

Seed Priming and Smoke Water Effects on Germination and Seed Vigor of Selected Low-Vigor  
Forage Legumes

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### ABSTRACT

A commercial solid matrix- and an osmotic-priming method were used to measure seed priming responses of birdsfoot trefoil, kura clover, and sericea lespedeza. Differences were not observed using standard germination tests, but both priming methods showed potential for increased germination rate ( $P>0.05$ ). Conflicting results for matrix and osmotic priming were found in terms of seed storage potential after priming: matrix primed seeds showed higher ( $P<0.05$ ) germination after accelerated aging; osmotic primed seeds showed lower germination ( $P<0.01$ ). Birdsfoot trefoil responses were positive, but varied by priming treatment, while kura clover showed less response to both priming treatments. In a field study comparing seedling emergence, matrix priming effects were small and these data suggest that solid matrix priming may be unlikely to improve the field establishment of either species.

Aqueous smoke solutions were also tested for effect on seed germination. Differences in final germination percent due to solution type (after exposure to liquid smoke solutions for 10- or 45-min) were not observed. Highest concentration of the 10-min solution treatment reduced ( $P<0.05$ ) birdsfoot trefoil germination. Greater germination was observed only for 'Perfect Fit' kura clover treated with low or intermediate concentrations of either solution. High concentrations of 10-min smoke water increased time to 50% germination ( $T_{50}$ ) for all seeds, but some reduction in  $T_{50}$  occurred for kura clovers treated with low (5%) solution concentrations. The 45-min treatments had little effect on germination rates. Applying aqueous smoke solution to seeds at germination did not improve germination responses of these forage legume species.

## **DEDICATION**

To Monte Anderson

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## Chapter 1: Introduction

Legumes can be an important component of pastures by supplying N, improving livestock nutrition and increasing biological and functional diversity. Despite these benefits, legume use in forage systems can be limited by the difficulty of establishing them in existing pastures. Although legumes can be established alone to avoid competition with forage grasses, this can be tedious, time consuming, and expensive if it requires added establishment inputs or comes with lower establishment-year forage yields. For both agronomic and economic success, it is imperative that high quality legumes be capable of rapid establishment when planted into existing pastures.

Low seed vigor is a very common problem in legume establishment. Low vigor results in sparse stands (i.e., low population densities) at establishment and may affect legume persistence within the stand. Poor seed vigor is a particular limitation for the use and production of legumes such as birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don), species that have important agronomic and nutritional traits.

Evaluating seed vigor is not a simple task because no one test gives a full indication of vigor. Since vigor is best determined when seeds are under a stress, tests measuring vigor are conducted under less than ideal conditions such as low or high germination temperatures, anaerobic conditions, or after seed aging. Thus, an understanding of various vigor test methods is necessary before any attempt is made towards a vigor measurement.

One potential way of improving establishment is to develop seed treatments that can increase seed vigor or germination rates. A common method employed is seed priming. Seed priming is a controlled hydration process followed by redrying that allows seeds to imbibe water and begin internal biological processes necessary for germination, but which does not allow the seed to actually germinate. The priming process gives the seed a “head-start” at germination and emergence when planted in the soil.

Another seed treatment which may increase seed vigor involves exposing seed to either aerosol or aqueous smoke. Butenolide, a compound found in smoke has been demonstrated as a cue for germination that promotes greater seed germination in comparison with untreated seeds.

The objective of this research was to test whether seed priming methods and smoke treatments would affect seed germination and vigor of three legume species in laboratory and in field (planting into existing pasture) settings. Three seed treatments, solid matrix priming, osmotic priming, and aqueous smoke, were tested in combination with birdsfoot trefoil, kura clover, and sericea lespedeza seed.

## **Chapter 2: Literature Review**

### **Benefits of Legumes in Pasture**

Legumes have the potential to increase pasture values to producers for both grazing livestock and in regards to soil-plant interrelationships. Forage legumes show potential for increasing productivity and quality of pastures in late summer as cool season grasses decline (Sheaffer et. al., 1990; Gerrish, 1991; Belesky and Wright, 1994; Lacefield et al., 1997).

Legumes in pastures support increased forage intake compared with grass monocultures (Moseley and Jones, 1979; Posler et al, 1993) and may increase animal performance (Burns and Standaert, 1985; Seo et. al., 1997). Additionally, up to 110 kg N ha<sup>-1</sup> (Matches, 1989; Burton and DeVane, 1992) may be supplied to grasses when legumes are in the sward.

More recently, interest in tannin-containing legumes has grown. Several studies have shown the potential of species such as birdsfoot trefoil and sericea lespedeza to benefit animal performance by increasing escape protein and reducing bloat (Waghorn and Jones, 1989; Nguyen, 2005). This occurs because tannins are capable of precipitating protein (Reed, 1995). Such species may also prove useful from an environmental perspective: by supporting increased N digestion efficiency in ruminants (Min et al., 2003), urinary N excretion may be reduced. In a review article by Min et al. (2003), additional benefits of tannin-containing legumes are discussed, including increased wool production in sheep grazing birdsfoot trefoil containing 22-38 g CT/kg DM, a 30% increase in milk production in dairy cattle due to CT in birdsfoot trefoil. Wang et al. (1996) demonstrated that whole milk secretion in ewes increased 21% in late lactation due to CT in birdsfoot trefoil and a 21 and 14% increase in lactose and protein content,

respectively. However, use of these species is limited in part due to their difficulty of establishment.

### **Legume Establishment**

One of the most important factors to consider when preparing to establish any plant in the field is the physical condition and performance ability of the seed. Perfect field conditions (weather, moisture, fertility, lack of competition, etc.) may exist, yet a suitable stand may never become established if poor quality seed is used. Use of high quality seed and knowledge of germination, viability, and vigor are essential for good establishment. Seed coatings and treatments may also be useful tools for supporting seed viability and seedling growth. Legume seed should be inoculated just prior to seeding to ensure the viability of the inoculum.

Several methods for introducing legumes into existing pastures are available, including reduced tillage, no-tillage, and frost seeding (Cosgrove and Collins, 2003). A fourth method, conventional seeding, may also be used. With conventional seedings soil is completely tilled and cultipacked for optimum seed to soil contact. However, this method may potentially increase soil erosion due to exposed soil surface and loose soil particles, and may be more difficult for first year grazing due to loose soil structure and limited plant root/thatch near the soil surface which may lead to potential of damage by animal traffic.

Reduced tillage utilizes some form of light soil disturbance, such as disking, field cultivating, or chiseling and disking, but should leave at least 35% of plant residue or ground cover on the soil surface (Cosgrove and Collins, 2003). No-till seeding does not turn soil over; rather, a specialized drill with a heavy, spring-loaded coulter in front of each seed drop/double

disk is used to open the soil and provide seed-to-soil contact in seed rows only, with minimum soil disturbance.

Legumes may be frost seeded into existing pastures when temperatures allow for soils to freeze at night and thaw during the day (Cosgrove and Collins, 2003). The repeated freeze-thaw cycle allows for seed to be incorporated into the surface of the soil with good seed-to-soil contact, before the spring growth period begins. Advantages to frost seeding include simple equipment needs (such as a simple broadcast seeder operated by hand or by tractor), flexibility in seeding date due to light-weight equipment, lower labor requirements, a shorter period between seeding and grazing, and limited or no soil disturbance (George 1984; Leep 1989; West and Undersander, 1997).

Successful establishment depends upon method of seeding and legume species, since variable results have been found within seeding method and legume species. Red clover (*Trifolium pratense* L.) is more easily established than alfalfa (*Medicago sativa* L.) when these legumes are frost seeded; birdsfoot trefoil response to frost seeding is variable (Cosgrove, 2001) due to low seed vigor. Generally the best results with frost seeding are obtained when seed are sown in early spring when the ground is still frozen, and grass competition is well controlled throughout the seeding year (Undersander, 1993).

George (1984) recommended frost seeding birdsfoot trefoil in combination with red clover. Under Iowa conditions, the red clover will establish rapidly and provide early legume production, with birdsfoot trefoil increasing production in the second year and beyond. A Michigan State study tested stand establishment of frost seeded red clover and birdsfoot trefoil. After seeding, the grass sod was suppressed by glyphosate or by mowing 1 to 4 times in the

establishment year. Increased mowing frequency resulted in greater stand density, with percentages of legume stand similar or slightly higher than with the glyphosate treatment (Leep, 1989).

Birdsfoot trefoil may also be established using a no-till drill, but it is important to control competition from either companion crops or existing grass stands (Undersander et al., 1993). McGraw and Nelson (2003) recommend close grazing or clipping in the fall grazing period to control existing vegetation and decrease surface residues which could pose a shade threat to young seedlings. Once seedlings have emerged, clipping companion plants is needed to help prevent excessive shading.

### **High-Potential, Low-Vigor Legumes**

*Trifolium ambiguum*: Kura clover is known for its winter hardiness, resistance to disease and insects, longevity and ability to spread by rhizomes to fill open spaces in pasture once it has been established (Taylor et al., 1998). Kura is very tolerant of growing conditions in the humid northeastern and north central regions of the United States, but appears less tolerant of conditions in the southeastern states (Taylor, et al., 1998). Kura clover is capable of rapid recovery following drought (Dear and Zorin, 1985; Woodman, 1983), most likely due to an extensive root and rhizome system (Daly and Mason, 1987).

Moore et al. (2004) reported that kura clover was more persistent than alfalfa, birdsfoot trefoil, and other legumes when compared in grazing systems: Taylor and Smith (1998) also confirmed its exceptional persistence. Kura is tolerant to grazing, probably due to the location of extensive roots, rhizomes and growing points, below the soil surface (Moorhead et al., 1980;

Scott, 1985). Its ability to persist is aided by heavy root biomass, up to 20 metric tons·ha<sup>-1</sup> and root to shoot ratios up to 4.6:1 in an established 13-yr old stand (Taylor, 1995). Compared with alfalfa, kura clover tends to have lower concentrations of neutral detergent fiber, acid detergent fiber, and acid detergent lignin, yet higher crude protein and in vitro digestibility (Allinson et al., 1985; Sequin et al., 2002).

The biggest factor limiting use of kura clover today is the difficulty of establishment (Lucas et al., 1980; Scott, 1985; Laberge et al., 2005). Kura clover displays both poor seed vigor and a slow rate of establishment of surviving seedlings. In a comparison of legume species establishment, kura clover had poorer stands than alfalfa, birdsfoot trefoil, or red clover when sod seeded (no-tilled) into pastures treated with glyphosate (0.62 kg a.i.ha<sup>-1</sup>) (Cuomo et al., 2001). However, over time it comprised more of the stand compared with alfalfa, birdsfoot trefoil, and red clover (Cuomo et al., 2003). These data match a common industry saying about kura clover: the first year it sleeps, the second year it creeps, and the third year it leaps. However, plantings often fail because “sleeping” and “creeping” growth patterns are insufficiently competitive to allow for future “leaping” when seeded into existing stands or when sown in combination with highly competitive companion species.

Kura can be successfully established in existing pastures using a no-till drill and herbicide. Moorhead et al. (1994) found acceptable emergence and stands for direct seeding into sod without herbicide application, while Woodman (1999) used glyphosate (1.08 kg a.i.ha<sup>-1</sup>) prior to seeding and achieved similar results. Gramoxone will suppress the existing grass stand for three to five wk, after which it should be mowed regularly to decrease shading and excess competition for the remainder of the summer. If glyphosate is used, it should be applied in the

fall preceding spring planting. A grass or cover crop may be seeded with kura clover, but competition must be controlled – especially from a small grain cover crop – by grazing or clipping or a combination of the two. Cuomo et al. (2001) argued that control or suppression of existing vegetation is much more important than planting method for kura clover establishment. In their studies, kura clover made up more than 31% of the whole stand if glyphosate was used to suppress the existing sod, but only 3% of the whole stand where glyphosate was not used.

Application of N to low fertility sandy soils has been shown to aid in establishment of kura clover by supporting the growth and survival of late-nodulating plants (Seguin et al., 2001). Labarge et al. (2005) reported that addition of N during establishment of sod-seeded kura clover gave inconsistent, yet non-detrimental results, while increasing total forage yield by approximately 40%.

*Lotus corniculatus*: Birdsfoot trefoil is a perennial legume, widely adapted to various soil types and regions (Kirkbride, 1999), and it can be used in areas with low-P soils (Formoso, 1993). Birdsfoot trefoil is of high value in forage mixtures because it does not cause bloat in cattle (Seaney and Henson, 1970), as well as for its positive nutritional attributes. For these reasons, birdsfoot trefoil has been recommended for use in both forage and pasture mixes (Grant and Marten, 1985).

In an intake study Wen et al., (2004) used four yearling Angus crossbred heifers with esophageal cannulas and allowed them to graze mixed swards of birdsfoot trefoil and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.). Pasture species composition was compared with diet selection (as boli collected via the cannula) for a fall and a spring grazing period. Wen

et al. (2004) found that while birdsfoot trefoil on-offer (in pasture) decreased over time by 73%, birdsfoot trefoil in the boli only decreased 22%. The authors concluded that despite the decreases as a percentage of the pasture, the value of birdsfoot trefoil is likely underestimated due to animal preference/selection for the species when its proportion in pastures is low.

Despite these positive attributes, birdsfoot trefoil is difficult to establish in pastures due to the slow growth of seedlings (Gregerson et al., 1999), especially when in competition with other faster growing seedlings such as alfalfa and red clover (Rhykerd and Mott, 1959). Birdsfoot trefoil also produces fewer and smaller seedlings compared with other forage legumes (Gist and Mott, 1957).

Early seedling growth and seed vigor are greatly affected by seed size (McKersie et al., 1981; Carleton and Cooper 1972; Henson and Tayman 1961; Stickler and Wassom 1963; Twamley 1967), thus considerable time was spent developing cultivars with larger seed size (Draper and Wilsie 1965; Twamley 1974). Despite these efforts, mixed results of establishment with large seeded cultivars have been obtained (Papadopoulos et al., 1994; Twamley et al., 1996) because many other factors besides seed size affect seed vigor (Nelson et al., 1994), such as growing conditions of seeds' mother plant, seed maturity at harvest, and storage conditions.

*Lespedeza cuneata*: Sericea lespedeza is a warm-season legume and the only perennial lespedeza of agronomic value in the United States (Cisco Seeds, 2005). Sericea is a perennial plant with multiple uses: pastures and hay, soil reclamation or control in erosion-prone areas, and is often used in wildlife areas to create a habitat for wild animals. The plants can be long-lived, dying back each year and forming new growth from the crown each spring. Sericea plants can

reach 0.6 to 1.2 m in height but the plant becomes very coarse and somewhat woody with maturity (Sollenberger and Collins, 2003). High levels of tannins in sericea lespedeza can be a major limitation for its use as a livestock feed because concentrations above 55 g CT/kg DM are sufficient to decrease intake and digestibility, and decrease body and wool growth in ruminants (Min et al., 2003). Recently, several low-tannin (below 55 g CT/kg DM) varieties have been released, including AU-Lotan, AU-Donnelly, and AU-Grazer (Mosjidis et al., 1990; Donnelly and Anthony, 1980).

### **Seed Vigor**

It is important to test the quality, vigor, and performance ability of a seed lot to know its true ability (AOSA, 1988), as sometimes the stated germination percentage on a seed tag may vary greatly from actual emergence in the field. This difference may often be attributed to seed vigor. The vigor of the seed can have consequences, positive or negative, on the emergence of the planted seed. The International Seed Testing Association (ISTA) defines seed vigor as “...the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence. Seeds which perform well are termed ‘high vigor seed,’ while those which perform poorly are called ‘low vigor seed’” (Perry, 1978). Principle known causes affecting the vigor of seeds include genetic constitution, environment and nutrition of the mother plant, stage of maturity at harvest, seed size, weight or specific gravity, mechanical integrity, deterioration, aging, and pathogens (Perry, 1978).

Seed vigor can be defined as: “Those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field

conditions” (AOSA, 2002). The underlying reason for testing seed vigor is to determine a more accurate value of a seed lot (AOSA., 2002). A vigor measurement is more than a germination test. Vigor tests should incorporate some stress factors that the seed may encounter when planted in the field. Stress factors may include excessive heat, drought, excess moisture, cold, aged seed due to prolonged storage, or lack of light. Performance aspects therefore related to seed vigor include the rate and uniformity of seed germination, field traits including extent, rate and uniformity of seedling emergence, and performance after storage and transport, particularly in regards to the retention of germination capacity.

*Measuring vigor:* Perry (1978) suggested using both direct and indirect tests to determine seed vigor. Direct vigor tests incorporate an expected environment stress that the seed may encompass in the field into a laboratory test. Indirect tests measure other characteristics of seed that are correlated to an aspect of field performance. Because no single method satisfies all requirements for understanding seed vigor, “a method or combination of methods should be chosen to suit the crop or the environment into which it will be sown” (Perry, 1978).

*Seed Vigor Tests:* The two most widely accepted vigor tests used or recommended by commercial laboratories are the conductivity test and the accelerated aging test (Hampton and TeKrony, 1995). However, other suitable vigor tests are used for research and in commercial seed testing laboratories. These other tests have not received the widespread acceptance largely due to the specialty of the species for which they have been developed and utilized. These other tests may be very useful, but research into exact procedures for various species is required. For

example, adjustment of temperature, germination time, or germination media may be needed for the test to be effective on a species. Additionally, some vigor tests, such as those that require scoring or classification of a seed, can be highly subjective and results can vary greatly between investigators within the same test.

*Accelerated Aging:* The accelerated aging test described by Delouch (1965) was initially developed to predict how a seed lot might perform after prolonged storage. It was later adopted as a vigor test (Woodstock, 1976; TeKrony and Egli, 1977). The two components of the test encompass an artificial aging period followed by a standard germination test. The artificial aging step exposes the seed to high temperatures (40-45°C) near 100% humidity for an amount of time, ranging from 48 to 96 hours, variable by the seed species. Higher vigor seeds are able to produce normal seedlings after exposure to the high temperatures and humidity. Originally, the accelerated aging test was performed in a chamber which maintained a constant prescribed temperature and humidity (Woodstock, 1976). The chamber is specialized equipment not readily available in all seed laboratories, which led to variable results across seed testing laboratories (McDonald, 1977; Tao, 1979) that did not possess the chamber. Several methodological modifications (Baskin, 1977; McDonald and Phaneendranath, 1978; Tao, 1979) have improved the reproducibility of the test (Tao, 1980a). Current methods include suspending seed on a wire mesh over a pool of water or saturated salt solutions inside a container. Care must be taken to avoid variation in the distance between seeds and the water surface, as variations in distance, water surface area and container size can affect test results (Tao, 1979, 1980a). Initial seed moisture content is another factor which causes variability in test results (McDonald, 1977b; Tao, 1979). Utilizing these methodological changes and using care to provide exact conditions

between accelerated aging containers, the accelerated aging test can and is now used widely in laboratories and can be done in a simple incubation chamber.

*Conductivity Test:* Conductivity testing measures leakage of electrolytes from tissues within the seed. The test was originally developed for measuring viability of cotton seed by Presley (1958) and later utilized as a vigor test for garden peas (Matthews and Bradnock, 1967, 1968). Cell membranes in seeds undergo a loss of integrity as they dry at maturity, but that these membranes can be repaired during imbibition (Simon and Raja Harun, 1972; Short and Lacy, 1976; Bramlage et al., 1978). The test is based on the ability of more vigorous seeds to repair cell membranes at a faster rate during imbibition than less vigorous seeds. Changes in membrane ultrastructure and permeability in aging seed can be detected by a leakage test and electron microscope (Harman and Granett, 1972; Gill and Delouche, 1973). The extent of leakage has been linked to incidence of seed rot, which has been directly related to the quantity of carbohydrates leaked from soybean, pea, and garden bean seeds (Schroth and Snyder, 1961; Matthews and Bradnock, 1968; Keeling, 1974). The conductivity test has been widely utilized for garden bean (Matthews and Bradnock, 1968) and soybean (Yaklick et al., 1979b). The Association of Official Seed Analysts referee programs have also shown that conductivity test results were highly correlated with field emergence both for corn and soybeans as well as providing uniformity of testing results within and across seed testing laboratories (Tao 1980a,b).

*Cold Test:* The cold test is one of the oldest and most widely adapted vigor tests in the United States. Seeds are exposed to cold temperatures and wet soil to simulate field planting in cold,

wet, spring-like conditions. Generally, seeds are planted in soil in plastic boxes (Clark, 1953; Svien and Isely, 1955) or on wet paper towels lined with soil (Hoppe, 1955; Crosier, 1957). The latter method is more commonly used in Europe and known as the Hoppe Test (Hoppe, 1955). Special care is made to assure each box contains the same amount of soil and the same amount of water. As a general rule, water is added to the soil to approximately 70% of its water holding capacity. Seed boxes are either sealed to prevent moisture loss, or care is taken to assure that the incubator door is not opened during the study, thus preventing large fluctuations in moisture content. Although traditionally used for corn, the cold test has been tested and documented for other seeds (McDonald, 1975), and results have been well-correlated to field performance for soybeans (Rice, 1960; Johnson and Wax, 1978; Tao, 1978b). The use of non-sterilized soil makes this test relate more closely to field performance due to the presence of soil microorganisms (Clark, 1953) which can affect seed germination (Svien and Isely, 1955; Crosier, 1957). Due to the range of differences in soils used across and within seed testing laboratories, only results of tests conducted within the same soil “lot” should be compared (McDonald, 1975). Optimum results are achieved when the soil in the test is the same in the field where the seed will be planted.

*Tetrazolium Test:* Tetrazolium testing was first developed by Lakon (1942) as a seed viability test. Upon noting a pattern of staining on the seeds, the test was later developed into a seed vigor test (Moore and Goodsell 1965, Moore 1972). The tetrazolium test is a measure of dehydrogenase enzyme activity within the seed. Dehydrogenase reacts with the tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) to form a red compound, known as formazan, which

cannot be dissolved in water. Formazan stains only the living cells. Vigor classification (high, moderate, or low) with this test is based on the amount of staining on the seed by the tetrazolium salt. Clear advantages to the test include its simplicity, rapidity, and limited equipment requirements. However, the test is highly subjective, which may make reproducibility difficult. Correlation between seed vigor and seed staining pattern was positive in oak acorns (Bonner 1974) but not with sorghum (Vanderlip et al., 1973) or cotton seeds (Metzer, 1961). To date, tetrazolium testing is commonly used to determine seed viability in a variety of agronomic and horticultural seeds, but its use specifically for determining seed vigor is less common (Peters, 2000).

*Birdsfoot Trefoil Vigor Test:* The birdsfoot trefoil vigor test was developed by Artola et al. (2003) to determine seed vigor of birdsfoot trefoil. It is similar to the cold test, a seed vigor test based on germination at cold temperatures. Since 4.7°C is considered the base temperature for birdsfoot trefoil seed germination (Hur and Nelson, 1985), seeds capable of germination at 5°C may be considered highly vigorous. For this test, seeds are germinated on filter paper moistened with distilled water, placed in plastic germination boxes that are sealed airtight and kept at a constant temperature of 5°C in complete darkness for 21 d. Seeds are evaluated every 7 d for germination, and results expressed as a percentage of germinated seeds. Because it does not rely on a soil medium, the results are more easily reproduced. Artola et al., (2003) reported that within seed lots this test was well-correlated with field emergence.

*Vacuum Test:* The vacuum test was developed to evaluate the capacity of birdsfoot trefoil seed to germinate when exposed to a stress created by anaerobic conditions (Artola et al., 2004).

Seeds are placed into Petri dishes lined with moistened filter paper and the dishes are then placed into a desiccator. Air is removed from the desiccator to a specified vacuum pressure (0.06 MPa), the desiccator is sealed and stored at 20°C, and the seeds are allowed to germinate. The vacuum test was well-correlated with field emergence, in addition to correlation with the birdsfoot trefoil vigor test, the electrical conductivity test, and a standard germination test (Artola et al., 2004).

### **Seed Priming**

Seed priming involves controlled hydration of seeds by exposure to water, either alone or in combination with solid media or osmotic agent, allowing seeds to imbibe, but removing the water and drying seeds to original moisture content prior to radicle emergence. (Murray and Wilson, 1987). Prior to germination, which is identified by radicle emergence, seeds absorb water in three phases (Bewley and Black, 1978). In phases one and three, water uptake is rapid while in the second phase water uptake is slow. By priming, allowing seed to take up water to a point just prior to radicle emergence, most steps necessary for germination can occur, but the seed can still be kept in a “seed” state until it is ready for planting. Once planted, seedlings can emerge from the soil faster due to a shortened tri-phasic water uptake pattern.

Priming seed has several advantages for crop production, with the greatest benefit being increased germination rate. Primed seeds germinate across a wider range of temperatures than unprimed seed (Valdes and Bradford, 1987; Ellis and Butcher, 1988) and are more tolerant of adverse field conditions such as salinity (Wiebe and Muhyaddin, 1987), extremes in temperature

– both high and low (Valdes et al., 1985; Bradford, 1986; Pill and Finch-Savage, 1988), and in places of decreased water availability (Frett and Pill, 1989). Primed seeds can have greater vigor, and the resultant increased yields of vegetable crops have led to increased profits, justifying the additional expense of priming (Warren and Bennett, 1997). Priming also may remove dormancy and substitute for after ripening (Welbaum et al., 1998). Several priming techniques have been developed, including hydropriming, matric priming, and osmopriming (McDonald, 2000). However, it is important to note that seed priming will not change poor quality seed into healthier, more germinable seed; priming is only effective with the best quality seed lots (Cantliffe, 1989).

*Hydropriming:* Hydropriming involves adding pure water to seed for a period of time by misting or soaking. Prior to radicle emergence, seeds are dried to initial (pre-primed) moisture content for storage prior to sowing. One such technique involves the spraying of a water mist over the seeds and allowing the moisture to equilibrate (Van Pijlen et al., 1996). With another hydropriming technique known as hydration, seeds are submersed in aerated water for the priming duration (Thornton and Powell, 1992). Seeds can also be soaked in water and then exposed to air maintained at near 100% relative humidity (Fujikura et al., 1993). Temperature and the duration of treatment must be carefully monitored and adjusted to prevent both radicle protrusion and microbial growth (Burgass and Powell, 1984; Coolbear et al., 1987).

Hydropriming has been utilized on a variety of plant species, including onion (*Allium cepa*; Caseiro et al., 2004), cauliflower (*Brassica oleracea* var. botrytis; Fujikura et al., 1993), watermelon (*Citrullus lanatus* (Thunb.); Demir and Van de Venter 1999), mustard (*Brassica*

*rapa* L.; Srinivasan et al., 1999), strawflower (*Helichrysum bracteatum* (Vent.) Andrews; Grzesik and Nowak, 1998), corn (Clark et al., 2001; Murungu et al., 2004), wheat (*Triticum aestivum* L.; Giri and Schillinger, 2003), and Kentucky bluegrass (*Poa pratensis* L.; Pill and Necker, 2001).

Onion was hydroprimed by means of imbibition on various thicknesses of paper towel soaked to 2.5 times its dry weight with demineralized distilled water for 48 or 96 h at 15°C (Caseiro et al., 2004). Priming did not increase seed germination percentage, but primed seeds germinated more rapidly than unprimed seeds, which is the main benefit to priming. Interaction between germination rate and priming medium/time length was also observed, with best results observed with longer priming (96 h).

Fujikura et al., (1993) hydroprimed cauliflower seeds by soaking five h then incubating in a closed container with 100% humidity at ambient temperature for three days. Seeds were air dried prior to germination tests. Hydropriming greatly improved the rate of seed germination, with the greatest improvement at 10°C. The authors concluded that this hydropriming treatment was more effective than osmopriming in polyethylene glycol (PEG) 8000 solutions (-1.5MPa; 20°C) for seven days in terms of increased germination rates, especially at lower than optimum germination temperatures. In contrast, Demir and Van de Venter (1999) found that osmotic priming (-0.9 MPa) of watermelon seeds produced significantly higher final germination percentage (97.5%) than did hydropriming (88.7%), but germination by both methods was significantly greater than for the unprimed control (80%) at 15°C. Additionally, watermelon seed primed at 25 and 38°C did not differ from the control (unprimed), and priming at any

temperature did not significantly improve root growth of seedlings after synchronization of radicle emergence.

A slow hydration technique was more successful in increasing germination rates of mustard seeds than was a soaking hydration technique (Srinivasan et al., 1999). Slow hydration was provided by an initial 3-h soaking of seeds in water, followed by 36 h of slow absorption of water from moist muslin wrapped around the seeds. This method was compared to full immersion of seeds in water for the entire hydration period.

On-farm seed priming is a common practice in the semi-arid tropics to increase germination rates and emergence rates of corn seeds. The method is also reported to increase the rate of crop development and to increase overall yield (Harris, 1996) due to faster plant growth and earlier flowering (Harris et al., 1999). Murungu et al. (2004) reported a 14% increase in crop establishment due to priming. The process is very simple: seeds are covered with de-mineralized water and imbibed water for 17 h at 20°C. There are disadvantages to priming, however. For example, Clark et al. (2001) reported decreased optimum and ceiling temperatures of hydroprimed corn seed, with fewer primed seeds germinating above 30°C than unprimed seeds. When planted in moist sand, priming advanced the time to 50% germination by 12 h at 30°C, only 5 h at 35°C, and delayed time to 50% germination at 40°C by 20 h. Clark et al. (2001) reported no favorable changes in corn growth or development at optimum temperatures, and no difference in primed vs. unprimed plants that emerged on the same day (but at different hours). However, when corn seeds were planted in progressively drier pots (simulating drought or dry soil conditions), primed seeds advanced the time to 50% germination by nearly 70 h in comparison to unprimed seed (Murungu et al., 2004). The authors reported 12- and 24-h

reductions in mean time to germination in two consecutive growing seasons as a result of hydropriming.

Less information about priming forage seeds is available. When seeds of Kentucky bluegrass (*Poa pratensis* L.) were soaked in water for five hours at 23°C, then surface dried and allowed to incubate in a sealed container at 100% relative humidity for three days (23°C), the treatment failed to improve germination over unprimed seeds (Pill et al., 2001). Germination rates were also not affected by the priming treatment.

Seeds of birdsfoot trefoil have been successfully hydroprimed using the aerated hydration method (Artola et al., 2003). While priming did not affect overall germination percentage or soil emergence, rates of seed germination and soil emergence were faster. Additionally, peak germination of primed seed occurred 24 to 36 h before that of unprimed seeds. These effects indicate that primed seeds exhibited greater vigor than unprimed seeds. The disadvantage to this method lies in the rather tedious and time-consuming procedure to determine priming duration. Seed must be soaked in aerated water then centrifuged (to remove excess water), weighed hourly and returned to the water. This process is repeated until no more water is imbibed. The weight gain shows the tri-phasic pattern of water uptake when plotted and the aeration period is then determined graphically.

*Drum Priming:* A specific method and device for hydropriming using water and a rotating cylinder has been developed (Rowse; 1991; Rowse, 1992). Seeds are placed into the rotating cylinder and water is added via mist in small amounts over time until the seed within the cylinder reaches a predetermined target moisture concentration. This process eliminates the concern for

the disposal of spent hydrating solution (as in osmotic priming) or spent solid carrier disposal (as in solid matrix priming) (Rowse, 1996). A device that can be assembled and utilized for drum priming of seed is described in detail by Warren and Bennett (1997). Both maize (Warren and Bennett, 1997) and onion (Caseiro et al., 2004) have been primed using a similar device. However, drum priming onion seeds was detrimental, as it resulted in lower final germination percentages and slower germination rates (Caseiro et al., 2004).

*Osmopriming:* Osmopriming, sometimes referred to as osmoconditioning, is similar to hydropriming. As with hydropriming, seeds are allowed to imbibe water, begin the germination process up to the second phase of the tri-phasic water uptake, and then dried to storage moisture before radicle emergence occurs (Bennett et al., 1992). However, various osmotica are added to the water during the imbibition period to prevent full hydration of the seeds. These osmolytes have included sugars, salts, polyethylene glycol, and mannitol. The solutions created provide osmotic potentials low enough to prevent germination and radicle emergence during priming. The concentration of the hydrating solution is an important factor in osmopriming. High levels of some osmotica may negatively affect seed water potential, may be toxic to the seed, or may be economically unsustainable (Taylor and Harman, 1990). Other limitations with osmopriming include difficulty in handling of polyethylene-glycol for priming large seed lots (Haydecker and Coolbear, 1977) and chemical waste disposal after priming.

No published data currently exist regarding the effects of osmopriming on birdsfoot trefoil, kura clover, or sericea lespedeza seeds. Responses to osmopriming have been reported for most vegetable and agronomic crops, including: onion (Caseiro et al., 2004), cauliflower

(Fujikura et al., 1993), watermelon (Demir and Van de Venter 1999), mustard (Srinivasan et al., 1999), soft white winter wheat (Giri and Schillinger 2003), Kentucky bluegrass (Pill and Necker, 2001; Yamamoto and Turgeon, 1998), broccoli (Jett and Welbaum, 1996), pepper (Thanos et al., 1989; Shakila et al., 2005), and sugar beet (Gummerson, 1986).

Osmopriming with PEG solutions was not successful for onion seed (Caseiro et al., 2004) but proved effective with watermelon (Demir and Van de Venter, 1999). Caseiro et al. (2004) soaked 6-g lots of onion seeds in 200 mL PEG 8000 solutions (-0.5 MPa or -1.0MPa) for 24 or 48 h at 15°C. Osmopriming did not affect final germination percentage or rate of germination. However, osmoprimed watermelon seeds soaked in PEG 6000 solution (-0.9MPa) at 25°C had 17% higher germination percentage than did unprimed seeds (Demir and Van de Venter, 1999). Root growth was also studied for watermelon, but no priming effects were observed after treatments were synchronized for radicle emergence. The conclusion was drawn that “Improved emergence after priming is not due to the beneficial effect on radicle emergence only, but also to improved hypocotyls growth” (Demir and Van de Venter, 1999).

Cauliflower seeds were responsive to hydropriming in a comparison of priming methods (Fujikura et al., 1993). In addition to increased germination rates over unprimed seeds at optimal and suboptimal temperatures, osmotic priming produced more normal seedlings (higher final germination percentage) compared with hydropriming. This was likely because osmoprimed aged seeds were better able to repair themselves than hydroprimed seed.

Srinivasan et al. (1999) found that osmopriming mustard seeds produced significant improvements in germination and vigor parameters over direct soaking of seeds in water. Osmoprimed seed leaked fewer electrolytes and UV absorbing materials and had increased

activity of free radical-scavenging and reserve-mobilizing enzymes. Seeds were osmoprimed in Petri dishes lined with Whatman No. 1 filter paper soaked with PEG 6000 (-0.75MPa) at 20°C for 72 h, after which seeds were thoroughly rinsed and air dried to initial moisture content.

Response of soft white winter wheat primed in either potassium chloride (KCl) or in PEG solutions varied by cultivar (Giri and Schillinger, 2003). Although one cultivar had greater germination in response to osmotic priming, neither field emergence nor grain yield was significantly benefited by any combination of priming media and cultivar, and it was implied that this method seed priming is of limited practical worth for soft white winter wheat.

Some benefit of osmotic priming Kentucky bluegrass was reported by Pill et al. (2001). The researchers exposed seed to PEG 8000 (-1.5 MPa; 20°C) for four days by holding in polystyrene boxes lined with two layers of saturated germination paper (354 g PEG per kg H<sub>2</sub>O). Germination rate was increased, but no difference in final germination percentage was observed. Drying seed after priming delayed germination when compared to immediate germination following the priming treatment (Pill et al., 2001).

*Matric priming:* Matric priming (also called matric conditioning, solid matric priming or solid matric conditioning) accomplishes the same type of controlled, limited hydration as hydropriming and osmopriming. However, unlike hydro- and osmopriming, matric priming utilizes a solid medium (the matrix) to deliver water and nutrients to the seed prior to emergence of the radicle (Taylor and Harman, 1990). Khan (1992) recommended the use of vermiculite or calcinated clay as the solid medium due to their good water holding capacity and ease of removal from the seed after the priming process is complete. A clear advantage of matric priming is the

ability to provide ample oxygen to the seed during the hydration process (John Easton, personal communication, 2005). However, as with osmopriming, waste disposal after priming may become an issue (Warren and Bennett, 1997).

Matric priming is a newer technique, and its effects on germination and vigor have been reported for fewer seed species. Matric priming has improved strawflower seed performance and seedling emergence (Grzesik and Nowak, 1998). The authors also demonstrated that matric priming can also increase seedling frost resistance and decrease effects of water stress on seed performance. Kentucky bluegrass exhibited the best results when matric primed and then germinated immediately following the priming treatment (without drying seed), compared to osmotic and hydropriming (Pill and Necker, 2001). Seeds were exposed to a growth regulator solution (-1.5 MPa for four d at 20°C) with a 50:50 matrix of No. 5 fine vermiculite and water (w/w) and in a sealed container to prevent evaporation. In a similar study by Yamamoto and Turgeon (1998), matric priming of Kentucky bluegrass resulted in faster, more uniform germination, but results varied in magnitude by seed lot/cultivar. Matric priming Kentucky bluegrass seed had greatest benefit with low-vigor seed lots.

### **Fire, Smoke, and Seed Germination**

For some time fire has been known to be a strong factor in seed biology and in stimulating the seed germination processes (Van Staden et al., 2000). Main areas of interest for fire as a germination cue occur in places where fire plays a vital role in ecosystem function, especially the temperate grasslands (Baxter et al., 1994; Blank & Young, 1998) and in the Mediterranean climate zones (Brown et al., 1993; De Lange & Boucher, 1993; Brown et al.,

1994; Dixon & Roche, 1995; Keeley & Bond, 1997). The initial observations of the positive effects of fire were noticed when seedlings of various species began rapidly germinating after forest fires. At first, this observation was attributed to the heat of the fire acting to break seed dormancy by fracturing or desiccating the seed coat (Brits et al., 1993; Jeffrey et al., 1998) or by direct stimulation of the embryo (Blommaert, 1972; Musil and De Witt, 1991; Van de Venter & Esterhuizen, 1998). It is now better understood that there may be more components to the fire-smoke-seed germination equation and further investigation is required (Roche et al., 1997), as there may be numerous chemical attributes of burning that may affect seed germination. Van de Venter & Esterhuizen (1998) showed that fire can produce ethylene and ammonia, known germination stimulants (Esashi & Leopold, 1969; Cairns & De Villiers, 1986), when fresh plant material is burned (Lewcick, 1937; Russell et al., 1974). Ash (Reyes and Casal, 1998) and nitrogenous substances (Christensen, 1973; Keeley and Fotheringham, 1997) can also be produced from burning plant material and may be beneficial stimuli to the seed bank.

Smoke has been shown to contain some form(s) of a chemical stimulant to seed germination (De Lange & Boucher, 1990; Brown, 1993; Brown et al., 1994; Brown and Botha, 1995). That property of smoke which stimulates seed germination is heat stable, volatile, water soluble, can adsorb to many types of surfaces, and can be readily stored for long time periods (Van Staden et al., 2000). Smoke readily adheres to fresh (Tieu et al., 1999) and charred (Keeley & Fotheringham, 1997) plant surfaces and to soil particles (Keeley & Fotheringham, 1998) and can remain viable in soil for extended periods (De Lange & Boucher, 1993). With this soil adherence and ability to remain in soil in mind, Egerton-Warburton (1998) reports on the ability for smoke to scarify seed surface in soil seed banks, such as the seeds of *Emmenanthe*

*penduliflora*. Smoke treatment can stimulate germination of light sensitive seeds held in dark conditions, such as *Erica sessiliflora* (Van Staden et al., 2000), and a combination of light and smoke treatments greatly enhanced the germination of lettuce seeds, (cv ‘Grand Rapids’) (Drewes et al., 1995). Germination response to smoke treatment of any type is highly variable among plant species. In South African studies testing response to smoke of 221 plant species from the families *Proteaceae*, *Ericaceae*, *Restionaceae*, *Bruniaceae*, *Asteraceae*, *Fabaceae*, *Mesembryanthemaceae*, *Poaceae*, *Rutaceae*, *Geraniaceae* and others, only 54% of these species showed a significant improvement in seed germination (Van Staden, et al., 2000; Brown, 1993; Brown, 1997). Numerous Western Australian species (Dixon & Roche, 1995; Dixon et al., 1995; Tieu et al., 1999) and matorral species (Baskin & Baskin, 1998) also display a positive response to some form of smoke treatment.

Early research attempted to determine which species needed burning in order to generate the active ingredient found in smoke that stimulates seed germination. At first, plant material of plant-species found in natural fire-prone areas was burned and the smoke captured (De Lange & Boucher, 1990; Brown; 1993a; Dixon et al., 1995). Baxter et al. (1995) gathered plant material from 27 montane grassland species, burned the same amount of each species, and tested their smoke effects on seeds of *Themeda triandra*. Smoke from 26 of the 27 species aided the germination of *Themeda triandra* seeds. Jager et al. (1996a) burned plant material from monocots, dicots, and gymnosperms and found that all had positive effects on seed germination. The authors also reported that an active ingredient in smoke could be produced by burning paper, cellulose, and agar but not by burning starch, glucose, galactose, or glucuronic acid. Given that cellulose may be found in all plants, it is interesting that all except one species in Baxter’s study

did not appear to yield the desired compound. Possible explanations to this are possible, however, including laboratory procedures in regards to burning (Van Staden et al., 2000) that may have resulted in concentrations too low or too high. More noteworthy is the strong effect of smoke concentration on seed germination (Drewes et al., 1995). Species respond differently to the same concentration of smoke, and some will display greater germination at specific concentrations.

*Aqueous Smoke Water:* Subsequent research found that smoke could be bubbled through water to create an aqueous smoke water treatment that could easily be applied to seeds prior to germination. This has simplified the smoke treatment process and made it possible to commercialize this treatment. Additionally, aqueous smoke solutions containing the active ingredient for stimulating germination could be readily stored in containers for long periods of time (Brown and Van Staden, 1997). The stored solution is very stable and holds activity for up to five years (Van Staden, 1999) and maintains its activity after autoclaving (Jager et al., 1996). De Lange and Boucher (1990) found that an aqueous smoke solution can be equally effective as an aerosol smoke treatment.

Another approach to producing aqueous smoke solutions involves exposing plant material to dry heat. Jager et al. (1996) heated *Themeda triandra* leaves to temperatures between 160 and 200°C for 30 min in an oven and then used water to extract compounds necessary to promote seed germination. Leachates from charred wood have also had similar activity with respect to seed germination (Keeley and Pizzorno, 1986; Keeley and Keeley, 1987; Jager et al., 1996; Keeley and Bond, 1997).

Sparg et al. (2006), tested effects an aqueous smoke solution on germinating corn seeds (cv 'PAN 6479'). Solutions were made by bubbling smoke through 500 mL water for 45 min following procedures of Baxter et al. (1994) and then diluted with water to 1:250, 1:500, 1:1000, and 1:2000. Corn shoot lengths were increased by all concentrations of smoke water compared to control (no smoke water), but no differences were observed among the smoke water concentrations. Additionally, root length was significantly increased over the control in increasing order from 1:2000, 1:1000, 1:500. Seed treated with the 1:250 concentration had roots shorter than that of the control, indicating that 1:250 may be too concentrated a solution of smoke water and may cause detrimental effects on corn.

Smoke water treatment (1:2000 dilution) had no effect on germination of *Albucca pachychamys* L (a species which requires light for germination). However, the treatment greatly increased the final germination percentage of *A.pachychamys* germinated under dark conditions (Sparg et al., 2005).

*Aerosol Smoke:* Aerosol smoke (also known as airborne smoke) for use as a germination stimulant is created by slowly burning plant material. Smoke created from burnt plant material can be vented into a tent and allowed to settle down onto the soil or onto trays of seeds beneath the tent (Roche et al., 1997), or it may be allowed to rise through a wire mesh where seeds of interest are placed for treatment (Sparg et al., 2006). Care should be given to place the mesh high enough that the smoke has cooled prior to contacting the seeds. Similar techniques for aerosol smoke treatments have been widely utilized (De Lange and Boucher, 1990; De Lange and Boucher, 1993; Brown, 1993a,b; Baxter et al., 1994; Dixon et al., 1995).

Sparg et al. (2006) exposed corn seeds to aerosol smoke by placing seeds on sieves 1.5 m above smoldering semidry spear grass (*Heteropogon contortus* L.) for 30, 60, or 90 min. Shoot length was increased over the control (un-smoked corn) when exposed to 30 min of aerosol smoke, but no difference was observed for seed exposed for either 60 or 90 min. However, rinsing the seeds after exposure to aerosol smoke increased shoot lengths for both 30- and 60-min smoking treatments, but not for the 90-min treatment. None of the three smoking time lengths affected root lengths in comparison to the control when seed was not rinsed after smoking. Root lengths were increased as a result of all three smoking lengths when seed was rinsed after smoking. In another study, Modi (2002) showed that whole ear corn stored over fireplaces, as in the indigenous storage method of farmers in South Africa, increases both the germination rates and final germination percentages of corn seed.

Benefits of smoke on seed germination and grain quality characteristics were also tested in a commercial setting with farm-scale grain drying units and grain storage bins (Paasonen et al. (2003) Grain from the same harvest was divided into control (un-smoked) and treatment (smoked in the grain drying process) groups. Grain exposure to smoke increased the germination of corn, rye (*Secale cereale* L.), barley wheat, and oats (*Avena sativa* L.). Additionally, aerosol smoke also decreased microbial contamination by endophytic species but had relatively low control against *Fusarium* toxins.

*Butenolide*: After years of exhaustive testing and extractions, the compound in smoke that promotes seed germination was identified as butenolide (3-methyl-2H-furo[2,3-c]pyran-2-one) (Flematti et al., 2004). Butenolide is a by-product of the burning of cellulose. The compound

was found by creating smoke from various plant sources and comparing the activity to that of the smoke from burning filter paper. Smoke from both plant and filter paper provided the same germination stimulation. Using mass spectrometry and two-dimensional nuclear magnetic resonance (NMR) techniques, the structure of the compound was determined. The structure was confirmed as butenolide by synthesis and the presence of butenolide in plant-derived smoke was verified by gas chromatography-MS analysis. Synthetically-derived butenolide has had similar activity to plant-derived sources in germination trials. “The identification of this natural molecule, the major germination cue from smoke, should now rapidly lead to a more comprehensive understanding of the role of smoke as a promoter of seed germination” (Van Staden et al., 2004). One such advancement that will benefit the seed industry would be a commercially available solution of butenolide and the ability to further test more seed species for response to butenolide on a larger, more economical scale.

### Chapter 3: Solid Matrix Priming Effects on Legume Seed Germination

#### ABSTRACT

Matric priming has been used with a wide range of species, and benefits include faster germination rates and increased range of germination temperatures. Thus, matric priming may support more rapid establishment when seeds are planted under less than ideal conditions. However, no data to confirm the utility of matric priming legume seeds is currently available. The objective of this study was to determine effects of matric priming on seeds of birdsfoot trefoil (*Lotus corniculatus* L.) and kura clover (*Trifolium ambiguum* Bieb.) both in the laboratory and in the field. Seed responses were tested over a range of germination temperatures, in seed vigor tests in the laboratory, and in seedling emergence studies in the field. Germination percentages under standard test conditions were not different by species and priming effects varied by test conditions. No differences were observed under standard germination conditions, but priming increased the germination rate of one cultivar of birdsfoot trefoil. Seeds tested after priming under accelerated aging, or under cold or anaerobic conditions generally responded to priming with numeric and sometimes significant increases in germination percentage. Greatest benefit to priming was observed at temperature extremes. In field studies, priming effects were small. Date had the greatest effect ( $P < 0.001$ ) on seedling numbers with highest values recorded on 29 September 2005 (the second measurement) and lowest values occurring the following spring. However, the decline in seedling numbers from fall to spring was greater for birdsfoot trefoil (species×date interaction;  $P < 0.01$ ). These data suggest that solid matrix priming may have condition-specific benefit but these are unlikely to be translated to improved establishment in the field with current methodologies.

## **Hypothesis**

H<sub>0</sub>: Priming treatments for low vigor legume seeds will not affect seedling germination rate, germination percent, range of germination temperatures, and field emergence.

## **Objectives**

- 1) Determine response (germination rate, percent, and temperature range) of birdsfoot trefoil and kura clover to a solid matrix priming treatment.
- 2) Determine seedling emergence in established pasture in response to priming and herbicide treatments.

## **MATERIALS AND METHODS**

### **Seeds**

Four forage seedlots – two lots each of birdsfoot trefoil and kura clover – were chosen to test germination responses to a proprietary commercial matrix priming method. Seed tag information is shown in Appendix 1. Seeds were evaluated during June 2005 for viability at University of Kentucky Seed Lab (Lexington, KY) by means of tetrazolium testing (at request of priming company) before priming (Kamterter, LLC, Lincoln, NE) during August 2005. Upon return from Kamterter, seeds were stored at ambient temperature prior to testing the effects of the treatment. Tests of primed vs. unprimed seed began in October 2005 and were completed by January 2006.

## Seed Testing

Unless otherwise noted, all seeds were germinated in four replications in either 9-cm plastic Petri-type dishes (Fisher Scientific) lined with one thickness of germination towel (Seedburo Equipment Co., Chicago, IL) moistened with 6 ml distilled water. After seeds were placed on the moistened germination towel, the dishes were closed and put into 3.8-L plastic zip-type freezer bags and placed into an incubator (Percival; Boone, IA) at the appropriate temperature. Counts of germinated seeds for both birdsfoot trefoil and kura clover were made for 12 consecutive days (recommended timeframe for birdsfoot trefoil, kura clover not specifically outlined, but similar clovers recommended at 7 d; 2 d period used to ensure adequate germination (AOSA, 1988) at approximately the same time each day (10:00 am). A seed was considered germinated when a normal radicle protruded from the seed coat, following the guidelines of AOSA (1988).

### *Accelerated Aging Test, Cold Test, Conductivity Test, and Standard Germination Test:*

Accelerated aging tests, cold tests, conductivity tests, and standard germination tests were conducted contiguously on both primed and unprimed seeds from each seed lot (September to October 2005) by the University of Kentucky Seed Testing Laboratory.

*Vacuum Test:* Seed lots were subjected to the vacuum test of Artola et al. (2004). Seeds were placed into Petri dishes which were immediately transferred to a vacuum desiccator (Pyrex, Corning, NY). The desiccator was then tightly sealed, and air was removed from the desiccator to a vacuum pressure of 0.06 MPa using a vacuum pump (Actron, Cleveland, OH). Vacuum was

held stable for 5 min prior to sealing the desiccator valve. The desiccator was then placed into a germination chamber (Percival; Boone, IA) at 20°C, and seeds were allowed to germinate for 72 h. The desiccator was then opened and normal germinated seedlings were counted. Results are expressed as percentage of germinated seeds.

*Gradient Table/Germination Temperature Tests:* Tests were run to compare the effects of seed priming over a range of temperatures. Primed and unprimed seeds within a cultivar were germinated simultaneously on a gradient table over a temperature range of 2.5 to 38°C. Round germination dishes were placed on a layer of petroleum jelly to ensure that the temperature inside the dish was as close as possible as on the table surface. Conditions in the gradient table caused water to move from the heated warm end and condense on the chilled cold end. Thus dishes subjected to warmer temperatures were re-watered periodically (4 ml) to maintain similar moisture content. Eight rows of eight dishes could be placed on the table. Four replicates of each primed and unprimed seed within a cultivar were tested across eight different temperatures (2.4, 7.2, 10.8, 16.8, 22.5, 28, 32.2, 37.2°C, +/-1.5°C). Seeds were counted for germination (emergence of a normal radicle) daily for 12 d. Dishes on the ends of each row were fitted with a thermocouple Type K thermometer probe (Sper Scientific, Ltd., Scottsdale, AZ) and temperatures recorded daily.

*Field Establishment:* Establishment in response to priming method and field (Hayter soil type) preparation treatment was tested in 2-m x 4-m plots replicated three times planted 1 September 2005 at Virginia Tech's Kentland Farm (Whitethorne, VA; 37° 11' N latitude and 80°

35 W longitude). Primed and unprimed seed were no-till drilled into an existing stand of tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) with or without herbicide suppression (band sprayed at time of planting). A no-till drill (Tye, AGCO, Batavia, IL) was modified to band-apply systemic herbicide (Roundup Pro Concentrate (Glyphosate, N-(phosphonomethyl) glycine) (Monsanto, St. Louis, MO) at 2.26 kg a.i. ha<sup>-1</sup> at seeding. Spray nozzles were set about 10 to 15 cm above the ground surface and in line with the drill row (Figure 3-1) creating a spray band of about 8 cm wide. Existing vegetation was mowed to a height of 8 cm on 31 August and seed was planted 1 September 2005. The sward was mowed again 30 days after planting to prevent tall fescue from forming a canopy over new seedlings. Inoculum was applied at twice the recommended rate just prior to seeding to ensure that it was not a limiting factor in establishment (Moorhead, 1994).

Three 0.61-m lengths of drill row were randomly selected and marked off with flags after planting. These rows allowed for emerged seedlings to be counted on a repeated basis. Due to dry soil conditions, initial emergence counts were not recorded until 22 September 2005. Measures were repeated 29 September and 13 October 2005. Plots were hand-watered (irrigated) between 9 September and 24 September at rates of about 2.5 cm week<sup>-1</sup> (Table 3-1). Precipitation and temperature data may be found in Figure 3-12. Counts of surviving seedlings were made on 17 May 2006. In 2006, no defoliation was imposed prior to measure collection.

A similar experiment was conducted in an existing orchardgrass (*Dactylis glomerata* L.) pasture at Wilmington College (Wilmington, OH). Primed and unprimed seeds were no-till drilled into plots using the same drill type and spacing as in the VA study. Herbicide (Roundup Pro Concentrate (Glyphosate, N-(phosphonomethyl) glycine) (Monsanto, St. Louis, MO) at 2.26

kg a.i. ha<sup>-1</sup>) was then applied to half of each plot using a CO<sub>2</sub>-type backpack sprayer instead of band spraying. Existing vegetation was primarily orchardgrass (*Dactylis glomerata* L.), which was initially mowed as in the VA study. Seeds were planted 7 September 2005 and seedling emergence was evaluated 23 and 30 September and 7, 14, and 21 October 2005.

## STATISTICS

Statistical analysis was performed on arcsine transformed germination percentage data and log transformed time data. Seed germination tests and vigor tests were analyzed individually by test using a completely randomized design with a two-factorial arrangement of treatments with cultivars nested within species. Treatment factors were priming and legume species. Analysis of variance was conducted with the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Data presented as mean germination percentage or mean time (days) to 50% germination (T<sub>50</sub>). Mean time to germination was calculated using the following equation:  $T_{50} = (H_p - L_p)^{-1} + L$ , where H<sub>p</sub> is the observed germination percentage on the day that 50% germination was reached or exceeded, L<sub>p</sub> is the observed germination percentage on day L, and L is the last day before 50% germination is reached.

The field experiment in Virginia was conducted as a randomized block design with a 2 × 2 factorial arrangement of treatments with cultivars nested within legume species. Data were square root transformed prior to analysis and the mixed procedure of SAS was used for the ANOVA with measurement date as the repeated measure and plot replicate used as the subject of interest. The experiment in Ohio was a randomized block design with herbicide application arranged as a split factor.

## RESULTS AND DISCUSSION

### Seed Germination Tests

When priming treatment was compared under optimal germination conditions in the standard germination test (Figure 3-2), no treatment differences were found ( $P=0.26$ ). Clark et al. (2001) also found that primed maize seeds germinated under ideal controlled conditions were not different from unprimed seeds in terms of final germination percentage.

Daily counts of germinated seeds for this study allowed mean germination rates ( $T_{50}$ ) to be calculated (Figure 3-3). Across species and cultivars, priming had no effect on  $T_{50}$  although priming did decrease time to  $T_{50}$  for 'Georgia One' birdsfoot trefoil by one day (cultivar effect;  $P<0.001$ ). Kura clover seed germinated about one day faster ( $P<0.01$ ) than birdsfoot trefoil seed.

These data stand in contrast to previously reported studies. For example, matric priming reduced germination time of strawflower (*Helichrysum bracteatum* L.) at both 10- and 20°C (Grzesik and Nowak, 1998); an effect that remained after five months storage in the laboratory. Kentucky bluegrass (*Poa pratensis* L.) required 49% less time to reach 50% emergence when matric primed (Pill and Necker, 2001). Additionally, matric priming increased total emergence by 13% and increased shoot dry weight by 84% over unprimed seed. Yamamoto and Turgeon (1998) demonstrated a similar germination rate benefit to priming bluegrass, but also noted that low-vigor seed lots responded more favorably to matric priming. Similarly, difference between cultivars of the same species were seen in this study, suggesting the natural variation in response to priming, perhaps even within seed lots.

No overall treatment effects ( $P=0.2$ ) were observed for the conductivity test (Figure 3-4), but differences were obscured by treatment×cultivar interaction ( $P<0.01$ ). Priming resulted in higher conductivity measures with ‘Dawn’ birdsfoot trefoil but had the opposite effect with ‘Georgia One’. Similar interaction was observed with kura clover cultivars. Differences between birdsfoot trefoil and kura clover were not significant ( $P>0.07$ ). ‘Perfect Fit’ had a much higher conductivity reading than ‘Cossack1’ kura clover. This higher reading is attributable to a seed coating on Perfect Fit seed which skewed the actual measures of the test (AOSA, 1983). Higher conductivity readings indicate greater electrolyte leakage from cell membranes and hence lower vigor seeds. Based on this measure, matric priming would lower seed vigor of Dawn, but both Georgia One and Cossack1 – with marked decreases in conductivity readings – would have increased seed vigor due to priming. No differences were observed for Perfect Fit as a result of priming ( $P>0.05$ ).

Matric priming significantly increased ( $P<0.05$ ) seed germination after seeds were subjected to accelerated aging (Figure 3-5). Species variation ( $P<0.002$ ), due to kura clover germinating at higher final percentages than birdsfoot trefoil, as well as cultivar effects ( $P <0.05$ ) were observed. No difference between primed and unprimed seed in terms of germination percentage after accelerated aging for Dawn birdsfoot trefoil or Cossack1 kura clover, but significantly increased the ability to germinate after a period of accelerated aging for both Georgia1 birdsfoot trefoil and Perfect fit kura clover. Final germination percentages for both birdsfoot trefoil cultivars were significantly lower than shown for standard germination test results in Figure 3-2. These data stand in contrast with several reports in the literature by suggesting that priming may actually increase seed shelf life. Grzesik and Nowak (1998)

reported *Helichrysum bracteatum* L. seed viability and quality deteriorated within five mo after priming. Similarly, Tarquis and Bradford (1992) found lettuce (*Lactuca sativa* L.) seeds deteriorated more rapidly in storage after priming, and Oluoch and Welbaum (1996) reported shortened shelf life for primed muskmelon seeds (*Cucumis melo* L.).

A cold test indicates how a seed may respond in the field to cold, wet soil conditions, and is different from a more standard germination test in that the seed is germinated in actual soil from the field containing microorganisms and other natural constituents. Under these conditions kura clover had greater ( $P < 0.05$ ) final germination percentages than birdsfoot trefoil (Figure 3-6). A cultivar effect ( $P < 0.002$ ) was driven by lower germination of Dawn birdsfoot trefoil. While no treatment differences ( $P > 0.2$ ) were observed, there was a numerical pattern for increased germination as a result of priming under cool, wet soil conditions for all cultivars except Dawn, where the response to priming was negative.

The vacuum test indicates germination responses of seeds under anaerobic stress (Figure 3-7). Across species, priming increased ( $P < 0.05$ ) germination of seeds held under a vacuum. Priming significantly increased final germination percentage of Georgia1 birdsfoot trefoil, while responses for all other seeds were smaller but numerically positive. Artola et al. (2004) correlated the results of the vacuum test to seed vigor performance in actual field emergence as well as with the birdsfoot trefoil vigor test (Artola et al., 2003), the conductivity test, and the standard germination test. This is the first known study to analyze seed vigor of kura clover using the vacuum test.

Priming had little effect on germination percentage of either legume species at temperature minimum and maximum (Figures 3-8, 3-9). This was likely because those

temperatures were too extreme for the seeds to readily germinate. However, a discrepancy exists between the temperature test for birdsfoot trefoil (Figure 3-8) and the cold test (Figure 3-6), where germination was increased for primed seed in Figure 3-8, but no difference in priming treatment (within cultivar) existed in the cold test. This may be explained by the conditions of the test; the temperature test (Figure 2-8) was conducted in sanitary Petri-dishes, while the cold test was conducted in moist soil, thus suggesting that priming may increase germination percentage in ideal laboratory conditions, but not in soil conditions. Solid matrix priming benefited birdsfoot trefoil germination at all temperatures between the extremes, but results for kura clover were variable. Kura clover seed germination responded positively to priming at 7.2 and 22.5°C with a similar tendency ( $P<0.10$ ) at 10.8°C. However, priming reduced germination percentage of kura clover seeds at greater temperatures (28 and 32.2°C) ( $P<0.01$ ).

### **Field Establishment**

The field trial in Virginia examined herbicide and priming effects on birdsfoot trefoil and kura clover seedling numbers when no-till drilled into an existing tall fescue sod. Data were recorded on all plots except kura clover at the third measurement period, and this date was omitted from the final model. Date had the greatest effect ( $P<0.001$ ) on seedling numbers with highest values recorded on 29 September 2005 (the second measurement) and lowest values occurring the following spring. However, the decline in seedling numbers from fall to spring was greater for birdsfoot trefoil (Figure 3-10; species×date interaction;  $P<0.01$ ).

Herbicide treatments benefited establishment of kura clovers regardless of priming treatment, but with birdsfoot trefoil, benefits were only observed with primed seed

(priming×herbicide×species interaction;  $P<0.05$ ) (Fig 3-11). Overall benefits from priming (3.72 vs 2.98 seedlings per 30 cm row;  $P<0.05$ ) and herbicide (3.98 vs 2.72 seedlings per 30 cm row;  $P<0.01$ ) treatments were small.

As in Virginia, date was the main source of variation ( $P<0.001$ ) in Ohio. Unlike the Virginia work, seedling numbers were greatest at the first measurement but declined until the final two seedling counts (2.28, 1.24, 0.63, 0.49, and 0.48 seedlings per 30-cm row for the five measurement dates). Seedling numbers were much lower in Ohio, but this was likely attributable to severe infestation of grubs in the pasture where the study was planted.

McGraw and Nelson (2003) proposed that establishment of a birdsfoot trefoil stand is “very successful if there are 10 plants/ft<sup>2</sup> (108 plants m<sup>2</sup>)<sup>-1</sup>.” Given the drill spacing in the present experiment was about 18 cm, there could be no more than five drill rows in a meter width. With this assumption, seeds per square meter could be estimated by multiplying seeds per linear meter by five. For example, the Dawn-primed seed + herbicide treatment, which had about 23 seedlings m<sup>-1</sup> on 29 September 2005, would have had about 116 seedlings m<sup>-2</sup>. On this basis, most both legume treatments in Virginia could be considered successfully established in fall 2005. However, low seedling counts at the 17 May 2006 measurement date suggests that they did not develop sufficiently for winter survival, that fall and spring clipping management were not frequent enough to provide sufficient resources for the seedlings, or both. Other possible, untested factors, may include insect damage or disease prevalence.

## CONCLUSIONS

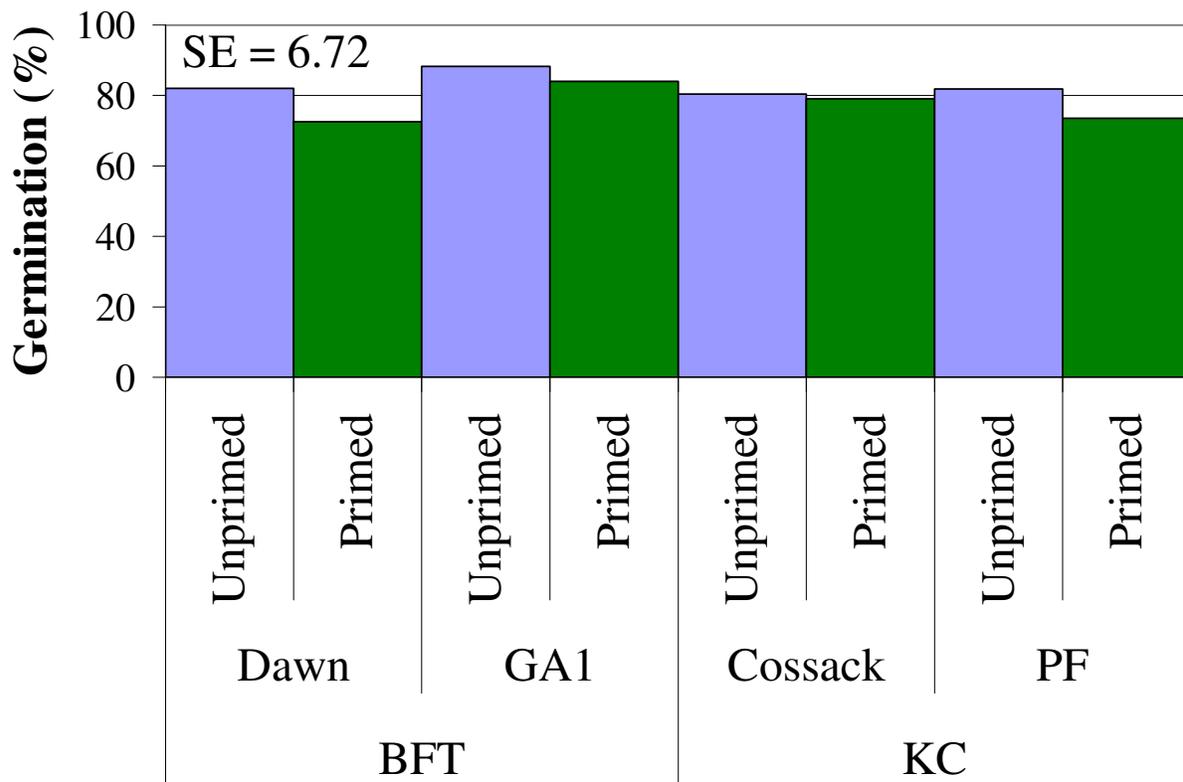
Seed responses to solid matrix priming are variable by species and seedlot. Only one seed source had greater rates of germination at 20°C with matrix priming. Priming increased total germination of both legume species at temperature extremes in comparison to unprimed seed at the same temperatures, although greater benefit existed for birdsfoot trefoil. However, priming did not have lasting effects on seedling emergence when seeds were sown into existing pastures. Priming's utility for establishment of new stands was undetermined, but it appears to have no practical benefits in the field with these legume species.

**Table 3-1: Irrigation scheduling and amounts of water applied for the VA legume establishment study.**

<b>Date Applied</b>	<b>Amount (cm)</b>
<b>9/7/2005</b>	<b>0.60</b>
<b>9/8/2005</b>	<b>0.80</b>
<b>9/13/2005</b>	<b>1.30</b>
<b>9/16/2005</b>	<b>1.30</b>
<b>9/19/2005</b>	<b>1.30</b>
<b>9/21/2005</b>	<b>1.30</b>
<b>9/24/2005</b>	<b>0.80</b>
<b>Total</b>	<b>7.40</b>



**Figure 3-1. No-till drill fitted for band herbicide application at seeding; used for VA seeding study**



**Figure 3-2: Priming effects on laboratory germination of birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and kura clover (*Trifolium ambiguum* Bieb.; KC) seed at 20°C using standard germination test procedures (AOSA, 1988). GA1 = ‘Gerogia One’; PF = ‘Perfect Fit’. Priming and species treatments had no effect on final germination percentage on day 12.**

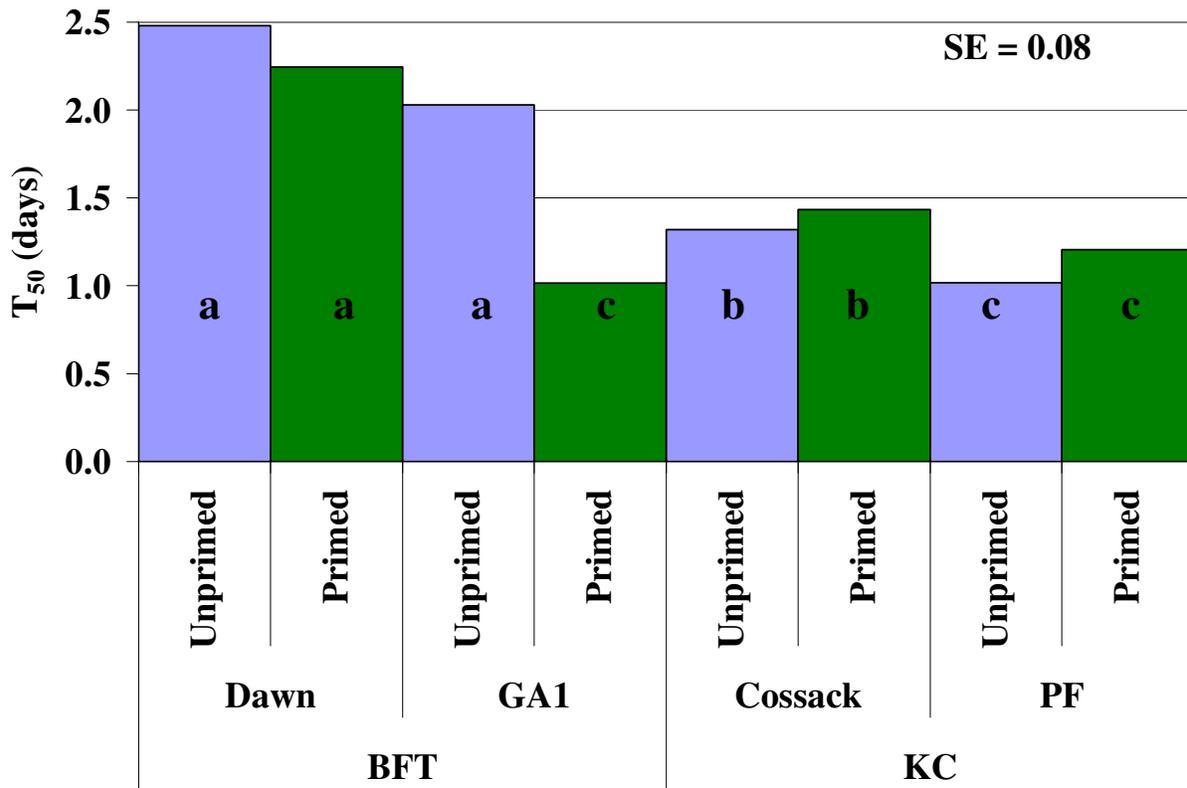
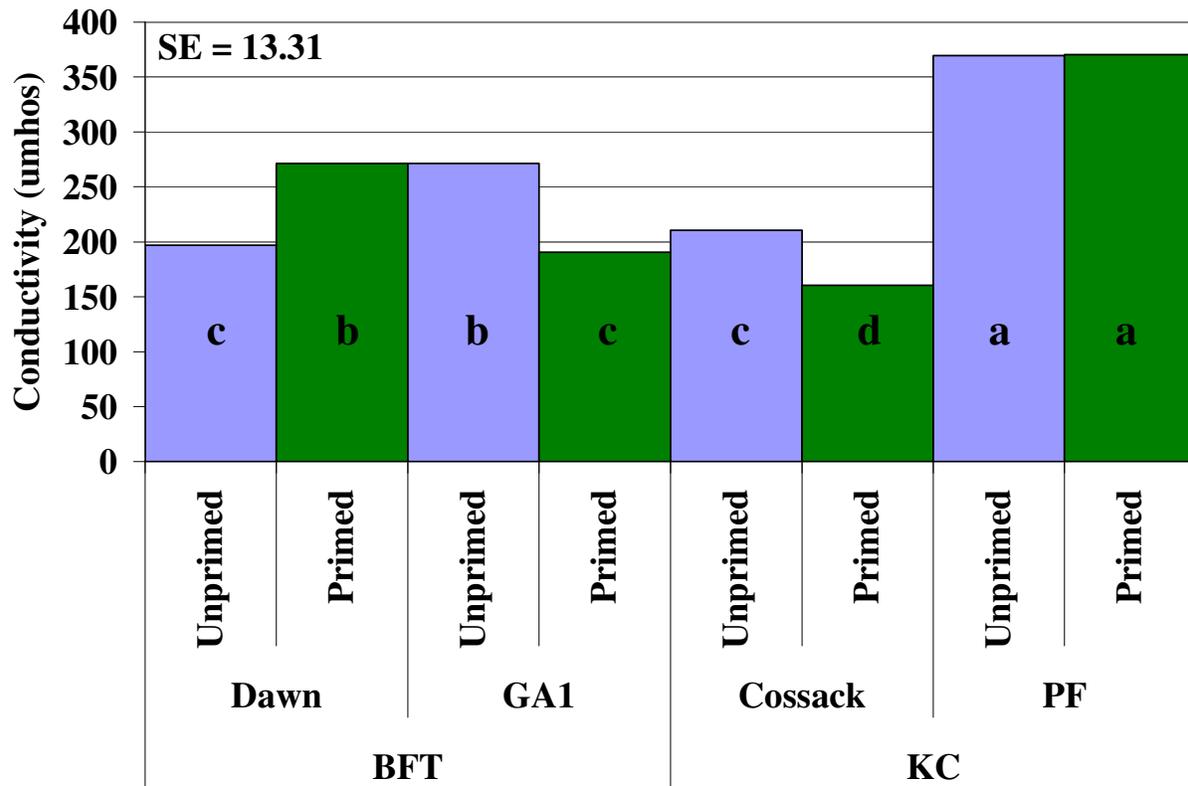


Figure 3-3: Priming effects on germination rates of birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and kura clover (*Trifolium ambiguum* Bieb.; KC) seed at 20°C using standard germination test procedures (AOSA, 1988). GA1 = ‘Gerogia One’; PF = ‘Perfect Fit’. Significant priming × species ( $P < 0.001$ ), species ( $P < 0.001$ ), priming ( $P < 0.05$ ) treatments were observed.



**Figure 3-4: Priming effects on solution conductivity within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and kura clover (*Trifolium ambiguum* Bieb.; KC) seed. Seeds were soaked for 24 h in distilled water prior to conductivity measurements. GA1 = ‘Georgia One’; PF = ‘Perfect Fit’. Significant priming × species ( $P < 0.005$ ), species ( $P > 0.08$ ), priming ( $P > 0.20$ ) treatments were observed.**

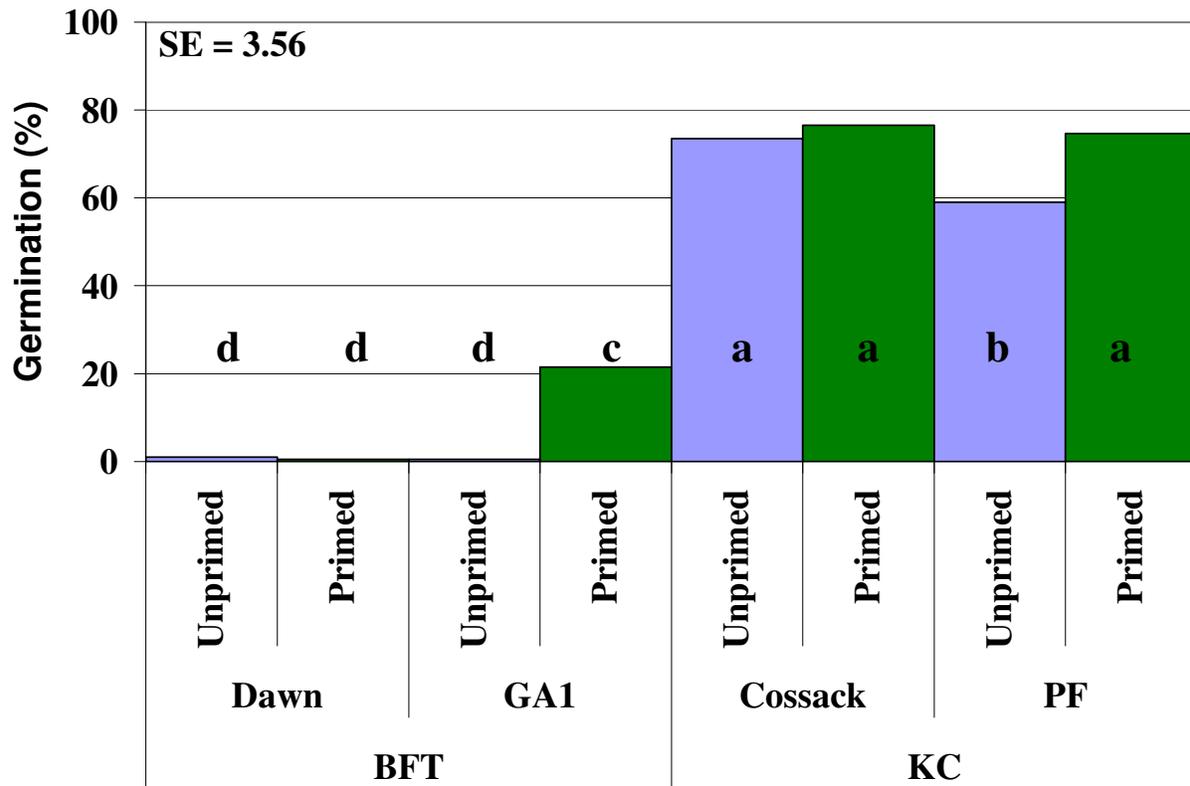


Figure 3-5: Priming effects on germination after accelerated aging within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and kura clover (*Trifolium ambiguum* Bieb.; KC) seed. GA1 = ‘Gerogia One’; PF = ‘Perfect Fit’. Significant priming × species ( $P < 0.05$ ), species ( $P < 0.002$ ), priming ( $P < 0.05$ ) treatments were observed.

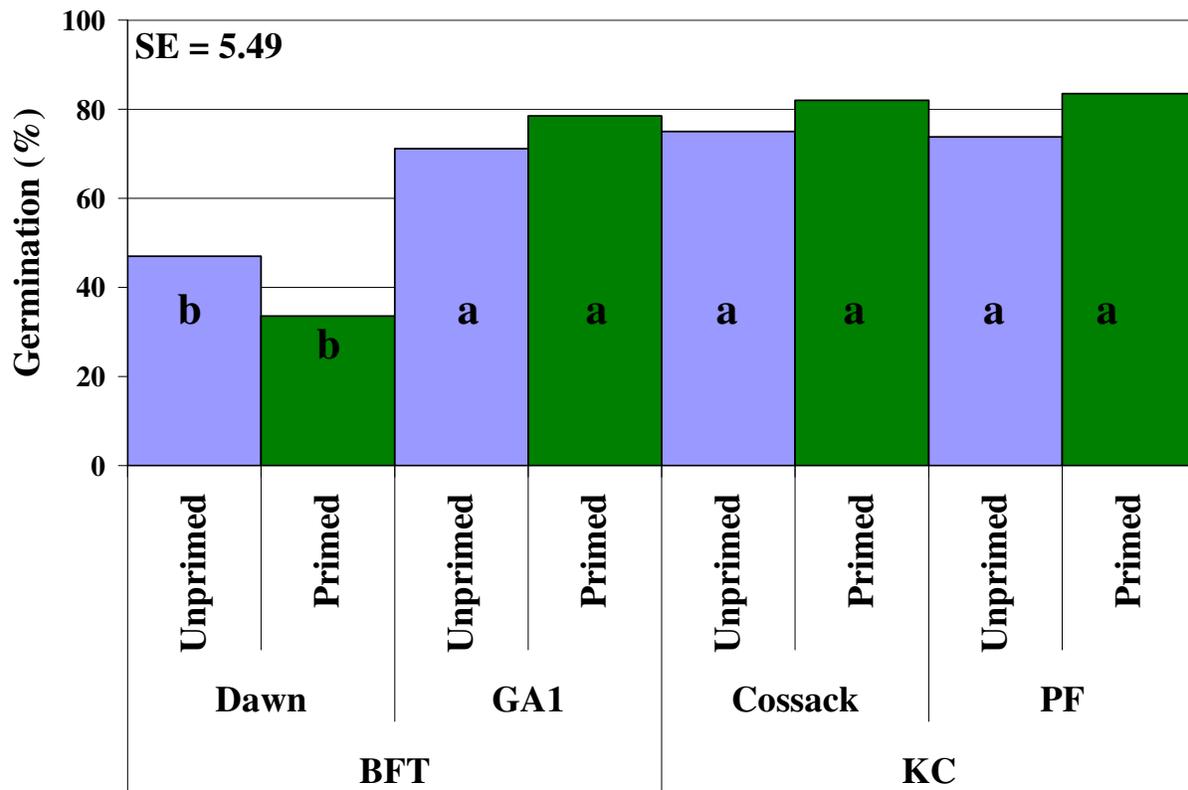
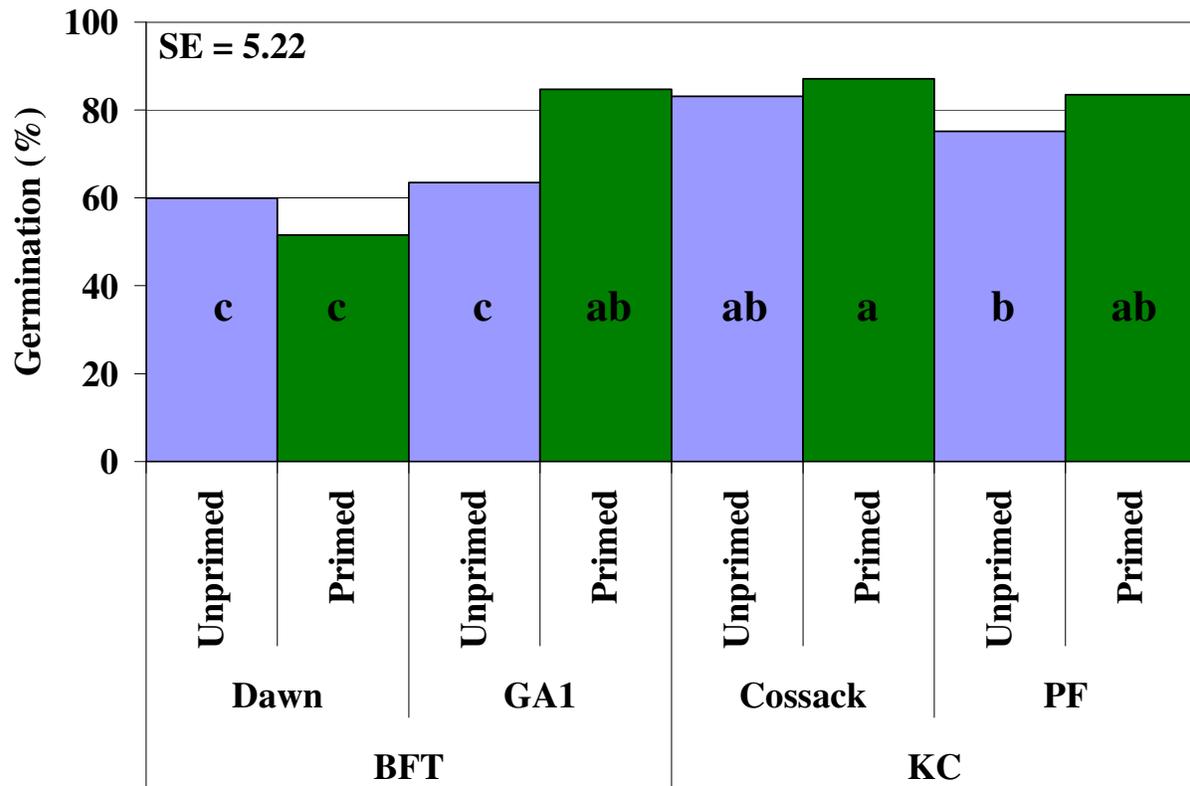
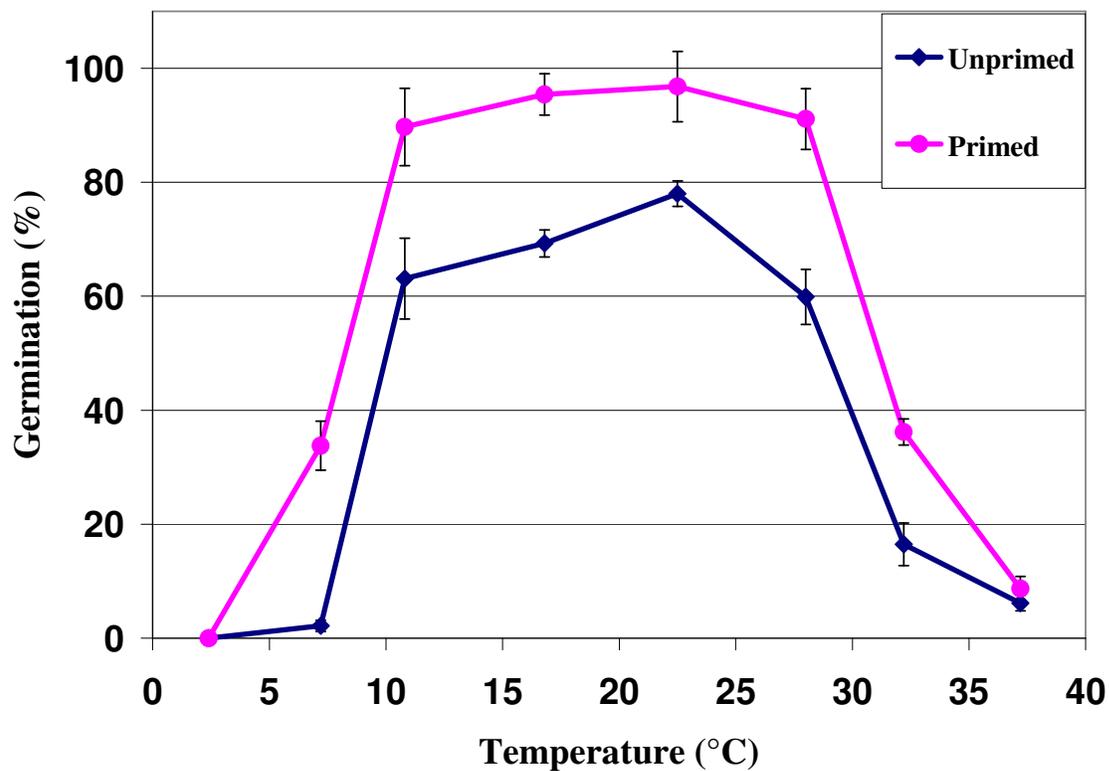


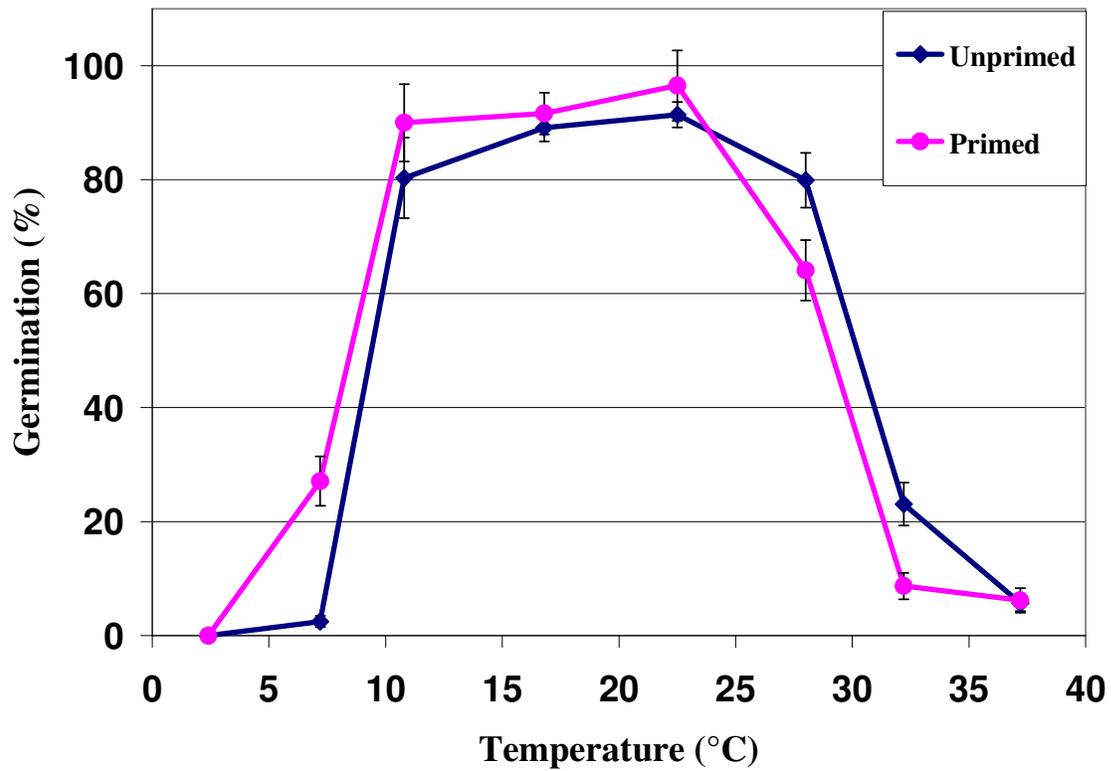
Figure 3-6: Priming effects on germination of seeds maintained at 10°C in a soil medium within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and kura clover (*Trifolium ambiguum* Bieb.; KC) seed as measured by the cold test (AOSA, 1988). GA1 = ‘Gerogia One’; PF = ‘Perfect Fit’. Significant priming × species ( $P > 0.10$ ), species ( $P < 0.04$ ), priming ( $P > 0.20$ ) treatments were observed.



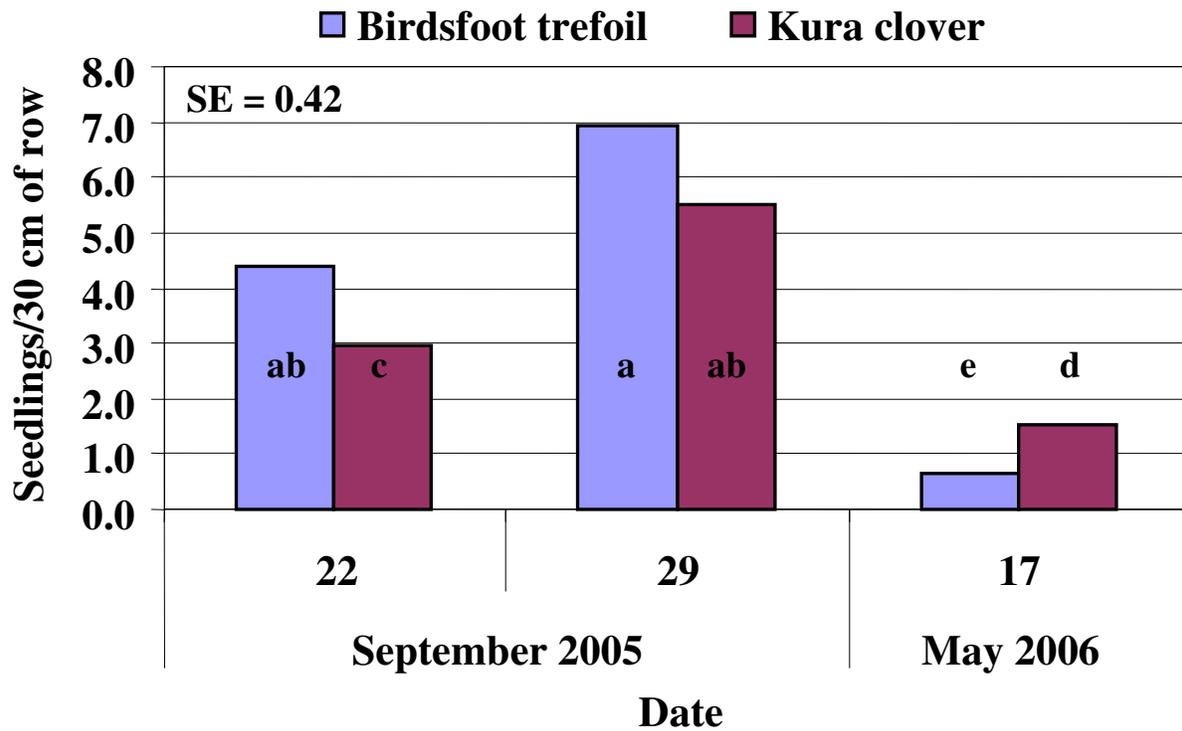
**Figure 3-7: Priming effects on germination of seeds held under anaerobic (vacuum) conditions within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and kura clover (*Trifolium ambiguum* Bieb.; KC) seed as measured by the vacuum test (Artola et al., 2004). GA1 = ‘Gerogia One’; PF = ‘Perfect Fit’. Significant priming × species ( $P>0.005$ ), species ( $P<0.001$ ), priming ( $P<0.05$ ) treatments were observed.**



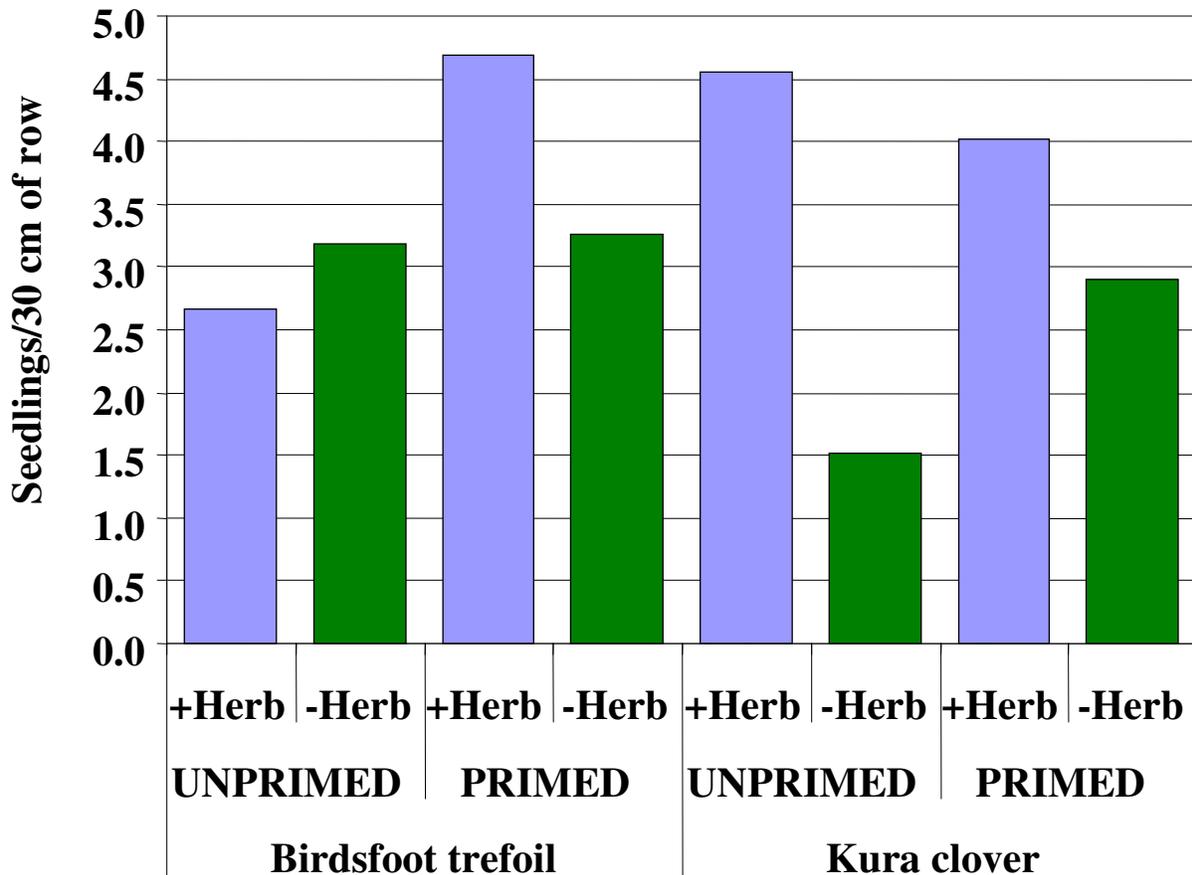
**Figure 3-8: Solid matrix priming effects for birdsfoot trefoil (*Lotus corniculatus* Bieb.) by temperature. The vertical bars represent the standard error of the mean of four replications.**



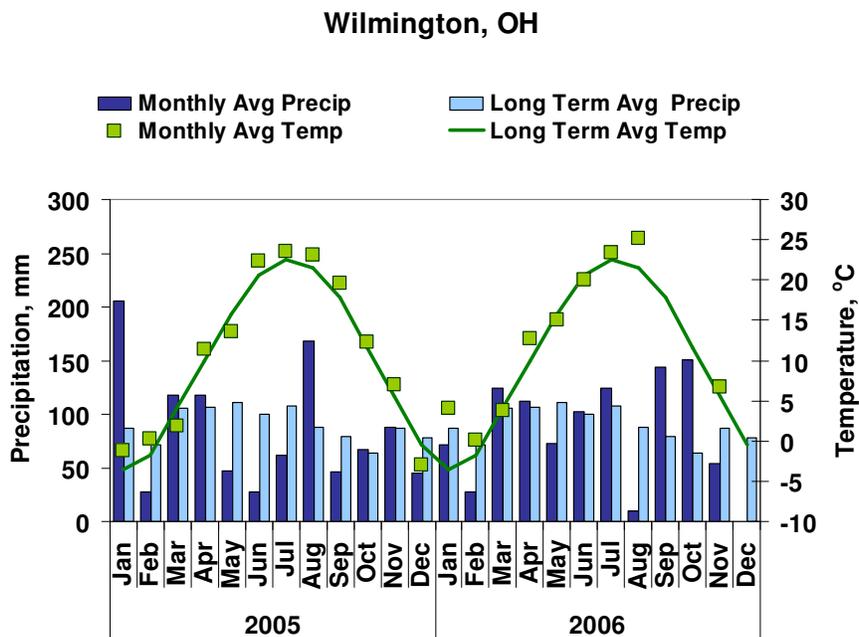
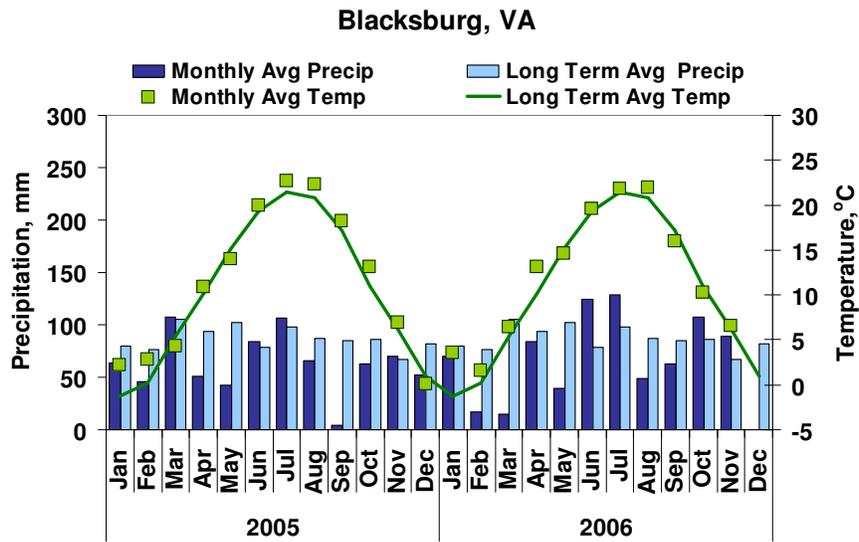
**Figure 3-9: Solid matrix priming effects for kura clover (*Trifolium ambiguum* L.) by temperature. The vertical bars represent the standard error of the mean of four replications.**



**Fig 3-10. Seedlings per 30 cm of row when measured at three dates after 01 September 2005 planting. Means across cultivar, herbicide, and priming treatments. Seeded no-till into existing sod with or without herbicide (2.26 kg a.i. ha<sup>-1</sup> Glyphosate, N-(phosphonomethyl) glycine) band applied in the row at time of seeding.**



**Fig 3-11. Seedlings per 30 cm of row when measured at three dates after 01 September 2005 planting. Means across three measurement dates (22 and 29 September 2005 and 17 May 2006). Seeded no-till into existing sod with or without herbicide (2.26 kg a.i. ha<sup>-1</sup> Glyphosate, N-(phosphonomethyl) glycine) band applied in the row at time of seeding.**



**Figure 3-12: Weather data for Blacksburg, VA and Wilmington, OH. Monthly average precipitation and temperatures recorded on Kentland Farm weather station (Blacksburg) and National Weather Service (Wilmington). Long term weather data for both locations from [www.worldclimate.com](http://www.worldclimate.com). Wilmington data not available for September and October 2006; no monthly average data available for December 2006.**

## Chapter 4: Osmotic Priming Effects on Legume Seed Germination

### ABSTRACT

The effects of osmotic priming have been well documented, and the treatment has been applied to a wide range of species. Benefits include faster germination rates and increased range of germination temperatures, which may allow more rapid germination under less than ideal conditions often encountered in the field. For this study, priming effects on germination and seed vigor of *Lotus corniculatus* L., *Trifolium ambiguum* Bieb., and *Lespedeza cuneata* (Dum. Cours.) G. Don. were tested under a wide range of conditions. Priming treatments generally did not affect final germination percentages ( $P>0.10$ ). However, there was some benefit to priming in terms of germination rates ( $P<0.01$ ), but varied by species with the most improvement seen in birdsfoot trefoil. Seed storage life was also decreased ( $P<0.01$ ), as indicated by the accelerated aging test. Increased vigor associated with vacuum stress was also indicated ( $P<0.001$ ), but less response from cold test ( $P<0.01$ ).

### Hypothesis

H<sub>0</sub>: Osmotic priming treatments for low vigor legume seeds will not affect seedling germination rate, final germination percent, and germination across a greater range of temperatures.

## **Objectives**

- 3) Determine response (germination rate and percent) of selected forage legumes – birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don – to two osmotic seed priming treatments over a range of temperatures.
- 4) Determine seed response to several seed vigor tests: conductivity test, cold test, accelerated aging test, and vacuum test.

## **MATERIALS AND METHODS**

### **Seeds**

Eight forage seedlots – four lots of birdsfoot trefoil, three lots of kura clover, and one lot of sericea lespedeza were chosen to test germination responses to two osmotic priming solutions. Seed tag information is shown in Appendix 1. Seeds were stored at ambient temperature in a laboratory (~25°C) prior to testing. Seed testing began in April 2006 and was completed by July 2006. All seed tests were conducted at Virginia Tech.

### **Osmotic Solutions and Priming Treatment**

Two osmotic solutions were made in the laboratory, monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and polyethylene glycol (PEG). Monopotassium phosphate solution (3%) was mixed (wt/wt basis) in distilled water by dissolving 7.5 g of  $\text{KH}_2\text{PO}_4$  in 250 ml distilled water. Polyethylene glycol solution was made by mixing 78.92 g of PEG 8000 and 250 ml distilled water based on the following equation of Michel (1983):

$$[\text{PEG}] = (4 - (5.16\Psi T - 560\Psi + 16)^{0.6}) / (2.58T - 280)$$

where [PEG] is the concentration of polyethylene glycol,  $\Psi$  is the water potential of the solution, and  $T$  is the temperature of the solution. The osmotic potentials of the priming solutions were -9.3 and -9.1 bars, for  $\text{KH}_2\text{PO}_4$  and PEG solutions, respectively, as measured by vapor pressure osmometer (Wescor 5500, Logan, UT).

Seeds were primed in 18-cm diameter round plastic Petri-type dishes lined with two sheets of germination paper. The paper was soaked with 15 ml of appropriate osmotic priming solution and then covered with a single layer of a seed. Lids were placed on top of dishes and the dishes were sealed air-tight with wax film to prevent evaporation and consequent increase in priming solution concentration. Dishes were stored at room temperature in a laboratory for three days. After day three, dishes were opened and seeds were thoroughly rinsed with distilled water, air dried on blotter paper for 12 h, and then placed in a sealed desiccator for 72 h to allow seeds to reach initial moisture content prior to priming. Primed seeds were stored in plastic test tubes fitted with lids until germination testing began.

### **Seed Testing**

Unless otherwise noted, four replications of all seeds were germinated in 9-cm plastic Petri-type dishes (Fisher Scientific) lined with one thickness of germination towel (Seedburo Equipment Co., Chicago, IL) moistened with 6 ml distilled water. After seeds were placed on the soaked germination towel, the dishes were closed with lids and put into 3.8-L plastic zip-type freezer bags and placed into an incubator (Percival; Boone, IA) at the appropriate temperature for the test. Counts of germinated seeds were made for 12 consecutive days (recommended

timeframe for birdsfoot trefoil, kura clover not specifically outlined, but similar clovers recommended at 7 d; 2 d period used to ensure adequate germination (AOSA, 1988). AOSA (1988) recommends a final seed count after 21 d of germination for sericea lespedeza; although, 12 d were used in this experiment to show seed vigor of these seeds in a short germination period. Seedlings were evaluated at approximately the same time each day (10:00 am). A seed was considered germinated when a normal radicle protruded from the seed coat, following the guidelines of AOSA (1988).

### **Vigor Tests**

*Accelerated Aging Test:* Metal cans (9 cm wide × 4.5cm deep) were filled with 40 ml of distilled water and covered with a single layer of plastic window screen. One layer sheet of Kimwipes™ (Kimberly-Clark, Neenah, WI) was placed on the screen mesh to prevent seeds from falling into the cans. Approximately 300 seeds were placed on top of the Kimwipes. The cans were then placed into an incubator at 40°C for 72 h to simulate an aging period on the seeds. After 72 h seeds were removed and four replicates of 50 seeds each were counted for each treatment and then transferred into germination dishes. Germination dishes were placed into an incubator at 20°C for 12 d, after which counts of normal germinated seeds were made.

*Cold Test:* A test medium was first prepared by mixing soil (type=Hayter) with sand (50:50 as-is basis) and a sample was oven dried (105°C) for 24 h to determine initial moisture content. Water holding capacity was then determined by placing the soil:sand mixture into a can (6 cm × 4 cm) with small holes in the bottom for drainage. The soil was saturated by adding water until

water ran through the holes in the bottom of the can. The can was then placed on a small rack within a sealed plastic bag to allow draining of the soil for 24 h in airtight conditions. After 24 h, the can was weighed and placed into an oven (105°C) for 24 h and reweighed. A calculation was made to determine the water holding capacity of the soil. Germination dishes were filled with 100 g of the soil mix, 50 seeds were pressed into each dish, and water was added to 70% water holding capacity of the soil mix. Germination dishes were then placed into 3.8-L (one-gallon) plastic freezer bags and placed into an incubator at 10°C for seven days. After seven days, the incubator temperature was raised to 20°C for five days to complete the 12-d total germination time. Normal germinated seeds were counted afterwards.

*Conductivity Test:* Four replicates of 25 seeds each were weighed and placed into 250 ml flasks, each containing 75 ml of distilled water. The seeds were gently stirred to ensure complete immersion and even distribution within the flask. Seeds were allowed to imbibe at 20°C for 24 h undisturbed. After 24 h, the seeds were gently stirred and the solution conductivity was measured using an electro-conductivity meter (Symphony, VWR, Batavia, IL) to determine the electrolyte leakage of the seeds. The dip cell was rinsed in distilled water after each measurement. Conductivity per gram of seed was then calculated.

*Standard Germination Test:* Germination dishes were placed into an incubator at 20°C for 12 d. Counts of normal germinated seedlings were made daily to determine mean time (days) to 50% germination ( $T_{50}$ ).

*Vacuum Test:* Seed lots were subjected to the vacuum test of Artola et al. (2004). Seeds were put in dishes then immediately placed into a vacuum desiccator which was then tightly sealed. Air was removed from the desiccator to a vacuum pressure of 0.06 MPa using a vacuum pump (Actron, Cleveland, OH). Vacuum was held stable for 5 min prior to sealing the desiccator valve. The desiccator was then placed into a germination chamber at 20°C, and seeds were allowed to germinate for 72 h. The desiccator was then opened and normal germinated seedlings were counted. Results are expressed as percentage of germinated seeds.

*Germination Temperature Tests:* Tests were run to compare the effects of seed priming over a range of temperatures. In the interest of time and the ability to control moisture, the gradient table was not utilized to test temperature range effects on osmotic primed seeds (as described with the matric priming study in Chapter 2). Instead, all seed lot × treatment combinations were tested simultaneously at each set temperature using one incubator. This allowed dishes to be sealed in zip-type plastic freezer bags (Kroger, Cincinnati, OH) to hold moisture, and therefore no additional water was required beyond the initial 6 ml. Incubators were set to test at one temperature at a time (5, 10, 15, 20, 25, 30, 35, or 40°C). Only three incubators were available in the laboratory for this use, therefore, as one temperature-test completed, another was begun, and therefore all germination-temperature-effects testing was completed within 36 d (3 incubators x 12 d per test). Temperature within incubators was recorded daily by a thermocouple Type K thermometer probe a thermocouple thermometer (Sper Scientific, Ltd., Scottsdale, AZ).

## STATISTICS

Statistical analysis was performed on arcsine transformed germination percentage data and log transformed time data. Seed germination tests and vigor tests were analyzed individually by test using a completely randomized design with a two-factorial arrangement of treatments for ANOVA utilizing the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Treatment factors were priming and legume species. Cultivars were nested within species. Data presented as mean germination (%) or  $T_{50}$  (mean germination time, days).

## RESULTS AND DISCUSSION

In the standard germination test, priming tended ( $P=0.10$ ) to reduce final germination of sericea lespedeza, and this effect was driven by reduced germination with the  $\text{KH}_2\text{PO}_4$  treatment. However, no priming effects (cultivar(species)\*treatment interaction) were observed with birdsfoot trefoil or kura clover (Figure 4-1). Caseiro et al. (2004) soaked onion seeds in PEG 8000 solutions (-0.5 or -1.0 MPa) for 24 or 48 h at 15°C and found that osmopriming did not affect final germination percentage or rate of germination. In contrast, Demir and Van de Venter (1999) found that osmotic priming (-0.9MPa) of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) seeds produced significantly higher final germination percentage (97.5%) than for the unprimed control (80%). Responses may also be dependent on cultivar as was observed with soft white winter wheat primed in KCl and PEG solutions (Giri and Schillinger, 2003). However, although one cultivar had greater germination in response to osmotic priming, neither field emergence nor grain yield was significantly benefited by any priming

media×cultivar combination, and the authors concluded that seed priming is of limited practical worth for soft white winter wheat.

Significant ( $P<0.002$ ) species×treatment interactions were observed for mean time to 50% germination ( $T_{50}$ ) (Figure 4-2). Both priming solutions stimulated birdsfoot trefoil to germinate approximately one day faster than unprimed seed. With kura clover, only the PEG treatment reduced  $T_{50}$  relative to unprimed seed, and  $T_{50}$  of sericea lespedeza was not affected by either priming solution. Although the magnitude of response is not as large, these data agree with those of Hardegree (1996), who reported a 4-d reduction in  $T_{50}$  for cheatgrass (*Bromus tectorum* L.) primed PEG 8000.

Significant species ( $P<0.01$ ) and species×treatment interactions ( $P<0.05$ ) on electrical conductivity were observed in the conductivity test (Figure 4-3). Higher conductivity readings indicate higher electrolyte leakage from cell membranes and hence lower vigor seeds. Birdsfoot trefoil seed had greater leakage than kura clover and sericea lespedeza (338 vs. 228 and 190 umhos, respectively). No differences in electrolyte leakage due to priming were observed with birdsfoot trefoil despite a large numerical increase for conductivity resulting from  $\text{KH}_2\text{PO}_4$  priming treatment. Treatment with PEG lowered ( $P<0.05$ ) conductivity readings for kura clover, suggesting PEG reduced membrane leakage below that of unprimed seeds. Priming with  $\text{KH}_2\text{PO}_4$  increased conductivity of sericea lespedeza solutions, probably due to leaching of some  $\text{KH}_2\text{PO}_4$  still in seeds into the solution, but no difference between PEG and unprimed seeds was observed. These results suggest  $\text{KH}_2\text{PO}_4$  damaged lespedeza seed membranes and would reduce storability after priming.

Results for the accelerated aging test (Figure 4-4) were affected by significant species ( $P<0.03$ ), priming treatment ( $P<0.001$ ), and species $\times$ priming treatment interactions ( $P<0.01$ ). Reductions in germinability were greater with  $\text{KH}_2\text{PO}_4$  for birdsfoot trefoil and kura clover.

Of the germination tests used in this study, the cold test is perhaps the best indicator of seed responses in the field in spring. This test is similar to field conditions, with seed germinated in actual soil from the field which contains microorganisms and other natural components. Significant species, treatment, and species $\times$ treatment interactions ( $P<0.0001$ ) were seen with the cold test (Figure 4-5). Kura clover had greater germination than birdsfoot trefoil and most sericea lespedeza-treatment combinations. Only priming with PEG increased germination and that only with sericea lespedeza seeds.

Significant effects of species, treatment, and species $\times$ treatment interaction ( $P<0.001$ ) were observed for seeds germinated under vacuum (Figure 4-6). The vacuum test is designed to mimic anaerobic stress effects on seed germination. Under these conditions, priming had no effect on kura clover. With birdsfoot trefoil, seeds responded positively to priming when the osmoticum was  $\text{KH}_2\text{PO}_4$  but not to PEG. However, sericea lespedeza seeds were very responsive to both priming solutions when seeds were germinated in vacuum..

When primed and unprimed seeds were germinated over a range of temperatures (5 to 40°C) in 5-degree increments, priming treatments generally affected germination percentage only at the temperature extremes (Figs. 4-7 and 4-8). Priming was ineffectual across the broad range of temperature optima (15 to 25°C). At 5°C, primed birdsfoot trefoil seeds had much greater ( $P<0.04$ ) germination percentage than unprimed seeds (Fig 4-7). Birdsfoot trefoil germination increased 50% over unprimed seeds at 5°C with no difference between priming

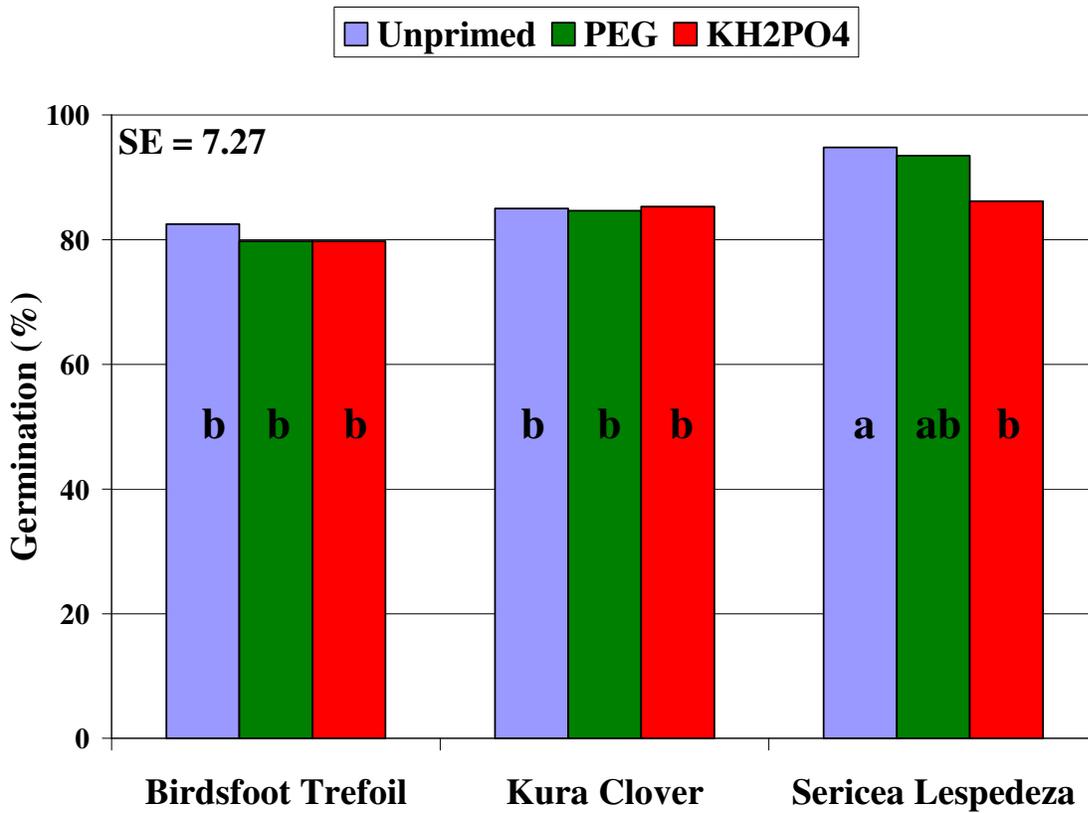
solutions. No differences for birdsfoot trefoil were seen at temperatures from 10° to 25°C ( $P>0.10$ ). At 30°C PEG-primed birdsfoot trefoil seed germination was greater by four percentage units. Germination percentage for  $\text{KH}_2\text{PO}_4$ -primed seed were not statistically different from either control or PEG-primed seeds. At 35°C this pattern for response to priming was reversed. Priming with  $\text{KH}_2\text{PO}_4$  increased ( $P<0.05$ ) birdsfoot trefoil germination by 10 percentage units. Although total germination with PEG or  $\text{KH}_2\text{PO}_4$  did not differ from each other, only values for  $\text{KH}_2\text{PO}_4$  treatments differed significantly from unprimed seeds. At 40°C, seed germination was greatly reduced relative to temperature optimum. At this extreme temperature, primed seed had two-fold or greater ( $P<0.001$ ) germination percentage relative to control seeds. Although germination percents were numerically 10 percentage units greater for PEG, priming solutions did not differ.

Similar results were observed with kura clover seed lots but the responses were smaller in magnitude (Fig 4-8). Both priming solutions increased ( $P<0.001$ ) germination over unprimed seeds at 5°C. At 30°C, germination of PEG- and  $\text{KH}_2\text{PO}_4$ -primed seed did not differ from each other, but only values for PEG-treated seed were greater ( $P<0.05$ ) than unprimed controls. Interestingly, no differences due to priming were observed at 35°C for kura clover, but  $\text{KH}_2\text{PO}_4$  priming did increase germination over unprimed seeds at 40°C.

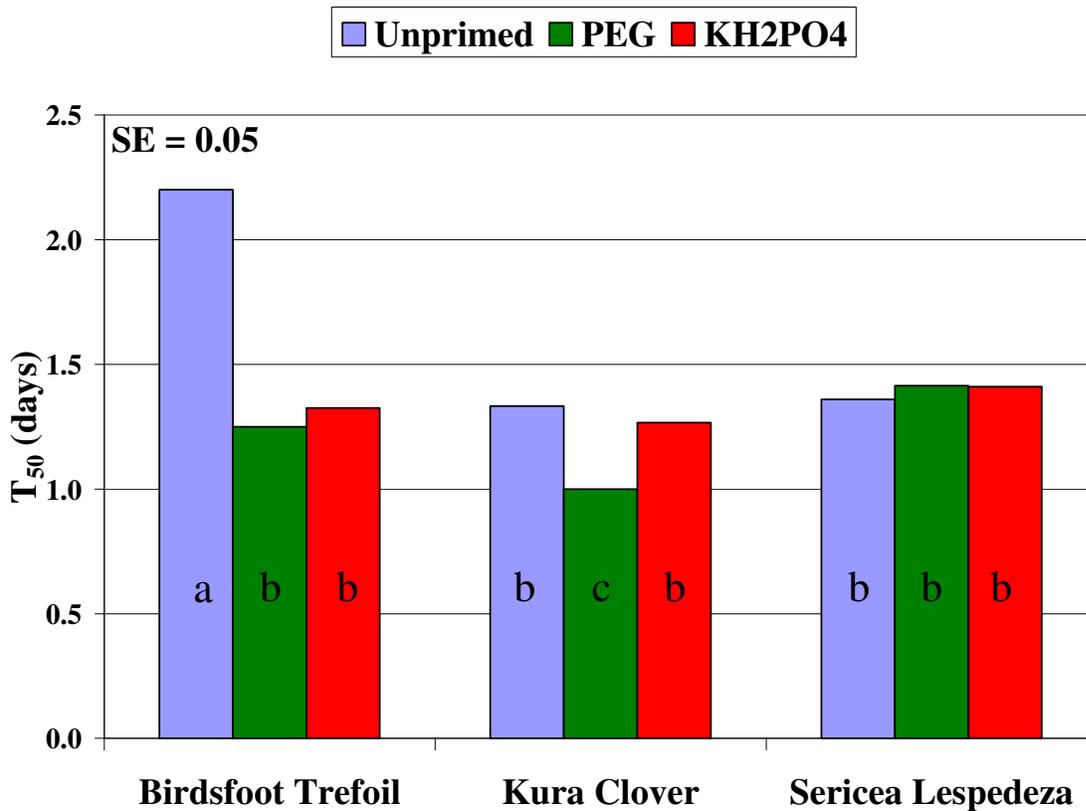
Across the range of temperatures, priming affected AuGrazer seed germination only at 10°C. Both priming solutions increased germination from 48% (unprimed) to 79% and 76% for PEG and  $\text{KH}_2\text{PO}_4$ , respectively ( $P<0.01$ ). Lack of response at higher temperatures may reflect the fact that lespedeza is a warm-season legume, while 5°C was too cold for priming to be effectual.

## **CONCLUSIONS**

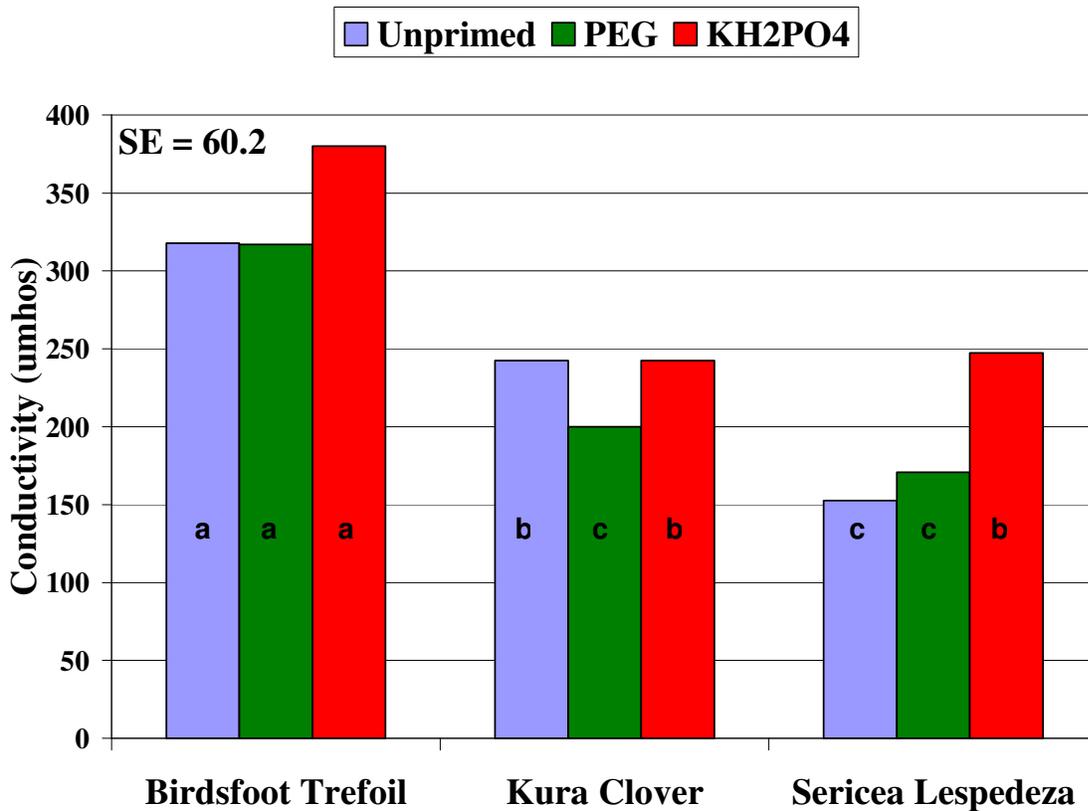
Priming treatments generally did not affect final germination percentages. However, there was some benefit to priming in terms of germination rates, though varied by species with most improvement seen in birdsfoot trefoil. Seed storage life was also decreased in response to priming (accelerated aging test). While priming can positively affect legume seed germination, the limited responses suggest this technique is unlikely to benefit legume establishment in existing pastures under most conditions.



**Figure 4-1: Priming effects on laboratory germination of birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) seed at 20°C using standard germination test procedures (AOSA, 1988). Significance of priming × species ( $P>0.20$ ), species ( $P<0.0001$ ), priming ( $P>0.30$ ) treatments were observed.**

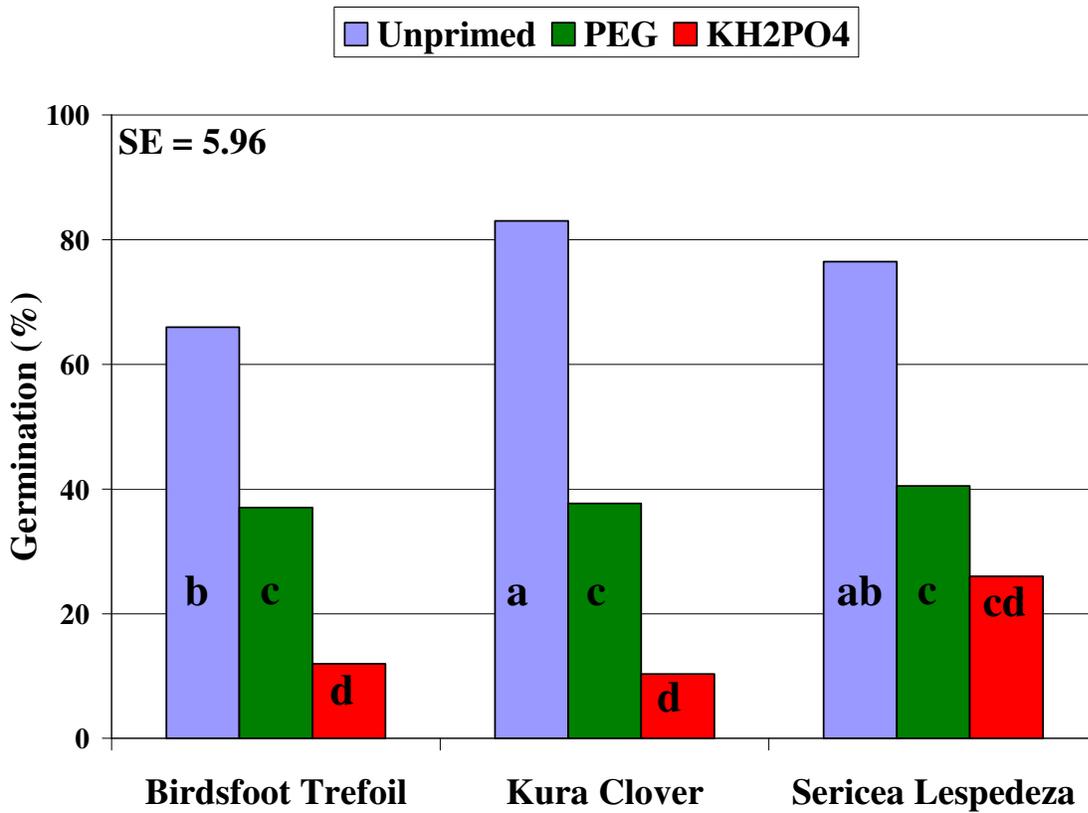


**Figure 4-2: Priming effects on germination rates of birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) seed at 20°C using standard germination test procedures (AOSA, 1988). Significance of priming × species ( $P < 0.002$ ), species ( $P < 0.001$ ), priming ( $P < 0.02$ ) treatments were observed.**

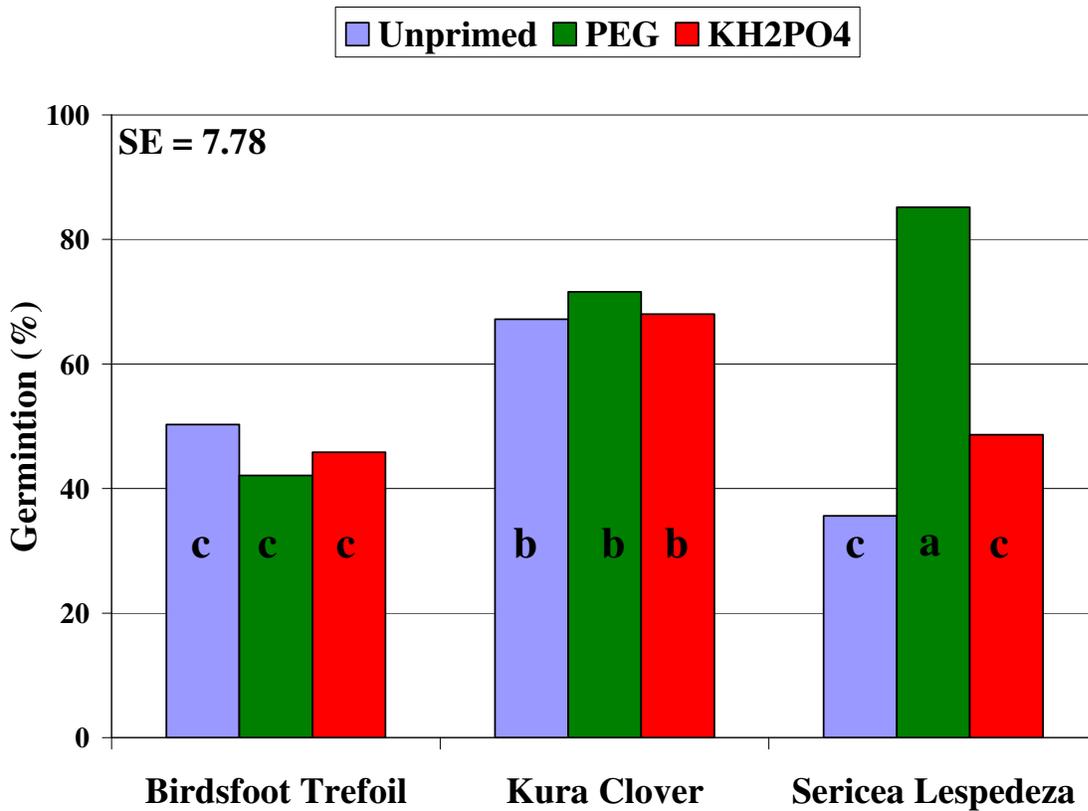


**Figure 4-3: Priming effects on solution conductivity within cultivars of birdsfoot trefoil**

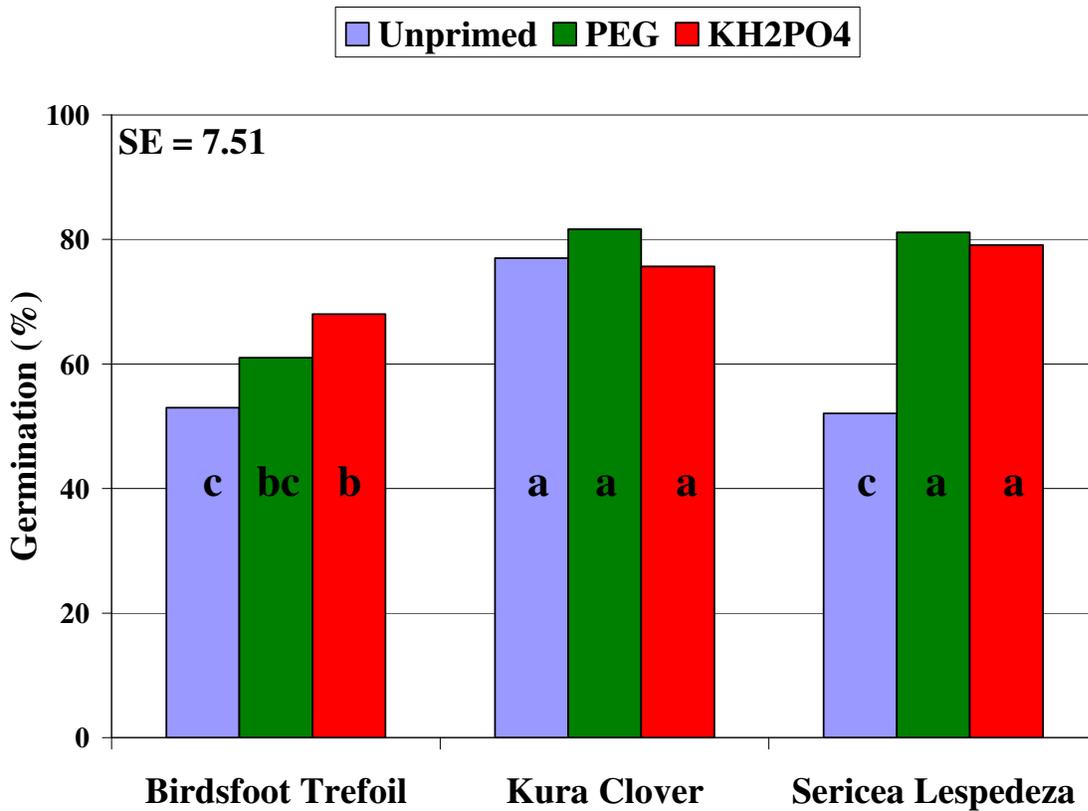
*(Lotus corniculatus L.)*, kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don). Seeds were soaked for 24 h in distilled water prior to conductivity measurements. Significant priming × species ( $P < 0.05$ ), species ( $P < 0.004$ ), priming ( $P > 0.20$ ) treatments were observed.



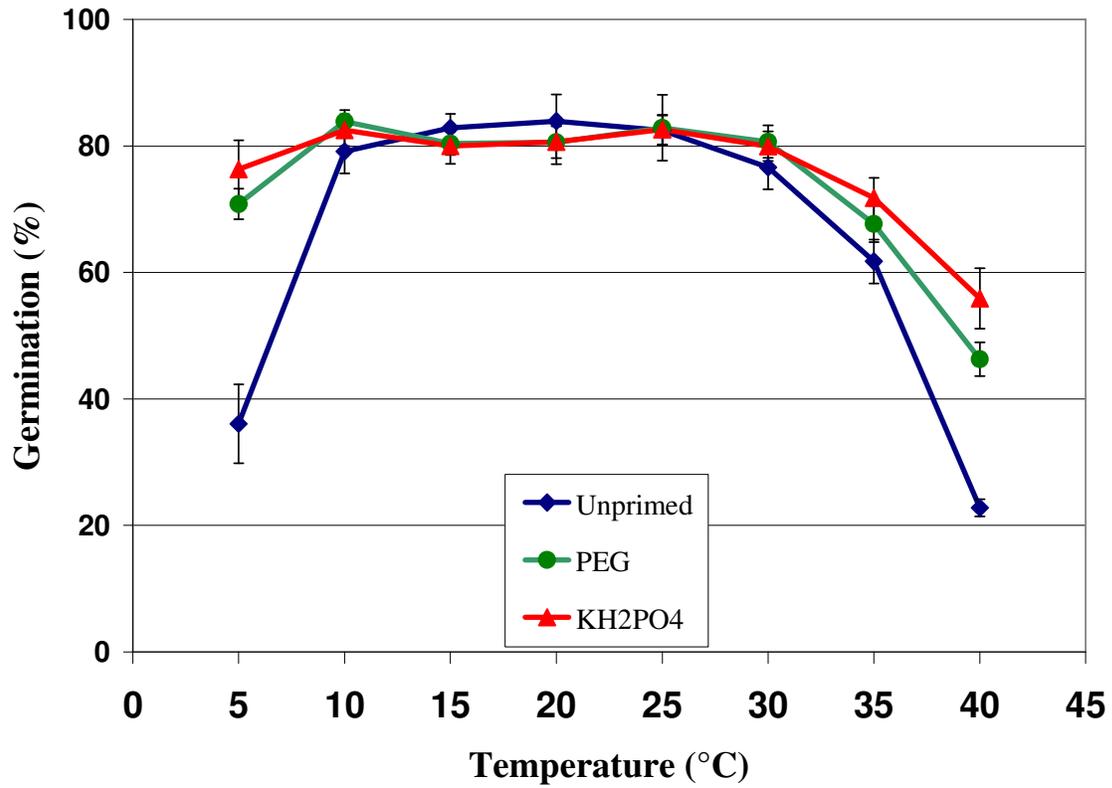
**Figure 4-4: Priming effects on germination after accelerated aging within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) seed. Significant priming × species ( $P < 0.01$ ), species ( $P < 0.02$ ), priming ( $P < 0.0001$ ) treatments were observed.**



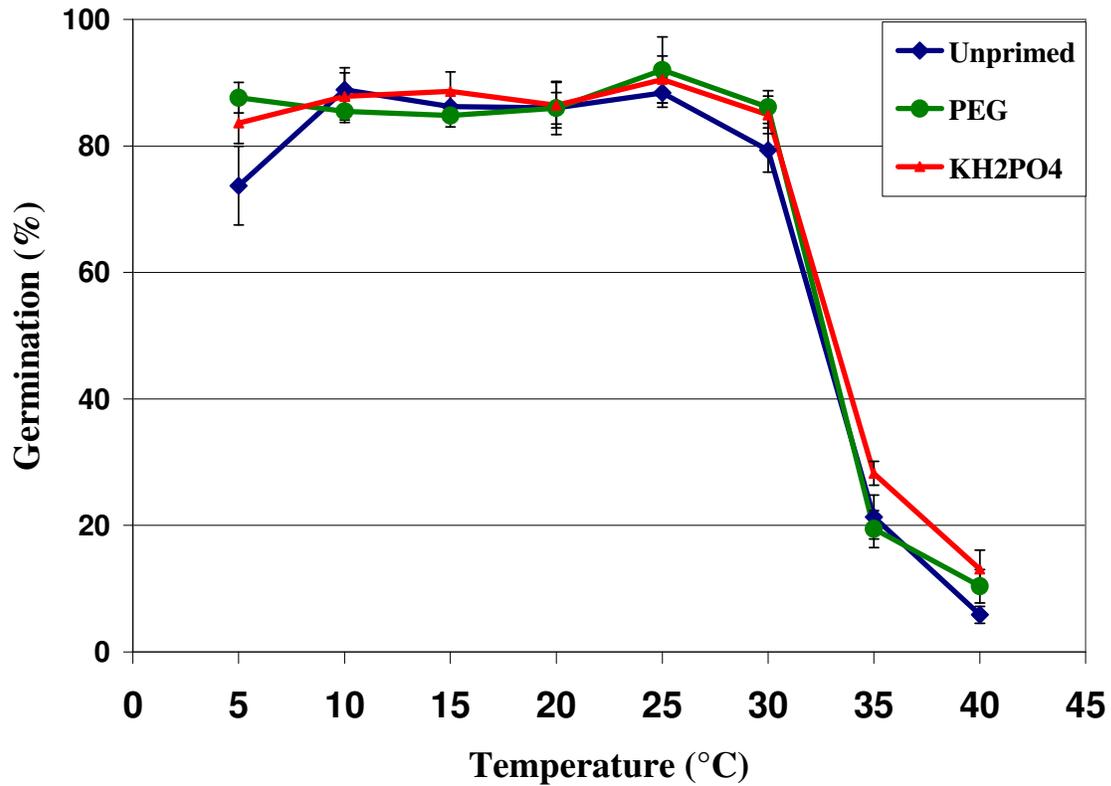
**Figure 4-5: Priming effects on germination of seeds maintained at 10°C in a soil medium within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) seed as measured by the cold test (AOSA, 1988). Significant priming × species ( $P < 0.01$ ), species ( $P < 0.02$ ), priming ( $P < 0.001$ ) treatments were observed.**



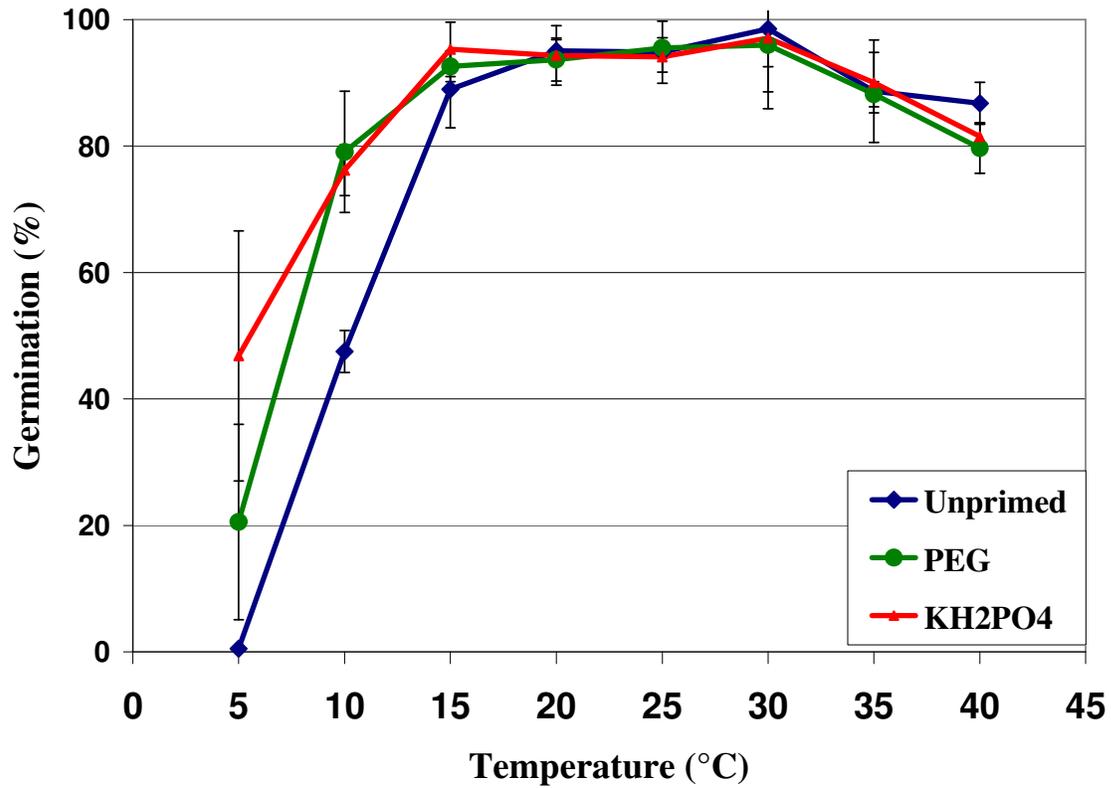
**Figure 4-6: Priming effects on germination of seeds held under anaerobic (vacuum) conditions within cultivars of birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) seed as measured by the vacuum test (Artola et al., 2004). Significant priming × species ( $P < 0.007$ ), species ( $P < 0.0001$ ), priming ( $P < 0.0001$ ) treatments were observed.**



**Figure 4-7: Birdsfoot trefoil (*Lotus corniculatus* L.) final (d-12) germination percentages in response to priming treatments. Germination was tested over a range of temperatures (5 to 40°C; 5° increments). Bars designate standard errors at each temperature.**



**Figure 4-8: Kura clover (*Trifolium ambiguum* Bieb.) final (d-12) germination percentages in response to priming treatments. Germination was tested over a range of temperatures (5 to 40°C; 5° increments). Bars designate standard errors at each temperature.**



**Figure 4-9: *Sericea lespedeza* (*Lespedeza cuneata* (Dum. Cours.) final (d-12) germination percentages in response to priming treatments. Germination was tested over a range of temperatures (5 to 40°C; 5° increments). Bars designate standard errors at each temperature.**

## Chapter 5: Smoke Water Effects on Legume Seed Germination

### ABSTRACT

An experiment was conducted to evaluate germination responses of three forage legumes species to aqueous smoke solution treatments. Commercially available cultivars of birdsfoot trefoil, kura clover, and sericea lespedeza were tested. Two smoke solutions were created by bubbling smoke from smoldering wheat straw through distilled water for either 10 or 45 min. Each solution was then diluted with distilled water to three concentrations prior to germination tests. Differences in final germination percent due to solution type (10- or 45-min) were not observed. Highest concentration of the 10-min solution treatment reduced ( $P<0.05$ ) birdsfoot trefoil germination percentage about 10 percentage units, and degree of response varied by cultivar. Greater germination with smoke water was only observed for 'Perfect Fit' kura clover treated with low or intermediate concentrations of either solution. High concentrations of 10-min smoke water increased time to 50% germination ( $T_{50}$ ) for all seeds, but some reduction in  $T_{50}$  occurred for kura clovers treated with low (5%) solution concentrations. The 45-min treatments had little effect on germination rates: only sericea lespedeza had greater  $T_{50}$  when germinated with these solutions. Applying aqueous smoke solution to seeds at germination did not improve germination responses of these forage legume species.

### Hypothesis

$H_0$ : Smoke water treatments for low vigor legume seeds will not affect seedling germination rate and germination percent.

## **Objectives**

- 5) Determine response (germination rate and percent) of three forage legumes – birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don – to smoke water treatment.
- 6) Compare seed response to differing smoke water solutions (based on synthesis time and solution concentrations).

## **MATERIALS AND METHODS**

### **Seeds**

Eight forage seedlots – four lots of birdsfoot trefoil, three lots kura clover, and one lot sericea lespedeza were chosen to test germination responses to a smoke water solution. Seed tag information is shown in Appendix 1. Seeds were stored at ambient temperature in a laboratory (~25°C) prior to testing the effects of the treatment. Seed testing began in April 2006 and was completed by July 2006. All seed tests were conducted at Virginia Tech.

### **Seed Testing**

Fifty seeds per treatment replicate were germinated in 9-cm plastic Petri-type dishes (Fisher Scientific) lined with one thickness of germination towel (Seedburo Equipment Co., Chicago, IL) moistened with 6 ml distilled water or appropriate concentration of a smoke solution. After seeds were placed on the soaked germination towel, the dishes were closed and put into 3.8-L plastic zip-type freezer bags and placed into an incubator (Percival; Boone, IA) at 20°C. Counts of germinated seeds were made for 12 consecutive days (recommended timeframe

for birdsfoot trefoil, kura clover not specifically outlined, but similar clovers recommended at 7 d; 2 d period used to ensure adequate germination (AOSA, 1988). AOSA (1988) recommends a final seed count after 21 d of germination for sericea lespedeza; although, 12 d were used in this experiment to show seed vigor of these seeds in a short germination period. Seedlings were evaluated at approximately the same time each day (10:00 am). A seed was considered germinated when a normal radicle protruded from the seed coat, following the guidelines of AOSA (1988).

### **Smoke Water**

An apparatus for creating the smoke solutions was assembled following the principles outlined by G. J. Farley (2005) (Figure 5-1). A Pyrex desiccator was fitted with a wire mesh bottom on which wheat straw (*Triticum aestivum* L.) was placed as a burning medium. The lid was set ajar to allow air to enter the desiccator. The lid of the desiccator was fitted with a rubber stopper with two glass tubes. A vacuum to pull smoke out of the desiccator was applied to one tube which extended into the desiccator above the burning wheat. The second tube extended below the wire mesh and wheat straw, allowing external air to enter below the burning wheat. A flask with 500 mL distilled water was placed near the desiccator and fitted with a rubber stopper and two glass tubes. The line from the desiccator was connected to a tube in the flask which extended below the water surface. The second tube remained above the water surface and was connected to a vacuum pump (W.M. Welch Manufacturing Co., Chicago, IL). This apparatus allowed the smoke created from smoldering wheat straw to bubble through the water in the flask.

Two initial solutions of smoke water were created by bubbling smoke through the water for either 10 min (Farley, 2005) or 45 min (following procedures of Sparg et al. (2005). The two solutions were then diluted to make three concentrations of each for use in seed germination studies. The 10-min solution was diluted with distilled water to make 5%, 10%, and 15% concentrations. The 45-min solution was diluted with distilled water to make 1:250 (0.4%), 1:500 (0.2%), and 1:1000 (0.1%) concentrations.

## **STATISTICS**

Analysis of variance was used to test the effects of smoke water preparation method and species on germination response using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). The experiment was conducted as a completely randomized design with a  $2 \times 3$  factorial arrangement of treatments with smoke water concentrations nested within preparation methods, and cultivars nested within legume species. Treatment factors were priming and legume species. Single degree of freedom contrasts for linear, quadratic, and cubic effects of increasing smokewater concentrations were also tested. Statistical analyses were performed on arcsine transformed germination percentage data and log transformed time data. Data are presented as final germination (%) or  $T_{50}$  (mean time (days) to 50% germination).

## **RESULTS**

Across all factors, smoke water effects on total germination were not observed ( $P=0.17$ ) but were obscured by treatment $\times$ species interaction ( $P<0.05$ ). Across all legume species, germination percentage generally was reduced ( $P<0.05$ ) with increasing smoke water

concentrations. Table 5-1 shows that germination percentage was greatest ( $P<0.001$ ) for sericea lespedeza (88.4%), followed by kura clover (75.4%) and birdsfoot trefoil (66.9%). Because cultivar effects ( $P<0.001$ ) were nested within species treatments, data were tested by cultivar to fully explore their relationships to treatment concentrations.

Of the eight seedlot×treatment solution combinations ( $n=16$ ), only six responded to treatment, and responses generally were negative. Germination percentage of ‘AU Grazer’ sericea lespedeza tended ( $P=0.08$ ) to decrease linearly with increasing concentration of the 10-min solution, but germination was unaffected by the 45-min solution treatments. ‘Georgia 1’ birdsfoot trefoil did not respond to treatment with 10-min solutions, but a quadratic ( $P<0.05$ ) pattern of lower germination at intermediate concentrations was observed for seeds germinated in 45-min solutions.

Only ‘Dawn’ birdsfoot trefoil and ‘Perfect Fit’ kura clover exhibited responses to both smoke water treatments (Table 5-1). Dawn germination percent was linearly decreased ( $P<0.01$ ) with increasing concentration of 10-min smoke water solution. This resulted in a 20 percentage unit reduction in germination at the highest smoke water concentration compared with untreated controls. With the 45-min solution, quadratic and cubic tendencies ( $P=0.07$ ) for germination of Dawn occurred due to much lower germination when seeds were treated with the 0.2% solution (Table 5-1).

Of all seeds tested, only ‘Perfect Fit’ kura clover seed exhibited a positive response to treatments. Perfect Fit tended ( $P=0.07$ ) to have a positive quadratic pattern of response to treatment with 10-min solutions. Highest germination occurred with the 10% solution concentration. With the 45-min treatment, germination patterns across solution concentrations

were quadratic and cubic ( $P<0.01$ ) due to elevated and intermediate germination totals with the 0.1 and 0.2 solution concentrations, respectively.

Equally important to final germination percentage is the rate at which seeds germinate. Germination rate comparisons were based on the time (in days) required for 50% of the seeds to germinate ( $T_{50}$ ). Treatments with smaller values require fewer days to reach  $T_{50}$ , and thus have faster rates of germination.

Treatment, species, and treatment×species interaction were significant ( $P<0.001$ ) sources of variation in the model. Across species, smoke water treatments increased  $T_{50}$  (Table 5-2) by about 0.5 d (1.82 vs. 2.35 d). Kura clover seeds reached  $T_{50}$  about a day faster than birdsfoot trefoil and sericea lespedeza (1.35 vs. 2.45 and 2.69 d, respectively). However, smoke water treatments did not affect  $T_{50}$  for kura clover (1.34 d) but increased  $T_{50}$  by 0.5 d for birdsfoot trefoil (2.19 vs 2.74 d) and 1.5 d for sericea lespedeza (2.03 vs. 3.56 d). Nested effects of solution concentration within smoke water treatments ( $P<0.001$ ) and cultivar within species ( $P<0.05$ ) were also observed. Thus data are presented for each cultivar by treatment combination (Table 5-2).

The 10-min smoke water solutions had much greater ( $P<0.0001$ ) effect on seed germination than the 45-min solutions. With 10-min solutions, linear increases ( $P<0.05$ ) for  $T_{50}$  were observed with all species and cultivars. This response was driven by a consistent, large reduction in germination rate associated with 15% solution treatment. No concentrations were beneficial to germination rates of birdsfoot trefoil or sericea lespedeza ( $P>0.10$ ). However, patterns of quadratic ( $P<0.10$ ) response for kura clover cultivars, – with reduced  $T_{50}$  at low concentrations – suggest low doses of smoke water may benefit germination rate of this species

The 45-min solution affected germination rate only for 'Empire' birdsfoot trefoil due to much slower germination with the 0.4% solution treatment. In contrast, kura clover seeds (Cossack' (seedlot 2) and Perfect Fit) responded positively to smoke water treatments. All smoke solutions increased  $T_{50}$  for sericea lespedeza.

## DISCUSSION

Germinating birdsfoot trefoil or sericea lespedeza in smoke water solutions generally failed to promote increased germination rates or final germination percentages. Limited benefits of smoke water treatments were observed with kura clover, although this was highly seedlot-dependent.

The medium used to make smoke (wheat straw) may have been a factor in the limited response to smoke water treatments. No other literature has reported use of wheat straw as a smoke source, thus its efficacy has not been proven. However, Jager et al. (1996) demonstrated that efficacious aqueous smoke extracts could be made from a variety of plant sources, and extracts derived by heating agar and cellulose also contained agents which stimulated germination of light-sensitive lettuce seeds (*Lactuca sativa* L.) The active germination stimulant was later identified as butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) by Flematti et al. (2004). Butenolide is derived from burning cellulose, and wheat straw, like all other plants, contains cellulose (Jager et al., 1996a). Thus, it is doubtful that the use of wheat straw limited the release of butenolide into smoke water solutions. However, whether there are other agents in wheat straw smoke which might affect germination or the efficacy remains unknown.

The lack of germination stimulation in this study in terms of final germination percentage in response to smoke treatment disagrees with much current literature. For example, the same 45-min smoke solution concentrations used in this experiment significantly increased the final germination percentage of corn (*Zea mays*) compared to unexposed (control) seeds (Sparg et al., 2006). Seeds of African flowers (*Othonna quinquedentata*, *Senecio grandifloris*, and *Syncarpha eximia*) also germinated faster than untreated seed when treated with an aqueous smoke solution, although final germination percentages were not different between treatments (Brown, 1993). Greater rate and total percent emergence of seedlings from soil have been reported for North American range grasses (Thurber's needlegrass (*Achnotherum thurberianum* (Piper) Barkworth) and needle-and-thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth)) treated with a smoke solution (Blank and James, 1998).

Despite these other findings, the lack of germination response to aqueous smoke treatments in this experiment should not be too surprising. In studies of 221 species from 10 plant families, only 54% of those treated with smoke had increased seed germination (Van Staden, et al., 2000; Brown, 1993; Brown, 1997). Further, some limited evidence of response for the legumes tested in this study indicates smoke water treatments may have efficacy if suitable parameters for smoke treatments and seed suitability can be defined. However, the variable response by and among these legume species, cultivars, and seedlots suggests this may be difficult.

**Table 5-1. Forage legume seed germination in response to smoke water concentrations within two preparation methods‡.**

Preparation method and species¶	Legume variety	Solution concentration, %				SE	P		
		0	5	10	15		Linear	Quadratic	Cubic
<b>10-min</b>		————Germination, %§————							
Birdsfoot trefoil	Dawn	73.3	73.1	65.1	53.3	0.17	**	NS	NS
	Empire	56.4	50.7	52.2	46.4	0.16	NS	NS	NS
	Georgia 1	83.8	82.0	84.9	78.4	0.14	NS	NS	NS
	Norcen	60.1	61.4	64.9	53.2	0.19	NS	NS	NS
Kura clover	Cossack††	75.7	81.4	77.8	79.4	0.30	NS	NS	NS
	Cossack2	77.7	80.9	82.5	76.1	0.41	NS	NS	NS
	Perfect Fit	68.2	72.6	77.6	71.4	0.09	NS	†	NS
Sericea lespedeza	AU Grazer	90.3	84.7	83.0	83.1	0.16	†	NS	NS
<b>45-min</b>		0	0.1	0.2	0.4	SE			
		————Germination, %§————							
Birdsfoot trefoil	DAWN	73.3	70.8	57.8	72.5	0.20	NS	†	†
	Empire	56.4	55.9	53.5	52.5	0.22	NS	NS	NS
	Georgia 1	83.8	78.1	67.1	80.5	0.19	NS	*	NS
	Norcen	60.1	67.0	61.8	60.2	0.11	NS	NS	NS
Kura clover	Cossack 1	75.7	71.7	79.8	78.6	0.33	NS	NS	NS
	Cossack 2	77.7	70.7	74.9	74.0	0.24	NS	NS	NS
	Perfect Fit	68.2	83.9	74.0	74.2	0.07	NS	**	**
Sericea lespedeza	AU Grazer	90.3	93.1	84.7	87.5	0.39	NS	NS	NS

†,\*,\*\* denote significance at  $P < 0.1$ ,  $0.05$ , and  $0.01$

‡Smoke from smoldering wheat straw was bubbled through water for 10 (10-min) or 45 (45-min) min. ¶Species tested include birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don.

§Statistical analysis was performed on arcsine transformed germination percentage data.

††Two different seed lots of Cossack kura clover were tested.

**Table 5-2. Forage legume seed time to 50% (T<sub>50</sub>) germination in response to smoke water concentrations within two preparation methods‡.**

Preparation method and species¶	Legume variety	Solution concentration, %				SE	P		
		0	5	10	15		Linear	Quadratic	Cubic
<b>10-min</b>		T <sub>50</sub> , d§							
Birdsfoot trefoil	Dawn	2.48	3.03	3.51	6.08	1.08	***	*	NS
	Empire	2.03	2.51	3.51	4.20	1.10	***	NS	NS
	Georgia 1	2.03	2.74	2.74	3.34	1.13	*	NS	NS
	Norcen	2.24	3.04	2.49	4.74	1.10	**	†	**
Kura clover	Cossack††	1.21	1.05	2.24	2.24	1.12	***	NS	**
	Cossack2	1.44	1.04	2.05	2.25	1.12	**	†	**
	Perfect Fit	1.44	1.05	1.46	2.51	1.16	*	*	NS
Sericea lespedeza	AU Grazer	2.03	4.04	3.98	5.03	1.05	***	***	**
<b>45-min</b>		T <sub>50</sub> , d				SE			
Birdsfoot trefoil	Dawn	2.48	3.25	2.48	2.48	1.11	NS	NS	NS
	Empire	2.03	2.03	2.04	2.49	1.06	*	NS	NS
	Georgia 1	2.03	2.03	2.03	2.02	1.00	NS	NS	NS
	Norcen	2.24	2.03	2.03	2.03	1.05	NS	NS	NS
Kura clover	Cossack1	1.21	1.22	1.03	1.03	1.13	NS	NS	NS
	Cossack2	1.44	1.21	1.03	1.03	1.14	†	NS	NS
	Perfect Fit	1.44	1.02	1.03	1.03	1.11	*	NS	NS
Sericea lespedeza	AU Grazer	2.03	2.74	3.03	3.03	1.05	***	*	NS

†, \*, \*\*, \*\*\* denote significance at  $P < 0.1, 0.05, 0.01,$  and  $0.001.$

‡Smoke from smoldering wheat straw was bubbled through water for 10 (10-min) or 45 (45-min) min. ¶Species tested include birdsfoot trefoil (*Lotus corniculatus* L.), kura clover (*Trifolium ambiguum* Bieb.), and sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don.

§Statistical analysis was performed on natural log transformed data for days to 50% germination.

††Two different seed lots of Cossack kura clover were tested.



**Figure 5-1: Apparatus for creating smoke solutions**

## Chapter 6: Conclusions

Seed responses to matric priming are variable by species and seedlot within species, as seen in the four seed vigor tests (conductivity test, cold test, accelerated aging test, vacuum test). Only one seed (Georgia1) showed a faster rate of germination at 20°C due to matric priming treatment. Some benefits in terms of final germination percentage exist for kura clover when germination is evaluated over a range of temperatures (2.4-37.2°C), while greater benefit exists for birdsfoot trefoil. In the field, however, solid matrix priming treatment had only limited effect on seedling emergence when sown in existing pastures.

Osmotic priming treatments generally did not affect final germination percentages for birdsfoot trefoil and kura clover as measured in standard germination and temperature-range tests. *Sericea lespedeza* final germination was negatively affected by the  $\text{KH}_2\text{PO}_4$  treatment. Both PEG and  $\text{KH}_2\text{PO}_4$  benefited birdsfoot trefoil by decreasing  $T_{50}$  by approximately one day. Only PEG affected  $T_{50}$  of kura clover, but the change was small (0.33 d) and *sericea lespedeza* germination was unaffected by priming. When evaluated under accelerated aging conditions, priming significantly reduced germination after storage, indicating a significant negative effect on shelf life of primed seeds. In contrast, two cultivars indicated a positive effect on shelf life of solid matrix primed seeds. While not always statistically significant,  $\text{KH}_2\text{PO}_4$  caused numeric reductions in germinability and vigor of seeds held under stressful conditions. Both birdsfoot trefoil and *sericea lespedeza* exhibited increased germination under vacuum stress as a result of priming, but kura clover did not. While priming can positively affect legume seed germination, the limited responses suggest priming is unlikely to be beneficial for legume establishment in existing pastures.

Seed response to smoke water treatments were very limited, and often negatively affected seed germination percentage and rates at greater concentrations. Different results might be possible if a known butenolide solution was used, as butenolide presence nor concentration were measured in this study. Additionally, a different technique for treating seeds with the smoke, either in solution or perhaps aerosol smoke may show different results, as well as testing at other concentrations of aqueous solution. Aqueous smoke solutions of the types used in this study are not suitable for increasing seed germination performance of birdsfoot trefoil, kura clover, or sericea lespedeza.

The practical benefits of either priming treatment on these legume species is of low value, and once the additional time, labor, and expense is paid for the priming procedures, any of these limited gains are quickly diminished. No results from any priming or smoke treatment in the laboratory suggested a practical benefit could be obtained for use with planting in the field. Under current conditions, these treatments could not be recommended.

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**Appendix 1: Seed Labels for Priming and Smoke Water Experiments**

	<b>Birdsfoot Trefoil</b>				<b>Kura Clover</b>			<b>Sericea Lespedeza</b>
	<u><b>Dawn</b></u>	<u><b>Georgia - One</b></u>	<u><b>Norcen</b></u>	<u><b>Empire</b></u>	<u><b>Perfect Fit</b></u>	<u><b>Cossack (#1)</b></u>	<u><b>Cossack (#2)</b></u>	<u><b>AuGrazer</b></u>
<b>Germination</b>	<b>95</b>	<b>88</b>	<b>94</b>	<b>81</b>	<b>84</b>	<b>81</b>	<b>84</b>	<b>80</b>
<b>Purity</b>	<b>99.91</b>	<b>99.86</b>	<b>98.45</b>	<b>99.76</b>	<b>98.88</b>	<b>98.87</b>	<b>99.95</b>	<b>99.00</b>
<b>Crop Seed</b>	<b>0.03</b>	<b>0.10</b>	<b>1.45</b>	<b>0.20</b>	<b>1.11</b>	<b>1.11</b>	<b>0.01</b>	<b>0.20</b>
<b>Inert Matter</b>	<b>0.01</b>	<b>0.01</b>	<b>0.07</b>	<b>0.03</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.30</b>
<b>Weed Seed</b>	<b>0.05</b>	<b>0.03</b>	<b>0.03</b>	<b>0.01</b>	<b>0.00</b>	<b>0.01</b>	<b>0.00</b>	<b>0.50</b>
<b>Test Date</b>	<b>09/2003</b>	<b>03/2005</b>	<b>01/2006</b>	<b>11/2005</b>	<b>01/2005</b>	<b>03/2005</b>	<b>02/2006</b>	<b>01/2003</b>
<b>Seed Origin</b>	<b>Idaho</b>	<b>Michigan</b>	<b>Wisconsin</b>	<b>Wisconsin</b>	<b>Idaho</b>	<b>Oregon</b>	<b>Oregon</b>	<b>Alabama</b>

**Germination, Purity, Crop Seed, Inert Matter, and Weed Seed given as percentage, as reported on original seed tag provided with seed from supplier.**

**Appendix 2: Seed Sources for Priming and Smoke Water Experiments**

<b>Cultivar</b>	<b>Species</b>	<b>Company</b>	<b>Address</b>	<b>Phone No.</b>
<b>Dawn</b>	<b>BFT</b>	<b>Deere Creek Seed Co.</b>	<b>PO Box 105, Ashland, WI 54806</b>	<b>715-278-3200</b>
<b>Georgia One</b>	<b>BFT</b>	<b>Deere Creek Seed Co.</b>	<b>PO Box 105, Ashland, WI 54806</b>	<b>715-278-3200</b>
<b>Norcen</b>	<b>BFT</b>	<b>Ernst Conservation Seed</b>	<b>9006 Mercer Pike, Meadville, PA 16335</b>	<b>800-873-3321</b>
<b>Empire</b>	<b>BFT</b>	<b>Ernst Conservation Seed</b>	<b>9006 Mercer Pike, Meadville, PA 16335</b>	<b>800-873-3321</b>
<b>Perfect Fit</b>	<b>KC</b>	<b>King's Agri Seed, LLC</b>	<b>96 Paradise Lane, Ronks, PA 17572</b>	<b>717-687-6224</b>
<b>Cossack*</b>	<b>KC</b>	<b>Geertson Seed Co.</b>	<b>1665 Burroughs Rd., Adrian, OR 97901</b>	<b>800-843-0390</b>
<b>AuGrazer</b>	<b>SL</b>	<b>Sims Brothers</b>	<b>3924 Cty Rd 87, Union Springs, AL 36089</b>	<b>334-738-2619</b>

**BFT = Birdsfoot trefoil; KC = Kura Clover; SL = Sericea Lespedeza**

**\* Two Cossack seed lots were evaluated.**

## VITA

Thomas M. Smith, son of Kathleen and Michael T. Smith, was born 5 December 1981 in the suburbs of Cincinnati, Ohio. His interest in agriculture developed late in high school. This interest prompted the completion of B.S. degree at Wilmington College, Wilmington, Ohio, and M.S. degree at Virginia Polytechnic Institute and State University in Blacksburg, Virginia. Research interests include animal production and forage systems, with special emphasis on grazing as the major source of livestock nutrition.