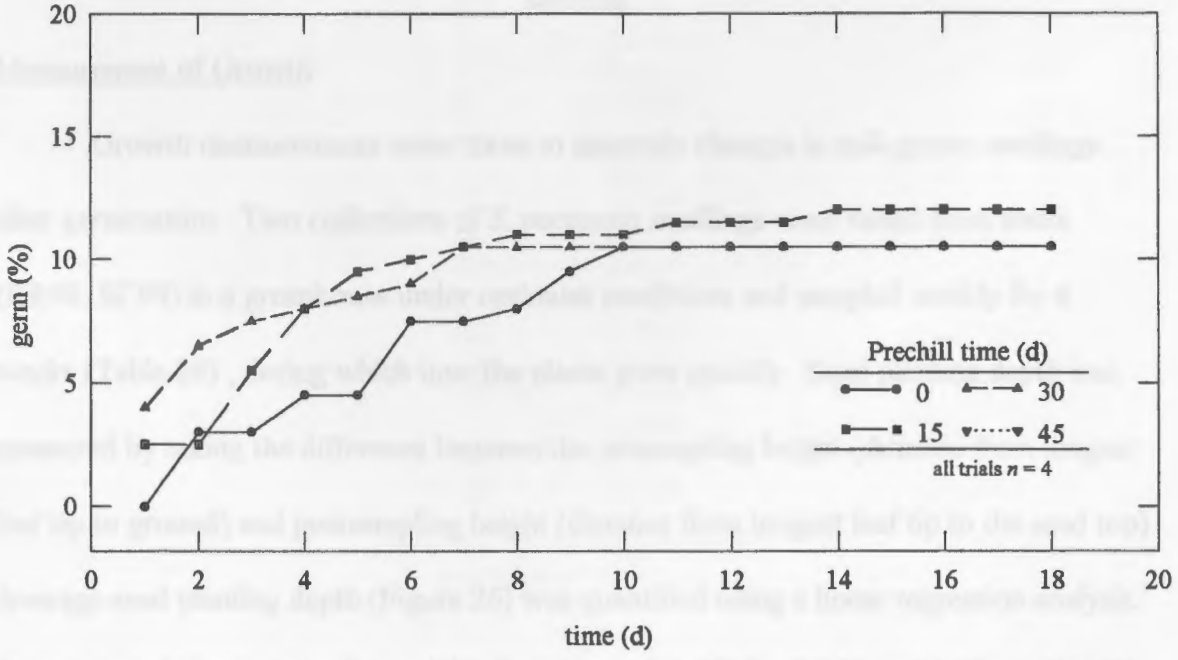


Figure 25 Average % emergence of imbibed-frozen BC99 seed from Emergence Trial 2

Table 24 shows the single ls-means comparison between the 2 seed collections (Bonferroni $\alpha = 0.05$ level of significance). Seed collections were distinctly different. Since the fixed effect *strat* (seed stratifying time) was not significant, there were no comparisons for stratifying times.

Table 25 shows an ls-means comparison within each seed stratifying treatment between each successive day (days 1-17) and the last day (day 18) (Bonferroni $\alpha = 0.0029$ (0.05/17) level of significance) for each seed collection. This test was used to estimate time to complete germination for seeds of any particular stratifying treatment and was a measure of the change in seed germination potential. The first nonsignificant difference is underlined. Complete emergence for both seed collections of imbibed-frozen RR99 and BC99 seed was reached after 4 days.

Growth

Measurement of Growth

Growth measurements were taken to ascertain changes in soil-grown seedlings after germination. Two collections of *S. pectinata* seedlings were raised from seeds (RR99, SC99) in a greenhouse under optimum conditions and sampled weekly for 6 weeks (Table 26) , during which time the plants grew quickly. Seed planting depth was measured by taking the difference between the presampling height (distance from longest leaf tip to ground) and postsampling height (distance from longest leaf tip to the seed top). Average seed planting depth (Figure 26) was quantified using a linear regression analysis. Average seed planting depth was initially 0.69 cm (±0.09) for RR98 and 0.62 cm (±0.11) for SC98. Average seed depth decreased throughout the sampling period.

Table 24 Least squares means difference between the imbibed-frozen seed collections in Emergence Trial 3

Least squares means comparison	difference
between RR99 & BC99	0.0305

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 99 = 1999; α = 0.05,
$n = 4$.

Table 25 Using least squares means differences between days 1-17 and 18 to estimate time to complete emergence for imbibed-frozen seed from Emergence Trial 3

Day	Seed
1	0.0001
2	0.0001
3	0.0003
4	0.0077
5	0.0168
6	0.1046
7	0.1967
8	0.2540
9	0.3839
10	0.5656
11	0.8068
12	0.7927
13	0.7745
14	1.0000
15	1.0000
16	1.0000
17	1.0000

$\alpha = 0.0029$ (0.05/17); $n = 4$.

Table 26 Growth measurements of *S. pectinata* seedlings, weeks 1-6

Measurement	Time (wk)	RR98 $\bar{x}$	RR98 ($S_{\bar{x}}$)	SC98 $\bar{x}$	SC98 ($S_{\bar{x}}$)
Presampling height (cm) (leaf tip to soil)	1	2.31	(0.16)	2.65	(0.30)
	2	4.98	(0.87)	5.92	(0.83)
	3	8.23	(0.76)	9.15	(0.81)
	4	8.88	(1.04)	9.77	(1.16)
	5	7.74	(1.16)	10.70	(1.09)
	6	9.74	(2.05)	11.71	(1.76)
Postsampling height (cm) (leaf tip to seed top)	1	3.00	(0.16)	3.27	(0.34)
	2	5.61	(0.90)	6.59	(0.85)
	3	8.72	(0.75)	9.26	(0.73)
	4	9.43	(1.02)	10.35	(1.20)
	5	8.13	(1.13)	11.07	(1.09)
	6	10.27	(2.02)	12.26	(1.75)
Seed planting depth (cm)	1	0.69	(0.09)	0.62	(0.11)
	2	0.63	(0.05)	0.67	(0.10)
	3	0.49	(0.08)	0.73	(0.09)
	4	0.55	(0.07)	0.58	(0.08)
	5	0.39	(0.06)	0.37	(0.11)
	6	0.53	(0.06)	0.55	(0.06)
Leaves	1	1.10	(0.10)	1.10	(0.10)
	2	1.70	(0.15)	2.00	(0.00)
	3	2.60	(0.16)	2.70	(0.15)
	4	3.10	(0.10)	2.90	(0.10)
	5	3.70	(0.30)	3.70	(0.15)
	6	4.10	(0.18)	4.00	(0.21)
Main root branches	1	1.20	(0.20)	1.00	(0.15)
	2	1.50	(0.27)	1.90	(0.23)
	3	0.30	(0.15)	3.10	(0.31)
	4	3.70	(0.21)	3.30	(0.52)
	5	3.80	(0.29)	4.10	(0.28)
	6	3.90	(0.31)	4.80	(0.49)

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error; n = 10.

(table continues)

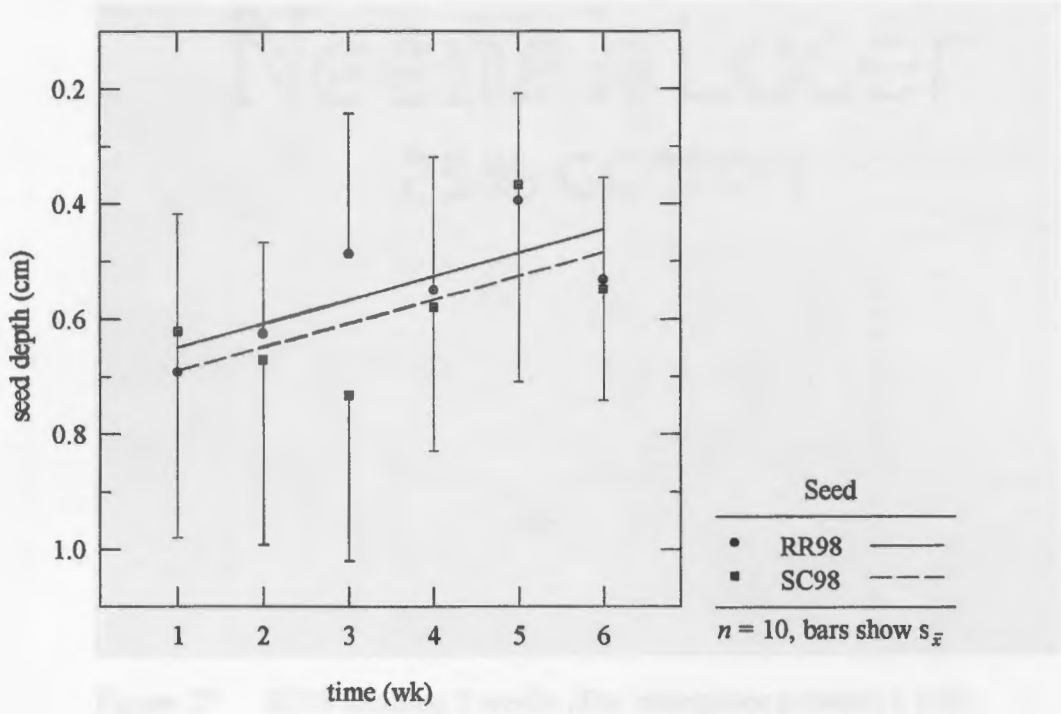
Table 26 Continued

Measurement	Time (wk)	RR98 $\bar{x}$	RR98 ($S_{\bar{x}}$)	SC98 $\bar{x}$	SC98 ($S_{\bar{x}}$)
IKI test result	1	0.00	(0.00)	0.00	(0.00)
	2	0.70	(0.15)	0.50	(0.17)
	3	0.60	(0.16)	0.40	(0.16)
	4	0.40	(0.16)	0.40	(0.16)
	5	0.00	(0.00)	0.00	(0.00)
	6	0.00	(0.00)	0.00	(0.00)
Root reticulation	1	1.20	(0.20)	1.00	(0.15)
	2	6.90	(1.69)	8.90	(1.43)
	3	17.10	(2.21)	19.60	(3.30)
	4	31.30	(5.09)	32.20	(8.86)
	5	25.70	(6.82)	49.50	(12.40)
	6	70.00	(26.46)	49.30	(20.30)
Total shoot length (cm)	1	3.29	(0.36)	3.61	(0.50)
	2	8.23	(1.51)	10.88	(1.40)
	3	15.78	(1.59)	19.04	(1.78)
	4	20.83	(2.22)	21.40	(2.41)
	5	20.53	(2.74)	27.35	(3.45)
	6	30.40	(5.89)	33.32	(4.71)
Total root length (cm)	1	1.69	(0.33)	1.67	(0.31)
	2	9.56	(2.59)	11.64	(2.43)
	3	34.15	(4.84)	32.74	(6.38)
	4	65.25	(9.88)	65.76	(18.56)
	5	68.41	(15.39)	107.43	(23.29)
	6	169.11	(52.42)	139.40	(39.40)

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998; $\bar{x}$ = mean, $S_{\bar{x}}$ = standard error; n = 10.

Actual planting depth ranged from 0 cm on the soil surface, to 1.36 cm below ground. The well-developed mesocotyl (Figure 27) is an example of seedling growth response to burying.

The seedlings produced leaves and major roots (Figure 28)(seminal and adventitious combined, higher order root branches excluded) in a steady progression.



Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998; $n = 10$.

Figure 26 Average seed planting depth

By week 6, RR98 seedlings averaged 4.1 leaves (±0.3) and 3.9 main roots (±0.2); SC98 seedlings averaged 4 leaves (±0.2) and 4.8 main roots (±0.5).

The depletion of seed starch is presented graphically (Figure 29), and pictorially (Figures 30 to 32). Endosperm starch was observed by staining it with iodine-potassium iodide (IKI). Starch reserves steadily declined from week 1 and were exhausted by week 5.

Root reticulation (Figure 33) steadily increased until week 4 or 5, then fell slightly. Average root reticulation for RR98 jumped from 25.7 branches (±6.8) at week 5 to 70

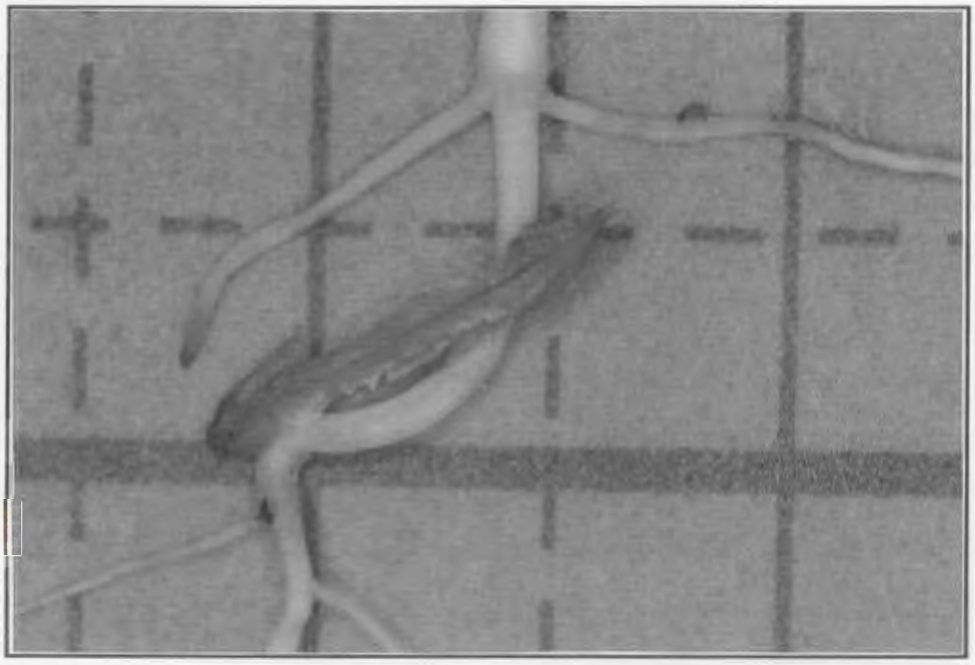


Figure 27 SC98 seedling 2 weeks after emergence presents a well-developed mesocotyl (10x)

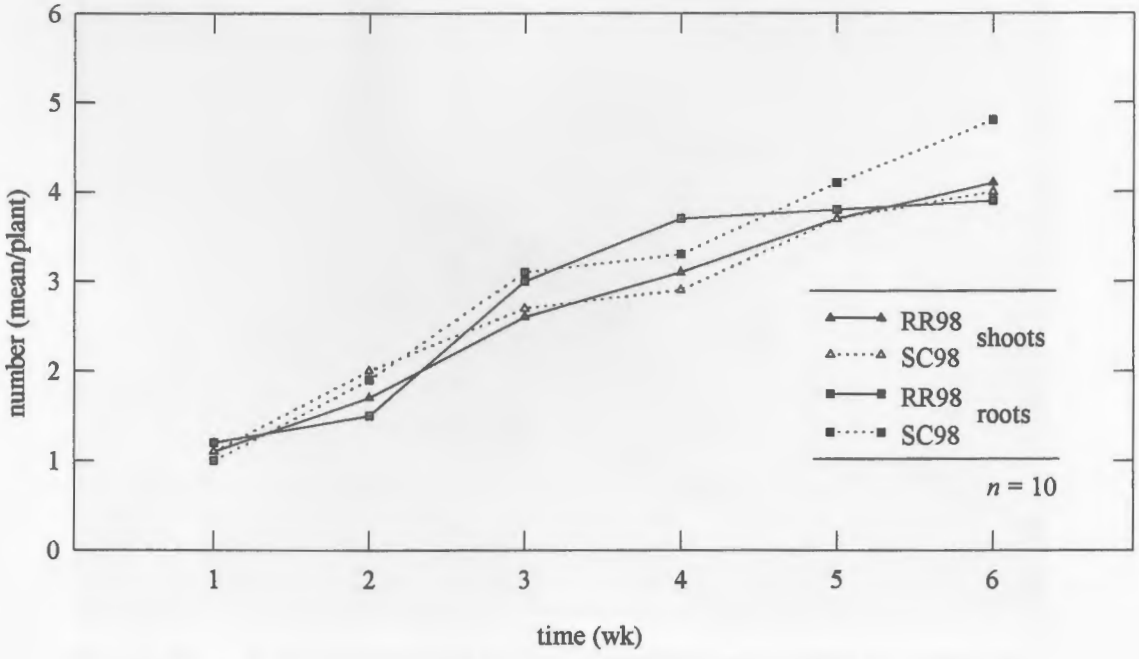


Figure 28 Production of leaves and major roots (includes seminal and adventitious root systems; no reticulation)

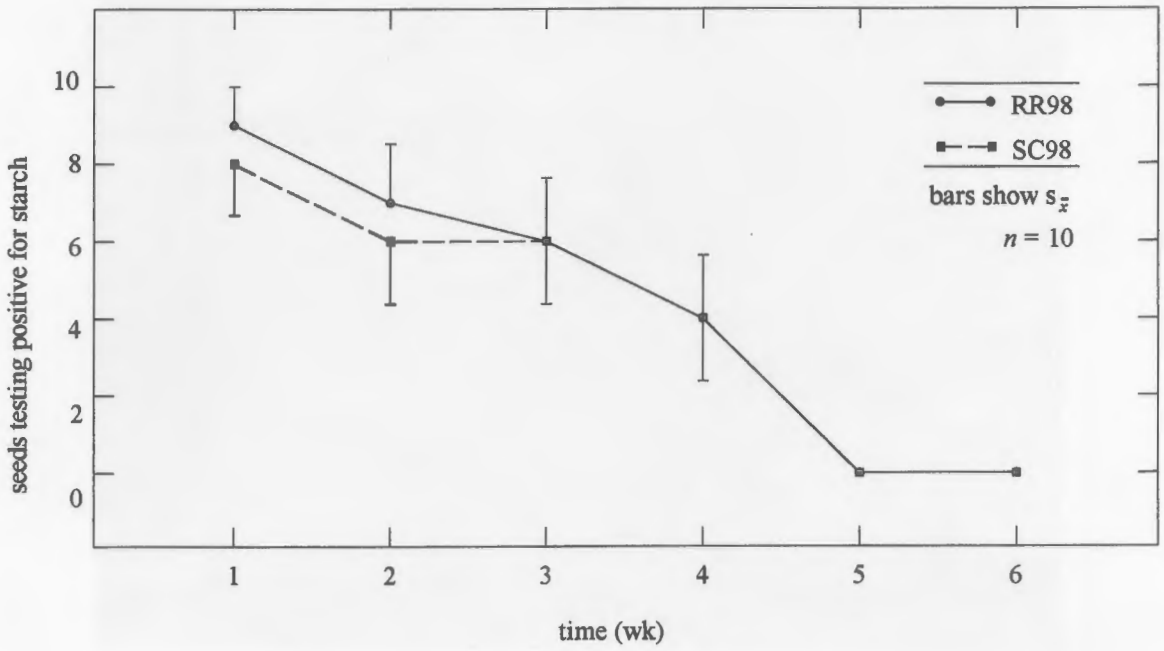


Figure 29 Endosperm consumption by developing seedling

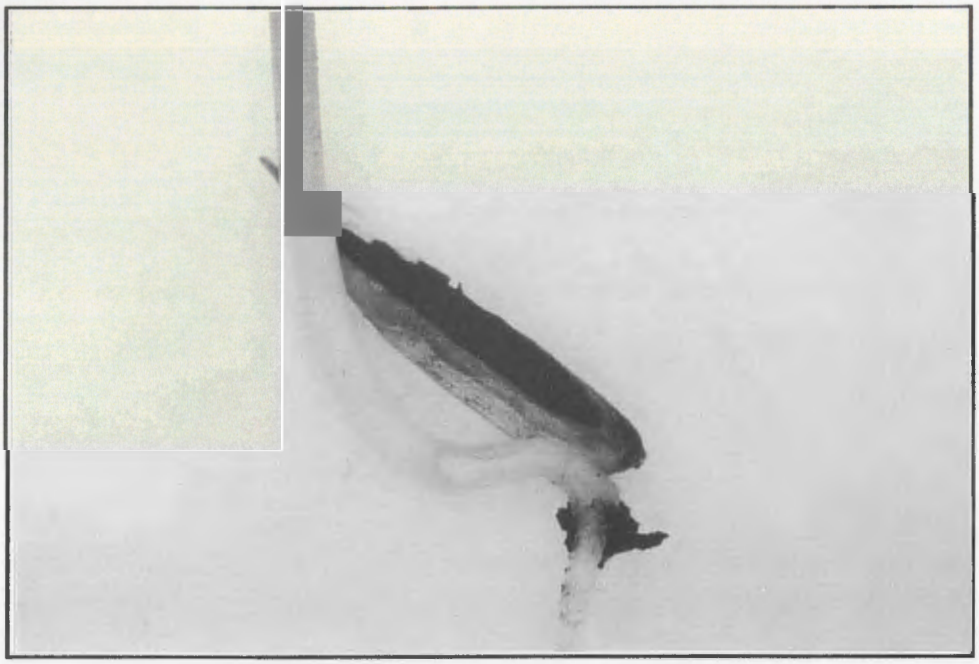


Figure 30 A SC98 caryopsis at day 0 (seedling emergence from the soil); the seed coat has been removed and the starch-rich endosperm visualized with IKI. (10x)

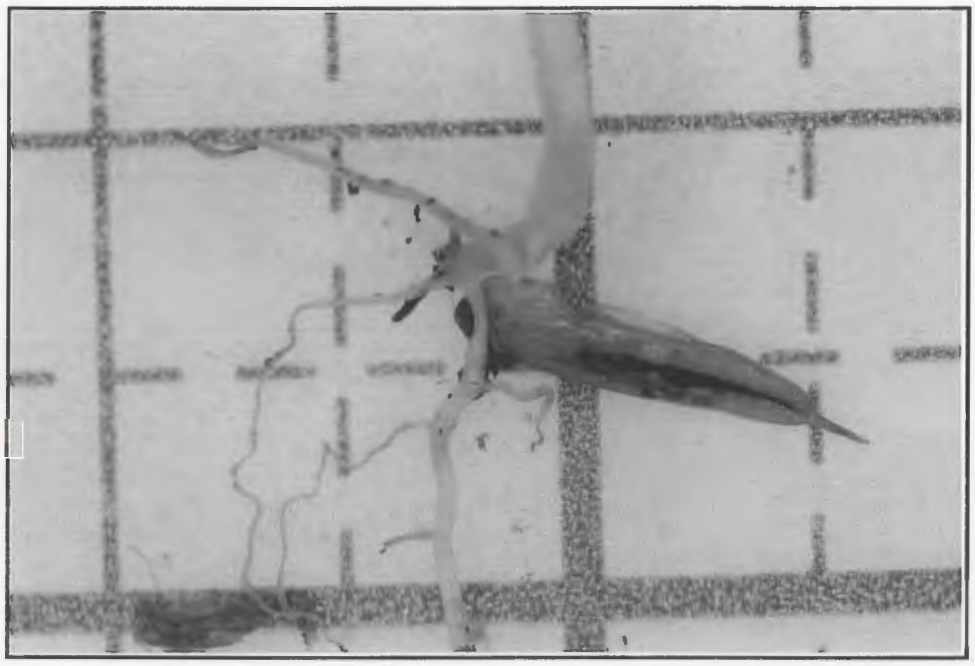


Figure 31 The partly depleted endosperm of a 2 week-old SC98 seedlings (10x)

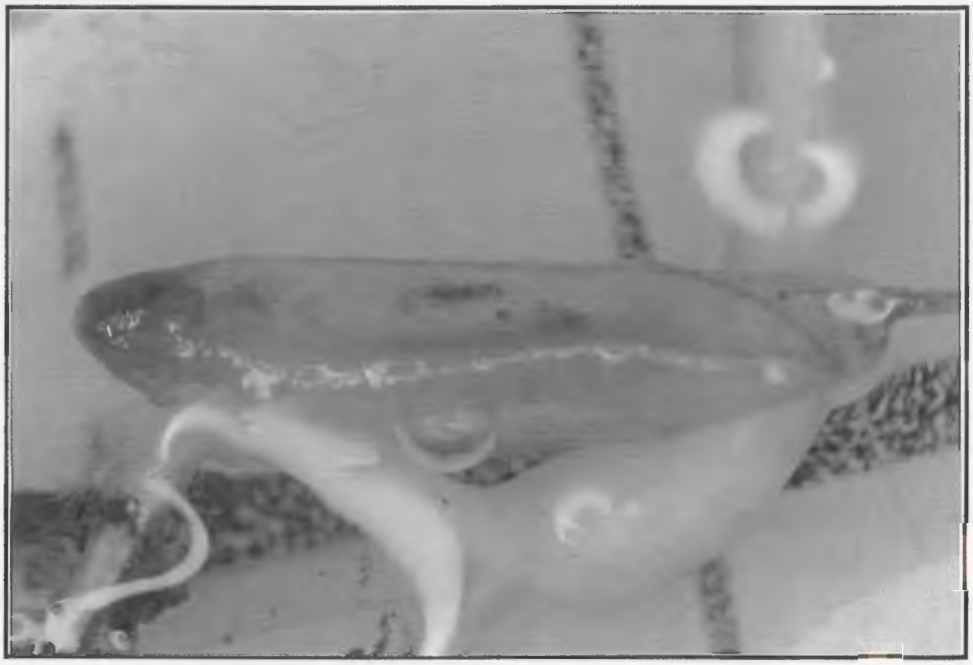


Figure 32 The mostly depleted endosperm of a 3 week-old SC98 seedling; note weak IKI staining (circled) (25x)

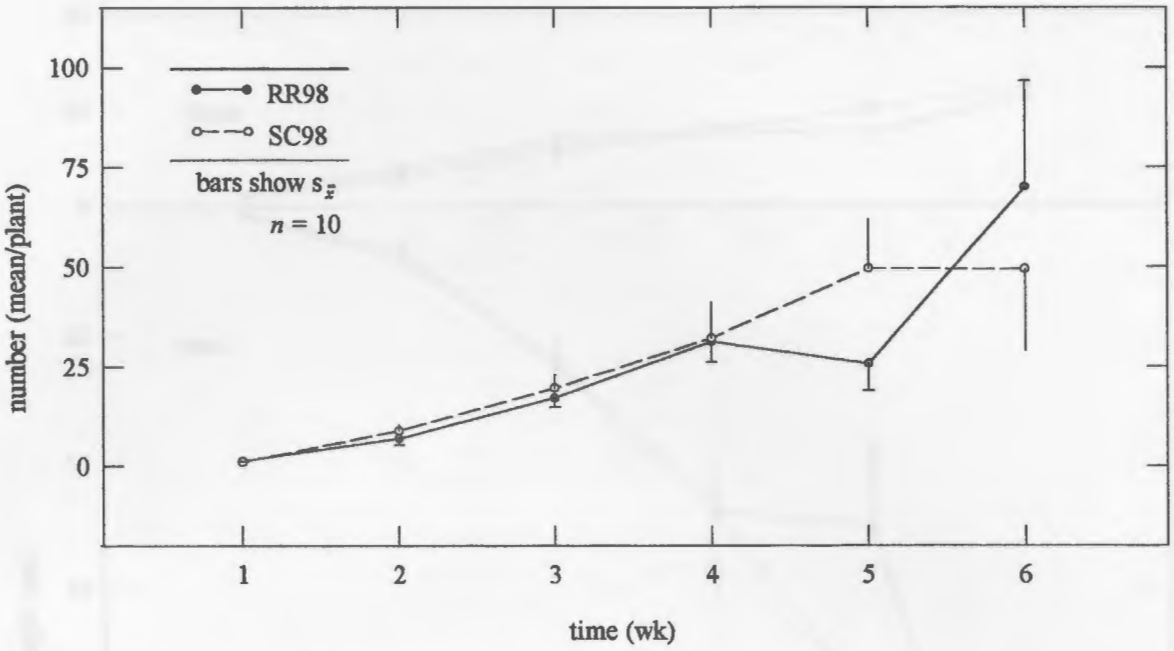


Figure 33 Root reticulation

branches (±26.5) at week 6, while SC98 declined slightly during the same period from 49.5 branches (±12.4) to 49.3 branches (±20.3).

Total root length exceeded total leaf length (Figure 34) by 4 to 5.5x. On week 6, total leaf length for RR98 seedlings was 30.4 cm (±5.9), and for SC98 seedlings was 33.3 cm (±4.7). Also on week 6, RR98 seedlings were found to have an average total root length of 169.1 cm (±52.4), and SC98 seedlings an average total root length of 139.4 cm (±39.4). There were 5 plants with a total root length greater than 200 cm, and 1 each greater than 300, 400, and 500 cm, respectively. Table 27 presents individual seedling having a total root length greater than 200 cm.

Initial assessment of covariance structure using the AR(1) heterogeneous model as described in the sections for Germination and Emergence (2-4 parameters) (not shown)

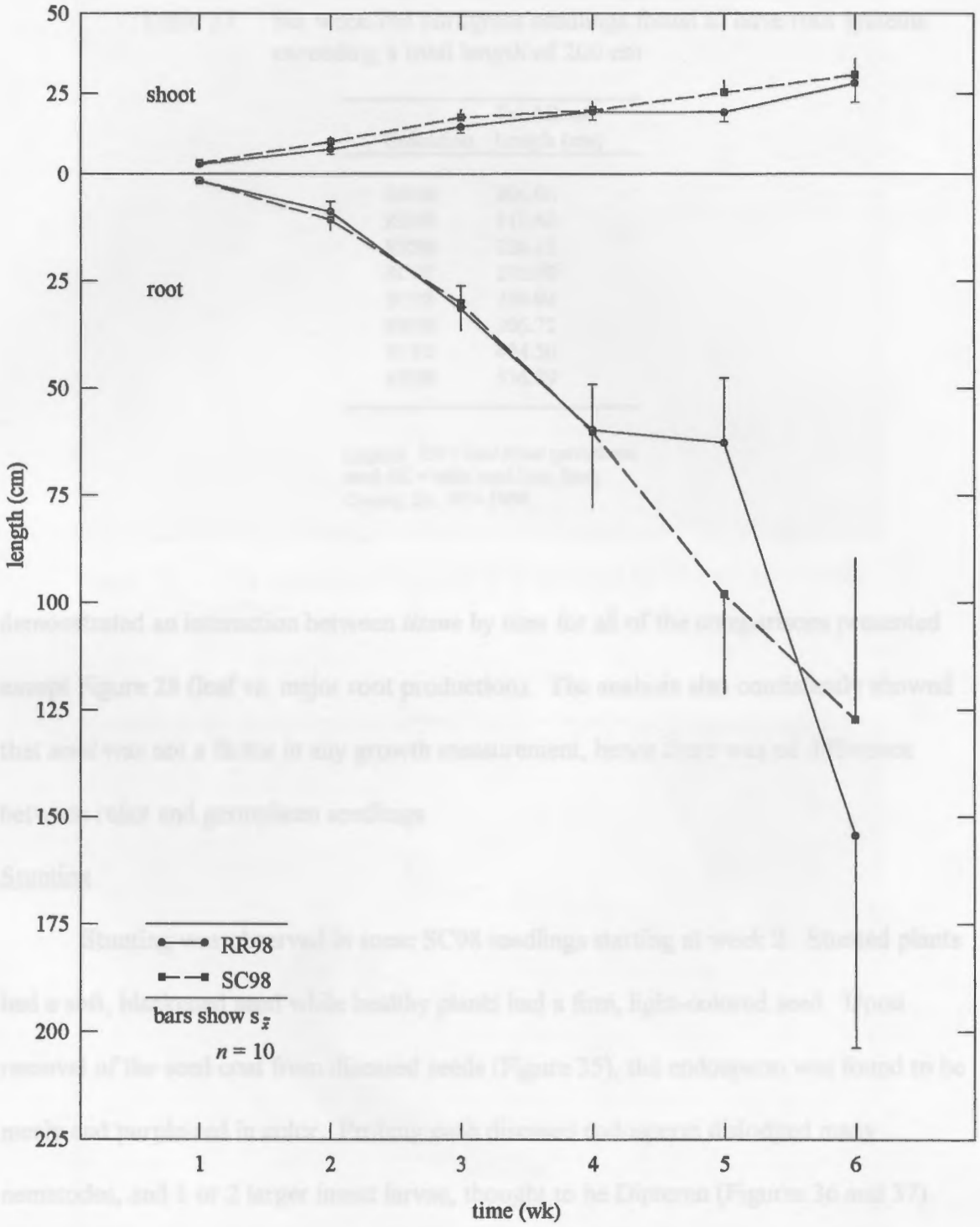


Figure 34 Total shoot and root length

Table 27 Six week-old cordgrass seedlings found to have root systems exceeding a total length of 200 cm

Collection	Total Root Length (cm)
RR98	206.06
RR98	212.88
RR98	226.12
SC98	232.09
SC98	280.04
RR98	306.72
SC98	424.50
RR98	536.89

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998.

demonstrated an interaction between *tissue* by *time* for all of the comparisons presented except Figure 28 (leaf vs. major root production). The analysis also consistently showed that *seed* was not a factor in any growth measurement, hence there was no difference between relict and germplasm seedlings.

Stunting

Stunting was observed in some SC98 seedlings starting at week 2. Stunted plants had a soft, blackened seed while healthy plants had a firm, light-colored seed. Upon removal of the seed coat from diseased seeds (Figure 35), the endosperm was found to be mealy and purple-red in color. Probing each diseased endosperm dislodged many nematodes, and 1 or 2 larger insect larvae, thought to be Dipteran (Figures 36 and 37). These insect larvae were only found in seedlings from weeks 2 to 4. Adult insects were never observed, despite growing stunted plants for several months. In attempt to grow

Figure 35 The digested endosperm of a diseased SC98 seed at 2 weeks (10x)

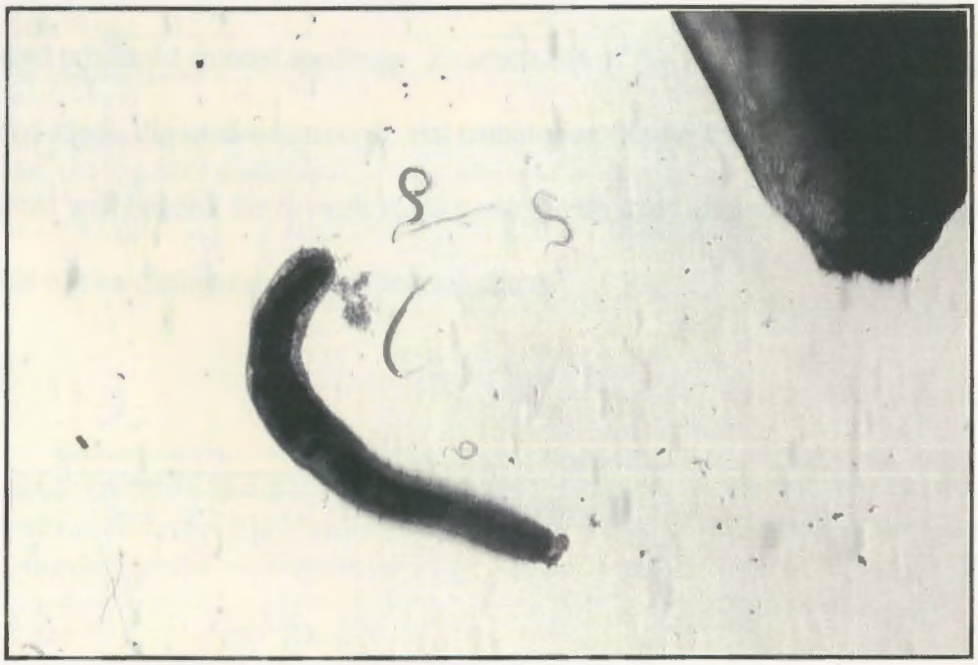


Figure 36 Contracted larvae, nematodes, and the tip of the host SC98 seed from a stunted SC98 seedling, 3 weeks (400x)

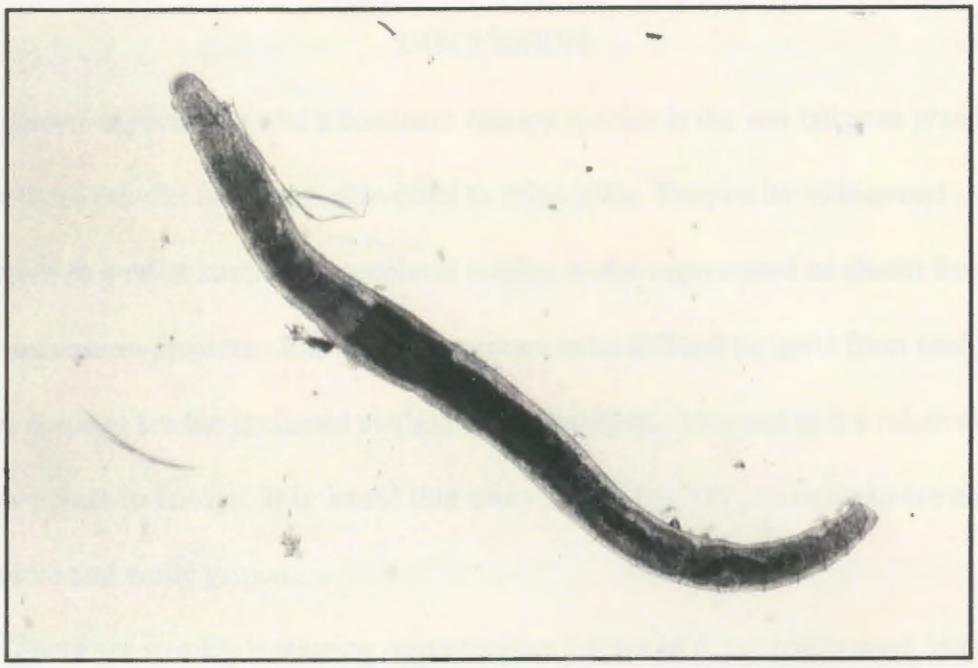


Figure 37 The larvae extended (400x)

more stunted seedlings, the experiment was repeated 6 months later. Once again, some SC98 seed produced stunted seedlings. Examination of the stunted seedlings revealed blackened seeds, digested endosperm, and nematodes, but no larger maggots. The stunted plants lived well beyond the 6-week study period, ultimately attained vigorous growth, and could not be distinguished from normal plants.

DISCUSSION

Spartina pectinata was a dominant canopy species in the wet tallgrass prairie, but most of these prairies have been converted to other uses. Despite its widespread distribution as a relict species, *S. pectinata* is often under represented or absent from prairie restoration projects. The plant has proven to be difficult to grow from seed, and plugs or cuttings are the preferred method of propagation. This makes it a relatively expensive plant to handle. It is ironic that many associated wet prairie forbs are already well known and easily grown.

There are steadily increasing opportunities for use of *S. pectinata* seed, but commercial supplies are limited. Anticipating a market, the USDA/NRCS has identified uses for *S. pectinata* seed in prairie restorations, soil conservation projects, and landscaping (Tober *et al.* 1998). Most importantly, the ability to grow this native, warm-season grass from seed would considerably advance the goal of achieving appropriate species composition when planting lowland prairies. Some nurseries have indicated a successful germination of the seed after stratification. Verification of stratification in improving *S. pectinata* seed germination could provide valuable information for prairie restoration. This study was designed to determine the effects of stratification on seed germination. Of lesser emphasis were emergence and early growth trials, which were conducted to observe seedling behavior after germination under ideal soil conditions.

The project was expedited by the availability of germplasm and relict seed. The USDA-NRCS has developed a germplasm for *S. pectinata*, and the Iowa Ecotype Project

has begun foundation seed production of native *S. pectinata* ecotypes from across Iowa. Both entities provided seed for the study.

The seed of *S. pectinata* proved somewhat difficult to work with. Despite the local abundance of relict cordgrass colonies, seed set was often low. The plant flowered infrequently, usually produced only a few panicles in one location, and insect predation on spikes was often nearly complete. In addition, spikelets held their seeds tightly, and caryopses were exacting to remove. Seed was harvested in late fall, after the first hard frost. Five collections of *S. pectinata* seed were examined (Table 1): RR97, RR98, RR99, SC98, BC99.

Germination

Central to this study was the assumption that the seed of *S. pectinata* required stratification before it would germinate. Most authors who worked with the issue of seed dormancy and *S. pectinata* assumed that seed stratification was needed to break dormancy. Only Eddleman (1977) reported otherwise, and he found that dormancy in *S. pectinata* was polymorphic, with 60% of the seed tested being dormant, while the remaining 40% had no apparent dormancy.

The expansive definition of stratification (Bewley and Black 1994; Hartmann *et al.* 1997) and after-ripening (Mayer and Poljakoff-Mayber 1982; Bewley and Black 1994; Hartmann *et al.* 1997) allowed some latitude in defining nomenclature for this study. The traditional object of seed stratification is to induce after-ripening within a seed, leading to the removal of seed dormancy. Hartmann *et al.* (1997) listed 2 types of stratification: moist-chilling, a synonym for stratification; and warm-moist stratification, used for double

dormant situations prior to moist-chill. This terminology has obviously evolved haphazardly with use. Since the main objective of this study was to systematically evaluate the germination of seeds after treatment in different conditions, the definition of stratification was expanded to include any deliberately contrived condition that would induce after-ripening and remove seed dormancy. Hence the seed treatments: imbibed-chilled, imbibed-frozen, aged, and dried-warmed seeds were all considered forms of seed stratification.

It was not surprising that there was a delay between the germination of *S. pectinata* seeds in the soil and their appearance as coleoptiles above ground level. The elongating coleoptiles had to bridge the distance between buried seed and sunlight, and this took time. Therefore while experiments conducted on naked seed in a germinating chamber lasted only 14 days, emergence experiments conducted on seeds buried in soil encompassed 18 days to allow 4 additional days for emergence. In many instances, coleoptiles continued to sporadically appear weeks after the majority of seed had sprouted. This strategy allows a species to survive hostile growing conditions by staggering the appearance of seedlings. It is attributed to the polymorphic nature of dormancy, and the interaction of dormant seeds with their environment (Simpson 1990; Raven *et al.* 1992; Bewley and Black 1994).

Stratifying imbibed seed up to 45 days at temperatures just above freezing produced germination and emergence at rates faster than unstratified seed. The advantage gained by this seed stimulation lasted only a few days, and the magnitude of the stimulation was directly proportional to the stratifying time. This seed stimulating effect is

observed in the data both qualitatively (as in Figure 5) and quantitatively (as in Figure 7). In Figure 5, it can be seen that stratified seed was quick to germinate within the first few days, while unstratified seed germinated slowly. By midtrial, these gains eroded as the germination of unstratified seed caught, then slightly exceeded results from the treated seed. In Figure 7, it can be seen that the unstratified RR98 seed took 6 days to reach completion, while the 30 and 45 day stratified RR98 seed completed germination in 2 days. Table 28 presents a summary of ls-means comparisons for germination and emergence trials within the same seed collection for which imbibed-chill was the method of stratification. It shows that one-half of the early-phase comparisons found significant differences for seed stratified for different lengths of time, however none of the end-of-trial comparisons found differences. Closer examination reveals that within each seed collection, late gains made by the germination or emergence of unstratified seed closed the gap on early gains made the stratified seed, regardless of the germinating medium. Therefore any advantage gained by this seed stimulating effect is probably limited to circumstances where coordinated seed germination is important, such as for the production of even aged plants as was done for growth experiments.

Ls-means comparisons of data collected from different collections of imbibed-chilled seeds produced different outcomes. Tables 28 and 29 present a summary of ls-means comparisons between 2 seed collections (RR98 vs. SC98, and RR99 vs. BC99 respectively) for all germination and emergence trials for which imbibed-chill was the method of stratification. Table 29 shows that slightly less than one-half of the early-phase ls-means comparisons found significant differences for seed stratified for different

Table 28 A comparison of least squares means differences *within* each seed collection for imbibed-chilled seed

Seed Collection	LS-Means Comparison	Rapid Event Day Germ (day 3)	Rapid Event Day Emerg (day 4)	Last Day Germ (day 14)	Last Day Emerg (day 18)
RR98	0 vs 15	0.0001	ns	ns	ns
	0 vs 30	0.0001	0.0001	ns	ns
	0 vs 45	0.0001	0.0001	ns	ns
	15 vs 30	0.0001	0.0001	ns	ns
	15 vs 45	0.0001	0.0001	ns	ns
	30 vs 45	ns	ns	ns	ns
SC98	0 vs 15	ns	ns	ns	ns
	0 vs 30	ns	0.0001	ns	ns
	0 vs 45	0.0003	0.0001	ns	ns
	15 vs 30	ns	0.0001	ns	ns
	15 vs 45	ns	0.0001	ns	ns
	30 vs 45	ns	ns	ns	ns
RR99	0 vs 15	0.0001	ns	ns	ns
	0 vs 30	0.0001	0.0001	ns	ns
	0 vs 45	0.0001	0.0001	ns	ns
	15 vs 30	0.0001	0.0001	ns	ns
	15 vs 45	ns	0.0001	ns	ns
	30 vs 45	ns	ns	ns	ns
BC99	0 vs 15	ns	ns	ns	ns
	0 vs 30	ns	ns	ns	ns
	0 vs 45	0.0012	ns	ns	ns
	15 vs 30	ns	ns	ns	ns
	15 vs 45	0.0013	ns	ns	ns
	30 vs 45	ns	ns	ns	ns

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA, SC = relict seed from Story County, IA; 98 = 1998, 99 = 1999; ns = not significant; ls = least squares; $\alpha = 0.0018$ (0.05/28); $n = 4$.

Table 29 A comparison of least squares means differences *between* seed collections RR98 and SC98 for imbibed-chilled seed

Seed Collections	LS-Means Comparison	Rapid Event Day Germ (day 3)	Rapid Event Day Emerg (day 4)	Last Day Germ (day 14)	Last Day Emerg (day 18)
RR98, SC98	0RR vs 0SC	0.0001	ns	ns	ns
	15RR vs 15SC	ns	ns	ns	ns
	30RR vs 30SC	ns	0.0003	ns	ns
	45RR vs 45SC	ns	ns	ns	ns
	0RR vs 15SC	0.0001	ns	ns	ns
	0SC vs 15RR	ns	ns	ns	0.0002
	0RR vs 30SC	0.0001	0.0001	ns	ns
	0SC vs 30RR	0.0001	0.0001	ns	0.0003
	0RR vs 45SC	0.0001	0.0001	ns	ns
	0SC vs 45RR	0.0001	0.0001	ns	ns
	15RR vs 30SC	ns	0.0001	0.0009	ns
	15SC vs 30RR	0.0003	0.0001	ns	ns
	15SC vs 45RR	0.0002	0.0001	ns	ns
	15RR vs 45SC	ns	0.0001	0.0009	ns
	30RR vs 45SC	ns	ns	ns	ns
	30SC vs 45RR	0.0009	0.0003	ns	ns

Legend: RR = *Red River* germplasm seed, SC = relict seed from Story County, IA; 98 = 1998, 99 = 1999; ns = not significant; ls = least squares; $\alpha = 0.0018$ (0.05/28); $n = 4$.

lengths of time, however few of the end-of-trial comparisons found differences. Table 30 shows that while ls-means differences for early-phase comparisons between the 2 seed collections were found to be nearly 90%, differences for end-of-trial comparisons had increased to nearly 100%. Viability was a qualitative predictor of seed stimulation: if the viability of seed collections was similar, resulting seed stimulation was similar; if the viability of seed collections was different, resulting seed stimulation was different. These

Table 30 A comparison of least squares means differences *between* seed collections RR99 and BC99 for imbibed-chilled seed

Seed Collections	LS-Means Comparison	Rapid Event Day Germ (day 3)	Rapid Event Day Emerg (day 4)	Last Day Germ (day 14)	Last Day Emerg (day 18)
RR99, BC99	0RR vs 0BC	ns	ns	ns	0.0001
	15RR vs 15BC	0.0001	ns	0.0001	0.0001
	30RR vs 30BC	0.0001	0.0001	0.0001	0.0001
	45RR vs 45BC	0.0001	0.0001	0.0001	0.0001
	0RR vs 15BC	ns	ns	0.0001	0.0001
	0BC vs 15RR	0.0001	ns	0.0001	0.0001
	0RR vs 30BC	ns	ns	0.0001	0.0001
	0BC vs 30RR	0.0001	0.0001	0.0001	0.0001
	0RR vs 45BC	0.0001	ns	0.0001	0.0001
	0BC vs 45RR	0.0001	0.0001	ns	0.0001
	15RR vs 30BC	0.0001	ns	0.0001	0.0001
	15BC vs 30RR	0.0001	0.0001	0.0001	0.0001
	15BC vs 45RR	0.0001	0.0001	0.0001	0.0001
	15RR vs 45BC	0.0001	ns	0.0001	0.0001
	30RR vs 45BC	0.0001	0.0001	0.0001	0.0001
	30BC vs 45RR	0.0001	0.0001	0.0001	0.0001

Legend: RR = *Red River* germplasm seed, BC = relict seed from Butler County, IA; 98 = 1998, 99 = 1999; ns = not significant; ls = least squares; $\alpha = 0.0018$ (0.05/28); $n = 4$.

lengths of time, however few of the end-of-trial comparisons found differences. Table 30 shows that while ls-means differences for early-phase comparisons between the 2 seed collections were found to be nearly 90%, differences for end-of-trial comparisons had increased to nearly 100%. Viability was a qualitative predictor of seed stimulation: if the viability of seed collections was similar, resulting seed stimulation was similar; if the viability of seed collections was different, resulting seed stimulation was different. These

results underscore the difference in potential numbers of seedlings that can be produced by these different collections of *S. pectinata* seed. From the results, one would expect that the quality of the seed collected from *S. pectinata* colonies will vary seasonally by location and growing conditions.

Post-emergence damping-off was not a problem for seedlings growing in steam-sterilized soil, despite optimal growing conditions for fungi. However mildew was a problem in closed boxes that contained imbibed-chilled seed, and was often more pronounced in boxes with seed that had been stratified for longer periods of time. In prescribing the experimental design, S. Goggi (personal communication, 1998 Sept 22) cautioned against treating seed with a fungicide, warning that this could suppress any naturally occurring fungi which might otherwise help the seed break dormancy. The blotter paper inside the boxes remained relatively fungus-free, but the incidence of mildew on seed after day 15 increased markedly, and seed mortality was nearly total by day 60. Because of this problem, both 60 and 75 day imbibed-chilled stratifying treatments were eliminated from this study. Eddleman (1977) treated *S. pectinata* seed with the fungicide *Captan* [(Z)-N-(trichloro methyl) thio-4-cyclohexene-1, 2-dicarboximide (Johnson *et al.* 1998)], stratified the seed 60 days, and still achieved an 80% seed germination rate. Evidently, *Captan* did not inhibit seed germination in these experiments. The high incidence of mildew on seed suggests the possibility that it could be a factor in the mortality of *S. pectinata* seeded in the fall, when air temperatures in winter and early spring hover around freezing although extrapolation from a closed container in the lab to natural conditions is risky at best. Mid-spring seeding could avoid mildew problems since

seed could germinate immediately in warm soil. Unfortunately, optimum *S. pectinata* habitat is seasonally wet, and seed drills may bog down in mud during the spring. Planting with machinery in late winter on frozen ground, or hand seeding in the spring may be other options. Treating *S. pectinata* seed with a fungicide before fall-planting is a question that deserves further study.

Freezing imbibed RR99 and BC99 seed up to 7 days on blotter paper had no effect on their germination. The same test of seed in soil failed as neither the stratified seed nor the unstratified seed grew. There were many differences between the germination and emergence trials. Germination data was modeled with AR(1), which found a 2-way fixed factor interaction between *time* (elapsed time within a trial) and *seed* (seed collection). Emergence data was modeled with heterogeneous AR(1), which found no interaction between fixed factors, though *time* (elapsed time within a trial) and *seed* (seed collection) were separately significant. The average germination of unstratified RR99 seed was 89% (±4.7), compared to 2% (±2) for average emergence. The average germination of unstratified BC99 seed was 68% (±2.5) compared to 10.5% (±8.5) for average emergence. An ls-means difference tests for both trials found a significant difference between the 2 seed collections, but for germination, RR99 seed outperformed BC99 seed, while emergence results were reversed. Seed viability favored the performance of RR99 seed, since it had a viability of 99% (±1) for unstratified seed, compared to 68% (±2.5) for unstratified BC99 seed. Both seed collections germinated completely after 8 or 9 days, while emergence of the 2 seedling collections ended after 4 days. It is not clear if the poor emergence resulted from the seed, the experimental design, or some other factor. Other *S.*

pectinata seedlings grown in the greenhouse emerged without incident. That included seedlings from other emergence trials, seedlings produced for growth experiments, and seedlings grown much earlier in cone-tainers (Stuewe 2001). The failure of these seedlings to grow was completely unexpected, and no reason for this failure was apparent.

Germination of *Red River* germplasm seed did not improve with age, but older seed appeared to retain most of its viability. A test of seed viability suggested some decline of viability with age: RR99 seed aged 6 months had a viability of 97% (±1.9); RR98 seed aged 18 months measured 92% (±1.4); and RR97 seed aged 30 months measured 84% (±3.2). An ls-means comparison of germination data found a difference between the 30 month-old RR97 seed and the younger RR98 and RR99 seed, but found no differences between the RR98 and RR99 seed. This test demonstrates that *S. pectinata* germplasm seed can retain viability for several years after harvest if properly stored.

Seeds of *S. pectinata* needed no special stratifying treatment to germinate. An overlooked bag of BC99 seed left to dry at room temperature for several months (termed dried-warmed stratification), germinated at the same rate as BC99 seed subjected to the more complicated dried-chilled storage. Seed from both treatments germinated completely after 9 days.

Growth

It is apparent from the literature that there has been little study of the early development of this species. For *Spartina pectinata* seeds, it was observed that the coleoptile emerged from the seed before the radicle (Figure 1). This is contrary to the sequence of the standard grass model, which was developed from corn and wheat seeds,

where the radicle is the first embryonic appendage to emerge from the seed (Langer 1979; Raven *et al.* 1992; Hartmann *et al.* 1997). It is possible that for *S. pectinata,* the pectinate grooves on the palea and lemma help anchor the spikelet in the soil while the coleoptile elongates.

Little is known about how depth affects germination of *S. pectinata* seed. The seed depth was not measured initially when it was planted. After 1 week, the average seeding depth for RR98 was measured as 0.69 cm (±0.09), and for BC98 was measured as 0.62 cm (±0.11). A few seeds, exposed by raindrop splash, germinated and set roots on the soil surface, while the maximum seeding depth was measured at 1.36 cm. The linear regression (Figure 26) shows that seed depth decreased by several seed thicknesses after 6 weeks. This may be significant for a seed the size of a rice grain. It is reasonable to assume that initially, seed was sown at a uniform depth. A nascent seedling is a dynamic organism, and forces generated by rapidly growing roots and shoots may have moved the shoot base/root crown to-and-fro underground. The soil, soft from water and pelted by raindrops, probably tended to settle and erode as time elapsed. The combination of dynamic seedling and eroding soil may account for decreased seed depth over time.

The model presented by Ries and Svejcar (1991) of a grass seedling depleting its endosperm and forming adventitious roots was generally upheld by this study, though their idealized model did not to allow for individual variation. It was difficult to discern the presence of starch in the endosperm of a caryopsis. During the first several weeks of seedling growth, starch reserves in seeds were moderately high, and stained readily with iodine-potassium iodide (Figures 29 and 30). But as those reserves were depleted, careful

staining sometimes produced only vague black patches (Figure 31) in an otherwise empty endosperm. To complicate matters, *S. pectinata* caryopses were sometimes naturally marked with black smudges. When it became impossible to discern between smudges, an endosperm was declared empty. The presence of nematodes or insect larva within the seed was also considered evidence for a positive starch test. The endosperm was apparently a food source for parasites found in the seeds of seedlings 2 to 4 weeks of age.

Similarly, despite the emphasis placed by Ries and Svejcar (1991), and Ries (1996, 1999) on the importance of adventitious root development in grass seedlings, it was difficult to distinguish whether a young root was seminal or adventitious. A microscopic examination of xylem elements may have been more revealing.

Growth of *S. pectinata* seedlings was rapid, with most growth occurring in the roots. There was little difference in growth between wild and germplasm collections. Both collections produced leaves and major roots at about the same rate, and roots quickly grew much longer than leaves. By week 5, when starch reserves were depleted, seedlings possessed 3 or 4 major leaves and roots. Root reticulation steadily increased until the time of starch depletion, and then fell slightly, although in RR98 it surged ahead by week 6. Total root and shoot length steadily increased throughout the measurement period, despite a downturn of RR98 at week 5. Growth experiments were terminated after 6 weeks because roots became too long and tangled in the cell-paks to be measured accurately. This was not unexpected. Weaver (1954) had observed mature roots to penetrate 2.5 to 4 meters almost vertically down into the soil profile. Being forced to

grow in tiny containers, the roots air-pruned, and were forced to coil and recoil back upon themselves, filling the cells in which they grew.

Stunting was observed in some SC98 seedlings. This stunting was apparently caused by nematodes and Dipteran larvae, which quickly consumed the endosperm reserves of the developing seedling. The below-ground interaction of nematodes and insects with the seed of *S. pectinata* is an intriguing question. It was a tantalizing puzzle to find that the only seed collection from a high quality prairie remnant (Doolittle Prairie State Preserve, Story County, Iowa) should produce plants that harbor seed parasites. The question remains, are these parasitizing species widespread generalists, common to agricultural crops, or specialists restricted to prairie remnants and *S. pectinata* in particular? More study of this phenomena is needed.

SUMMARY AND CONCLUSION

Control (unstratified) seed ranged in average viability from 66.5% (±7) to 99% (±1), in average germination from 59.5% (±7) to 94% (±2.2), and in average emergence from 38.5% (±3.8) to 84.5% (±6.8). Stratification did not improve these values. The imbibed-chilled stratification (1°C for 15 to 45 d) reduced germination time by a maximum average of 4 d (±0.4), and soil emergence time by a maximum average of 8.3 d (±0.6). Longer stratification periods increased the germination percentage in the early phase, this increase was also reflected in coleoptile emergence from the soil. In spite of the early phase increases, there was no difference in germination and emergence from the controls at the end of the trials.

Stratifying seed by imbibed-freezing, dried-warming, or aging up to 1.5 years had no effect on its germination, though control seed germinated significantly better than aged 2.5-year old seed. Germination varied in seed collections from different sites. Post-emergence damping-off was not observed for any seed collection, but mildew became a problem for seed stratified by imbibed-chill beyond 45 days. The imbibed-frozen emergence trial failed due to unknown causes for both treated and control seed.

During the first 6 weeks for relict and germplasm plants, the total length of leaves and roots steadily increased. The majority of growth was below ground. Average seed depth decreased during early growth, rising several seed thicknesses from an average planting depth of 0.62 cm (±0.11) to 0.69 cm (±0.09). Endosperm starch levels in 2 seed collections steadily decreased through week 4 and were depleted by week 5. Six week-

old seedlings had an average total shoot length of 30.40 cm (±5.89) to 33.32 cm (±4.71) and an average total root length of 139.40 (±39.40) cm to 169.11 cm (±52.42).

It can definitely be concluded from this study that *Spartina pectinata* should be included in prairie restoration projects. If viable seed is used, it will germinate readily with or without stratification.

OTHER FACTORS

Perhaps other factors were involved in the failure of *S. pectinata* to grow in restorations and production plots. A partial list is provided below, and these might be considered for further study:

1) Improper seed assessment. Any fall harvest of *S. pectinata* should include a caryopsis count and tetrazolium test to ascertain the fill rate and viability of seed. Most spikelets produced by *S. pectinata* appeared normal at first glance, but closer inspection revealed that the fill rate was often low. In addition, insect damage can be easily overlooked. The piercing, sucking mouthparts of insects produced tiny holes in the spikelets, and seeds so marked were usually dry and crumbled.
2) Aggressive seed cleaning. Mechanical brushing has shown a tendency to damage caryopses, which may produce deformed seedlings that do not grow properly. By contrast, a uniform air blowing procedure can quickly, gently separate filled seed from partly filled and empty seed without injury to the caryopses.
3) Spring drought. This species is adapted to seasonally wet soil, and seedlings may be drought intolerant.
4) Cool-season plant competition. *S. pectinata* is slow to begin growth in the spring, and is shade-intolerant (Weaver 1954). Any planting dominated by cool season weeds and grasses may be too competitive for *S. pectinata* seedlings.

LITERATURE CITED

[AOSA] Assoc of Official Seed Analysts. 1992. Seedling evaluation handbook: contribution number 35 to the handbook on seed testing. Lincoln, NE: AOSA. 101 p.

[AOSA] Assoc of Official Seed Analysts. 1998. Rules for Testing Seeds. Revised 1999. Lincoln, NE: AOSA. 126 p.

Baker B. 2000. Basic principles of statistical analysis. Saskatoon, Saskatchewan, Canada: Department of Plant Science, University of Saskatchewan. <http://duke.usask.ca/~rbaker/stats.html>. Accessed 2000 Aug 13.

Bewley JD, Black M. 1994. Seeds: physiology of development and germination. 2nd ed. New York: Plenum. 445 p.

Bingham E, Halvorson C, editors. 2000. The old farmer's almanac: www.almanac.com. Dublin, NH: Yankee. <http://www.almanac.com/garden/garden.frostcanada.html>. Accessed 2000 Oct 2.

Britton NL, Brown A. 1913. An illustrated flora of the northern United States and Canada: from Newfoundland to the parallel of the southern boundary of Virginia, and from the Atlantic Ocean westward to the 102d meridian. 2nd ed. Volume 1, Ferns to Buckwheat. New York: Dover. 680 p.

Broome SW, Woodhouse WW, Seneca ED. (Departments of Soil Science and Botany, North Carolina State University, Raleigh, NC). 1973 Jul. An investigation of propagation and the mineral nutrition of *Spartina alterniflora*. Raleigh, NC: Department of Commerce (US), National Oceanic and Atmospheric Administration, Office of Sea Grant; Department of Defense (US), Army Corps of Engineers, Costal Engineering Research Center; North Carolina Costal Research Program. Sea Grant Publication nr UNC-SG-73-14. Grants nr 2-35178 and 04-3-158-40. 121 p.

Brown LB. 1979. Grasses: an identification guide. Boston, MA: Houghton Mifflin. 240 p.

Buchanan BB, Gruissem W, Jones RL, editors. 2000. Biochemistry and molecular biology of plants. Waldorf, MD: Am Soc of Plant Physiologists. 1408 p.

Budy JA, Evans RA, Young JA. 1986. Technical paper number 42: understanding seed handling for germination. Arlington, VA: Volunteers in Technical Assistance. 9 p.

Campbell CS, Quinn JA, Cheplick GP, Bell TJ. 1983. Cleistogamy in grasses. Annu Rev of Ecology and Systematics 14:411-441.

Chapman GP. 1996. The biology of grasses. United Kingdom: CAB Int. 273 p.

Christensen CM. 1972. Microflora and seed deterioration. In: Roberts EH, editor. Viability of seeds. Great Britain: Syracuse Univ Pr. p 59-93.

Christiansen P, Müller M. 1999. An illustrated guide to Iowa prairie plants. Iowa City, IA: Univ of Iowa Pr. 237 p.

Cobb GW. 1998. Introduction to design and analysis of experiments. New York: Springer. 795 p.

Crist A, Glenn-Lewin DC. 1978. The structure of community and environmental gradients in a northern Iowa prairie. In: Glenn-Lewin DC, Landers RQ Jr, editors. Proceedings of the 5th Midwest prairie conference; 1976 Aug 22-24; Ames, Iowa. Ames IA: Iowa State University. p 57-64.

Currier PJ, Davis CB, van der Valk AG. 1978. A vegetation analysis of a wetland prairie marsh in northern Iowa. In: Glenn-Lewin DC, Landers RQ Jr, editors. Proceedings of the 5th Midwest prairie conference; 1976 Aug 22-24; Ames, Iowa. Ames IA: Iowa State University. p 65-9.

Eddleman LE (Montana Forest and Conservation Experiment Station, School of Forestry, University of Montana, Missoula, MT) 1977 Nov. Indigenous plants of southeastern Montana. Part 1, Viability and suitability for reclamation in the Fort Union Basin. Missoula, MT: Department of Agriculture (US), Forest Service, Intermountain Forest and Range Experiment Station; Energy Research and Development Administration (US); MFCES. Special publication nr 4. Agreement nr 12-11-204-1, supplement nr 56, amendment nr 1; Contract nr EY-76-S-06-2232, task agreement nr 2. 232 p.

Eddleman LE, Meinhardt PL. 1981. Seed viability and seedling vigor in selected prairie plants. In: Stuckey RL, Reese KJ, editors. Proceedings of the 6th North American prairie conference: The prairie peninsula--in the "shadow" of Transeau; 1978 Aug 12-17; Columbus, OH. Columbus, OH: College of Biological Sciences, Ohio State University. p 213-7. (Ohio Biological Survey, Biological Notes Number 15).

Ehley AM. 1989 Aug. Integrated roadside vegetation management [brochure]. Cedar Falls, IA: Iowa Roadside Management Program, Center for Energy and Environmental Education, University of Northern Iowa.

Eilers LJ, Roosa DM. 1994. The vascular plants of Iowa: an annotated checklist and natural history. Iowa City, IA: Univ of Iowa Pr. 304 p.

Ellis RH, Hong TD, Roberts EH. 1985. Handbook of seed technology for genebanks. Volume 2, Compendium of specific germination information and test recommendations. Rome: International Board for Plant Genetic Resources. 667 p.

Emal JG, Conard EC. 1973. Seed dormancy and germination in Indian grass as affected by light, chilling, and certain chemical treatments. Agronomy J 65:383-5.

Englert JM, White RS. 1997 Oct 28. Improved conservation plant materials released by NRCS and cooperators through December 1996. Beltsville, Maryland: Department of Agriculture (US), Natural Resource Conservation Service. <http://plant-materials.nrcs.usda.gov:90/pmc/MDPMC/rel96_contents.html>. Accessed 2000 Aug 30.

Galinato MI, van der Valk AG. 1986. Seed germination traits of annuals and emergents recruited during drawdowns in the Delta Marsh, Manitoba, Canada. Aquatic Bot 26:89-102.

Gleason HAA, Cronquist A. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. 2nd ed. New York, NY: New York Botanical Garden. 910 p.

Gould FW, Shaw RB. 1983. Grass systematics. 2nd ed. College Station, TX: Texas A&M Univ Pr. 397 p.

Hartmann HT, Kester DE, Davies FT, Geneve RL. 1997. Plant propagation: principles and practices. Upper Saddle River, NJ: Prentice Hall. 770 p.

Holder MD. 1990. The sap-feeding guild of prairie cordgrass, *Spartina pectinata* [thesis]. Warrensburg, (MO): Central Missouri State University. 37 p.

Holder MW, Wilson SW. 1992. Life history and descriptions of the immature stages of the planthopper *Prokelisia crocea* (Van Duzee) (Homoptera: Delphacidae). J of New York Entomol Soc 100(3):491-7.

Holm RE, Miller MR. 1972. Hormonal control of weed seed germination. Weed Sci 20:209-12.

Johnson SR, Knapp AK. 1996. Impact of *Ischnodemus falicus* (Hemiptera: Lygaeidae) on photosynthesis and production of *Spartina pectinata* wetlands. Environ Entomol 25(5):1122-7.

Johnson WE, Kroll RB, Pait AS, Plimmer JR. 1998 Jan 28. Captan. In: Gardner MA, coordinator. Data base of the occurrence and distribution of pesticides in Chesapeake Bay. Beltsville, MD: Department of Agriculture (US), Information Systems Division, Agricultural Network Information Center, National Agricultural Library. <http://www.agnic.nal.usda.gov/cbp/pest/captan.html>. Accessed 2000 Sept 19.

Koss WJ, Owenby JR, Steurer PM, Ezell DS. 1988. Climatography of the United States: number 20, supplement number 1: freeze/frost data. Ashville, NC: Department of Commerce (US), National Oceanic and Atmospheric Administration, National Climatic Data Center. <http://www.victoryseeds.com/frost/>. Accessed 2000 Oct 2.

Kromray E. 2000. Development of experimental equipment to address the unique problems of harvesting and planting mix tallgrass prairie seed [abstract]. In: Schutte C, Zoellner C, editors. Proceedings of the 17th North American prairie conference; 2000, Jul 16-20; North Iowa Area Community College, Mason City, IA. Mason City, IA: North Iowa Area Community College. Abstract M&R (session 2).

Kucera CL. 1961. The grasses of Missouri. Columbia, MO: Univ of Missouri Pr. 305 p.

Ladd D, Oberle F. 1995. Tallgrass prairie wildflowers: a Falcon field guide. Helena, MT: Nature Conservancy and Falcon Pr. 262 p.

Langer RHM. 1979. Institute of biology's studies in biology: number 34: how grasses grow. 2nd ed. London, England: Edward Arnold. 66 p.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD. 1996. SAS systems for mixed models. Cary, NC: SAS Institute. 656 p.

Long SP, Potter L, Bingham MJ, Stirling CM. 1990. An analysis of limitations to the production of C4 perennials as ligno-cellulosic biomass crops, with reference to trials in eastern England. In: Grassi G, Gosse G, dos Santos G, editors. Volume 1, Biomass for energy and industry. London, England: Elsevier Applied Science. p 235-41.

Madson J. 1982. Where the sky began: land of the tallgrass prairie. Boston, MA: Houghton Mifflin. 319 p.

Mayer AM, Poljakoff-Mayber A. 1982. The Germination of seeds. 3rd ed. Elmsford, NY: Permagmon Pr. 211 p.

McCarthy, P. 2000 July 24. Complete sun and moon data for one day. Washington: Naval Observatory (US), Astronomical Applications Department. <http://riemann.usno.navy.mil/AA/data/docs/RS_OneDay.html#formb>. Accessed 2000 Oct 8.

McGregor RL, Barkley TM, and the Great Plains Flora Association. 1986. Flora of the Great Plains. Lawrence, KS: Univ Pr of Kansas. 1392 p.

McLean RA, Sanders WL, Stroup WW. 1991. A unified approach to mixed linear models. Am Statistician 45:54-65.

Mobberley, D. 1956. Taxonomy and distribution of the genus *Spartina*. Iowa State Coll J of Sci 30:471-574.

Morgan JP. 1997. Plowing and seeding. In: Packard S, Mutel CF, editors. The tallgrass restoration handbook: for prairies, savannas, and woodlands. Washington: Island Pr. p 193-215.

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. 1996. Applied linear statistical models. 4th ed. Irwin, Chicago: Irwin. 720 p.

Nicholson JWG, Langille JE. 1965. The comparative nutritional value of *Spartina pectinata* and timothy at several stages of growth. Can J of Animal Sci 45:157-163.

Owen DD. 1852. Report of a geological survey of district of Wisconsin, Iowa, and Minnesota: and incidentally a portion of the Nebraska territory: made under instructions from the United States Treasury Department. Philadelphia: Lippincott and Grambo. 638 p.

Packard S, Mutel CF, editors. 1997. In: The tallgrass restoration handbook: for prairies, savannas, and woodlands. Washington: Island Pr. p xii-xvii.

Pammel LH, Ball CR, Lamson-Scribner F (Iowa Geological Survey). 1904. The grasses of Iowa. Part 2, A descriptive and geographical study of the grasses of Iowa. Des Moines, IA: FR Conaway, Iowa State Printer. 136 p.

Pohl, RW. 1966. The grasses of Iowa. Iowa State J of Sci 40(4):341-566.

Proctor M, Yeo P, Lack A. 1996. The natural history of pollination. Portland, OR: Timber Pr. 479 p.

Raven PH, Evert RF, Eichhorn SE. 1992. Biology of plants. 5th ed. New York: Worth. 791 p.

Ries RE. 1996. Predicting grass stand adequacy from early stand parameters. In: Daniels WL, Burger JA, Zipper CE, editors. Proceedings of the 13th annual meeting of the American Society for Surface Mining and Reclamation: Successes and failures: Applying research results to insure reclamation success; 1996 May 18-23; Knoxville, TN. Blacksburg, VA: Virginia Tech Research Division, Powell River Project. p 462-71.

Ries RE. 1999. Early grass seedling growth stage improves explanation of future stand success. In: Bengson SA, Bland DM, editors. Proceedings of the 16th national meeting of the American Society for Surface Mining and Reclamation; Mining and reclamation for the next millennium; 1999 Aug 13-19; Scottsdale, AZ. Lexington, KY: ASSMR. p 66-71.

Ries RE, Svejcar TJ. 1991. The grass seedling,: when is it established? J of Range Management 44:574-6.

Ritchie SW, Hanway JJ, Benson GO. 1992. Special report number 48: how a corn plant develops. Ames, IA: Iowa State University of Science and Technology, Cooperative Extension Service. <http://www.ag.iastate.edu/departments/agronomy/corngrows.html>. Accessed 2000 Aug 13.

Roberts EH, editor. 1972. Introduction. In: Viability of seeds. Great Britain: Syracuse Univ Pr, Chapman and Hall. p 1-13.

Runkle ST, Roosa DM. 1989. Wildflowers of the tallgrass prairie: the upper Midwest. Ames, IA: Iowa State Univ Pr. 279 p.

Santos GB. 1968. Sterility in *Spartina pectinata* [thesis]. Ames (IA): Iowa State University. 40 p.

Shipley B, Peters RH. 1990. A test of the Tilman model of plant strategies,: relative growth rate and biomass partitioning. American Naturalist 136:139-53.

Shipley B, Parent M. 1991. Germination responses of 64 wetland species in relation to seed size, minimum time to reproduction and seedling relative growth rate. Functional Ecology 5(1):111-8.

Shirley S. 1994. Restoring the tallgrass prairie: an illustrated manual for Iowa and the upper Midwest. Iowa City, IA: Univ of Iowa Pr. 330 p.

[SI] SAS Institute. 1999. The mixed procedure. In: SAS/STAT user's guide: SAS online-doc, version 8. Cary, NC. <http://sasdocs.ats.ucla.edu/stat/chap41/index.htm>. Accessed 2000 November 17.

Silveus WA. 1933. Texas grasses: Classification and description of grasses, descriptive systematic agrostology. San Antonio, TX: Silveus WA and Clegg 782 p.

Simpson GM. 1990. Seed dormancy in grasses. New York: Cambridge Univ Pr. 297 p.

Smith RS Jr, Smith S, editors. 1997. Native grass seed production manual (including selected forbs). Winnipeg, Manitoba, Canada: A cooperative publication of the Plant Materials Program, United States Department of Agriculture, Natural Resources Conservation Service, Ducks Unlimited Canada, Manitoba Forage Seed Assoc and the University of Manitoba. Ducks Unlimited, Canada. 155 p.

Spector PE. 1993. SAS Programming for Researchers and Social Scientists. Newbury Park, CA: Sage. 167 p.

Stuewe E. 2001. Ray Leach cone-tainers. Corvallis, OR: Stuewe and Sons. <http://www.stuewe.com/contact.html>. Accessed 2001 Feb 28.

Taiz L, Zeiger E. 1991. Plant physiology. Redwood City, CA: Benjamin/Cummings. 565 p.

Thomson A, Dennis J, Trotter D, Shaykewich D, Banfieldet R. 2000. Damping-off: diseases and insects in British Columbia forest seedling nurseries. [place unknown]: Forest Health Network, Canadian Forest Service, Natural Resources Canada. <http://www.pfc.forestry.ca/nursery/pests/damping_f.htm>. Accessed 2000 Sept 1.

Tober DA, Jensen NK, Knudson MJ, Haas RJ. 1998. The United States Department of Agriculture - Natural Resources Conservation Service, Minnesota Agricultural Experiment Station, North Dakota Agricultural Experiment Station, and South Dakota Agricultural Experiment Station announce the release of selected class Red River "natural" germplasm prairie cordgrass. Bismarck, ND: USDA-NRCS Plant Materials Center. Release announcement, 1998 Sept. 6 p.

[USDA-NRCS] Department of Agriculture (US) - Natural Resources Conservation Service, Bismarck, ND Plant Materials Center. 1992-3. Initial evaluation planting: technical report. Part 1, Grasses, forbs, and legumes. [Bismarck, ND: USDA-NRCS Plant Materials Center]. Initial evaluation of prairie cordgrass (*Spartina pectinata*). Project nr 38I021W. p 45-9.

[USDA-NRCS] Department of Agriculture (US) - Natural Resources Conservation Service, Plant Materials Program. 1997 Dec 22. Fact sheet for prairie cordgrass. Washington: USDA-NRCS. <http://plant-materials.nrcs.usda.gov/pmc/grasses/sppe.html>. Accessed 2000 Aug 30.

[USDI-NPS-JENA] Department of Interior (US), National Park Service, Jefferson National Expansion Memorial, Museum of Westward Expansion. 2000 Sept 1. The sod house. St. Louis, MO: USDI-NPS-JENA. <http://www.nps.gov/jeff/mowe-sodhouse.htm>. Accessed 2000 Oct 1.

Weaver JE. 1954. North American prairie. Lincoln, NE: Johnsen. 348 p.

Weaver JE. 1960. Flood plain vegetation of the central Missouri Valley and contacts of woodland with prairie. Ecological Monogr 30(1):37-64.

Weaver JE. 1991. Prairie plants and their environment. 2nd printing. Lincoln, NE: Univ of Nebraska Pr. 277 p.

Weaver JE, Zink E. 1945. Extent and longevity of the seminal roots of certain grasses. Plant Physiol 20:359-79.

Wilson GL. 1917. Buffalo Bird Woman's garden: Agriculture of the Hidatsa Indians as told to Gilbert L. Wilson. St. Paul, MN: Minnesota Historical Soc Pr. 142 p.

Wilson SW, Smith JL, Calvert PD. 1993. Planthoppers of a Missouri tallgrass prairie (Homoptera: Fulgoroidea). J Kansas Entomol Soc 66(1): 75-80.

Wynia RL. 1994. *Spartina pectinata* Link. In: Everett HW, compiler. Species guide for wetland plantings in the southeast United States. Fort Worth, TX: Department of Agriculture (US), Soil Conservation Service, South National Technical Center. p 1-7.

APPENDIX 1
PLANT MATERIAL CLASSES

The USDA-NRCS lists 5 classes of plant materials. These are arranged from least to most domesticated:

Source identified (*ecotype, relict*) seeds or plants are those which were randomly collected, or agronomically grown, from a naturally occurring population occupying a known or defined geographic area.

Selected seeds or plants are those which show some desirable trait, but which have not been tested to discover if that trait is stable over multiple generations.

Tested seeds or plants have been tested for a specific trait beyond 1 generation at multiple sites with replicated plots to verify performance and heritability of that trait, but whose area of adaptation is unknown.

Germplasm seeds or plants are shown to possess 1 or more heritability stable, potentially desirable characteristics which may be of value for plant breeding, but which are still under study for some reason and not ready for commercial use.

Cultivated variety (*cultivar*) seeds or plants have documented, heritability stable, desirable characteristics of superior performance with a known range of adaptation and are available for commercial use.

after Englert and White (1997)

APPENDIX 2
RECIPE FOR 0.8% TETRAZOLIUM CHLORIDE SOLUTION CONTAINING 0.05 MOLAR PHOSPHATE BUFFER

Prepare:

(A) Dissolve 1.36 g 0.1-M KH_2PO_4 in 100 ml distilled H_2O

(B) Dissolve 2.68 g 0.1-M Na_2HPO_4 in 100 ml distilled H_2O

Combine:

20 ml (A)
80 ml (B)
100 ml distilled H_2O
+ 1.6 g tetrazolium chloride (fresh, kept refrigerated)

200 ml tetrazolium chloride solution in 0.05 M phosphate buffer

We found this recipe to prepare a 7.5 pH solution consistently without buffer adjustment. Refrigerate in a brown glass bottle.

APPENDIX 3
SAS SAMPLE DATA SET AND MIXED LINEAR REGRESSION MODELS 1-5

To illustrate how data was input to SAS, a partial seed germination data set is presented (Table 31). These data were collected from 8 sandwich boxes, during the 4th and 5th day of the germination trial. Sandwich boxes contained 1 of 2 seed collections, and each had been exposed to 1 of 4 different vernalizing times.

Table 31 Excerpts from a SAS seed germination data set

BOX	TIME	SEED	STRAT	GERM
1	4	0	0	7
1	5	0	0	17
5	4	0	15	44
5	5	0	15	47
9	4	0	30	47
9	5	0	30	47
13	4	0	45	37
13	5	0	45	37
18	4	1	0	5
18	5	1	0	7
21	4	1	15	8
21	5	1	15	11
25	4	1	30	11
25	5	1	30	22
29	4	1	45	27
29	5	1	45	31

Legend

BOX = sandwich box

TIME = 4th or 5th day in germination chamber

SEED = 0 *Red River* germplasm
1 Butler County relict

STRAT = seed imbibed-chilled 0, 15, 30, or 45 days

GERM = seed germinated that day

The data was input to SAS as:

```
DATA[1] TEST;
INPUT[2] BOX TIME SEED STRAT GERM;
CARDS[3];
```

where:

[1] The DATA command instructed SAS to create the file *test.*

[2] INPUT identified the variables *box, time, seed, strat, and germ* to the file *test* and defined the order of data input to follow.

[3] CARDS alerted SAS that data follows, and numbers are in the order specified by INPUT.

A generalized SAS mixed model program was written as:

```
PROC MIXED[4];
    CLASS[5] STRAT TIME SEED BOX;
    MODEL[6] GERM = STRAT|TIME|SEED /S[7];
    REPEATED TIME/TYPE[8] = AR(1) SUBJECT[9] = BOX GROUP[10] =
    STRAT;
    LSMEANS STRAT*TIME*SEED[11] / PDIFF[12];
```

where:

[4] PROC MIXED invoked the procedure.

[5] CLASS identified the variables *strat, time, seed,* and *box.*

[6] MODEL identified the dependant *x* variable as *germ.*

[7] STRAT|TIME|SEED /S directed SAS to calculate all fixed effect parameters and their 2- and 3-way interactions on the subject (effects singly or in combination).

[8] The REPEATED TIME/TYPE specified the covariance structure. Without this line, SAS defaulted to the traditional general liner regression model.

[9] The sampling unit or SUBJECT was defined as *box.*

[10] GROUP made the model heterogeneous by adding extra *strat* parameters (the model becomes much more detailed).

[11] LSMEANS directed SAS to compute least squares means (ls-means) for the fixed effect parameters.

[12] PDIFF directed SAS to compute and display ls-means differences (SI 1999).

Sample programs of mixed linear regression models 1 to 5 follow:

Model 1 Standard General Linear Regression Model

```
TITLE "Title.";
* Strat  =  days seed was stratified;
* Seed  =  0 Red River germplasm,  1  Story County relict,  2 Butler County relict;
* Box  =  sandwich box;
* Time  =  day (1 - 14);
* Germ  =  number of seeds germinated out of 50;
OPTIONS LINESIZE = 80;
DATA [file name];
INPUT STRAT  SEED BOX  TIME  GERM;
CARDS;
[data]
PROC MIXED;
        CLASS  STRAT  SEED  TIME;
        MODEL GERM  =  STRAT|TIME|SEED /S;
PROC GLM;
        CLASS  STRAT  TIME  SEED;
        MODEL GERM  =  STRAT|TIME|SEED /S;
```

Model 2 Homogeneous Compound Symmetry Model

```
TITLE "Title.";
* Strat  =  days seed was stratified;
* Seed  =  0 Red River germplasm,  1  Story County relict,  2 Butler County relict;
* Box  =  sandwich box;
* Time  =  day (1 - 14);
* Germ  =  number of seeds germinated out of 50;
OPTIONS LINESIZE = 80;
DATA [file name];
INPUT STRAT  SEED BOX  TIME  GERM;
CARDS;
[data]
PROC MIXED;
        CLASS  STRAT  TIME  BOX  SEED;
        MODEL GERM = STRAT|TIME|SEED /S;
        REPEATED TIME/TYPE = CS  SUBJECT = BOX;
```

Model 3 Homogeneous Autoregressive Order (One) Model

```
TITLE "Title.";
* Strat  =  days seed was stratified;
* Seed  =  0 Red River germplasm,  1  Story County relict,  2 Butler County relict;
* Box  =  sandwich box;
* Time  =  day (1 - 14);
* Germ  =  number of seeds germinated out of 50;
OPTIONS LINESIZE = 80;
DATA [file name];
INPUT STRAT  SEED BOX  TIME  GERM;
CARDS;
[data]
PROC MIXED;
        CLASS  STRAT  TIME  BOX  SEED;
        MODEL GERM = STRAT|TIME|SEED /S;
        REPEATED TIME/TYPE = AR(1)  SUBJECT = BOX;
```

Model 4 Heterogeneous Compound Symmetry Model

```
TITLE "Title.";
* Strat  =  days seed was stratified;
* Seed  =  0 Red River germplasm,  1  Story County relict,  2 Butler County relict;
* Box  =  sandwich box;
* Time  =  day (1 - 14);
* Germ  =  number of seeds germinated out of 50;
OPTIONS LINESIZE = 80;
DATA [file name];
INPUT STRAT  SEED BOX  TIME  GERM;
CARDS;
[data]
PROC MIXED;
        CLASS  STRAT  TIME  BOX  SEED;
        MODEL GERM = STRAT|TIME|SEED /S;
        REPEATED/TYPE = CS  SUBJECT = BOX  GROUP = STRAT;
```

Model 5 Heterogeneous Autoregressive Order (One) Model

```
TITLE "Title.";
* Strat  =  days seed was stratified;
* Seed  =  0 Red River germplasm,  1  Story County relict,  2 Butler County relict;
* Box  =  sandwich box;
* Time  =  day (1 - 14);
* Germ  =  number of seeds germinated out of 50;
OPTIONS LINESIZE = 80;
DATA [file name];
INPUT STRAT  SEED BOX  TIME  GERM;
CARDS;
[data]
PROC MIXED;
        CLASS  STRAT  TIME  BOX  SEED;
        MODEL GERM = STRAT|TIME|SEED /S;
        REPEATED/TYPE = AR(1)  SUBJECT = BOX  GROUP = STRAT;
```