WEED CONTROL EFFECTS ON NATIVE SPECIES, SOIL SEEDBANK CHANGE, AND BIOFUEL PRODUCTION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

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Aphthona spp. flea beetles were released in the Little Missouri National Grasslands (LMNG) in western North Dakota in 1999 to control leafy spurge (*Euphorbia esula* L.). The changes in soil seedbank composition and leafy spurge density were evaluated on two ecological sites five (2004) and ten years (2009) after *Aphthona* spp. release to monitor the effectiveness of the insects on weed control and associated change in plant communities. In 2009, leafy spurge stem density averaged 2 and 9 stems m^{-2} in the loamy overflow and loamy sites, respectively, compared to 110 and 78 stems m^{-2}, respectively, in 1999 and 7 and 10 stems m^{-2}, respectively, in 2004. Leafy spurge constituted nearly 67% of the loamy overflow seedbank in 1999 compared to 17% in 2004 and 2% in 2009. In the loamy seedbank, the weed represented nearly 70% in 1999 compared to approximately 11% in 2004 and 15% in 2009. As leafy spurge was reduced, native species diversity and seed count increased ten years following *Aphthona* spp. release. High-seral species represented 17% of the loamy overflow seedbank in 2009, an increase from 5% in 1999. However, Kentucky bluegrass, a non-target weedy species, increased over 250% in the loamy overflow seedbank from 2004 to 2009. The reestablishment of native plant species has often been slow in areas where leafy spurge was controlled using *Aphthona* spp. A bioassay was completed to evaluate native grass establishment when grown in soil from *Aphthona* spp. release and non-release sites throughout North Dakota. Native grass production was not affected when grown in soil collected from established *Aphthona* spp.

sites (1.5 g per pot) compared to soil without insects (1.6 g per pot). The cause of reduced native grass production in sites with *Aphthona* spp. previously observed is unknown but may have been due to a chemical inhibition caused by the insects within the soil that no longer exists. The native warm-season switchgrass (*Panicum virgatum* L.) may be an alternative to corn for efficient biofuel production; however, control of cool-season grassy weeds has been a problem in switchgrass production. Various herbicides were evaluated for smooth bromegrass (*Bromus inermis* Leyss.) and quackgrass [*Elymus repens* (L.) Gould] control in an established switchgrass stand near Streeter, ND and a weed-infested field in Fargo, ND. Switchgrass yield was higher than the control 14 mo after treatment (MAT) when aminocyclopyrachlor or sulfometuron were applied early in the growing season, but no treatment provided satisfactory long-term grassy weed control. Herbicides were reevaluated at increased rates for smooth bromegrass or quackgrass control in Fargo. Sulfometuron provided 99% smooth bromegrass control when applied at 280 g ha^{-1} in the fall but injured other grass and forb species as well. Sulfometuron would likely be injurious to switchgrass and could not be used for biofuel production. Aminocyclopyrachlor did not injure other grass species but only reduced smooth bromegrass control by 76% when applied at 280 g ha^{-1} in the fall. No treatment provided satisfactory long-term quackgrass control.

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INTRODUCTION

Weed control is important for the removal of unwanted pests that could be harmful to native plants, animals, and humans (Randall et al. 2008). Weed control methods include biological, chemical, cultural, and mechanical, each with various benefits and limitations. Biological control agents such as *Aphthona* spp. (Coleoptera: Chrysomelidae) have successfully controlled leafy spurge (*Euphorbia esula* L.), but may have a negative effect on native grass recovery. Switchgrass (*Panicum virgatum* L.) is a potential biofuel agent to be used in North Dakota, but weed control can be difficult during establishment.

Leafy spurge is listed as a noxious weed throughout North America (USDA-NRCS 2011) and was first detected in North Dakota in 1909 (Hanson and Rudd 1933). Leafy spurge has an extensive root system, emerges in early spring, and produces an abundant amount of seed (Dunn 1979), which gives the weed a competitive advantage over native plant species.

Chemical, cultural, and biological control methods have been used to control leafy spurge (Lym 1998), but even a successful integrated pest management system will not eliminate viable seed from the seedbank (Foley 2004). Herbicides have been the most common and successful control method; however, chemicals are not always practical due to the potential for groundwater contamination, chemical and application costs, and prohibition in environmentally sensitive areas (Lym and Tober 1997). Biological control agents, *Aphthona* spp., have successfully reduced leafy spurge infestations (Cline et al. 2008; Juricek 2006; Mico and Shay 2002), are economical, and can be used in many environments where other control methods are inadequate (Lym 2005). However, following *Aphthona* spp. release and leafy spurge reduction, non-desirable species diversity has increased and the recovery of native species has been slow compared to chemical control methods (Cline et al. 2008; Kirby et al. 2000; Mico and Shay 2002). The reason for slow native plant reestablishment is unknown, but may be caused by an undesirable element produced by the *Aphthona* spp. (Juricek 2006).

Biofuel production is an important potential contributor to reduce greenhouse gas emissions and the overall ecological impact of fossil fuel use (Ragauskas et al. 2006). Currently, corn (*Zea mays* L.) is the prevalent crop used for biofuel production; however, the use of corn for biofuel has resulted in increased food costs (Taylor et al. 2006) and requires large amounts of energy and resources to produce (Perrin and Beckman 2008). Switchgrass, a perennial grass native to North America, may be a better alternative than corn for biofuel production (Sokhansanj et al. 2009). As a biofuel energy source, switchgrass will benefit the economy and environment from reduced net carbon emissions, enhanced water and soil quality, improved wildlife habitat, and increased farm revenues (McLaughlin and Walsh 1998).

Weed competition can severely reduce or prevent switchgrass establishment (McLaughlin and Adams Kszos 2005; Vogel and Masters 1998). Perennial forbs and cool-season grasses compete with switchgrass for important nutrients and resources (Wolf and Fiske 1995). Few herbicides are labeled for weed control in switchgrass and the efficacy of many herbicides on this species is unknown (Nyoka et al. 2007).

The objectives of this research were to evaluate: 1) the change in soil seedbank composition 10 yr after *Aphthona* spp. were released to control leafy spurge in the Little

Missouri National Grasslands (LMNG); 2) native grass establishment when grown in soil from *Aphthona* spp. release and non-release sites; and 3) various herbicides for smooth bromegrass (*Bromus inermis* Leyss.) and quackgrass [*Elymus repens* (L.) Gould] control in an established switchgrass stand and weed-infested field. All studies were continuations from previous graduate student work at North Dakota State University (NDSU).

LITERATURE REVIEW

Leafy Spurge Biology. Leafy spurge is native to Europe and Asia and was introduced to the United Sates through ship ballasts and seed stocks (Dunn 1979, 1985). The first known leafy spurge infestation in North America was recorded in Massachusetts in 1827 (Britton 1921), and by 1909, the weed was found in North Dakota (Hanson and Rudd 1933). The weed infested areas across the U.S. from Maine to Washington and was noted as a serious problem by the early 1930s. The widespread distribution and continuous spread of leafy spurge was likely caused by the dispersion of contaminated crop seed (Messersmith and Lym 1983).

Leafy spurge is a perennial plant that emerges in the early spring. The plant flowers from May to mid-summer and produces seed from July until initial frost (Messersmith 1983; Watson 1985). Distinct yellow-green bracts surround reduced true flower parts and the plant reproduces sexually and asexually from seed and root buds (Dunn 1979). Insects are the main source of pollination and the plant rarely self pollinates. Leafy spurge consists of many flowering shoots, each capable of producing 30 to 150 seeds (Bakke 1936). The plant can propel seeds up to 5 m at maturity (Bakke 1936) and seed can be viable for over 8 yr (Bowes and Thomas 1978).

Leafy spurge has a widespread root system that consists of many course and fine roots (Hanson and Rudd 1933). The roots produce new shoots, and many nutrients are stored in the root system (Dunn 1979; Hanson and Rudd 1933). The stems are erect, unbranched, and become woody at the base (Hanson and Rudd 1933). The leaves are entire or slightly sinuate, alternate, and whorl beneath the inflorescence (Watson 1985). The plant also contains a milky latex that can be toxic to livestock (Dunn 1979).

Economic Impact. Leafy spurge infestations negatively impact the economy (Lym and Messersmith 1983). The plant infested 657,435 ha within Montana, North Dakota, South Dakota, and Wyoming by the mid-1990s (Leitch et al. 1994), and within North Dakota, about 6% of the non-cropland area was infested with the weed. In North Dakota, the total cost of direct and indirect leafy spurge impacts was approximately $87 million dollars in 1990 (Leistritz et al. 1993), which, with inflation, would equate to nearly $152 million dollars in 2011. Leafy spurge has affected grazing capacity, income of stock growers and landowners, wildlife habitat, and native vegetation. Specific expenditures for leafy spurge control include the cost of chemicals, application equipment, and the need to control the weed on a variety of terrain such as farmland, roadsides, railroads, public utilities, and government land (Messersmith and Lym 1983).

Infested grazing areas have reduced hay production, which negatively impacts the beef industry (Messersmith and Lym 1983). Leafy spurge reduced cattle carrying capacity over 30% from a 100-cow herd to 69 head when 25% of a grazing allotment was infested (Leistritz et al. 1992). In 1990, nearly 255,760 ha of grazing land were infested with leafy spurge and about 583,000 animal unit months were lost in North Dakota alone (Leistritz et al. 1993). As a result, income for ranchers and landowners was reduced nearly $9 million dollars in 1990, which would correspond to over $15 million dollars with inflation by 2011.

Leafy Spurge Control Methods. Various control methods, including chemical, cultural, and biological, have been used to manage leafy spurge (Lym 1998). These methods have not successfully eliminated the weed, but a combination of these techniques has been more effective than any one method used alone (Lym 2005; Lym and Messersmith 1985a). The combined use of various control methods to suppress invasive weeds is known as Integrated Pest Management (IPM). IPM has been used in critical and sensitive ecological habitats, such as in national and state parks, where methods other than chemicals must be used for weed control (Larson et al. 2007).

Herbicides have been the most successful and common single method used for leafy spurge control (Lym and Messersmith 1985a). Typical herbicides for leafy spurge control include dicamba, picloram, 2,4-D, and quinclorac (Kuehl and Lym 1997; Lym and Messersmith 1985a; USDA–ARS-TEAM Leafy Spurge 2002). Picloram has been the most common herbicide used to control the weed (Lym and Messersmith 1985a); however, this chemical has had a negative effect on surrounding ecosystems (Lym and Messersmith 1988). Picloram has a high water solubility, lengthy soil persistence, and high leaching potential, which has led to groundwater contamination.

Picloram applied annually at 0.28 kg ae ha^{-1} plus 2,4-D at 1.1 kg ae ha^{-1} was the most cost effective treatment to both control leafy spurge and maximize forage production in North Dakota (Lym and Messersmith 1987). Picloram applied at 2.2 kg ai ha^{-1} controlled leafy spurge 77%, 27 mo after treatment (MAT) when spring-applied as the liquid formulation (Lym and Messersmith 1985b). A combination of picloram at 0.3 kg ae ha^{-1} to

0.6 kg ha^{-1} applied with 2,4-D at 0.3 kg ha^{-1} to 1.1 kg ha^{-1} improved leafy spurge control compared to picloram alone at the same rate.

Application of just 2,4-D provided short-term leafy spurge control (Lym and Messersmith 1985a). In North Dakota, control was less than 40% after 1 yr when 2,4-D was spring- or fall-applied at 4.5 kg ha^{-1}. Leafy spurge was reduced and forage production increased when 2,4-D was applied in June; however, control was enhanced when combined with other herbicides (Lym and Messersmith 1985b). A mixture of 2,4-D plus picloram and imazapic provided better long-term leafy spurge control than any of the herbicides used alone (Lym 2000). In eastern North Dakota, this three-way mixture provided 98% leafy spurge control 15 MAT when applied once in the spring.

Dicamba plus 2,4-D controlled leafy spurge 91% compared to 47% with dicamba applied alone (Lym and Messersmith 1985a). Leafy spurge control decreased the following year after treatment when dicamba was applied at less than 9.0 kg ai ha^{-1}. Additionally, leafy spurge control was better when dicamba granules were applied compared to the liquid formulation.

Glyphosate reduced leafy spurge on average 76% when fall-applied at 0.8 kg ai ha^{-1} to 2.2 kg ai ha^{-1}, but efficacy rapidly decreased after 1 yr (Lym and Messersmith 1985b). However, 2,4-D applied at 0.3 to 0.6 kg ha^{-1} the following spring increased leafy spurge control by reducing seedling growth. Although herbicides have proven to be a successful method for leafy spurge control, chemical treatment is not appropriate in all areas where leafy spurge is found (Lym and Tober 1997).

Sheep (*Ovis aries* L.) and angora goat (*Capra hircus* L.) grazing have successfully suppressed leafy spurge populations, especially when combined with other control methods (Lym 2005; Lym et al. 1997). Sheep and goat grazing can be economical and used in areas where herbicide use may be restricted (Bangsund et al. 2001; Lym et al. 1997). Continuous grazing has suppressed leafy spurge densities and slowed the rate of spread by reducing top growth and seed production (Bowes and Thomas 1978). However, when sheep were removed from the grazing area, the weed has reestablished from root growth within 2 yr.

Seeded grass species in leafy spurge infestations have successfully competed with the weed (Lym and Tober 1997; Masters et al. 2001). Native grasses have reduced leafy spurge seed production, rate of spread, and cover (Selleck et al. 1962). In North Dakota, a competitive mixture of grasses included western wheatgrass (*Agropyron smithii* Rydb.), green needlegrass [*Nassella viridula* (Trin.) Barkworth], and slender wheatgrass [*Elymus trachycaulus* (Link) Shinners] (Lym and Tober 1997).

Many biological control agents have been released for leafy spurge control. The leafy spurge hawk moth (*Hyles euphorbiae* L.) was released in 1965 in North America (Harris 1984). Larvae feed on the leaves and flowers of leafy spurge, but establishment has been relatively low with minimal reduction of leafy spurge infestations (Joshi and Olson 2009). The leafy spurge gall midge (*Spurgia esulae* Gaǵne) was first released in 1985 (Poritz 1989). The larvae feed on terminal leaves and flower buds of leafy spurge; however, this does not stop vegetative growth (Joshi and Olson 2009). The stem- and root-boring beetle (*Oberea erythrocephala* Shrank) was released in North Dakota in 1985 and was the first leafy spurge biological control insect to become established (Lym and Messersmith 1990). The larval stage grows within the crown and stem, and the adults feed on leaves and flowers (Joshi and Olson 2009). This beetle has relatively low reproductive potential and has not reduced leafy spurge infestations at any release site (Progar et al. 2011).

The most successful biological control agents released for leafy spurge control are several *Aphthona* spp. The beetles are native to Eurasia, and were introduced to the U.S. in the mid-1980s (Julien and Griffiths 1999). Six species were introduced into the Northern Great Plains, including *Aphthona abdominalis* Duftschmid, *Aphthona cyparissiae* Weise, *Aphthona czwalinae* Weise, *Aphthona flava* Guill, *Aphthona lacertosa* Rosenhauer, and *Aphthona nigriscutis* Foudras (Carlson and Mundal 1990). *A. abdominalis* has a reddish-yellow head, prothorax, and mesothorax with a black metathorax and abdomen (Fornasari 1993). *A. cyparissiae* and *A. nigriscutis* are brown in color with a dark scutellum (Gassman et al. 1996). *A. czwalinae* and *A. lacertosa* are both black and can be distinguished by the hind femur that is entirely black in *A. czwalinae* and partly brown in *A. lacertosa*. *A. flava* has a reddish tint and a pale underside.

Aphthona spp. require leafy spurge to complete their life cycle (Gassman et al. 1996). All species except *A. abdominalis* are univoltine (Jackson 1997). Adults are 3 to 4 mm in length and emerge in early summer to feed on above-ground foliage (Joshi and Olson 2009). Eggs are laid at the base of the stem throughout the summer and larvae feed on the root system (Gassman et al. 1996). Larvae prefer former feeding sites over undamaged areas and cause leafy spurge injury by disrupting nutrient flow and providing an entryway for disease and infection (Larson and Grace 2004). The larvae overwinter beneath the soil and pupate in late spring of the following year (Gassman et al. 1996).

A. nigriscutis and *A. lacertosa* have been the most successful leafy spurge biological control agents to establish in North Dakota (Lym 1998; Lym and Carlson 2002). *A. lacertosa* adapted well to most habitats infested with leafy spurge, but has been less successful in sandy soils (Larson and Grace 2004). *A. nigriscutis* adapted to more open and drier habitats, has tolerated sandier soil, and is more likely to disperse from release points than *A. lacertosa*. However, *A. lacertosa* may cause more leafy spurge damage as this species tends to remain in a more localized area until the weed is depleted.

Aphthona spp. establishment has been challenging and varied by location (Kirby et al. 2000). Survival and establishment depended on slope, soil type, density and type of surrounding vegetation, ground cover, temperature, and moisture (Gassman et al. 1996; Kirby et al. 2000). Adult flea beetles are subject to little predation, but eggs and larvae are susceptible to attack by general predators within the soil (Gassman et al. 1996). Common predators include protozoa, acari, and hymenopterous parasitoids. Nevertheless, primary mortality factors for *Aphthona* spp. are temperature and extreme humidity.

Aphthona spp. have substantially reduced leafy spurge cover, density, and biomass (Kirby et al. 2000). Leafy spurge was reduced nearly 70% over a 14 yr period after *Aphthona* spp. were released in north-central Montana (Lesica and Hanna 2009a). Significant reductions of leafy spurge root biomass occurred within 2 to 3 yr after release in North Dakota (Kirby et al. 2000). Additionally, leafy spurge infestations were further reduced when controlled with *Aphthona* spp. and picloram plus 2,4-D applied in the fall compared to either method used alone (Lym and Nelson 2002).

Soil Seedbank. Soil seedbank analysis provides insight on the historical and future composition of above-ground vegetation in an ecosystem (Cavers 1994; Thompson and Grime 1979). Soil seedbanks have been analyzed around the world in many types of environments including agricultural lands, grassland pastures, forests, prairies, tundra, and wetlands. The seedbank can be separated into three types including transient, short-term persistent, and long-term persistent, components which were proposed by Bakker (1989) and explained by Thompson (1992). A transient seedbank is comprised of seeds that persist in the soil for less than 1 yr. Short-term persistent seedbanks include seeds that remain viable in the soil for at least 1 yr, but less than 5 yr, while long-term persistent seedbanks include seeds that live longer than 5 yr. Seed from many pioneer species and weedy communities persist up to several decades, whereas seed from woodland species remain less than 1 yr, and seed from most grassland species remain viable up to a few years (Bekker et al. 1998).

Soil seedbank evaluation is valuable for effective weed control and land management to help preserve native biodiversity, reduce management costs, and enhance forage diversity (Cardina and Sparrow 1996). The timing of weed seed germination is an important aspect for successful weed control (Buhler et al. 1997) that affects herbicide application and cultivation timing (Ogg and Dawson 1984). Seedbank composition assessment can also help determine whether restoration, such as seeding, is necessary to improve the development and quality of an ecosystem, as above-ground vegetation does not always correspond to seed composition within the soil (Thompson and Grime 1979). The weed seed composition and competitive ability of invasive species within the seedbank are important aspects to determine which native species should be seeded for restoration (Cardina and Sparrow 1996). An effective mixture of native species should provide adequate competition against invasive species within the soil to help prevent and reduce weed seed production (Buhler et al. 1997). In Fargo, ND, various grass species were evaluated for competitiveness with leafy spurge. 'Rebound' smooth bromegrass, 'Rodan' western wheatgrass, and 'Bozoisky' Russian wildrye [*Psathrostachys juncea* (Fisch.) Nevski.] each reduced leafy spurge stem density 63% compared to 25% for 'Reliant' intermediate grass [*Elymus hispidus* (P. Opiz) Melderis] and T-17596 mountain rye (*Secale montanum* Guss.), 3 yr following seeding (Lym and Tober 1997).

Breaking seed dormancy can be difficult during seedbank analysis and there are many factors that affect germination, such as light (Forcella et al. 1992), oxygen, competition, seed depth (Perez et al. 1998), and soil temperature. For leafy spurge, the seed coat is a major factor that inhibits germination, but moist conditions and abrasion can help break dormancy (Foley 2004). Seeds chilled prior to sowing have improved germination compared to seeds that did not undergo a cool period (Perez et al. 1998). Additionally, seedling removal is important to stimulate germination and reduce competition for light, water, and nutrients (Forcella et al. 1992). A thin layer of seeds sowed near the soil surface increased seed germination compared to seeds planted deeper within the soil (Perez et al. 1998; Ter Heerdt et al. 1996).

Extensive seedbank analysis can require a lot of time and greenhouse space. Sieves are commonly used to speed up seedbank analysis and separate seed from excess soil and plant debris (Cardina and Sparrow 1996; Forcella et al. 1992; Ter Heerdt et al. 1996). Soil washed through a course (4-mm) and fine (0.2-mm) sieve to form a concentrated seed mixture improved seed germination, enhanced observed species diversity, and reduced required greenhouse space compared to when seeds were hand-sorted and the soil mixture was not concentrated (Ter Heerdt et al. 1996). The 0.2-mm sieve was small enough to catch seeds of *Juncus* spp., which were the smallest seeds expected to be found within the study area.

A leafy spurge soil seedbank study was first conducted in the LMNG in western North Dakota in 1999 (Cline 2002). *Aphthona* spp. were released to control leafy spurge while the change in seedbank composition over time was evaluated. Soil samples were extracted in the spring and fall from each site and the seedbank analyzed following methods by Ter Heerdt et al. (1996). A total of 43 species germinated from the loamy overflow sites and 40 species from the loamy sites (Cline 2002). The most abundant species included leafy spurge, Kentucky bluegrass (*Poa pratensis* L.), prairie Junegrass [*Koeleria macrantha* (Ladeb.) J. A. Schultes.], little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and green needlegrass.

The study was repeated in 2004, 5 yr after *Aphthona* spp. were released to control leafy spurge (Cline et al. 2008; Juricek 2006). The same procedure and methods were used from Cline (2002), except the flea beetles had spread throughout the entire study area and the soil cores were taken exclusively in the late summer. Leafy spurge stem density was reduced approximately 90% by the *Aphthona* spp. 5 yr following release. Leafy spurge seed in the soil seedbank also decreased 66% in the loamy overflow sites and 79% in the loamy sites. The most abundant species were still leafy spurge and Kentucky bluegrass and native plant species had not recovered following leafy spurge reduction. A similar soil seedbank study was completed in Theodore Roosevelt National Park, ND, near the LMNG (Travnicek et al. 2005). The seedbank primarily consisted of low-seral species, such as Canada thistle [*Cirsium arvense* (L.) Scop] and Kentucky bluegrass, which accounted for more than 80% of the total germinated seed. A total of 74 species germinated across all soil cores including 56 forbs, 13 grasses, and 5 mesic species.

A soil seedbank study was analyzed in the Nebraska Sandhills to evaluate the relationship among seedbank composition, seedbank depth, seed dormancy, and vegetative expression (Perez et al. 1998). Species with a dense above-ground composition, such as hairy grama (*Bouteloua hirsuta* Lag.) and prairie sandreed [*Calamovilfa longifolia* (Hook.) Scribn.] appeared sparsely or were absent in the seedbank. Germination was greatest from seed that was excavated 0 to 5 cm compared to 15 to 20 cm, and seed chilling increased germination for most species.

Native Plant Recovery. Native plant species diversity increased following *Aphthona* spp. release and leafy spurge reduction in the LMNG, but the recovery rate was slower than expected (Juricek 2006). At several locations where leafy spurge was reduced by *Aphthona* spp., native plant reestablishment had not increased to the same levels prior to weed invasion, while non-native species diversity increased (Juricek 2006; Lesica and Hanna 2009b; Mico and Shay 2002). In east-central North Dakota, grass production was not fully recovered 7 to 8 yr following *Aphthona* spp. release (Kirby et al. 2000). Additionally, in Montana, no substantial change in species diversity or richness was indicated after *Aphthona* spp. reduced leafy spurge populations (Butler and Wacker 2010; Lesica and Hanna 2004). In contrast, species richness was higher at flea beetle release sites compared to non-release sites in Canada (Mico and Shay 2002). The reason for slow native grass recovery and low species diversity at various locations is unknown, but may be caused by different mechanisms such as competition from the undesired target plant or damage-induced allelopathy by the weed (Callaway et al. 1999).

Biological control insects may negatively affect native plant recolonization (Callaway et al. 1999). In the LMNG in North Dakota, the reestablishment of native grasses following leafy spurge control with *Aphthona* spp. beetles was evaluated 5 yr following beetle release (Juricek 2006). Switchgrass production was reduced approximately 50% when grown in soil from *Aphthona* spp. release sites compared to non-release sites (Cline et al. 2008; Juricek 2006). Leafy spurge was present at insect-release and non-release sites, suggesting slow native species reestablishment may not be caused by leafy spurge. Grass seedling production was also less in loamy overflow sites compared to loamy sties, which proposes landscape and soil type have an impact on grass recovery as well (Juricek 2006). Loamy overflow sites have better fertility, greater moisture availability, and more organic matter compared to upland sites (Wolf 1987).

Slow native species reestablishment following leafy spurge control with *Aphthona* spp. may also be affected by remaining leafy spurge plants. Invasive species have modified soil, physically and chemically, to increase fitness by inhibiting growth of surrounding plant life (Jordan et al. 2008). Aggressive plant invaders with proficient colonizing abilities have out-competed native plant species and transformed environments to become more susceptible to invasion by other undesirable species (Maron and Marler 2008). Leafy spurge above- and below-ground plant material that have survived control methods may slow the recovery rate of native vegetation through competition for limited resources (Kirby et al. 2000; Kirby et al. 2003). The weed may also produce phytotoxins that accumulate in the soil and allelopathically influence surrounding plant life (Qin et al. 2006; Steenhagen and Zimdahl 1979). For example, tomato (*Lycopersicon esculentum* Mil.) seedling growth was inhibited when grown in soil that was previously infested with leafy spurge plants (Qin et al. 2006). In addition, tomato and crabgrass (*Digitaria sanguinalis* L.) growth was slowed when grown in soil that consisted of small pieces of leafy spurge plant material.

Kentucky bluegrass is one of the main plant invaders of the Northwestern Great Plains (DeKeyser et al. 2009) and has established in various perennial and tall grass prairie communities throughout the U.S. similar to leafy spurge (Ferell et al. 1998; Hulbert 1986). Kentucky bluegrass is a cool-season grass that is native to Europe, but has adapted to a variety of environments in North America including meadows, open woodlands, pastures, open ground, and disturbed sites (Stubbendieck et al. 2003). Kentucky bluegrass grows in various soil textures and favors a moist environment. Grass production is low compared to high valued grasses; however, forage value is good for livestock and wildlife in early spring when other plants are unavailable.

Kentucky bluegrass has prevented native plant growth and reduced species diversity on rangeland habitat (DeKeyser et al. 2009; Ferell et al. 1998; Hulbert 1986). Kentucky bluegrass infestations increased an average of 260% at two different non-use management sites in North Dakota from 1983 to 2007, while native forbs and grasses decreased approximately 54% and 83%, respectively (DeKeyser et al. 2009). In southeastern Montana, Kentucky bluegrass invaded and dominated the surrounding plant community after leafy spurge was controlled using *Aphthona* spp. (Butler and Wacker 2010). Additionally, in Crook County, WY, 'Critana' thickspike wheatgrass [*Elymus lanceolatus* (Scribn. & J. G. Smith) Gould] and mountain rye were seeded to provide competition against leafy spurge, but the grasses were replaced by Kentucky bluegrass (Ferrell et al. 1998).

Biofuel Production. Concerns of finite petroleum supplies, high energy prices, and environmental consequences from fossil fuel production have stimulated the search for an efficient alternative renewable energy source (Hill et al. 2006; Parrish and Fike 2005). Biofuel production has become an important energy source to reduce dependence on foreign oil, provide alternate farm income, and enhance energy security (Hahn and Cecot 2009; Ragauskas et al. 2006). A reduction of foreign oil imports into the U.S. would decrease the world price of oil, making the cost of the remaining oil imported into the U.S. less expensive (Hahn and Cecot 2009). Biofuel has the potential to become a more efficient alternative energy source compared to fossil fuel (Rinehart 2006).

Various forms of biofuel are used today. Ethanol can be produced from food crops, which primarily include corn and sugarcane (Solomon et al. 2007). Corn-based ethanol production converts the starch portion of the crop into ethanol with a seven step process, which includes milling (wet and dry), liquefaction, saccharification, fermentation, distillation, dehydration, and denaturation (Hohmann and Rendleman 1993; Solomon et al. 2007). Ethanol production from sugarcane involves only four steps including milling, pressing, fermentation, and distillation (Solomon et al. 2007). Another biofuel source is cellulosic or lignocellulosic ethanol. The woody part of trees, plants, grasses, or residues is converted into sugars, which are then fermented into ethanol.

Currently, corn is the major crop grown for ethanol production. About 95% of biofuel produced in the U.S. is corn-based ethanol (Magdoff 2008). Corn-based ethanol production technologies are much more advanced than the current technologies for cellulosic ethanol (Haque and Epplin 2010). Cellulosic ethanol production is still in a research and development phase but has potential to become a successful, efficient energy source (Rinehart 2006; Solomon et al. 2007).

The use of corn for biofuel has stimulated many economic and environmental concerns. Corn-based ethanol has indirectly caused an increase in food prices in soybean, wheat, and high fructose corn syrup (Koo and Taylor 2008) and increased the cost of feedstock for livestock and poultry industries (Mol 2007). The overall production and distribution cost of corn-based ethanol is higher than the cost of gasoline. Additionally, corn-based ethanol production has negatively impacted the environment. Ethanol production from corn caused an increase in nitrogen and sulfur oxide emissions, particulate matter, volatile organic compounds, and ground-level ozone and water contamination compared to gasoline use (EPA 2007). Corn-based ethanol production and distribution also require sufficient energy and resources, including frequent irrigation and fertilizer and herbicide applications (Perrin and Beckman 2008).

Biofuel Laws and Regulations. Various governmental laws have stimulated biofuel production and encouraged research for an alternative energy source. In the Energy Security Act of 2005, Congress required 28 billion L of renewable biofuel be produced by 2012 (Johnson and Runge 2007). The Energy Independence and Security Act of 2007 mandated 136 billion L of ethanol be produced by 2022 and a minimum of 61 billion L must be from cellulosic ethanol (Dicks et al. 2009; Haque and Epplin 2010). These mandates have resulted in an expansion of governmental incentive programs and regulations that have increased biofuel production, such as grants and loans, renewable fuel standards, and corn subsidies (Hahn and Cecot 2009). Tariffs are also placed on imported ethanol to help prevent a price reduction from competing foreign countries. Additionally, the Volumetric Ethanol Excise Tax Credit is a federal subsidy that provides a 51 cent credit against gasoline taxes for every 4 L of ethanol blended with gasoline.

The progression of biofuel incentive programs and regulations resulted in an increase of U.S. ethanol production from approximately 4 billion L in 1990 to nearly 30 billion L in 2007 (Koo and Taylor 2008). By September 2007, there were 128 ethanol plants throughout the U.S. that produced nearly 27 billion L of ethanol per year (Hahn and Cecot 2009). Ethanol production within the U.S. is expected to continue to increase in the future (Koo and Taylor 2008).

Switchgrass Production. Switchgrass has economic potential and environmental benefits for efficient cellulosic biofuel production with minimal inputs within the Northern Great

Plains (Sokhansanj et al. 2009). Switchgrass is a perennial warm-season grass that is native to Central and North America (Keshwani and Cheng 2009). Switchgrass is a dominant grass species within the North American tall grass prairie and is found growing with other native plant species including big bluestem (*Andropogon gerardii* Vitman), indiangrass [*Sorghastrum nutans* (L.) Nash], little bluestem, sideoats grama [*Bouteloua curtipendula* (Michx.) Torr], and various broadleaf species (Rinehart 2006). Switchgrass has adapted throughout various regions in the U.S. from the Atlantic coast to the eastern Rocky Mountains. The grass grows well in fine to coarse textured soils and in regions where annual precipitation falls above 37 cm per year and can reach over 3 m tall in wet areas (Rinehart 2006). Switchgrass emerges in the spring, flowers during summer, and reproduces from seed, tillers, and rhizomes (Stubbendieck et al. 2003).

Switchgrass is a C_4 species and can withstand very hot, cold, or arid conditions (Casler et al. 2007). Genetic and phenotypic variation in switchgrass has resulted from evolutionary processes (genetic drift, mutation, and natural selection) and environmental adaptation (soil type, precipitation, and longitude and latitude). Two major switchgrass types have evolved, which include upland and lowland ecotypes (Porter 1966). The upland types prefer drier soils and are found in semi-arid climates and the lowland types establish best in heavier soils and wetter environments. Breeding programs throughout the U.S. have produced many switchgrass cultivars (McLaughlin et al. 1999, Rinehart 2006). Each cultivar was adapted to specific environments and chosen for planting based on ecotype (upland or lowland) and latitude of origin, which enhanced the survival rate and productivity of the switchgrass establishment (Rinehart 2006).

Switchgrass can provide many benefits as a potential biofuel crop compared to fossil fuel and other species used for biofuel, such as reduced net carbon emissions, enhanced water and soil quality, improved wildlife habitat, and increased farm revenue (Cassida et al. 2000; McLaughlin and Walsh 1998). Switchgrass used for biofuel production would reduce soil erosion 95% compared to corn (Hohenstein and Wright 1994) as the crop only needs tillage the first year of establishment (Ma et al. 2000). Switchgrass production also requires less fertilizer and fewer herbicide applications than corn (McLaughlin et al. 1999) and is estimated to reduce pesticide use 90% compared to annual row crops (Hohenstein and Wright 1994). Additionally, switchgrass is tolerant to dry and wet conditions; can establish on shallow, rocky soils; is resistant to many pests and diseases; and has adapted to a wide range of soil pH (Rinehart 2006; Wolf and Fiske 1995).

Switchgrass establishment has been challenging, but a few sowing methods have been successful. These include conventional tillage and drill planting; no-till planting onto crop stubble, pasture, or Conservation Reserve Program land; and frost seeding (Rinehart 2006). Switchgrass seeded 0.6 to 1.3 cm deep at 4 to 11 kg ha ^{1} provided the most successful establishment. Wide row widths produced the highest yields with rows about 81 cm apart. Additionally, seed stratification (periods of cool or moist conditions) triggers germination and improves establishment (Rihehart 2006; Shen et al. 2001). Maximum yearly switchgrass production typically is achieved the third year after planting and switchgrass should be harvested once a year in the fall for maximum production (Rinehart 2006). A switchgrass field can survive up to 10 yr or longer before reestablishment becomes necessary (Genera Energy 2010).

Weed Control in Switchgrass. Switchgrass stands can become weedy if not properly managed. Cool-season weeds can be problematic and have out-competed warm-season grasses, such as switchgrass, in cool soils (Rinehart 2006). Common weedy grasses include witchgrass (*Panicum capillare* L.), stinkgrass (*Eragrostis cilianensis* All.), yellow foxtail (*Setaria glauca* L.), green foxtail (*Setaria viridis* L.), smooth bromegrass, and quackgrass[1].

Weed control in switchgrass can be achieved with various cultural, mechanical, and chemical methods. Annual cropping with small grains and field pea (*Pisum arvesnse* L.) for 1 or 2 years helped control weeds and increase organic matter in the soil (Rinehart 2006). Switchgrass should be clipped or mowed only to 20 to 25 cm during July or August of the establishment year and grazing should be avoided (George et al. 1979). Fertilizers such as lime, phosphorus, or potassium may be applied as needed, but nitrogen application should be avoided during the year of establishment to inhibit excessive weed growth. Nitrogen applied at 67 to 112 kg ha ^{1} the following year will improve forage quality and quantity.

Few herbicides are available for weed control in switchgrass (Nyoka et al. 2007). Atrazine is labeled for use in switchgrass, but has been injurious when applied at high rates (Parrish and Fike 2005) and does not effectively control rhizomatous weeds with deep root systems such as Johnsongrass [*Sorghum halepense* (L.) Pers.] (Minelli et al. 2004). Weed control has been most successful when atrazine was applied prior to

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[1] Dwight Tober 2008, USDA-NRCS, PO Box 1458, Bismarck, ND 58502, Personal communication

switchgrass seeding (George et al. 1979). In Mead, NE, a variety of grasses including big bluestem, indiangrass, sand lovegrass [*Eragrostis trichodes* (Nutt.) Alph. Wood], side-oats grama, and switchgrass were evaluated for atrazine tolerance (Martin et al. 1982). Switchgrass yield increased when atrazine was applied at 1.1 kg ai ha ^{1} and 3.4 kg ai ha ^{1}, suggesting switchgrass is tolerant to the herbicide. Switchgrass and big bluestem had the highest yield when herbicides were not used for weed control, which indicated they are better competitors than the other grasses. A variety of other herbicides have been evaluated for weed control in switchgrass; however, many have reduced switchgrass density (Peters et al. 1989).

Glyphosate has been used to control weeds in a switchgrass establishment prior to seeding (Sampson and Moser 1982). In southeastern Nebraska, glyphosate was applied in early spring for cool-season grassy weed control in a warm-season grass establishment (Waller and Schmidt 1983). Cool-season grasses were suppressed nearly 90%, while big bluestem, indiangrass, little bluestem, and switchgrass yield increased (Sanderson et al. 2004). Timing of glyphosate application was important to prevent decreased warm-season grass production. Glyphosate reduced switchgrass seed yield approximately 75% when applied in May compared to mid-April.

Herbicides from the sulfonylurea family have been used to control weeds in switchgrass but can cause crop injury. Sulfonylurea herbicides are acetolactate synthase (ALS) inhibitors that can be applied pre- or post-emergence (Beyer et al. 1988). ALS inhibitors prevent the synthesis of amino acids necessary for protein and cell formation. Post-applied sulfonylurea herbicides were evaluated for switchgrass injury in Concord and

Mead, NE during switchgrass establishment (Peters et al. 1989). Switchgrass yield was greatest when sulfometuron, chlorsulfuron, or metsulfuron were applied compared to the control. Similar results were reported in southeastern Colorado when switchgrass was treated with sulfonylurea herbicides. Metsulfuron and chlorsulfuron reduced switchgrass basal cover 61% to 71% (Lair and Redente 2004).

Imidazolinone herbicides had not been thoroughly evaluated for native warm-season grass tolerance until the late 1990s (Masters et al. 1994; Washburn and Barnes 2000). Imazapyr is registered for pre- and post-emergence application for total vegetation control, and imazaquin and imazethapyr are registered for preplant incorporation, pre-emergence, and post-emergence control for annual broadleaf and grass weeds in soybean (*Glycine max* L.). Big bluestem and little bluestem production increased when imazethapyr was applied at the time of planting compared to seeding without herbicide (Masters et al. 1996). Additionally, a study in Columbus, NE, indicated that switchgrass was not injured when imazaquin or imazethapyr were applied at 0.07 kg ae ha^{-1} to 0.28 kg ae ha^{-1} in the fall (Masters et al. 1994).

Aminocyclopyrachlor is a synthetic auxin herbicide that is currently in development by E. I. du Pont de Nemours and Company (Dupont) (Bukun et al. 2010). Aminocyclopyrachlor is a pyrimidine carboxylic acid herbicide with a chemical structure similar to the pyridine herbicides picloram, clopyralid, aminopyralid, and fluroxypyr (Wood 2011). The herbicide was proposed for use in pasture and rangeland, non-cropland, and natural areas for broad-spectrum control (Bukun et al. 2010). The herbicide is expected to be less harmful to native prairie species compared to picloram and clopyralid (Edwards 2008). Aminocyclopyrachlor will control weeds resistant to ALS, PPO, and glyphosate such as kochia [*Kochia scoparia* (L.) Schrad.], ragweed species (*Ambrosia* spp.), and horseweed [*Conzya Canadensis* (L.) Cronq.] (Turner et al. 2009). Cool-season grasses were more sensitive when aminocyclopyrachlor was foliar-applied at the seedling stage compared to warm-season grasses; however, both grass types were tolerant to the herbicide when applied pre-emergence in June (Vassios et al. 2009). Application timing was more important than rate for effective weed control.

Smooth Bromegrass Control. Smooth bromegrass is a cool-season perennial grass that is native to Eurasia. The first record of smooth bromegrass found in the U.S. was in 1880 along the West Coast (Dibbern 1947). Smooth bromegrass spreads vegetatively through rhizomes (Stubbendieck et al. 2003) and is a widespread invader of the Northern Great Plains (Jordan et al. 2008). The persistence of smooth bromegrass has contributed to a reduction in native species diversity throughout the U.S.

Various cultural and mechanical methods have been used to control smooth bromegrass. Grazing and cutting after shoot elongation easily damaged the grass (Reynolds and Smith 1962; Stacy et al. 2005) and prescribed fire has been successful at reducing smooth bromegrass stands (Willson 1992; Stacy et al. 2005). Prescribed burning in mid-May reduced tiller density 50% when conducted soon after the start of tiller elongation (Willson 1992).

Herbicides also have been used to control smooth bromegrass. Paraquat applied at 0.57 kg ai ha^{-1} reduced smooth bromegrass cover 56% the first yr of application (Stacy et al. 2005). Smooth bromegrass was injured but not killed when metsulfuron, chlorsulfuron, or sulfometuron were applied to plants 1 to 5 cm tall (Peters et al. 1989). Jacobs and Denny (2006) reported similar results when chlorsulfuron was applied to smooth bromegrass. Additionally, atrazine and glyphosate applied in the late spring reduced smooth bromegrass densities and stimulated recovery of warm-season grasses (Waller and Schmidt 1983).

Imazaquin and imazethapyr applied in the fall at 0.14 kg ae ha^{-1} and 0.28 kg ae ha^{-1} had little to no adverse effect on smooth bromegrass (Masters et al. 1994). Additionally, Peters et al. (1989) found that smooth bromegrass produced a higher yield than the control following chlorsulfuron, fenoxaprop, and sethoxydim treatments.

Quackgrass Control. Quackgrass is an invasive perennial grass that has become a problem weed throughout many areas of North America (Dekker and Chandler 1985). The grass has spread throughout the U.S. through seed and aggressive rhizomes and is persistent in moist soils and cool to moderate climates (Buchholtz 1963). Quackgrass infestations can severely reduce crop yield and quality.

Various methods including tillage and/or competitive crops have been used to control quackgrass, but in the last 50 yr, herbicides have been a more common method. Triazine herbicides effectively controlled quackgrass, with atrazine providing better control than simazine and simetone (Buchholtz 1963). Sethoxydim, glyphosate, haloxyfop-methyl, and fluazifop-butyl were evaluated for quackgrass control in a controlled environment and all herbicides were more effective when applied on younger (3-leaf) plants than older (5-leaf) plants, but none of the herbicides completely eliminated the grass (Dekker and Chandeler 1985). Rimsulfuron reduced quackgrass when applied at any growth stage and was 100%

effective when applied at 27 g ai ha^{-1} to the 2-to 4-leaf stage (Mitra and Bhowmik 1999). Additionally, quackgrass control was more effective when herbicide application occurred on an undisturbed field in the spring or fall compared to an application on a plowed field.

Biofuel Research at North Dakota State University. A preliminary greenhouse study was conducted at NDSU to evaluate 27 herbicides from various families for grassy weed control (Eken et al. 2010). Herbicides were assessed for efficacy on 'Dacotah'[2] switchgrass and 'Rodan'[2] western wheatgrass and weed control on smooth bromegrass[3], quackgrass[4], and green and yellow foxtail. The foxtail species were collected from wild infestations and herbicide application rates were applied at the lowest recommended label rate.

Herbicides with the most potential to control smooth bromegrass and quackgrass without injury to switchgrass were further evaluated in 2009 in an established switchgrass stand near Streeter, ND, and a smooth bromegrass- and quackgrass-infested field in Fargo, ND (Eken et al. 2010).

Switchgrass yield was similar among treatments when applied in May and decreased when applied in June (Eken et al. 2010; Tables 16 and 17). At Streeter, pyroxsulam, sulfometuron, and sulfosulfuron controlled smooth bromegrass and aminocyclopyrachlor and propoxycarbazone controlled quackgrass when applied in May. Propoxycarbazone, sulfometuron, and tebuthiuron controlled smooth bromegrass when applied in June, but none of the treatments controlled quackgrass. At Fargo, aminocyclopyrachlor,

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[2]Plant Materials Center, USDA, NRCS, 3308 University Drive, Bismarck, ND 58504.
[3]Agassiz Seed and Supply, 445 7th Street NW, West Fargo, ND 58078.
[4]Norfarm Seeds, Inc., 100 Minnesota Avenue NW, Bemidji, MN 56601.

flucarbazone, propoxycarbazone, and sulfosulfuron controlled quackgrass when applied in May, but none of the treatments controlled smooth bromegrass. Weedy grasses from treatments applied in June at Fargo were not harvested due to little visible difference among treatments. The weedy grass infestations were uneven and patchy, which may have resulted in variable yield that may not have been due to herbicide treatments alone.

MATERIALS AND METHODS

Soil Seedbank. A soil seedbank study was established in the LMNG in western North Dakota to evaluate species composition change 10 yr following leafy spurge control by *Aphthona* spp. The landscape in the LMNG consists of many gullies, ravines, and buttes and covers 500,000 ha in western North Dakota (Hopkins et al. 1986). The predominant soils include well-drained loams, clay loams, and sandy loams. The soils derived from soft clayey shales and sandstones, are unstable, and are highly susceptible to erosion. The annual precipitation near the LMNG in Medora has fluctuated over the last 50 yr, but has withheld a constant trend of approximately 39 cm per year (USDC-NOAA-NRCDC 1949-2010). Historically, grazing was the primary land use in the LMNG (Hopkins et al. 1986), but now, oil and gas development have increased dramatically (NDIC-OGD 2011).

There were 24 sites established in 1999 for seedbank analysis as described by Cline (2002). A mixture of 3,000 *Aphthona lacertosa* and *A. czwalinae* and 3,000 *A. nigriscutis* flea beetles were released for leafy spurge control. Each site was recorded with a global positioning system (GPS) and marked on topographic maps. Sites were also marked with labeled PVC posts and two plastic surveyor stakes. One stake was located in the center of the site, and another stake was located at 90-degrees to the right of the center and perpendicular to the maximum water flow of the slope.

Five sites were located in Township 141 N, Range 102 W, Section 25 (47.0°N, 103.5°W; elevation 821 m) Golden Valley County; three sites in Township 141 N, Range 102W, Section 31 (46.98°N, 103.6°W; elevation 802 m) Billings County; seven sites in Township 141 N, Range 102 W, Section 36 (46.98°N, 103.5°W; elevation 823 m) Golden Valley

County; six sites in Township 141 N, Range 103 W, Section 28 (47.0°N, 103.7°W; elevation 800 m) Billings County; and three sites in Township 141 N, Range 103 W, Section 33 (46.98°N, 103.7°W; elevation 792 m) Billings County (Cline et al. 2008).

The major vegetation type was a mixed-grass prairie dominated by blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], western wheatgrass, needle-and-thread grass [*Hesperostipa comata* (Trin & Rupr.) Barkworth], green needlegrass, prairie sandreed [*Camalmovilfa longifolia* (Hook.) Scrib.], and little bluestem (Cline 2002; Juricek 2006). Woody vegetation species among the prairie species included cottonwood (*Populus deltoides* Marsh.), silver buffaloberry [*Shepherdia argentea* (Pursh) Nutt.], western snowberry (*Symphoricarpos occidentalis* Hook.), and creeping juniper (*Juniperus horizontalis* Moench).

The seedbank study was conducted on 12 loamy overflow and 12 loamy sites, as defined by the USDA-NRCS Ecological Site Description System (USDA-NRCS 2007). Locations were originally chosen in 1999 based on leafy spurge density (uninfested, light, moderate, and heavy), soil type, and vegetation composition (Cline 2002). The light, moderate, and heavy leafy spurge infestations averaged approximately 87, 127, and 224 stems m^{-2}, respectively, from the loamy overflow sites and 46, 83, and 183 stems m^{-2}, respectively, from the loamy sites.

Leafy spurge stem density was counted and soil cores were collected for seedbank analysis in mid-August 2009 from each of the 24 original sites. The sites were 255 m^2 in size and separated into eight equal transects radiating clockwise from the center at 45-degree angles with transect three always pointing north from the center point (Cline

2002). Stem density was determined by counting the number of stems in four 0.25-m^2 quadrats placed at 1-m intervals on the cardinal directions. Additionally, four soil cores at 1-m intervals were excavated from each of five transects that were chosen at random. Each soil core was removed using a standard golf-cup cutter to a depth of 5 cm with a 10-cm diameter for a total of 480 soil samples. Soil samples were refrigerated at 3 C prior to seedbank evaluation for at least 14 d to overcome dormancy as suggested by Perez et al. (1998).

Seedbank analysis was conducted by seed germination methods outlined by Ter Heerdt et al. (1996). A mixture of steam-sterilized soil and commercial plant-growth media[5] was added to 28- by 56-cm greenhouse trays to a 2.5 cm depth and topped with a 3- to 5-cm thick layer of sterile silica sand. Four soil cores from each transect were combined and washed with tap water through a coarse (4-mm) and fine (0.2-mm) sieve to remove debris and unwanted plant material. Tap water was added to the soil samples to form a slurry and the mixture was poured into a tray as the top layer. All trays were placed in the greenhouse and watered daily. Greenhouse temperature was maintained between 20 and 28 C and natural light was supplemented with halide lamps at 450 μE m^{-2} s^{-1} for a 16-h photoperiod. Once seedlings emerged, they were identified, recorded, and removed. Unknown seedlings were transplanted to allow further growth until proper identification was possible. The study was conducted for approximately 24 wk.

The soil seedbank data were analyzed as a completely random design utilizing the

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[5] Sunshine Mix No. 1®; Sun Gro Horticulture, 15831 NE 8th St., Bellevue, WA 97008.

Generalized Linear Models (GLM) procedure of SAS[6]. A Fischer's protected least significant difference (LSD) ($p < 0.1$) was calculated for mean separation to evaluate the change in species composition within the soil seedbank 5 and 10 yr following *Aphthona* spp. release in 1999. Soil cores were excavated in the spring and fall of 1999, but only the fall data were used for comparison. A factorial arrangement was used to compare seedling densities in seven vegetation categories among four levels of leafy spurge infestation (uninfested, light, moderate, and heavy) between two ecological sites (loamy overflow and loamy). Seedlings were placed into one of seven categories including major invasives, high- and low-seral forbs, high- and low-seral grasses, hydric/mesic species, and unknown species. There were three replicates and five sub-samples for each level of leafy spurge infestation, and each ecological site was analyzed separately.

Coefficients of Conservatism values (C-value) were assigned to every plant species based on an assessment by the Northern Great Plains Floristic Quality Assessment Panel (2001). The C-value ranges from 0 to 10, with 0 for plant species that inhabit highly disturbed (low-seral) areas and 10 for undisturbed, natural (high-seral) areas. Low-seral species had a C-value of 3 or less, and high-seral species had a C-value of 4 or greater (Cline 2002).

Native Grass Recovery. A grass seedling emergence bioassay was conducted with soil excavated from sites at least 10 yr following the release of *Aphthona* spp. to control leafy spurge. A mixed population of *A. czwalinae* and *A. lacertosa* were released near Valley

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[6] [SAS] Statistical Analysis Systems; Statistical Analysis Software 2004, Version 9.1.2; SAS, Inc., 100 SAS Campus Drive, Cary, NC 27513.

City, ND in 1988 (Lym and Olson 1999), and were then distributed throughout the state. Flea beetles were also released in the LMNG near Medora in 1999 (Cline 2002). In 2010, soil samples were collected throughout North Dakota at six different locations and excavated from *Aphthona* spp. release and non-release sites (Table 1). Similar field methods were followed from Juricek (2006). Release-sites were chosen based on active leafy spurge control by *Aphthona* spp. and were marked with a GPS unit. The non-release sites were adjacent to the release sites and determined by the presence of leafy spurge without evidence of active biological control. A bulk soil sample was excavated with a spade from an approximate 100- by 100- by 15-cm area and passed through a 6-mm sieve to remove plant material and debris in the field. Soil was returned to Fargo and then air-dried for 72 h at 26 C and refrigerated at 3 C for 2 to 4 wk. Soil was pre-weighed (300 g) and then added to 3.5- by 5-cm deep pots, which were held in foam plastic trays to allow surface and sub-surface irrigation.

Native grasses were used to compare seedling emergence from insect-release and non-release soils. The seeded grasses included 'Rodan' western wheatgrass, 'Lodorm' green needlegrass, 'Badlands' little bluestem, and 'Dakotah' switchgrass. Grasses were over-seeded and thinned to 8 seedlings per pot, as necessary. Pots were placed in the greenhouse and rotated weekly, and grasses were watered daily through surface and subsurface irrigation. Greenhouse temperature was maintained between 20 and 28 C and natural light was supplemented with halide lamps at 450 μE m^{2} s^{1} for a 16 h photoperiod.

Table 1. Location of sample sites and soil type collected in September 2010 from *Aphthona* spp. release and non-release sites at various locations throughout North Dakota.

Location	Geographic coordinates	Soil series	Soil type
Buffalo	46° 55′ 04.2″N 97° 32′ 06.9″ W	Barnes-Svea	Fine-loamy, mixed, superactive, frigid Calcic Hapludolls; Fine-loamy, mixed, superactive, frigid Pachic Hapludolls
Gladstone	46° 47″ 49.7″ N 102° 34′ 05.4″ W	Shambo	Fine-loamy, mixed superlative, frigid Typic Haplustolls
Lake Tschida	46° 35′ 54.6″ N 101° 50′ 41.7″ W	Schaller-Cabba	Sandy, mixed, frigid Entic Haplustolls; Loamy, mixed, superactive, calcareous, frigid, shallow Typic Ustorthents
Medora	46° 59′ 54.3″ N 103° 37′ 20.3″ W	Lonna-Cabbart	Fine-silty, mixed, superactive, frigid Aridic Haplustepts; Loamy, mixed, superactive, calcareous, frigid, shallow Ardic Ustorthents
Pipestem Dam	46° 57′ 50.48″ N 98° 44′ 45.93″ W	Barnes-Svea	Fine-loamy, mixed, superactive, frigid Calcic Hapludolls; Fine-loamy, mixed, superactive, frigid Pachic Hapludolls
Standing Rock Reservation in Sioux County	46° 47′ 49.7″ N 98° 44′ 49.42″ W	Desart-Ekalaka-Telfer	Coarse-loamy, mixed, superactive, frigid Typic Natrustolls; Coarse-loamy, mixed, superlative, frigid Typic Natrustolls; Sandy, mixed, frigid Entic Haplustolls

Pots were weeded as needed and fertilized 2 and 4 wk after emergence equivalent to 170 kg ha $^{-1}$ nitrogen using a water-soluble fertilizer[7]. Approximately 15 wk after emergence,

[7] Peters Professional® 19-20-18 Peat-Lite Special®, Peters Fertilizer Products, P.O. Box 789, Fogelsville, PA 18051.

the grasses were counted, removed at the soil surface, dried at 60 C for 36 h, and weighed.

The experimental design was completely random with six replicates and a factorial arrangement comparing four native grass species between *Aphthona* spp. release and non-release sites at six locations. Data were analyzed by the PROC GLM procedure of SAS and treatment means were separated using probability of difference (PDIFF) ($p < 0.05$). The experiment was repeated and the error mean squares from each run were compared for homogeneity of variance. A combined analysis was conducted when error mean squares for each run differed by less than a factor of 10.

Weed Control in Switchgrass. Herbicides with the most potential to control smooth bromegrass and quackgrass without injury to switchgrass were determined in a greenhouse study and evaluated in the field (Eken et al. 2010). The field locations included an established switchgrass stand at the Central Grassland Research Extension Center (CGREC) near Streeter, ND and a smooth bromegrass- and quackgrass-infested field at the NDSU Experiment Station in Fargo, ND. The switchgrass stand was originally established in 2001 and since then quackgrass and smooth bromegrass had invaded. Nine herbicides were applied on May 21 or June 25, 2009 at the lowest recommended label rate and twice that rate (Appendix Tables 16 and 17). Herbicides were applied with a hand-held boom sprayer at 240 kPa with 8002 flat-fan nozzles delivering 160 L ha^{-1}. Plots were 9- by 3-m in a randomized complete-block design with four replicates.

Herbicide efficacy was evaluated approximately 14, 30, 60, and 90 d after treatment. Visual evaluations were completed on a scale of 0 to 100, with 0 representing no visible injury and 100 corresponding to all surface vegetation dead. Grasses were harvested between July 27 and July 29, 2009 at the NDSU Experiment Station and August 2 and August 3, 2009 at the CGREC. In each plot, samples were harvested from three 0.25-m^2 quadrats placed every 0.75 m down the center of each plot. All living plant material above 5 cm was hand harvested and vegetation was separated into quackgrass, smooth bromegrass, other grasses, or forbs at the NDSU Experiment Station or switchgrass, quackgrass, smooth bromegrass, other grasses, or forbs at the CGREC. The three subsamples from each plot were combined for analysis and harvested material was dried for 72 h at 45 C and weighed. Data were analyzed using a PROC GLM of SAS and a Fischer's protected LSD ($p < 0.05$) was used for mean separation of switchgrass injury and weedy grass control.

Herbicide efficacy was evaluated 1 yr after treatment (YAT) as previously described for switchgrass injury and grassy weed control at the CGREC and grassy weed control at Fargo. Vegetation from selected treatments was harvested as previously described on July 29, 2010 at the CGREC near Streeter, ND. Grasses were not harvested at Fargo. Data were analyzed using the PROC GLM procedure of SAS, and a Fischer's protected LSD ($p < 0.05$) was calculated for mean separation of switchgrass injury and weedy grass control.

Three herbicides were further evaluated for smooth bromegrass control and four herbicides for quackgrass control based on results from a 2008 greenhouse study and previous fieldwork (Table 2). Separate smooth bromegrass and quackgrass studies were located on pastureland adjacent to the NDSU campus and approximately 8 km north on

Table 2. Post-emergent herbicides applied on October 6, 2010 or May 16, 2011 at the North Dakota State University campus or October 16, 2010 or June 6, 2010 at the NDSU Experiment Station in Fargo, ND for smooth bromegrass or quackgrass control.

Smooth bromegrass		Quackgrass	
Herbicide	Rate[a]	Herbicide	Rate[a]
	g ha^{-1}		g ha^{-1}
Aminocyclopyrachlor	140	Aminocyclopyrachlor	140
	280		280
Chlorsulfuron	23	Imazaquin	196
	47		392
Sulfometuron	140	Metsulfuron	14
	280		28
		Sulfometuron	140
			280

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.

NDSU Experiment Station land. At the NDSU campus, primary forb species included alfalfa, Canada thistle, and plumeless thistle (*Carduus acanthoides* L.), and other grass species mainly consisted of Kentucky bluegrass. At the NDSU Experiment Station site, grass and forb species primarily included Kentucky bluegrass and leafy spurge, respectively.

Herbicide application, visual evaluation, and harvest methods were completed as previously described for grassy weed control. At the NDSU campus, herbicides were applied on October 6, 2010 or May 16, 2011 for smooth bromegrass and quackgrass control. At the NDSU Experiment Station, herbicides were applied on October 16, 2010 or June 6, 2011 for smooth bromegrass control or October 16, 2010 for quackgrass control. The quackgrass experiment at the NDSU Experiment Station was not treated in the spring due to flooding. Visual evaluations for grassy weed control were completed on May 31, June 17, and September 2, 2011 at NDSU campus and June 17, July 5, and September 2, 2011 at the Experiment Station. Grasses were harvested on July 7, 2011 at NDSU campus and July 29, 2011 at the Experiment Station as previously described, except only one quadrat was harvested in the center of each plot. Vegetation was separated into smooth bromegrass or quackgrass, other grasses, and forbs.

Data were analyzed as a randomized complete block design with four replicates and a factorial arrangement comparing herbicides and rates. Visual evaluations and harvest yield were analyzed using PROC GLM and MIXED procedures of SAS and a Fischer's protected LSD ($p < 0.05$) was used for mean separation for weedy grass control. Error mean squares from each location were compared for homogeneity of variance, but were not combined as the error mean squares differed by greater than a factor of 100.

RESULTS AND DISCUSSION

Soil Seedbank. Leafy spurge stem density decreased 98% and 89% from the loamy overflow and loamy sites, respectively, in the LMNG 10 years following *Aphthona* spp. release in 1999 (Table 3). In 2009, leafy spurge stem density averaged 2 and 9 stems m^{-2} in the loamy overflow and loamy sites, respectively, compared to 110 and 78 stems m^{-2}, respectively, in 1999 and 7 and 10 stems m^{-2}, respectively, in 2004. By 2009, stem density was similar within the four original leafy spurge categories (uninfested, low, moderate, and high). *Aphthona* spp. have also substantially reduced leafy spurge infestations in other areas throughout the Northern Great Plains (Butler et al. 2006; Kirby et al. 2000; Lesica and Hanna 2004; Lym and Nelson 2002; Mico and Shay 2002).

Leafy spurge seed was reduced more than 96% from 3,358 seedlings in 1999 to 127 seedlings in 2009 in the loamy overflow seedbank as the above-ground stem density decreased (Table 4). Leafy spurge constituted nearly 67% of the loamy overflow seedbank in 1999 compared to 17% in 2004 and 2% in 2009. For the loamy sites, leafy spurge was reduced approximately 90% from 1,429 seedlings in 1999 to 146 seedlings in 2009 (Table 5). The weed represented nearly 70% of the loamy seedbank in 1999 compared to approximately 11% in 2004 and 15% in 2009.

The total number of seeds in the loamy overflow seedbank increased from 1999 to 2009 (Table 4), but decreased in the loamy seedbank (Table 5). From the loamy overflow sites, 5,966 seedlings emerged in 2009 compared to 5,042 and 6,798 seedlings in 1999 and 2004, respectively. A total of 977 seedlings emerged from the loamy sites in 2009 compared to 2,052 and 2,788 seedlings in 1999 and 2004, respectively.

Table 3. Leafy spurge stem density across various categories in 1999 and 5 and 10 yr following *Aphthona* spp. release on loamy overflow and loamy sites in the Little Missouri National Grasslands in western North Dakota.

Year and leafy spurge density classification	Loamy overflow	Loamy
	Stems m^{-2}	
1999		
Uninfested	0	0
Low	87	46
Moderate	127	83
High	224	183
LSD (0.05)	12	11
2004		
Uninfested	5	3
Low	1	0
Moderate	16	16
High	7	20
LSD (0.05)	10	9
2009		
Uninfested	3	9
Low	2	10
Moderate	2	4
High	1	15
LSD (0.05)	NS	NS
1999 Mean	110	78
2004 Mean	7	10
2009 Mean	2	9

Species diversity in the LMNG increased 33% and 35% in the loamy overflow and loamy seedbank, respectively, 10 yr following *Aphthona* spp. release for leafy spurge control (Tables 4 and 5). A total of 57 species emerged from the loamy overflow seedbank in 2009 compared to 43 species in 1999 and 54 species in 2004. From the loamy sites, 54 species emerged in 2009 compared to 40 species in 1999 and 51 species in 2004. The increasing trend in species diversity generally came from an increase in high-seral forbs at both ecological sites from 1999 to 2004.

Table 4. Scientific name, common name, Coefficient of Conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in mid-August, 5 and 10 yr after *Aphthona* spp. release for leafy spurge control in 1999, from 12 loamy overflow sites in southwestern North Dakota.

			1999		2004		2009		
Scientific Name[a]	Common name	C-C[b]	No.[c]	%[d]	No.	%	No.	%	LSD[e]
Major Invasives									
Euphorbia esula L.	Leafy spurge	NV	3,358	67	1,135	17	127	2	333
Poa pratensis L.	Kentucky bluegrass	NV	1,066	21	1,226	18	3,783	64	453
Subtotal			4,424	88	2,361	35	3,910	66	440
High-Seral Forbs									
Allium textile A. Nelson & J.F. Macbr.	Wild onion	7	-	-	-	-	19	0.3	
Androsace occidentalis Pursh	Western rock-jasmine	5	21	0.4	693	10	16	0.3	
Arabis holboelli var. *collinsii* (Fern) Rollins	Rock cress	5	-	-	9	0.1	-	-	
Artemisia frigida Willd.	Fringed sage	4	30	1	508	8	8	0.1	
Aster oblongifolis Nutt.	Aromatic aster	8	-	-	13	0.2	-	-	
Astragalus agrestis Don	Purple milkvetch	6	-	-	-	-	1	0.1	
Chenopodium gigantospermum Aellen	Maple-leaved goosefoot	5	-	-	1	0.1	-	-	
Erysimum inconspicuum (S. Watson) MacMill.	Shy wallflower	7	-	-	-	-	1	0.1	
Fragaria virginiana Duchesne	Wild strawberry	4	42	0.8	-	-	-	-	
Galium boreale L.	Northern bedstraw	4	3	0.1	11	0.2	-	-	
Galium triflorum Michx.	Fragrant bedstraw	7	-	-	-	-	21	0.4	
Gaura coccinea var. *glabra* (Lehm.) T. & G.	Scarlet gaura	4	-	-	1	0.1	-	-	
Gutierrezia sarothrae (Pursh) Britt. & Rusby	Broom snakeweed	6	-	-	1	0.1	1	0.1	
Hedeoma drummondii Benth.	Drummond false pennyroyal	4	67	1	252	4	-	-	
Lesquerella arenosa var. (Richardson) Rydb.	Great Plains bladderpod	6	-	-	3	0.1	-	-	
Linum perenne Pursh var. *lewisii*	Prairie flax	6	-	-	5	0.1	37	0.6	
Lithospermum canescens Lehm.	Hoary puccoon	7	-	-	7	0.1	9	0.2	
Mondarda fistulosa L.	Wild bergamot	5	-	-	1	0.1	-	-	

Table 4. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Oxytropis spp. DC.	Locoweed	NV	-	-	-	-	1	0.1	
Potentilla arguta Pursh	Tall cinquefoil	8	-	-	122	2	3	0.1	
Senecio plattensis (Nutt.) Weber & A. Löve	Prairie groundsel	6	-	-	-	-	1	0.1	
Solidago rigidum L.	Stiff goldenrod	4	-	-	-	-	1	0.1	
Solidago spp.	Goldenrods	NV	2	0.1	-	-	-	-	
Subtotal			165	3	1,627	25	119	3	342
Low-Seral Forbs									
Achillea millefolium L.	Common yarrow	3	34	0.1	104	2	136	2	
Ambrosia artemisiifolia L.	Common ragweed	0	-	-	1	0.1	-	-	
Artemisia ludoviciana Nutt.	White sage	3	25	1	30	0.4	5	0.1	
Aster ericoides L.	White aster	2	2	0.1	7	0.1	-	-	
Brassicaceae spp.	Mustard family	NV	-	-	-	-	296	5	
Cerastium arvense L.	Prairie chickweed	2	-	-	4	0.1	4	0.1	
Chamaesyce serpyllifolia Small	Thymeleaf sandmat	0	-	-	-	-	193	3	
Chenopodium album L.	Common lambsqaurters	NV	-	-	5	0.1	-	-	
Chenopodium glaucum L.	Oak-leaved goosefoot	NV	-	-	7	0.1	1	0.1	
Chenopodium rubrum L.	Alkali blight	2	1	0.02	-	-	-	-	
Cirsium arvense (L.) Scop.	Canada thistle	NV	1	0.02	1	0.01	1	0.1	
Convolvulus arvensis L.	Field bindweed	NV	1	0.02	-	-	-	-	
Conyza canadensis (L.) Cronq.	Horseweed	0	19	0.4	4	0.1	1	0.1	
Descurainia pinnata (Walt.) Britt.	Tansy mustard	1	5	0.1	4	0.1	163	3	
Descurainia sophia (L.) Prantl	Flixweed	NV	-	-	-	-	3	0.1	
Draba nemorosa L.	Yellow whitlowort	1	-	-	1,719	25	89	2	
Epilobium ciliatum Raf.	Willow-herb	3	-	-	1	0.1	-	-	
Erigeron philadephicus L.	Daisy fleabane	2	3	0.1	-	-	-	-	
Erysimum asperum (Nutt.) DC.	Western wallflower	3	-	-	-	-	1	0.1	
Erysimum cheiranthoides L.	Wormseed wallflower	NV	10	0.2	65	1	-	-	
Euphorbia glyptosperma Engelm.	Ridge-seeded spurge	0	2	0.04	42	1	-	-	

Table 4. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Euphorbia maculata L.	Prostrate spurge	1	-	-	2	0.1	7	0.1	
Grindelia squarrosa (Pursh) Dun.	Curly-cup gumweed	1	-	-	6	0.1	5	0.1	
Hedeoma hispida Pursh	Rough false pennyroyal	2	4	0.1	-	-	-	-	
Lappula redowski Greene	Low stickseed	2	4	0.1	40	1	-	-	
Lepidium densiflorum Schrad.	Greenflower peppergrass	0	1	0.1	86	1	21	0.4	
Melilotus officinalis (L.) Lam.	Yellow sweet clover	NV	-	-	-	-	4	0.1	
Nepeta cataria L.	Catnip	NV	-	-	4	0.1	1	0.1	
Neslia paniculata (L.) Desv.	Ball mustard	NV	-	-	145	2	-	-	
Plantago elongata Pursh	Slender plantain	3	-	-	3	0.1	-	-	
Plantago major L.	Common plantain	NV	4	0.1	-	-			
Plantago patagonica Jacq.	Woolly plantain	1	-	-	-	-	5	0.1	
Polygonum convolvulus L.	Wild buckwheat	NV	7	0.1	-	-	-	-	
Potentilla norvegica L.	Norwegian cinquefoil	0	-	-	-	-	3	0.1	
Ratibida columnifera (Nutt.) Woot. & Standl.	Prairie coneflower	3	15	0.3	41	1	23	0.4	
Rosa arkansana Porter	Prairie wild rose	3	1	0.1	-	-	-	-	
Rumex crispus L.	Curly dock	NV	-	-	1	0.1	-	-	
Silene noctiflora L.	Night-flowering catchfly	NV			8	0.1	1	0.1	
Sonchus spp.	Sowthistles	NV	13	0.3	-	-	-	-	
Taraxacum officinale Weber	Common dandelion	NV	128	3	127	2	45	1	
Tragopogon dubius Scop.	Goat's beard	NV	-	-	3	0.1	2	0.1	
Verbascum thapus L.	Common mullein	NV	16	0.3	-	-	-	-	
Verbena bracteata lag. & Rodr.	Prostrate vervain	0	1	0.1	-	-	-	-	
Subtotal			297	7	2,460	38	1,010	18	147
High-Seral Grasses									
Bouteloua gracilis (Willd. Ex Kunth) Lag. ex Griffiths	Blue grama	7	4	0.1	-	-	-	-	
Calamovilfa longifolia (Hook) Scribn.	Prairie sandreed	5	-	-	4	0.1	-	-	

Table 4. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Elymus trachycaulus (Link) Gould ex Shinners	Slender wheatgrass	6	-	-	-	-	2	0.1	
Hesperostipa comata (Trin. & Rupr.) Barkworth	Needle-and-thread	6	3	0.1	-	-	2	0.1	
Hesperostipa spartea Trin.	Porcupine grass	8	-	-	-	-	2	0.1	
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	7	39	1	-	-	758	13	
Muhlenbergia cuspidata (Torr. ex Hook) Rydb.	Plains muhly	8	1	0.1	-	-	-	-	
Nassella viridula (Trin.) Barkworth	Green needlegrass	5	34	0.1	112	2	24	0.4	
Poa palustris L.	Fowl bluegrass	4	-	-	44	1	-	-	
Puccinellia nuttalliana (Schult.) A. Hitchc.	Nuttall's alkaligrass	4	-	-	5	0.1	-	-	
Schizachyrium scoparium (Michx.) Nash-Gould	Little bluestem	6	1	0.1	12	0.2	1	0.1	
Sporobolus cryptandrus (Torr.) A. Gray	Sand dropseed	6	19	0.4	3	0.1	15	0.3	
Subtotal			101	2	180	4	804	14	251
Low-Seral Grasses									
Agrostis scabra Willd.	Rough bentgrass	1	-	-	-	-	1	0.2	
Bromus inermis Leyss. Ssp. *inermis*	Smooth bromegrass	NV	-	-	96	1	-	-	
Bromus tectorum L.	Cheatgrass	NV	-	-	-	-	84	1	
Digitaria ischaemum (Schreb.) Schreb. Ex Muhl	Smooth crabgrass	NV	13	0.3	-	-	-	-	
Distichlis spicata (L.) Greene	Saltgrass	2			-	-	2	0.1	
Echinochola crus-galli (L.) Beauv.	Barnyardgrass	NV	7	0.1	-	-	-	-	
Elymus repens (L.) Gould	Quackgrass	NV	-	-	-	-	2	0.1	
Poa compressa L.	Canada bluegrass	NV	-	-	1	0.1	9	0.2	
Schedonnardus paniculatus (Nutt.) Trel.	Tumblegrass	1	21	0.4	13	0.2	-	-	
Setaria viridis (L.) beauv.	Green foxtail	NV	1	0.1	-	-	-	-	
Vulpia octoflora (Walter) Rydb.	Sixweeks fescue	0	-	-		-	1	0.1	
Subtotal			42	1	110	1	99	2	NS

Table 4. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Hydric/Mesic Species									
Carex spp. L.	Sedges	NV	12	0.2	34	1	5	0.1	
Juncus balticus Willd.	Baltic rush	5	-	-	16	0.2	-	-	
Juncus spp. L.	Rushes	NV	-	-	-	-	2	0.1	
Typha spp. L.	Cattails	NV	1	0.1	10	0.2	1	0.1	
Subtotal			13	0.3	60	1	8	0.3	5
Unknowns									
Lamiaceae spp.	Mint family	NV	-	-	-	-	12	0.2	
Unknown spp. 1		NV	-	-	-	-	1	0.1	
Unknown spp. 2		NV	-	-	-	-	3	0.1	
Subtotal			0	0	0	0	16	0.4	NS
Total			5,042	100	6,798	100	5,966	100	650

[a] Scientific nomenclature follows the Flora of the Great Plains (Great Plains Flora Association 1986), except as amended according to the United States Departmnet of Agriculture (USDA)/Natural Resources Conservation Service (NRCS) Plants Database (2006) (http://plants.usda.gov/index.html).

[b] C-C = Coefficient of Conservatism. A coefficient value of 'NV' (*no value*) is *indictive of an introduced or unidentified* species; 0-3 is indicative of species that flourish in highly disturbed habitats; and values of 4 10 are assigned to species from less disturbed, natural areas (Northern Great Plains Floristic Quality Assessment Panel 2001).

[c] Total number of seedlings that emerged per 0.5 m^2 from soil samples collected to a depth of 5 cm.

[d] Percent of total seedlings that emerged across all soil cores.

[e] A Fischer's protected LSD ($p < 0.1$) was calculated to evaluate the change in seedling number across years.

There was a greater tendency for increased species diversity and seedling emergence in the loamy overflow sites compared to the loamy sites in 2009 (Tables 4 and 5). Loamy overflow soils are characterized by greater moisture availability from surface runoff and subsurface water movement, greater organic matter content, and higher fertility than loamy site soils (Hanna et al. 1982; Malo and Worcester 1975; Wolf 1987). Cline (2002) also reported twice as many seedlings emerged from the loamy overflow seedbank than the loamy seedbank due to a more favorable habitat for growth and seed production.

Table 5. Scientific name, common name, Coefficient of Conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in mid-August, 5 and 10 yr after *Aphthona* spp. release for leafy spurge control in 1999, from 12 loamy sites in southwestern North Dakota.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Major Invasives									
Euphorbia esula L.	Leafy spurge	NV	1,429	70	299	11	146	15	207
Poa pratensis L.	Kentucky bluegrass	NV	160	8	182	7	99	10	NS
Subtotal			1,589	78	481	17	245	25	303
High-Seral Forbs									
Allium textile A. Nelson & J.F. Macbr	Wild onion	7	-	-	1	0.1	1	0.1	
Androsace occidentalis Pursh	Western rock-jasmine	5	12	1	46	2	14	1	
Arabis holboelli var. *collinsii* (Fern) Rollins	Rock cress	5	-	-	51	2	-	-	
Artemisia campestris L. subsp. *caudata* (Michx.) Hall & Clem.	Field sagewort	5	3	0.2	3	0.1	1	0.1	
Artemisia dracunculus L.	Silky wormwood	4		-	3	0.1	-	-	
Artemisia frigida Willd.	Fringed sage	4	42	2	265	10	21	2	
Aster oblongifolis Nutt.	Aromatic aster	8	-	-	5	0.2	-	-	
Campanula rotundifolia L.	Harebell	7	-	-	47	2	53	5	
Erysimum inconspicuum (S.Watson) MacMill.	Shy wallflower	7			-	-	1	0.1	
Fragaria virginiana Duchesne	Wild strawberry	4	3	0.2	-	-	-	-	
Galium boreale L.	Northern bedstraw bedstraw	4	2	0.1	-	-	-	-	
Gutierrezia sarothrae (Pursh) Britt. & Rusby	Broom snakeweed	6			1	0.1	1	0.1	
Hedeoma drummondii Benth.	Drummond false pennyroyal	4	21	1	252	9	-	-	
Lesquerella arenosa var. *arenosa* (Richardson) Rydb.	Great Plains bladderpod	6	-	-	22	1	2	0.2	
Linum perenne Pursh var. *lewisii*	Prarie flax	6	-	-	15	1	7	1	
Lithospermum canescens (Michx.) Lehm.	Hoary puccoon	7	-	-	2	0.1	103	11	

Table 5. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Orthocarpus luteus Nutt.	Yellow owl's-clover	6	-	-	-	-	1	0.1	
Senecio plattensis (Nutt.) W.A. Weber & A. Löve	Prairie groundsel	6	-	-	-	-	2	0.2	
Solidago rigidum L.	Stiff goldenrod	4	-	-	-	-	1	0.1	
Solidago spp.	Goldenrods	NV	6	0.3	-	-	-	-	
Subtotal			89	5	730	29	209	21	163
Low-Seral Forbs									
Achillea millefolium L.	Common yarrow	3	2	0.1	2	0.1	3	0.3	
Artemisia ludoviciana Nutt.	White sage	3	2	0.1	-	-	-	-	
Aster ericoides L.	White aster	2	2	0.1	3	0.1	1	0.1	
Chamaesyce serpyllifolia (Pers.) Small	Thymeleaf sandmat	0	-	-	-	-	1	0.1	
Chenopodium album L.	Common lambsqaurters	NV	-	-	1	0.1	12	1	
Cirsium arvense (L.) Scop.	Canada thistle	NV	-	-		-	2	0.2	
Conyza canadensis (L.) Cronq.	Horseweed	0	23	1	10	0.4	16	2	
Descurainia pinnata (Walt.) Britt.	Tansy mustard	1	1	0.1	2	0.1	-		
Descurainia sophia (L.) Webb ex Prantl	Flixweed	NV	-	-	-	-	11	1	
Draba nemorosa L.	Yellow whitlowort	1	-	-	822	30	216	22	
Epilobium ciliatum Raf.	Willow-herb	3	-	-	8	0.3	1	0.1	
Erigeron philadephicus L.	Daisy fleabane	2	2	0.1	11	0.4	-	-	
Erysimum asperum (Nutt.) DC.	Western wallflower	3	3	0.2	-	-	5	1	
Erysimum cheiranthoides L.	Wormseed wallflower	NV	1	0.1	133	5	-	-	
Euphorbia glyptosperma Engelm.	Ridge-seeded spurge	0	3	0.2	5	0.2	-	-	
Euphorbia maculata L.	Prostrate spurge	1	-	-	2	0.1	-	-	
Grindelia squarrosa (Pursh) Dun.	Curly-cup gumweed	1	-	-	4	0.1	-	-	
Hedeoma hispida Pursh	Rough false pennyroyal	2	-	-		-	3	0.3	
Lactuca serriola L.	Prickly lettuce	NV	2	0.1	-	-	-	-	
Lappula redowski (Hornem.) Greene	Low stickseed	2	3	0.2	8	0.3			

Table 5. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Lepidium densiflorum Schrad.	Greenflower peppergrass	0	1	0.1	3	0.1	2	0.2	
Medicago lupulina L.	Black medick	NV	-	-	-	-	2	0.2	
Medicago sativa L.	Alfalfa	NV	-	-	-	-	1	0.1	
Melilotus officinalis (L.) Lam.	Yellow sweet clover	NV	-	-	-	-	4	0.4	
Neslia paniculata (L.) Desv.	Ball mustard	NV	-	-	140	5	-	-	
Oenothera biennis L.	Common evening primrose	0	-	-	8	0.3		-	
Plantago elongata Pursh	Slender plantain	3	-	-	9	0.3	-	-	
Plantago major L.	Common plantain	NV	4	0.2	-	-	-	-	
Plantago patagonica Jacq.	Woolly plantain	1	-	-	-	-	1	0.1	
Potentilla norvegica L.	Norwegian cinquefoil	0	-		-	-	1	0.1	
Ratibida columnifera (Nutt.) Woot. & Standl.	Prairie coneflower	3	6	0.2	22	1	5	1	
Silene noctiflora L.	Night-flowering catchfly	NV	-	-	1	0.1	-	-	
Sisymbrium altissimum L.	Tumble mustard	NV	6	0.3	-	-	-	-	
Sonchus spp.	Sowthistles	NV	2	0.1	-	-	-	-	
Taraxacum officinale Weber	Common dandelion	NV	43	2	119	4	43	4	
Tragopogon dubius Scop.	Goat's beard	NV	-	-	1	0.1	3	3	
Verbascum thapus L.	Common mullein	NV	74	4	-	-	-	-	
Subtotal			180	9	1,314	48	333	37	271
High-Seral Grasses									
Agropyron spicatum (Pursh) Scribn. & Sm.	Beardless wheatgrass	9	-	-	3	0.1	-	-	
Bouteloua gracilis (Willd. Ex Kunth) Lag. ex Griffiths	Blue grama	7	3	0.2	-	-	-	-	
Calamovilfa longifolia (Hook) Scribn.	Prairie sandreed	5	32	2	117	4	8	0.8	
Hesperostipa comata (Trin. & Rupr.) Barkworth	Needle-and-thread	6	4	0.2	-	-	1	0.1	
Hesperostipa spartea Trin.	Porcupine grass	8	-	-	3	0.1	3	0.3	

Table 5. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	7	85	4	-	-	138	14	
Muhlenbergia cuspidata (Torr. ex Hook) Rydb.	Plains muhly	8	1	0.1	-	-		-	
Muhlenbergia richardsonis (Trin.) Rydb.	Mat muhly	10	-	-	4	0.1	·	-	
Nassella viridula (Trin.) Barkworth	Green needlegrass	5	9	0.4	2	0.1	3	0.3	
Poa palustris L.	Fowl bluegrass	4	-	-	41	2	·	-	
Puccinellia nuttalliana (Schult.) A. Hitchc.	Nuttall's alkaligrass	4	-	-	10	0.4	·	-	
Schizachyrium scoparium (Michx.) Nash-Gould	Little bluestem	6	19	0.9	31	1	7	0.7	
Sporobolus compositus (Poir.) Merr.	Composite dropseed	4	-	-	-	-	1	0.1	
Sporobolus cryptandrus (Torr.) A. Gray	Sand dropseed	6	15	0.73	2	0.1	2	0.2	
Subtotal			168	8	213	8	163	17	NS
Low-Seral Grasses									
Bromus inermis Leyss. Ssp. *inermis*	Smooth bromegrass	NV	-	-	2	0.1		-	
Bromus japonicus Thunb. Ex Murr.	Japanese brome	NV	2	0.1	-	-		-	
Echinochola crus-galli (L.) Beauv.	Barnyardgrass	NV	1	0.1	-	-		-	
Elymus repens (L.) Gould	Quackgrass	NV	-	-	-		3	0.3	
Poa compressa L.	Canada bluegrass	NV	-	-	-	-	1	0.1	
Schedonnardus paniculatus (Nutt.) Trel.	Tumblegrass	1	3	0.2	3	0.1	-	-	
Subtotal			6	0.4	5	0.2	4	0.4	NS
Hydric/Mesic Species									
Carex spp. L.	Sedges	NV	19	0.9	14	0.1	2	0.2	
Juncus balticus Willd.	Baltic rush	5	-	-	1	0.1		-	
Juncus spp. L.	Rushes	NV	-	-	-	-	2	0.2	
Typha spp. L.	Cattails	NV	1	0.5	28	1	6	0.6	
Subtotal			20	1	43	2	10	1	NS
Unknowns									
Lamiaceae spp.	Mint family	NV	-	-	-	-	8	1	

Table 5. Continued.

Scientific Name[a]	Common name	C-C[b]	1999 No.[c]	1999 %[d]	2004 No.	2004 %	2009 No.	2009 %	LSD[e]
Unknown spp. 1		NV	-	-	-	-	1	0.1	
Unknown spp. 2		NV	-	-	-	-	1	0.1	
Unknown spp. 3		NV	-	-	-	-	2	0.2	
Unknown spp. 4		NV	-	-	-	-	1	0.1	
Subtotal			0	0	0	0	13	2	NS
Total			2,052	100	2,788	100	977	100	423

[a] Scientific nomenclature follows the Flora of the Great Plains (Great Plains Flora Association 1986), except as amended according to the United States Departmnet of Agriculture (USDA)/Natural Resources Conservation Service (NRCS) Plants Database (2006) (http://plants.usda.gov/index.html).

[b] C-C = Coefficient of Conservatism. A coefficient value of 'NV' (no value) is indictive of an introduced or unidentified species; 0-3 is indicative of species that flourish in highly disturbed habitats; and values of 4-10 are assigned to species from less disturbed, natural areas (Northern Great Plains Floristic Quality Assessment Panel 2001).

[c] Total number of seedlings that emerged per 0.5 m^2 from soil samples collected to a depth of 5 cm.

[d] Percent of total seedlings that emerged across all soil cores.

[e] A Fischer's protected LSD ($p < 0.1$) was calculated to evaluate the change in seedling number across years.

Species that constituted the majority of the loamy overflow and loamy seedbank in 2009 included Kentucky bluegrass (64%) and yellow whitlowort (*Draba nemorosa* L.) (22%), respectively (Tables 4 and 5). Leafy spurge was a prevalent species in the loamy seedbank (15%), but only represented 2% of the loamy overflow seedbank. Prairie Junegrass also appeared frequently and constituted 13% and 14% in the loamy overflow and loamy seedbank, respectively. In 1999, leafy spurge was the most dominant species and represented 67% and 70% of the loamy overflow and loamy seedbank, respectively (Cline 2002). Yellow whitlowort was the most prevalent species in 2004 and constituted 25% and 30% of the loamy overflow and loamy seedbank, respectively (Juricek 2006).

Kentucky bluegrass seedling density increased more than 250% in the loamy overflow seedbank, 10 yr following Aphthona spp. release, from 1,066 seedlings in 1999 to

1,226 and 3,783 seedlings in 2004 and 2009, respectively (Table 4). The rapid increase of Kentucky bluegrass in the soil seedbank from 2004 to 2009 was most likely due to reduced competition from leafy spurge, which provided the grass with an opportunity to invade. Precipitation and heavy grazing may have also contributed to the invasion or provided the grass with a favorable habitat for growth and seed production. Kentucky bluegrass tends to favor a wet environment (Stubbendieck et al. 2003) and precipitation was greater from April to June in 2009 (15 cm) than 2004 (5 cm)[8] (NDAWN 2011), which is conducive for Kentucky bluegrass growth. Annual precipitation has fluctuated over the last 50 yr, but the overall average precipitation has remained constant (USDC-NOAA-NRCDC 1949-2010). The grass also emerges in the early spring and is tolerant of drought, excessive flooding, and poorly drained soils (USDA-NRCS 2004). Kentucky bluegrass frequently has replaced native species in grazed pastures as the grass is tolerant to overgrazing whereas native species often cannot withstand the removal of above-ground vegetation and reduced root growth caused by continuous grazing (Bailey et al. 2010).

Kentucky bluegrass has been a persistent species within the nearby Theodore Roosevelt National Park and other perennial and tall grass communities (DeKeyser et al. 2009; Ferell et al. 1998; Hulbert 1986). The grass has proved competitive against or even replaced leafy spurge following *Aphthona* spp. release in both North Dakota and Montana (Butler and Cogan 2004; Butler and Wacker 2010).

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[8] Precipitation was averaged between Beach and Dickinson, ND the year of soil collection.

Kentucky bluegrass seedling emergence from the loamy seedbank was similar across study years as 160 seedlings emerged in 1999 compared to 182 and 99 seedlings in 2004 and 2009, respectively (Table 5). The grass prefers a moist environment and is less competitive in drier, windier sites. The loamy overflow sites are characterized as lowland sites that experience increased water movement and wind deposition compared to the upland or loamy sites.

High-seral forbs constituted approximately 3% of the loamy overflow seedbank in 1999 compared to 25% in 2004, but then declined to 3% again in 2009 (Table 4). Even though the total number of high-seral forb seed was similar 10 yr following *Aphthona* spp. release, there was a trend for increased species diversity. Six high-seral forb species appeared in the loamy overflow seedbank in 1999 compared to 14 and 13 species in 2004 and 2009, respectively. However, many species that emerged in 2004 were not found in 1999 or 2009 and included rock cress [*Arabis holboelli* var. *Collinsii* (Fern) Rollins], maple-leaved goosefoot, scarlet gaura [*Gaura coccinea* var. *glabra* (Lehm.) T. & G.], Great Plains bladderpod [*Lesquerella arenosa* var. *arenosa* (Richardson) Rydb.], and wild bergamot (*Mondarda fistulosa* L.). Additionally, some species appeared more frequently in 2004 compared to 1999 or 2009. For example, western rockjasmine (*Androsace occidentalis* Pursh) constituted more than 10% of the loamy overflow seedbank in 2004, but less than 1% in 1999 and 2009. Fringed sage (*Artemisia frigida* Willd.) represented 8% of the seedbank in 2004, but decreased to less than 1% in 2009. Increased species diversity and seedling emergence in 2004 may be explained by the temporary increase in nutrient and moisture availability following the removal of vegetation (Redente et al. 1992), which was caused by the reduction of leafy spurge prior to the large increase in Kentucky bluegrass.

High-seral forbs that appeared in the loamy overflow seedbank in 2009 but were absent in 1999 and 2004 included wild onion (*Allium textile* A. Nelson & J. F. Macbr.), shy wallflower [*Erysimum inconspicuum* (S. Watson) MacMill.], locoweed (*Oxytropis* spp. DC.), and prairie groundsel [*Senecio plattensis* (Nutt.) Weber & A. Love] (Table 4). Shy wallflower is found in every county in North Dakota and mostly occurs in areas that are light to moderately grazed (USGS-NPWRC 2006b), while prairie groundsel is found on moderately to heavily grazed areas (USGS-NPWRC 2006a).

High-seral forb species increased in the loamy seedbank during the 10 yr study, and constituted 5% in 1999 compared to 29% in 2004 and 21% in 2009 (Table 5). For instance, harebell (*Campanula rotundifolia* L.) was absent in 1999 and constituted 2% and 5% of the seedbank in 2004 and 2009, respectively. Hoary puccoon [*Lithospermum canescens* (Michx.) Lehm] was also absent in 1999 and represented less than 1% and 11% of the seedbank in 2004 and 2009, respectively. Species that were present in 2009 but absent in both 1999 and 2004 included shy wallflower, yellow owl's clover, and prairie groundsel.

High-seral forbs declined in the loamy seedbank from 2004 to 2009 (Table 5). Drummond false pennyroyal (*Hedeoma drummondii* Benth.) was reduced 100% as 252 seedlings emerged in 2004, but the plant was absent in 2009. This was the greatest reduction of any high-seral forb. Other species such as western rockjasmine, rock cress, fringed sage, and Great Plains bladderpod were also reduced from 2004 to 2009. The substantial reduction of high-seral forb species in the loamy seedbank (and loamy overflow seedbank) from 2004 to 2009 was most likely due to the invasion of Kentucky bluegrass following leafy spurge control. Native forbs also decreased 46% over 23 yr in North Dakota as Kentucky bluegrass invaded non-use management areas (DeKeyser et al. 2009).

Low-seral forb seedling emergence in the loamy overflow seedbank increased from 7% in 1999 to 38% in 2004, but then decreased in 2009 to 18%, (Table 4). A total of 24 species emerged in 1999 compared to 26 species in 2004 and 23 species in 2009. The largest increase of any low-seral forb was from a Brassicaceae spp., which was absent in 1999 but 296 seedlings emerged in 2009. This plant could not be positively identified, but was likely a low-seral invasive forb. Yellow whitlowort was reduced to a greater extent than any other low-seral forb. The plant constituted over 25% of the loamy overflow seedbank in 2004, but less than 2% in 2009. Yellow whitlowort had the greatest seedling emergence among all species in 2004.

Low-seral forbs represented approximately 9% of the loamy seedbank in 1999, but then increased to 48% and 37% in 2004 and 2009, respectively (Table 5). Species that appeared in 2009 but were absent in 1999 and 2004 included thymeleaf sandmat [*Chamaesyce serpyllifolia* (Pers.) Small], Canada thistle, rough false pennyroyal (*Hedeoma hispida* Pursh), black medick (*Medicago lupulina* L.), alfalfa (*Medicago sativa* L.), yellow sweet clover [*Melilotus officinalis* (L.) Lam.], woolly plantain (*Plantago patagonica* Jacq.), and Norwegian cinquefoil (*Potentilla norvegica* L.). Increased precipitation in 2009 may have contributed to the presence of Canada thistle and sweet-clover seed in the loamy seedbank. Canada thistle can be found on all soil types except peat and grows best when water is abundant (Gordon 2010). Sweet clover seed can remain viable within the soil for up to 20 yr (Stoa 1933) and remain dormant during dry, cool conditions, but becomes permeable with added moisture (Helgeson 1932).

High-seral grass seedling emergence from the loamy overflow seedbank increased 10 yr following *Aphthona* spp. release from representing 2% of the seedbank in 1999 to 14% by 2009 (Table 4). Species diversity remained the same, but only green needlegrass, little bluestem, and sand dropseed were present at all three evaluations. Kirby et al. (2000) also reported that grass and grass-like production increased approximately 50% on sites with *Aphthona* spp. compared to insect-free sites 7 to 8 yr following leafy spurge reduction in a northern mixed-grass prairie in North Dakota. However, yields were still lower at sites with leafy spurge than sites without leafy spurge.

The largest high-seral grass increase in the loamy overflow seedbank was from prairie Junegrass, which constituted 1% of the seedbank in 1999 but was 13% in 2009 (Table 4). Prairie Junegrass is a native cool-season perennial grass that emerges in the early spring (Stubbendieck et al. 2003), providing the grass with a competitive advantage over other species. Walter and Quinton (1995) reported that prairie Junegrass seed increased atop the soil surface following heavy grazing as other species decreased in southwestern Alberta, Canada, but seed numbers were still less than Kentucky bluegrass.

High-seral grasses constituted only 8% of the loamy seedbank in 1999 and 2004 compared to 17% in 2009 (Table 5). High-seral grass species diversity in the loamy seedbank remained the same 10 yr following *Aphthona* spp. release, as eight species emerged in 1999 and 2009. Prairie sandreed represented over 4% of the seedbank in

2004, but less than 1% in 2009. Reece et al. (1996) reported that prairie sandreed seed reserves diminished in areas with heavy grazing; however, the LMNG was not observed to be overgrazed.

High-seral grass species that commonly appeared in both the loamy overflow and loamy seedbanks in 1999, 2004, and 2009 included green needlegrass, little bluestem, and sand dropseed (Tables 4 and 5). These species represented a similar percentage of the total seedbank over time. Green needlegrass is a cool-season native species within the Northern Great Plains that is very resistant to disease and has good forage value (USDA-NRCS 2005); however, the grass had low germination percentages due to inadequate moisture and air temperature (Fulbright et al. 1983). Little bluestem is one of the most widely distributed native grasses in North America. The plant grows well on a variety of soils and has excellent drought tolerance (USDA-NRCS 2002b). Sand dropseed is a warm-season grass that can become weedy or invasive within the Northern Great Plains, but is widely used for restoration purposes in disturbed areas (USDA-NRCS 2010). Perez et al. (1998) reported that sand dropseed was one of the most common perennial grass species in a seedbank study at the Nebraska Sandhills and consistently emerged prior to other grass species in a greenhouse trial. All of these high-seral grasses are used in seed mixtures for restoration purposes (Applewood Seed Company 2011) as they are well adapted to local environmental conditions and can maintain or improve soil fertility (VDCR 2011).

Low-seral grasses represented 2% or less of the total seedbank regardless of ecological site in 2009 (Tables 4 and 5). Similar results were reported in 1999 and 2004. A total of 41, 110, and 99 seedlings emerged from the loamy overflow seedbank in 1999, 2004, and 2009, respectively, but only 6, 5, and 4 seedlings emerged from the loamy seedbank, respectively. Additionally, six species germinated from the loamy overflow seedbank in 2009 compared to only two species in the loamy seedbank.

Species from the 'unknown' and hydric/mesic categories were difficult to identify and represented a small percentage of the seedbank for both ecological sites (Tables 4 and 5). The 'unknown' category included plant species that were not identified as the plant died soon after germination or transplanting. Some hydric/mesic species were only identified to the genus name and included sedges (*Carex* spp.), rushes (*Juncus* spp.), and cattails (*Typha* spp.). These species have primarily adapted to wet habitats (Stubbendieck et al. 2003); however, many seedlings emerged from the loamy seedbank, which is a drier habitat than the loamy overflow seedbank. Cline (2002) reported *Typha* spp. grow well in the greenhouse, but not in the field, and were carried to the loamy sites by wind dispersal.

Overall, the major invasive species represented 88% of the loamy overflow seedbank in 1999 and were reduced to 66% in 2009 (Figure 1a). Kentucky bluegrass constituted the majority (64%) of the loamy overflow seedbank in 2009; whereas leafy spurge represented only 2%. High-seral species (forbs and grasses) represented 17% of the loamy overflow seedbank in 2009, an increase from 5% in 1999. The low-seral grass, hydric/mesic, and 'unknown' categories constituted a low percentage of the loamy overflow seedbank, and changes in seedling emergence minimally impacted the overall seedbank composition. Furthermore, 31, 40, and 39 native species and 13, 14, and 14

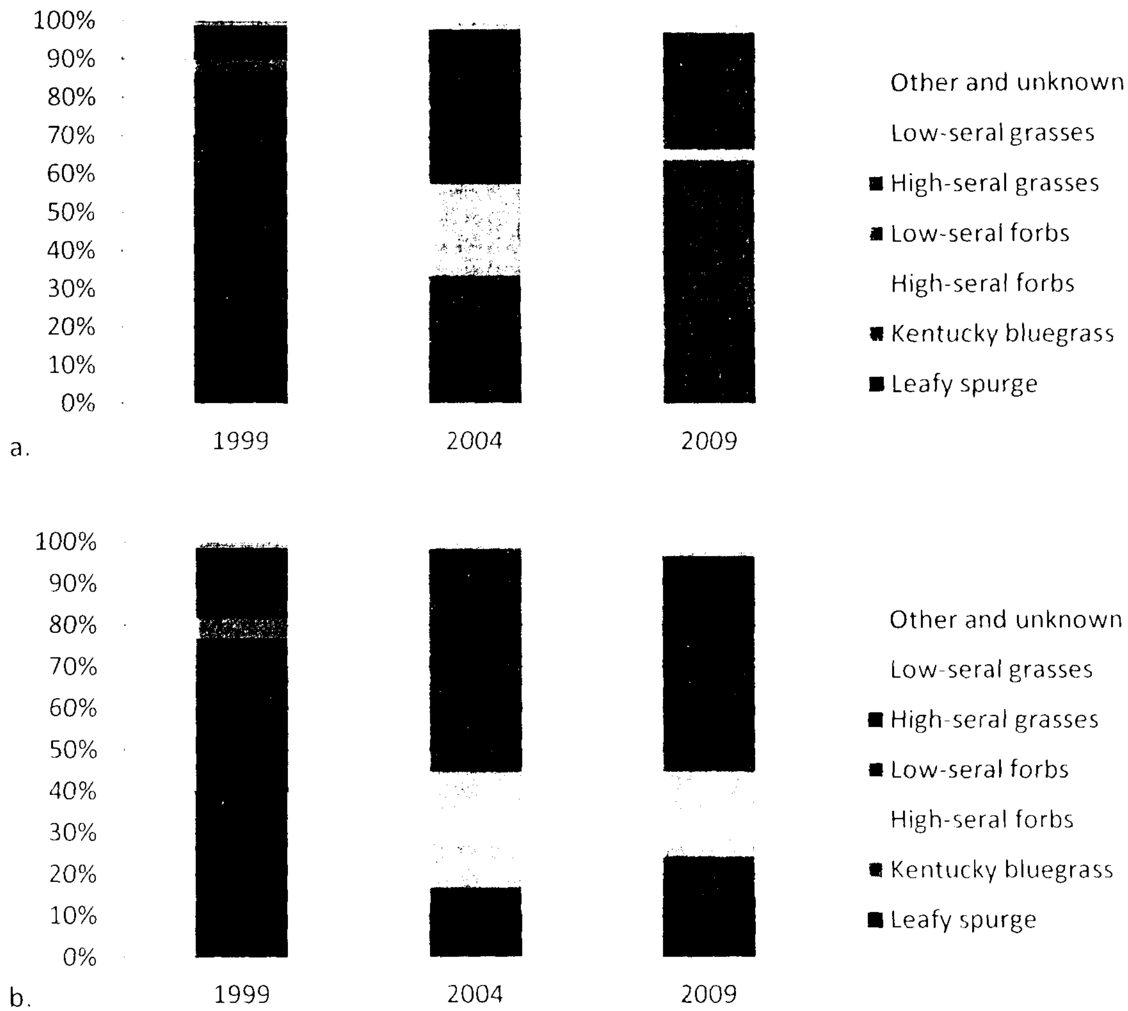

Figure 1. Seedbank composition in 1999 and five (2004) and ten (2009) years following *Aphthona* spp. release to control leafy spurge on loamy overflow (a) and loamy (b) sites in the Little Missouri National Grasslands in western North Dakota.

introduced species emerged from the loamy overflow seedbank in 1999, 2004, and 2009, respectively. For the loamy seedbank, the major invasive species represented 78% in 1999 and decreased to 25% in 2009. Leafy spurge and Kentucky bluegrass constituted only 15% and 10%, respectively, of the loamy seedbank in 2009, while low-seral forbs represented 37% (Figure 1b). High-seral species made up over 38% of the loamy seedbank in 2009 compared to 13% in 1999. The low-seral grass, hydric/mesic, and 'unknown' category represented a small percentage of the overall seedbank composition. In addition, 32, 42, and 34 native species and 12, 10, and 12 introduced species emerged from the loamy seedbank in 1999, 2004, and 2009, respectively.

Leafy spurge was successfully controlled in the LMNG, 10 yr following *Aphthona* spp. release, while native species seed increased in the soil seedbank. Prairie Junegrass was a prevalent native species in the loamy overflow seedbank in 2009 (Table 4). Some high-seral species that appeared in 2009 but were absent in the previous studies included wild onion, shy wallflower, locoweed, and prairie groundsel (Tables 4 and 5). However, from 2004 to 2009, there was a substantial increase in Kentucky bluegrass in the loamy overflow seedbank and a decreasing trend in native species diversity. The increase in Kentucky bluegrass was most likely enabled by the reduction in leafy spurge, which provided a favorable habitat for growth and invasion.

The transition of one major invasive species (leafy spurge) to another (Kentucky bluegrass) in the LMNG is not ideal, but may have some positive attributes. First of all, leafy spurge is listed as a noxious weed in North Dakota, whereas Kentucky bluegrass is an invasive species that is often considered naturalized throughout North America (USDA-NRCS 2004). Kentucky bluegrass provides habitat and forage for wildlife, is included in seed mixes for road ditch revegetation, and prevents soil erosion due to a dense, vigorous root system (USDA-NRCS 2002a). Many ranchers may benefit from the transition as leafy spurge is not palatable to livestock and Kentucky bluegrass forage value is good in the spring. However, decreased plant diversity in grassland communities has been linked to lower production and forage yield (Naeem et al. 1994; Tilman et al. 1996), reduced stability following a disturbance (McNaughton 1977; Tilman and Downing 1994), and increased invasion from exotic species (Tilman 1997; Tracy and Sanderson 2004).

Kentucky bluegrass is a difficult weed to control, and total replacement of the weed is labor intensive and impractical (USDA-NRCS 2004). Spring and fall prescribed burns can help control Kentucky bluegrass and atrazine and glyphosate are effective herbicides to reduce the weed when applied prior to native seeding. Desirable forb species and native grasses such as prairie Junegrass could be seeded within 5 yr following leafy spurge reduction before Kentucky bluegrass seed becomes too prevalent in the soil seedbank. Although Kentucky bluegrass may become a problem species in the LMNG, an increasing trend in native species diversity was observed following the reduction of leafy spurge.

Native Grass Recovery. Native grass production was similar when grown in soil from *Aphthona* spp. release sites compared to non-release sites. Total grass production was 1.5 g per pot when grown in insect-release soil compared to 1.6 g per pot from non-release soil (Table 6). While native grass production averaged across all species at each location did not differ, there was a difference in grass yield for green needlegrass at Buffalo and little bluestem at Gladstone. Green needlegrass production was greater when grown in soil from Buffalo without flea beetles (1.73 g per pot) compared to soil with flea beetles (1.40 g per pot). In contrast, little bluestem yield was greater when grown in soil with flea beetles (2.49 g per pot) compared to soil without flea beetles (2.14 g per pot). The yield difference for green needlegrass was most likely due to inconsistent seed germination in the greenhouse rather than the presence of *Aphthona* spp.

Table 6. Dry weight of four native grass species grown for 15 wk in the greenhouse in soil excavated from six locations throughout North Dakota with or without *Aphthona* spp.

	Buffalo		Gladstone		Lake Tshida		Medora		Pipestem Dam		Standing Rock	
Grass species	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
	g per pot[a]											
Green needlegrass	1.40 a	1.73 b	1.40 a	1.09 a	1.04 a	0.88 a	1.08 a	1.42 a	1.23 a	1.44 a	1.05 a	1.07 a
Little bluestem	2.62 a	2.57 a	2.49 a	2.14 b	1.18 a	1.23 a	1.58 a	1.68 a	2.44 a	2.42 a	1.54 a	1.85 a
Switchgrass	1.45 a	1.68 a	1.69 a	1.86 a	0.90 a	0.97 a	1.60 a	1.31 a	1.33 a	1.70 a	1.16 a	1.24 a
Western wheatgrass	2.04 a	1.98 a	1.66 a	1.67 a	0.98 a	0.95 a	1.85 a	1.68 a	1.68 a	1.48 a	1.04 a	1.23 a
Mean[b]	1.87 a	1.99 a	1.81 a	1.69 a	1.02 a	1.01 a	1.53 a	1.53 a	1.67 a	1.76 a	1.21 a	1.34 a

[a]Values followed by the same letter within a site do not differ ($P < 0.05$).

[b]Mean calculated over the four grass species in each column.

The results of this study contradict those of Juricek (2006) who reported that grass production was reduced nearly 50% when native grass species were grown in soil from *Aphthona* spp. release sites (0.15 g per pot) compared to non-release sites (0.29 per pot), 5 yr following flea beetle release (Appendix Table 18). Switchgrass production was greater when grown in non-release soil compared to insect-release soil and was reduced to a greater extent than any other grass species. Juricek stated there were no differences in dry weight for green needlegrass, little bluestem, or western wheatgrass; however, when combined across species, production was reduced 53% when grown in release soil compared to non-release soil at loamy sites.

The total average grass production was greater in 2010 (1.55 g per pot) than 2004 (0.22 g per pot) (Table 6, Appendix Table 18). The 2010 native grass recovery study was an extension from Juricek (2006) and similar field methods were followed. However, Juricek (2006) inadvertently reported that the grasses were grown in the greenhouse for two different time periods, 8 and 15 wk, and the grasses from the 2010 study were grown in the greenhouse for 15 wk. The considerably higher yield in 2010 compared to 2004 suggests that the grasses were not grown in the greenhouse for 15 wk in 2004, but rather only 8 wk. Whether a longer growth period in the greenhouse in 2010 affected grass yield when grown in soil with or without *Aphthona* spp. is unknown.

Native species recovery in the LMNG has been slow since *Aphthona* spp. were released in 1999, and the cause is unknown. Qin et al. (2006) and Steenhagen and Zimdahl (1979) reported that leafy spurge has allelopathic abilities and can inhibit growth of surrounding vegetation. However, leafy spurge was present at insect-release and non-release sites, which excludes leafy spurge as the cause of reduced grass production when grown in non-release soil (Juricek 2006). Furthermore, native grass recovery has been slower when controlled with *Aphthona* spp. than herbicides (Kirby et al. 2003; Lym and Messersmith 1985a). *Aphthona* spp. may affect native grass recovery 5 yr following release possibly due to an unknown chemical inhibition within the soil caused by the insects; however, at least 10 yr following *Aphthona* spp. release, that reaction within the soil may no longer exist. The slow recovery of desirable species in the LMNG 10 yr following *Aphthona* spp. release may no longer be due to a chemical inhibition within the soil, but rather competition from a new invader, Kentucky bluegrass. The cause of slow native species recovery following leafy spurge control with *Aphthona* spp. remains unknown, but is a concern for land managers when trying to improve native biodiversity.

Weed Control in Switchgrass. None of the herbicides provided satisfactory weedy grass control 1 YAT when applied in May or June 2009 to an infested switchgrass stand near Streeter, ND or a weedy grass-infested field at Fargo, ND (Tables 7 and 8). At Streeter, sulfometuron and aminocyclopyrachlor provided 57% and 26% quackgrass control, respectively, 1 YAT when applied at 210 g ha^{-1} in May. Smooth bromegrass and quackgrass were reduced an average of 63% when sulfometuron was applied at 210 g ha^{-1} in May at Fargo, but control was only 44% when applied in June. Only vegetation from sulfometuron and aminocyclopyrachlor treatments applied in May at Streeter were harvested to evaluate switchgrass production even though weed control was poor.

Switchgrass yield was greater than the untreated control 14 MAT when aminocyclopyrachlor or sulfometuron were applied early in the growing season (Table 9).

Table 7. Switchgrass injury and quackgrass or smooth bromegrass control approximately 1 yr after treatment from various herbicides applied on May 21, 2009 at Streeter or Fargo, ND based on visual evaluations with 0 = no visible injury and 100 = all surface vegetation dead.

		Streeter			Fargo[d]	
Treatment	Rate	Switch[c]	Quack[c]	Brome[c]	Quack	Brome
	— g ha^{-1} —	% injury		% control		
Aminocyclopyrachlor+MSO[a]	105 + 1%	3	5	7	8	7
	210 + 1%	9	26	17	16	16
Atrazine+NIS[b]	560 + 0.25%	3	7	7	4	4
	1120 + 0.25%	1	16	13	9	9
Flucarbazone+NIS	15 + 0.25%	0	12	16	6	6
	29 + 0.25%	3	9	3	6	6
Propoxycarbazone+NIS	29 + 0.25%	1	18	10	10	10
	59 + 0.25%	3	12	9	10	10
Pyroxsulam+NIS	9 + 0.25%	1	7	6	9	9
	18 + 0.25%	1	14	2	12	12
Sulfometuron+NIS	105 + 0.25%	1	26	18	29	27
	210 + 0.25%	19	57	36	60	67
Sulfosulfuron+NIS	18 + 0.5%	4	21	9	8	8
	35 + 0.5%	4	18	12	4	4
Tebuthiuron+NIS	140 + 0.25%	0	10	0	7	7
	280 + 0.25%	2	7	1	6	6
Topramezone+MSO	12 + 1%	1	7	17	5	5
	25 + 1%	1	16	18	6	6
Control		0	0	0	0	0
LSD (0.05)		NS	25	NS	17	16

[a]MSO = methylated seed oil was Scoil by AGSCO, 1168 12 St NE Grand Forks, ND 58201.
[b]NIS = non-ionic surfactant was Activator 90 surfactant by Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632.
[c]Abbreviations: Switch = switchgrass, Quack = quackgrass, Brome = smooth bromegrass.
[d]Switchgrass was not established in Fargo.

Switchgrass yield was greater than the untreated control 14 MAT when aminocyclopyrachlor or sulfometuron were applied early in the growing season (Table 9). In 2010, switchgrass yield averaged 2,145 kg ha^{-1} when sulfometuron was applied at 210 g ha^{-1} compared to 735 kg ha^{-1} in the control. Switchgrass yield was 97% greater than the

Table 8. Switchgrass injury and quackgrass or smooth bromegrass control approximately 1 yr after treatment from various herbicides applied on June 25, 2009 at Streeter or Fargo, ND based on visual evaluations with 0 = no visible injury and 100 = all surface vegetation dead.

		Streeter			Fargo[d]	
Treatment	Rate	Switch[c]	Quack[c]	Brome[c]	Quack	Brome
	— g ha^{-1} —	% injury		% control		
Aminocyclopyrachlor+MSO[a]	105 + 1%	0	4	3	27	23
	210 + 1%	8	43	26	75	72
Atrazine+NIS[b]	560 + 0.25%	0	1	0	7	7
	1120 + 0.25%	0	2	1	7	7
Flucarbazone+NIS	15 + 0.25%	0	7	6	5	5
	29 + 0.25%	0	9	6	3	3
Propoxycarbazone+NIS	29 + 0.25%	0	9	7	6	6
	59 + 0.25%	0	11	11	5	5
Pyroxsulam+NIS	9 + 0.25%	0	8	4	8	8
	18 + 0.25%	0	8	5	7	7
Sulfometuron+NIS	105 + 0.25%	3	31	28	8	8
	210 + 0.25%	23	43	37	44	43
Sulfosulfuron+NIS	18 + 0.5%	0	5	5	16	14
	35 + 0.5%	1	11	11	7	7
Tebuthiuron+NIS	140 + 0.25%	0	4	4	9	9
	280 + 0.25%	0	8	7	6	6
Topramezone+MSO	12 + 1%	0	7	6	4	4
	25 + 1%	0	5	1	8	8
Control		0	0	0	0	0
LSD (0.05)		7	12	13	17	16

[a]MSO = methylated seed oil was Scoil by AGSCO, 1168 12 St NE Grand Forks, ND 58201.
[b]NIS = non-ionic surfactant was Activator 90 surfactant by Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632.
[c]Abbreviations: Switch = switchgrass, Quack = quackgrass, Brome = smooth bromegrass.
[d]Switchgrass was not established in Fargo.

control when aminocyclopyrachlor was applied at 210 g ha^{-1}. None of the treatments provided satisfactory weedy grass control the season after treatment, despite a greater crop yield.

Table 9. Switchgrass, quackgrass, and smooth bromegrass yields 2 and 14 mo after treatment from various herbicides applied on May 21, 2009 in an established switchgrass stand near Streeter, ND.

		2009			2010		
Treatment	Rate	Switch[a]	Quack[a]	Brome[a]	Switch	Quack	Brome
	$g\ ha^{-1}$			$kg\ ha^{-1}$			
Aminocyclopyrachlor + MSO[b]	105	615	325	0	1,870	895	25
	210	365	220	0	1,450	990	95
Sulfometuron + NIS[c]	105	625	5	15	1,580	1,485	315
	210	790	5	15	2,145	880	595
Control		350	525	15	735	795	20
LSD (0.05)		NS	55	NS	51	NS	NS

[a]Abbreviations: Switch = switchgrass, Quack = quackgrass, Brome = smooth bromegrass.
[b]MSO = methylated seed oil. Treatments applied with Scoil at 1% v/v, by AGSCO, 1168 12 St NE Grand Forks, ND 58201.
[c]NIS = non-ionic surfactant. Treatments applied with Activator 90 at 0.25% v/v, by Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632.

Herbicides examined in the previous research were reevaluated at higher rates for smooth bromegrass and quackgrass control because of poor results at initial rates. Switchgrass was not further evaluated as weedy grass control was the main concern. Locations included NDSU campus and Experiment Station north of campus. Data could not be combined for the smooth bromegrass studies and are discussed separately. The quackgrass study at the Experiment Station was lost due to flooding, so only the NDSU campus location was evaluated.

Smooth bromegrass control. None of the treatments provided satisfactory smooth bromegrass control 4 or 11 MAT based on visual evaluation at campus, despite initial reduction (Table 10). For example, sulfometuron applied at 280 g ha^{-1} in October reduced smooth bromegrass 91% 7 MAT (May), but only 54% 11 MAT (September).

Table 10. Smooth bromegrass control with aminocyclopyrachlor, chlorsulfuron, or sulfometuron applied October 6, 2010 or May 16, 2011 in an infested pasture at North Dakota State University campus in Fargo.

		Evaluation 2011		
Application date/herbicide	Rate[a]	31 May	17 June	2 September
	g ha^{-1}	% control		
October 6, 2010				
Aminocyclopyrachlor	140	8	13	1
	280	10	15	16
Chlorsulfuron	23	0	0	0
	47	0	3	0
Sulfometuron	140	84	66	29
	280	91	79	54
May 16, 2011				
Aminocyclopyrachlor	140	0	11	19
	280	0	12	40
Chlorsulfuron	23	0	8	3
	47	0	5	0
Sulfometuron	140	29	46	28
	280	32	49	57
Control		0	0	0
Trt LSD (0.05)[b]		8	11	22
Timing LSD (0.05)[c]		4	4	NS

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.
[b]Means are compared across all treatments.
[c]Means are compared by timing within treatment.

Sulfometuron provided the best smooth bromegrass control at campus compared to aminocyclopyrachlor and chlorsulfuron based on biomass evaluation. Sulfometuron reduced smooth bromegrass an average of 92% from 658 kg ha^{-1} in the untreated control to 50 kg ha^{-1} when applied at 280 g ha^{-1} in the spring or fall (Table 11). Smooth bromegrass was reduced 85% when aminocyclopyrachlor was applied at 280 g ha^{-1} in the fall. Chlorsulfuron treatments did not provide smooth bromegrass control. Peters et al.

Table 11. Smooth bromegrass, other grass, and forb yields harvested on July 7, 2011 with aminocyclopyrachlor, chlorsulfuron, or sulfometuron applied October 6, 2010 or May 16, 2011 in a smooth bromegrass infestation at the North Dakota State University campus in Fargo.

Application date/herbicide	Rate[a]	Smooth bromegrass	Other grasses	Forbs	Total yield
	$g\ ha^{-1}$	$kg\ ha^{-1}$			
October 6, 2010					
Aminocyclopyrachlor	140	460	611	99	1,170
	280	96	622	0	718
Chlorsulfuron	23	551	654	64	1,270
	47	576	519	69	1,163
Sulfometuron	140	233	3	15	251
	280	51	65	72	187
Mean					793
May 16, 2011					
Aminocyclopyrachlor	140	341	482	0	823
	280	292	387	0	679
Chlorsulfuron	23	341	498	0	839
	47	350	416	2	767
Sulfometuron	140	138	99	15	252
	280	49	98	6	153
Mean					589
Control		658	373	63	1,094
Trt LSD (0.05)[b]		363	330	NS	287
Timing LSD (0.05)[c]		NS	NS	NS	NS

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.

[b]Means are compared across all treatments.

[c]Means are compared by timing within treatment.

(1989) also reported that smooth bromegrass was tolerant to chlorsulfuron in Nebraska.

Aminocyclopyrachlor and chlorsulfuron did not injure other grass species 2 or 9 MAT when applied in the spring or fall at campus, respectively, whereas sulfometuron caused nearly 100% injury when applied at 140 g ha^{-1} in the fall (Table 11). Sulfometuron most likely would be injurious to switchgrass as well and not be used for production.

Smooth bromegrass was reduced 99% 3 MAT (September) at the Experiment Station when sulfometuron was applied at 280 g ha^{-1} in the spring compared to only 77% when the same treatment was fall-applied (Table 12). Aminocyclopyrachlor applied at 280 g ha^{-1} in the fall reduced smooth bromegrass 95% 11 MAT (September) compared to only 23% when spring-applied. In contrast, smooth bromegrass yield was similar regardless of treatment or application timing 1.5 or 9 MAT based on biomass evaluation (Table 13). The smooth bromegrass was stressed due to excess moisture, which inhibited the formation of an inflorescence and resulted in a lower biomass than the smooth bromegrass at campus. This may be the reason for no treatment differences for smooth bromegrass yield at the Experiment Station and why location data could not be combined.

Other grass species production was greater than the untreated control 1.5 or 9 MAT when aminocyclopyrachlor was applied at 280 g ha^{-1} in the spring or fall, respectively, whereas sulfometuron provided 100% grass injury 9 MAT when fall-applied at 280 g ha^{-1} (Table 13). Forb species were reduced 100% 1.5 or 9 MAT when sulfometuron or aminocyclopyrachlor were applied in the fall or spring, whereas chlorsulfuron did not affect forb production.

Quackgrass control. None of the treatments provided satisfactory quackgrass control 4 or 11 MAT (September) at campus, despite initial control in May, based on visual evaluation (Table 14). Sulfometuron reduced quackgrass 99% 9 MAT from 771 k g ha^{-1} in the untreated control to 6 kg ha^{-1} when applied at 280 g ha^{-1} in the fall (Table 15). However, sulfometuron injured other grass species to a greater extent than any other treatment, and is not likely a useful treatment to be used in switchgrass production.

Aminocyclopyrachlor improved other grass species production, but provided poor quackgrass control.

Table 12. Smooth bromegrass control with aminocyclopyrachlor, chlorsulfuron, or sulfometuron applied October 15, 2010 or June 6, 2011 in an infested pasture at the North Dakota State University Experiment Station in Fargo.

		Evaluation 2011		
Application date/herbicide	Rate[a]	17 June	5 July	2 September
	$g\ ha^{-1}$	% control		
October 15, 2010				
Aminocyclopyrachlor	140	50	48	53
	280	93	93	95
Chlorsulfuron	23	0	0	0
	47	0	0	6
Sulfometuron	140	92	73	43
	280	97	83	77
June 6, 2011				
Aminocyclopyrachlor	140	0	5	36
	280	4	6	23
Chlorsulfuron	23	0	0	4
	47	1	0	0
Sulfometuron	140	6	13	92
	280	10	17	99
Control		0	0	0
Trt LSD (0.05)[b]		18	21	12
Timing LSD (0.05)[c]		7	9	31

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.

[b]Means are compared across all treatments.

[c]Means are compared by timing within treatment.

Table 13. Smooth bromegrass, other grass, and forb yields harvested on July 29, 2011 from aminocyclopyrachlor, chlorsulfuron, or sulfometuron applied October 15, 2010 or June 6, 2011 in an infested pasture at the North Dakota State University Experiment Station in Fargo.

Application date/herbicide	Rate[a]	Smooth bromegrass	Other grasses	Forbs	Total yield
	$g\ ha^{-1}$	kg ha^{-1}			
October 15, 2010					
Aminocyclopyrachlor	140	77	213	0	289
	280	49	227	0	275
Chlorsulfuron	23	69	167	77	313
	47	102	224	14	340
Sulfometuron	140	60	3	0	63
	280	2	0	3	5
Mean					214
June 6, 2011					
Aminocyclopyrachlor	140	95	104	0	199
	280	66	261	0	327
Chlorsulfuron	23	71	175	10	256
	47	77	179	0	254
Sulfometuron	140	8	46	0	54
	280	40	99	13	151
Mean					207
Control		148	132	1	281
Trt LSD (0.05)[b]		NS	178	43	157
Timing LSD (0.05)[c]		NS	NS	NS	NS

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.

[b]Means are compared across all treatments.

[c]Means are compared by timing within treatment.

Table 14. Quackgrass control with aminocyclopyrachlor, imazaquin, metsulfuron, or sulfometuron applied October 6, 2010 or May 16, 2011 in an infested pasture at the North Dakota State University campus in Fargo.

		Evaluation 2011		
Application date/herbicide	Rate[a]	31 May	17 June	2 September
	g ha^{-1}	% control		
October 6, 2010				
Aminocyclopyrachlor	140	1	6	0
	280	5	6	0
Imazaquin	196	0	0	0
	392	0	1	0
Metsulfuron	14	3	3	0
	28	0	0	0
Sulfometuron	140	89	83	23
	280	95	93	31
May 16, 2011				
Aminocyclopyrachlor	140	1	7	10
	280	1	19	24
Imazaquin	196	7	30	5
	392	11	26	1
Metsulfuron	14	7	11	0
	28	11	15	3
Sulfometuron	140	19	53	23
	280	18	55	56
Control		0	0	0
Trt LSD (0.05)[b]		6	8	13
Timing LSD (0.05)[c]		2	3	5

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.

[b]Means are compared across all treatments.

[c]Means are compared by timing within treatment.

Table 15. Quackgrass, other grass, and forb yields harvested on July 7, 2011 from aminocyclopyrachlor, imazaquin, metsulfuron, or sulfometuron applied October 6, 2010 or May 16, 2011 in an infested pasture at the North Dakota State University campus in Fargo.

Application date/herbicide	Rate[a]	Quackgrass	Other grasses	Forbs	Total yield
	g ha^{-1}	kg ha^{-1}			
October 6, 2010					
Aminocyclopyrachlor	140	425	516	1	942
	280	475	318	0	793
Imazaquin	196	783	99	84	966
	392	373	322	21	716
Metsulfuron	14	506	325	53	884
	28	528	164	1	692
Sulfometuron	140	117	51	63	231
	280	6	4	6	16
Mean					655
May 16, 2011					
Aminocyclopyrachlor	140	410	322	11	743
	280	240	269	0	509
Imazaquin	196	203	122	109	434
	392	311	133	160	604
Metsulfuron	14	385	369	7	762
	28	408	342	2	751
Sulfometuron	140	46	89	12	147
	280	68	75	2	145
Mean					512
Control		771	159	85	1,015
Trt LSD (0.05)[b]		370	262	NS	312
Timing LSD (0.05)[c]		NS	NS	NS	NS

[a]All treatments applied with 0.25% v/v non-ionic surfactant, Induce®, Helena Chemical Company, 225 Shilling Boulevard, Suite 300, Collierville, TN 38017.

[b]Means are compared across all treatments.

[c]Means are compared by timing within treatment.

SUMMARY

Leafy spurge stem density and seed were reduced an average of 93% from two ecological sites (loamy overflow and loamy) in the Little Missouri National Grasslands 10 yr following *Aphthona* spp. release in 1999. Native species seed increased in the soil seedbank as leafy spurge was reduced. High-seral species represented 17% of the loamy overflow seedbank in 2009, which increased from 5% in 1999. However, from 2004 to 2009 there was a substantial increase in Kentucky bluegrass in the loamy overflow seedbank and a slight decline in native species, which was most likely caused by increased precipitation, grazing, and the competitiveness of Kentucky bluegrass.

Native species recovery in the LMNG has been slow since *Aphthona* spp. were released in 1999. The cause is unknown, but may be influenced by factors such as leafy spurge allelopathy or Kentucky bluegrass invasion. Greenhouse research indicated that *Aphthona* spp. may also affect native grass recovery 5 yr following release possibly due to an unknown chemical inhibition within the soil caused by the insects. However, 10 yr following *Aphthona* spp. release, there was no reduction in native grass production. The cause of slow native species recovery following leafy spurge control with *Aphthona* spp. remains unknown, but is a concern for land managers when trying to improve native biodiversity.

Aphthona spp. are still cost effective biocontrol agents for long-term leafy spurge control in the LMNG. *Aphthona* spp. have successfully established and reduced leafy spurge throughout the Northern Great Plains and can be used in environmentally sensitive habitat or large areas where herbicide use is too costly and impractical.

Cool-season grassy weed control can be a problem in switchgrass production used for biofuel. Various herbicides at common-use rates were evaluated for smooth bromegrass and quackgrass control in a switchgrass establishment near Streeter, ND and weed-infested field in Fargo, ND. Switchgrass yield was greater than the untreated control 14 MAT when aminocyclopyrachlor or sulfometuron were applied in May at Streeter, but none of the treatments provided satisfactory long-term grassy weed control.

Selected herbicides were reevaluated at increased rates for smooth bromegrass or quackgrass control in Fargo. Sulfometuron provided 99% smooth bromegrass control 9 MAT when fall-applied at 280 g ha^{-1}, but other grass and forb species were injured as well. This treatment would most likely not be used for switchgrass production. Aminocyclopyrachlor applied at 280 g ha^{-1} in the fall did not injure other grass species, but smooth bromegrass control only averaged 76%. None of the treatments provided satisfactory quackgrass control.

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APPENDIX

Table 16. Switchgrass, quackgrass, or smooth bromegrass yield 2 mo after various herbicide treatments were applied May 21, 2009 in an established switchgrass stand near Streeter, ND or a weed-infested pasture at Fargo, ND.

		Streeter			Fargo	
Treatment	Rate	Switch[a]	Quack[a]	Brome[a]	Quack	Brome
	—— g ha^{-1} ——	kg ha^{-1}		—— % of control ——		
Aminocyclopyrachlor+MSO[a,b]	105 + 1%	623	1	88	9	43
	210 + 1%	790	1	86	0	25
Atrazine+NIS[a,c]	560 + 0.25%	577	154	268	220	124
	1120 + 0.25%	737	108	280	21	135
Flucarbazone+NIS	15 + 0.25%	404	52	12	65	173
	29 + 0.25%	588	42	207	4	132
Propoxycarbazone+NIS	29 + 0.25%	529	1	94	18	73
	59 + 0.25%	833	0	112	3	70
Pyroxsulam+NIS	9 + 0.25%	552	78	110	15	70
	18 + 0.25%	559	35	0	305	35
Sulfometuron+NIS	105 + 0.25%	615	62	0	52	130
	210 + 0.25%	365	42	0	19	98
Sulfosulfuron+NIS	18 + 0.5%	711	44	0	0	51
	35 + 0.5%	814	10	321	33	52
Tebuthiuron+NIS	140 + 0.25%	409	164	333	64	113
	280 + 0.25%	717	89	450	386	109
Topramezone+MSO	12 + 1%	317	115	278	9	171
	25 + 1%	408	102	79	25	190
Control		352	-	-	-	-
LSD (0.05)		NS	55	NS	NS	79

[a]Abbreviations: Switch = switchgrass; Quack = quackgrass; Brome = smooth bromegrass; MSO = methylated seed oil; NIS = non-ionic surfactant.

[a]MSO was Scoil by AGSCO, 1168 12 St NE Grand Forks, ND 58201.

[b]NIS was Activator 90 surfactant by Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632.

Table 17. Switchgrass, quackgrass, and smooth bromegrass yield 1.5 mo after various herbicide treatments were applied June 25, 2009 in an established switchgrass stand near Streeter, ND.

Treatment	Rate	Switchgrass	Quackgrass	Smooth bromegrass
	—— g ha^{-1} ——	kg ha^{-1}	—— % of control	——
Aminocyclopyrachlor+MSO[a]	105 + 1%	288	131	43
	210 + 1%	145	86	186
Atrazine+NIS[b]	560 + 0.25%	435	66	22
	1120 + 0.25%	288	83	28
Flucarbazone+NIS	15 + 0.25%	112	129	15
	29 + 0.25%	138	402	40
Propoxycarbazone+NIS	29 + 0.25%	272	111	59
	59 + 0.25%	233	84	0
Pyroxsulam+NIS	9 + 0.25%	377	70	133
	18 + 0.25%	372	89	11
Sulfometuron+NIS	105 + 0.25%	312	132	5
	210 + 0.25%	630	87	4
Sulfosulfuron+NIS	18 + 0.5%	388	74	4
	35 + 0.5%	244	124	7
Tebuthiuron+NIS	140 + 0.25%	156	198	0
	280 + 0.25%	140	160	0
Topramezone+MSO	12 + 1%	168	169	6
	25 + 1%	258	163	7
Control		258	-	-
LSD (0.05)		263	NS	NS

[a]MSO = methylated seed oil from Scoil by AGSCO, 1168 12 St NE Grand Forks, ND 58201.

[b]NIS = non-ionic surfactant was Activator 90 by Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632.

Table 18. Dry weight of four native grass species grown for 8 wk in soil excavated in mid-August 2004 from loamy overflow and loamy sites with or without *Aphthona* spp. released in 1999 for leafy spurge control in southwestern North Dakota (Juricek 2008).[a]

	Loamy overflow sites		Loamy sites	
Grass species	Insect-release	Non-release	Insect-release	Non-release
	g per pot[b]			
Green needlegrass	0.08 a	0.12 a	0.09 a	0.26 a
Little bluestem	0.21 a	0.25 a	0.17 a	0.26 a
Switchgrass	0.29 a	450. b	0.23 a	0.68 b
Western wheatgrass	0.09 a	0.10 a	0.05 a	0.12 a
Mean[c]	0.16 a	0.23 b	0.14 a	0.33 b

[a] A similar table was reported in Cline et al. 2008; however, yields were listed incorrectly.

[b] Values followed by the same letter within a site do not differ ($P < 0.05$).

[c] Mean calculated over the four grass species in each column and a separate LSD ($P < 0.05$) for this row.