

Persistence and Productivity of Orchardgrass (*Dactylis glomerata* L.) Hay Stands

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Abstract (Academic)

Persistence of perennial grass crops is essential to their profitable management. Recently, orchardgrass producers in the Mid-Atlantic have reported a reduction in the persistence and regrowth vigor of their swards. The overall objective was to evaluate which factors play a major role in controlling the persistence of orchardgrass harvested for hay in the Mid-Atlantic. A survey of orchardgrass fields, growth chamber experiment, and field experiment were conducted to that end. The objectives were to: (1) assess soil fertility, management practices, disease status, and climate in relation to producer perceived stand persistence rating, orchardgrass biomass, and soil test thresholds in orchardgrass hayfields in 4 states, (2) examine the interactions of high temperature and low cutting height on the physiology and regrowth of orchardgrass in controlled environments, and (3) evaluate yield, composition, and size/density compensation-corrected productivity of orchardgrass and orchardgrass/alfalfa mixtures harvested to four cutting heights over three years. The survey of hayfields indicated that the sward age, soil organic matter, grazing, manure application, and historical average high temperature were main determinants of stand persistence score. In the growth chamber experiment, regrowth was significantly reduced by the 35°C treatment as compared to 20°C. Low cutting height significantly reduced regrowth in the cool temperature treatment, but no effect of cutting height was detected under heat stress. In the field experiment, yields were highest from plots cut to 5 cm, but orchardgrass cover in these plots thinned through the experiment. Tiller size and density measurements indicated that cutting heights of 10 cm or greater were able to achieve and maintain optimal leaf area while productivity was reduced for the 5 cm treatment. Overall, it is apparent that excessively low cutting heights are a major cause of reduced persistence in orchardgrass swards and that high temperature stress will limit regrowth. These factors likely interact with fertility and disease

status, and together cause the premature loss of orchardgrass stands. Efforts should be made to communicate the importance of increased cutting height to producers. Breeding of orchardgrass resistant to fungal pathogens and heat stress may be required to sustain an orchardgrass hay industry in the Mid-Atlantic.

Abstract (Public)

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Chapter 1. Introduction

Orchardgrass is one of the most valuable cool-season forage grasses globally. It is a high quality and highly productive perennial and is described to be long-lived. Recently, however, many orchardgrass hay producers in the Mid-Atlantic States have reported that the longevity and vigor of their swards are much reduced. The overall objective of this research is to elucidate the causes of this poor persistence and regrowth.

This dissertation is organized in 6 chapters. This introduction is followed by a review of the literature (Chapter 2) on the persistence and productivity of orchardgrass and other cool-season grasses. Chapter 3 is a survey of 53 orchardgrass hayfields in Virginia, West Virginia, Maryland, and Pennsylvania with the objective of determining the relationships among soil fertility, harvest management, disease status, and climate with the persistence rating and orchardgrass biomass of swards. Chapter 4 examines the effects and interactions of high temperature and low cutting height on the physiology and regrowth of orchardgrass in controlled environments. Chapter 5 describes the effects of four cutting heights on the yield, composition, and size/density compensation-based productivity of orchardgrass and orchardgrass/alfalfa mixtures grown in the field over three years. The sixth chapter presents the overall conclusions of this research.

Chapter 2. Literature Review

Orchardgrass and its Recently Observed Decline in the Mid-Atlantic

Orchardgrass (*Dactylis glomerata* L.) is one of the most important cool-season forage grasses globally. Orchardgrass is a high yielding, high quality, shade-tolerant, and long-lived C3 bunchgrass and because of these characteristics is a desired species both for pasture and for conserved feed. It was first cultivated from an orchard in Virginia in 1760 (Van Santen and Sleper 1996). Since then, it has been grown for forage on all seven continents, including the subantarctic island of Kerguelen (Bourzat and Monie 1977; Van Santen and Sleper 1996; Frenot et al. 2003). A measure of its value for cultivation is that it ranks fourth in terms of global cool-season grass seed sales [after: perennial ryegrass (*Lolium perenne* L.), tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.), and timothy (*Phleum pratensis* L.)] (Stewart and Ellison 2010).

Lack of persistence of orchardgrass hay stands has become a concern in recent decades. While it is challenging to quantify a decrease in persistence across the region, it has been stated that orchardgrass stands used to persist for 6 – 8 years and recently stands persist for 3 – 4 years or fewer (Clark 2009). In a survey of producers in the Mid-Atlantic in 2010, 74% felt that their stands had declined more quickly than expected (Tracy 2011). Soil fertility, harvest management, especially cutting height, and disease pressure are most commonly noted as potential causes (Clark 2009; Smith and Saylor 2012; Taylor 2013) while heat and drought stress may also play a role (Tracy 2011). Given the range of potential causes, it is likely that interactions among several factors are responsible for a decline in orchardgrass persistence.

Persistence of Grasses

One of the advantages of the use of perennial grasses in forage systems is the economic benefit of establishment costs being amortized over a number of years of production. Thus, persistence is a goal of many perennial forage systems. In this context, persistence might be thought of as the ability to continue to produce economic yield over the course of years. At the biological level, persistence is usually defined as the capacity of a sward of tillers to be able to replace themselves each year and thus to maintain a stable tiller density (Langer 1973; Edwards and Chapman 2011; Culvenor and Simpson 2014). Difficulty arises, however, because tiller density does not correspond to yield in many cases and many studies evaluate tiller density of swards as a measure of persistence rather than conducting experiments for long enough periods to observe yield reduction. Further discussion of this relationship between tiller dynamics and yield is discussed in the Competition section of this review. Following is a review of literature related to five main factors related to persistence (soil fertility, harvest management, pests, climate, and competition) and, importantly, their interactions.

Soil Fertility

The short-term yield response of forage grasses to N, P, and K fertilization is well understood (Wedin 1974; Havlin et al. 2014). However, there has been less study of the effects of fertilization on the persistence of perennial grasses.

Nitrogen

The expected response of orchardgrass to amended N differs between spaced plants or those grown in pots and plants grown in swards. Nitrogen fertilization has been shown to increase orchardgrass tiller density when grown in pots in a greenhouse (Langer 1963; MacLeod

1965a; MacLeod 1965b; Auda et al. 1966). At low rates of N, the response in the field is similar with increasing tiller number (Kim and Lee 1990). However, high rates of N fertilizer cause intraspecific competition among orchardgrass tillers and leads to the death of small tillers and thus a reduction in persistence. Examples and discussion of the loss of tiller density associated with large fertility amendment are discussed briefly here but in more detail in the Competition section of this review. The self-thinning of orchardgrass fertilized heavily with N can be seen in many studies (Griffith and Teel 1964; Washko et al. 1967; George et al. 1973; Jung et al. 1974; Marten et al. 1979). Griffith and Teel (1964) measured 52% stand loss at a low cutting height with high N and low K. Winter injury of orchardgrass has also found to be greater in N fertilized stands than stands without added N (Jung and Kocher 1974). High rates of N fertilization have also been attributed to increased disease susceptibility for forages (Leath and Radcliff 1974) and other crops (Huber et al. 2012), although this has not been specifically studied for orchardgrass.

Potassium

Potassium is likely the most important nutrient controlling persistence of grasses other than N. Potassium plays an important role in plant-water relations, enzyme activation, protein synthesis, photosynthesis, osmoregulation, and biotic and abiotic stress resistance (Hawkesford et al. 2012). In a controlled pot study, increasing rates of K as KCl caused increased tiller production regardless of the N supplied (MacLeod 1965a). Blaser and Kimbrough (1968) reported that usually long-lived perennials, including orchardgrass, showed stand reductions when K limitation occurred. In a 14-year fertilizer response experiment in Orange, VA, Singh et al. (1967) found that orchardgrass yield responded to N during the first two years, to N and K during the next three years, and to N, P, and K during the final eight years. They concluded that

the yield was more limited by K than by P, but that response to K was dependant on the rate of N applied.

Several other studies have documented the interaction of N and K on the yield, persistence, and competitiveness of orchardgrass. Griffith et al. (1964) found that large applications of N increased K deficiency and that added K decreased the fructose content of the tissue across rates of N. Blaser and Kimbrough (1968) described an experiment with mixtures of orchardgrass, bluegrass, and tall fescue with various rates of N and K fertilizer, they found that orchardgrass was the most competitive species when fertilized with high rates of N and K, but when high rates of N and low rates of K were applied, tall fescue dominated the stand and orchardgrass was nearly eliminated. Griffith and Teel (1965) found a third order interaction among the effects of N rate, K rate, and cutting height on orchardgrass persistence. Stand reduction was nearly 60% in plots fertilized with 300 lbs N acre⁻¹ when no K was applied and the cutting height was 2 inches, but that when 332 lbs K acre⁻¹ was applied, stand reduction was only about 25% (Griffith and Teel 1965). Potassium also plays a role in the disease resistance of crops. While high rates of N increase disease occurrence, increased rates of K have been shown to reduce disease, although, to my knowledge, this has not been tested in orchardgrass (Leath and Radcliff 1974; Huber et al. 2012).

Phosphorus

Adequate phosphorus is required to maintain yields of crops, however, its role in the persistence of perennial grasses has not been well explored. Phosphorus is known to have several important physiological roles (Havlin et al. 2014), and early spring growth of grass is stimulated by P (Sheil et al. 2016). A long-term study investigating fertilization on the persistence of

timothy in Canada found no effect of P fertilization on persistence, but that added P increased yield during the final years of the experiment (Bélanger et al. 1989).

Other Nutrients

Secondary macronutrient and micronutrients are rarely deficient in grass forage systems especially when appropriate soil pH is maintained (Snyder and Leep 2007; Havlin et al. 2014). No studies of the secondary or micronutrient application or deficiency on the persistence of grasses could be found.

Sulfur nutrition is not commonly associated with tillering or the persistence of grasses. However, given the observed reduction in atmospheric sulfur input to eastern soils over the last several decades and the lack of a consistently accurate soil S test (Havlin et al. 2014), it is possible that orchardgrass stands could be yield-limited by soil S supply. Research by Reneau (1982) showed a yield response of Virginia Common orchardgrass planted on Groseclose silt loam with an application of 17 kg S / ha as CaSO_4 in two out of three years. Sulfur is required for certain amino acids (cystine, cysteine, and methionine) and thus sulfur's main function is as a substrate for protein (Havlin et al. 2014). While yield and protein content can increase with increased soil S, there is not a clear physiological link between soil S and orchardgrass persistence. However, the decline in atmospheric S deposition has occurred over a similar period as the recent decline in orchardgrass persistence.

Harvest Management

The quantity and quality of regrowth following mechanical harvest is dependent on the presence of apical meristems, stubble leaf area and carbohydrate content, root mass, and environmental conditions (Pearson and Ison 1997). All of these factors can be affected by cutting

date, cutting frequency, and cutting height of swards. These harvest management factors interact with each other as well as with climate, soil fertility, and pathogen pressure. Some interactions will be discussed here and some in later sections of this review.

Cutting Height

It has been suggested that cutting height is an important factor in the recently observed decline in orchardgrass stands (Smith and Saylor 2012; Clark 2009). Over the past several decades, rotary-disc mowers have been adopted by most hay producers (Adams 1996), and the design of these mowers allows for a lower cutting height. Thus, a common recommendation has been to increase cutting height to increase leaf area index (LAI) and water-soluble carbohydrate (WSC) reserves to improve regrowth and orchardgrass persistence. Producers, however, face a trade-off in selecting a cutting height. Low cutting height will maximize yield for a single cutting while higher cutting height should improve persistence.

It is known that defoliation directly affects LAI and WSC and that these influence regrowth rate (Ward and Blaser 1961; Davidson and Milthorpe 1965). It is commonly thought that increasing cutting height should decrease plant stress and thus improve persistence. The literature, however, does not consistently indicate that increasing cutting height improves either yield or persistence.

One example is a 3-year experiment by Washko et al. (1967), in which Potomac orchardgrass was grown in 6 Northeastern states. After taking the first cutting to 6.35 cm, the aftermath was harvested at either 3.8 or 8.9 cm. Yields were greater at 3.8 cm in RI, PA, and MD, while the yield was greater at 8.9 cm in NY and WV. Stand persistence ratings were made in the spring of the third year, and the lower cutting height resulted in stands in PA and MD with significantly greater stand ratings, while the higher cutting height in WV resulted in greater stand

ratings and there was no difference in NY. Some interactions were noted with a fertilizer N treatment, but there was not a consistent trend among sites. Growth stage at harvest and weather may have played a role in the varied results.

In Virginia, Bryant and Blaser (1961) found 2-cm cutting height to produce higher orchardgrass yields than 6.3 cm cutting height over a 3-year experiment. In Wales, stands harvested at 5 cm yielded better in the first and second years of the experiment than an 8-cm cutting treatment, but yields were higher at 8 cm in the third year (Jones 1983). A greenhouse experiment measuring the location of stored WSC indicated that orchardgrass should be persistent with defoliation to 3 cm (Turner et al. 2007).

These examples are in contrast to several experiments that find lower cutting height reduce yield (Stapleton and Milton, 1930; Harrison and Hodgson, 1939; Volesky and Anderson 2006) and persistence (Brink et al. 2010; Smith and Saylor 2012). Harrison and Hodgson (1939) clipped orchardgrass weekly in the greenhouse to three cutting heights; the frequency of their defoliation likely compounded the effects of cutting height.

Interactions among cutting height and N and K fertilization have also been found (Griffith and Teel 1964; Raese and Decker 1966; Washko et al. 1967; Smith and Saylor 2012). Smith and Saylor (2012) found 75% reduction in orchardgrass stands cut to 1.25 cm, but found that stands fertilized with N and K and cut to 5 cm had similar stand ratings as those cut to 10 cm.

Interactions, though sometimes poorly defined, between harvest management treatments and temperature and moisture have also been reported to impact yield and persistence of orchardgrass (Jäntti and Heinonen 1957; Griffith and Teel 1965; Washko et al. 1967; Seo et al. 1988; Malinowski et al. 2012). In other cool-season grass species, increased cutting height can

mitigate some effects of stressful weather conditions (Youngner and Nudge 1976; Liu et al. 2003; Song et al. 2015).

Cutting Date & Frequency

The dates and frequency of cutting will cause differences in yield, regrowth, and persistence but these responses are usually dependent on cutting height, fertility, and other factors. Persistence responses to harvest frequency are non-linear. When cutting is infrequent and fertility is applied, self-thinning occurs among tillers and a reduction in tiller density is observed (Smith et al. 1973; Marten et al. 1979). Smith et al. (1973) found slightly increased ground cover of orchardgrass when harvest was increased from two to three to four times per year and was improved with a higher cutting height as compared to lower. Marten et al. (1979) found the same pattern, but it was more evident when high rates of fertility were applied as wastewater.

Harvest can become too frequent, however, if the plants do not have sufficient time to put on leaf area and reaccumulate non-structural carbohydrates. Jung et al. (1974) found that increasing cutting frequency from 3 to 5 to 8 times per year increased orchardgrass ground cover but reduced the yield harvested. Stapledon and Milton (1930) found that the yield of orchardgrass was better when harvested for hay twice per season rather than being harvested to 15 cm every three weeks. When cut every week, orchardgrass yielded about 4 times more when cut to 3 inches rather than 2.5 cm, and yielded about 6 times more when cut to 15 cm instead of 2.5 cm (Harrison and Hodgson 1939).

The date of cutting can also play an important role in the yield and persistence of orchardgrass. Cherney et al. (1986) found that delaying first harvest improved yield but thinned tillers during the second year of their experiment. Ward (1961) studied cutting height and first cutting date on orchardgrass in Virginia. He found lower cutting height reduced residual leaf

area, as expected but also found that delaying cutting from the leafy stage (April 25) to seed (June 16) reduced remaining leaf area index from 1.7 to 0.04 (Ward 1961). In that experiment, initial and total yield increased by delaying cutting date, but the yield of 28-day regrowth following first cutting was reduced from 2234 lbs acre⁻¹ when first cut at the leafy stage to only 134 lbs acre⁻¹ when first cut at the seed stage even though fructan content was highest at the later dates. These results showed the degradation of leaf area in the lower strata of the sward when cutting is delayed and indicated that leaf area is more important than fructan/non-structural carbohydrates in determining the rate of regrowth.

Pests

Fungal Pathogens

It has been long known that foliar fungal pathogens infect orchardgrass (Abbott 1917; Sprague 1950; Kreitlow 1953; Elliot 1962; O'Rourke 1976). Fungal pathogens which damage leaf area will reduce the whole plant photosynthetic capacity (Agrios 2005) and reduce non-structural carbohydrate content (Mainer and Leath 1978). While these two factors will degrade the plant's ability to maintain positive carbon balance and likely lead to mortality and thus a reduction in persistence, no examples of the direct effects of disease on orchardgrass persistence could be found.

There are very few examples of research that have assessed the occurrence or severity of disease in orchardgrass swards (e.g. Elliot 1962). This is a limitation to understanding which pathogens are most detrimental to swards in the region and to knowing if the occurrence or severity of diseases has changed. One source for pathogen assessment in crops is through university plant pathology clinic reports. While these are not scientific research and only contain

samples which someone chose to submit, they can still provide some useful information. An analysis of reports from the Virginia Tech Plant Pathology Clinic from 2004 to 2014 found that 67 orchardgrass samples were positively confirmed as having pathogen infection. Of those 67 samples, 33 had leaf streak/brown stripe (*Cercosporidium graminis*), 26 had anthracnose (*Colletotrichum graminicola*), 4 had *Rhizoctonia solani*, 2 had *Stagonospora arenaria*, 1 had scald (*Rhynchosporium orthosporium*), and 1 had *Drechslera dactylidis* (Virginia Tech Plant Pathology Clinic 2004-2014). While there is not much basis of comparison for these identifications, it is noteworthy that all of these pathogens had been identified prior to 1990 in Virginia (Roane and Roane 1996) and that all of these species, less *Drechslera dactylidis*, were found in an orchardgrass field near Morgantown, WV in 1960 (Elliot 1962). This provides some evidence that new or novel diseases of orchardgrass have not appeared in the recent past to drive persistence problems. While, the frequency and severity of these diseases may have changed, there is no clear strategy to provide evidence for that. It is clear that leaf streak and anthracnose are the most commonly identified pathogens submitted to the VT Plant Pathology Clinic.

Control strategies for foliar pathogens of orchardgrass have not been well developed. Fungicides have been used to control disease on orchardgrass for seed production (Welty 1991), but they are considered uneconomical for forage production. Numeric evidence of this was not found, and no fungicides are presently labeled to control fungal diseases on pure stands of orchardgrass. It is often stated that genetic resistance is the only practical means of control of many pathogens in forage grasses (Berg and Sherwood, 1994). There are, however, surprisingly few scientific assessments of disease resistance among orchardgrass cultivars (Yan et al. 2013; Tsukiboshi and Shimanuki 1999; Berg and Sherwood 1994; Braverman 1986; Miller and Carlson 1982). It should be noted that many of the resistance traits are to rust diseases which are mostly

associated with seed production (Yan et al. 2013; Miller and Carlson 1982) and have not recently been an economic problem in Virginia. A thorough search of the literature to identify varieties resistant to the main fungal pathogens in Virginia (leaf streak and anthracnose) yielded only one study from Japan evaluating resistance to anthracnose (Tsukioboshi and Shimanuki 1999) and none were found resistant to leaf streak. Brummer and Muray (1996) noted that adoption of disease resistant cultivars, particularly for orchardgrass, has been slow because resistant cultivars do not yield significantly better than more susceptible ones.

Fungal pathogens of orchardgrass are frequently observed in the field, but little is known about the economic thresholds for infection and, even if there were, no practical management solutions exist. There are few, if any, forage pathologists working in the eastern U.S., and the few remaining cool-season forage breeding programs have prioritized forage yield, seed yield, and forage quality over disease resistance (P. Ballerstedt, personal communication). Given this lack of attention and the disjointed priorities of forage breeding and production, it is not clear how a solution to disease problems in orchardgrass will be developed.

Following are descriptions and possible control strategies for the two most common pathogens of orchardgrass identified by the Virginia Tech Plant Pathology Clinic between 2004 and 2014.

Anthracnose: *Colletotrichum cereale*

Anthracnose is a common disease of forage grasses. It occurs on a wide range of warm- and cool-season grass species (Crouch and Beirn 2009). It can first be observed as water-soaked spots on leaves. Tan lesions with red or brown edges form on leaves as the season progresses (Braverman et al. 1986). The fungus can spread to the crown and roots of plants and can kill

perennial grasses after a few seasons of infection. Infection is associated with warm, wet weather. Infection can occur from diseased seed or spores and mycelia on dead crop residues. Once the pathogen has developed lesions on a plant, it can spread secondarily from spores and mycelia.

There are limited methods of control for anthracnose in grasses. Spores and mycelia survive in stubble and residue and re-infect swards during the following growing season (Braverman et al 1986). Crop rotation, with non-susceptible crops, is recommended. Recently it has been noted that two species of *Colletotrichum* infect grass crops; *C. graminicola* infects C4 grasses while *C. cereale* infects certain C3 grasses (Crouch and Beirn 2009). Previously, rotations were recommended that excluded grass crops to control *Colletotrichum* in orchardgrass. Since it is now known that *C. cereale* only infects certain C3 crops, using C4 grasses in rotation should help to provide control by breaking the pest cycle. Maintaining soil fertility can reduce infection. Resistant cultivars are recommended (Braverman et al. 1986), but only one study of orchardgrass resistance to leaf streak has been found (Tsukiboshi and Shimanuki 1999) and included few varieties adapted to North America.

Leaf Streak: *Cercosporidium graminis*

Leaf streak, also known as brown stripe, is a common foliar fungal pathogen of orchardgrass and numerous other grasses. Brown stripe infections occur throughout the growing season but are particularly severe in mid-summer (Braverman et al. 1986). Small elliptical lesions form which are brown to dark purple in color. As lesions age and grow larger they often become gray to whitish in color. As severity increases leaves wither and die reducing forage yield and quality (Braverman et al. 1986). The brown and shriveled forages caused by the

infection can give the appearance of drought stress (Abbott 1918). A distinguishing feature for identification of leaf streak is parallel rows of black fasciculate, bundled, conidiophores on lesions (Sprague 1950).

Planting of resistant varieties is the recommended control method (Braverman 1984), but no studies of orchardgrass variety resistance to leaf streak could be found. Burning of grass residue has also been recommended to control inoculum spread (Sprague 1950).

Viral Pathogens

The issue of viral pathogens of orchardgrass in Virginia has not been well studied, and the information available is not clear. There are a number of viruses that infect orchardgrass; cocksfoot mottle virus and cocksfoot streak virus are known to cause significant yield losses in Europe and the United Kingdom (Sutic et al. 1999).

Viruses of orchardgrass in Virginia have been mentioned twice in recent literature. First by, Sforza et al. (2000) in an on-farm survey of barley yellow dwarf virus (BYDV). The second and only mention of the viral pathogen of orchardgrass in Virginia is by Stromberg (2008) in his presentation of orchardgrass diseases wherein he includes BYDV and mentions that “infection adds to stress of orchardgrass.” A search of the literature for negative pathogenic effects of BYDV in orchardgrass did not yield any clear evidence that BYDV causes yield reductions or other problems in orchardgrass. *Dactylis* is an alternate host for BYDV on small grains (Sutic et al. 1999). Stromberg (2008) has been cited several times (Myers 2011; Hall 2014), and it reported the BYDV has caused significant economic damage to orchardgrass in Virginia (R. Clark, personal communication) – this is yet unconfirmed.

Insects

More than 30 insect species have been identified infesting orchardgrass stands (Hardee et al. 1963). Insect damage is known to reduce the yield, quality, and persistence of orchardgrass, but these effects have not been well studied or quantified (Jung and Baker 1973; Christie and McElroy 1995).

Recently, the hunting (*Sphenophorus venatus vestitus* Chittenden) and bluegrass billbugs (*Sphenophorus parvulus* Gyllenhal) have been found to be a pest of orchardgrass (Kuhn et al. 2013). The paired feeding holes left by adults in leaves provides for straightforward identification of damage and has been found in numerous locations in Virginia (Kuhn et al. 2013; G. Jones, unpublished). It has been reported that billbugs can cause 40 – 100% loss of orchardgrass stands in Virginia but the extent of the pests and their damage potential has not been quantified (Kuhn et al. 2013). Appropriate control strategies have not been well developed (Kuhn et al. 2013). Insecticides labeled for use on billbugs in orchardgrass provides suppression only rather than control (Laub 2016).

Climate

Role of Climate and Weather on the Persistence of Perennial Grasses

The climate of a region and weather which occurs during the growing season are major determinants of the adaptation and persistence of perennial forage grasses (Baron and Belanger 2007; Culvenor and Simpson 2014). High and low temperatures and precipitation accumulation and distribution all play important roles in the adaptation of a forage species to a region (Baron and Belanger 2007). Many studies have evaluated the effects of temperature and moisture on the

physiology, morphology, and agronomic performance of cool-season grasses but often in controlled environments. This generally leaves the challenge of integrating these responses to other authors or to readers, and the results presented may not have much bearing on the complex interactions which occur in managed forage swards. There are, however, several studies which have investigated or observed important interactions of weather and management. These will be discussed here and a review and discussion of the effects of climate change will follow.

Researchers in Massachusetts investigated N fertilization, cutting height and first cutting date on the regrowth and non-structural carbohydrates of orchardgrass (Drake et al. 1963; Colby et al. 1965; Colby et al. 1966). They showed that high rates of N reduced stubble carbohydrates, likely because of utilization of photosynthate for growth rather than storage, and because of this when harvest was delayed to early flowering and was followed by high temperature and low soil moisture, that significant injury to that stand occurred. They suggest that the hot, dry conditions following cutting is the main cause of stand decline as evidenced in inter-annual variation in conditions and the lack of plant injury following close cutting of high N treatments in the greenhouse (Drake et al. 1963). Later, Stringer et al. (1981) hypothesized a similar set of interactions could occur to cause stand loss of tall fescue. They did not find a detrimental effect of N and cutting management during their two site-year study, but they did observe temperatures as high as 37°C at 2.5 cm above the soil surface following cutting in mid-June as compared to 23°C in unharvested control plots (Stringer et al. 1981). Korean scientists harvested orchardgrass and tall fescue at 3, 6, and 9 cm on two dates in mid-summer (Seo et al. 1988). They found that regrowth was improved at high cutting heights regardless of cutting date and that low cutting heights and high temperature caused a relative increase in the summer depression of growth (Seo et al. 1988). In a recent study of orchardgrass and white clover (*Trifolium repens* L.) swards in

West Virginia, it was concluded that productivity and persistence of the sown species were less dependent on management practices than the effects of inter- and intra-annual variation in weather (Malinowski et al. 2012). Stand loss of orchardgrass cut at the late bloom stage has been reported in southern Missouri, and this effect is stated to be exacerbated by high temperature conditions following cutting (Henning and Risner 1993). These effects of weather and interactions with management appear to be important determinants of the persistence of orchardgrass. No reports of randomized, replicated heat stress treatments and management interactions for orchardgrass have been found. This is not surprising given the complexity and cost of field-based warming experiments. However, given the changing climate, the effects of high temperature stress and its interactions with the management of orchardgrass may become more apparent.

Predicted Future Climate for Virginia¹

The International Panel on Climate Change (2013) predicts that global mean surface temperatures for 2081 – 2100 will be 0.3 – 4.8°C warmer than mean surface temperature for 1986 – 2005 depending on emissions scenario and region of the globe. It is also predicted that dry regions of the Earth will become drier and wet regions wetter (IPCC 2013). What then is the predicted climate for Virginia?

To predict future climate for Virginia, the coarse resolution of global climate prediction models must be downscaled (Thrasher et al. 2013). A dataset, NASA NEX-DCP30, which is a downscaled version of the 5th Climate Model Intercomparison Program model provides temperature and precipitation predictions under four potential warming scenarios called

¹ This subsection and the following three were adapted from a final project written by the author for BIOL 5114G: Advanced Global Change Ecology, Spring 2014.

Representative Concentration Pathways (RCPs) (Thrasher et al. 2013). An online tool, NEX-DCP30 Viewer, was used to determine predicted maximum and minimum temperatures and precipitation for Virginia for 2075 – 2099 and these results were compared to the values for 1980 – 2004 (Alder et al. 2013). Both average maximum and minimum temperature are predicted to be about 2.8 or 5°C warmer in 2075-2099 than 1980-2004 for RCP 4.5 and RCP 8.5, respectively. Precipitation is predicted to increase by about 0.3 mm day⁻¹ or about 11 cm year⁻¹ for 2075 – 2099 regardless of RCP.

Response to Changing Carbon Dioxide

Cool-season plants, by their leaf structure and C3 photosynthetic apparatus, are not saturated with CO₂, thus these plants respond to increases in CO₂ concentration, while C4 plants do not (Drake et al. 1997; Nowak et al. 2004). It has been shown that the yield of short grass prairie, especially C3 species, increased with elevated CO₂ but the nutritional quality of the forage declined (Morgan et al. 2004). In a chamber experiment with tall fescue, photosynthetic rate, and dry matter yields were greater with elevated CO₂ with both high and low rates of N (Newman et al. 2003). Other authors have not found consistent yield increase with elevated CO₂ (Soussana and Lüscher 2007; Piva et al. 2013).

While an increase in CO₂ generally slightly increases cool-season forage yield, a meta-analysis of Free Air Carbon Enrichment (FACE) experiments found that N fertilization of elevated CO₂ plots results in greater net primary production and greater aboveground production as compared to elevated CO₂ treatments or high N treatments with ambient CO₂ (Nowak et al. 2004). It is thought that CO₂ enriched systems often become nutrient limited and thus respond to added N (Norwak et al. 2004, Piva et al. 2013)

Response to Changing Temperature

It is known that growth of cool-season grasses decreases with increasing temperature above the optimum (Brown 1939; Baker and Jung 1968, Su et al. 2007). Baker and Jung (1968) found that day temperature was more important than nighttime temperature to the yield for four cool-season grasses. Orchardgrass, timothy, and Kentucky bluegrass yields declined at daytime temperatures above 28.2 or 31.5°C, while smooth bromegrass (*Bromus inermis* Leyss.) yield was somewhat more resistant to decline at higher temperature (Baker and Jung 1968).

In a study modeling timothy growth under the predicted future climate, Jing et al. (2013) found that the growing season could start three weeks earlier in 2040-2069 and as a result of the increased length of the growing season, it is possible that one additional harvest of forage could be possible. The effect of the future climate on forage quality was inconsistent depending on the parameter measured and which of two harvest dates was analyzed (Jing et al. 2013).

An author studying the heading dates of orchardgrass between 1972 and 2002 in Poland found that over that period average heading date had advanced by 11 days and this response was correlated with increasing average annual temperature (Grzegorz 2011). The impact of this change in phenology on forage management and persistence has not been studied. It is unknown whether orchardgrass producers in the Mid-Atlantic base their decision to make first cutting on the growth stage of the plant, calendar date, weather, or some combination thereof. It has been shown in meadow foxtail (*Alopecurus pratensis*) swards in Germany that the advance in average first cutting date has not kept pace with the advancing heading date of the species between 1991 and 2011 such that swards are not harvested at somewhat later maturity (Bock et al. 2013).

Response to Changing Precipitation

Adequate soil moisture is important for plant growth. A simulation of alfalfa production across 12 U.S. regions was conducted and moisture, temperature, and CO₂ parameters were manipulated. Across three climate models, moisture was the most important variable controlling yield at the regional scale (Izaurre et al. 2011).

Intraspecific variation in forage plants can result in different response to drought. Poirier et al. (2012) compared temperate and Mediterranean varieties of tall fescue and orchardgrass, and they found that the yield of temperate varieties showed a significantly greater decrease in annual aboveground production for both species as compared to Mediterranean varieties under summer drought stress.

In a field experiment in which a semi-natural cool-season grassland (dominated by *Alopecurus pratensis* L., meadow foxtail, and *Arrhenatherum elatius* L., tall oatgrass) had several drought severity and variability treatments applied, forage yield decline and forage quality increase with increasing drought variability (Grant et al. 2014). The authors note that N fertilization offset some of the effects of drought variability, but that fertilization also changes species composition, and thus recommended fertilization only in cases of exceptional stress for semi-natural grasslands (Grant et al. 2014).

Several studies have shown that elevated CO₂ can help improve the drought resistance of grasses (Yu et al. 2012) and plants in general (Drake 1997; Soussana and Lüscher 2007). Yu et al. (2012) showed that increased CO₂ improve tall fescue's resistance to drought and increased temperature by enhancing plant water status, cell membrane stability, and enhanced photosynthetic capacity.

Discussion of Impacts of Changing Climate

The impacts of climate change on temperate grasslands are complex and varied. For many cool-season forage plants, increased atmospheric CO₂ has a fertilization effect and increases yields. This increase can be offset by increased temperature or reduced moisture caused by climate change. In some cases, N fertilization improved yield response to elevated CO₂. Forage quality and sward species composition can also be affected by changes in CO₂, temperature, and moisture. It is difficult to make long-term predictions of direction and magnitudes of impacts of climate change on global or specific grasslands because of the interactions among many factors.

Little literature was found on forage systems common to the humid eastern U.S., but there is some research on forage species used in the region, though not in the mixtures commonly found. It is predicted that Virginia will see an increase in temperature, precipitation, and atmospheric CO₂ by the end of the century. These are not the most stressful conditions expected from climate change globally because some regions will see a decrease in precipitation concomitant to increase temperature and CO₂ (IPCC 2007).

Warmer conditions could cause growth initiation to begin earlier in the year in Virginia, as suggested for Canada by Jing et al. (2013), and this could reduce the winter-feeding requirements. With higher temperatures, the decline in summer production of cool-season pastures could be exacerbated. Presently it can be difficult to feed livestock from pasture through the summer (Ball et al. 2007), and this could become more difficult in the future with higher summer temperatures. Planting of warm-season (C4) species has been recommended to alleviate summer forage deficiencies (Ball et al. 2007). Under climate change, the benefits of C4

species could be heightened during summer. It is difficult to discern what impact a slight increase in precipitation would have on the cool-season pasture in Virginia.

Without additional N inputs, it is possible that forage quality will decline with increased CO₂. If research from other climates and grassland systems holds true there could be an increase in legume content of pastures in Virginia (Soussana and Lüscher 2007). It is expected that an increase biomass of legumes will fix proportionally more N (Ball et al. 2007) such that increase legume content could provide the needed N to grass plants in the sward to improve yield and quality under climate change.

Given research from other regions and climate projections for Virginia, forage production and quality may not be as severely altered if precipitation increases rather than decreases as predicted. A better understanding of the seasonal growth distributions of forage species during climate change could help to predict the timing and magnitude of feed deficiencies for livestock. With the importance of forage-livestock systems to agriculture in Virginia and the surrounding region, there is a need to better understand the impacts and interactions of temperature, moisture, and CO₂ on these agroecosystems.

Competition

It is not clear that grassland practitioners have an adequate understanding the role of competition, especially for light, in infrequently mown perennial grass swards. The yield and persistence of swards together represent their productivity. However, a vast literature exists indicating that yield and persistence are negatively correlated – as higher yields are obtained the tiller density of swards declines. The deleterious effects of the self-thinning that occurs in swards

which have been allowed to accumulate for conserved feed have been reviewed for perennial ryegrass (Dorrington Williams 1970) and have been discussed in relation to the historic breeding orchardgrass in Europe (Donald 1958), but little review of research conducted in North America has occurred.

Donald (1958) described a flaw in the early selection and breeding of orchardgrass and a misunderstanding of the relationship between persistence and productivity. In a classic study, Stapledon (1928) collected and compared ecotypes of orchardgrass from fence lines and old grazed pastures. Fence line ecotypes were tall and sparsely tillered whereas ecotypes from grazed pastures were short, dense, and highly tillered. Based on that work, it was concluded that the traits associated with the pasture ecotypes would confer improved forage, meat, and milk production. However, later trials comparing short, dense ecotypes with and tall, sparse ones found improved yield and productivity in the tall, sparse ecotypes rather than the short and dense ones. A main conclusion drawn was that traits for persistence are not necessarily related, and are sometimes the opposite, of those associated with high productivity (Donald 1958).

Modern breeding programs in North America have focused their selection on high yielding and highly-digestible varieties (Casler et al. 2000) with relatively little concern for persistence longer than four years (P. Ballerstadt; S. Reid; personal communication). To maximize yield harvested, one must remove a maximum of plant biomass. Varieties that are highly erect allow the maximal herbage to be removed by cutting and contribute to yield. However, this will leave minimal leaf area in swards following harvest and could contribute to limited regrowth.

Indeed, there are many cases in which practices shown to increase yield also reduce the persistence of swards. Fertilization with N is commonly shown to increase yield but also causes

thinning of tillers at ground level including in experiments with orchardgrass (Griffith and Teel 1965; Rease and Decker 1966; George et al. 1973; Singer 2002). Raese and Decker (1966) found that increasing annual N application from 75 to 225 lbs N acre⁻¹ yr⁻¹ increased Potomac orchardgrass yield from 3.1 t acre⁻¹ to 4.3 t acre⁻¹ but also that percent orchardgrass cover declined from 93% for the lower N treatment to 65% for the higher N treatment after three years of that management. Griffith and Teel (1965) found a similar response from orchardgrass in Iowa. Plots fertilized with 25, 150, and 300 lbs N acre⁻¹ showed increasing yield with increasing N, but also showed increase in stand reduction with increasing rates of N. Stand reduction was further exacerbated by low cutting (2 inches) and in some cases by a lack of amended K. Donohue et al. (1981) studied Potomac orchardgrass and found increasing yield but no reduction in tiller density with increasing N rates up to 2,100 N kg ha⁻¹. This effect was likely seen because individual plants were grown in pots in a controlled chamber and only for three weeks; thus competition for light and self-thinning appeared not to have occurred.

Initial date of cutting and cutting frequency also tie competition for light to persistence and regrowth. Cherney et al. (1986) found that delaying cutting of orchardgrass from mid-May until late July caused a reduction in tiller number while either increasing or having no effect on yield. A similar effect was found for tall fescue in that study, and this has been proposed as a reason why the long rest periods associated with high-density stocking of mature swards may be deleterious to long-term stand persistence (E. Rayburn, personal communication). Papadopolis et al. (1995) found higher tiller density in rotationally-grazed orchardgrass swards as compared to those harvested thrice annually for hay.

With the notion of competition for light in mind, it would be expected that an N supply × cutting frequency interaction would occur. This has been shown in several studies (Marten et al.

1979; Jung et al. 1974; Griffith and Teel 1965). Marten et al. (1979) found that high rates of wastewater effluent ($668 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) caused orchardgrass ground cover to decline to 28% as compared to 81% for a fertilizer control ($308 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) after four years of being harvested twice per year. When harvested three times per year the OG ground cover was 90% for the fertilizer control and 55% for the high rate of effluent, and when harvested four times per year OG ground cover was 91% for the fertilizer control and 80% for the high rate of effluent treatment.

Competition for light within swards explains these effects. As the sward canopy closes, individual tillers will compete to reach their leaves into the light. Large tillers tend to have an advantage in this regard; it has been shown in perennial ryegrass the youngest tillers are most likely to die in the event of stress (Ong 1987). Mortality of small tillers increases in low light. This allows the largest tiller to dominate that sward (Ong 1978).

Mitchell (1967) conducted an experiment with S-37 orchardgrass in Delaware. He manipulated cutting height (1 vs. 3 inches), N rate (0, 100, 200, 300 lbs N acre⁻¹) and irrigation (irrigated vs. non-irrigated). Yields were increased with increasing N rate, irrigation, and low cutting. He concluded, "Each of these practices, however, can contribute to stand depletion unless counter-balanced by adjustments to the other two. Heavy applications of nitrogen applied in split-applications, coupled with irrigation and a 1-inch harvest height, can destroy an orchardgrass stand in one season."

Size/Density Compensation and Self-Thinning Theory and Their Application

The concept of size/density compensation of tillers in a grass sward is a well-understood response to defoliation (e.g. closely-grazed pasture will have many tillers each of small mass, while swards conserved for forage contain relatively few tillers each with high mass)(Stapledon

1929; Bougham 1957; Langer 1963; Morris 1969; Davies 1988; Briske 2007). Further, valuable, and likely underexploited, insight can be gained from application of the ecological theory of self-thinning to size/density compensation in swards. Yoda et al. (1963) described the phenomenon that regardless of initial density, plants would grow up to and then self-thin along a line with slope $-3/2$ on a plot of log tiller density by log average tiller weight. They termed this ‘the $3/2$ power law of self-thinning.’ while there has been some controversy about the exact slope of this line and its basis (Lonsdale 1990; Sackville Hamilton et al. 1995; Enquist et al. 1998), it has been shown to describe the self-thinning of species varying in size by 12 orders of magnitude (Enquist et al. 1998). This ecological theory of self-thinning can be applied to the management of grassland systems and provides an intuitive link between tiller size and density and productivity (Matthew et al. 1995; Matthew et al. 1996; Hernandez Garay et al. 1999).

The $-3/2$ slope size/density compensation line is the upper limit of leaf area assuming constant tiller geometry (Hernandez Garay et al. 1999). Matthew et al. (1995) proposed that a relative shift in the y-intercept of the self-thinning line is a relative shift in leaf area production potential as compared to other otherwise similar swards. The distance between the apparent self-thinning line and the coordinates of a sward or treatment is the quantitative measure of this productivity index (Matthew et al. 1995; Hernandez Garay et al. 1999). This has been shown in perennial ryegrass mini-swards (Hernandez Garay et al. 1999), rhodesgrass (*Chloris gayana* Kunth.) mini-swards (Martinez Calsina et al. 2012), and field-grown tall fescue and prairie grass (*Bromus catharticus* Vahl) (Scheneiter and Assuero 2010).

No references have been found for this use of an SDC-based approach to sward productivity in North America. Application of this approach could provide clarity into the actual effects of treatments after correcting for expected changes in size/density compensation. The

difference in leaf area potential is a reasonable measure to assess relative persistence among treatments and would help in discerning the relationships among management factors and productivity and persistence.

Summary

Clearly there are numerous factors which affect the growth, yield, and persistence of orchardgrass and other cool-season grasses. The interactions among those factors are particularly important. The loss of stands based on management factors is likely a response to high order cutting height \times cutting frequency \times N supply \times K supply \times disease severity \times temperature \times soil moisture interactions. While it is likely impossible to conduct and interpret an experiment investigating such high order interactions, an understanding of the lower order interactions based on previous literature and experiments conducted for this dissertation should help to improve management.

It is also valuable to consider the complex responses to management in terms of the plant biology and physiology rather than management practices. In order to refoliate quickly and resume tillering after cutting, swards require an adequate number of tillers with sufficient photosynthetic capacity of leaf area, adequate non-structural stubble reserves and adequate temperature and moisture.

Some management factors are easily controlled by producers while others are not. The clear and dry weather conditions required to dry hay are likely antithetical to the weather conditions which would yield maximal regrowth. Also, presently, management of disease in orchardgrass swards is nearly impossible. With no fungicides available, and questionable economic returns therefrom, and a lack of available cultivars and breeding and evaluation for the

diseases present in the Mid-Atlantic, it is not clear how disease problems will be resolved in the near future.

Management recommendations and practices tend to be based around the optimization of yield and forage quality or solely maximizing yield rather than optimization for persistence.

While there is an extensive history of experiments measuring yield and tiller density or stand score, generally the interpretation of those do not adequately distinguish the expected changes in tiller density cause by size/density compensation and that caused by stress-induced tiller loss.

The ecological theory of self-thinning and the use of size/density-corrected productivity calculations appears to be a valuable approach for making that distinction.

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Chapter 3. Survey of Orchardgrass Hayfields in Four Mid-Atlantic States

Abstract

Orchardgrass (*Dactylis glomerata* L.) is an important species for hay production and livestock feed in the Mid-Atlantic; however recently, producers have observed a marked decline in orchardgrass persistence in hay fields. The objective of this survey was to assess relationships among soil fertility, management practices, disease, and climate to producer-perceived stand persistence rating of orchardgrass hay fields, OG biomass present, and established critical thresholds. Fifty-three orchardgrass fields were sampled in VA, PA, MD, and WV in spring 2014. Planting information and management details were collected about each stand. Soil samples were collected for routine analysis, standing biomass was harvested, sorted, and green tissue was analyzed for mineral nutrient content, and tillers were evaluated for leaf disease. An information theoretic approach to multi-model inference was used to determine the most important explanatory variables. Available soil P, soil OM, manure application, planting year, and historic average maximum temperature were related to perceived OG persistence. Growth stage and soil P were related to green OG biomass at sampling, and average soil test pH, P, and K were adequate relative to critical values for those factors. Soil fertility does not appear to be the main driver of OG persistence problems, but climatic adaptation seems to play an important role in persistence.

Introduction

Orchardgrass is one of the most commonly planted perennial cool-season forage grasses in the world. It provides an important source of feed to beef, dairy, and equine industries in the Mid-Atlantic United States (Ball et al. 2007). Since about 2000, producers in the Mid-Atlantic have noticed a reduction in persistence and vigor of their orchardgrass stands (Tracy, 2011; Clark, 2009). Reduced persistence of this perennial grass increases costs of production when frequent replanting is required. Numerous factors affect the ability of a sward to maintain a population of growing tillers or to persist. Soil fertility, harvest management, and foliar disease have all been associated with persistence problems in forage grasses (Culvernor and Simpson 2014; Smith and Saylor, 2012; Griffith and Teel 1965).

Proper crop nutrition is required to initiate and maintain tiller and leaf growth and development. Nitrogen fertilization is essential to the production of high yielding orchardgrass (Van Santen and Sleper 1996; Jung et al. 1974). However, N fertilization can have varied effects on tillering and persistence. In controlled environments, the addition of N improved tiller production (Auda et al. 1966), but in field settings high agronomic rates of N can cause stands to thin (Raese and Decker 1966). Both N and K supplies are important factors affecting persistence of orchardgrass. Several studies have shown that additions of fertilizer N and K reduce the deleterious effects of low cutting height on persistence of orchardgrass (Smith and Saylor, 2012; Griffith and Teal, 1965). If nutrient deficiencies, especially K, are common in soils of orchardgrass fields, this could be a cause of the recent observed reduction in persistence.

Cutting date, cutting frequency, and residual stubble height after mowing are important factors in tiller demographics and the regrowth potential of forage grasses. Cutting date and cutting frequency both play a role in the available carbohydrate reserve and in the residual

photosynthetic area of orchardgrass following cutting (Griffith and Teel 1965; Ward 1961). Low residual height can remove meristematic tissue which then must be regrown before leaf regrowth can commence. When all green leaves are removed at harvest, non-structural carbohydrates are the main source of energy for regrowth until sufficient area of photosynthetic tissue is achieved (Ward and Blaser 1961). With the adoption of disk-type mowers in the 1980s, producers can achieve lower residual cutting heights than previously possible with sickle-bar type mowers (Adams 1996). Low cutting by disk-type mowers has been a primary hypothesis as to the cause of decreased persistence observed in orchardgrass.

Infection by pathogens is known to reduce the persistence of perennial forage crops (Leath 1996). Foliar pathogens reduce photosynthetic leaf area, crude protein, and non-structural carbohydrates of orchardgrass (Mainer and Leith 1978). Numerous foliar pathogens have been found infecting orchardgrass in Virginia (Roane and Roane 1996) and the surrounding region (Elliott 1962; Braverman et al. 1986), but to our knowledge no attempt has been made to relate reduced persistence of orchardgrass to disease incidence in mown swards in the Mid-Atlantic.

Several factors are known to affect and interact the persistence of forage grasses. However, these factors are so varied that without additional information to characterize the problem, recommending practices to ameliorate the persistence concerns is difficult. A regional survey of orchardgrass fields will allow for the quantification of factors related to persistence and should elucidate the driving cause. Thus, the objective of this survey was to assess relationships among soil fertility, management practices, disease status, and climate to producer perceived stand persistence rating of orchardgrass hay fields, OG biomass, and established critical thresholds.

Methods

Site Selection

Orchardgrass fields in Virginia, West Virginia, Pennsylvania, and Maryland were identified with the help of local Extension agents. The aim in selecting sites was to include fields comprised of >50% orchardgrass located in a wide range of environments under varied management strategies and to include fields perceived to be experiencing persistence problems as well as those which were more long-lived.

Data Collection

Each site was visited and sampled between March 19-April 7, 2014. A representative location in each field was selected and if major variation was visually observed or reported by the producer, a second location representative of the differing area was also selected for sampling. The point was georeferenced by GPS and a 5 m diameter circle was flagged around that point; all sampling was conducted within the flagged circle.

Six soil cores (1.5 cm diameter by 15 cm depth) were collected, divided into 0-5 cm and 5-15 cm depth samples, and the cores for the respective depths were aggregated for further analysis. Soil pH, extractable nutrient concentrations, and loss on ignition organic matter were determined for both sample depths by the Virginia Tech Soil Testing Laboratory. Phosphorus and K were extracted using the Mehlich 1, 0.05N HCl in 0.025N H₂SO₄, extracting solution before analysis by inductively-coupled plasma atomic emission spectrometry (ICP-AES). Soil micronutrient concentrations were not evaluated here. Soil pH is a main determinant of the availability of micronutrients (Havlin et al. 2005) such that a persistence or biomass response to pH could indicate a micronutrient deficiency, but no reports of micronutrient deficiency in mown

orchardgrass have been found in the literature. A stratification ratio was calculated to assess the effect of vertical nutrient stratification which is often caused by fertilizer application in reduced tillage production systems (StratRatio; SoilP(0-5cm):SoilP(5-15cm)). Since soil was analyzed separately for the 0-5 cm and 5-15 cm, the weighted average of the two samples was calculated to provide results for 0-15 cm depth of the soil profile. Weighted average soil P, K, pH and OM for 0 -15 cm depth were calculated as: $\text{Result 0-5 cm} + (2 * \text{Result 5-15 cm}) / 3$ for variables SoilP, SoilK, SoilpH, and SoilOM respectively.

At each site, three 0.25 m² quadrats of herbage were harvested at 2.5 cm above the soil surface when the sites were visited in March and April 2014. Samples were hand-sorted into three classes: green orchardgrass, legume/broadleaf/grass weeds, and dead material. All samples were dried at 60°C for at least 48 h and were weighed. Green orchardgrass dry matter was averaged for the three quadrats (OG Biomass). Tissue N and S concentrations of the green OG fraction were determined using microwave-assisted acid extraction and ICP-AES at A & L Eastern Labs, Richmond, VA. The ratio of tissue N to S is a useful tool in determining sulfur adequacy or deficiency (Reneau 1982; Baker et al. 1972). The ratio of tissue N to tissue S was calculated (NSRatio; TissueN:TissueS).

Orchardgrass tillers were collected at each site for disease injury evaluation. Leaves from 9 haphazardly collected tillers were visually scored for foliar disease injury (necrotic spots) as a percentage of leaf area using aids from Saylor et al. 2013. The presence/absence of leaf disease was determined for each site (Disease). The developmental growth stage of each of the 9 tillers was determined (Moore et al. 1991) and the mean stage by count was calculated (GrowthStage).

Historic average high temperature was determined for each field in a geographic information system using a locally-weighted temperature model based on data from 1981-2010 and applied in an 800 m grid (AvgMaxTemp)(PRISM 2016).

The producer who managed each field was queried to determine relevant management practices. Year of planting (PlantYear), variety planted (Variety), cutting height used, number of cuttings yr⁻¹, application of manure (Manure), and the use of grazing livestock (Grazing) were determined. Producers were also asked to respond to the question, “Considering the past three growing seasons, how would you rate the yield, vigor, and persistence of this orchardgrass stand as compared to others you have seen or managed during that time?” Responses were quantified using a 1 – 7 Likert scale where 1 was poor and 7 was exceptional (Score).

Statistical Analysis

The statistical analysis for the study was conducted using R 3.0.2 (R Development Core Team, 2013). Summary statistics were calculated for soil nutrients and were compared to established critical values (Maguire and Heckendorn 2015) for the respective nutrients using one-sided one-sample t-tests ($\alpha < 0.05$).

An information theoretic approach (Burnham and Anderson 2002) was used to select models to explain Score and to explain the OG biomass. A reduced set of 14 explanatory variables was developed to minimize multicollinearity and assess pertinent hypotheses (Table 1). Several variables were excluded because of missing values for several fields. Cutting height and number of cuttings were removed from the variable set because of incomplete data; preliminary subset analysis found these to not be important variables in explaining Score or OG Biomass (Data not shown).

An all-possible regressions algorithm, *dredge*, from the R package MuMIn was used (Bartoń 2015), and the resultant models were ranked by sample size-corrected Akaike Information Criteria (AICc), where lower AICc indicates a relative improvement in the model, for both the persistence score and for the OG Biomass. Models with an AICc < 10 units greater than the top model were averaged. Akaike weight of a model is the probability that that model is the actual best fitted model in the set (Anderson 2008) The importance of explanatory variables was determined based on the sum of Akaike weights across models containing that explanatory variable (Burnham and Anderson 2002). Models with an AICc < 2 units greater than the top model were considered ostensibly equivalent to the top model and adjusted R² and overall significance were determined for each. Residuals for top models for Score and OG biomass were evaluated for normality and outlying values, and no transformations were made.

Results

Summary Statistics

Fifty-three orchardgrass fields were sampled in Virginia, West Virginia, Maryland and Pennsylvania (Figure 3.1). These fields were located in 19 counties and were farmed by 35 producers. Producers scores of perceived stand persistence ranged from 2 to 7 with an average of 4.5. Producers reported taking between 0 and 5 hay cuttings during 2013, and the average was 2.54 cuttings per field during that year. The average cutting height reported by producers was 8 cm above ground-level. The range of cutting heights was 2.5 to 20 cm. Fields were planted with 10 varieties (Table 3.2). The most commonly planted varieties were Benchmark, WP300, and Benchmark Plus.

SoilpH, SoilP, and SoilK varied considerably but means were greater than critical value thresholds ($p < 0.001$) (Table 3.3).

Persistence Score Model Selection

The multi-model selection process and averaging of top models are tools that allow for the determination of which variable should be included in the top models and the relative importance of those variables. The model selection process yielded top models, those with AICc < 2 , which explained approximately half of the variation in persistence score ($R^2 = 0.47-0.52$). Variables in the top model included SoilOM, SoilP, Grazing, Manure, PlantYear, and AvgMaxTemp (Table 3.4). SoilpH and StratRatio were also included in some models with an AICc < 2 units greater than the top model (Table 3.5). Variables of lesser importance are shown in Figure 3.2.

OG Biomass Model Selection

The minority of variation in OG Biomass was explained by variables studied here ($R^2 = 0.28-0.34$). Model selection for evaluating OG Biomass yielded a top model which included SoilP and GrowthStage as explanatory variables (Table 3.6). SoilK, SoilpH, StratRatio, N:SRatio, and AvgMaxTemp were also included some models with an AICc < 2 units greater than the top model (Table 3.7). Variables of lesser importance are shown in Figure 3.2.

Discussion

Summary Statistics

Average soil test pH, P, and K were all above established critical thresholds. While several fields had soil test results which might indicate a yield-limiting deficiency, the majority of fields should not be limited by pH or these nutrients. However, the critical soil nutrient concentrations used were developed for optimizing crop yield rather than persistence and as such may not necessarily be good indicators of adequate fertility for stand longevity.

Persistence Score Model Selection

The model selection process yielded top models which explained about half of the variation in persistence score. Other factors, not measured here, related to the biology or management of the fields or the perception of the producers must also be related to persistence score.

Manure is an important fertility and organic matter source in some cropping systems. Here, reported additions of poultry litter or other types of manure (beef, dairy, swine) were both related to increased persistence score. However, Manure was not correlated to SoilP, SoilK, or SoilOM from these fields (Data not shown). Poultry litter application was strongly related to persistence score, but only 8 fields had litter applied and 7 of these were located in one county in the Shenandoah Valley of VA. Either poultry litter confers some little-understood benefit for persistence or producers from that one county have somewhat different expectations of OG persistence and also happen to have access to and apply poultry litter.

As available soil P increases from low levels, forage yield and tillering increase to a plateau (Havlin et al. 2005). Plant toxicity from very high soil P is rarely a concern although high soil P concentrations can lead to environmental degradation. Unexpectedly, increasing soil P was negatively related to persistence score. Since most soils from the survey met the

minimum P concentration to support hay production it might be expected that there would be no significant positive relationship with SoilP. One explanation might be that high soil P concentration has induced a Zn deficiency in some fields (Havlin et al. 2005). Reports of this effect could not be found in the literature for forage grasses, and a simplified analysis with persistence score and both tissue and soil Zn and P found no correlation among those variables (data no shown). Another possible explanation is that the high soil P is a legacy of historic fertilization or manurial practices. If high fertility had been previously applied as blended NPK fertilizer or animal manure, an imbalance of nutrients may have been applied relative to crop removal. Large inputs of available N have been shown to decrease the persistence of OG (Raese and Decker 1966; Griffith and Teel 1967), and if the fertility source also included P, accumulation of soil P might be expected with decreased persistence score.

Persistence score increased as soil OM increased. While organic matter is not usually specifically implicated in persistence of perennial forages, it does confer numerous agronomic benefits to cropping systems such as increased watering-holding capacity, aggregate stability, cation exchange capacity, and N, P, and S supply, all of which may improve general growing conditions and thus persistence (Brady and Weil 2010). The capacity of a soil to hold organic matter varies with texture. Increased clay and silt content are associated with increased organic matter. Laboratory texture was not determined here. Soil texture based on UDSA NRCS Official Soil Description was determined for each field and used in preliminary variable selection. Because soil texture was included as a variable of only very few models of those with AICc < 20 units better than the top model, it was excluded from further analysis (Data not shown).

Average historic maximum temperature was negatively related to persistence score in the top models. This is an expected result as OG employs C₃ photosynthesis which operates

inefficiently at high temperatures. Both summer heat stress (Colby et al. 1966; Drake et al. 1963) and planting at low latitudes in the eastern U.S. (Van Santen and Sleper 1996) have been attributed to reduced OG persistence.

The year of stand establishment was positively related to persistence score. While orchardgrass is generally considered a long-lived perennial (Van Santen and Sleper 1996), stands do thin with time, especially when management practices to maximize yield are applied (Alexander and McCloud 1962; Mitchell 1967).

Other variables were less well related to persistence score. Grazing, StratRatio and SoilpH were moderately important variables. Grazing rather than or in addition to mechanically harvesting forage can improve nutrient cycling (Haynes and Williams 1993), apply somewhat less severe defoliation, and may allow for improved tiller development (Brink et al 2010; Bryant and Blaser 1966), so might be expected to improve persistence. Nutrient stratification is a concern associated with surface application of nutrients in no-tillage and conservation-tillage systems and perennial crops, but it is generally not associated with yield reduction (Karlen et al. 1991). Soil pH is an important master variable in soil and can be a cause of nutrient deficiency (Havlin et al. 2005), although here the pH of most soils was appropriate to allow adequate supply of nutrients.

Least important variables in top models were SoilK, N:SRatio, Disease, Variety, and GrowthStage. Additions of K have been implicated in improved persistence of OG (Griffith and Teel 1965) and are generally required for high yield (Havlin et al. 2005), but here soil K was not related to perceived persistence indicating that the K fertility management used is generally adequate to support stands. Nitrogen:sulfur ratio is an indicator of S adequacy, but S did not appear to be important to OG persistence in this survey. Disease presence is known to reduce the

carbohydrate status of OG (Mainer and Leith 1978) and thus could reduce yield or persistence. However, in this case, disease presence was not related to persistence. This could be because diseases in spring are different and generally less severe than those during summer (Elliott 1962). Summer disease evaluation could have more bearing on persistence. Variety of orchardgrass planted might be expected to relate to persistence because some commercial varieties would be better adapted to the Mid-Atlantic States than others, but here a relationship with persistence score was not found. The stage of growth is an important variable in explaining the mass of forage present at a moment in time, but it is expected that it is not related to persistence.

OG Biomass Model Selection

The relationship between explanatory variables and OG biomass was weaker than with Score. The increased number of models < 2 AICc greater than the top model indicates the relative weakness of all of the best models. Nonetheless, GrowthStage and SoilP were the most important variables in explaining OG biomass, appearing in $> 90\%$ of top models.

Stage of growth was the most important variable explaining OG biomass. During vegetative growth, the number of leaves per tiller or stage of growth is positively correlated to forage mass (Turner et al. 2006) such that the results seen here would be expected.

Orchardgrass biomass also increased as SoilP increased. Increased available P has been shown to increase forage growth during the early spring in ryegrass (*Lolium perenne* L.) pasture in New Zealand (Gillingham et al. 1998; Saunders and Metson 1970) and Ireland (Sheil et al. 2016). It has been suggested that P demand in early spring may be greater than can be supplied in low P soils because of low soil temperature limiting rooting volume (Sheil et al. 2016). This

would explain the increase in aboveground OG Biomass with increasing SoilP found in this survey.

Conclusions

Most producers found their OG persistence to be approximately average as compared to other fields they observed in 2013. Nutrient deficiencies did not appear to drive OG persistence problems across the region. While the climatic adaptation of OG must vary depending on variety, here we found that planting in cooler environments appears to improve persistence without significant effect of variety. If climate continues to change as predicted, the range of OG adaptation may move northward and upward in elevation. Local variety trials of orchardgrass should help producers select the best locally-adapted OG cultivar, but these trials generally focus on short-term yield and rarely evaluate longer-term persistence. While soil fertility and disease status are important factors often associated with stand persistence, these were not found to be important variables in explaining OG persistence in this survey. It was not possible to include cutting height in this analysis, but based on a large body of literature, cutting height is an important determinant of the yield and persistence of swards. Large-scale surveys of forage systems can help to elucidate production challenges, but the high cost and labor requirement for repeated measurements of fields can be prohibitive. Future research of this type may benefit from aerial or satellite remote sensing to quantify changes in biomass throughout the growing season. A more complete set of temporal biomass production data in combination with ground-truthed plant, soil, and management information could offer a more complete evaluation of the drivers controlling persistence.

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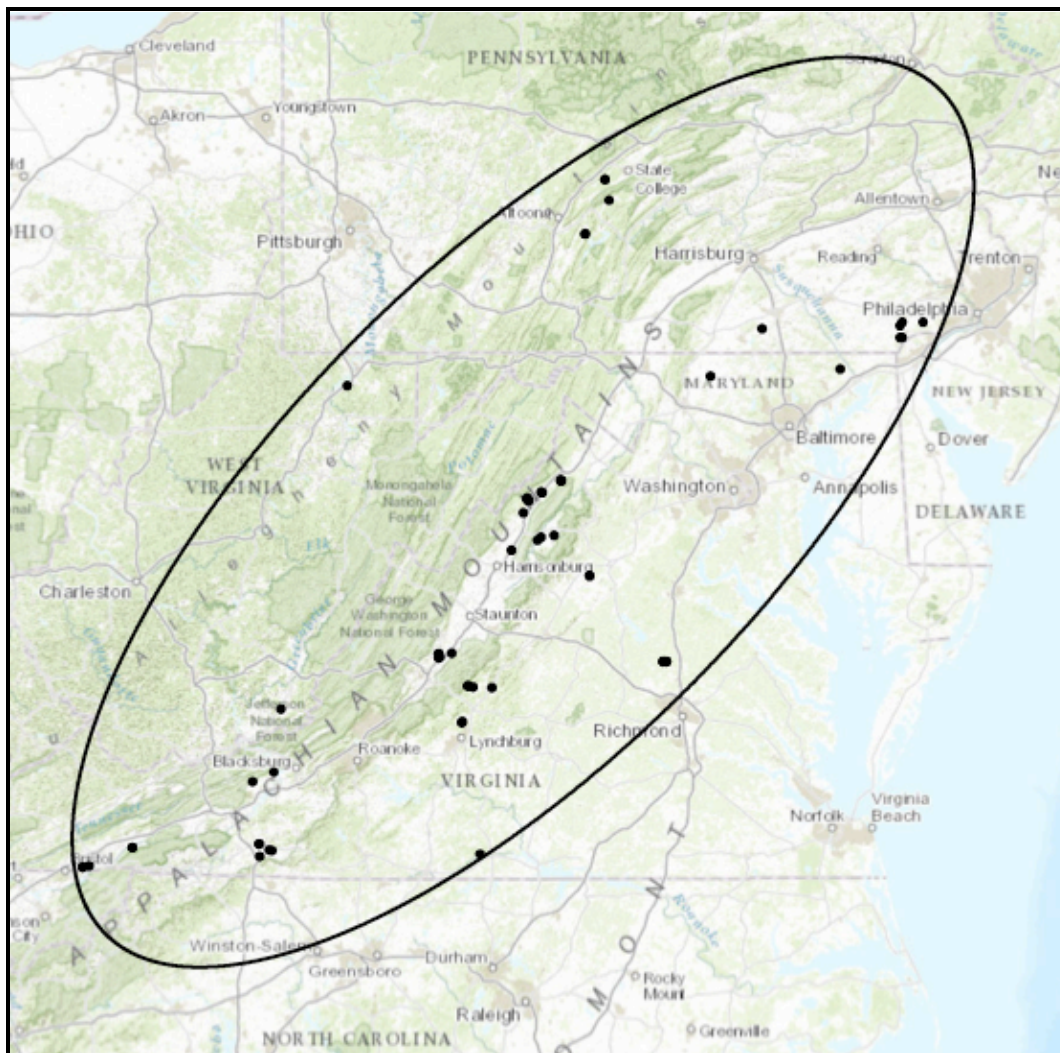


Figure 3.1: Fields sampled during survey (Mar. 19 –Apr. 7, 2014)

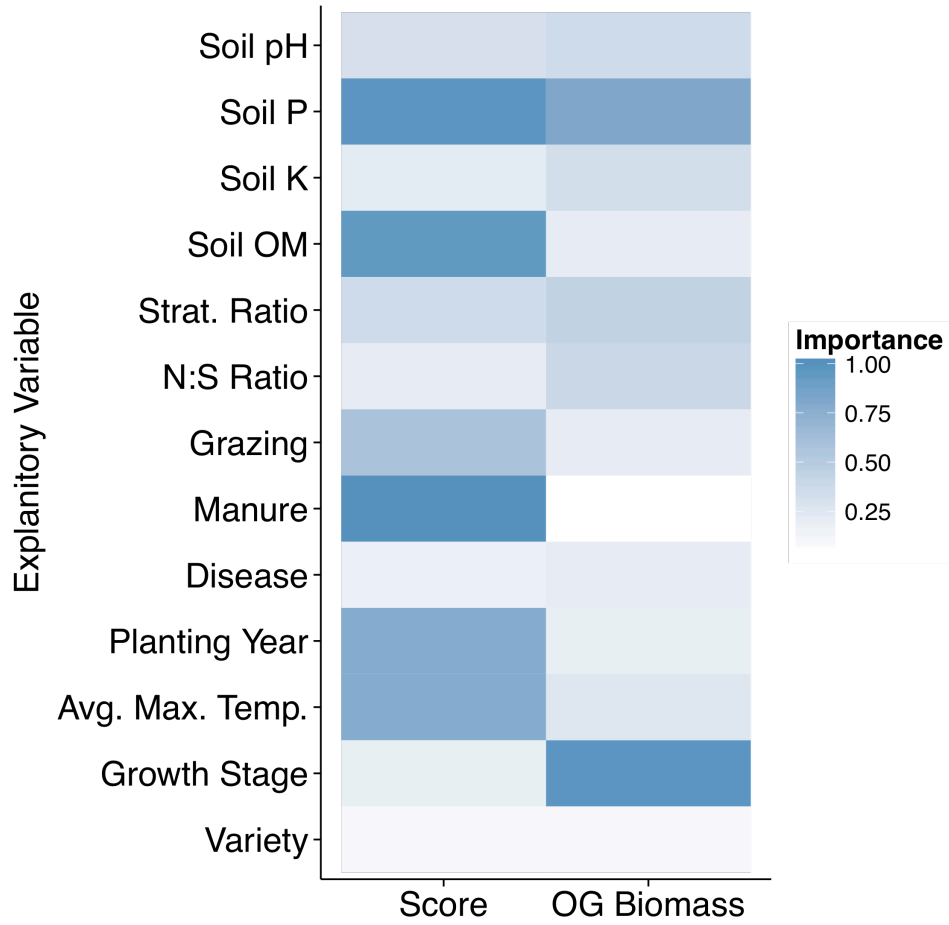


Figure 3.2: Variable importance in explaining persistence score and OG biomass. Averaged from models with AICc < 10 units greater than the top model.

Table 3.1: Variable set used in model selection

Variable	Type	Description
SoilpH	Continuous	1:1 water pH (0-15cm depth)
SoilP	Continuous	Mehlich 1 extractable phosphorus (0-15cm depth)
SoilK	Continuous	Mehlich 1 exchangeable potassium (0-15cm depth)
SoilOM	Continuous	Loss on ignition soil organic matter (0-15cm depth)
StratRatio	Continuous	Soil nutrient stratification. Soil P (0-5cm): Soil P (5-15cm)
N:SRatio	Continuous	OG tissue nitrogen:sulfur. Indicator of sulfur deficiency
Grazing	Categorical	Any grazing by livestock during 2013 (No, Yes)
Manure	Categorical	Application of manure during 2013 (No, Poultry Litter, Other)
Disease	Categorical	Presence of foliar pathogen/necrotic spots (No, Yes)
PlantYear	Continuous	Year of stand establishment
AvgMaxTemp	Continuous	30-year annual average high temperature (1981-2010) (PRISM Climate Group 2016)
GrowthStage	Continuous	Growth stage (Moore et al. 1991)
Variety	Categorical	Orchardgrass variety planted (Table 2)

Table 3.2: Orchardgrass varieties planted as reported by producers

Variety	Number of Fields	Breeding Program
Benchmark	10	FFR
WP300	6	Hancock Seed
Benchmark Plus	5	FFR
Hallmark	4	FFR
Common	2	Public
Intensiv	2	Barenbrug
Liberty	2	Unknown
Athos	1	DLF
Baridana	1	Barenbrug
Pennlate	1	Penn State
Unknown/Unreported	19	-

Table 3.3: Summary statistics for soil test pH, P, and K (0-15cm), critical values, and p-value for one-sided one-sampled t-tests

Soil Variable	Minimum	1 st Quartile	Mean	3 rd Quartile	Maximum	Critical Value	p-value
pH	5.4	5.9	6.3	6.7	7.5	5.5	<0.0001
P (ppm)	4.7	11.3	36.6	42.0	157.0	6	<0.0001
K (ppm)	16.0	35.0	67.4	92.3	253.3	28	<0.0001

Table 3.4: Top model explaining persistence score based on AICc.

Term	Coefficient Estimate	p-Value
Intercept	-243	0.0212
SoilOM	0.270	0.0226
SoilP	-0.011	0.0023
Grazing	0.625	0.0698
Manure (Litter)	1.36	0.0002
Manure (Other)	0.772	0.0409
PlantYear	0.124	0.0179
AvgMaxTemp	-0.197	0.0250

Table 3.5: Model significance, R^2 , and AICc for the top model explaining persistence score and models < 2 AICc units greater than the top model.

Model Terms	p-Value	R^2	AICc
SoilOM + SoilP + Grazing + Manure + PlantYear + AvgMaxTemp	<0.0001	0.51	137.22
SoilOM + SoilP + Manure + PlantYear + AvgMaxTemp	<0.0001	0.47	138.23
SoilOM + SoilP + SoilpH + Grazing + Manure + PlantYear + AvgMaxTemp	<0.0001	0.52	138.44
SoilOM + SoilP + Grazing + Manure + PlantYear + StratRatio + AvgMaxTemp	<0.0001	0.52	139.20
SoilOM + SoilP + Manure + PlantYear + StratRatio + AvgMaxTemp	<0.0001	0.49	139.43

Table 3.6: Top model explaining persistence score based on AICc.

Term	Estimate	p-Value
Intercept	-5.46	0.0734
SoilP	0.019	0.0110
GrowthStage	7.02	0.0065

Table 3.7: Model significance, R^2 , and AICc for the top model explaining green OG biomass and models < 2 AICc units greater than the top model.

Model Terms	p-Value	R^2	AICc
SoilP + GrowthStage	0.00013	0.30	220.61
SoilP + Strat. + GrowthStage	0.00022	0.33	221.07
SoilP + N:SRatio + GrowthStage	0.00026	0.32	221.47
SoilK + SoilP + GrowthStage	0.00027	0.32	221.48
SoilP + SoilpH + GrowthStage	0.00027	0.32	221.54
Strat. + GrowthStage	0.00027	0.28	222.07
SoilK + SoilP + Strat. + GrowthStage	0.00037	0.34	222.07
SoilK + SoilP + N:SRatio + GrowthStage	0.00037	0.34	222.08
SoilP + SoilpH + Strat. + GrowthStage	0.00038	0.34	222.16
SoilP + SoilpH + N:SRatio + GrowthStage	0.00039	0.34	222.23
SoilP + GrowthStage + AvgMaxTemp	0.00039	0.31	222.38
SoilP + Strat. + N:SRatio + GrowthStage	0.00042	0.34	222.41
SoilK + SoilP + N:SRatio + GrowthStage	0.00037	0.34	222.41

Chapter 4. Physiological effect of high temperature and cutting height on regrowth vigor in orchardgrass

Abstract

Producers of orchardgrass (*Dactylis glomerata* L.) hay in the Mid-Atlantic U.S. have experienced a reduction in regrowth vigor and a decline in the persistence of their swards. The common management practice for the region is to harvest the first growth of hay by cutting at 2.5 – 7.5 cm height in May or June. We hypothesize that high temperature and low cutting height interact to limit the regrowth rate. To test this, orchardgrass plants were cut to either 2.5 or 7.5 cm and then placed into environmentally-controlled chambers with a constant temperature of 20 or 35°C. Stubble was harvested on days 0, 1, 3, and 11 following cutting and subjected to metabolite analysis. Photosynthetic parameters were measured in the regrown leaves on days 3 and 11, and regrowth biomass was recorded on day 11. Under optimal growth temperature (20°C), vegetative regrowth upon defoliation was significantly enhanced when more stubble tissue remained (7.5 cm cutting height). However, the positive effect of high cutting height on regrowth vigor was not observed under heat stress. Carbohydrate and nitrogen reserves in stubble are major resources to support regrowth of vegetative tissue upon defoliation. The abundance of starch, water-soluble and ethanol-soluble carbohydrates decreased upon defoliation at 20°C, relative to their levels on day 0. However, high temperature stimulated the accumulation of starch and ethanol-soluble carbohydrates in plants cut to 7.5 cm. Heat-activated sugar accumulation was not observed at low cutting height, which is most likely related to reduced photosynthetic performance, stomatal conductance, and photosystem II photochemistry. Similar to carbohydrates, the levels of protein, amino acids, nitrate, and ammonium were elevated under

high temperature, especially in plants cut to 7.5 cm. It is anticipated that these metabolic responses to heat stress result in stabilization of membrane structure and detoxification of reactive oxygen species. However, modified allocation of carbohydrate and nitrogen reserves under heat stress leads to the inhibition of vegetative regrowth upon defoliation. These data suggest that cutting height management for orchardgrass may be more effective for its regrowth vigor and productivity in cool seasons or when cool weather follows hay harvest.

Introduction

Orchardgrass is a high yielding cool-season forage grass which is a valuable feedstuff for several classes of livestock. Globally, orchardgrass is the 4th most produced perennial cool-season forage grass seed and is widely planted in North America, Europe, and East Asia (Stewart and Ellison, 2011). Although orchardgrass was first cultivated and has been grown in the eastern United States since 1760 (Van Santen and Sleper, 1996), producers in this region have recently observed a decrease in the persistence and regrowth vigor of swards. Too low of a cutting height, a practice partially allowed by the adoption of disk-type hay mowers in the 1980s (Adams, 1996), has been implicated as a possible cause of the recent orchardgrass persistence problem.

Cutting height is a major determinant of quantity and quality of stubble from which the sward will regrow. The energy for regrowth is supplied by carbohydrates generated in the remaining photosynthetic tissue and non-structural carbohydrates (NSC) stored in lower stems (Ward and Blaser, 1961; Morvan-Bertrand et al., 1999). Harvest at a low cutting height will remove the majority or all photosynthetic tissue and some of the stem tissue containing NSC, reducing the energy sources for regrowth. For regrowing tillers to survive and contribute to whole-plant photosynthesis, they must be supplied with adequate mineral nutrients and energy to grow leaf area and be recruited into the canopy (Ong, 1978; Davies, 1988).

Environmental factors, such as temperature, play an important role in the energy status of grasses regrowing following defoliation. The optimum growth temperature for orchardgrass is approximately 21°C (Brown, 1939; Baker and Jung, 1968). At temperatures above optimum, a reduction in NSC is generally observed in cool-season (C₃) grasses (Auda et al., 1966; Brown and Blaser, 1970; Youngner and Nudge, 1976). This decrease in NSC is caused by increased

maintenance respiration which, especially at night, demands more energy than is produced by photosynthesis.

While previous studies have examined the effects of cutting height and temperature on orchardgrass and other cool-season grasses independently, we are not aware of any experiments to study these stress components together. Evaluation of stubble carbon and nitrogen status along with measurements of photosynthetic parameters in regenerated leaves provides a unique view into the physiological processes of regrowth in orchardgrass under supraoptimal temperature conditions and cut to a low cutting height. This information will help to explain management \times environment interactions in orchardgrass hay production and will inform hay producers about the regrowth implications of defoliation height under differing temperature conditions.

Materials and methods

Plant growth and stress treatment

Seeds of orchardgrass (*Dactylis glomerata* L. cv. Benchmark Plus) were germinated in moist paper towels under ambient conditions for 3 days. Seedlings were transplanted to 2.25 L pots (13 cm d × 17 cm h) containing soil mix (Metro Mix 300; SunGro, Agawam, MA, USA) with three seedlings pot⁻¹. Plants were grown in the Virginia Polytechnic Institute and State University greenhouse (Blacksburg, Virginia, USA) at 28°C day / 23°C night under ambient light conditions. Plants were watered daily to field capacity and supplied with 15 mL of slow-release fertilizer (Osmocote Plus; The Scotts Company, Marysville, OH, USA) twice during the establishment phase. After 10 weeks, plants were clipped to 10 cm and allowed to regrow. Plants were vernalized in the greenhouse during winter at 14-7°C under ambient light conditions for 8 weeks. Flowering was initiated with 23°C day / 18°C night and supplemental lighting (14 h light/10 h dark), and plants were allowed to mature to anthesis—stage R4-R5 (Moore et al., 1991). Plants were then transferred to environmentally-controlled chambers set to 20°C constant temperature for five days. Following this acclimatization, plants were cut to either 2.5 cm or 7.5 cm above the soil surface and were regrown at 20°C or 35°C constant temperature. Both chambers maintained 70% relative humidity, 14 h light/10 h dark at 400 μmol m⁻² s⁻¹ of photosynthetically active radiation. On days 0, 1, 3, and 11 d following cutting, stubble tissue was harvested, separated from regrowth, immediately frozen in liquid nitrogen, and stored at -80°C.

Chlorophyll measurements

Chlorophyll content was estimated in regenerating leaf blades using a portable chlorophyll meter (Opti-Sciences CCM-300, Hudson, NH, USA).

Gas exchange measurements

On days 3 and 11 following defoliation, gas exchange measurements were made on regrowing leaf blades. A portable photosynthesis analysis system (LI-6400XT; LI-COR Lincoln, NE, USA) was used to determine the rates of CO₂ assimilation, stomatal conductance, and transpiration. The system maintained the measurement conditions of 400 μmol CO₂ mol⁻¹ under 400 μmol m⁻² s⁻¹ of photosynthetically active radiation at 20°C and 60-70% relative humidity.

Chlorophyll fluorescence analysis

Chlorophyll fluorescence parameters were measured in regrowing leaf blades on days 3 and 11 using a chlorophyll fluorometer (MINI-PAM-II; WALZ, Effeltrich, Germany) and associated leaf-chip holder (2035-B). Leaf areas used for dark-adapted F_v/F_m were exposed to dark conditions for 30 minutes using a dark leaf clip. Light adapted F_v/F_m was determined after exposure to continuous actinic light (400 μmol m⁻² s⁻¹) for 5 min. Calculations for effective quantum yield of PS II photochemistry (Φ_{PSII}), photochemical quenching (qP), and non-photochemical quenching (NPQ) were $(F'_m - F')/F'_m$, $(F'_m - F)/(F'_m - F'_0)$, and $F_m/F'_m - 1$, respectively (Murchie and Lawson, 2013).

Carbohydrate assays

Water- and ethanol-soluble carbohydrates and starch were analyzed using methods adapted from Fukao et al. (2012). Stubble tissue (30 mg) was homogenized in 1 mL of 80% (v/v) ethanol and incubated at 80°C for 20 min. After centrifugation, the supernatant was collected. The ethanol extraction was repeated twice more, and the three extracts were pooled. The pellet was then suspended in 1 mL water and incubated at 80°C for 20 min. Water extraction was repeated and the supernatants from both water extractions were combined. The ethanol and water extracted solutions were dried using a vacuum concentrator and then re-dissolved in 1 mL of water. Soluble carbohydrates were measured by the anthrone method. Glucose was used as a standard. The carbohydrate extracts were incubated with 1 mL of 0.14% (w/v) anthrone solution in 100% H₂SO₄ at 100°C for 20 min. Samples were cooled, and their absorbance at 620 nm was determined with a spectrophotometer. Starch content was quantified in the pellet used for ethanol- and water-soluble carbohydrate extraction. The pellet was re-suspended in 1 mL water containing 10 units of heat-resistant α -amylase. The suspension was incubated for 30 min at 95°C. The reactant was then mixed with 25 μ L of 1M sodium citrate (pH 4.8) and 5 units of amyloglucosidase and incubated at 55°C for 1 h. The mixture was centrifuged for 30 min, and the glucose content of 100 μ L of the supernatant was determined by the anthrone method as described above.

Nitrate, ammonium, and total amino acid assays

Frozen tissue (75 mg) was homogenized in 450 μ L of 0.83 M perchloric acid on ice. After centrifugation, 300 μ L of the supernatant was neutralized with 75 μ L of 1 M bicin (pH 8.3) and 70 μ L of 4 M KOH. Following centrifugation, the supernatant was used for nitrate, ammonium, and amino acid assays as described in van Veen et al. (2013) and Alpuerto et al. (2016). For

nitrate, the extract (10 μ L) was incubated with 40 μ L of 5% (w/v) salicylic acid in 100% H₂SO₄ at 25°C for 20 min. The solution was mixed with 950 μ L of 2 M NaOH, and the absorbance at 410 nm was determined with a spectrophotometer. Potassium nitrate was used as the standard. For ammonium, 25 μ L of the extract was mixed with 375 μ L of 8.8% (w/v) salicylic acid, 10 M NaOH, 21.5 mM EDTA, and 6.7 mM sodium nitroferricyanide (III) dehydrate. The mixture was added to 625 μ L of 70 mM NaH₂PO₄ and 45-mM sodium dichlorisocyanurate and incubated for 2 h at 25°C. Following incubation, the absorbance at 660 nm was determined. Ammonium sulfate was used as the standard. For total amino acid, 80 μ L of the extract was added to 20 μ L of 3 M MgO and incubated in an opened 1.5 mL tube for 16 h at 25°C. After 16 h, 80 μ L of the solution was mixed with 50 μ L of 0.2 mM sodium cyanide resolved in 8 M sodium acetate and 50 μ L of 168 mM ninhydrin resolved in 100% 2-methoxyethanol. The mixture was incubated at 100°C for 15 min, and 1 mL of 50% isopropanol was immediately added to the solution. After cooling, the absorbance at 570 nm was measured with a spectrophotometer. Glycine was used as the standard.

Protein assay

Total protein was extracted from 50 mg of stubble tissue in a buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2 mM EDTA, 10% (v/v) glycerol, 0.5% (v/v) IGEPAL CA-360, and 1 mM phenylmethanesulfonyl fluoride on ice. Protein concentration was determined by Coomassie Plus protein assay reagent (Thermo Fisher Scientific, Waltham, MA, USA). Bovine serum albumin was used as the standard.

Statistical Analysis

Statistical analysis was conducted using JMP Pro (ver. 11.0.0) (SAS Institute, Cary, NC, USA). ANOVA was used to determine treatment differences at each time point. Tukey's honest significant difference (HSD) was used to determine mean separation ($\alpha < 0.05$).

Results

Low cutting height and high temperature suppress regrowth upon defoliation

This study broadly simulated the environmental conditions during regrowth following the first harvest of spring growth for orchardgrass hay/silage. Plants were excised at the early heading stage at 7.5 cm or 2.5 cm above the soil surface - a commonly recommended growth stage and cutting height range for hay harvest. Following defoliation, plants were regrown under control (20°C) or high temperature (35°C) conditions for up to 11 days. On day 11 the most vigorous regrowth was observed for the 20°C treatment cut to 7.5 cm with diminishing regrowth for the 20°C - 2.5 cm, 35°C - 7.5 cm, and 35°C - 2.5 cm treatments (Figure 4.1A). Consistently, the biomass of regrown tissue differed significantly with cutting height and temperature treatments (Figure 4.1B). At optimal temperature (20°C), 7.5 cm defoliation height supported more regrowth than 2.5 cm. Under heat stress (35°C), however, the advantage of high cutting height on regrowth vigor was not observed. When plants were cut to 7.5 cm, high temperature significantly suppressed regrowth. This temperature effect was also detected at 2.5 cm cutting height. These results indicate that the impact of heat stress on biomass yield following defoliation is apparent regardless of cutting height, whereas the effect of cutting height is temperature-dependent.

The influence of cutting height and high temperature on photosynthetic parameters in regenerating leaves

Chlorophyll abundance in regrowing laminar tissue was quantified as a determinant of photosynthetic performance (Figure 4.2A). Three days following defoliation, chlorophyll was significantly greater in 35°C treatments as compared to 20°C treatments, irrespective of cutting height. In addition, chlorophyll abundance was reduced by low cutting height in heat-treated plants, but not in non-stressed plants. On day 11 following defoliation, a lower chlorophyll abundance was measured in the 35°C - 2.5 cm, but the other treatments did not differ.

Direct measurements of leaf gas exchange determined photosynthetic activity as well as stomatal conductance and transpiration rate in regrowing leaves. On day 3, the photosynthetic rate was greater at 35°C than 20°C in plants cut to 7.5 cm (Figure 4.2B). The same pattern was observed in plants cut to 2.5 cm. We also observed that short cutting height reduced photosynthetic activity in regrowing leaves under both non-stress and high temperature conditions. On the 11th d of recovery, photosynthesis rates were clearly augmented in plants cut to 7.5 and 2.5 cm at 20°C, and their values did not differ significantly. High temperature suppressed photosynthetic activity regardless of mowing height, with a more severe reduction in plants trimmed to 2.5 cm. Stomatal conductance of regrowing leaves was significantly increased by low cutting height only at 35°C, not at 20°C (Figure 4.2C). High temperature did not affect stomatal conductance at either cutting height. On day 11, heat stress repressed stomatal conductance at both mowing heights. However, the negative effect of low cutting height on stomatal conductance was detected only under high temperature. On day 3, transpiration was stimulated in response to high temperature at both defoliation levels, with higher induction in plants cut to 2.5 cm (Figure 4.2D). At 20°C, cutting height did not influence transpiration. On

day 11, heat-induced transpiration was not observed at either mowing level; the 35°C - 2.5 cm treatment had a significantly lower transpiration rate than the other treatments.

Chlorophyll fluorescence measurements provided the insight into the performance of PSII in regrowing leaves under non-stress and heat stress conditions. Maximum quantum efficiency of dark-adapted PSII photochemistry (F_v/F_m) did not differ among treatments on day 3, but it was significantly lower in leaves of the 35°C – 2.5 cm treatment on day 11 (Figure 4.3A). The operating efficiency of PSII (Φ_{PSII}) was enhanced by high temperature in plants cut to 7.5 cm and 2.5 cm on day 3 but did not differ between the cutting heights (Figure 4.3B). On day 11, the positive effect of high temperature on Φ_{PSII} was canceled at both defoliation levels. Low cutting height suppressed Φ_{PSII} under heat stress. Photochemical quenching (qP) did not differ among the treatments on day 3 (Figure 4.3C). On day 11, qP was reduced at low mowing height only at high temperature. Heat stress restrained non-photochemical quenching (NPQ) only in plants cut to 2.5 cm on day 3 (Figure 4.3D). This trend was also observed on day 11.

The effect of cutting height and heat stress on carbohydrate accumulation in stubble

Concentrations of three classes of carbohydrates were measured to elucidate how cutting height and heat stress influence the status of carbon reserves in the stubble during regrowth following defoliation. Water-soluble carbohydrate (WSC) extracts contain primarily fructan, while ethanol-soluble carbohydrate (ESC) extracts include mono- and disaccharides (Kagan et al., 2014). On days 1 and 3 following defoliation, the levels of WSC were low relative to the values on day 0 in all treatments (Figure 4.4A). The effect of cutting height and temperature on relative WSC concentration was not detected on day 1. However, on day 3, relative WSC content was greater under high temperature in plants cut to 2.5 cm. On day 11, no significant difference among the

relative WSC concentrations was detected. The relative concentration of ESC was positively affected by high temperature only in plants cut to 7.5 cm on day 1 (Figure 4.4B). The effect of high temperature on ESC continued on days 3 and 11. In addition, low cutting height suppressed the relative ESC content under high temperature at these time points. For relative starch concentration, there was no significant difference among the treatments on day 1 (Figure 4.4C). On day 3, the relative starch concentration was greater at high temperature only when cut to 2.5 cm, which is consistent with the observation in WSC. This trend was not observed on day 11, but low defoliation height suppressed the relative starch content at high temperature, which is in accordance with the observation in ESC at the same time point.

Cutting height and high temperature affect the abundance of nitrogen compounds in stubble

To determine the status of nitrogen reserves in stubble tissue, the relative concentrations of total soluble protein, total free amino acids, nitrate, and ammonia were measured. At all of the three time points, high cutting height increased the relative protein content at 20°C (Figure 4.5A). Under high temperature, the positive effect of high cutting height was observed only on day 11. The protein content was also induced by high temperature only in plants cut to 7.5 cm on day 11. Relative total amino acids did not differ significantly among treatments on day 1 (Figure 4.5B). On days 3 and 11, high temperature increased the abundance of total amino acids regardless of mowing levels. However, low cutting height limited the accumulation of relative total amino acids under heat stress at these time points. Relative nitrate concentrations were suppressed by low cutting height under non-stress and heat stress conditions on day 1 (Figure 4.5C). This effect is not observed on day 3, but high temperature increased the relative nitrate content in plants cut

to 7.5 cm. The positive effect of high temperature on nitrate was also detected on day 11 at both defoliation levels. Ammonium concentrations followed a similar pattern to that of nitrate (Figure 4.5D).

Discussion

Supraoptimal temperatures reduce the growth rate and biomass accumulation of various C₃ species (Brown, 1939; Sullivan and Sprague, 1949; Baker and Jung, 1968, Youngner and Nudge, 1976; Rutledge et al., 2012; Song et al., 2014). We also observed that heat stress significantly suppressed the biomass yield following defoliation both in plants cut to 7.5 cm and 2.5 cm (Figure 4.1). Cutting height of forage grasses is a critical factor determining hay/silage yield and subsequent productivity (Mislevy et al., 1977; Sheffer et al., 1978; Fulkerson and Michell, 1987; Garay and Hodgson, 1999). In this study, increased mowing height improved regrowth of orchardgrass at 20°C. However, the positive effect of high cutting height was canceled under heat stress. These results were in accordance with the observation by Youngner and Nudge (1976) who grew Kentucky bluegrass at 21°C and 32°C with 2 cm and 3.75 cm cutting heights. They found that taller stubble tissue left following defoliation resulted in greater biomass regrowth at the lower air temperature but not at the higher. These data suggest that cutting height management for C₃ grasses may be more effective in increased regrowth vigor and productivity during cool seasons or when cool weather follows hay harvest.

Chlorophyll content is an important component that affects photosynthetic capacity in plants. Studies of heat stress in cool-season grasses reported a reduction in chlorophyll content caused by high temperature in wheat (Pradhan et al., 2012) and bentgrass (*Agrostis* spp.) (Liu

and Huang, 2000; Jespersen et al., 2016) However, Yu et al. (2014) found that undefoliated, heat-stressed (35°C) tall fescue had higher chlorophyll content than control plants (25°C) during the initial 21 days of heat stress. Consistently, we observed that plants regrown at 35°C contained more chlorophyll than those at 20°C (Figure 4.2A). This initial rise in leaf chlorophyll could be caused by an increased rate of substrate mobilization from the stubble tissue as a result of the elevated temperature. It appears that cutting height is also crucial for chlorophyll generation in regrowing leaves. Indeed, low defoliation height suppressed the amount of chlorophyll under high temperature on days 3 and 11 (Figure 4.2A). A study manipulating cutting height in frequently-mown bermudagrass (*Cynodon dactylon* L.) found increased total shoot chlorophyll in the higher cutting height treatments when measured after 4 weeks (Bunnell et al., 2005).

A reduction in net photosynthesis has been observed at supraoptimal temperatures in numerous C₃ turf and forage grass species including Kentucky bluegrass (*Poa pratensis* L.) (Song et al., 2014), tall fescue (*Schedonorus phoenix* (Scop.) Holub) (Jiang and Huang, 2001; Yu et al., 2014), Chinese rye grass (*Leymus chinensis* L.) (Xu and Zhou, 2006), perennial ryegrass (*Lolium perenne* L.) (Jiang and Huang 2001), and creeping bentgrass (*Agrostis stolonifera* L.) (Pote et al., 2006). We found that photosynthetic rate initially increased on day 3 for the 35°C treatments as compared to the 20°C counterparts, which is likely related to leaf chlorophyll content (Figure 4.2A, 4.2B). On day 11, heat stress did not alter the abundance of chlorophyll in plants cut to 7.5 cm. However, net photosynthesis was significantly suppressed by high temperature in the same plants. Removal of more vegetative tissue (2.5 cm cutting height) further reduced photosynthesis under heat stress. This can be caused by the observed reduction in stomatal conductance (Figure 4.2C) and presumably increased photorespiration under prolonged

heat stress. Increased transpiration is an adaptation response to heat stress wherein transpirational cooling reduces the surface temperature in leaves (Jagadish et al., 2015). We observed that the rate of transpiration was elevated in response to heat on day 3 regardless of defoliation height (Figure 4.2D). Greater transpiration in plants at low cutting height may reflect more severe heat stress in leaves that emerged from the shorter stubble. Under prolonged heat stress (day 11), increased transpiration was not detected; low mowing height even reduced the rate of transpiration. It seems that the cooling effect from elevated transpiration does not last for a prolonged period of heat.

The functioning of photosystem II is an indicator of plant stress and a determinant of photosynthetic performance. High temperature stress has been shown to reduce the maximum efficiency of PSII photochemistry (F_v/F_m) and effective quantum yield of photosystem II (Φ_{PSII}) in C₃ plants (Liu and Huang, 2000; Jiang and Huang, 2001; Xu and Zhou, 2006; Wang et al., 2010). In this study, we found that F_v/F_m was reduced for the 35°C – 2.5 cm treatment on day 11 (Figure 4.3A). Heat stress for 3 days increased Φ_{PSII} regardless of cutting height (Figure 4.3B). This changing pattern was similar to that in leaf chlorophyll content at day 3 (Figure 4.2A), suggesting that the amount of chlorophyll can be a major cause for heat-activated quantum yield of photosystem II at this time point. Under prolonged high temperature, low cutting height reduced Φ_{PSII} , which is consistent with the observations in chlorophyll, net photosynthesis, stomatal conductance, transpiration, F_v/F_m , and qP (Figure 4.2, 4.3A, 4.3C). These data indicate that defoliation height or the amount of stubble tissue remained following defoliation significantly influences photosynthetic capability, stomatal regulation, and photosystem II photochemistry in newly emerged leaves under prolonged heat stress. In general, non-photochemical quenching (NPQ) is elevated with increasing temperature in leaves of non-

defoliated plants (Haldimann and Feller, 2004; Salvucci and Crafts-Brandner, 2004). However, we observed that NPQ was reduced at 35°C in leaves regrowing from the plants cut to 2.5 cm on days 3 and 11 (Figure 4.3D). Leaves emerging from defoliated and non-defoliated plants may respond differentially to high temperature in terms of NPQ-dependent stress adaptation.

Heat stress generally reduces non-structural carbohydrate contents in leaves of non-defoliated C3 plants (Auda et al., 1966, Brown and Blaser, 1970; Youngner and Nudge, 1976). However, we observed that high temperature induced the accumulation of ethanol-soluble carbohydrates in the stubble of plants cut to 7.5 cm. Under the stress, starch content was also greater in the 7.5 cm treatment than the 2.5 cm treatment. The abundance of soluble carbohydrates and starch is positively correlated with the degree of heat tolerance in tomato (Pressman et al., 2002; Firon et al., 2006). In rice, continuous mild heat stimulates sugar transporters in reproductive organs, resulting in increased starch accumulation in pollen (Chung et al., 2014). Based on these data, induction of carbohydrate accumulation under prolonged heat stress in stubble tissue may be an adaptive response to the stress in defoliated plants. However, the modification of carbohydrate allocation can lead to reduced regeneration of vegetative tissue under high temperature. Heat-mediated accumulation of carbohydrates was not obvious when most photosynthetic tissue was removed (2.5 cm cutting height), probably due to the lack of or reduced carbon assimilation capability.

As observed in carbohydrate assays, prolonged heat stress induced the accumulation of protein, amino acids, nitrate, and ammonium in the stubble of plants cut to 7.5 cm (Figure 4.5). Shorter cutting height limited these stress responses, but we still observed significant elevations in the amino acid, nitrate, and ammonium contents as compared to its counterpart (20°C - 2.5 cm treatment). Free amino acids are known to accumulate during heat and other abiotic stresses such

as drought and flooding for membrane stabilization, free radical scavenging, and osmotic adjustment (Rai et al., 2002; Seki et al., 2007; Du et al., 2011; Rutledge et al., 2012; Tamang et al., 2014; Alpuerto et al., 2016). An increase in amino acid content in stubble would be an acclimation response to the stress. However, the significance of heat-induced nitrate and ammonium accumulation in stress adaptation is unknown. It is expected that promoted preservation of major nitrogen compounds in stubble can lead to reduced translocation of these reserves into sink tissue, resulting in slow vegetative regrowth.

Conclusions

At optimal temperature for cool-season grasses (20°C), leaving more stubble tissue resulted in greater biomass regrowth upon defoliation. However, this advantage was negated under prolonged heat stress (35°C). It appears that high temperature triggers changes in carbon and nitrogen allocation in stubble, resulting in the accumulation of mono- and disaccharides, starch, amino acids, nitrate, and ammonium in the source tissue. This can contribute to the enhanced protection in stubble against heat stress, but inhibit the translocation of energy resources into growing leaves (sinks). Heat-induced accumulation of carbohydrates and nitrogen compounds were more prominent in plants cut to 7.5 cm than 2.5 cm, which can explain in part the cancelation of the positive effect of high cutting height on vegetative regrowth. It is likely that greater net photosynthesis and photosystem II photochemistry in taller stubble under high temperature can facilitate the metabolic adjustment to the stress but does not benefit the formation of new leaves.

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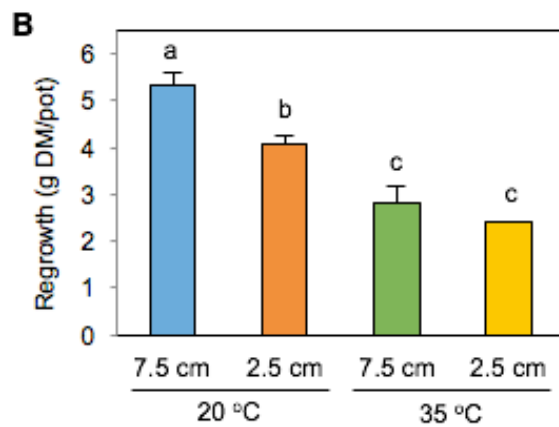
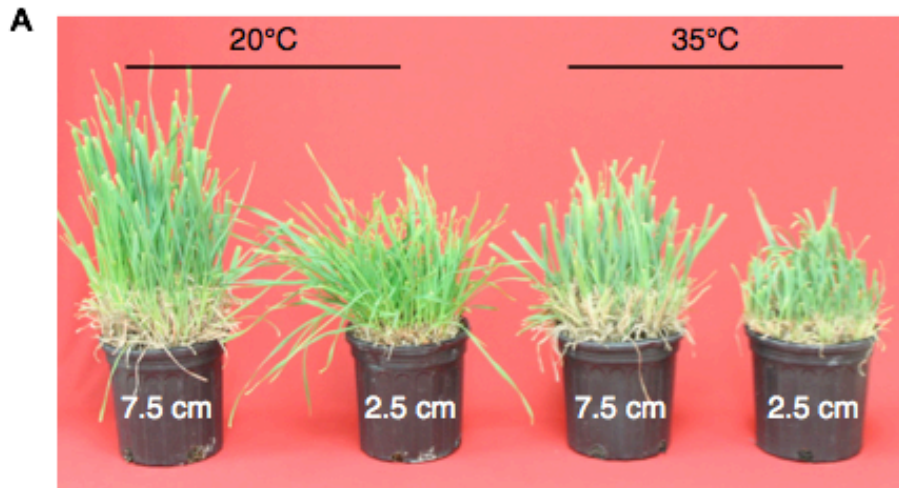


Figure 4.1: The effect of cutting height and high temperature on regrowth of leaves from the stubble. (A) Photos of orchardgrass 11 d after defoliation. Orchardgrass was exposed to clipping at 7.5 or 2.5 cm cutting height at the heading stage and placed under 20 or 35 °C for regrowth for 11 d. (B) Dry biomass of regrown leaves following defoliation. Data represent mean \pm SE (n=3). Bars not sharing the same letter are significantly different ($P < 0.05$).

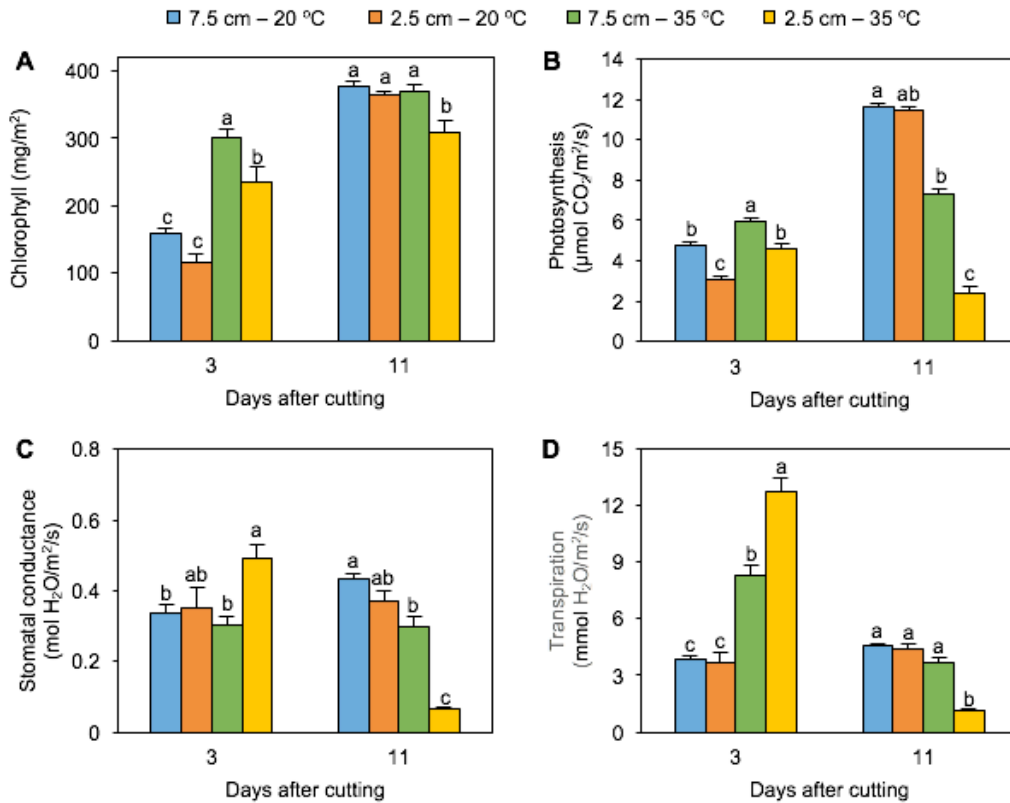


Figure 4.2: The effect of cutting height and high temperature on photosynthetic parameters in re-emerging leaves following defoliation. Orchardgrass was exposed to clipping at 7.5 or 2.5 cm cutting height at the heading stage and placed under 20 or 35 °C for regrowth. After 3 or 11 d, chlorophyll (A), photosynthesis (B), stomatal conductance (C), and transpiration (D) were monitored in newly emerged leaves. Data represent mean \pm SE [$n=20$ in (A); $n=18$ in (B)-(D)]. Bars not sharing the same letter are significantly different ($P < 0.05$).

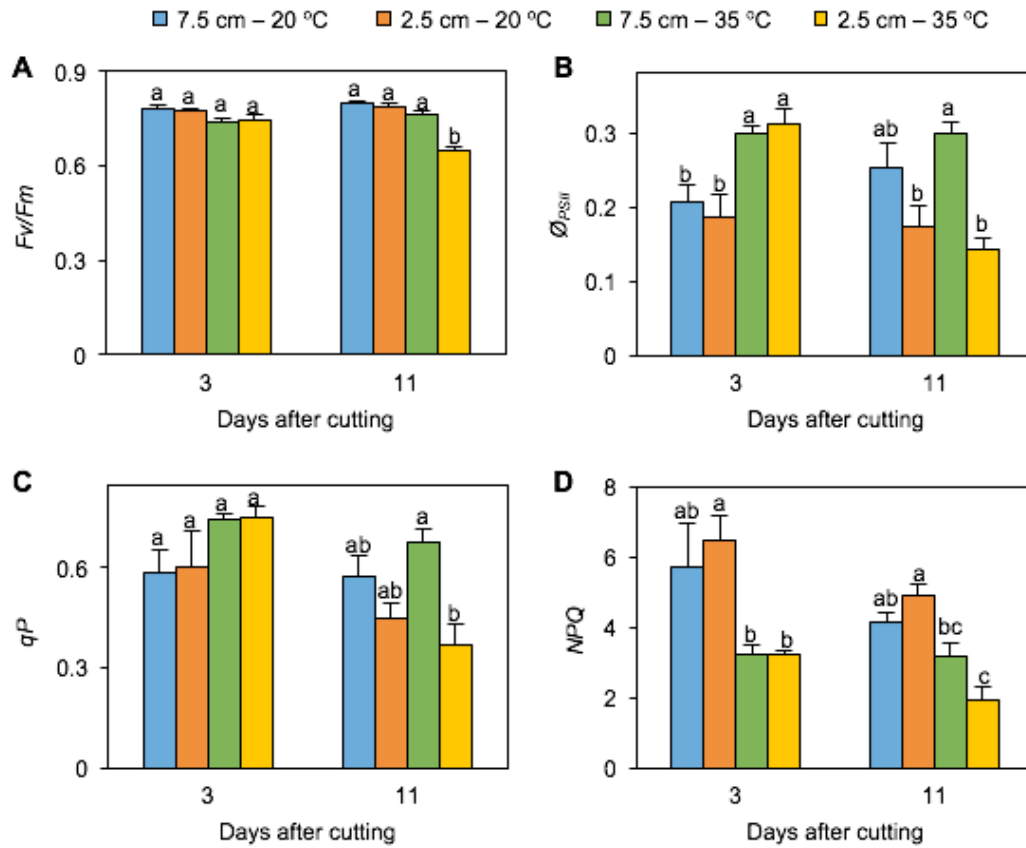


Figure 4.3: The effect of cutting height and high temperature on photosystem II photochemistry in re-emerging leaves following defoliation. Orchardgrass was exposed to clipping at 7.5 or 2.5 cm cutting height at the heading stage and placed under 20 or 35 °C for regrowth. After 3 or 11 d, dark-adapted F_v/F_m (A), effective quantum yield of photosystem II (Φ_{PSII}) (B), photochemical quenching (qP) (C), and non-photochemical quenching (NPQ) (D) were monitored in newly emerged leaves. Data represent mean \pm SE (n=4). Bars not sharing the same letter are significantly different ($P < 0.05$).

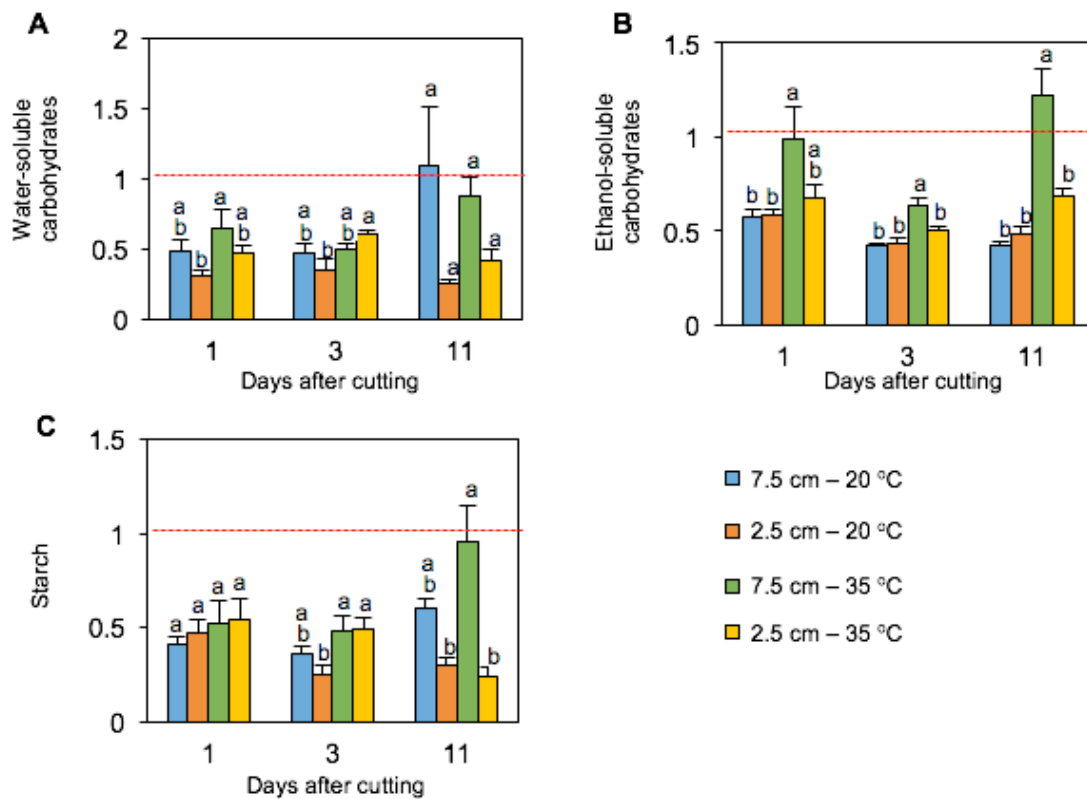


Figure 4.4: The effect of cutting height and high temperature on the alterations in carbohydrate reserves in stubble during recovery from defoliation. Orchardgrass was exposed to clipping at 7.5 or 2.5 cm cutting height at the heading stage and placed under 20 or 35 °C for regrowth. After 0, 1, 3 or 11 d, the concentrations of major carbon reserves were quantified in stubble. Leaf tissues newly emerged during regrowth was not included in this analysis. Data represent the change in water-soluble carbohydrates (A), ethanol-soluble carbohydrates (B), and starch (C) relative to their levels on day 0. The error bars indicate SE (n=9). Bars not sharing the same letter are significantly different ($P < 0.05$).

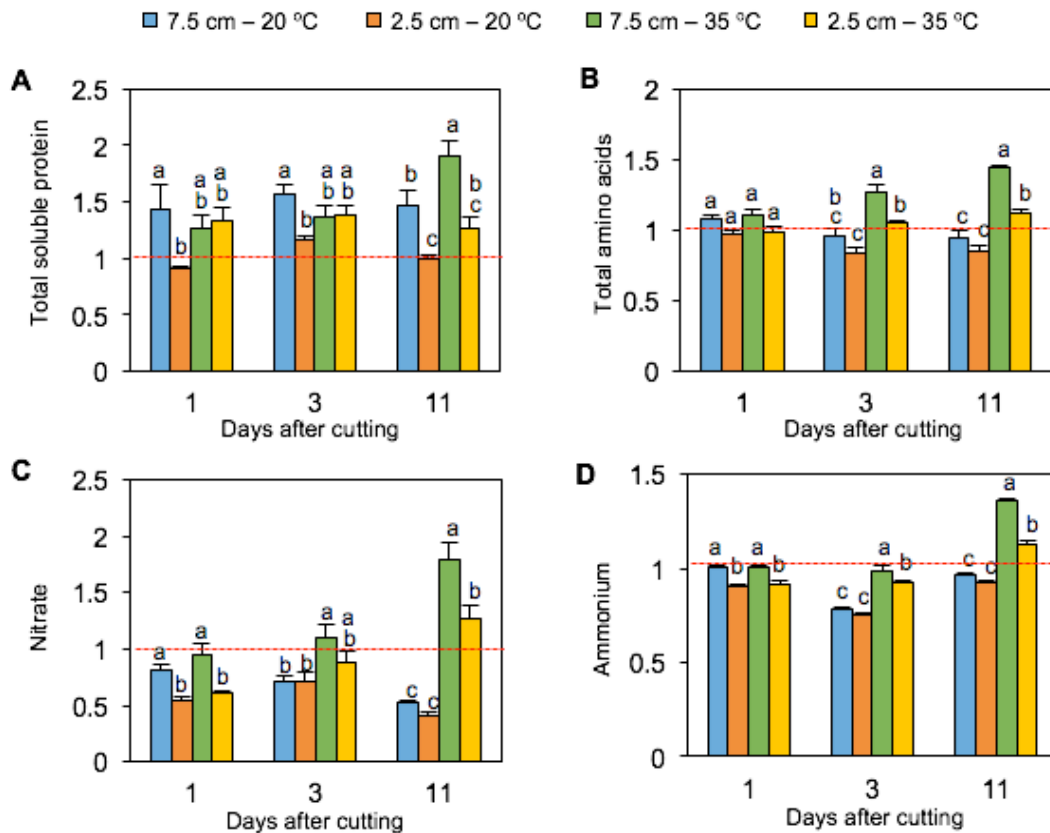


Figure 4.5: The effect of cutting height and high temperature on the alterations in nitrogen reserves in stubble during recovery from defoliation. Orchardgrass was exposed to clipping at 7.5 or 2.5 cm cutting height at the heading stage and placed under 20 or 35 °C for regrowth. After 0, 1, 3 or 11 d, the concentrations of major N reserves were quantified in stubble. Leaf tissues newly emerged during regrowth was not included in this analysis. Data represent the changes in total protein (A), total free amino acids (B), nitrate (C), and ammonium (D) relative to their levels on day 0. The error bars indicate SE [n=9 in (A), (C), and (D); n=6 in (B)]. Bars not sharing the same letter are significantly different ($P < 0.05$).

Chapter 5. Persistence and productivity of orchardgrass and orchardgrass/alfalfa mixtures as affected by cutting height

Abstract

Cutting height is an important factor controlling the yield and persistence of grass swards harvested for conserved feed. The objective of this experiment was to determine the effect of four cutting heights (5, 10, 15, and 20 cm) on the yield, composition, and productivity based on deviation from a size/density compensation line for swards of orchardgrass (*Dactylis glomerata* L.) and an orchardgrass/alfalfa (*Medicago sativa* L.) mixture harvested nine times over three growing seasons. Yield was greatest for the 5 cm cutting height through the course of the experiment but ground cover of orchardgrass declined. Prior to the final harvest, tiller weight and density were determined. The 10, 15, and 20 cm treatments fell on an apparent size/density compensation line with slope -1.779 ($R^2 = 0.99$; $p = 0.008$) while the 5-cm treatment fell considerably below that line indicating a reduction in productivity or relative persistence. Harvest at 10 cm appears to optimize yield while maintaining stand productivity in infrequently harvested orchardgrass swards.

Introduction

It is well known that excessively low cutting height can have deleterious effects on mown perennial grass swards (Stapledon and Milton 1930; Volesky and Anderson 2007; Brink et al. 2010). It has been suggested that cutting height is a major factor linked to recently observed decline in orchardgrass stands in the Mid-Atlantic (Smith and Saylor 2012; Clark 2009). This may be because of the adoption of rotary-disc mowers by many hay producers in recent decades (Adams 1996), and because the design of these mowers allows for a lower cutting height. In determining mowing height for perennial grasses, producers face a trade-off between increased yield at lower heights and increased persistence at higher heights. Thus, a common recommendation has been to increase cutting height to increase stubble leaf area index and water-soluble carbohydrate (WSC) content to improve regrowth and sward persistence. This, however, may be at the expense of short-term yield.

Persistence of perennial grasses is defined as the ability of each tiller to produce on offspring annually such that members of the population replace themselves (Edwards and Chapman 2011). Many studies use either tiller density (Rease and Decker 1966; Volesky and Anderson 2007) or percent ground cover (Smith et al. 1973; Brink et al. 2010) to determine the effects of various treatments on the persistence of swards. It is also known, however, that tiller density alone is not always related to the yield of swards (Davies 1988). Tiller weight is required in addition to density in order to determine the productivity of grass stands. For example, swards with low tiller weight need to achieve high tiller number in order to maintain high productivity, while swards with low tiller number need to reach a high tiller weight to maintain high productivity

Size-density compensation (SDC) theory dictates that swards of various tiller densities and weights will maximize leaf area along a $-3/2$ self-thinning line (Sackville Hamilton et al. 1995; Matthew et al. 1995). It has been proposed that deviation from the apparent SDC line for otherwise similarly managed swards can be used as a measure of relative stand productivity (Hernandez Garay et al. 1999). This has been implemented in several forage systems including perennial ryegrass (*Lolium perenne* L.) mini-swards (Hernandez Garay et al. 1999), rhodesgrass (*Chloris gayana* Kunth.) mini-swards (Martinez Calsina et al. 2012), and field-grown tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.), and prairie grass (*Bromus catharticus* Vhal) (Scheneiter and Assuero 2010).

A better understanding of the trade-off between persistence and productivity of mown perennial grass swards is required for improved management decisions. To our knowledge, the SDC of mown swards has not been evaluated in North America. Deviation from an SDC line may provide clarity in the effects of cutting height on orchardgrass productivity. Thus the objective of this experiment was to evaluate the yield, composition, and SDC-corrected productivity of pure orchardgrass and an orchardgrass/alfalfa mixture harvested to four cutting heights over three growing seasons.

Methods:

Site and Establishment

This experiment was conducted during the 2014 – 2016 growing seasons at the Northern Piedmont Center near Orange, VA, USA (38° 13' 26.10" N, 78° 07' 13.08" W, 156 m a.s.l.). Weather data were collected at a weather station on the site. The soil was Davidson loam (Fine, kaolinitic, thermic Rhodic Kandiudults). Paired plots (12 m × 4 m) of pure orchardgrass (cv.

‘Benchmark Plus’) at 15 kg seed ha⁻¹ and orchardgrass/alfalfa mixture (15 kg OG and 15 kg Alf (cv. ‘Evermore’) ha⁻¹) were planted in September 2012 into a tilled seedbed where the previous crop was switchgrass (*Panicum virgatum* L.). Plots were fertilized and limed according to Virginia Tech Soil Test recommendation at establishment. During the 2013 growing season, the plots were allowed to establish and were mown to 15 cm twice (May and September) but no data were collected.

For 2014-2016, pure OG plots were fertilized with 67 kg N ha⁻¹ as urea (CO(NH₂)₂) and 165 kg K ha⁻¹ as KCl during mid-March. An additional 67 kg N ha⁻¹ was applied to pure OG plots following first cutting. The OG/Alf plots received 165 kg K ha⁻¹ year⁻¹ as KCl in mid-March but no N was applied.

Harvest and Composition

Four cutting height treatments (5, 10, 15, and 20 cm) were designated for each block. First cutting was made near the emergence of the seedhead (R1; Moore et al. 1991) which corresponded to May 19, 12, and 16 for 2014, 2015, and 2016, respectively. Second cutting was made approximately 60 days later (July 11, 20, and 18 for 2014, 2015, and 2016, respectively), and third cutting was made approximately 90 days thereafter (October 24, 8, and 20 for 2014, 2015, and 2016, respectively). Harvest of a 1.3 m × 3 m swath was made at the designated cutting height with a Swift Forage Harvester (Swift Current, SK, Canada) with height adjustment made at each block. At harvest, a grab sample of forage was collected, then dried and weighed for dry matter determination. Yields are presented on a dry matter basis but were not corrected for inclusion of non-planted species.

Prior to each harvest, visual percent cover was determined for a 0.5 m² (50 cm × 100 cm) quadrat in each plot using a modified Daubenmire method (1968). Cover was classed in to orchardgrass, alfalfa, weeds, and bare ground. The major weed species observed was *Securigera varia* L. with rare observance of *Panicum virgatum* L., *Rumex* sp., *Apocynum cannabinum* L., and *Convolvulus arvensis* L.

Tiller Harvest

Tillers and stems were destructively harvested prior to the final cutting in 2016 to assess the cumulative effect of the cutting treatments on those populations. A 0.1 m² (20 cm × 50 cm) quadrat was randomly selected from each plot in six of the blocks. Orchardgrass and alfalfa were harvested at ground level with a razor blade. Samples were initially sorted into orchardgrass, alfalfa, and dead material categories. Phytomers were removed from orchardgrass tillers if more than 50% of the leaf area was senescent or necrotic from pathogen damage. Orchardgrass tillers and alfalfa stems were counted. Fifty randomly-selected orchardgrass tillers per OG plot were dissected into leaf blade and pseudostem (mostly leaf sheaths and stem) fractions. All tissue was dried at 60°C for 48 hr in a forced air oven. Leaf:pseudostem and leaf:non-leaf (non-leaf material included the pseudostem and senescent fractions) ratios were calculated following Hernandez Garay et al. (1999).

To evaluate the effect of cutting height on tiller size and density, the log₁₀ tiller weight (living + senescent) and log₁₀ tiller density were plotted. It has been theoretically shown (Matthew et al. 1995) and experimentally confirmed (Hernandez Garay 1999) that the distance a treatment lies from an arbitrarily placed SDC line with slope -3/2 provides a measure of relative stand productivity. The slope of the SDC line is also known to be steeper (closer to -5/2) in

defoliated swards (Matthew et al. 1995; Martinez Calsina et al. 2012). The slope of the apparent SDC line was calculated by major axis regression of the log tiller weight by log tiller density for the 10 – 20 cm cutting height treatments. The distance each point lies from this apparent SDC line was calculated.

Statistical Analysis

Statistical analysis was conducted with JMP Pro 13 (SAS Inst., Cary, NC, USA) and R version 3.02 (R Development Core Team 2013). The experiment was designed as a split-block design (n = 8). Within each replication, cutting heights were blocked across the OG and OG/Alf treatments to control for error associated with adjusting the mower height. The main ANOVA model was as follows:

$$Y_{ijklm} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + \beta_k + (\beta B)_{kj} + (\alpha\beta)_{ik} + \gamma_l + \lambda_m + (\alpha\gamma)_{il} + (\alpha\lambda)_{im} + (\beta\gamma)_{kl} + (\beta\lambda)_{km} + (\gamma\lambda)_{lm} + R_{ijk} + E_{ijklm}$$

where Y = dry matter yield; α = cutting height effect (i = 1, 2, 3, 4); B = block effect (j = 1, 2, ...8); β = mixture effect (k = 1, 2); γ = cutting effect (l = 1, 2, 3); λ = year effect (m= 1, 2, 3); R = plot block effect; $(\alpha B)_{ij}$ = split-block error I; $(\beta B)_{kj}$ = split-block error II; E = experimental error. A similarly constructed model without provision for the effect of multiple cuttings was used to analyze tiller data. Model residuals were evaluated and found to be normally distributed. Effects with p < 0.05 were considered significant. Tukey's HSD was used to determine mean separation.

Results

Weather Data

The average monthly temperature and precipitation accumulation were measured and compared to 20-year averages (1995-2015) (Figure 5.1). Average temperature during the 2014 growing season was slightly less than the historic average, close to the historic average for 2015 and slightly greater than the historic average for 2016. Early season precipitation exceeded the historic average in 2014 but was well below the historic average for June to September. Accumulated precipitation exceeded the historic average for most months of the 2015 growing season except for August. In 2016, March and April were relatively dry, May-July had precipitation exceeding the historic average and August, September, and October were dry again.

Herbage Yield

Herbage yield was significantly affected ($p < 0.0001$) by cutting height (Figure 5.2) but did not differ significantly between OG and OG/Alf treatments ($p = 0.587$) and there was no cutting height \times mixture interaction (Table 5.1). The yield differed and interacted both by cutting and year (Table 5.1). The slope of the linear effect of year was 522.7. The effect of cutting height differed by the cutting but not by year (Figure 5.3), and the effect of mixture differed by cutting ($p = 0.003$) but not by year ($p = 0.769$) (Figure 5.4). The lowest cutting height removed a greater proportion of standing biomass throughout the experiment. While the degree of separation among the treatment means varied among the 9 harvests, in every case the 5 cm cutting height yielded significantly more than the 20 cm height (Figure 5.3). Third cutting yielded less across years than did cuttings 1 and 2 (Figure 5.4). For Cutting 1 of 2014 and Cutting 3 of 2016 OG significantly outyielded OG/Alf, but for the three cuttings of 2015 OG/Alf outyielded the OG

plots (Figure 5.4). Cumulative yield also differed by cutting height but not mixture. The 5 cm cutting height had the greatest cumulative yield and the 15 and 20 cm treatments had the lowest (Figure 5.2).

Composition

The proportion of orchardgrass ground cover was the focus of evaluation of sward composition. The proportion of orchardgrass cover differed significantly with cutting height, mixture, and harvest ($p < 0.05$). Significant interactions of cutting height \times harvest and mixture \times harvest were detected ($p < 0.01$) but a cutting height \times mixture interaction was not found. Individual ANOVA within each harvest indicated that the proportion of orchardgrass present in OG plots was significantly reduced for the lowest cutting height as compared to the highest height for third cutting in 2014 and 2015 and first and third cuttings in 2016 (Figure 5.5). Similarly, individual ANOVA within each harvest indicated that the proportion of orchardgrass present in OG/Alf plots was significantly reduced for the lowest cutting height as compared to the highest for third cuttings of 2014 and 2015 and first and second cuttings of 2016 (Figure 5.6). ANOVA assessing cutting height differences in alfalfa cover within harvests found increased alfalfa cover in the 5 cm treatment as compared to the 20 cm treatment for harvest 3 2014 and harvest 1 2016 (Figure 5.6).

Sward Components and Size/Density Compensation

Sward and tiller components were evaluated from the OG plots prior to the final harvest of the experiment. The mass of living + senescent orchardgrass differed among the cutting heights with the 5 cm treatment having lower mass than the other 3 treatments (Table 5.2). Tiller density did

not differ significantly among the treatments but tended ($p = 0.18$) to be highest for the 10 cm treatment and lowest for the 5 cm treatment (Table 5.2). The weight of individual tillers was reduced in the 5 cm treatment as compared to 15 and 20 cm treatments, and the 20 cm treatment had the greatest tiller weight (Table 5.2). The orchardgrass leaf mass differed among the treatments with the lowest leaf mass for the 5 cm treatment, the highest leaf mass for the 15 cm treatment and intermediate mass for 10 and 20 cm treatments (Table 5.2). Leaf:pseudostem ratio was greatest for the 5 cm treatment and lowest for the 20 cm treatment (Table 5.2). Leaf:non-leaf ratio was significantly higher for the 5 cm treatment as compared to the others (Table 5.2).

The mean tiller weight and tiller density by cutting height were plotted in log-log space (Figure 5.7). The apparent slope of the SDC line was -1.778 based on the 10 – 20 cm treatments. The distance between treatment means and the apparent SDC line were calculated. The 10, 15, and 20 cm treatments fell on the apparent SDC line while the 5 cm showed a large negative deviation from the line (Table 5.3).

Discussion

Cutting to 5 cm resulted in the highest yields in this system for both pure orchardgrass and the orchardgrass/alfalfa mixture over 3 years. It is clear that harvesting to a low cutting height will remove a greater proportion of aerial biomass and this has been shown in several experiments with orchardgrass where yields were greater at lower cutting heights (Sprague and Garber 1950; Raese and Decker 1966; Mitchell 1967; Volesky and Anderson 2007; Brink et al. 2010). Low cutting heights, however, remove a greater proportion of photosynthetic leaf area and non-structural carbohydrates (Ward and Blaser 1961). In some cases the stress caused by low cutting has reduced sward yield over the course of experiments (Stapledon and Milton 1930;

Harrison and Hodgson 1939; Volesky and Anderson 2007) though this effect is often related to harvest frequency and the number of years of treatment application.

The differences in yield among harvests and years are likely related to the weather conditions present during the regrowth from the previous harvest and also to N supply. Nitrogen fertilizer was applied to the pure OG plots prior to first and second cutting each year but additional N was not amended prior to third cutting. This explains the reduced yield for third cutting. The linear effect of year had a positive slope indicating that any change in sward dynamics or weather did not deleteriously effect yield during the course of the experiment. Yield was greater for OG/Alf plot only during 2015. This may be explained by the greater proportion of alfalfa in the swards during that year (Figure 5.5). Dry weather in August may have been partly related increased proportion of alfalfa to orchardgrass. Deep taproots of alfalfa allow the plants to be relatively more drought tolerant than many forage grasses (Barnes and Sheaffer 1995).

Proportions of orchardgrass and alfalfa cover differed among harvest dates which would be expected with varying weather conditions. Cutting height only had an effect on orchardgrass cover in 4 of 9 harvests for both pure OG and OG/Alf stands. In all cases, the proportion of orchardgrass was reduced in the 5 cm treatment as compared to the 20 cm treatment. A reduction in ground cover or tiller density at low cutting heights has been observed in other experiments with orchardgrass (Mitchell 1967; Zolesky and Anderson 2007; Brink et al. 2010), and is likely due to stress caused by the removal of photosynthetic leaf area and non-structural carbohydrates. Alfalfa cover differed among the cutting heights in 2 of 9 harvests and was significantly greater for the 5 cm treatments as compared to 20 cm treatments in both cases. The differences in morphology of orchardgrass and alfalfa explain this response. The meristematic tissue and non-

structural carbohydrates in tillers of orchardgrass are located above the soil surface (Turner et al 2007; Christie and McElroy 1995), while the buds of alfalfa are located near or just below the soil surface and non-structural carbohydrates are located in the roots (Barnes and Sheaffer 1995)). Because of this, Blaser et al. (1986) predicted improved regrowth for orchardgrass at higher cutting heights and improved regrowth for alfalfa lower cutting heights. It is expected that this is the main cause of the differences seen here as well.

Orchardgrass yields remained high during three years of cutting at 5 cm, but ground cover of orchardgrass was reduced at low cutting heights. A finer level of observation, than stand scores, is required to evaluate relative changes in productivity and persistence of these swards. Several parameters were measured from destructively harvested tillers prior to the final cutting. Total orchardgrass mass was reduced at the 5 cm cutting height because a greater proportion of those plants were removed at each harvest while leaves and pseudostem were allowed to accumulate below the mowing height of the higher height treatments. Tiller density was not significantly affected by cutting height but tended to be lowest at the 5 cm height and greatest at the 10 cm height. This matches to some extent the ground cover observation from these treatments. The mass per tiller was proportional to the cutting height treatments. Leaf mass was reduced for the 5 cm cutting height but the leaf:pseudostem ratio was greatest for the lowest cutting height. The leaf:non-leaf ratio was also highest for the 5 cm treatment. Higher cutting heights allows for senescence and accumulation of leaves below that cutting height and also causes a shift in morphology wherein the collar region of tillers is elevated causing an increased proportion of pseudostem to leaves. This trend was also found in perennial ryegrass mini-swards harvested to several cutting heights (Hernandez Garay et al. 1999).

Neither tiller size nor tiller density alone provides adequate information to be able to infer sward productivity or persistence. This is because of the phenomenon of the size/density compensation in which optimal sustainable leaf area for a site can be achieved with many small tillers or a few large ones. The solutions for the expected size/density compensation of unmanaged swards grow up to and then along a $-3/2$ line (Yoda et al. 1963; Davies 1988) assuming constant tiller geometry. Matthew et al. (1999) proposed a modification to this self-thinning equation for defoliated swards which will differ in tiller geometry (leaf area cm^2 tissue). They proposed that a steeper SDC line ($-5/2$) is expected for defoliated swards if not corrected for differing tiller volume. Here, we found the slope of the apparent SDC line to be -1.778 , somewhat steeper than $-3/2$. This matches with SDC slopes greater than $-3/2$ found for perennial ryegrass (Hernandez Garay et al. 1999) and rhodesgrass (Martinez Calsina et al. 2012).

The distance that otherwise similar treatments fall from a SDC line can be used as a measure of relative sward productivity (Hernandez Garay et al. 1999; Martinez Calsina et al. 2012). The 10, 15, and 20 cm treatments were located along the apparent SDC slope found in this experiment while the 5 cm treatment deviated from this line in the negative direction. This indicates that even after approximately 90 days of regrowth, the 5 cm cutting height was not able to achieve the expected tiller weight given the tiller density. This limitation to accumulation of tiller mass and leaf area indicates that low cutting height caused the orchardgrass plants to reach their limit of phenotypic plasticity; this caused a reduction in tiller weight and density and thus a reduction in productivity of the 5 cm treatment and a relative reduction in persistence of those swards.

Conclusions

Cutting height is an important determining factor in the yield and persistence of orchardgrass swards. We found higher yields at the lowest cutting height through the duration of the experiment even as orchardgrass ground cover declined. The use of tiller mass and density to determine the apparent slope of the size/density compensation line for these swards indicated that cutting heights of 10 cm or greater were required to maintain productivity, and that the productivity of swards repeatedly harvested to 5 cm was reduced. Were this experiment carried out for further growing seasons, it would be expected that yields would decline for plots cut to 5 cm. It was also observed that orchardgrass/alfalfa mixtures without fertilizer N yielded as well as pure orchardgrass fertilized with 120 kg N ha⁻¹ yr⁻¹. While forage quality, not measured in this experiment, is an important determinant of the economic value of harvested feed, the similar yields without the additional cost of added N fertilizer may help to reduce the cost of production while continuing to provide forage mass to livestock or for sale. These results reaffirm the recommendation to harvest orchardgrass at 10 cm to optimize yield and persistence when harvested infrequently for conserved forage.

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Table 5.1: Abbreviated ANOVA table for dry matter yield (n = 8).

Source	Df	F Ratio	p Value
Height	3	28.64	< 0.0001 ***
Mix	1	0.33	0.587
Cutting	2	353.29	< 0.0001 ***
Year (linear)	1	208.20	< 0.0001 ***
Height × Mix	3	0.56	0.646
Height × Cutting	6	2.78	0.012 *
Height × Year	3	1.16	0.323
Mix × Cutting	2	5.89	0.003 **
Mix × Year	1	0.09	0.769
Cutting × Year	2	27.05	< 0.0001 ***
Plot	63		
Block	7		
Block × Height	21		
Block × Mix	7		
Experimental Error	453		

Table 5.2: Effect of cutting height on orchardgrass mass (living + senescent)(OG mass; g m⁻²), tiller population density (Density; tillers m⁻²), Leaf mass (g m⁻²), tiller weight (g tiller⁻¹), leaf:pseudostem ratio (L:S ratio), and leaf:non-leaf ratio (L:NL ratio) where ‘leaf’ included green leaf, and ‘non-leaf’ included pseudostem and senescent components. Rows with different letters with a column differ significantly (p < 0.05; n = 6).

Cutting Height (cm)	OG mass	Density	Tiller weight	Leaf mass	L:S ratio	L:NL ratio
5	200.8 b	733	0.303 c	44.3 b	2.277 a	0.380 a
10	453.6 a	1050	0.454 bc	58.6 ab	1.792 ab	0.183 b
15	503.9 a	913	0.581 ab	62.2 a	1.428 ab	0.172 b
20	572.8 a	802	0.746 a	55.0 ab	1.326 b	0.131 b
P-value	0.002	0.182	< 0.001	0.036	0.029	< 0.001

Table 5.3: The effect of cutting height on the distance from the apparent SDC line ($y = -1.779x + 11.563$; $p = 0.008$) (See Figure 5.7). Negative values indicate a relative reduction in sward productivity.

Cutting Height (cm)	Distance to the apparent SDC Line
5	-0.537
10	0.001
15	0.004
20	-0.001

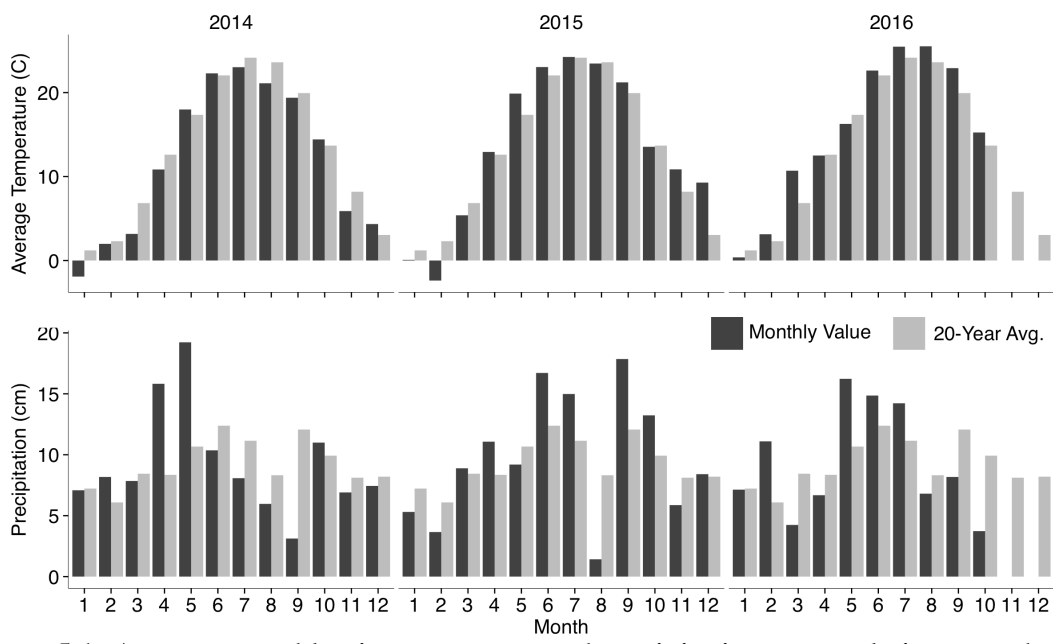


Figure 5.1: Average monthly air temperature and precipitation accumulation over the experimental period and 20-year averages in Orange, VA

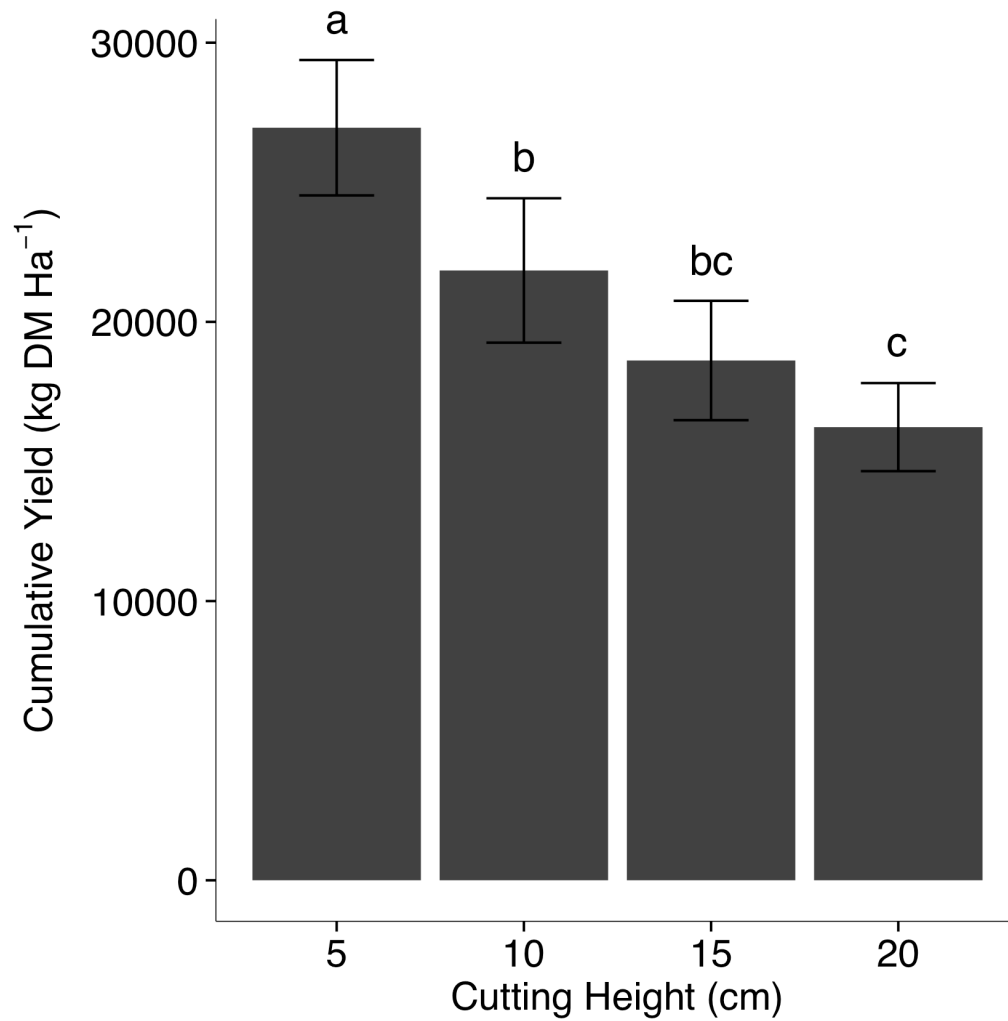


Figure 5.2: Effect of four cutting heights on cumulative yield on 9 harvests. Average of pure orchardgrass and orchardgrass/alfalfa treatments. Bars marked with differing letter indicated significant differences ($p < 0.5$).

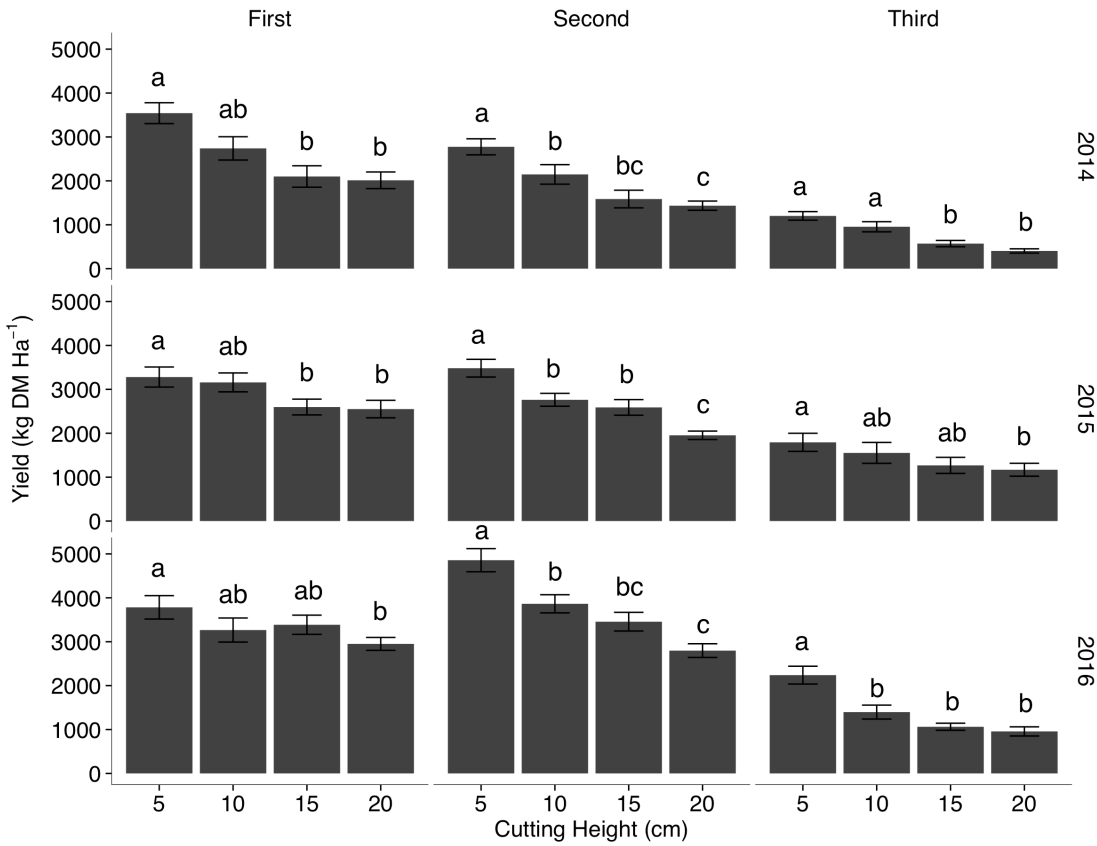


Figure 5.3: The effect of cutting height on the dry matter yield of 9 harvests of orchardgrass and orchardgrass/alfalfa plots. Bars with different letters differ significantly within a time point ($p < 0.05$; $n = 8$).

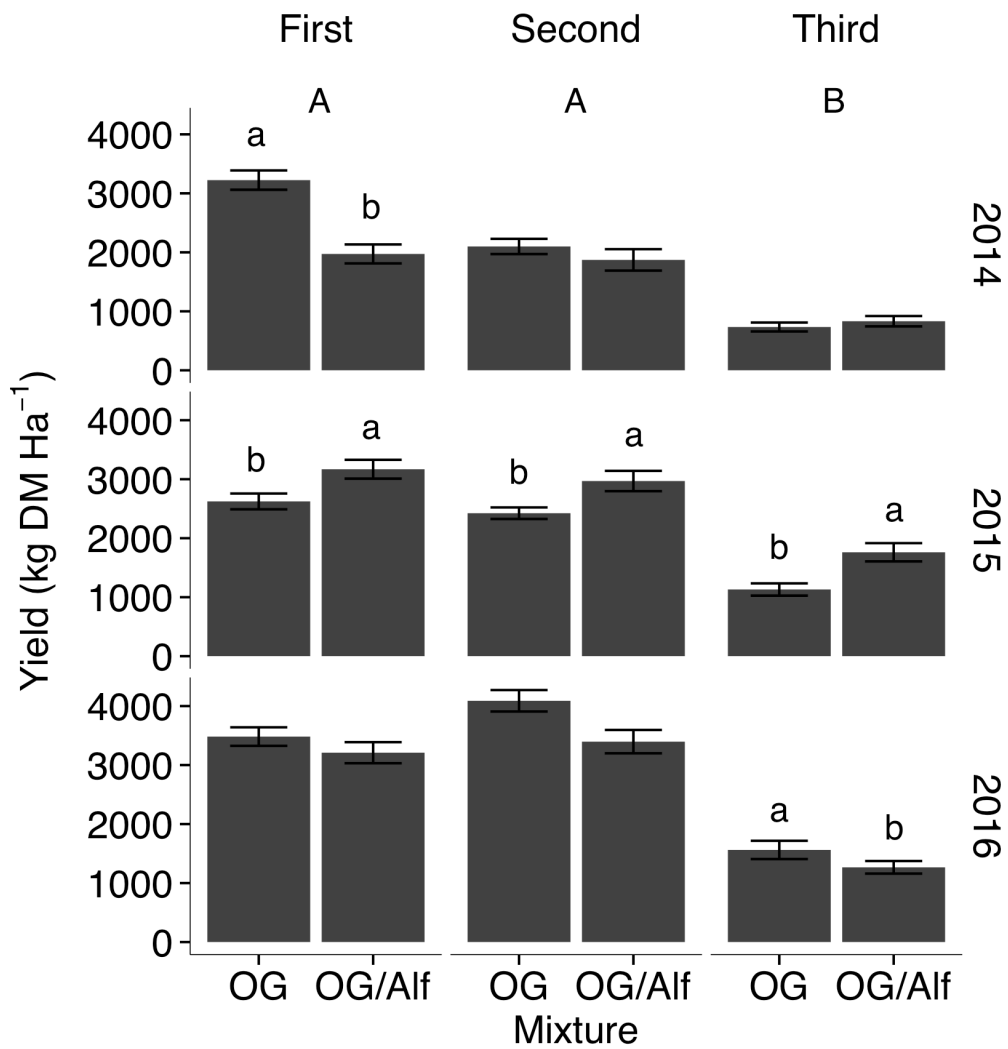


Figure 5.4: The effect of planted mixture and cutting on the dry matter yield of 9 harvests. Bars with different lowercase letters differ significantly within a time point ($p < 0.05$). Columns with different uppercase letters indicate significant differences among cuttings ($p < 0.05$).

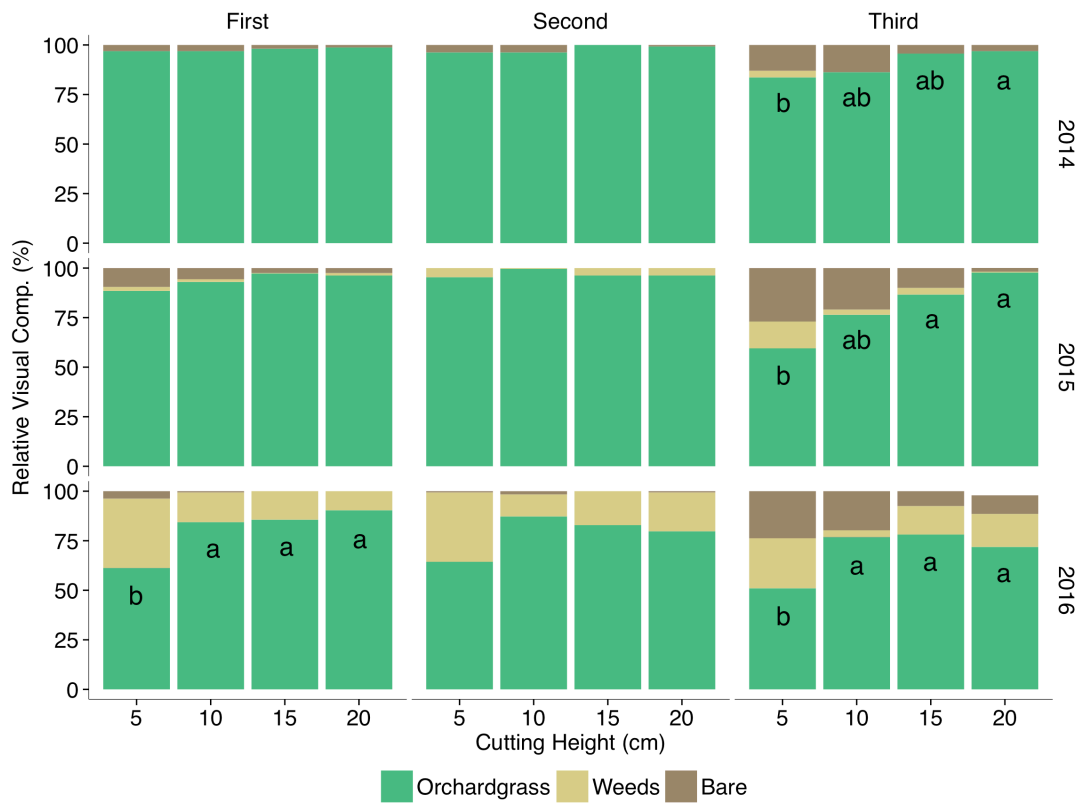


Figure 5.5: Effect of cutting height on visual composition of orchardgrass plots prior to each harvest. Bars with different letters indicate significantly different proportions of orchardgrass within a time point ($p < 0.05$, $n = 8$).

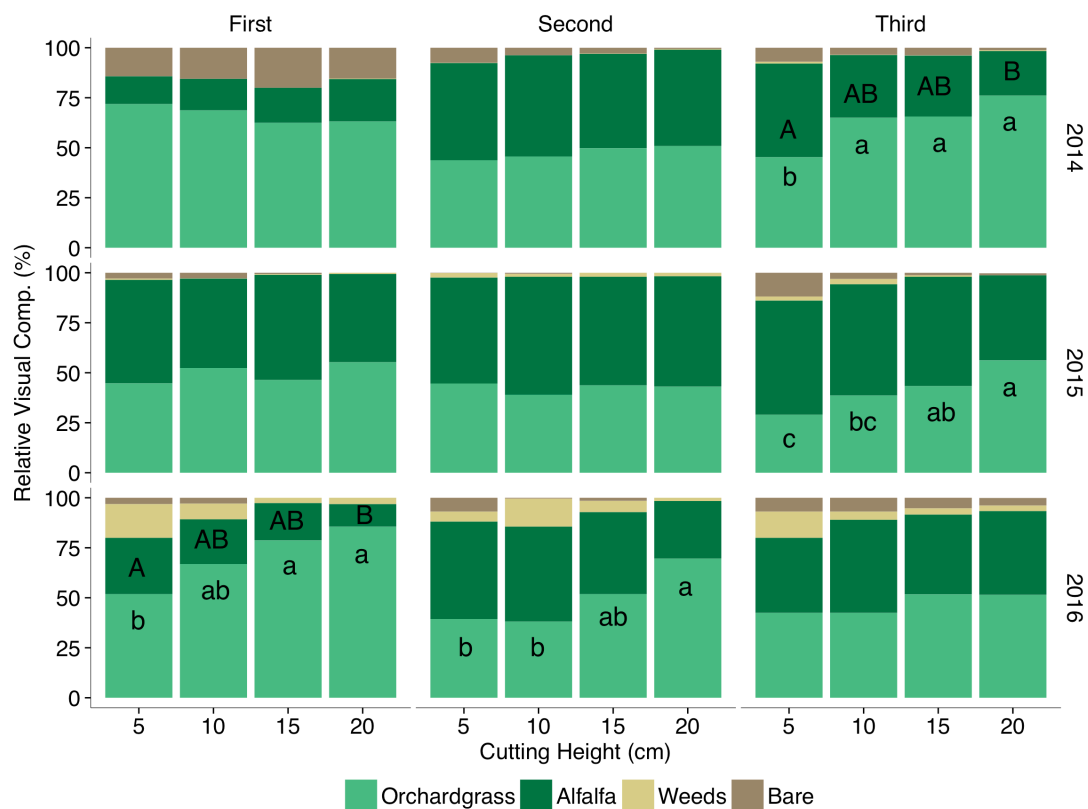


Figure 5.6: Effect of cutting height on visual composition of orchardgrass/alfalfa plots prior to each harvest. Bars with different lowercase letters indicate significantly different proportions of orchardgrass within a time point ($p < 0.05$; $n = 8$). Bars with different uppercase letters indicate significantly different proportions of alfalfa within a time point ($p < 0.05$; $n = 8$).

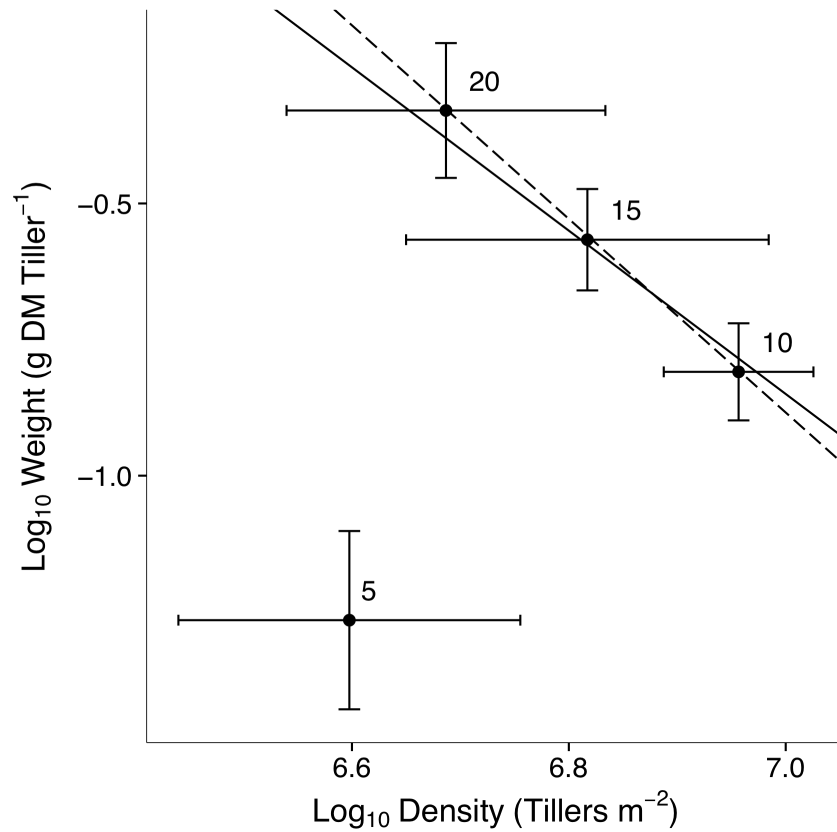


Figure 5.7: Log tiller weight (living + senescent) and log tiller density (\pm SEM) for OG plots as affected by cutting height (cm) prior to the final harvest in 2016. The dashed line is the apparent SDC line for the system fit by major axis regression ($y = -1.779x + 11.563$; $p = 0.0082$; $n = 3$; $R^2 = 0.999$). The solid line is an arbitrarily placed SDC line with slope $-3/2$.

Chapter 6. Conclusions

The persistence and productivity of perennial grasses grown for hay are essential to their economic management. Numerous factors and their interactions are known to control the persistence of swards, but continued study is required to provide management recommendations to address the recently observed poor persistence of orchardgrass in the Mid-Atlantic U.S. A field survey, a growth chamber experiment, and a field experiment were conducted to elucidate the causes of poor persistence and regrowth vigor of orchardgrass. The main conclusions were as follows:

1. The survey of Mid-Atlantic orchardgrass fields indicated that orchardgrass persists less well in warmer parts of the region and that most fields had adequate soil fertility relative to soil test thresholds. Foliar disease was not related to perceived stand persistence score here and cutting height was not evaluated, but it is expected that both of these factors are important determinates of persistence and regrowth vigor.
2. Growth chamber experiments revealed that regrowth of orchardgrass at 35°C was significantly reduced as compared to 20°C conditions. At the cool temperature, higher cutting height increased regrowth, whereas the benefit of high cutting was not present under heat stress. Cutting height may be a more important factor during spring harvest rather than summer because high temperature stress may be affecting photosynthesis in remaining stubble leaves and appears to cause accumulation of carbohydrates rather than regrowth of leaf area.

3. The yield of orchardgrass and orchardgrass/alfalfa mixtures was maximized by 5 cm cutting height over three years, but orchardgrass cover declined significantly for those swards.

Evaluation of size/density compensation and deviation from an expected size/density compensation line clarified the relationships among tiller size, tiller density, and productivity.

When cut to 5 cm, Benchmark Plus orchardgrass was unable to produce enough tillers to achieve the optimum leaf area at the final harvest, while 10 cm and higher cutting heights allowed the swards to achieve optimal leaf area. The deviation from an expected size/density compensation line should be used in future research evaluating the persistence of grasses.

4. While not experimentally evaluated here, infection by foliar pathogens of orchardgrass was observed in numerous swards across the region. Fungicides for disease control are not labeled for orchardgrass, and the use of fungicides is generally not economical in forage systems. Breeding for resistance to anthracnose and leaf streak will be essential to reducing the deleterious effects of these fungi.