SMOOTH BROME (*BROMUS INERMIS*) PHENOLOGY IN THE NORTHERN TALLGRASS PRAIRIE

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Smooth brome (*Bromus inermis*) invasion into tallgrass prairie has led to development of methods of control. Prescribed burning is used by the US Fish and Wildlife Service (USFWS) to manage prairie according to a provisional model developed by Willson and Stubbendieck (2000). The model recommends conducting a prescribed burn at the onset of elongation of smooth brome. The USFWS uses the 5-leaf stage as a phenological cue, signaling the initiation of elongation. Variability in smooth brome development limits the reliability of this method. Our objective was to develop an alternative method to determine when smooth brome populations reach the targeted 50% elongation by correlating accumulated growing degree days and population level plant phenological stages (mean stage count) throughout sites in North Dakota, South Dakota, and Minnesota. A linear regression model was used to determine the onset of elongation in the smooth brome population, regardless of leaf stage variation. Field and greenhouse studies confirmed accumulated growing degree days predicted the initiation of elongation. We also compared smooth brome response to different seasonal burn treatments, determining it could be decreased by burning at other times. As part of the USFWS Native Prairie Adaptive Management program, results will be used to assist management decisions regarding the timing of control.

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SMOOTH BROME INTRODUCTION AND BACKGROUND

The genus *Bromus* contains more than 60 species (Walton 1980, Saarela et al. 2007), all of which are cool-season grasses. *Bromus inermis*, smooth brome, is a Eurasian grass that was initially introduced in the 1880's as a perennial forage grass and for erosion control. Its wide use throughout the northern Great Plains and its highly competitive nature have presented land managers of native prairies with a problem as smooth brome invades native landscapes (USDA 2002).

Description

Smooth brome is a perennial cool-season grass (Otfinowski et al. 2007). Individual plants can vary in height, reaching between 0.5 and 1.25 meters tall. The leaf blades, between ten and twenty-five centimeters long, are easily identified by a distinct constriction mark resembling a "W" or "M" below the tip (USDA 2002). The inflorescence is an erect open panicle with minimal awns (Stubbendieck et al. 2011), developing a purplish-brown color when mature (USDA 2002). Plants produce seeds but are also very rhizomatous, with the majority of reproduction occurring through vegetative tillers (Palmblad 1968), forming thick sod through vegetative reproduction (Otfinowski et al. 2007, Stubbendieck et al. 2011).

Phylogeny

The genus *Bromus* is nested in the tribe Bromeae, subfamily Pooideae, and the family Poaceae. The nearest phylogenetic relative to Bromeae is the tribe Triticeae, which contains wheat and rye, and consists of 18 genera (Kellogg 1998). Bromeae contains only a single genus but *Bromus* is further broken into six sections, each section being identified by morphological characters such as floret and spikelet descriptions (Saarela et al. 2007). *B. inermis* is a member of the section Bromopsis. All the species in this section, most of which are considered Old World species, are long-lived, rhizomatous perennials with large anthers (Saarela et al. 2007). The closely related *Bromus pumpellianus* is a North American native species, however studies suggest that it is the result of divergent evolution from *B. inermis* and possibly a *B. inermis* subspecies (Elliott 1949). The likely hybridization of *B. pumpellianus* and *B. inermis* through cross-pollination (Elliott 1949) complicates the phylogeny of wild populations of *B. inermis*.

History

Originating from regions of Europe and Asia, smooth brome was introduced in the late 1800s as forage grass and for erosion control (Newell and Keim 1943, Hitchcock 1950). There are numerous cultivated varieties, each slightly different from the other, making the different cultivars more suited to regional variations such as temperature and moisture (Newell and Keim 1943, Casler et al. 2000, Coulman 2006). However Lamp (1952) determined that cultivars from different regions all behaved similarly when grown under identical conditions. This may speak to the phenotypic plasticity of the species and contribute to its aggressive nature. If different cultivars have a great deal of phenotypic plasticity, they are more likely to grow well in a wide range of conditions.

Uses and Distribution

B. inermis is a highly palatable, high protein-containing forage grass for livestock and wildlife (USDA 2002; Stubbindieck et al. 2011). Its rapid growth and massive root systems make it useful in erosion control. Seed spread over bare ground will quickly grow and form sod that is valued for stabilizing bare earth (Walton 1980). As a drought tolerant species, it has adapted to survive in a wide range of ecological settings. Additionally, *B. inermis* has been used in the past for wildlife cover and feed. However, this practice has generally been discontinued as its aggressive nature threatens invasion into native grasslands (USDA 2002).

As stated, one reason smooth brome was introduced to the United States in the 1880s was as a forage grass to provide a highly nutritive and palatable feed source for livestock (Newell and Keim 1943, Hitchcock 1950). It was planted throughout the US and managed to maximize its growth, with livestock grazing timed to minimize impact on the plant population and to allow livestock to obtain the maximum nutritional value (Frank and Hofmann 1989). When grazing is complete, the open canopy created by grazing animals allows the plants to rebound, sending out new tillers for the remainder of the season (Dibbern 1947, Mitchell et al. 1998).

Because smooth brome is an aggressive grower and forms rhizomatous mats, it is very desirable as an erosion control mechanism (Walton 1980). The rapid tillering creates a root mat that holds the soil in place (Walton 1980). This makes smooth brome a popular reseeding species for roadways and construction sites. Human introduction of smooth brome throughout the US was done with the best of intentions. However, as roadways and other disturbed areas adjacent to natural ecosystems were planted with smooth brome, the tillering of smooth brome allowed the plants to begin to creep into native prairies and continue to encroach upon these ecosystems.

Ecological Impacts

Smooth brome is now found throughout much of the United States and its range continues to expand throughout the native prairies of the Great Plains (Otfinowski et al. 2008). As a drought tolerant species, it is able to survive extreme variations in precipitation (Walton 1980). The early growth activity as a cool season grass allows it to produce carbohydrates in early spring when other plants are still dormant. These conditions favor smooth brome and give it the competitive edge over the warm season native plants. By the time warm season plants are coming out of dormancy, resources have already been intercepted – nutrients, water, and sunlight are no longer as abundant because smooth brome has been growing for several weeks (Vinton and Goergen 2006).

Smooth brome invasion into native prairie becomes an issue because it creates a monoculture as it sends out tillers. The plants are aggressive growing and get a jump start on the native warm season grasses by beginning to grow very early in the spring. By the time the native grasses start growing, smooth brome has already grown taller and out competes for sunshine by shading the underlying species and seeds (Otfinowski et al. 2007). Established smooth brome plants use moisture and nutrients in the soil, passing resources from plant to plant via rhizomes (Otfinowski and Kenkel 2008), effectively starving other plant species that will start growing later. This rhizome connection between plants makes smooth brome especially tolerant of patchy nutrient availability as plants in high nutrient areas can support those connected plants that have low nutrient availability (Otfinowski and Kenkel 2008).

The problem with smooth brome invasion and monoculture formation is that, by limiting the number of plant types or the abundance of those species, the biodiversity of the ecosystem is deteriorated (Otfinowski et al. 2007). As the swaths of smooth brome successfully choke out the surrounding native plants, there is a decline in biodiversity (Willson 1990). Native grassland ecosystems typically have a high degree of biodiversity, supporting the needs of a large number of other species: plants, insects, microbes, and wildlife. Declining plant biodiversity results in fewer resources available for native wildlife (Trammell and Butler 1995), as vital links within the ecosystem's food web are broken. Additionally, low biodiversity limits the ability of an ecosystem to respond to stressors and rebound after catastrophic events. Invasion of smooth brome may occur gradually enough that the reliant fauna is able to adapt to the new conditions and resources. However, when a smooth brome monoculture develops, it is more susceptible to the impact of disease and other events (Otfinowski et al. 2007).

Invasion of smooth brome has a cascading effect on the native prairie grasslands. Its rapid and rhizomatous growth allow it to spread once it has gained a foothold, increasing in above- and below-ground biomass (Piper et al. 2015), and ultimately resulting in decreased plant species biodiversity (Fink and Wilson 2011, Piper et al. 2015). In addition to declining biodiversity, invasive species can alter aspects of the ecosystem, including productivity and nutrient-cycling (Vitousek et al. 1996, Vinton and Goergen 2006). Increased litter production with expanded smooth brome growth results in declining plant diversity through shading and competition for other resources (Fink and Wilson 2011, Piper et al. 2015). Soil moisture decreases beneath smooth brome (Fink and Wilson 2011), making it unavailable to native plants. Soil dynamics that favor smooth brome are created through microbial (Jordan et al. 2008, Sherrard and Maherali 2012) and bacterial (Piper et al. 2015, Sherrard and Maherali 2012) population changes. Smooth brome expansion is further enhanced by rapid decomposition of litter and efficient nitrogen cycling (Vinton and Goergen 2006), as well as human supplemented nitrogen sources (Peterson and Moser 1985, Vinton and Goergen 2006). It is interesting to note, however, that contrary to expectations, Cully et al. (2003) found fragmented prairie did not experience a decline in species richness even though cool-season invasive grasses were abundant in their study area. This could be due to a number of factors, including the scale of the study, seasonal variations or class of vegetation (i.e. grass, forb, etc.).

Methods of Control

Conventional methods which target smooth brome in order to control its spread throughout the native tallgrass prairie include herbicides, grazing or mowing, and fire.

Application of atrazine and other herbicides were found to decrease smooth brome in warm-season pastures (Dill et al. 1986, Willson and Stubbendieck 1996, Bahm et al. 2011), and glyphosate showed long-term control of smooth brome in rangeland restoration attempts in southeastern North Dakota (Link et al. 2017). However herbicide application creates additional environmental and public health concerns, most notably as an endocrine disruptor (Taylor and Harrison 1999, Fan et al. 2007, Orton et al. 2009).

Much research has been completed on the effective management of smooth brome as a forage grass, focusing on methods to enhance growth and nutritional value of the smooth brome (Newell and Keim 1943, Petersen and Moser 1985). For example, ranchers and livestock managers would normally pull grazers from pastures in order to minimize the damage to the population, leaving sufficient leaf area to maximize photosynthetic capability and subsequent regrowth (Johnson and Parsons 1985). When looking to control the spread of smooth brome, it seems that an investigation into those methods of enhancement would identify the ways that the plant is able to survive. The opposite action, to continue or start grazing at the time that grazers would normally be pulled, would cause damage to the plants' ability to survive the remainder of the season. To do the opposite seems like an effective way to manage smooth brome growth. Research has shown that under various grazing treatments, smooth brome production on loamy sites in the Northern Great Plains is the greatest under light grazing while heavy and extreme grazing treatments resulted in less than 2% smooth brome frequency (Patton and Nyren 2015). However, the same study found that loamy overflow sites did not experience as dramatic a difference between the grazing treatments. Heavy and extreme grazing treatments resulted in lower smooth brome frequency but it was between approximately 20 and 35%, compared to 2%

on the loamy site. Interestingly smooth brome in the loamy overflow non-grazed exclosure increased to anywhere between a frequency of 70-80% (Patton and Nyren 2015).

Fire has shaped ecosystems since the beginning of time. Recent use of prescribed burning has sought to restore some of the historical conditions that have been altered by human use, development, and fire suppression (DiTomaso et al. 2006). An effective burn for perennial grasses, like smooth brome, will destroy the above ground plant tissue and the reproductive capability of the plant, whether it is below ground shoots or seeds (DiTomaso et al. 2006). Willson and Stubbendieck (2000) created a provisional model for burning to control smooth brome in native prairie ecosystems. This model recommends there be at least 20% native component in the grassland in order to have successful decreases in smooth brome. The destruction of smooth brome opens the canopy for new native plants to grow and fire raises the soil temperature, setting the stage for rapid revegetation. If there is no native component, the rapid revegetation that occurs will be that of smooth brome, further securing its foothold. However, the successful control of smooth brome relies on the ability to repeatedly burn; single burns, even at the appropriate time of year, did not have long term success (Willson and Stubbendieck 1996). Because annual burning can be cost-prohibitive, the solution may lie with a combination of all three methods (DiTomaso et al. 2006). Additionally, the variability of fire duration, temperature, and intensity can affect the success of the fire to have a long-term impact on smooth brome (DiTomaso et al. 2006). The result of burning may not be a decrease in the overall number of smooth brome tillers, but rather a general increase in species diversity (DiTomaso et al. 2006), an outcome that may be acceptable depending on management goals.

Developmental Physiology and Phenology

Successful germination of seed is dependent on sowing density, with increased density leading to decreased germination rates (Palmblad 1968). When smooth brome grows from a seed, new plants emerge with a coleoptile (Moore et al. 1991). The first true leaf emerges and lengthens followed by the 2nd, 3rd, 4th, and so on. The plant will reach a point at which it will stop adding additional leaves, but will begin to lengthen between the nodes (internodal elongation). Just prior to elongation, it is possible to feel the nodes "plumping" with your fingers. This palpable identification of nodes is the point at which the plant is considered to move from the vegetative state to the elongation state, corresponding to the increase of carbohydrate reserves within the above-ground plant tissue and a decline of carbohydrate reserves within in the below-ground crown tissue (Eastin et al. 1964).

A reproductive stalk may be sent out after elongation bearing an inflorescence and, eventually, seeds. When the inflorescence reaches anthesis, large pollen-laden anthers will extrude from the florets and be visible to the naked eye (Moore et al. 1991), positioning the pollen to be carried to neighboring plants. Unlike many other *Bromus* species, smooth brome does not self-pollinate, instead relying on wind driven cross-fertilization to produce seeds (McKone 1985). Relatively few tillers will actually produce a reproductive stalk while 100% of tillers will reproduce through vegetative tillers via rhizomes (Palmblad 1968). In spite of producing an inflorescence, it was determined that virtually 0% produced seed (Palmblad 1968).

Vegetative reproduction, via tillering, occurs at two distinct times: 1) mid-March through early May, and 2) mid-June through mid-July (Lamp 1952). Relatively few tillers emerge between the two periods (Lamp 1952). Lamp (1952) found that tillers emerging in the spring did not develop completely, likely due to the damage caused by cold temperatures over winter, when the pre-emergent growth was below ground.

Perennial grass plants grow from a single crown, with one plant consisting of several tillers that are all genetically similar but may include several generations of tillers (Moore and Moser 1995); each tiller is considered a clone of the parent plant. However, tillers develop at different rates, allowing one plant to have tillers of different phenological development (emergent, vegetative, elongating, and/or reproductive) at the same time (Moore and Moser 1995). Tillering makes identifying individual plants difficult in the field, as they grow together and amongst other species.

A system of describing and quantifying perennial grass growth stages developed by Moore et al. (1991) lists universal descriptors for forage grasses and a corresponding continuous numerical index. This system, designed to be easily memorized and used in the field for practical management decisions, consists of five life growth stages and the corresponding numerical indices allow for quantitative manipulation and comparison of data. Identification of the phenological stages of development in smooth brome is accomplished by inspection of each individual tiller to determine if palpable nodes are present. If there are no palpable nodes present, then the number of fully collared leaves is counted. If a leaf is present but not fully collared, it is not counted according to Moore et al. (1991). A fully collared leaf that is more than 50% dead (Lamp 1952) is not counted either. A tiller with no fully collared leaves would be considered vegetative 0, one fully collared leaf is vegetative 1, and so on. Once the node is palpable, classification switches from vegetative stage to elongation stage beginning at 1 node through N nodes. The reproductive stage begins when the inflorescence stalk emerges in the boot phase, and is classified as reproduction 0. As the inflorescence begins to emerge from the boot, it is considered reproduction 1. The reproductive stages are further classified by progression of anthesis followed by seed formation (Moore et al. 1991). A partial list of stages is included in Table 1.1. According to the Moore et al. (1991) protocol, each tiller is treated as an individual plant, eliminating the need to identify the parent plant.

Table 1.1. Phenological development stage, index and description (Moore et al. 1991).

Stage	Index	Description
Vegetative-Leaf development		
Emergent or V_O	1.0	Emergence of first leaf
V_1	(1/N)+0.9	First leaf collared
V_2	(2/N)+0.9	Second leaf collared
V_n	(n/N)+0.9	Nth leaf collared
Elongation-Stem elongation		
E_O	2.0	Onset of stem elongation
E_l	(1/N)+1.9	First node palpable/visible
E_2	(2/N)+1.9	Second node palpable/visible
E_n	(n/N)+1.9	Nth node palpable/visible
Reproductive-Floral development		
R_O	3.0	Boot stage
R_1	3.1	Inflorescence emergence/1st spikelet visible
R_2	3.3	Spikelets fully emerged/peduncle not emerged
R_3	3.5	Inflorescence emerged/peduncle fully elongated
R_4	3.7	Anther emergence/anthesis
R_5	3.9	Post-anthesis/fertilization

USFWS Management

USFWS seeks to maintain biodiversity of plant communities in the tallgrass prairie because the wildlife that they are responsible for depends on these plant communities for habitat (Grant et al. 2009, Gannon et al. 2013). In order to meet their mission as stewards of the wildlife, they must also become stewards of the required habitat. It is to that end this research is being conducted. Controlling the spread of smooth brome into the native tallgrass prairie is a tool to maintain the biodiversity that is imperative to achieving their mission (Murphy and Grant 2005).

Based on Willson and Stubbendieck's (2000) model, USFWS uses prescribed burning to control the smooth brome and limit its advance in tallgrass prairie ecosystems (Gannon et al. 2013). This is completed in the spring in order to both damage the growth of the smooth brome and allow the native species to effectively recover over the new growing season. The model uses phenological cues to determine when fire damages the carbohydrate reserves in smooth brome, specifically signaled by the majority of the population being at the five-leaf stage. Unfortunately, within our region, smooth brome exhibits a great deal of variability. (Sara Vacek, USFWS, Pers. comm.). Some locations will reach the pivotal five-leaf stage while others seem to skip straight to the elongation phase. This variability in phenological development makes the model difficult to use, as managers wait for the five-leaf stage to develop.

The following chapters report on each of 3 separate studies. The first project involved a field study that investigated the phenological development of smooth brome through elongation and compared that development to the number of accumulated growing degree days (AGDD). The correlation between development and AGDD was used to create a model for USFWS, allowing personnel to estimate burn timing based on the number of AGDD. The variability in phenological development reported by USFWS was also observed in the field study, prompting a second project that involved a greenhouse study to determine if this variability was characteristic of smooth brome. Finally, the third project was a small scale study investigating several burn regimes to determine if Willson and Stubbendieck's (2000) recommended spring burn is the only opportunity for USFWS decrease smooth brome populations. The results of this last study could be used as guidance to create a large scale investigation to further research the effectiveness of different burn regimes on smooth brome populations in the northern tallgrass prairies. Each chapter was written as separate articles for submission to peer-reviewed journals and, as a result, duplicates some of the background information.

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IDENTIFYING SMOOTH BROME ELONGATION USING THE CORRELATION OF MEAN STAGE COUNT AND ACCUMULATED GROWING DEGREE DAYS

Abstract

The US Fish and Wildlife Service (USFWS) uses the number of leaves as a phenological cue, in which development of the five-leaf stage serves as a signal to the initiation of elongation in smooth brome (*Bromus inermis*). In areas where certain plant community criteria are met, conducting a prescribed burn at the onset of elongation has shown to reduce smooth brome populations. However, leaf stage identification presents USFWS managers with challenges, due to the variability of smooth brome development in tallgrass prairies of the northern Great Plains. The objective of this research was to develop an alternative method to determine when smooth brome populations reach the targeted 50% elongation by linking growing degree days and population level plant phenological stages (mean stage count). Sites in North Dakota, South Dakota, and Minnesota were identified and smooth brome phenological stages were determined, as well as the corresponding number of growing degree days, calculated using the base temperature of 0 °C (32 °F). The correlation between phenological stage and growing degree days allowed for development of the linear regression model to determine onset of elongation in the smooth brome population, regardless of leaf stage variation. The average number of accumulated growing degree days (1256 AGDD) and corresponding standard deviation (+/- 155 AGDD) can be used to predict when 95% of smooth brome populations in northern tallgrass prairies will reach 50% elongation between 946 AGDD and 1566 AGDD. As part of the USFWS Native Prairie Adaptive Management program, results will be used to assist in management decisions regarding the timing of control in an effort to enhance the native plant communities where smooth brome is the dominant invader.

Introduction

Smooth brome (*Bromus inermis*) is a perennial cool-season grass (Newell and Keim 1943, Lamp 1952). Individual plants vary in height, reaching between 0.5 and 1.25 meters tall. The leaf blades, between ten and twenty-five centimeters long, are easily identified by a distinct constriction mark resembling a “W” or “M” below the tip (USDA 2002). The inflorescence is an erect open panicle with minimal awns (Stubbendieck et al. 2011), developing a purplish-brown color when mature (USDA 2002). Plants produce seeds but are also very rhizomatous, forming thick sod through vegetative reproduction (Stubbendieck et al. 2011).

Smooth brome was introduced from regions of Europe and Asia in the late 1800s as forage grass and for erosion control (Newell and Keim 1943, Hitchcock 1950). There are numerous cultivated varieties, each slightly different from the other, making the different cultivars more suited to regional variations such as temperature and moisture (Newell and Keim 1943, Casler et al. 2000, Coulman 2006). However Lamp (1952) determined that cultivars from different regions all behaved similarly when grown under identical conditions. This may speak to the phenotypic plasticity of the species and contribute to its aggressive nature. If different cultivars have a great deal of phenotypic plasticity, they are more likely to grow well in a wide range of conditions.

Perennial grass plants are collections of tillers growing from the same crown, all of the same genotype. Because a single plant can have several generations of tillers, the developmental stage of the tillers can vary, containing both vegetative and reproductive tillers at the same time (Moore et al. 1991). While genetic variability can be limited by growth through rhizomatous tillers, a population of perennial grass plants is still able to contain genetic variation due to cross-pollination, resulting in developmental variability (Moore and Moser 1995).

When smooth brome grows from a seed, new plants emerge with a coleoptile (Moore et al. 1991). Successful germination of seed is dependent on sowing density, with increased density leading to decreased germination rates (Palmblad 1968). The vegetative stage is the period of leaf development and growth, in which the first true leaf emerges and lengthens followed by the 2nd, 3rd, 4th, and so on. The plant will reach a point at which it will stop adding additional leaves but will begin to lengthen between the nodes (internodal elongation). The elongation stage, often referred to as jointing, begins when the stem or culm is elongating. As elongation begins, it is possible to feel the first nodes "plumping" with your fingers. The first palpably identifiable node is the point at which the plant is considered to move from the vegetative stage to the elongation stage, corresponding to the increase of carbohydrate reserves within the above-ground plant tissue and a decline within in the crown tissue (Eastin et al. 1964).

Following elongation, a reproductive stalk may develop that will bear an inflorescence and, eventually seeds. When the inflorescence reaches anthesis, large pollen-laden anthers will extrude from the florets and be visible to the naked eye (Moore et al. 1991), positioning the pollen to be carried to neighboring plants. Unlike many other *Bromus* species, smooth brome does not self-pollinate, instead relying on wind driven cross-fertilization to produce seeds (McKone 1985). Relatively few tillers will actually produce a reproductive stalk while 100% of tillers will reproduce through vegetative tillers via rhizomes. In spite of producing an inflorescence, it was determined that virtually 0% produced seed (Palmblad 1968).

During the vegetative and elongation stages, management decisions must consider both the nutritive value of the plants and the effect that treatment will have on their ability to recover. Brueland et al. (2003) concluded that early grazing on smooth brome after the presence of one fully collared leaf per tiller, followed by a recovery period, would not be detrimental to the plant or have a negative effect on forage quality. Mitchell et al. (1998) suggested when tillers are in the elongation and reproductive stages, opening the canopy by grazing could recruit new tillers. Both of these situations would stimulate smooth brome growth. Conversely, reverse strategies could inhibit that growth if controlling the spread of smooth brome is the objective. Opening the canopy in a smooth brome invaded tallgrass prairie could encourage the recruitment of native species (Willson and Stubbendieck 2000), restoring the diversity of this threatened ecosystem (Murphy and Grant 2005).

Moore et al. (1991) created a system to identify the developmental stage of forage grasses at the population level, with morphological descriptors for each stage and a corresponding numerical index (mean stage count, MSC) that can be mathematically manipulated to calculate various statistics on species populations. The system, which uses the five growth stages (germination, vegetative, elongation, reproductive and seed-ripening), was designed to be easily memorized and used in the field for practical management decisions. When in the vegetative stage, only fully collared leaves are counted. If a leaf is present but not fully collared, it is not counted according to this method (Moore et al. 1991). A fully collared leaf that is more than 50% dead (Lamp 1952) will not be counted either. Once the first node is palpable, the plant is categorized in the elongation stage until such a time as the reproductive shoot can be observed (Moore et al. 1991).

As a highly palatable, high protein-containing forage grass smooth brome was widely planted as a food source for livestock (USDA 2002, Stubbindieck et al. 2011). Its rapid growth and massive root systems also make it useful in erosion control. Seed spread over bare ground will quickly grow and form sod that is valued for stabilizing bare earth. As a drought tolerant species, it has adapted to survive in a wide range of ecological settings. Additionally, smooth brome has been used in the past for wildlife cover and food; a practice that has generally been discontinued as its aggressive nature threatens invasion into native grasslands (USDA 2002).

Invasion of smooth brome has a cascading effect on the native prairie grasslands. Its rapid and rhizomatous growth allow it to spread once it has gained a foothold, increasing in above- and below-ground biomass (Piper et al. 2015), and ultimately resulting in decreased plant species biodiversity (Fink and Wilson 2011, Piper et al. 2015). In addition to declining biodiversity, invasive species can alter aspects of the ecosystem, including productivity and nutrient-cycling (Vitousek et al. 1996, Vinton and Goergen 2006). Increased litter production with expanded smooth brome growth results in declining plant diversity through shading and competition for other resources (Fink and Wilson 2011, Piper et al. 2015). Soil moisture decreases beneath smooth brome (Fink and Wilson 2011), making it unavailable to native plants. Soil dynamics that favor smooth brome are created through microbial (Jordan et al. 2008, Sherrard and Maherali 2012) and bacterial (Piper et al. 2015, Sherrard and Maherali 2012) population changes. Smooth brome expansion is further enhanced by rapid decomposition of litter and efficient nitrogen cycling (Vinton and Goergen 2006), as well as human supplemented nitrogen sources (Peterson and Moser 1985, Vinton and Goergen 2006).

Herbicides have been successfully used to control smooth brome. Dill et al. (1986) found that high rates of atrazine applied in spring decreased the smooth brome population on invaded warm-season pastures. However, endocrine disruption caused by herbicides can pose a risk to wildlife and humans (Fan et al. 2007, Orton et al. 2009), jeopardizing the very populations that prairie habitat restoration seeks to protect. Therefore, it is necessary for land managers to seek out alternatives that minimize future damage to prairie inhabitants.

The answer lies with naturally occurring phenomena of grazing and fire that shaped the grassland ecosystem prior to human intervention and the partitioning and development that came with settlement (Fuhlendorf and Engle 2001). The science of range management incorporates grazing and fire as tools to maintain the health of grasslands, the timing of which plays a critical role in achieving the desired management goals (Fuhlendorf and Engle 2004). Numerous studies have been done on the impact of grazing or mowing and fire on smooth brome. Reynolds and Smith (1962) found that carbohydrate reserves in smooth brome were highest in mid-July, during seed formation. Mowing or grazing at this time depletes carbohydrate reserves and inhibits recovery of smooth brome. Prescribed burning in late spring decreased smooth brome (Blankespoor and Larson 1994, Willson and Stubbendieck 1997) but there is evidence that burning too frequently can reduce the fuel load, and thus, the effectiveness of the fire (Ohrtman et al. 2015).

In an effort to control smooth brome in the native Tallgrass prairie and maintain a diverse habitat that supports a wide variety of species, the US Fish and Wildlife Service (USFWS) uses both grazing and prescribed burning in their management plans (Grant et al. 2009). USFWS has adopted Willson and Stubbendieck’s (2000) provisional model, a tool for land managers, designed to assist them in determining when prescribed burning should be applied to tallgrass prairies in order to control smooth brome. Having determined that the most effective time to burn smooth brome is during tiller elongation (Willson and Stubbendieck 1997), the model outlines a decision-making matrix. After verifying that the invaded tallgrass prairie includes at least 20% native tall grasses, a requirement to support the competitive exclusion of smooth brome, it is necessary to identify the developmental stage of the population (Willson and Stubbendieck 2000). If more than 50% of the smooth brome population has begun elongating but not yet reached the inflorescence stage then it is recommended to burn the site (Willson and Stubbendieck 2000). In some cases Willson and Stubbendieck (2000) noted it may not be possible to determine elongation. Their recommended alternative is to begin prescribed burning when the majority of smooth brome has reached the 5-leaf vegetative state, a benchmark that corresponds with the beginning of tiller elongation (Willson 1990), allowing prescribed burning to have maximum detrimental impact on the smooth brome by destroying the carbohydrate reserves required by the plant to survive, especially over winter (Willson and Stubbendieck, 2000).

USFWS personnel (Sara Vacek, USFWS, Pers. comm.) in the Northern Great Plains Tallgrass prairies use the 5-leaf method of Willson and Stubbendieck's (2000) provisional model and have found populations of smooth brome that appear to not reach the 5-leaf stage. USFWS' current seasonal monitoring requires repeated surveys during the spring to determine the developmental stage of smooth brome in order to burn. Following their policy of adaptive management (Grant et al. 2009, Gannon et al. 2013), USFWS is focusing on alternative methods to identify the appropriate timing for prescribed burning of smooth brome that does not rely on the population reaching the 5-leaf stage threshold. The objective of this research is to develop an alternative method to determine when smooth brome populations have reached the targeted 50% elongation using the phenological stages and population mean stage count developed by Moore et al. (1991), thereby minimizing repeated field surveys. The relationship between growing degree days and phenological development has been used by other researchers (Hendrickson et al. 1998, Frank et al. 1985) to determine population developmental stages. The number of accumulated growing degree days has the potential to serve as a cue to the onset of elongation within a smooth brome population.

Methods

Transects were located throughout North Dakota, South Dakota and Minnesota within the region's Tallgrass prairie (Figure 2.1, Appendix A). While the majority of the sites were located on USFWS managed land, two transects were located at The Nature Conservancy's Bluestem Prairie (Glyndon, MN), one transect on Minnesota State Parks property (east of Crookston, MN), two transects on US Forest Service Sheyenne National Grassland (north of Milnor, ND) and one transect on the NDSU Development Foundation's Ekre Grassland Preserve (Richland County, ND).

Sites were selected based on the visual presence of smooth brome. Transects, 50 m in length, were placed within the site to intersect as much smooth brome as possible. In order to minimize variables caused by microhabitat changes, transects were placed in areas with relatively uniform topography and underlying soil structure. If a site had topographical variation (for example, obvious sloping), multiple (2 or 3) transects were placed at that site to account for the variation.

The starting point of each transect was marked with a pin made of a lag bolt and washers, hammered flush with the ground, enabling location with a metal detector in future sampling seasons. The GPS coordinates of the pin and compass bearing were recorded for each transect.

Five 1-square meter quadrats were centered on the transect line, using restricted randomization to determine the placement of the quadrat within each 10-m subsection of the transect line. For each 10-m subsection, a random number generator was used to determine the location of the quadrat. For example, if the number 3 was randomly generated for the transect section from 30 to 40 m then the quadrat was placed from 33 to 34 m.

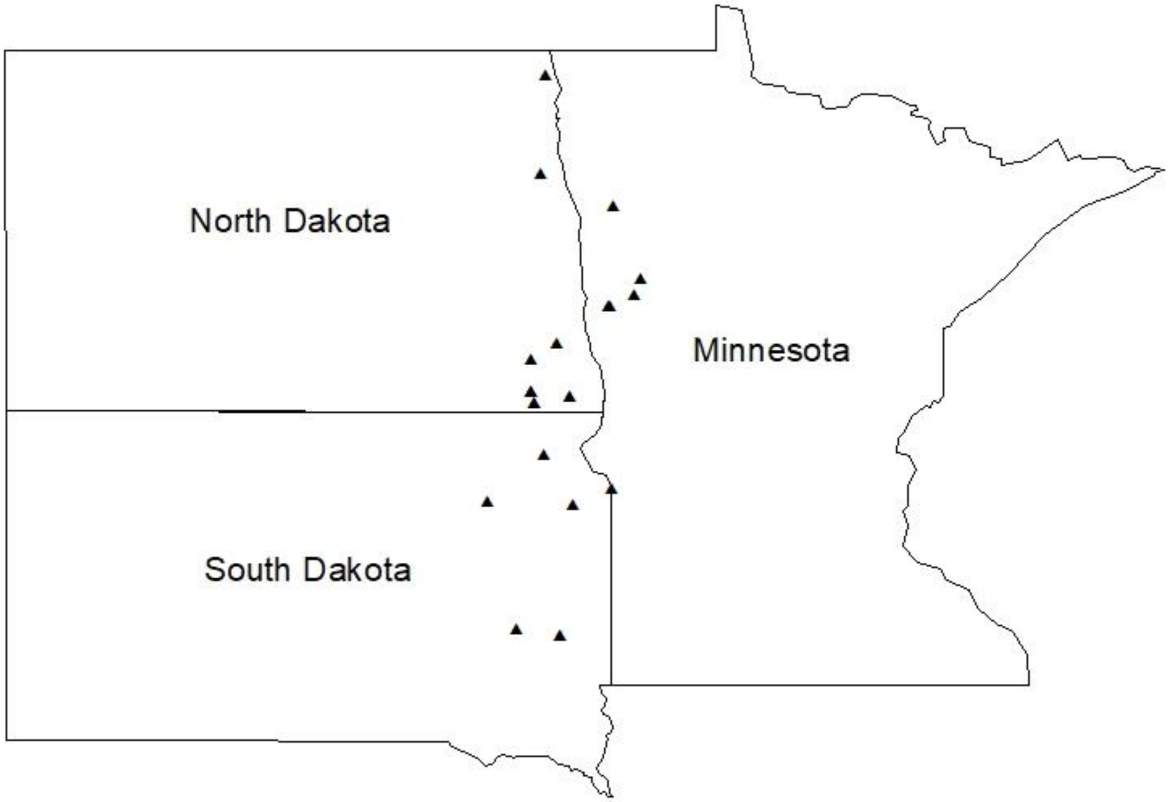

Figure 2.1. Locations of transect sites throughout North Dakota, South Dakota and Minnesota. Some sites include multiple transects.

Each quadrat, divided into ten 20-cm X 50-cm subplots, was sampled on a weekly basis. A different subplot was sampled each week to limit disturbance effects of sampling. Sampling consisted of placing a 20-cm X 50-cm frame in one of the ten subplots and identifying the phenological stage of each smooth brome tiller rooted within the frame. Phenological stage identification was completed using the Moore et al. (1991) method, described previously and summarized in Table 2.1. All smooth brome tillers within the subplot were identified and recorded. Subplot counts were tallied for each transect to obtain the number of tillers at each phenological stage for the transect sample. Data collection began in early May and continued for at least 6 weeks or until at least 50% of the sample population reached the elongation stage. A single soil sample was collected at each subplot for future measurement of soil nitrogen levels and soil temperature, litter depth and soil moisture were recorded.

This method was repeated in 2014 and 2015, using the same transects. If a transect was located on a site that experienced a disturbance (i.e. grazing, burning, etc.) the data was collected up to the date of the disturbance and discontinued following the disturbance. A notation was made on the data collection form to indicate the reason for discontinuing data collection. In some cases, lack of sufficient data required a transect be eliminated from the study.

Each phenological stage was assigned an index value according to Moore et al. (1991). The values for germination have been omitted because, as a perennial grass, it is difficult to distinguish between a germinating seed and a new emergent tiller. The index values for seed ripening were also omitted because the sampling concluded prior to seed ripening. For each stage, the index value was calculated by multiplying the total number of tillers at that stage by its index value (Table 2.1). Mean stage count (MSC) was then determined according to the following equation (Moore et al. 1991):

$$\text{MSC} = (\sum \text{Index Values for each stage encountered})/\text{total sampled tillers}.$$

Accumulated growing degree days (AGDD) were calculated, beginning January 1 of each year, using the following equation (Akyuz and Ransom 2015):

$$\text{AGDD} = \sum[(\text{Maximum temperature} + \text{Minimum temperature})/2 - \text{Base temperature})].$$

Maximum and minimum temperatures for each site were determined using the Applied Climate Information System Query Builder (Regional Climate Centers 2016). GPS coordinates and desired output were entered into the query builder to create a list of maximum and minimum daily temperatures from Jan 1 through July 31 of each year for each transect. Any temperature, maximum or minimum, lower than 0 °C (32 °F) was adjusted to the effective temperature of 0 °C (32 °F), as described by Akyuz and Ransom (2015). Effective maximum and minimum daily temperatures were then used to calculate the accumulated growing degree days with a base temperature of 0 °C. Because this model was developed to be used in the United States, actual GDD calculations were completed using Fahrenheit scale.

Table 2.1. Phenological development stage, index and description (Moore et al. 1991).

Stage	Index	Description
Vegetative-Leaf development		
Emergent or V_O	1.0	Emergence of first leaf
V_1	(1/N)+0.9	First leaf collared
V_2	(2/N)+0.9	Second leaf collared
V_n	(n/N)+0.9	Nth leaf collared
Elongation-Stem elongation		
E_O	2.0	Onset of stem elongation
E_l	(1/N)+1.9	First node palpable/visible
E_2	(2/N)+1.9	Second node palpable/visible
E_n	(n/N)+1.9	Nth node palpable/visible
Reproductive-Floral development		
R_O	3.0	Boot stage
R_1	3.1	Inflorescence emergence/1st spikelet visible
R_2	3.3	Spikelets fully emerged/peduncle not emerged
R_3	3.5	Inflorescence emerged/peduncle fully elongated
R_4	3.7	Anther emergence/anthesis
R_5	3.9	Post-anthesis/fertilization

For each transect, AGDD and MSC were plotted against one another and analyzed using linear regression (Hendrickson et al. 1998, Frank et al. 1985). Additionally, the percentage of each tiller stage was calculated for each sampling date to determine the approximate AGDD at which the sampled population reached 50% elongation. Using the slope of the line between 2 points that included 50% elongation, AGDD was calculated for each transect to determine the point at which the sample population reached this critical value of 50% elongation. This estimated AGDD at 50% elongation was calculated for each transect during both years and the average for all sites and both seasons was calculated with their corresponding standard deviations.

Following completion of the initial study, a model validation trial was set up to determine the accuracy of the model. In 2016, six previously unstudied sites (Figure 2.2) were selected, transects were set up at each site, and weekly phenological stage sampling was performed in mid- to late-May thru early June using the same methods described above.

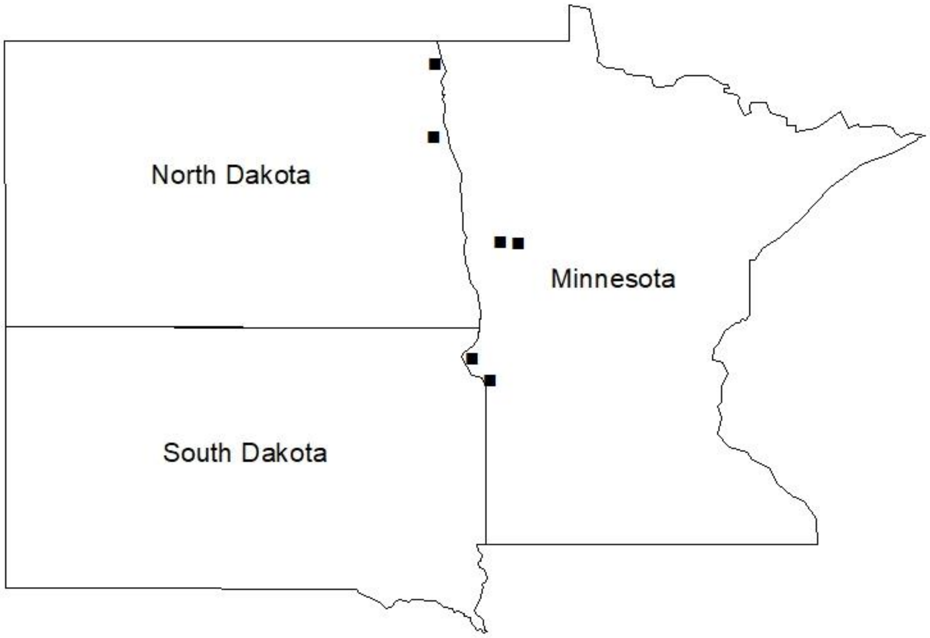

Figure 2.2. Location of six unknown sites selected for model validation study.

Linear regression analysis, non-linear regression analysis and bootstrap analysis were performed, both for individual years and combined data. Linear regression analysis was also performed on model validation data.

Results and Discussion

The results of the identification of phenological development stages were tallied and used to determine the percentage of the population that was at elongation phase or beyond and to calculate the MSC for each transect. These results can be found in Appendix B, C, and D. It is of note that the percentage of tillers that had begun elongation could jump significantly from one week to the next, progressing from no sampled tillers in elongation to greater than 50% tillers in elongation in the time between sampling. Additionally, the sample population rarely achieved 5-leaf stage before beginning elongation, more commonly elongating when the tillers had only three or four leaves. This supports the USFWS's reported difficulty in identifying 5-leaf stage because our sampled populations exhibited similar development.

There are several different methods for identifying the developmental stage of grasses, with a great deal of focus on annual cereal grasses (i.e. Large 1954, Haun 1973). Annual cereal grasses start as seeds in the spring of a season and complete their life cycle within that season, culminating with harvest. This differs from perennial grasses, like smooth brome, in which new plants may grow from seeds but can also develop from existing plants that have survived the winter. The method proposed by Moore et al. (1991) focuses on perennial grasses, identifying growth from seeds and from existing plants. The corresponding numerical index allows researchers to quantify the entire population by calculating index values for each stage, regardless of individual plant origin. The Haun method (1973) and Feekes method [described by Large (1954) and Miller (1999)] identify the developmental stage for individual plants but do not include an index by which to monitor overall development of the population. Additionally, the Haun and Feekes methods were specifically intended to identify developmental stages of wheat, although they have been applied to other grasses. Moore et al (1991) created their method to identify a more generic category of perennial forage grasses so it can more accurately be applied to a wider range of species.

Linear regression analysis shows a positive linear relationship between MSC and AGDD for each transect in 2014 and 2015 (Table 2.2; Appendix D). Early production of smooth brome, and the corresponding mean stage count, increases in a linear fashion as seedlings and early tillers emerge, develop leaves, and begin to elongate (Moore et al. 1991). However, later in the season, as more plants in the population begin to vegetatively reproduce, sending out new tillers, the population will have plants at all stages of development. The resulting mean stage count will begin to level out or even decrease. Because Willson and Stubbendieck's (2000) provisional model recommends control measures be performed during the elongation phase for maximum detrimental impact, production and MSC index values through elongation phase are the focus of this study. At some sites, populations may have passed this initial production and began secondary production, resulting in more variability in MSC and a lower R^2 value.

Growing degree day (GDD) refers to the thermal heating units required for plant development and is generally thought to be a more accurate method of measuring time of development than calendar days (Cross and Zuber 1972). There are a number of formulas to use when calculating growing degree days but a lack of consensus among researchers as to which formula is better than the rest (Cross and Zuber 1972). Cross and Zuber (1972) compared 22 different methods of calculating growing degree days in corn and found that using daily temperature measurements was similar in accuracy to hourly temperature measurements. Likewise, Frank and Hofmann (1989) used GDD calculations based on maximum and minimum daily temperatures, concluding that a linear relationship exists between AGDD and developmental stages in several native grass species. In considering which formula to use for our study, we chose a formula that included maximum and minimum daily temperature measurements. While some formulas use a constant temperature (Romo and Eddleman 1995), suitable for laboratory controlled environments, the formula selected uses variable temperatures, a requirement for populations studied in situ.

Table 2.2. Results of linear regression analysis and calculated accumulated growing degree days when sampled populations reached at least 50% elongation for 2014 & 2015.

Tran-sect #	Transect Name	R^2 (2014)	R^2 (2015)	AGDD @ 50% >E1 (2014)	AGDD @ 50%>E1 (2015)
1	Tewaukon/Wyum A	0.8354	0.7788	1279	1298
2	Tewaukon/Wyum B	0.7533	0.6661	1250	1258
3	Tewaukon/Wyum C	0.7736	0.6166	1221	1237
4	Ekre/NDAWN	0.9271	0.6916	1235	1250
5	Sheyenne National Grasslands A	0.7064	0.7308	1232	1286
6	Sheyenne National Grasslands B	0.8670	0.6861	1271	1419
7	Tewaukon/Pool 4 A	0.5724	0.6792	1146	1266
8	Tewaukon/Pool 4 B	0.6229	0.7441	1042	1134
10	Hartleben C B	0.6586	0.5827	1342	1231
11	Helliksen	0.8051	0.7726	1242	1286
12	Marks	0.7248	Na	1276	Na
13	Mekinock	0.9301	0.9130	1209	1299
14	Pembina Prairie	0.6583	0.9167	970	1239
16	Hepner	0.7592	Na	1386	Na
17	Wolfe A	0.7106	0.5063	1348	1358
18	Wolfe B	0.5744	0.4781	1163	1531
19	Sanderson	0.6766	0.9205	1285	Na
20	Overland	0.6166	0.8266	1270	1398
21	Gerber	0.6887	0.8204	1169	1322
22	Big Stone NWR	0.5055	Na	788	Na
23	Tympie	0.8932	0.8874	783	1325
26	Bluestem Prairie West A	0.8077	Na	1502	Na
27	Bluestem Prairie West B	0.9144	Na	1518	Na
	Annual Average			1214	1309
	Standard Deviation			182	92
	Combined Average			1256	
	Standard Deviation			155	

Growing degree days are commonly used for agricultural purposes to identify developmental stages of crops in order to determine the timing of events like fertilizer, pesticide or herbicide application and harvest. In these cases, growing degree days are accumulated beginning at the date of seed sowing. When considering perennial grasses, there is not a definite date when the seed was applied, or they may not require seeds at all as they are able to grow new plants from existing plants via tillers. Growth can potentially occur any time the temperature rises above the species' base temperature. April 1st is a common calendar day to begin accumulating growing degree days but because there are periods of time in the Northern Great Plains when the temperature can rise above 0 °C, the base temperature of smooth brome, earlier in the year and outside the typical growing season, we chose to start accumulating growing degree days on January 1st. In some years there may be no growing degree days during the winter months. However, during the years that our study was conducted, there were several periods of unseasonably warm temperatures, exceeding the base temperature for several days in a row. Some degree of photosynthetic activity could be seen as early as February and March. Therefore, similar to Hendrickson et al. (1998), we chose to begin accumulating growing degree days beginning on January 1st of each year. In any given year, it is possible that there are few, if any, growing degree days during the first 2 to 3 months of the year. If this is the case, the accumulated growing degree days during this time would be minimal so starting on January 1st would not add to the total. However, during those years when there is an early warm up, beginning on January 1st will provide a more accurate estimation of accumulated growing degree days for those plants that are already developed. Targeting specific AGDD values allows for identification of the burn window no matter how severe or mild the winter and spring conditions were. Regardless of the date chosen, it is important to remain consistent from year to year.

Different types of plants have different temperature requirements for growth to occur and this difference is accounted for by including the base temperature (minimum temperature for biological activity of that species) in the growing degree day equation (Hatfield et al. 2011). Unlike Cross and Zuber (1972), who recommended using a base temperature of 10 °C in their preferred model for corn, we used a base temperature of 0 °C. Frank and Hofmann (1989) used 0 °C as the base temperature for their study of Northern Great Plains native grasses. Romo and Eddleman (1995) used 0 °C as the base temperature in their smooth brome germination greenhouse trials to determine the number of AGDD required for germination at a variety of temperatures, as established by Frank and Hofmann (1989) and other researchers. We chose 0 °C as the base temperature for our comparisons because smooth brome is a cool season grass, like those studied by Frank and Hofmann (1989), that will begin growing early in the season and does not necessarily require germination of seeds as it is perennial and rhizomatous. The minimum temperature required for germination of smooth brome seeds was determined by Jordan and Haferkamp (1989) to be 4.9 °C. Because smooth brome is a cool-season perennial grass, we used the base temperature of 0 °C to account for the possibility photosynthesis could occur in existing plants when the temperature rises above freezing.

Using the ACIS Query Builder provided a uniform method to determine the temperature. Other temperature data was available but not consistently located near the transect. In some instances, there was temperature data available at the transect site, while other transects were anywhere from 8 to 32 km from the nearest recording station. The greater the distance between the transect and the weather gauging station, the less reliable the data becomes. Therefore, the ACIS Query Builder was chosen, using interpolated data from National Oceanic and Atmospheric Administration (NOAA) and increasing the accuracy of the maximum and minimum temperatures used for the AGDD equation, while also having a consistency in methodology.

Nonlinear regression analysis (Microsoft EXCEL 2013) between AGDD and % > E1 for the combined years showed a polynomial relationship (R^2=0.8301) (Figure 2.3), allowing for estimation of the number of AGDD elapsed when the population reached 50% elongation, even though sampling may not have occurred on the day of 50% elongation. Using the extrapolated AGDD at 50% elongation for each transect, the average number of AGDD for 2014, 2015, and the combined years were each calculated, along with their respective standard deviations. In 2014, data showed populations reaching 50% elongation at 1214 AGDD (standard deviation of 182). Based on these 2014 results, we expected populations to reach 50% elongation at roughly the same number of AGDD. During the 2015 season, sampling began early enough to ensure that each site was sampled before the population reached the 50% elongation benchmark. The average AGDD at which the population reached 50% elongation in 2015 was 1309 (standard deviation of 92). The overall average AGDD for both years combined was 1256 (standard deviation of 155).

During the model validation sampling, the expectation that 50% of the smooth brome population would reach elongation at approximately 1250 AGDD, as predicted by the model, was confirmed. There was a strong linear correlation between MSC and AGDD, for both the individual sites and for the combined site data. The average number AGDD at which 50% of the smooth brome tillers reached elongation or higher was 1160 for the validation sites, well within the 95% interval predicted by the model. This data is summarized in Table 2.3 and the correlation between MSC and AGDD for all the validation sites is shown in Figure 2.4. Individual validation site correlation data is available in Appendix E.

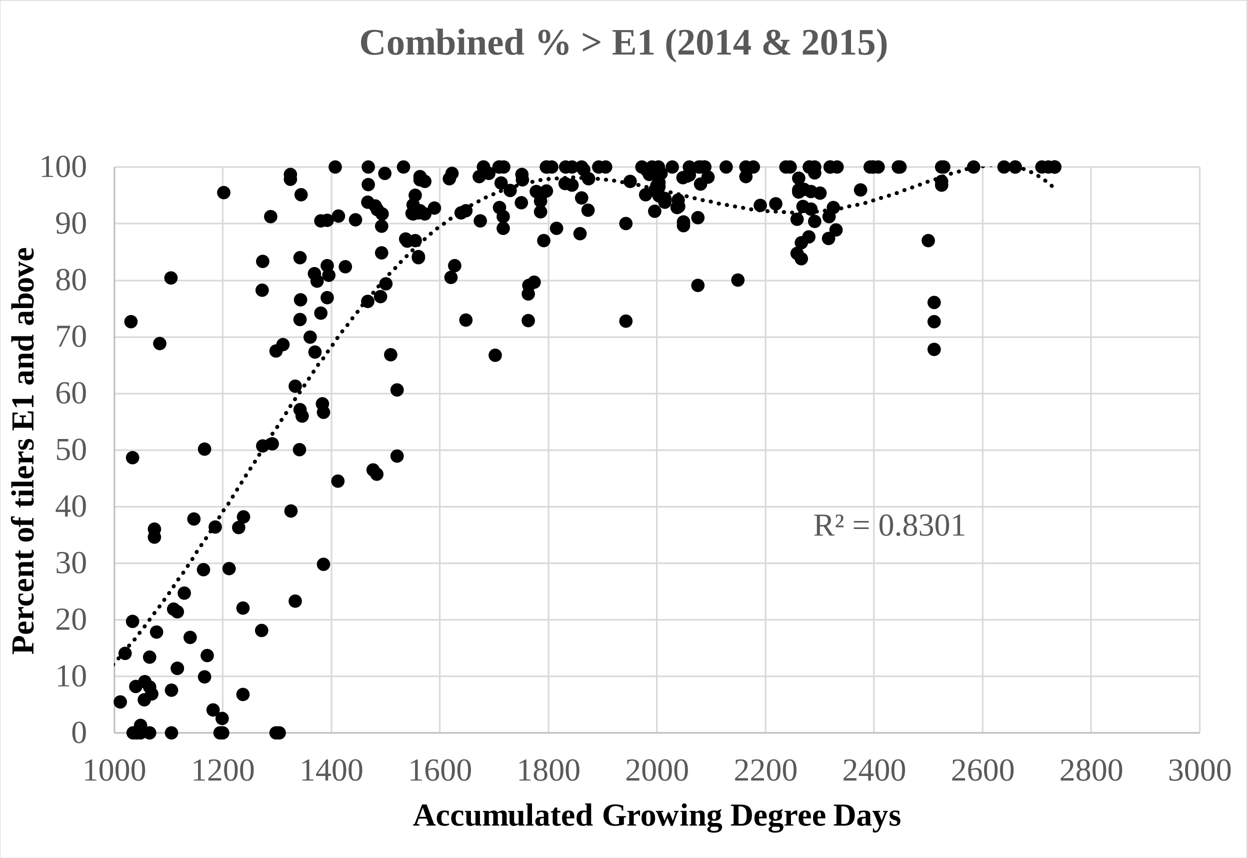

Figure 2.3. Nonlinear regression analysis shows a polynomial relationship between the percent of tillers in elongation and the number of accumulated growing degree days of all sites for both years.

Table 2.3. Summary of R^2 values and the number of accumulated growing degree days calculated when the sampled population reached at least 50% elongation for each of the model validation sites.

Transect Name	R^2 (2016)	AGDD @ 50% >E1 (2016)
Arneson	0.8976	1179
Olson	0.9546	1111
Kelly Slough	0.9716	1137
Kemp	0.9797	1214
Diekmann	0.7854	1157
Twin Lakes	0.8741	1159
Annual Average		1160
Standard Deviation		35

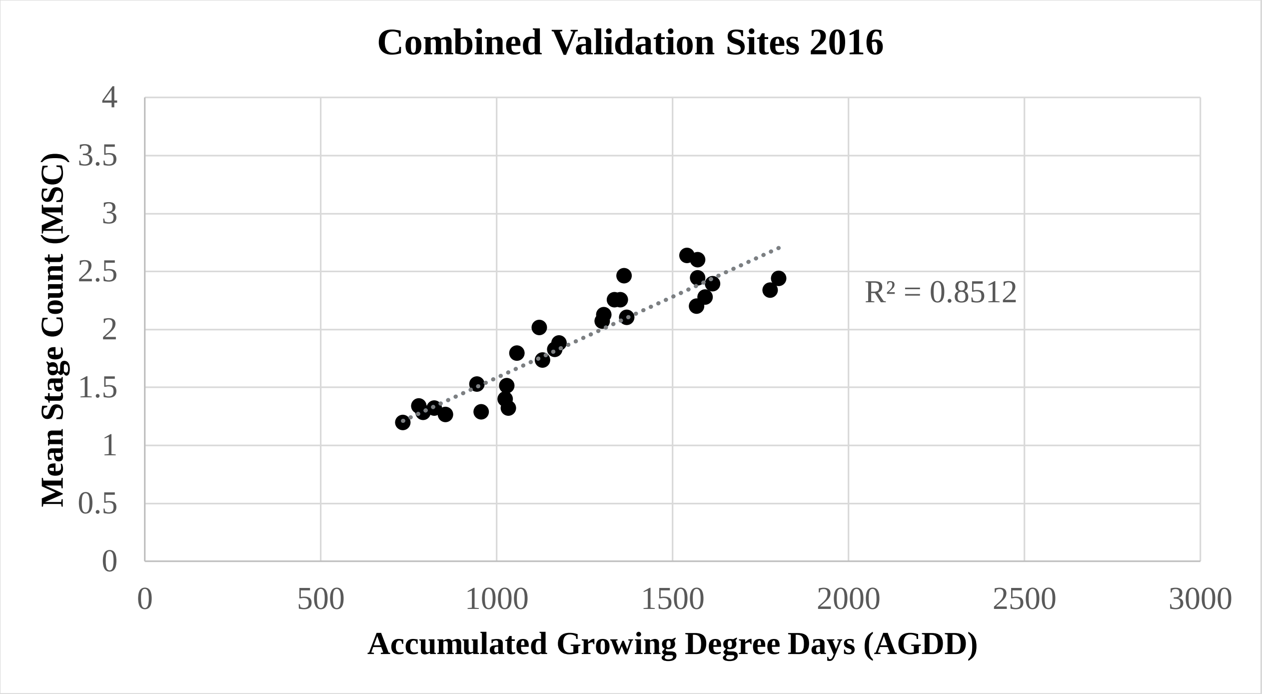

Figure 2.4. Positive linear relationship between Mean Stage Count and Accumulated Growing Degree Days for the combined data in the model validation. Individual site data can be found in Appendix D.

Bootstrap analysis (Microsoft EXCEL 2013) of the combined year's data was also performed on the AGDD at 50% elongation to confirm the average number of AGDD at which these smooth brome populations reached 50% elongation. The resulting value was 1255 AGDD (*n*=500) but, because the bootstrap analysis eliminates the highest and lowest 2.5% of the data set, the 95% confidence interval is bound much closer to the mean than when the raw data is averaged ([1205, 1301]).

For practical management purposes, it is better to use the raw data average and standard deviation, giving managers more time to respond to the phenological cues and schedule prescribed burns during the most effective window. Calculating AGDD can be done quickly on a daily or weekly basis without the resources required to perform phenological staging in the field environment. When AGDD nears the beginning of the 95% confidence interval (946 AGDD), field verification is required to confirm that the population has reached the targeted development of 50% or greater elongation. Prescribed burning can then be implemented according to Willson and Stubbendieck's (2000) provisional model for controlling smooth brome in native Tallgrass prairie.

Timing of prescribed burning is an important consideration. Burning early in the season, prior to elongation, removes litter from the young smooth brome plants, gives them better access to sunlight, and allows for increased growth of smooth brome (Willson and Stubbendieck 1997). On the other hand, burning during this early season could deplete soil moisture (Willson and Stubbendieck 1997), affecting the growth of both smooth brome and the native plants. Because smooth brome is drought tolerant, drier conditions could simply slow its growth and decrease its biomass, but not impact overall survival of the plants. Soil temperature is also affected by burning, increasing when smooth brome dominated plots are burned (Willson and Stubbendieck 1995). Late season burning, following elongation, was found to have a negative impact on smooth brome but not as effectively as during elongation (Willson and Stubbendieck 1997). Waiting to burn until later in the season could also be detrimental to the native species.

Additional ad hoc analysis could be applied to historical USFWS burn data, calculating elapsed AGDD of previous burns, to strengthen the accuracy of our AGDD model. The model could be made more precise, narrowing the prediction, by identifying other variables that affect smooth brome growth (i.e. moisture, nitrogen, etc.). However, narrowing the window to burn will not make the model any more useful to USFWS as they will be required to respond quicker with management treatments, increasing the possibility of missing the burn window. Continued review and adaptability is crucial for model success.

Conclusion

The results of this research show that there is a linear relationship between mean stage count and accumulated growing degree days for smooth brome. This relationship allows us to determine the average number of accumulated growing degree days required for smooth brome populations in our study area to reach 50% tillers in the elongation stage. With this average (1256 AGDD) and corresponding standard deviation (+/- 155 AGDD), we expect that 95% of smooth brome populations in northern Tallgrass prairies will reach 50% elongation between 946 AGDD and 1566 AGDD. This period can be identified as the window in which to expect smooth brome populations to best respond to prescribed burning as described in Willson and Stubbendieck's (2000) provisional model for controlling smooth brome. Monitoring sites for the target window via temperature reports requires fewer resources than the repeated field surveys currently performed by USFWS personnel. Resources can be allocated more effectively by adapting Willson and Stubbendieck's (2000) model to use AGDD instead of 5-leaf stage to identify smooth brome elongation and to determine the timing of prescribed burning.

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OBSERVATION OF SMOOTH BROME PHENOLOGICAL VARIABILITY UNDER CONTROLLED GREENHOUSE CONDITIONS

Abstract

Smooth brome (*Bromus inermis*) has been widely used as a forage grass and erosion control plant. Its competitive nature and rapid growth have allowed it to become an invasive species in the grasslands of the Northern Great Plains. Prescribed burning is a common method to control smooth brome in the native grasslands. Burning when smooth brome populations are most vulnerable to fire, at the initiation of elongation, destroys the carbohydrate reserves that allow the plants to recover. Identifying 5-leaf stage in phenological development has been recommended as a simple cue, signaling the start of elongation. However, local populations have been observed to begin elongating prior to reaching the 5-leaf stage. This study was designed to observe phenological development of smooth brome in a controlled greenhouse environment and determine if phenological development exhibited the same variability observed in the field. Under varying treatments of nitrogen supplementation, phenological development of smooth brome plants was monitored. It was determined that, while nitrogen treatment did affect the biomass and number of tillers produced, it did not affect the phenological progression of development. Using the correlation between mean stage count and accumulated growing degree days, it was determined that both greenhouse and field populations progressed through the phenological stages at approximately the same rate, reaching elongation at roughly 2500 to 3000 accumulated growing degree days.

Introduction

First introduced to the United States from Eurasia in the late 1800s, smooth brome is valued both as a forage grass and for soil stabilization in disturbed areas (Newell and Keim 1943, Hitchcock 1950). Its rhizomatous growth pattern (Stubbendieck et al. 2011) allows for rapid colonization under suitable conditions. Smooth brome is a highly adaptable species, due in part to the numerous cultivars (Newell and Keim 1943, Casler et al. 2000, Coulman 2006), allowing it to respond to a variety of climatic and environmental conditions.

Throughout the Northern Great Plains smooth brome has spread from agricultural fields and disturbed roadsides into grasslands. Increasing expansion of smooth brome into the northern tallgrass prairies poses a threat to the native grassland ecosystems and the biodiversity found within these regions (Murphy and Grant 2005, Fink and Wilson 2011, Piper et al. 2015). As this cool season perennial grass advances, its early season growth allows it become established earlier than native plants using the valuable resources of water, nutrients, and sunlight before the native plants are able to be competitive (Fink and Wilson 2011).

Early studies on smooth brome found both seed yield and forage yield increased to varying degrees with increased nitrogen application (Harrison and Crawford 1941). These results have been confirmed by other researchers (Levang-Brilz and Biondini 2002, Vinton and Goergen 2006). Nitrogen supplementation increased production of sterile tillers (Harrison and Crawford 1941), suggesting that increased nitrogen allows smooth brome to be more competitive because it produces more and larger tillers in response to the increased nitrogen. Increased smooth brome canopy (Vinton and Goergen 2006) allows individual plants to produce more carbohydrates through photosynthesis, the excess of which can be stored in seeds of fertile tillers for the next season (Harrison and Crawford 1941). Additionally, rapid decomposition of smooth brome litter allows for further supplementation of nitrogen via nutrient cycling (Vinton and Goergen 2006), resulting in favorable conditions for smooth brome to thrive.

The United States Fish and Wildlife Service (USFWS) uses prescribed burning to control smooth brome as part of their native prairie adaptive management program (Gannon et al. 2013). USFWS personnel follow Willson and Stubbendieck's (2000) provisional model for burning smooth brome to guide their decision making with regards to burn timing, which recommends prescribed burning occur once 50% of a smooth brome population has reached elongation, using the development of the 5-leaf stage as an easily identifiable cue to the onset of elongation. The model further recommends that burning be completed prior to the maturation of inflorescence. However, field application of this method in the Northern Great Plains Tallgrass prairies has been problematic due to the apparent lack of 5-leaf development and further development of reproductive inflorescence, resulting in missed opportunities for optimal impact with prescribed burning (Sara Vacek, USFWS, personal comm.). Current seasonal monitoring by USFWS requires repeated surveys during the spring to determine the developmental stage of smooth brome in order to burn. Following their policy of adaptive management (Grant et al. 2009, Gannon et al. 2013), USFWS is focusing on alternative methods to identify the appropriate timing for prescribed burning of smooth brome that does not rely on the population reaching the 5-leaf stage threshold.

When smooth brome grows from a seed, new plants emerge with a coleoptile (Moore et al. 1991). The vegetative stage is the period of leaf development and growth, in which the first true leaf emerges and lengthens followed by the 2nd, 3rd, 4th, and so on. The plant will reach a point at which it will stop adding additional leaves, but will begin to lengthen between the nodes (internodal elongation). The elongation stage, often referred to as jointing, begins when the stem or culm is elongating. The first palpably identifiable node is the point at which the plant is considered to move from the vegetative stage to the elongation stage, corresponding to the increase of carbohydrate reserves within the above-ground plant tissue and a decline within in the crown tissue (Eastin et al. 1964). Following elongation, a reproductive stalk may develop that will bear an inflorescence and eventually seeds. When the inflorescence reaches anthesis, large pollen-laden anthers will extrude from the florets and be visible to the naked eye (Moore et al. 1991), positioning the pollen to be carried to neighboring plants. Moore et al. (1991) developed an index to calculate the mean stage count (MSC) of a population on a given day using the following equation (Moore et al. 1991):

$$\text{MSC} = (\sum \text{Index Values for each stage encountered})/\text{total sampled tillers},$$

where sampled tillers at each developmental stage are tallied and multiplied by the index value for that stage (Table 3.1).

Chapter 2 of this document discusses alternative ways to determine elongation of smooth brome using accumulated growing degree days (AGDD) instead of the 5-leaf stage that had been used previously. While collecting data for that project, we confirmed USFWS observations that the 5-leaf phenological stage was rarely observed in situ. The purpose of this project was to observe smooth brome phenological development in a controlled setting. Following smooth brome development under more controlled conditions, the goal was to identify phenological trends and determine if any source of variability could be identified. In this study, nitrogen levels were manipulated to determine if nitrogen supplementation affects phenological development or biomass of smooth brome as has been noted by others in field plots (Vinton and Goergen 2006) and greenhouse studies (Levang-Brilz and Biondini 2002)

Methods

For each of the 3 trials, 400 7.25-cm round unglazed clay pots were filled with commercial potting mix and a single smooth brome seedling, grown from an agricultural seed source of smooth brome. In trials 1 and 3, seeds were allowed to germinate in growth tubes and robust seedling plants were selected to be transplanted into the pots. In trial 2, several seeds were planted directly into the pots and the seedling that appeared most robust remained while the others were removed from the pots. Pots were randomly assigned 1 of 4 nitrogen supplementation treatments: control (no addition), low (28 kg/ha), medium (56 kg/ha), or high (112 kg/ha) with UFLEXX® granular 46% stabilized nitrogen fertilizer (Koch Agronomic Services, LLC, Wichita, KS) added the day following transplant of individual seedlings in trials 1 and 3. For trial 2, nitrogen supplementation treatments were randomly assigned and applied the day of seed planting. A second identical nitrogen application was added to all trials approximately 21 days following initial treatment to maintain sufficient nitrogen levels. Greenhouse conditions were monitored and controlled by computer with the temperature held

Table 3.1. Phenological development stage, index and description (Moore et al. 1991).

Stage	Index	Description
Vegetative-Leaf development		
Emergent or V_O	1.0	Emergence of first leaf
V_1	(1/N)+0.9	First leaf collared
V_2	(2/N)+0.9	Second leaf collared
V_n	(n/N)+0.9	Nth leaf collared
Elongation-Stem elongation		
E_O	2.0	Onset of stem elongation
E_l	(1/N)+1.9	First node palpable/visible
E_2	(2/N)+1.9	Second node palpable/visible
E_n	(n/N)+1.9	Nth node palpable/visible
Reproductive-Floral development		
R_O	3.0	Boot stage
R_1	3.1	Inflorescence emergence/1st spikelet visible
R_2	3.3	Spikelets fully emerged/peduncle not emerged
R_3	3.5	Inflorescence emerged/peduncle fully elongated
R_4	3.7	Anther emergence/anthesis
R_5	3.9	Post-anthesis/fertilization

between 21 °C and 23 °C. During periods of warmer weather, the greenhouse conditions were maintained at ambient temperature minus 10 degrees. Computer controlled hourly temperature readings were automatically logged and recorded. Greenhouse lights, set on computer controlled timers, supplemented daylight as needed from 0600 to 2200 daily. Plants were watered on a regular basis. Plants were monitored daily for growth and phenological development, according to Moore et al. (1991), as described in Table 3.1.

In the event that rogue seeds may have been mixed in with the smooth brome seed, plants were allowed to mature and removed from the study when it was positively determined to be a species other than smooth brome. These individuals were excluded from the calculations and summaries.

At the end of each trial, all tillers in each pot were clipped at the soil level, placed in individual bags (one bag/pot) and dried in an industrial dryer for 7 days. Dried samples were stored in the North Dakota State University herbarium until they were to be weighed, at which time samples were returned to the dryer for an additional 48 hours to remove any possible atmospheric moisture that may have been added while in storage. Immediately following the second drying, the clipped biomass of each pot was determined by weighing the entire sample including the bag, then the bag tare weight was subtracted to calculate the sample's clipped biomass weight. One-way ANOVA (SAS Enterprise Guide Version 7.1, SAS Institute, Cary, NC) was performed on biomass to identify differences in treatment response, followed by a Tukey's studentized test of $P \leq 0.05$.

Each treatment within a trial was treated as an independent sample population, with the phenological stages totaled and mean stage count (MSC) calculated for each trial. Accumulated growing degree (AGDD) days were also calculated for each trial using the minimum and maximum daily temperature and a base temperature of 0 °C (32 °F). The equation used for AGDD was the same as that used for the field study (Akyuz and Ransom 2015):

$$\text{AGDD} = \sum[(\text{Maximum temperature} + \text{Minimum temperature})/2 - \text{Base temperature})].$$

However, because greenhouse plants were grown from seed and not perennial growth, AGDD began on day 1 of planting the seeds, as opposed to January 1 in the field study.

Linear regression analysis was used to compare MSC and AGDD for each treatment and combined data for each trial. Percent elongation was also calculated for each treatment and trial, identifying the number of AGDD at which the populations reached 50% elongation or higher. This AGDD and corresponding % elongation were used, along with nearest previous % elongation point, to determine the slope of the line and then extrapolate the value of AGDD at 50% for that treatment. Average AGDD for all treatments and trials were calculated and one-way ANOVA (SAS Enterprise Guide Version 7.1, SAS Institute, Cary, NC) was performed, using treatment as the independent variable and AGDD at 50% elongation as the dependent variable.

Additionally, the percentage of observations of plants at the 5-leaf stage was calculated to determine how frequently the study population reached the 5-leaf stage. This was compared to the percentage of 5-leaf observations during the field studies reported in chapter 2.

Results

One-way ANOVA confirmed a significant difference between nitrogen supplementation treatments on biomass ($P < 0.0001$) and on number of tillers per pot ($P < 0.0001$). A Tukey's studentized (hsd) test applied to both biomass (Figure 3.1) and tiller count (Figure 3.2) data showed which treatments had a significant difference. Comparison of mean biomass showed that all treatments were statistically different than the control and high nitrogen treatment was statistically different from the low nitrogen treatment (Figure 3.1). Tukey's results on mean tiller count show that all treatments were statistically different than the control but, unlike the biomass results, medium nitrogen treatment was statistically different from the low nitrogen treatment (Figure 3.2).

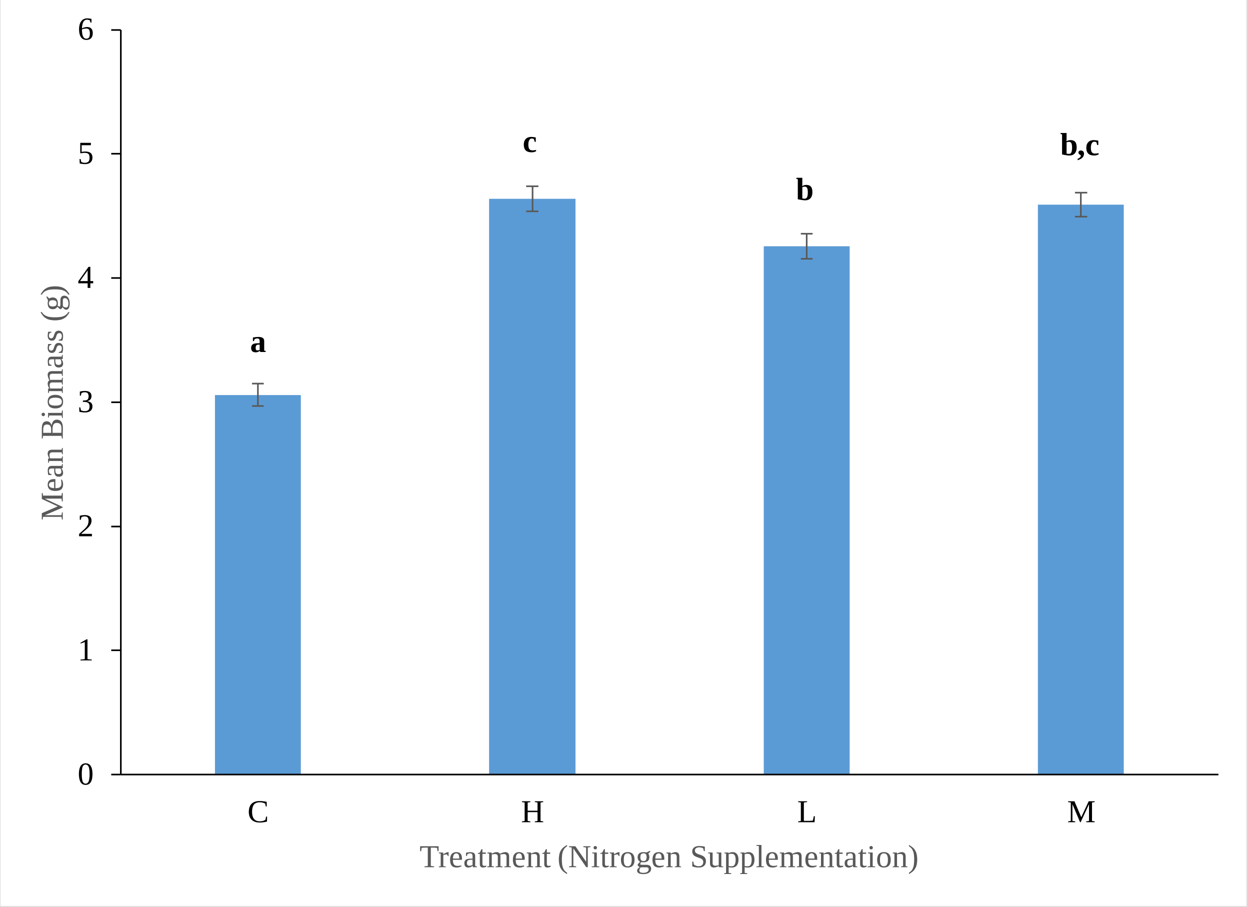


Figure 3.1. Mean (±SE) biomass for each treatment. Letters indicate significant differences ($P < 0.05$).

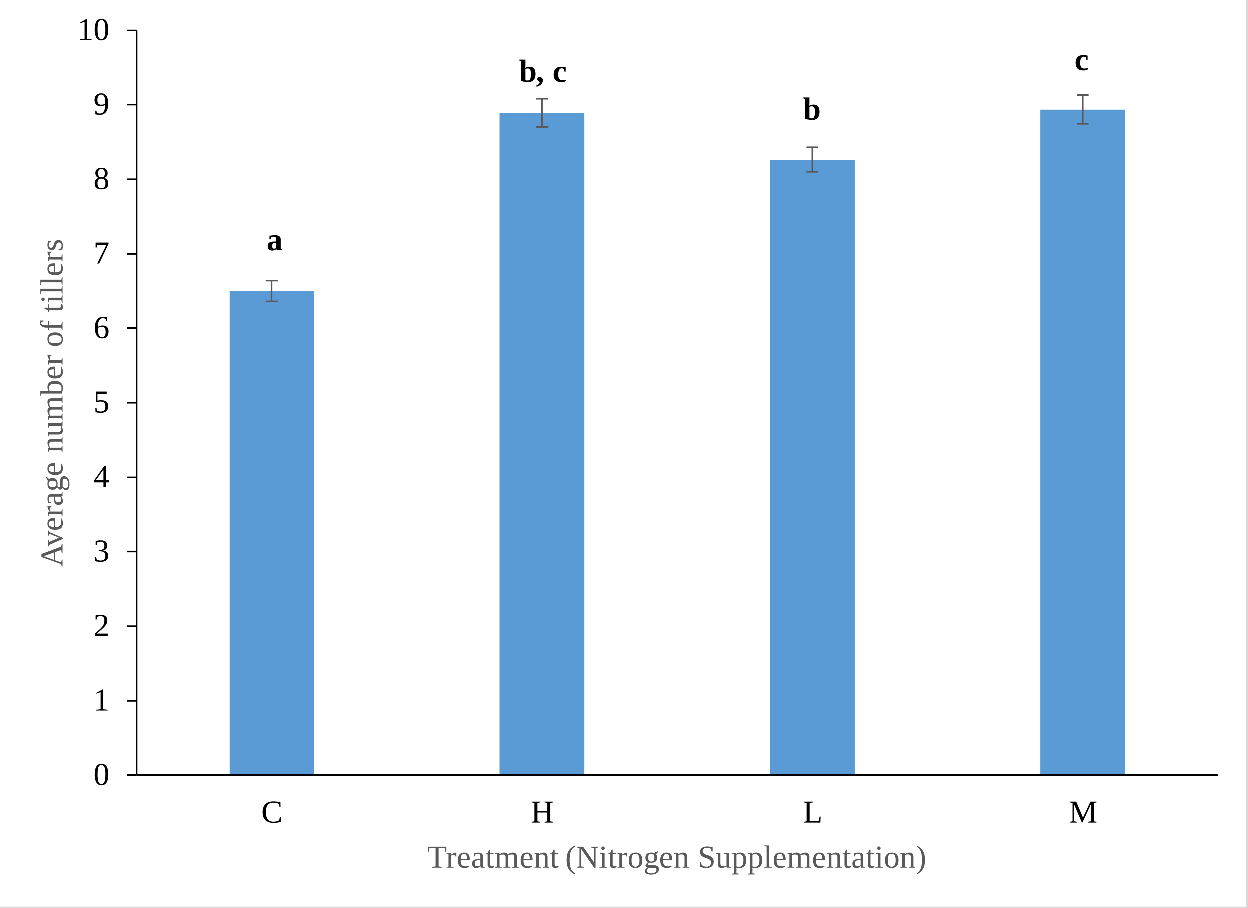


Figure 3.2. Mean (±SE) tiller count for each treatment. Letters indicate significant differences ($P < 0.05$).

For each treatment level and trial, MSC and AGDD were calculated for each observation. Linear regression analysis was applied to MSC and AGDD for each treatment within the trials (Table 3.2, Appendix F) and the combined data for each trial (Figures 3.3a, b, c). Each greenhouse trial showed a linear relationship between combined treatment MSC and AGDD but not as strong as the field survey described in Chapter 2. One-way ANOVA applied to the extrapolated AGDD at 50% elongation (Table 3.2) showed no significant difference between treatment effects on the AGDD at 50% elongation ($P = 0.6464$).

Willson and Stubbendieck (2000) recommended using the 5-leaf stage to determine elongation and population readiness for prescribed burning. The percentage of observed 5-leaf stage plants was calculated for the population, using both total observations and only those observations after elongation was initiated (Table 3.3).

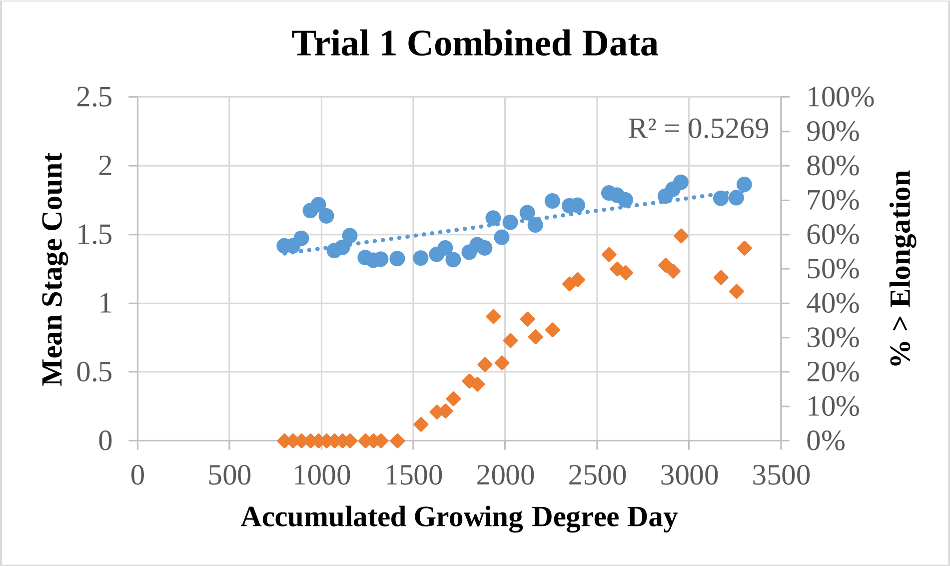
Table 3.2. Results of linear regression analysis (R^2) and extrapolated AGDD at 50% elongation for each trial and treatment.

Trial	Treatment	R^2	AGDD @ 50% E
1	C	0.1998	2597
1	L	0.3367	2208
1	M	0.3367	2244
1	H	0.5269	2468
2	C	0.6301	2077
2	L	0.5667	2160
2	M	0.6349	2079
2	H	0.6334	2029
3	C	0.5720	2163
3	L	0.6175	2215
3	M	0.5937	2357
3	H	0.6296	2848
		Average AGDD:	2287
		Std Dev:	243

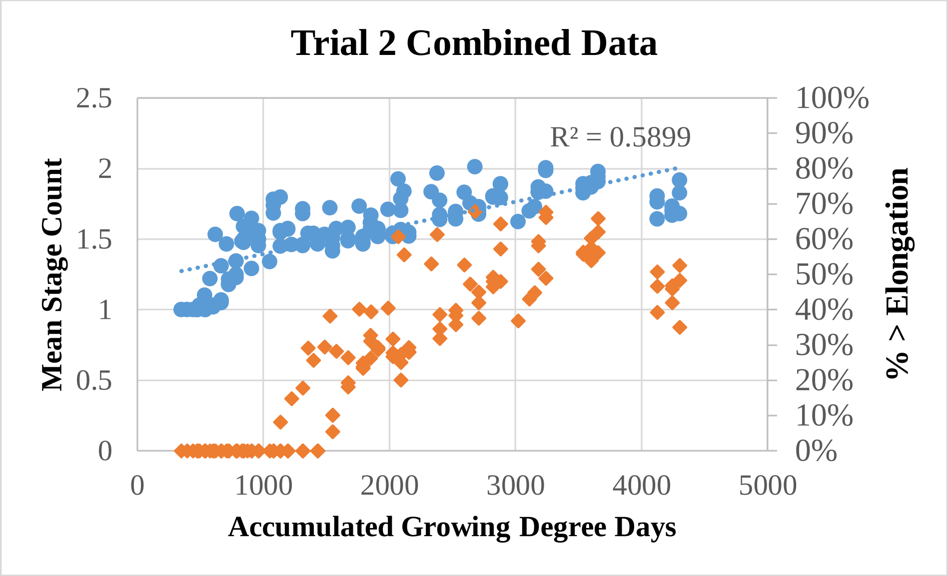
Table 3.3. Percent of observed 5-leaf tillers of total observations and observations following the initiation of elongation.

Trial	Treatment	% 5-leaf total	% 5-leaf after elongation
1	C	*4.1%	*4.0%
1	L	2.6%	2.5%
1	M	3.1%	3.0%
1	H	2.5%	2.5%
2	C	1.7%	1.7%
2	L	1.8%	1.8%
2	M	1.6%	1.6%
2	H	1.4%	1.3%
3	C	**0.9%	**0.9%
3	L	**0.9%	**0.9%
3	M	1.0%	1.0%
3	H	**0.9%	**0.9%

*Maximum, ** Minimum.

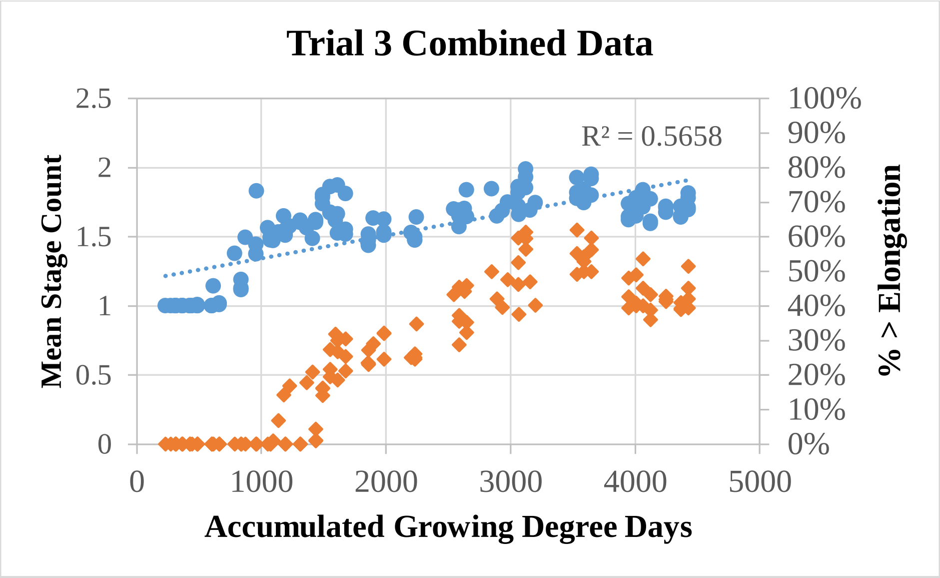


(a)



(b)

Figure 3.3. Linear regression analysis for each trial's combined data, comparing accumulated growing degree days and mean stage count, indicated by circles on the primary axis. The percentage of daily observations in elongation phase or higher, indicated by diamonds on the secondary axis. (a) Trial 1, (b) Trial 2 and (c) Trial 3.



(c)

Figure 3.3. Linear regression analysis for each trial's combined data, comparing accumulated growing degree days and mean stage count, indicated by circles on the primary axis (continued). The percentage of daily observations in elongation phase or higher, indicated by diamonds on the secondary axis. (a) Trial 1, (b) Trial 2 and (c) Trial 3.

Discussion

Significant treatment effects on smooth brome biomass and tiller counts at time of harvest are consistent with research performed by Frank and Hofmann (1989) and Levang-Brilz and Biondini (2002). Frank and Hofmann (1989) stated that air temperature primarily controlled the rate of morphological development in Northern Great Plains grasses, while nutrients impacted the quantity of production. Levang-Brilz and Biondini (2002) determined that increased nitrogen resulted in increased aboveground biomass in smooth brome. Increased biomass results in more canopy to intercept solar radiation and produce more litter that rapidly decomposes, perpetuating a nitrogen-rich environment favorable for smooth brome invasion (Vinton and Goergen 2006).

Willson and Stubbendieck's (2000) provisional model recommends burning only when there is at least a 20% native plant component in order to allow those native plants to utilize the newly available resources following the burning of smooth brome, resulting in increased competition for smooth brome. Without enough competition from other species, smooth brome will simply grow back, perhaps more vigorous than before, on the disturbed site. Unlike a native prairie environment, this greenhouse experiment used smooth brome seeds grown in pots without any other species to provide competition.

The average AGDD in the field survey sites (described in Chapter 2) for the population to reach 50% elongation was 1256 (standard deviation of 155). The greenhouse population reached 50% elongation at an average of 2287 AGDD (standard deviation of 243). The greenhouse population average does not fall within the 95% confidence interval of the field study model and the difference between the two averages could be hypothesized to be the result of a number of factors. The greenhouse populations were planted from seeds with all pots in each trial at approximately the same chronological age. In a field setting, perennial plants grow from existing plants that potentially have very different chronological ages or from seeds that were produced during a number of possible seasons. This variation in growth could be reproduced in the greenhouse if the study was extended over a number of growing seasons but because our study was completed in one season, we were unable to reproduce the perennial growth variation. The greenhouse environment allowed us to control a number of factors that would not be as uniform in the natural prairie setting, such as length of daylight, temperature and moisture. Greenhouse plants did not experience the more extreme temperature dips that may occur naturally or the variation in sunlight caused by a cloudy day. The greenhouse lights automatically turn on to supplement sunlight on cloudy days and create a uniform span of “daylight” hours, even when naturally occurring daylight hours would be increasing or decreasing, depending on season. Additionally, the populations planted in the greenhouse were seeded during the first 3 weeks of

July, after field populations of smooth brome had already begun to flower and produce seed. Starting the seeds after the field populations had completed their initial growth could contribute to the variation in the average AGDD at 50% elongation.

Linear regression analysis between MSC and AGDD were not as strongly correlated in the greenhouse study as they were in the field study (Chapter 2). Again, the lack of competition, environmental and climate variations, and lack of perennial growth in the greenhouse populations may have reduced the correlation.

USFWS personnel (Sara Vacek, personal comm.) had reported a notable lack of field observations in which smooth brome plants reached 5-leaf vegetative stage or higher. The 2014 field surveys also observed minimal plants at 5-leaf stage. Following the initial field survey season, we decided to monitor phenological development in the greenhouse to determine if the lack of 5-leaf stage was an anomaly. The greenhouse population showed a higher incidence of 5-leaf stage observations than the field survey (average observation of 2%, and less than 1%, respectively) but the total observation of 5-leaf stage was quite low in both cases. Statistical significance was not calculated for the greenhouse treatments because all treatments had so few 5-leaf stage tillers observed throughout the experiment.

Smooth brome cultivars exhibited a variety of characteristics and a great deal of phenotypic plasticity (Otfinowski et al. 2006). Lack of 5-leaf stage observations could be attributed to the cultivars that have been used throughout this region and in this study.

Conclusions

Phenological variability was seen in field (Chapter 2) and greenhouse populations. The linear correlation between AGDD and phenological development, measured by MSC, exists in both instances and can be used to predict the approximate number of AGDD required for a population to reach elongation. This greenhouse study confirmed smooth brome populations may not reach the5-leaf stage before initiating elongation. The contributing factors to this developmental progression are yet to be determined, but the results of our study showed that nitrogen supplementation treatments did not significantly affect the number of AGDD required for a smooth brome population to reach the elongation stage. As such, we recommend the use of elongation, as determined by the formation of palpable nodes, be used as a cue for smooth brome population control by prescribed burning. Palpable node identification is a simple and more consistent signal to the onset of elongation than the 5-leaf stage.

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SMOOTH BROME RESPONSE TO VARIED PRESCRIBED FIRE REGIMES

Abstract

Smooth brome invasion into native tallgrass prairie has led to the need to develop methods of control. Prescribed burning is commonly used throughout the Northern Great Plains and US Fish and Wildlife Service actively manage their native grasslands according to a provisional model developed by Willson and Stubbendieck (2000). Model recommendations rely on prescribed burning in the spring, when smooth brome has initiated elongation. However, given variable climatic conditions, prescribed burning may not be possible at this recommended time. This preliminary small scale study observed smooth brome response to burning throughout the growing season to determine if there are other times when smooth brome decreases following prescribed burns. Burn treatments were randomly assigned to 10 m X 10 m plots with pre-burn and post-burn sampling of smooth brome completed to determine smooth brome survival at different times of the growing season. Preliminary results suggest that smooth brome decreases when burned during alternative seasons, such as late fall, but further research is necessary to determine the size of the effect and the impact on other species.

Introduction

The severe decline of native Tallgrass prairie, due in part to agricultural expansion, has left US Fish and Wildlife Service (USFWS) with the government mandated role of protecting this important habitat for the wildlife that depend on it (Grant et al. 2009). It is estimated that as little as 13% of the historic Tallgrass prairie remains in the American Great Plains (Samson et al. 2004). Smooth brome (*Bromus inermis*) is one of the invasive cool-season grass species that threatens the remaining Tallgrass prairie under USFWS management (Murphy and Grant 2005, Grant et al. 2009). Merely protecting the Tallgrass prairie has not been sufficient to prevent invasion of introduced species (Grant et al. 2009). Active and ongoing management must be engaged in order to slow and potentially reverse the impact that smooth brome invasion has on the native Northern Tallgrass prairie (Willson and Stubbendieck 2000, Grant et al. 2009).

Control of smooth brome in Northern Tallgrass prairies by prescribed burning was recommended by Willson and Stubbendieck (2000) and included specific guidelines in a decision-making model to determine when to burn. In the presence of at least 20% native plants, burning should be postponed until at least 50% of the smooth brome population has reached 5-leaf vegetative stage in order to have the greatest damaging effect on the smooth brome (Willson and Stubbendieck 2000). This provides a three-fold impact on smooth brome by exposing the meristem to lethal heat after it has been elevated above the ground, destroying the carbohydrate reserves that are required for tiller growth and removing smooth brome as a competitor for native plants (Willson and Stubbendieck 1997, 2000).

Willson and Stubbendieck (1997) found that early spring burning, during emergence, resulted in smooth brome populations increasing. Burns at all other tested times of year were effective in decreasing smooth brome but burns completed at elongation, prior to development of an inflorescence, were the most effective (Willson and Stubbendieck 1997). However, an earlier study concluded that burning smooth brome was not an effective control mechanism because, although there was a decline in smooth brome density, it was not considered significantly different from the control population (Willson and Stubbendieck 1996). This apparent contradiction was possibly due to a lack of native plants to out compete the remaining smooth brome (Willson and Stubbendieck 1996).

The previous two chapters investigated an alternative method of identifying the burn window, using the correlation between mean stage count (MSC) (Moore et al. 1991) and accumulated growing degree days (AGDD) (Akyuz and Ransom 2015) to predict elongation. We determined that the 5-leaf vegetative stage was a morphological cue that was rarely met in this region and that AGDD is an effective method of predicting when smooth brome populations have begun elongation and, thus, the transfer of carbohydrate stores to the above ground biomass in preparation for reproduction via seed (Eastin et al. 1964, Jensen et al 2013). Burning after elongation has been initiated destroys those carbohydrates reserves and decreases the chance of survival, ultimately reducing smooth brome (Willson 1990). However, in the event that conditions are not met for prescribed burning during this window, we must then ask if we forego burning for that year or if there is another opportunity to damage the smooth brome population. This study looked at the question of burn timing on Tallgrass prairie sites currently under USFWS management. Does smooth brome respond differently under differently timed burn treatments? Are burn treatment responses different at different locations? To further investigate the use of fire to control smooth brome populations in the Northern Tallgrass prairie, this study was conducted to compare smooth brome response to burning at different times of the year and at different phenological stages. The goal of this small scale study was to determine if time of year affects the impact that burning has on smooth brome survivability and if current USFWS protocol for burn timing should be adjusted in order to have a greater detrimental impact on smooth brome populations in the native prairie. The results of this study will be used to guide USFWS in the development of a broader scaled study if it is warranted.

Methods

Site locations were selected based on the following criteria: smooth brome dominated, native grassland component, underlying soil conditions predominantly loamy, and subject to minimal management with no burn or grazing for the past three to five years. Both sites were located on USFWS property. The participating USFWS properties were Arrowwood National Wildlife Refuge (-98.8486, 47.25595; Pingree, ND) and Thompson Waterfowl Production Area (-99.2572, 45.34634; Sand Lake National Wildlife Refuge District, Roscoe, SD).

Thompson WPA plots were laid out in a grid pattern, 6 plots per replicate with 3 replicates (Figure 4.1). At the Arrowwood NWR, 1 of the 3 replicates was separated from the other two in order to maximize the inclusion of smooth brome in the replicate (Figure 4.2). Individual plots were 10 m X 10 m squares with a 2 m mowing buffer between plots. Each replicate plot was randomly assigned one of six treatment methods: 1) Control, 2) Early Spring Burn – up to and including May 15, 3) Late Spring Burn – May 16 through June 15, 4) Summer Burn– June 16 through August 15, 5) Early Fall Burn – August 16 through September 15, or 6) Late Fall Burn – September 16 through October 15. Burn treatments were performed by USFWS fire trained personnel within the defined windows for each treatment category according to USFWS prescribed burning protocol (Table 4.1), resulting in moderate ground char (Ryan and Noste 1985). Late fall burn treatments were administered in 2015, while all other burns occurred during 2016.

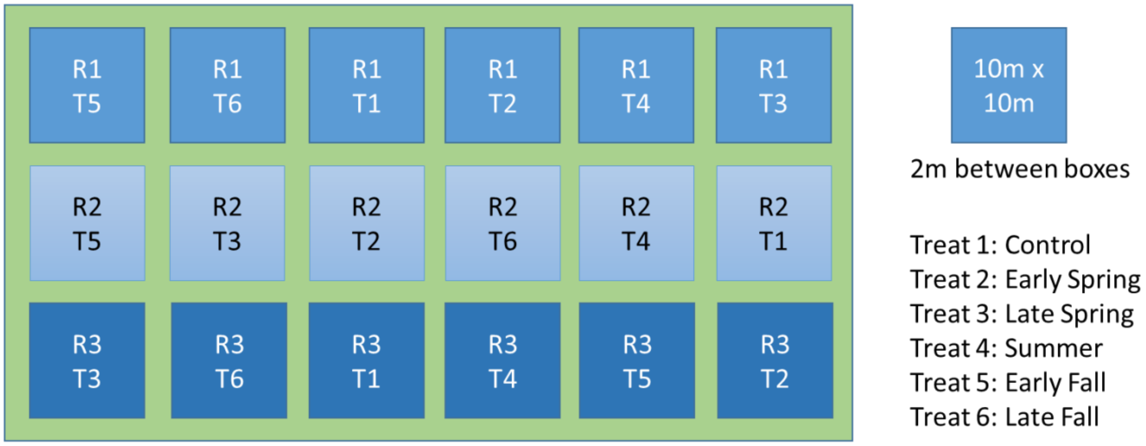

Figure 4.1. Thompson Waterfowl Production Area, Roscoe, SD, treatment plan.

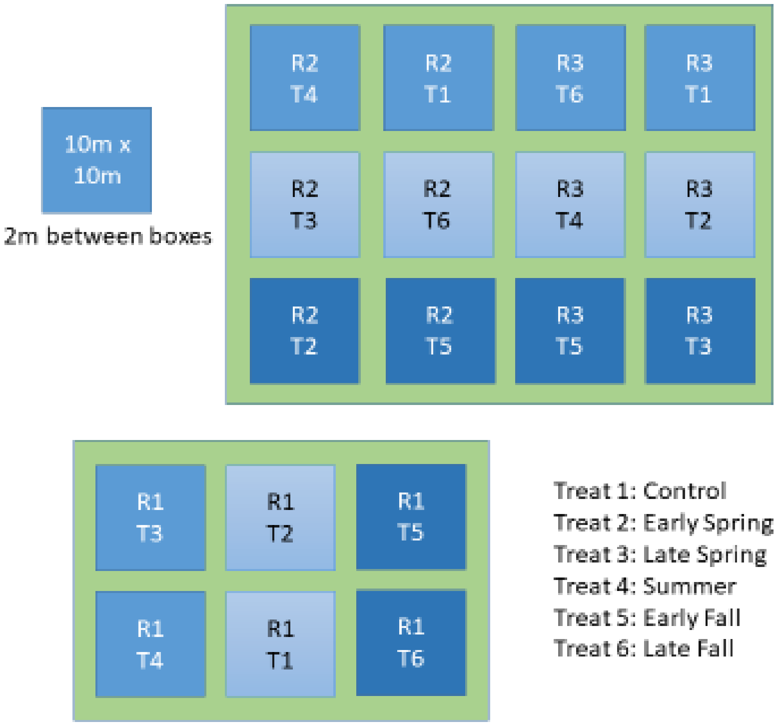

Figure 4.2. Arrowwood National Wildlife, Pingree, ND, treatment plan.

Table 4.1. US Fish and Wildlife Service burn protocol parameters for each site (USFWS 2015, 2017).

	Arrowwood	Thompson
Temperature, °C	4.4-37.2	4.4-32.2
Relative Humidity, %	15-70	20-60
Wind Speed (at 20 feet), mph	3-20	1-20

Pre-treatment sampling was performed within 24 to 48 hours prior to the burn treatment. Each plot was divided into four sections along bisecting diagonal lines from opposite corners. Five 20 cm X 50 cm quadrats were sampled within the plot using the phenological stage identification method (Moore et al. 1991). Placement of the 20 cm X 50 cm sample frames was determined by visually identifying smooth brome near the intersection of the two lines and within each of the four subplots, for a total of five sample frames. Within each of the five quadrats, smooth brome tillers rooted within the frame were identified and each tiller's phenological stage determined and recorded. The mean stage count for the entire plot was calculated by tallying the number of tillers at each phenological stage and multiplying by the corresponding index value (Moore et al. 1991) in the following equation:

$$\text{MSC} = (\sum \text{Index Values for each stage encountered})/\text{total sampled tillers.}$$

Accumulated growing degree days (AGDD) were calculated, beginning January 1 of each year and using the following equation (Akyuz and Ransom 2015):

$$\text{AGDD} = \sum[(\text{Maximum temperature} + \text{Minimum temperature})/2 - \text{Base temperature})].$$

Maximum and minimum temperatures for each site were determined using the Applied Climate Information System Query Builder (Regional Climate Centers 2016). GPS coordinates for both sites were entered into the query builder to create a list of maximum and minimum daily temperatures from Jan 1 through the end of each sample year. These maximum and minimum daily temperatures were then used to calculate the accumulated growing degree days with a base temperature of 0 °C, as described in Chapter 2.

Additionally, within each plot, two 20-cm diameter rings were placed at ground level in areas with smooth brome. The center of the circle was marked for post-treatment sampling with a steel washer and bolt. The number of smooth brome tillers rooted within each circle were counted and recorded to track survivorship rate. Tiller counts were repeated following the burn, at the end of the growing season and in the following spring. Pre-treatment and post-treatment tiller counts were compared and analyzed using one-way ANOVA (SAS Enterprise Guide Version 7.1, SAS Institute, Cary, NC), at a significance level of $\alpha = 0.05$, to determine if there was a significant change in the number or percent of tillers depending on treatment.

Results

MSC and AGDD were calculated to identify the phenologic development and seasonal timing just prior to the burn (Table 4.2). MSC was not calculated for the control plots because these plots were not burned. The differences between pre-burn and post burn tiller count and tiller percent were calculated for each plot, as described previously. Additionally, the percent difference was calculated for each plot (Table 4.3).

One-way ANOVA compared before and after treatment tiller counts and percent change between treatments. While the difference between end of season tiller counts and pre-burn tiller counts was not significant ($P = 0.3525$), the difference between tiller counts completed in the spring of the next growing season and pre-burn was significant ($P = 0.0024$, Figure 4.3). Additionally, the difference between the tiller counts completed in spring of the next growing season and the end of season were significantly different ($P = 0.0002$, Figure 4.4). Tukey’s studentized test was performed to determine which treatments were significantly different from one another, indicating that there were significantly more tillers following the summer burn compared to early spring, late spring, and late fall burns.

Table 4.2. Mean stage count and accumulated growing degree days at the time of pre-burn tiller counts and treatment.

Site Location	Replicate	Treatment	MSC	AGDD
Arrowwood	1	1 Control		--
Arrowwood	1	2 Early Spring	1.10	522
Arrowwood	1	3 Late Spring	2.43	1460
Arrowwood	1	4 Summer	2.61	3476
Arrowwood	1	5 Early Fall	2.06	4468
Arrowwood	1	6 Late Fall	1.31	5864
Arrowwood	2	1 Control		--
Arrowwood	2	2 Early Spring	1.14	522
Arrowwood	2	3 Late Spring	2.29	1460
Arrowwood	2	4 Summer	2.40	3476
Arrowwood	2	5 Early Fall	2.05	4468
Arrowwood	2	6 Late Fall	1.88	5864
Arrowwood	3	1 Control		--
Arrowwood	3	2 Early Spring	1.14	522
Arrowwood	3	3 Late Spring	2.52	1460
Arrowwood	3	4 Summer	2.37	3476
Arrowwood	3	5 Early Fall	1.92	4468
Arrowwood	3	6 Late Fall	1.64	5864
Thompson	1	1 Control		--
Thompson	1	2 Early Spring	1.36	848
Thompson	1	3 Late Spring	1.78	1168
Thompson	1	4 Summer	2.55	3412
Thompson	1	5 Early Fall	2.26	5463
Thompson	1	6 Late Fall	1.64	6127
Thompson	2	1 Control		--
Thompson	2	2 Early Spring	1.39	848
Thompson	2	3 Late Spring	2.14	1168
Thompson	2	4 Summer	2.64	3412
Thompson	2	5 Early Fall	2.07	5463
Thompson	2	6 Late Fall	1.73	6127
Thompson	3	1 Control		--
Thompson	3	2 Early Spring	1.40	848
Thompson	3	3 Late Spring	1.93	1168
Thompson	3	4 Summer	2.52	3412
Thompson	3	5 Early Fall	2.10	5463
Thompson	3	6 Late Fall	1.78	6127

Table 4.3. Differences in the number of tillers before treatment and after treatment, by number of tillers and percentage of tillers.

Site Location	Replicate	Treatment	Diff1[a]	%Diff1	Diff2[b]	%Diff2	Diff3[c]	%Diff3
Arrowwood	1	1 Control	14	53.8	5	19.2	-9	-22.5
Arrowwood	1	2 Early Spring	10	71.4	4	28.6	-6	-25.0
Arrowwood	1	3 Late Spring	9	22.5	-2	-5.0	-11	-22.4
Arrowwood	1	4 Summer	2	5.0	30	75.0	28	66.7
Arrowwood	1	5 Early Fall	-2	-5.7	-2	-5.7	0	0.0
Arrowwood	1	6 Late Fall	--	--	--	--	-31	-44.3
Arrowwood	2	1 Control	5	55.6	6	66.7	1	7.1
Arrowwood	2	2 Early Spring	8	61.5	9	69.2	1	4.8
Arrowwood	2	3 Late Spring	-10	-23.8	4	9.5	14	43.8
Arrowwood	2	4 Summer	9	22.0	23	56.1	14	28.0
Arrowwood	2	5 Early Fall	-10	-24.4	8	19.5	18	58.1
Arrowwood	2	6 Late Fall	--	--	--	--	-24	-48.0
Arrowwood	3	1 Control	10	90.9	27	245.5	17	81.0
Arrowwood	3	2 Early Spring	2	10.5	-8	-42.1	-10	-47.6
Arrowwood	3	3 Late Spring	12	109.1	8	72.7	-4	-17.4
Arrowwood	3	4 Summer	25	83.3	50	166.7	25	45.5
Arrowwood	3	5 Early Fall	14	233.3	23	383.3	9	45.0
Arrowwood	3	6 Late Fall	--	--	--	--	-20	-23.3
Thompson	1	1 Control	-2	-15.4	7	53.8	9	81.8
Thompson	1	2 Early Spring	-8	-32.0	-2	-8.0	6	35.3
Thompson	1	3 Late Spring	-8	-26.7	-3	-10.0	5	22.7
Thompson	1	4 Summer	9	25.0	15	41.7	6	13.3
Thompson	1	5 Early Fall	-3	-9.7	9	29.0	12	42.9
Thompson	1	6 Late Fall	-1	-5.9	-11	-64.7	-10	-62.5
Thompson	2	1 Control	-4	-10.0	-9	-22.5	-5	-13.9
Thompson	2	2 Early Spring	-12	-26.1	-12	-26.1	0	0.0
Thompson	2	3 Late Spring	9	39.1	7	30.4	-2	-6.3
Thompson	2	4 Summer	2	7.1	8	28.6	6	20.0
Thompson	2	5 Early Fall	1	4.0	9	36.0	8	30.8
Thompson	2	6 Late Fall	1	2.9	-4	-11.4	-5	-13.9
Thompson	3	1 Control	2	8.0	11	44.0	9	33.3
Thompson	3	2 Early Spring	4	18.2	12	54.5	8	30.8
Thompson	3	3 Late Spring	13	34.2	3	7.9	-10	-19.6
Thompson	3	4 Summer	7	29.2	19	79.2	12	38.7
Thompson	3	5 Early Fall	-5	-11.4	30	68.2	35	89.7
Thompson	3	6 Late Fall	-3	-8.3	-2	-5.6	1	3.0

[a] *Diff1 is the difference between end of season and pre-burn.*

[b] *Diff2 is the difference between the following spring and pre-burn.*

[c] *Diff3 is the difference between the following season and end of season.*

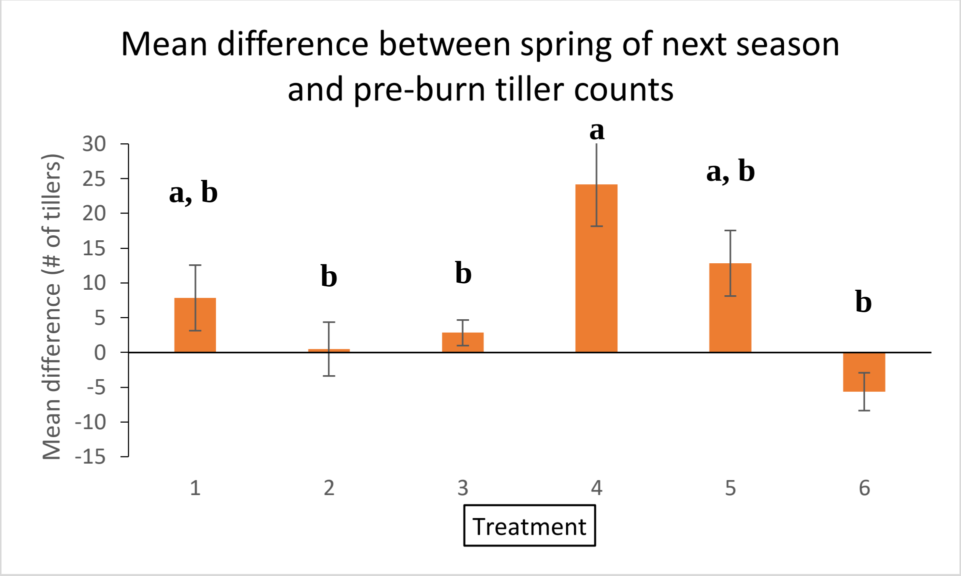

Figure 4.3. Mean difference between spring of next season and pre-burn tiller counts. Letters indicate significant differences ($P < 0.05$). Treatments: (1) Control, (2) Early Spring, (3) Late Spring, (4) Summer, (5) Early Fall, and (6) Late Fall.

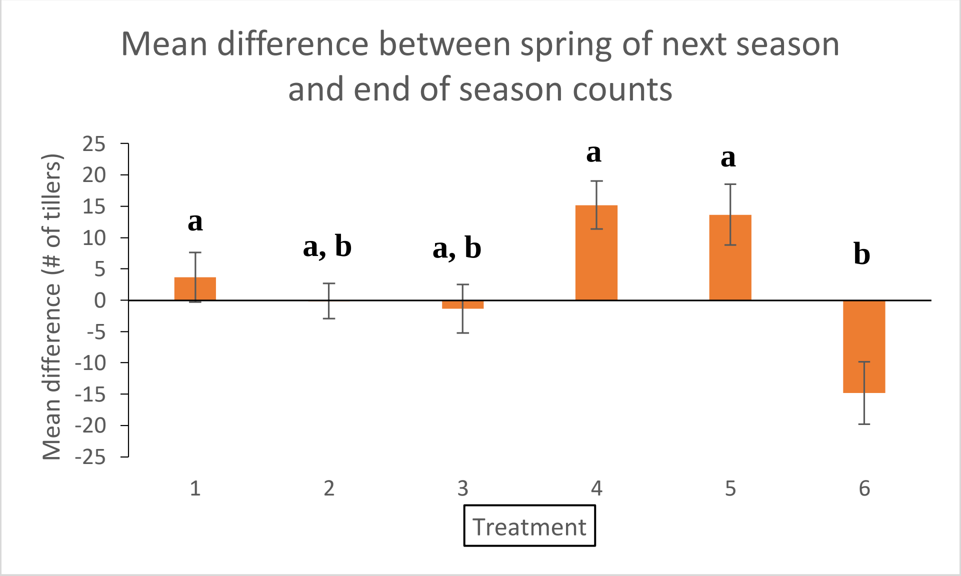

Figure 4.4. Mean difference between spring of next season and end of season counts. Letters indicate significant differences ($P < 0.05$). Treatments: (1) Control, (2) Early Spring, (3) Late Spring, (4) Summer, (5) Early Fall, and (6) Late Fall.

One-way ANOVA applied to percent difference determined the treatment effect on percent difference between the end of season and pre-burn tiller counts or the spring of next season and pre-burn tiller counts were not significant (*P* = 0.9553, *P* = 0.2596, respectively). Treatment effect on the percent difference between spring of next season and end of season tiller counts was found to be significant (*P* = 0.0017). Tukey's studentized test determined that there was a significant difference between late fall burn and early fall burn, late fall burn and summer burn, and late fall burn and the control (Figure 4.5).

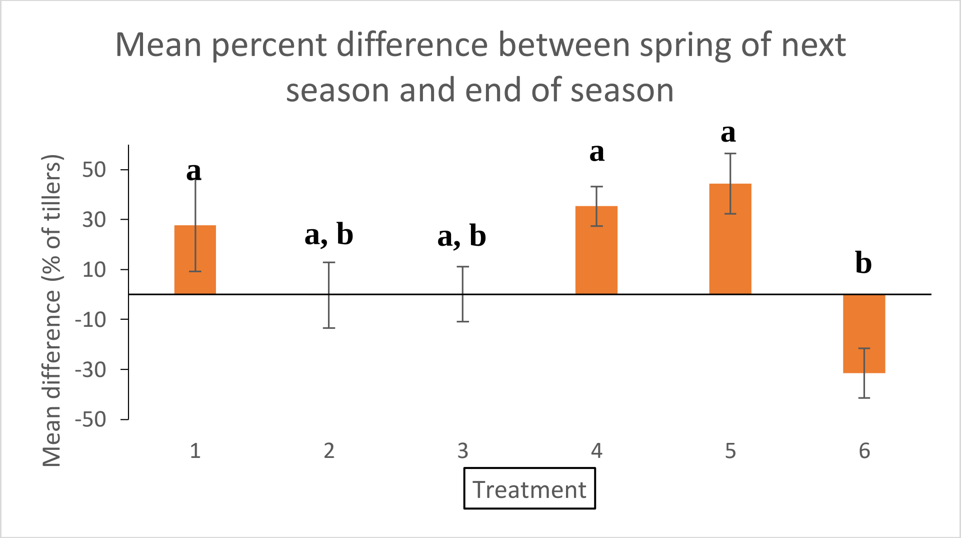

Figure 4.5. Mean percent difference between spring of next season and end of season counts. Letters indicate significant differences (P < 0.05). Treatments: (1) Control, (2) Early Spring, (3) Late Spring, (4) Summer, (5) Early Fall, and (6) Late Fall.

Additionally, one-way ANOVA was applied to the same data sets, separating the data by sites (Table 4.4). At Arrowwood, treatment effect on the difference in percent tillers was not significant when comparing the end of season with pre-burn percentages (*P* = 0.9688) or spring of the next season with pre-burn percentages (*P* = 0.6877). However, treatment effect on the difference between spring of next year and end of season percentage did show significance (*P* = 0.0491). In contrast, at Thompson, none of the change in percentages showed any significant treatment effect. Comparison of the two sites with regards to percent difference in tiller count did show a significant location effect between the two sites when only the site was considered.

Table 4.4. Summary of treatment effect on percent difference for individual sites, corresponding p-value within the sites, and p-value between the sites.

	Arrowwood						
Trt:	Control	Early spring burn	Late spring burn	Summer Burn	Early fall burn	Late fall burn	Within site p-value
%Diff1	66.77	47.83	35.93	36.76	67.74	--	0.9688
%Diff2	110.45	18.57	25.75	99.25	132.38	--	0.6877
%Diff3	21.87	-22.62	1.30	46.71	34.35	-38.51	0.0491
	Thompson						
Trt:	Control	Early spring burn	Late spring burn	Summer Burn	Early fall burn	Late fall burn	Within site p-value
%Diff1	-5.79	-13.30	15.56	20.44	-5.68	-3.79	0.3221
%Diff2	25.12	6.82	9.44	49.80	44.40	-27.23	0.1038
%Diff3	33.75	22.02	-1.04	24.01	54.46	-24.45	0.0883
	Across site p-value						
%Diff1	0.0046						
%Diff2	0.0414						
%Diff3	0.4051						

Discussion

Calculating MSC and AGDD provides information about the phenological development of smooth brome at the time of the burn. Treatments were defined by day of the year but determining the effectiveness of the treatment based on AGDD would eliminate some uncertainty with regards to climatic variability. For example, the early burn treatment was defined in this study as up to and including May 15th. However, other studies have defined early burn periods as those prior to the start of elongation (Willson and Stubbendieck 1997). Based on the conclusions of Chapter 2, the early spring burn treatment window would be up to 945 AGDD and late spring burn treatment would be from 946 AGDD and 1566 AGDD. Using these parameters, treatment windows could be calculated using the AGDD for each of the corresponding dates in the study. Additional years' AGDD data could be used to determine an average AGDD for those dates over several years and recommend treatment windows that account for seasonal variability.

With the exception of treatment 6, treatments were applied in the growing season of 2016. Treatment 6 was applied in late fall of 2015 with little growth occurring after burning. All treatments were analyzed together at the end of 2016 since the majority of the growth occurred during the 2016 growing season. Because the recovery time for 2016 post treatment is varied depending on the timing of the treatment, we also surveyed the recovery growth in the spring of 2017 to determine if there was a difference between treatment response at that time. The late fall burn showed the greatest decrease in smooth brome during the spring 2017 survey but it is difficult to determine if this result is due to the treatment or due to the fact this sample had been subjected to two winters following the treatment. Additionally, this study focused on a single growing season but it is possible an initial decrease in smooth brome after a single year would be followed by resurgence in following years (Willson and Stubbendieck 1997). To better assess the effectiveness of the treatments, we recommend that the surveys be repeated after additional growing seasons.

10 x 10 meter plot size was chosen as suitable size to allow the fire to burn thoroughly but still be manageable for a small fire crew. There are numerous studies using a range of plot sizes for burn studies: 3 x 6 meters (Smart et al. 2013), 6 X 6 meters (Ohrtmann et al. 2015), 20 x 20 meters (Strong et al. 2013), 30 x 30 meters (Willson and Stubbendieck 1997), for example. Fires were able to burn hot enough to result in a moderate ground char (Ryan and Noste 1985) based on visual inspection but the temperature, duration, and intensity of the fire were not monitored. Because the vegetation was burned to bare ground, we can assume that the lethal temperature of >60 °C (Whelan 1995) was met but how much hotter it burned cannot be determined. We cannot definitively state that the fire burned hot enough to damage the crown tissue or seeds below the soil surface. We recommend future research employ thermocouples to

measure temperature and duration of the fire (Ohrtman et al 2015), confirming the intensity of the fire.

Although there were significant differences between treatments, the late fall burn was the only treatment that showed a decline in the overall number of smooth brome tillers. Tiller production is most abundant during the period after initial flowering, from late June through the fall (Lamp 1952). If burn treatments are applied in late fall, there is potential to damage those tillers that will become active again in the spring, both by the fire itself and by winter kill due to exposure to harsh temperature (Walton 1980).

While this study was designed to observe only the tiller survival of the smooth brome, it is interesting to note that there were some distinct anecdotal morphological observations. At the Thompson site, the late fall burn plots had noticeably more inflorescence the following growing season that any of the other plots. This suggests that the burn encouraged sexual reproduction in those plants that survived the treatment, either via surviving crown tissue or seeds, possibly the result of volatilization and consequent decrease in nitrogen, leading to fewer sterile tillers (Harrison and Crawford 1941). In this study, we only considered the MSC and AGDD prior to the burn and did not follow morphological development of regrowth. The question to be considered by future research is whether burn time affects the timing or type of morphological development of the regrowth.

This method only considers the effect that the treatments have on smooth brome survivorship. It does not take other plants into consideration. It would be beneficial to complete an inventory of the sites and determine the survivorship of all species, native and non-native, following each burn treatment. Control of smooth brome can result in expansion of another invasive species like Kentucky bluegrass (Grant et al. 2009), so care needs to be exercised in order to balance the control of both invasive grasses. Additionally, the recommended timing of burns should not interfere with wildlife during critical times in their life cycles, such as nesting and hatching, in order to limit the impact on resident populations. There is a delicate balance between preserving the native prairie, providing biodiversity and habitat, and maintaining wildlife populations in these habitats.

Conclusion

Based on this preliminary study, the widely used spring burn following the initiation of elongation is not the only time that a burn treatment will result in a decline in smooth brome. A late fall burn could adversely affect smooth brome as well. Additional research is recommended, both to confirm these results and to consider the impact it has on other species.

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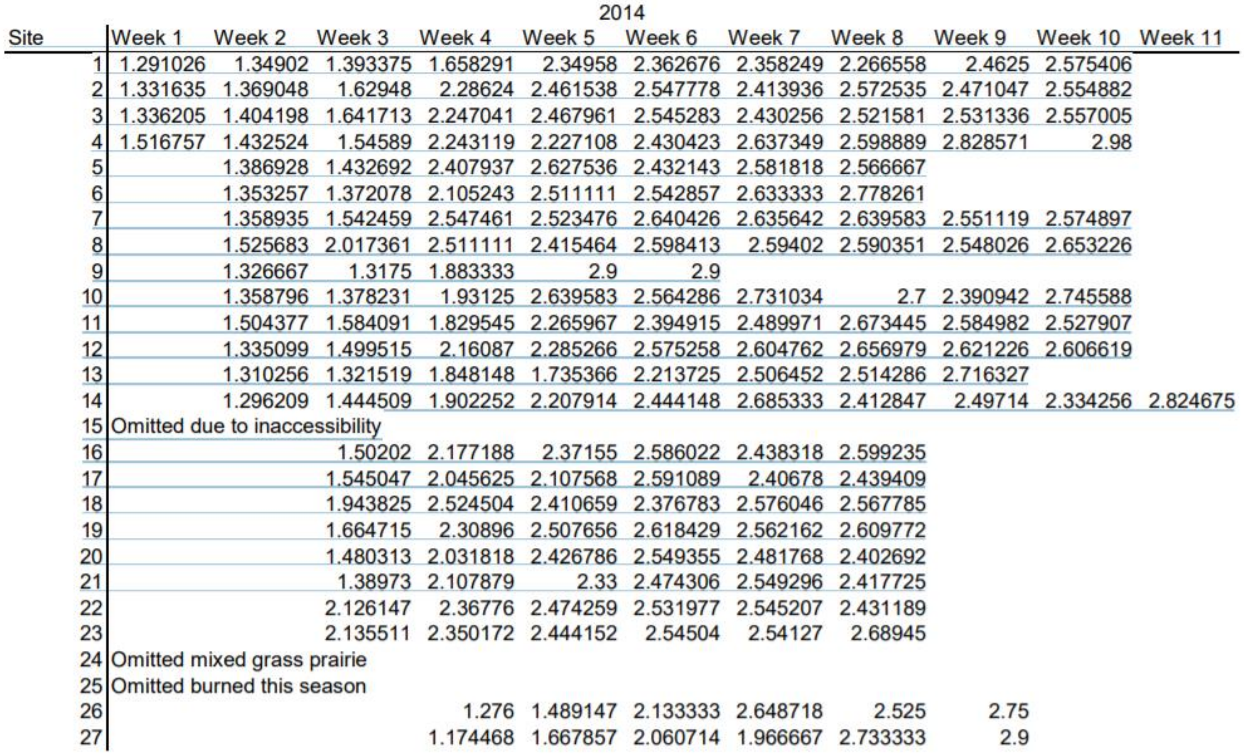
APPENDIX A. LIST OF 2014/2015 TRANSECT SITES

Transect #	Transect Name	County	State	Managed by
1	Tewaukon/Wyum A	Sargent	ND	USFWS: Tewaukon NWR
2	Tewaukon/Wyum B	Sargent	ND	USFWS: Tewaukon NWR
3	Tewaukon/Wyum C	Sargent	ND	USFWS: Tewaukon NWR
4	Ekre/NDAWN	Richland	ND	NDSU
5	Sheyenne National Grasslands A	Ransom	ND	USFS: Lisbon
6	Sheyenne National Grasslands B	Ransom	ND	USFS: Lisbon
7	Tewaukon/Pool 4 A	Sargent	ND	USFWS: Tewaukon NWR
8	Tewaukon/Pool 4 B	Sargent	ND	USFWS: Tewaukon NWR
9	Hartleben C A	Richland	ND	USFWS: Tewaukon NWR
10	Hartleben C B	Richland	ND	USFWS: Tewaukon NWR
11	Helliksen	Becker	MN	USFWS: Detroit Lakes
12	Marks	Becker	MN	USFWS: Detroit Lakes
13	Mekinock	Grand Forks	ND	USFWS: Devils Lake
14	Pembina Prairie	Pembina	ND	USFWS: Devils Lake
16	Hepner	Miner	SD	USFWS
17	Wolfe A	Lake	SD	USFWS
18	Wolfe B	Lake	SD	USFWS
19	Sanderson	Spink	SD	USFWS
20	Overland	Covington	SD	USFWS
21	Gerber	Marshall	SD	USFWS
22	Big Stone NWR	Big Stone	MN	USFWS: Big Stone NWR
23	Tympanuchus WMA	Polk	MN	MN DNR
26	Bluestem Prairie West A	Clay	MN	The Nature Conservancy
27	Bluestem Prairie West B	Clay	MN	The Nature Conservancy

APPENDIX B. MEAN STAGE COUNT FOR EACH SITE DURING 2014 AND 2015

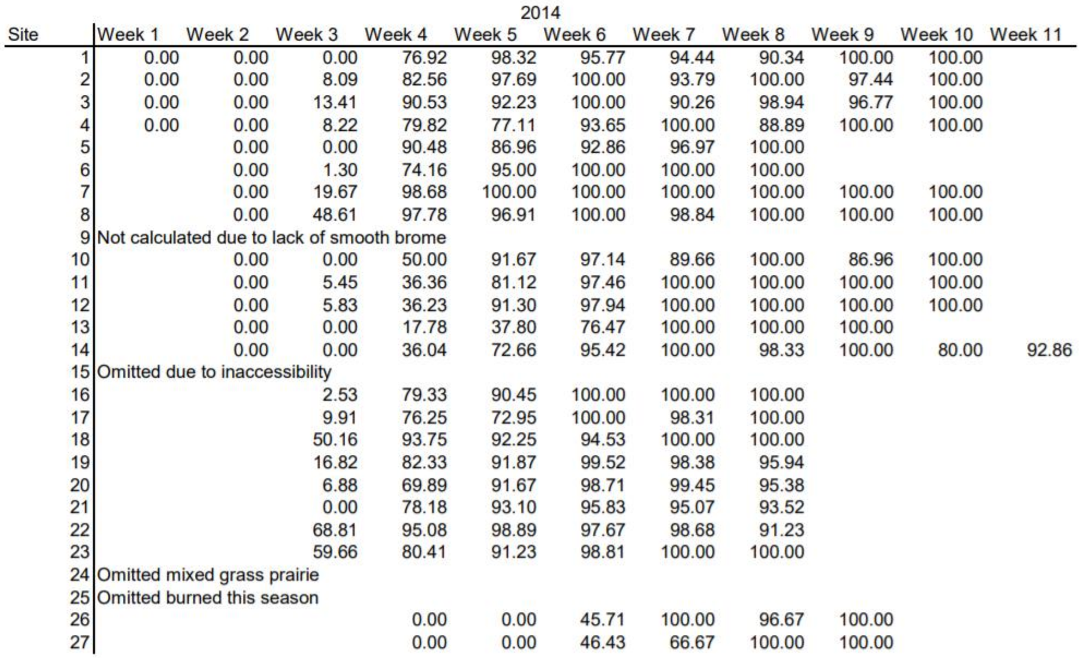


2014

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11
1	1.291026	1.34902	1.393375	1.658291	2.34958	2.362676	2.358249	2.266558	2.4625	2.575406	
2	1.331635	1.369048	1.62948	2.28624	2.461538	2.547778	2.413936	2.572535	2.471047	2.554882	
3	1.336205	1.404198	1.641713	2.247041	2.467961	2.545283	2.430256	2.521581	2.531336	2.557005	
4	1.516757	1.432524	1.54589	2.243119	2.227108	2.430423	2.637349	2.598889	2.828571	2.98	
5		1.386928	1.432692	2.407937	2.627536	2.432143	2.581818	2.566667			
6		1.353257	1.372078	2.105243	2.511111	2.542857	2.633333	2.778261			
7		1.358935	1.542459	2.547461	2.523476	2.640426	2.635642	2.639583	2.551119	2.574897	
8		1.525683	2.017361	2.511111	2.415464	2.598413	2.59402	2.590351	2.548026	2.653226	
9		1.326667	1.3175	1.883333	2.9	2.9					
10		1.358796	1.378231	1.93125	2.639583	2.564286	2.731034	2.7	2.390942	2.745588	
11		1.504377	1.584091	1.829545	2.265967	2.394915	2.489971	2.673445	2.584982	2.527907	
12		1.335099	1.499515	2.16087	2.285266	2.575258	2.604762	2.656979	2.621226	2.606619	
13		1.310256	1.321519	1.848148	1.735366	2.213725	2.506452	2.514286	2.716327		
14		1.296209	1.444509	1.902252	2.207914	2.444148	2.685333	2.412847	2.49714	2.334256	2.824675
15	Omitted due to inaccessibility										
16			1.50202	2.177188	2.37155	2.586022	2.438318	2.599235			
17			1.545047	2.045625	2.107568	2.591089	2.40678	2.439409			
18			1.943825	2.524504	2.410659	2.376783	2.576046	2.567785			
19			1.664715	2.30896	2.507656	2.618429	2.562162	2.609772			
20			1.480313	2.031818	2.426786	2.549355	2.481768	2.402692			
21			1.38973	2.107879	2.33	2.474306	2.549296	2.417725			
22			2.126147	2.36776	2.474259	2.531977	2.545207	2.431189			
23			2.135511	2.350172	2.444152	2.54504	2.54127	2.68945			
24	Omitted mixed grass prairie										
25	Omitted burned this season										
26				1.276	1.489147	2.133333	2.648718	2.525	2.75		
27				1.174468	1.667857	2.060714	1.966667	2.733333	2.9		

	2015							
Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1			1.446679	2.015863	2.021	2.297205	2.269419	2.356425
2			1.566122	2.030311	2.430816	2.40051	2.463318	2.414212
3			1.516495	2.28019	2.236604	2.374035	2.433665	2.432671
4		1.366864	1.579213	2.274772	2.32298	2.482456	2.421205	2.401764
5			1.469892	2.005	2.423611	2.580645	2.560476	2.521118
6			1.361364	1.532686	2.427778	2.445455	2.597143	2.36875
7		1.480435	1.709545	1.987662	2.324429	2.336125	2.442583	2.236885
8		1.646748	1.780793	2.229688	2.30942	2.451471	2.47443	2.429124
9	Omitted due to lack of smooth brome							
10			1.519063	2.026961	2.389091	2.303101	2.546092	2.314241
11		1.341623	1.452431	1.904501	2.426823	2.483721	2.412429	2.48961
12	Omitted due to burning							
13	1.273293	1.363492	1.42335	1.83107	2.162242	2.327985	2.329332	2.483193
14	1.209821	1.393955	1.471667	1.65131	2.114762	2.45	2.461111	2.485271
15								
16	Omitted due to proximity to wetland							
17		1.48	2.006593	1.933235	2.074503	2.14497	2.196386	2.086258
18		1.382034	1.742191	1.820149	2.184813	2.361643	2.180935	2.014356
19	Omitted due to grazing							
20			1.44308	1.835078	1.998667	2.338288	2.379048	2.362262
21			1.372098	1.523474	1.9196	2.144615	2.279242	2.164091
22	Omitted due to grazing							
23	1.459971	1.396359	1.403822	1.909792	2.153535	2.148601	2.293919	2.386791
24								
25								
26	Omitted due to lack of smooth brome							
27	Omitted due to lack of smooth brome							

APPENDIX C. PERCENTAGE OF TILLERS IN ELONGATION STAGE OR HIGHER AT EACH SITE FOR 2014 AND 2015



	2014										
Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11
1	0.00	0.00	0.00	76.92	98.32	95.77	94.44	90.34	100.00	100.00	
2	0.00	0.00	8.09	82.56	97.69	100.00	93.79	100.00	97.44	100.00	
3	0.00	0.00	13.41	90.53	92.23	100.00	90.26	98.94	96.77	100.00	
4	0.00	0.00	8.22	79.82	77.11	93.65	100.00	88.89	100.00	100.00	
5		0.00	0.00	90.48	86.96	92.86	96.97	100.00			
6		0.00	1.30	74.16	95.00	100.00	100.00	100.00			
7		0.00	19.67	98.68	100.00	100.00	100.00	100.00	100.00	100.00	
8		0.00	48.61	97.78	96.91	100.00	98.84	100.00	100.00	100.00	
9	Not calculated due to lack of smooth brome										
10		0.00	0.00	50.00	91.67	97.14	89.66	100.00	86.96	100.00	
11		0.00	5.45	36.36	81.12	97.46	100.00	100.00	100.00	100.00	
12		0.00	5.83	36.23	91.30	97.94	100.00	100.00	100.00	100.00	
13		0.00	0.00	17.78	37.80	76.47	100.00	100.00	100.00		
14		0.00	0.00	36.04	72.66	95.42	100.00	98.33	100.00	80.00	92.86
15	Omitted due to inaccessibility										
16			2.53	79.33	90.45	100.00	100.00	100.00			
17			9.91	76.25	72.95	100.00	98.31	100.00			
18			50.16	93.75	92.25	94.53	100.00	100.00			
19			16.82	82.33	91.87	99.52	98.38	95.94			
20			6.88	69.89	91.67	98.71	99.45	95.38			
21			0.00	78.18	93.10	95.83	95.07	93.52			
22			68.81	95.08	98.89	97.67	98.68	91.23			
23			59.66	80.41	91.23	98.81	100.00	100.00			
24	Omitted mixed grass prairie										
25	Omitted burned this season										
26				0.00	0.00	45.71	100.00	96.67	100.00		
27				0.00	0.00	46.43	66.67	100.00	100.00		

Site	2015							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1			21.34	57.14	83.92	93.98	94.90	95.54
2			11.43	73.06	91.84	94.90	97.26	95.90
3			11.34	84.00	84.11	92.11	96.52	98.01
4		3.55	24.72	80.82	92.42	97.89	98.21	87.65
5			7.53	61.25	93.33	96.77	98.57	95.65
6			0.00	23.30	91.67	100.00	100.00	92.50
7		10.14	34.55	50.65	89.50	89.16	92.15	84.70
8		30.08	35.98	83.33	84.78	91.18	95.80	90.72
9	Omitted due to lack of smooth brome							
10			21.88	68.63	87.27	79.07	98.11	87.34
11		0.00	9.03	51.09	90.63	98.84	92.37	93.18
12	Omitted due to burning							
13	0.00	0.00	0.00	29.01	67.26	91.79	97.04	94.12
14	0.00	0.00	2.08	28.82	67.43	92.74	95.65	93.02
15								
16	Omitted due to proximity to wetland							
17		22.04	56.59	60.59	72.85	72.78	83.73	76.09
18		6.78	29.72	48.88	77.57	89.95	86.59	67.75
19	Omitted due to grazing							
20			4.02	39.15	66.80	86.94	92.86	93.00
21			0.00	13.62	56.00	80.51	88.18	79.09
22	Omitted due to grazing							
23	0.00	3.40	14.01	38.13	58.18	82.52	89.19	91.07
24								
25								
26	Omitted due to lack of smooth brome							
27	Omitted due to lack of smooth brome							

APPENDIX D. LINEAR REGRESSION ANALYSIS OF ACCUMULATED GROWING DEGREE DAYS AND MEAN STAGE COUNT AND PERCENTAGE OF TILLERS AT THE ELONGATION PHASE OR HIGHER FOR 2014 AND 2015

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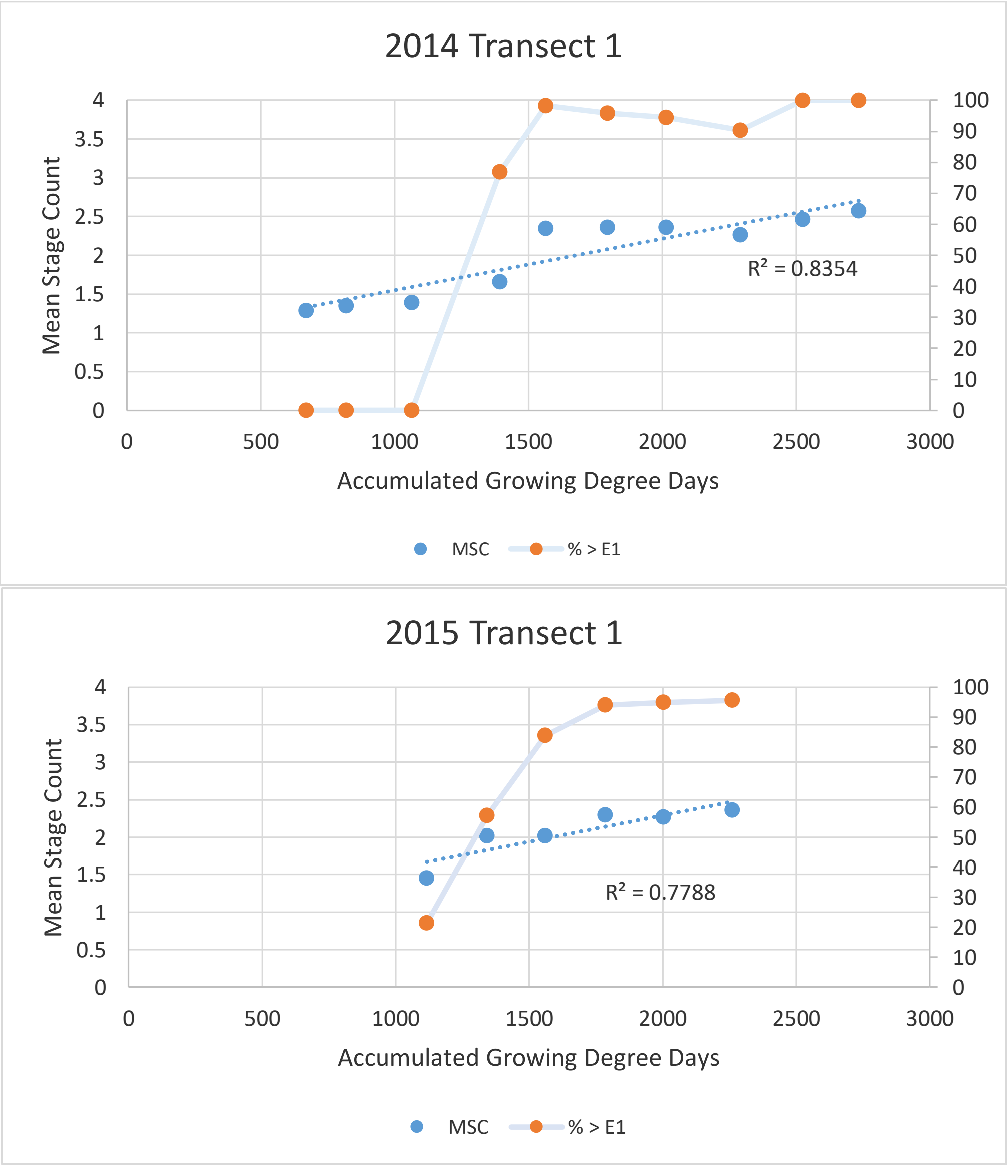
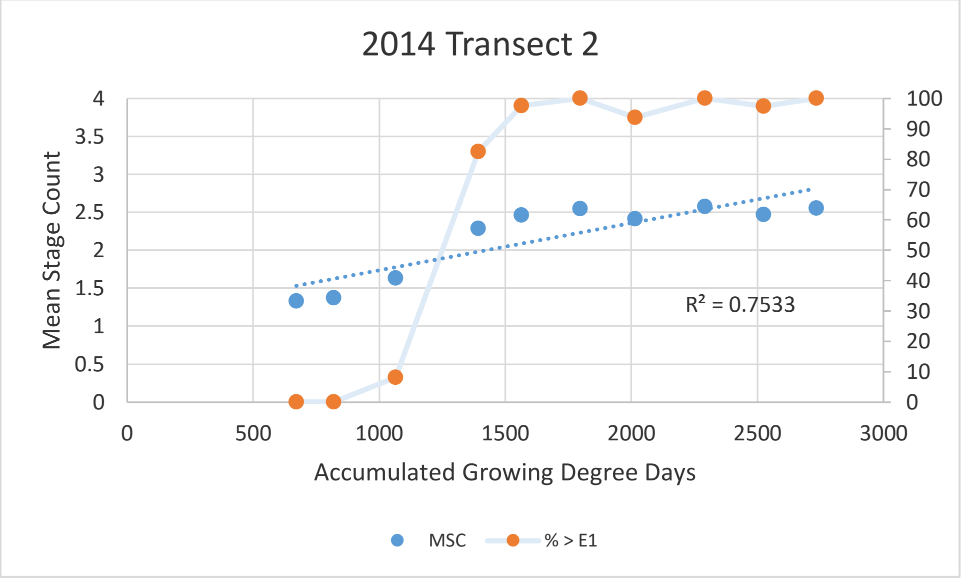


Figure D1. Transect 1, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.



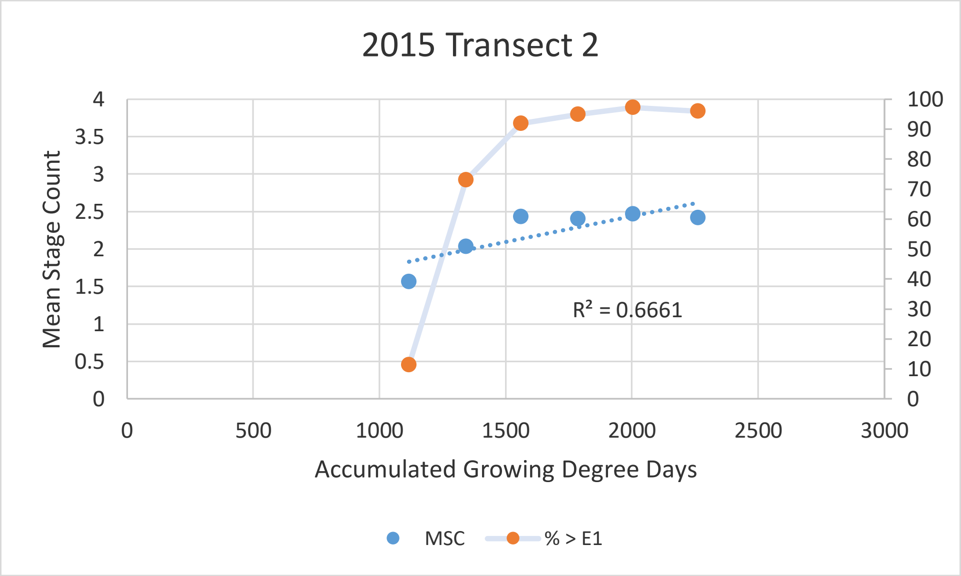


Figure D2. Transect 2, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

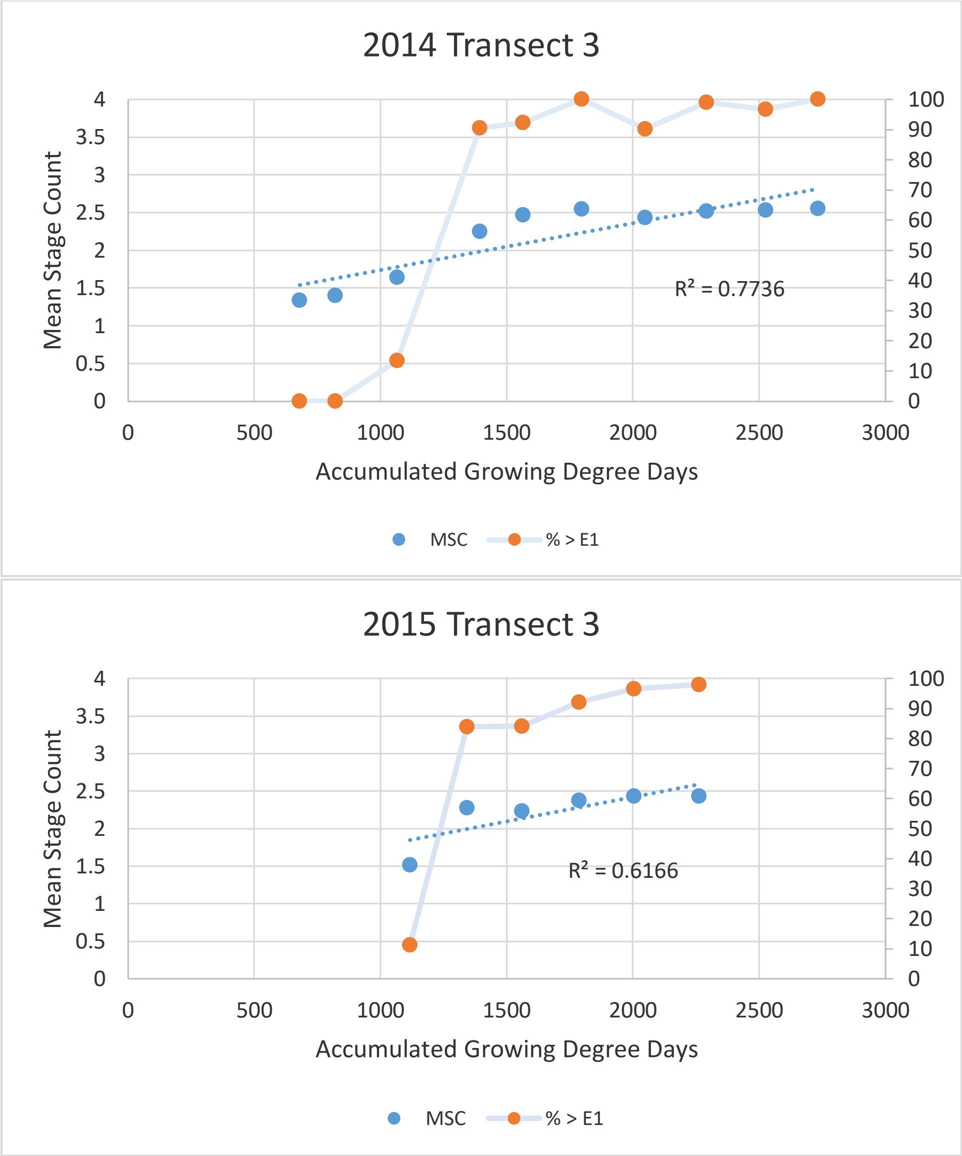


Figure D3. Transect 3, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

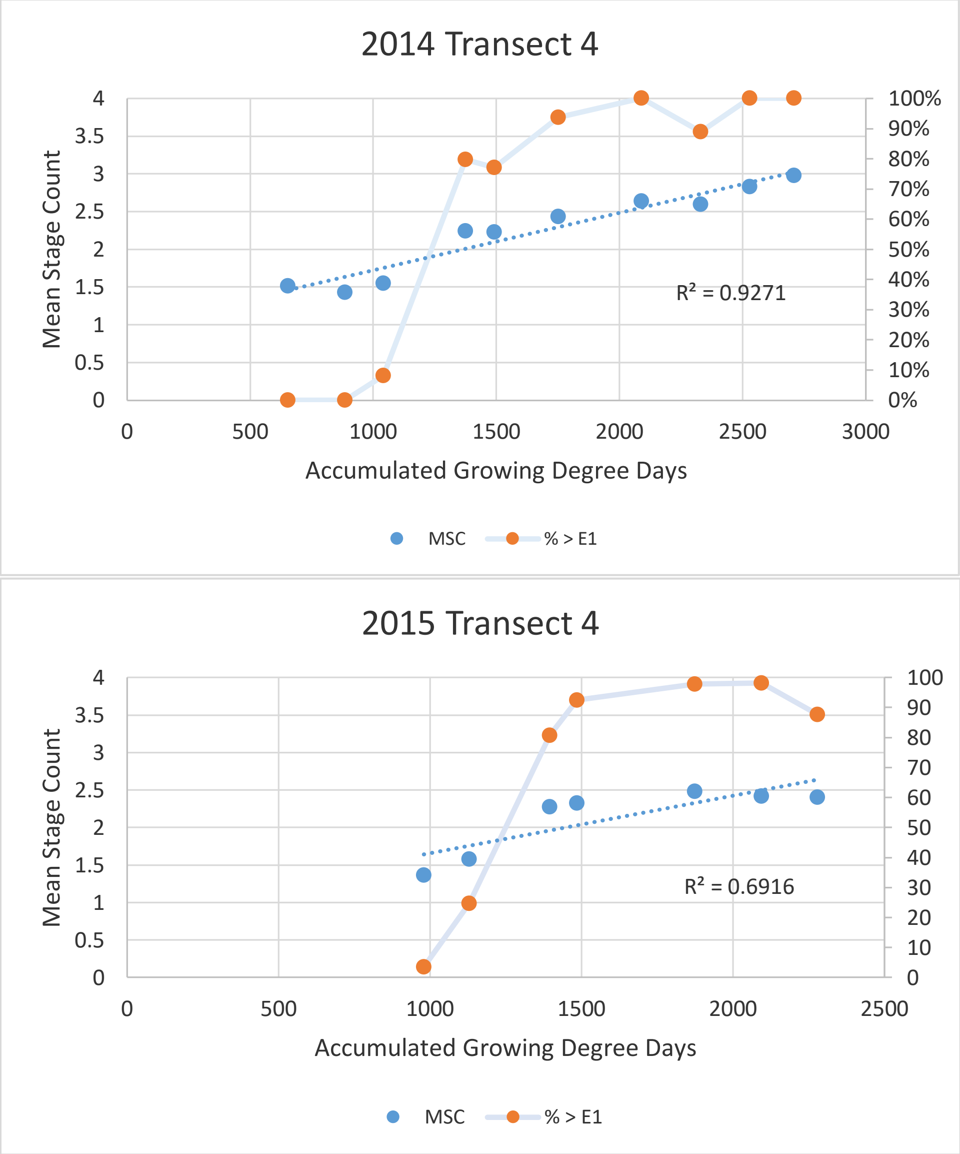


Figure D4. Transect 4, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

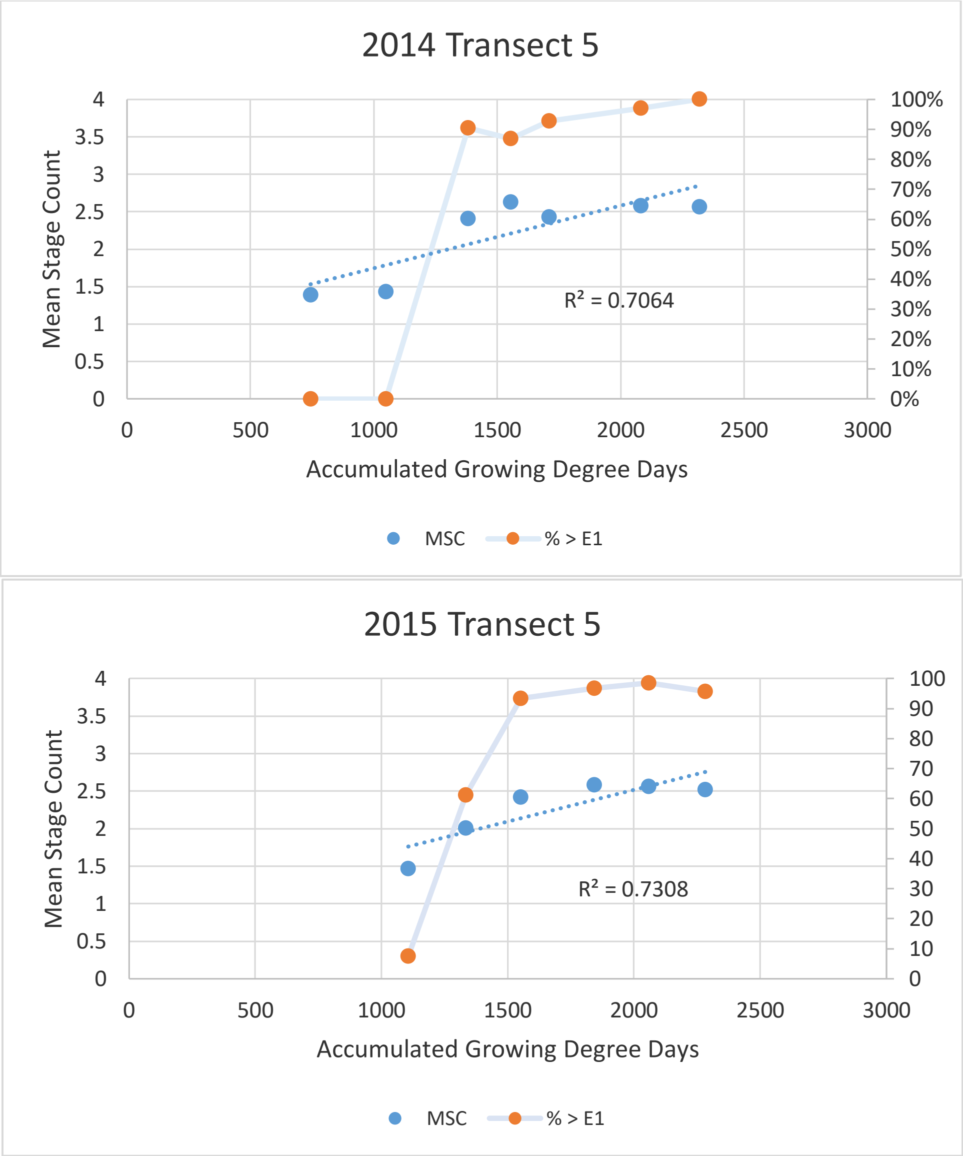
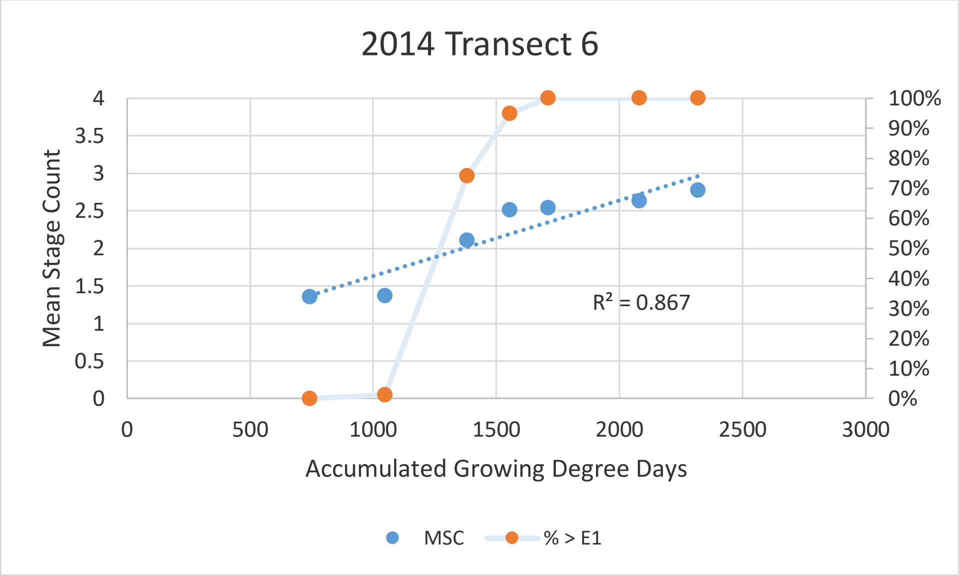


Figure D5. Transect 5, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.



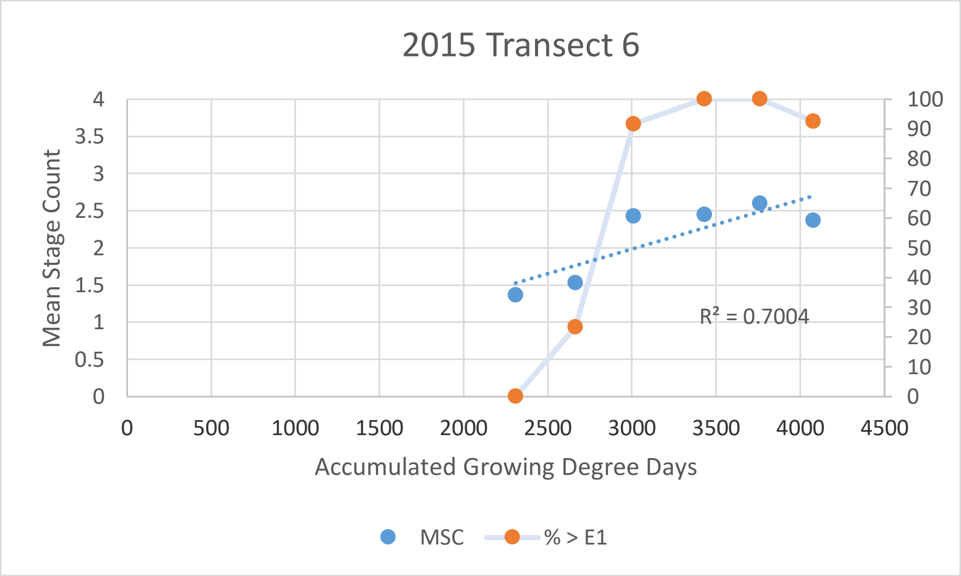


Figure D6. Transect 6, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

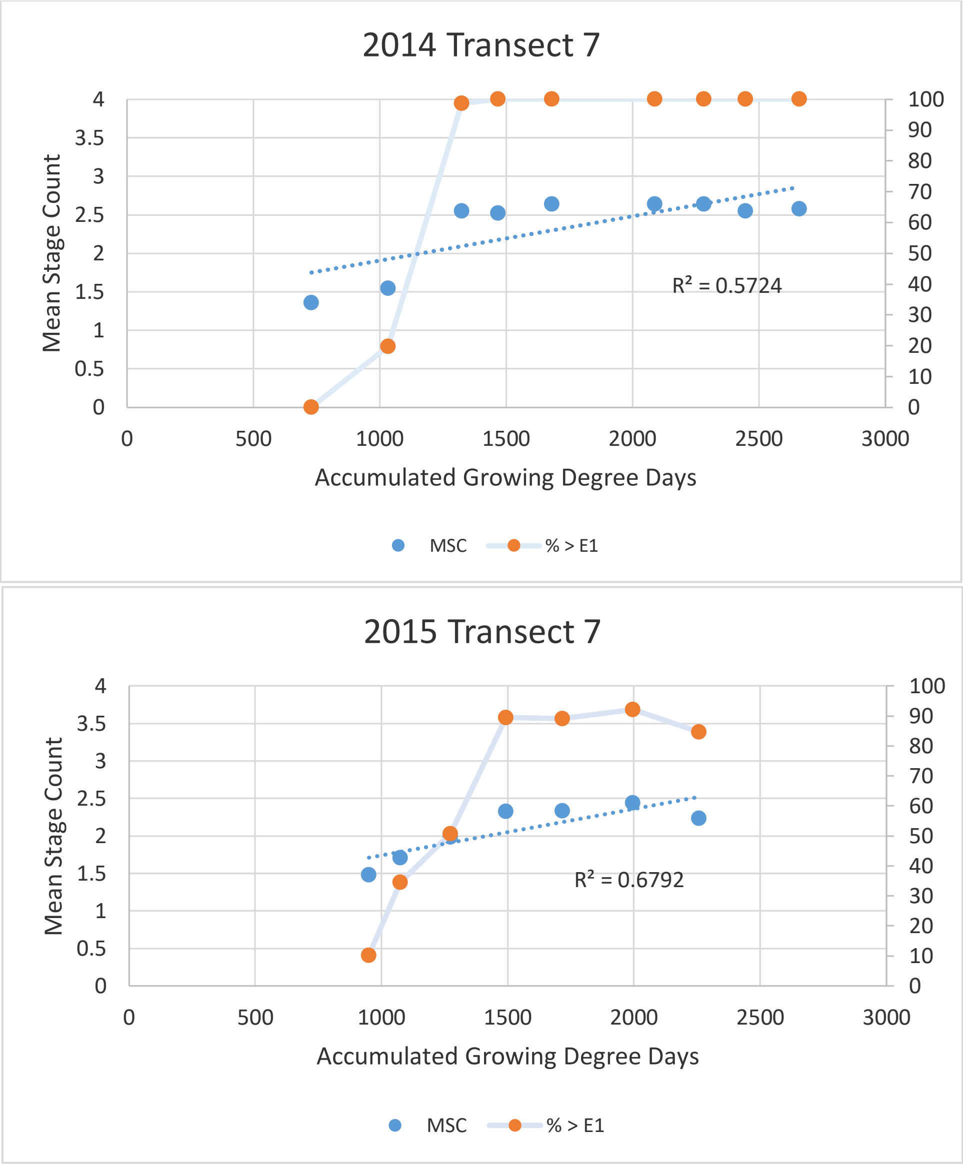


Figure D7. Transect 7, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

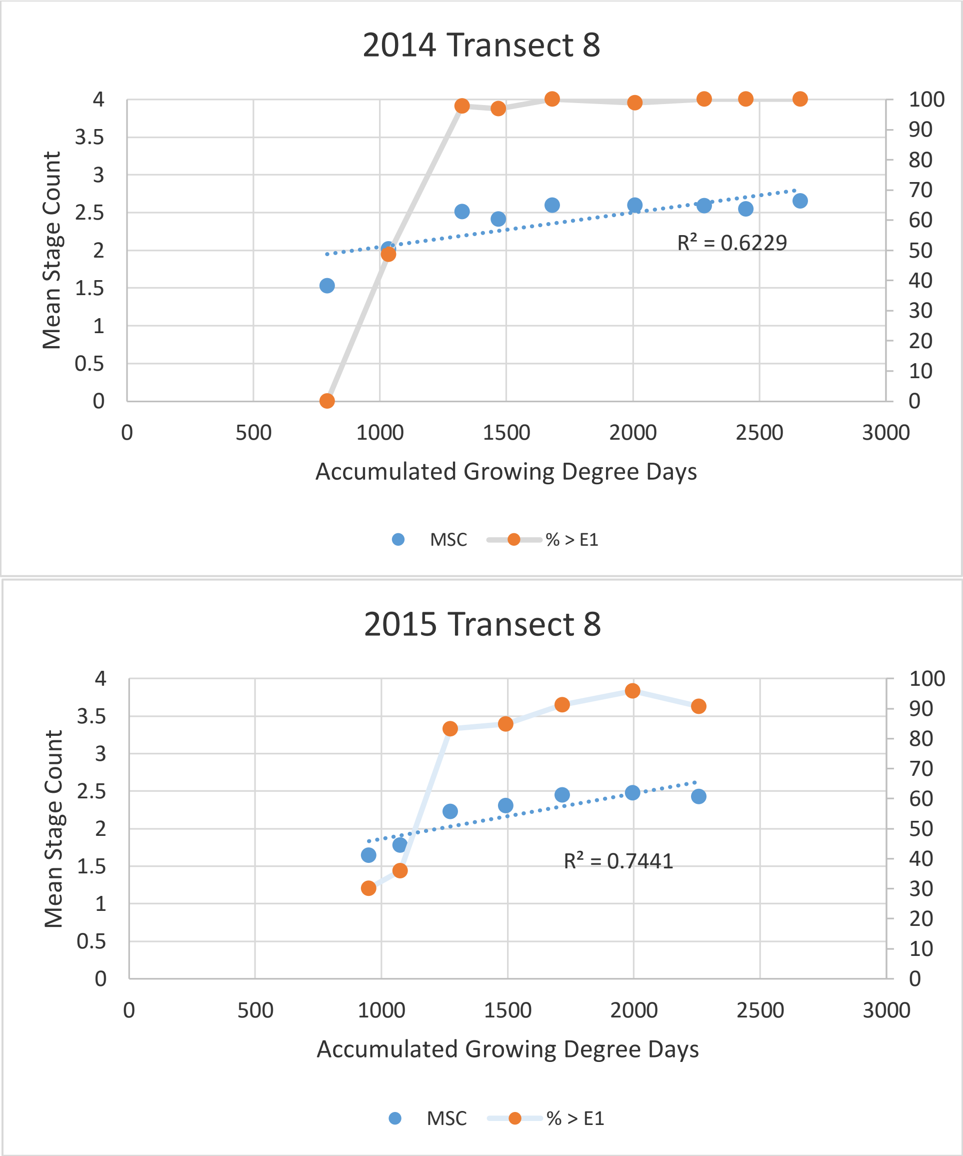
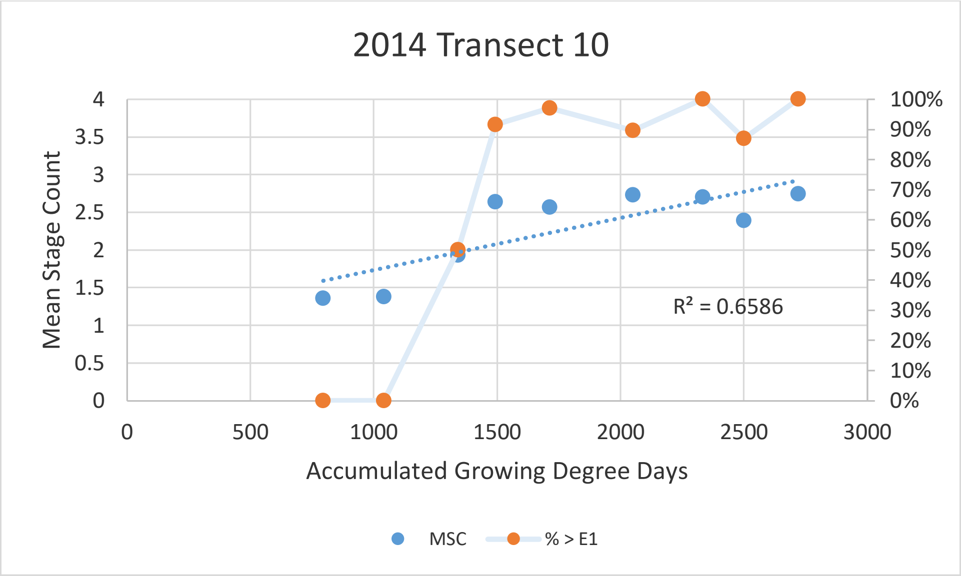


Figure D8. Transect 8, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.



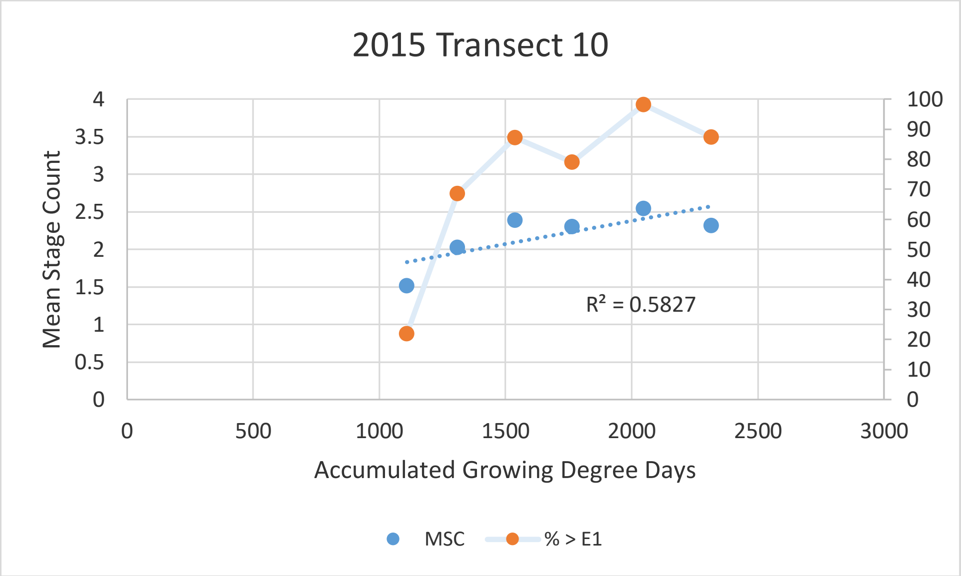


Figure D9. Transect 10, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

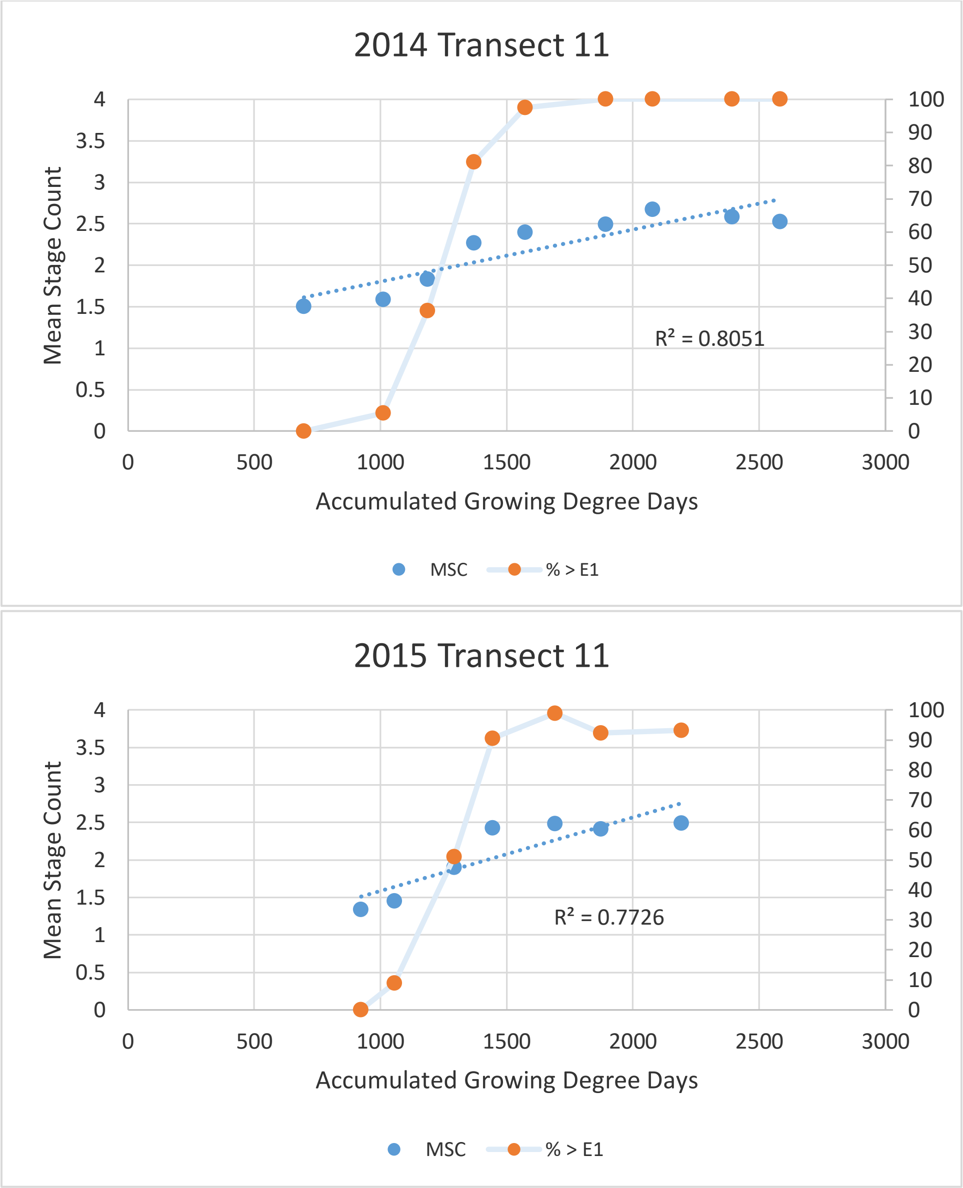


Figure D10. Transect 11, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

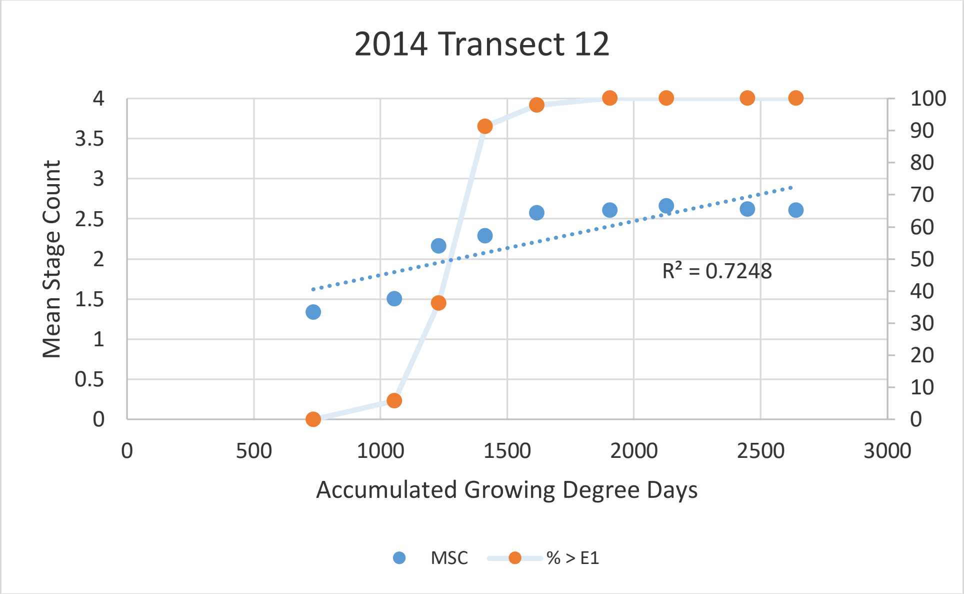


Figure D11. Transect 12, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

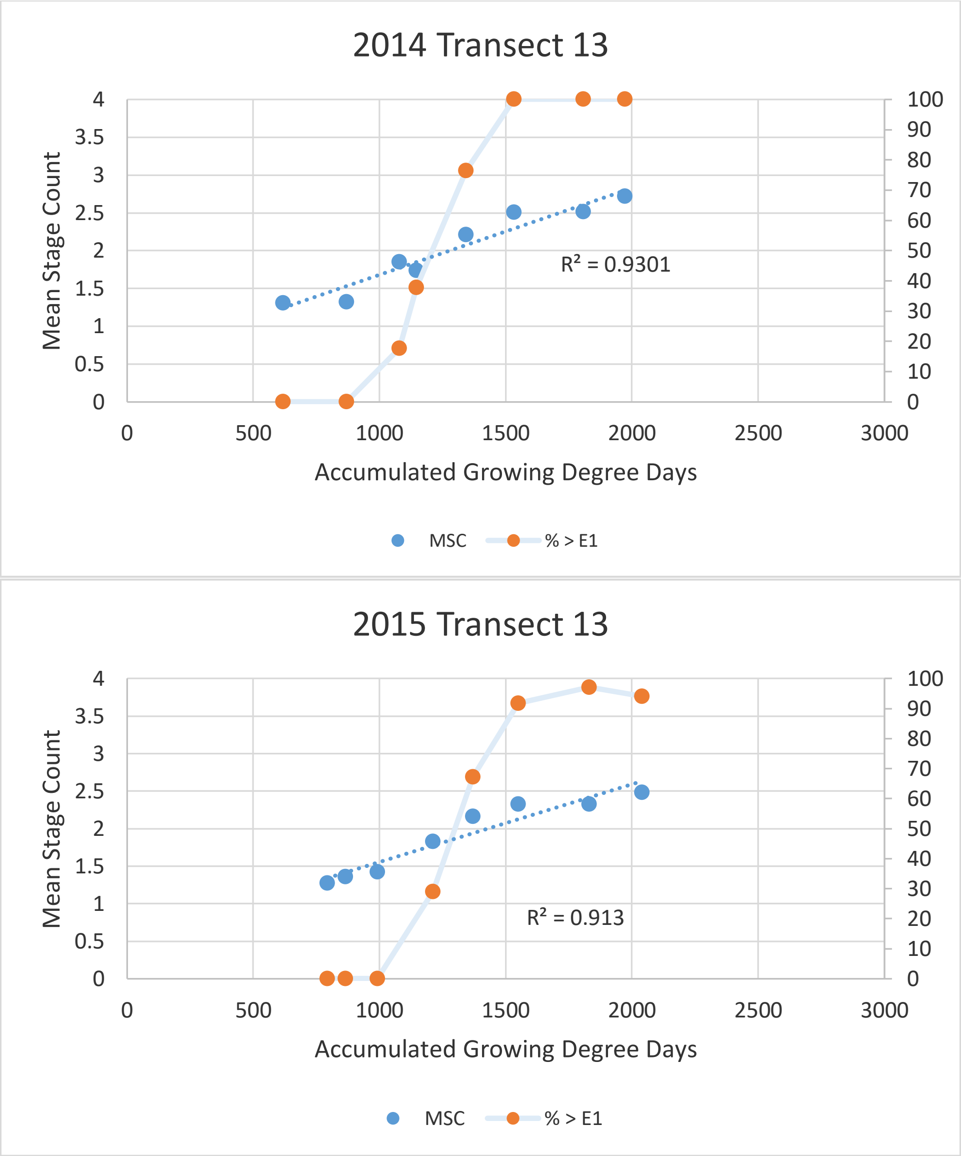
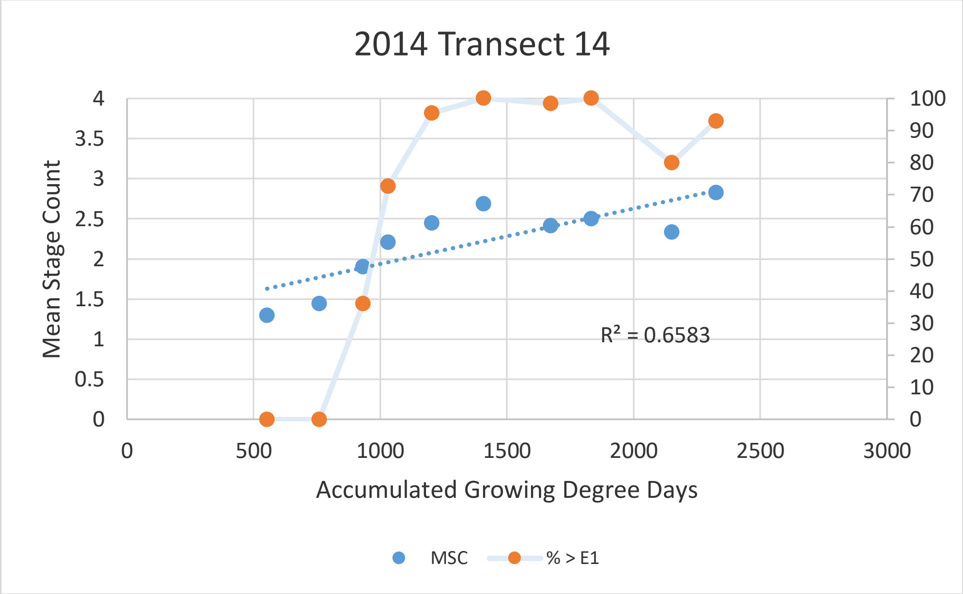


Figure D12. Transect 13, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.



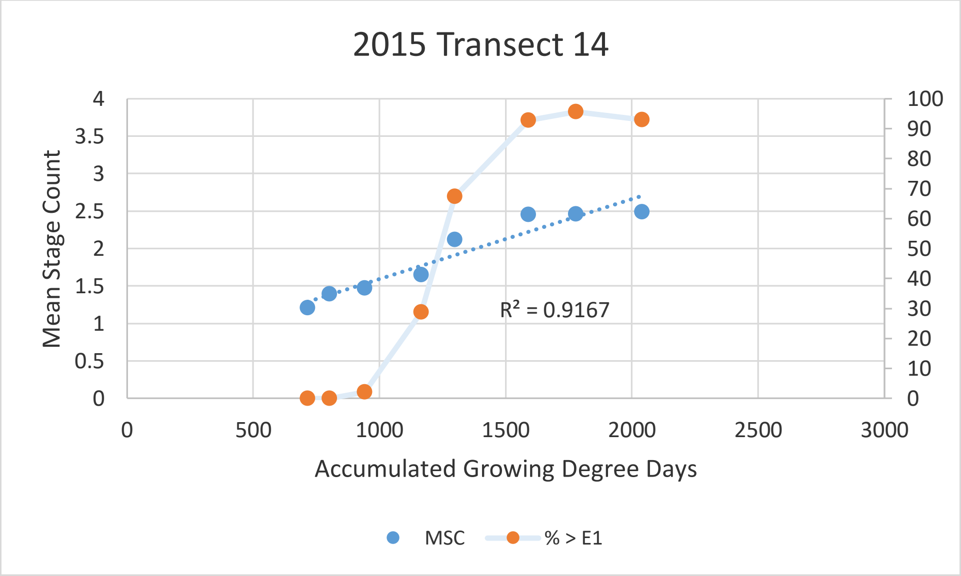


Figure D13. Transect 14, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

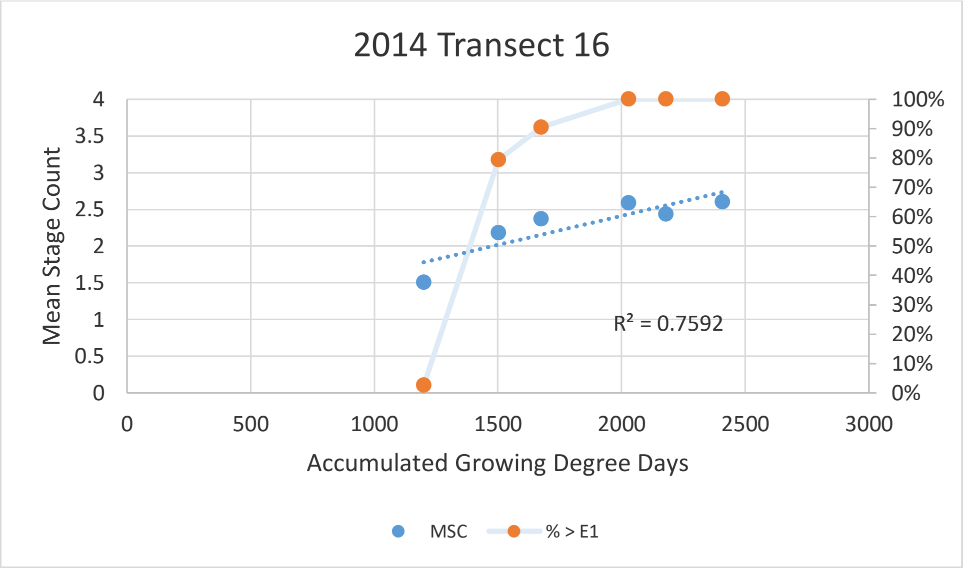


Figure D14. Transect 16, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

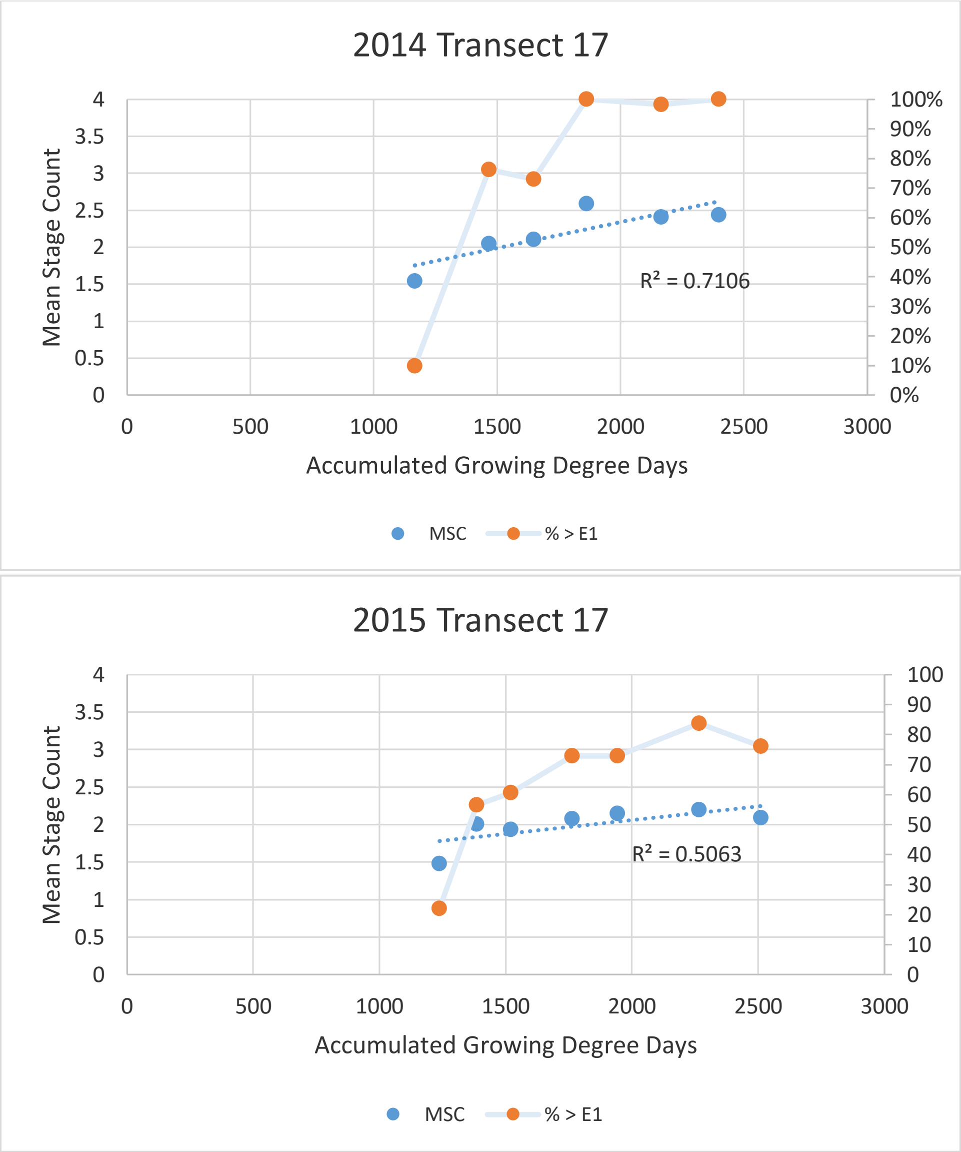
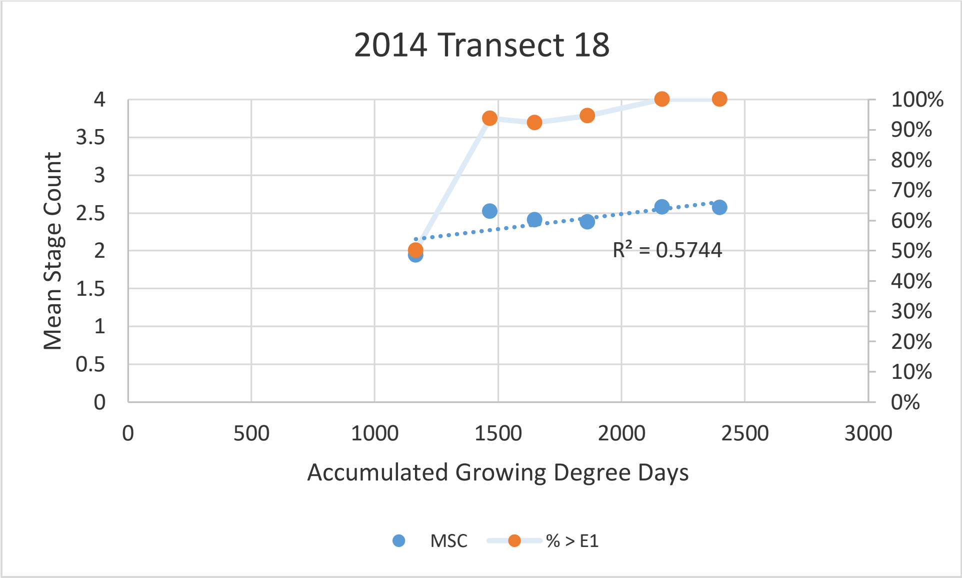


Figure D15. Transect 17, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.



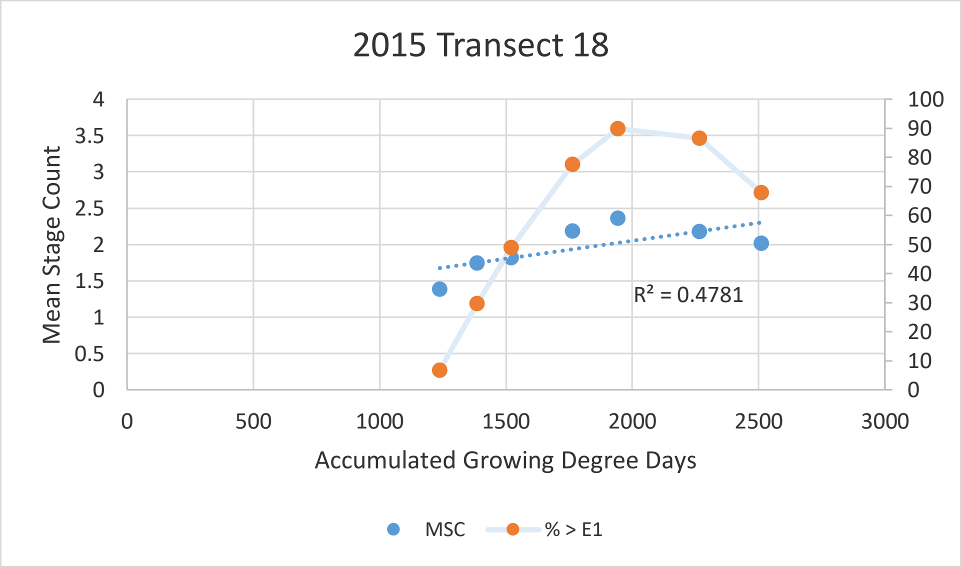


Figure D16. Transect 18, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

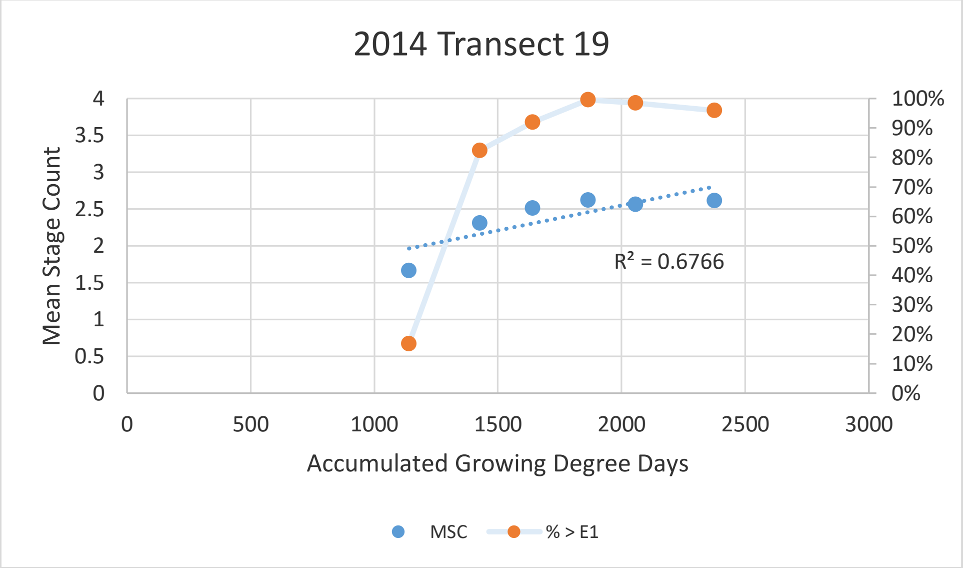
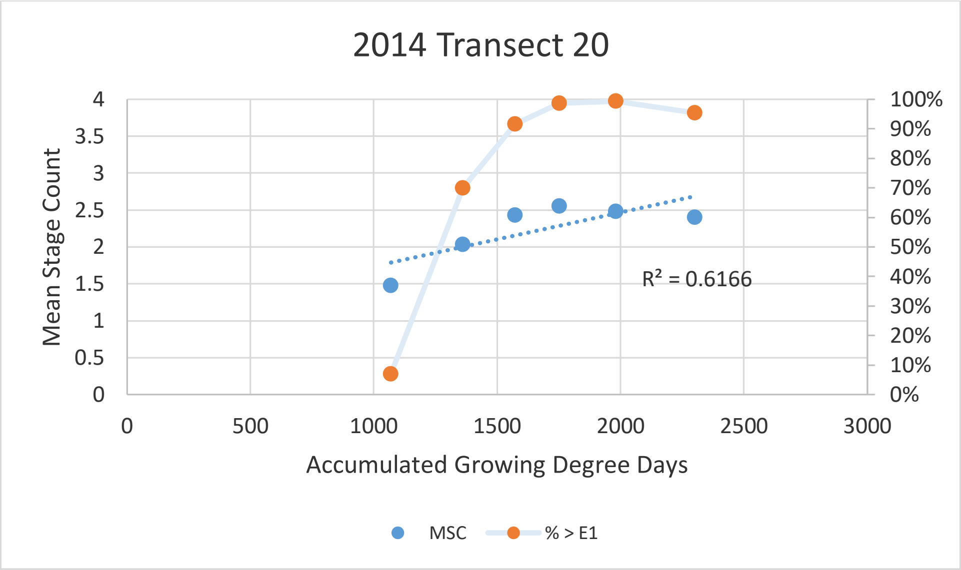


Figure D17. Transect 19, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.



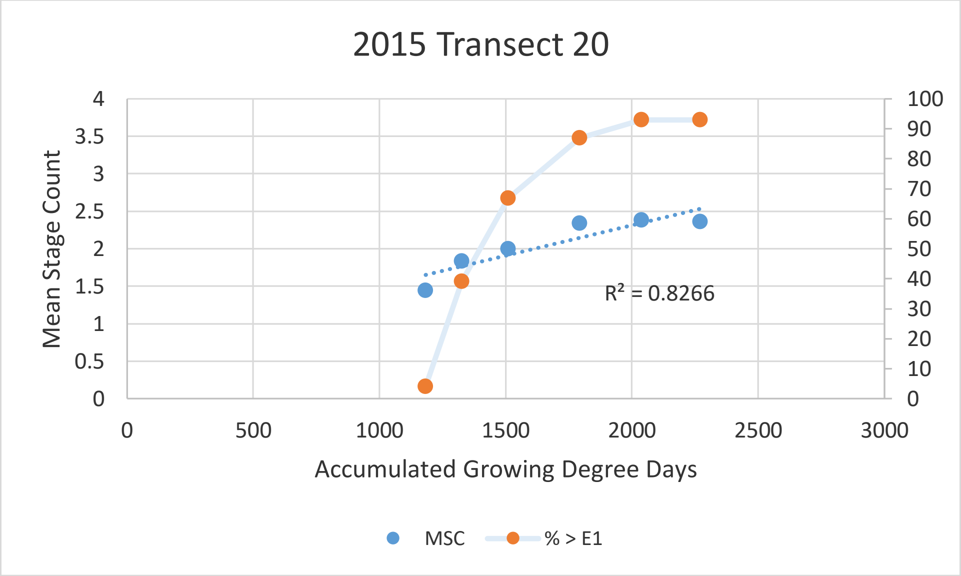


Figure D18. Transect 20, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

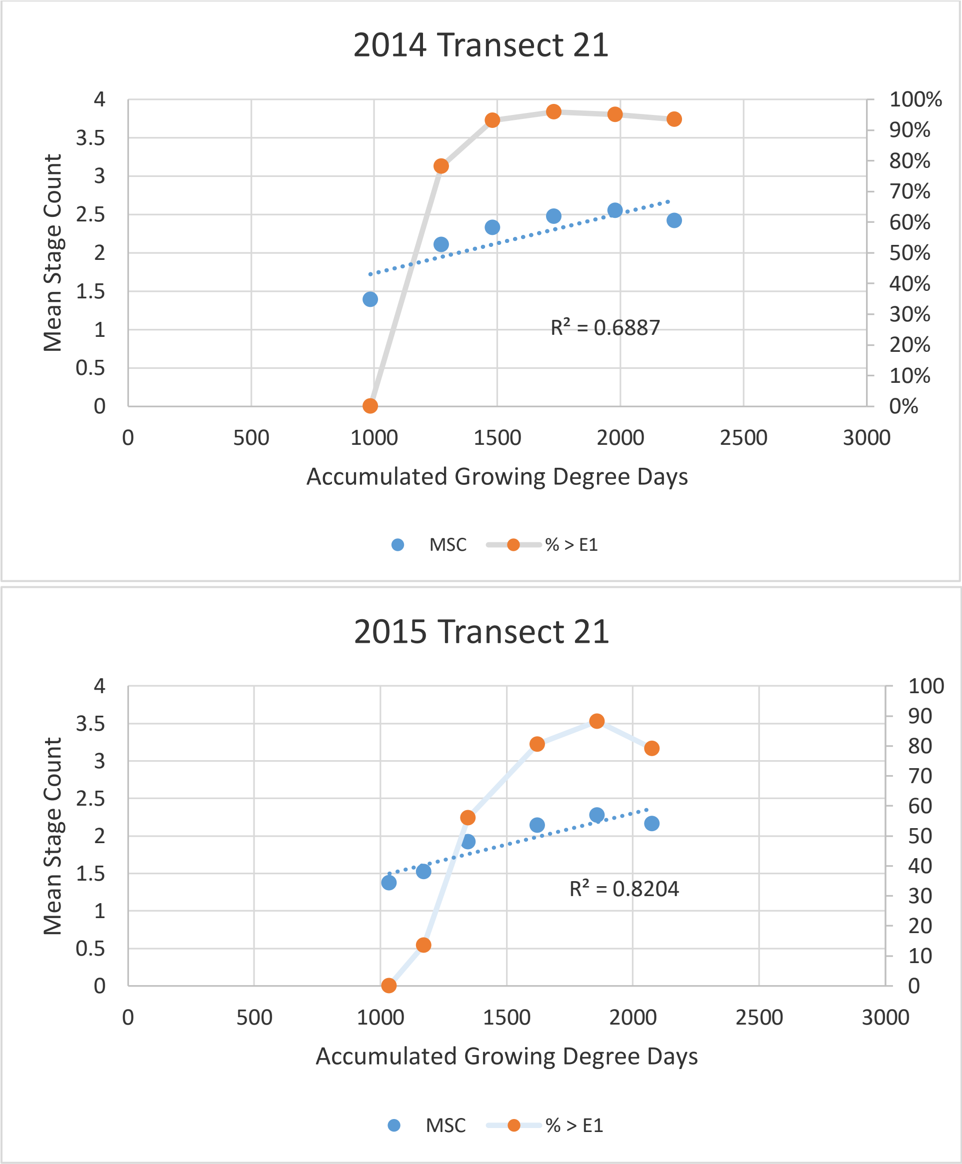


Figure D19. Transect 21, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

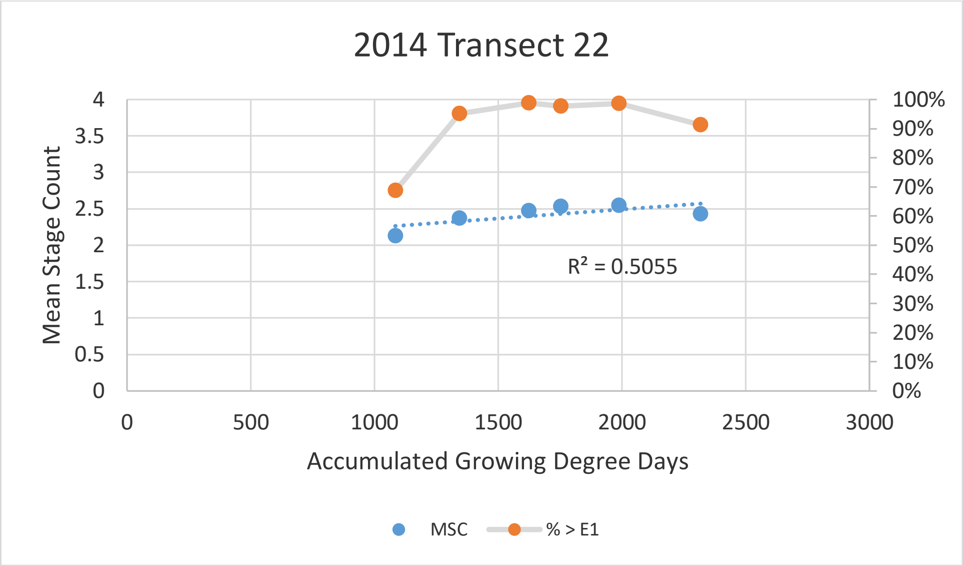


Figure D20. Transect 22, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

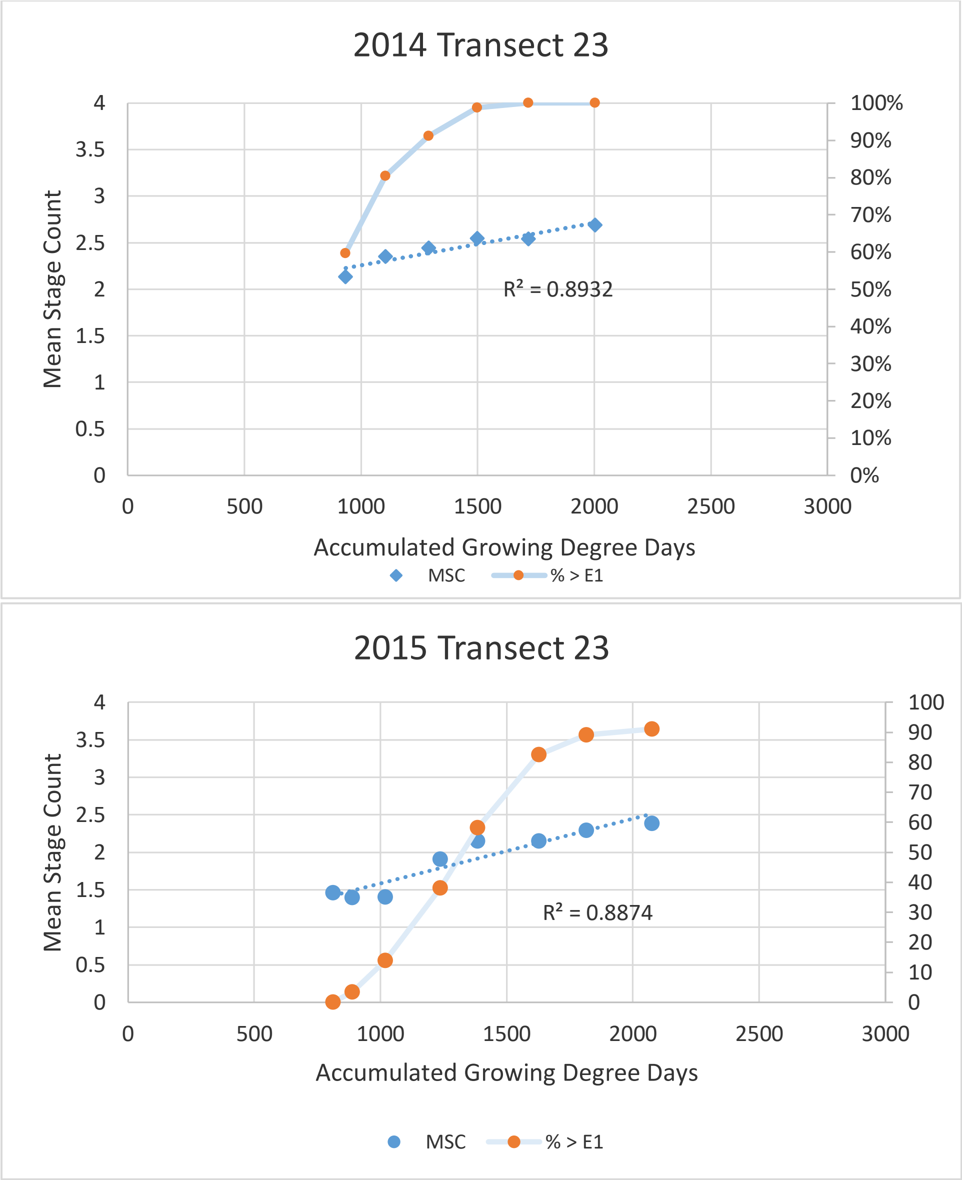


Figure D21. Transect 23, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

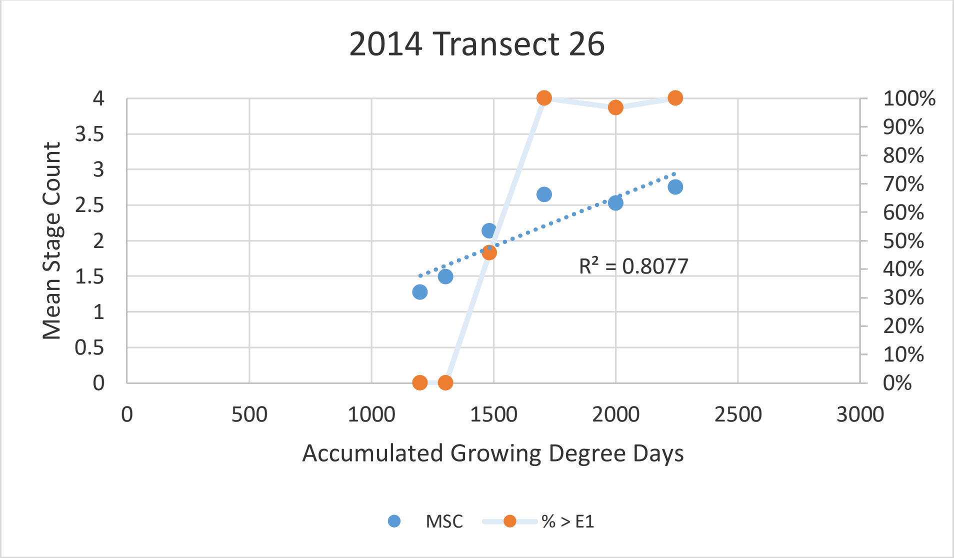


Figure D22. Transect 26, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

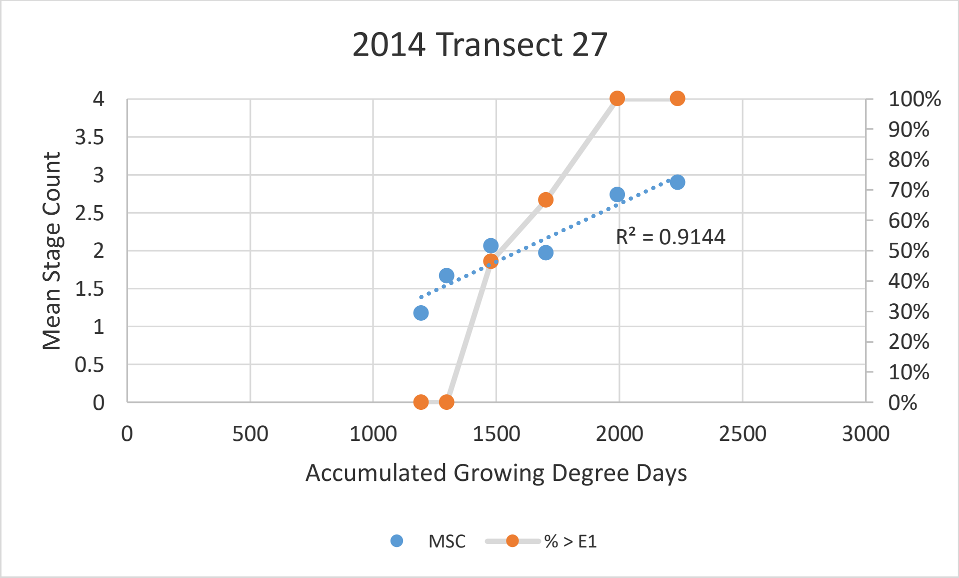


Figure D23. Transect 27, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

APPENDIX E. LINEAR REGRESSION ANALYSIS OF ACCUMULATED GROWING DEGREE DAYS AND MEAN STAGE COUNT AND PERCENTAGE OF TILLERS AT THE ELONGATION PHASE OR HIGHER FOR MODEL VALIDATION SITES IN 2016

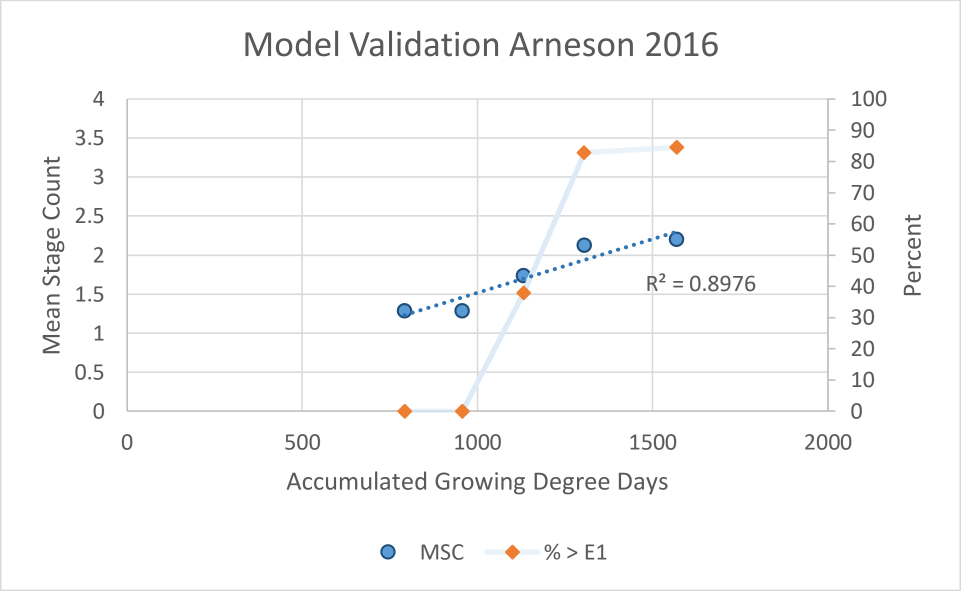


Figure E1. Arneson model validation site, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

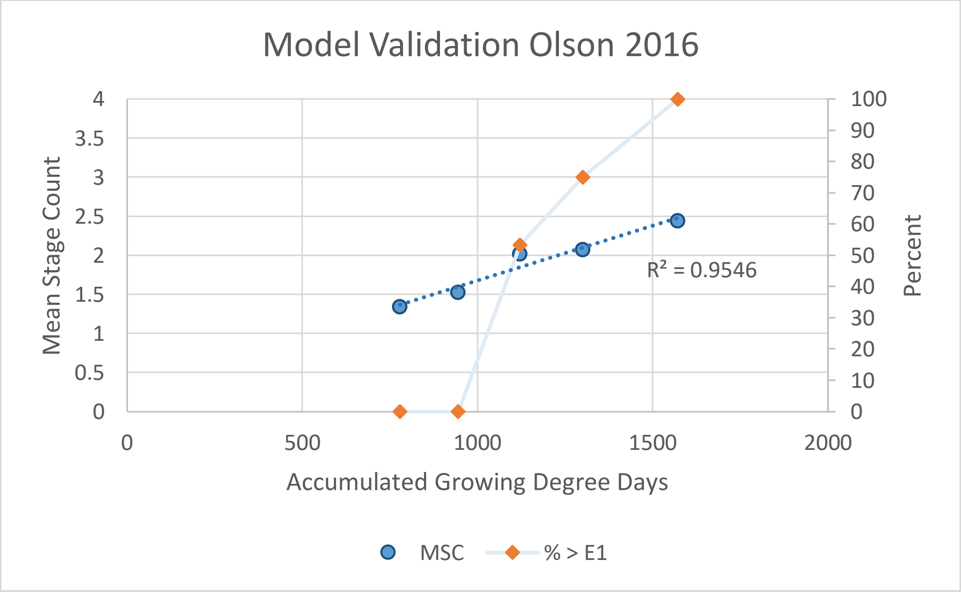


Figure E2. Olson model validation, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

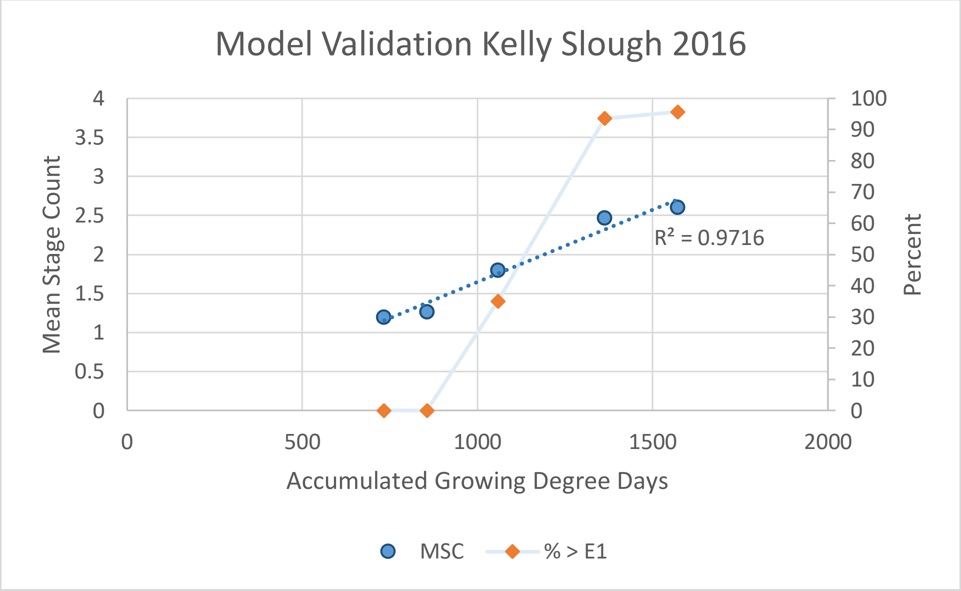


Figure E3. Kelly Slough model validation, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

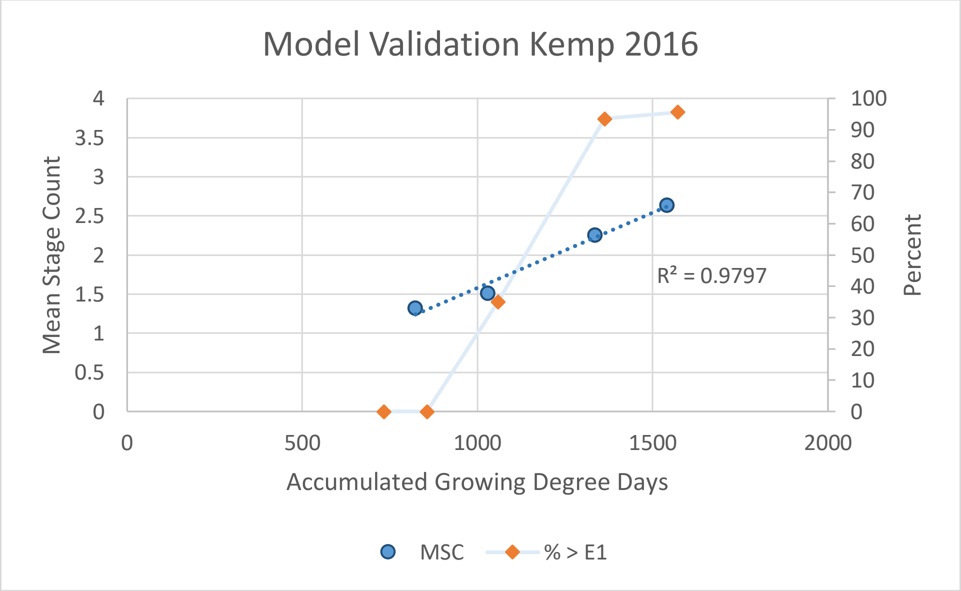


Figure E4. Kemp model validation, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

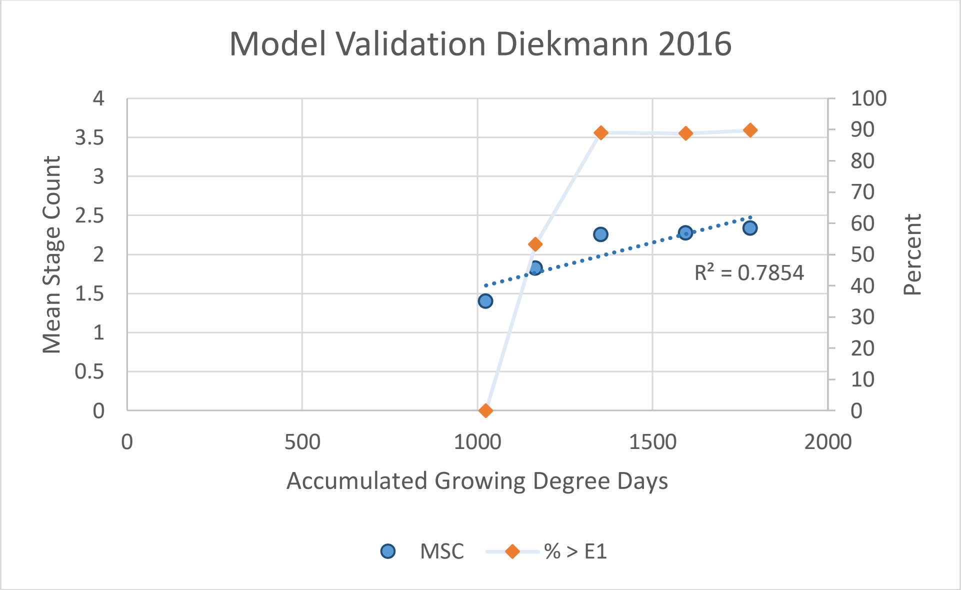


Figure E5. Diekmann model validation, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

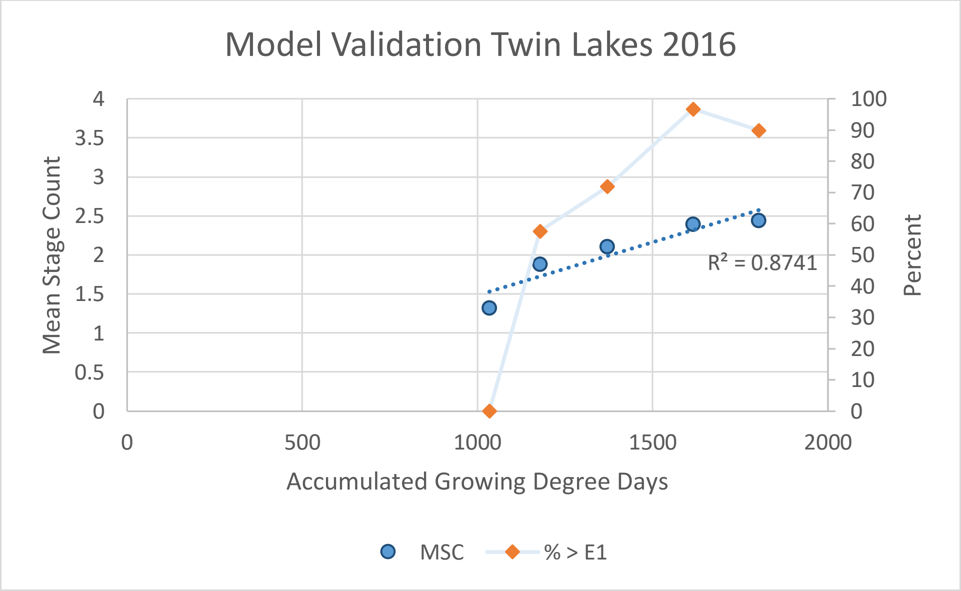


Figure E6. Twin Lakes model validation, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

APPENDIX F. LINEAR REGRESSION ANALYSIS OF ACCUMULATED GROWING DEGREE DAYS AND MEAN STAGE COUNT AND PERCENTAGE OF TILLERS AT THE ELONGATION PHASE OR HIGHER FOR GREENHOUSE TRIALS

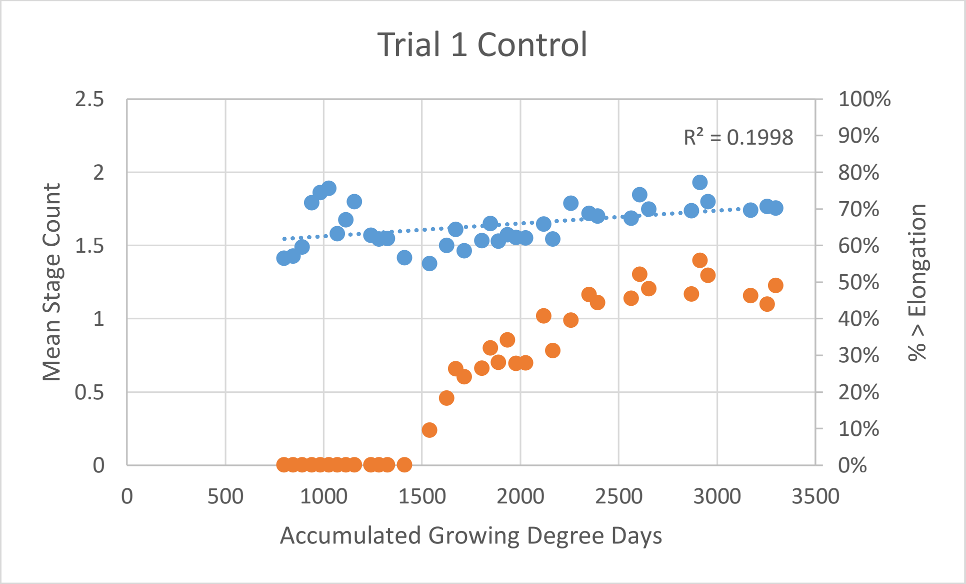


Figure F1. Greenhouse trial 1, control treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

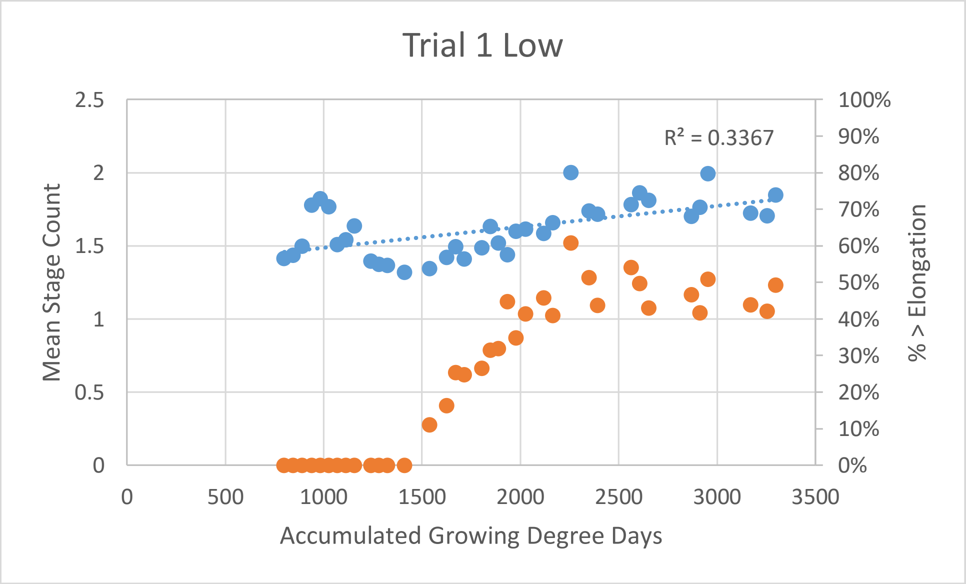


Figure F2. Greenhouse trial 1, low treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

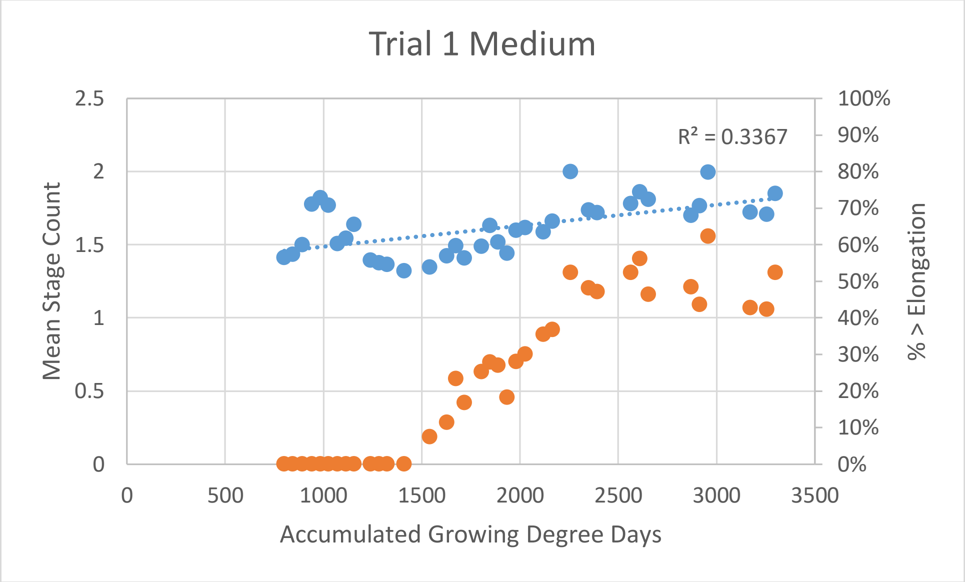


Figure F3. Greenhouse trial 1, medium treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

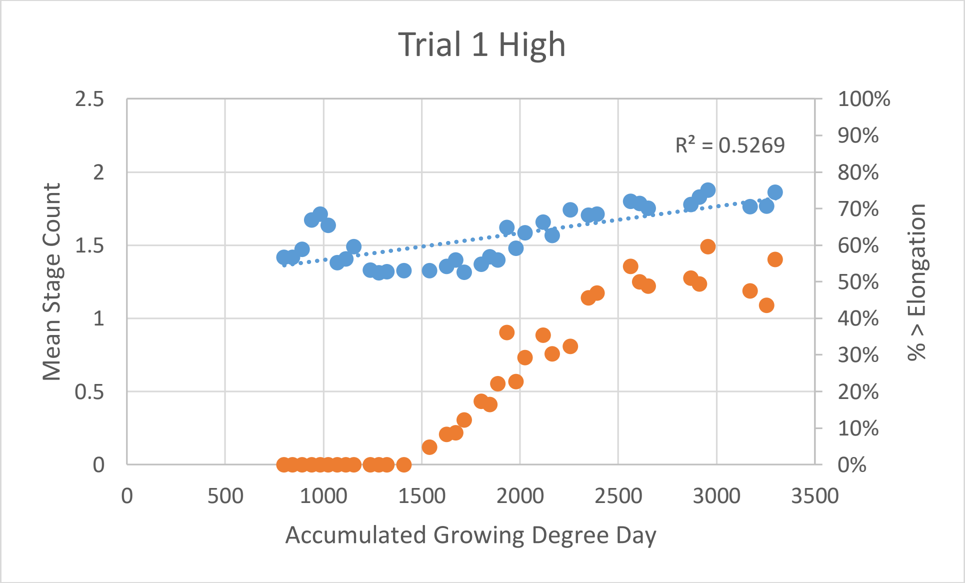


Figure F4. Greenhouse trial 1, high treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

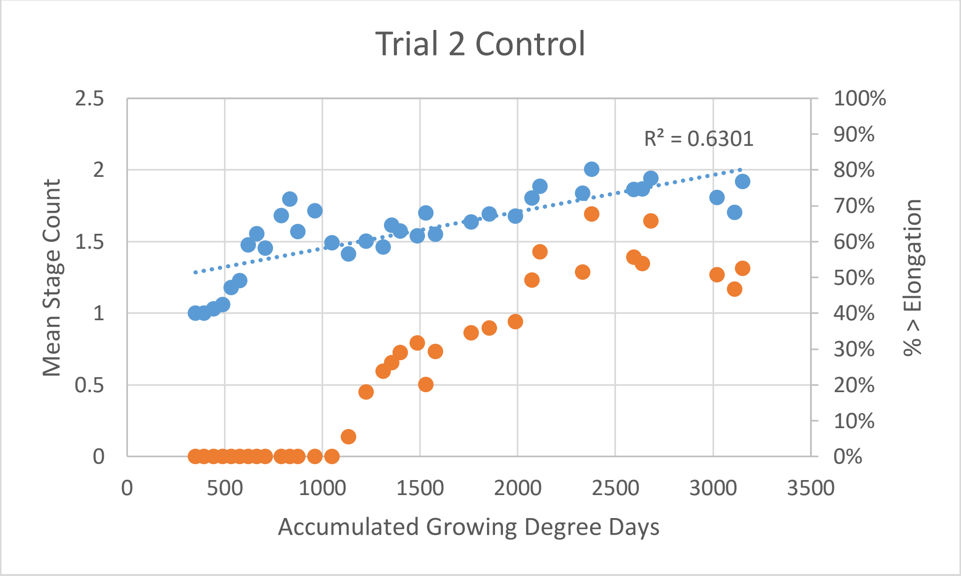


Figure F5. Greenhouse trial 2, control treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

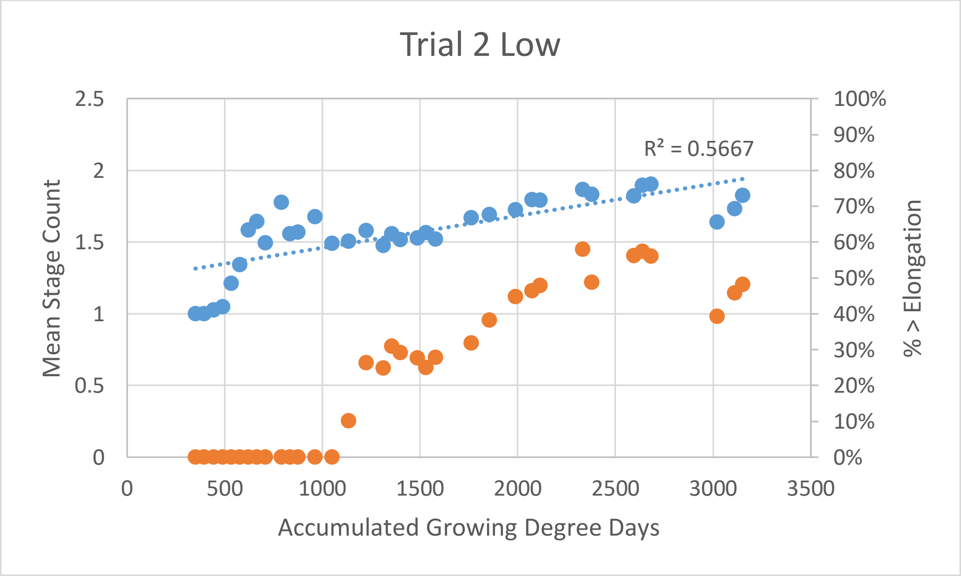


Figure F6. Greenhouse trial 2, low treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

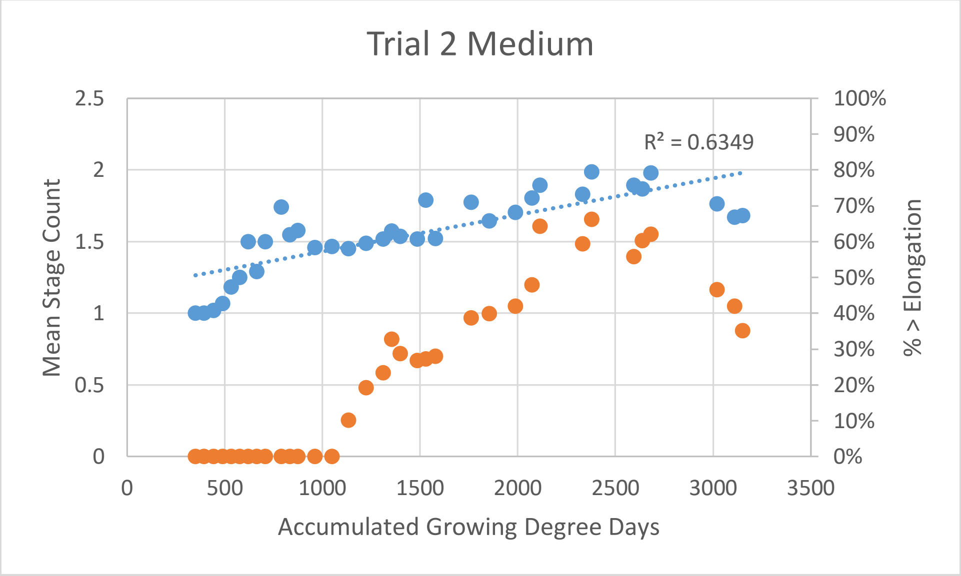


Figure F7. Greenhouse trial 2, medium treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

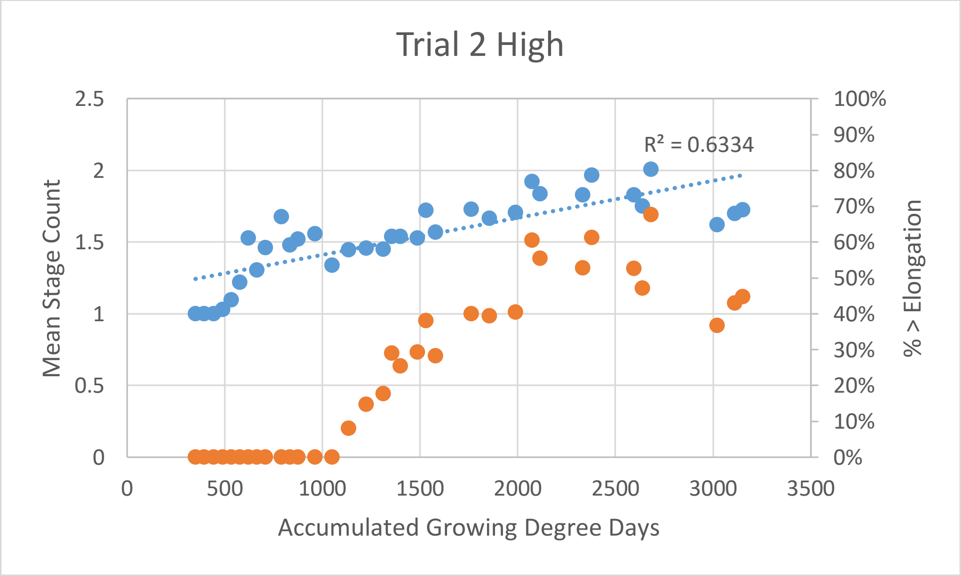


Figure F8. Greenhouse trial 2, high treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

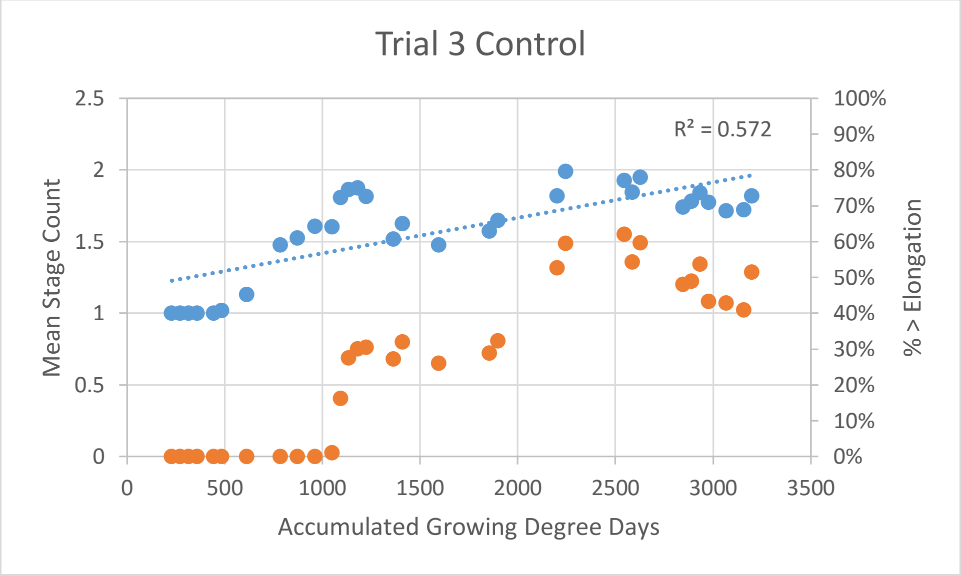


Figure F9. Greenhouse trial 3, control treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

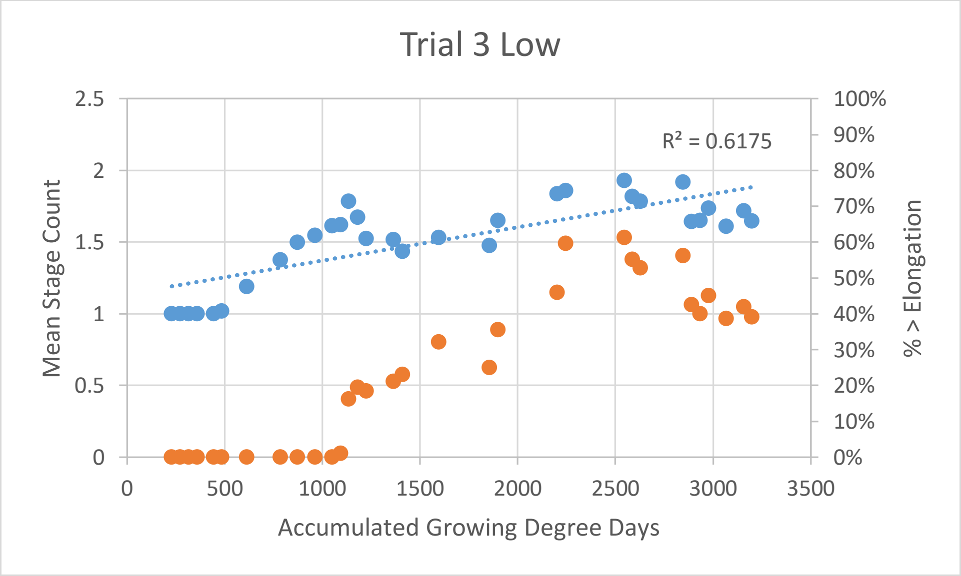


Figure F10. Greenhouse trial 3, low treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

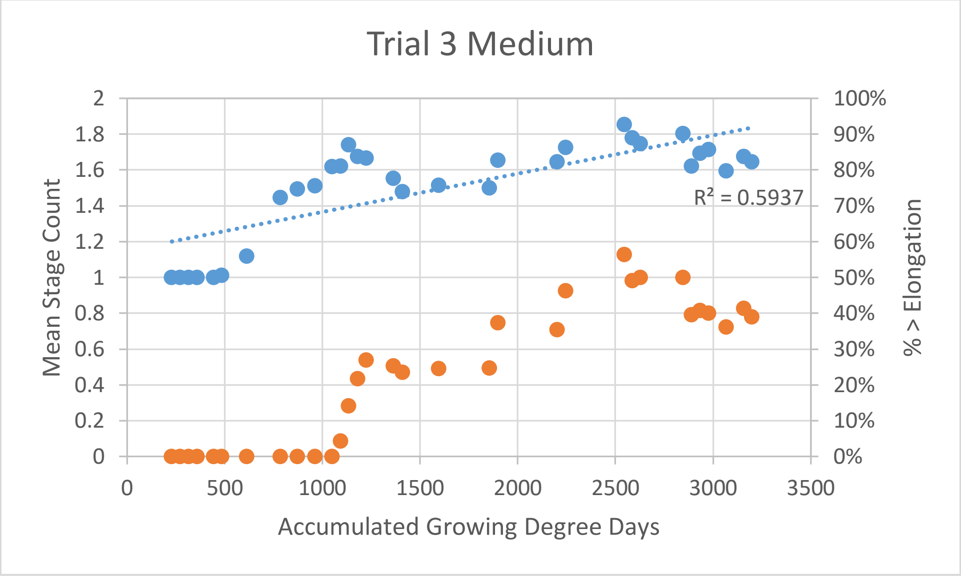


Figure F11. Greenhouse trial 3, medium treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.

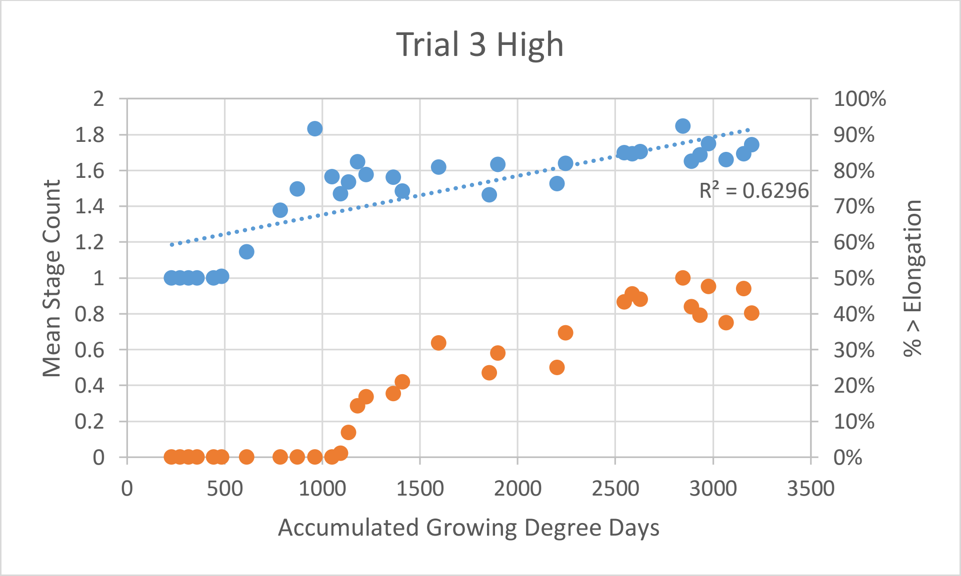


Figure F12. Greenhouse trial 3, high treatment, mean stage count on the left axis and percentage of tillers at the elongation phase or higher on the right axis.