# BUD BANK MORPHOLOGY, DYNAMICS, AND PRODUCTION IN PERENNIAL GRASSES

by

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# Abstract

Perennial grasses on tallgrass prairie primarily reproduce vegetatively via the belowground bud bank, yet the production, dynamics, and morphology of belowground buds is largely unexplored. Since the two main photosynthetic pathway guilds ($C_3$ and $C_4$) on tallgrass prairie vary in their aboveground phenology, their belowground phenology would also be expected to vary. Differences in bud production, development, and spatial arrangement result in different growth forms. Therefore, an extensive biweekly examination of a dominant tallgrass prairie $C_4$ rhizomatous grass *Andropogon gerardii* and $C_3$ caespitose grass *Dichanthelium oligosanthes* was conducted over an entire year.

*Andropogon gerardii* and *D. oligosanthes* have multiple distinctive bud developmental stages. *Andropogon gerardii* was synchronous in its bud development and its bud bank was composed of multiple annual cohorts. The bud bank of *D. oligosanthes* was developmentally asynchronous and was comprised of a single bud cohort since its bud bank underwent a complete turnover in early summer. The different roles of buds in the life history of each species reflected their differences in bud longevity, quality, and dormancy. In *D. oligosanthes*, belowground buds enabled plant survival over the $C_3$ summer dormant period whereas juvenile tillers overwintered during the longer winter dormant period. In contrast, *A. gerardii* survived its single, winter dormant period as dormant buds. The higher-order bud production observed in *D. oligosanthes* multiplied its tiller production potential and, along with its shortened internodes, contributed to its caespitose growth form. The rhizomatous growth form of *A. gerardii* resulted from its lack of higher-order bud production and its elongated internodes.

Differences in production of buds per vegetative and flowering tiller were quantified in *A. gerardii*. Flowering tillers of *A. gerardii* produced larger numbers of buds per tiller and transitioned a larger proportion of their buds to tillers than did vegetative tillers. Therefore, no tradeoff between sexual and vegetative reproduction was evident. Developmental constraints likely prevented such a tradeoff.

Bud bank dynamics offer insight into the control of grass population dynamics, production, and ultimately aboveground net primary production (ANPP) and will be useful in understanding the underlying mechanisms by which management practices and environmental change can alter perennial grasslands.

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# Dedication

## *Soli Deo Gloria*

I dedicate this work to the glory of God who has given me salvation through Jesus Christ His Son and to the wonderful people, my family and friends, He has given me on this earth.

To my family and friends, who have supported me in numerous ways over the course of my life and especially during this research. Their constant encouragement and love have kept me going. I especially dedicate this work to my parents and my brother. They have provided inspiration, counseling, laughter, and IT support. Dad, thanks for showing me the joys data can bring and helping me learn to be a positive thinker. Mom, thanks for not giving up on my math skills in grade school and your listening ears. Paul, thanks for all the reality checks and of course the IT help.

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# CHAPTER 1 - Bud Bank Dynamics of a $C_3$ and a $C_4$ Grass


## Abstract

Annual regeneration and sustainability of perennial grass populations rely heavily on the belowground population of meristems (the bud bank), yet the production and dynamics of grass bud banks and the stages of bud development have not been explored. Since the two major photosynthetic guilds of grasses vary in their aboveground dynamics, their belowground bud bank dynamics would likely vary as well. Therefore, the bud banks of *Dichanthelium oligosanthes* (Schult.) Gould, a representative $C_3$ grass, and *Andropogon gerardii* Vitman, a representative $C_4$ grass, were examined in tallgrass prairie throughout an entire annual cycle. *Andropogon gerardii* was synchronous in its bud development and maintained viable buds from multiple annual cohorts. The bud bank of *D. oligosanthes* was asynchronous in its development and was comprised of a single bud cohort since it experienced a complete turnover of its bud bank during late June. The interspecific differences in bud bank development and age distribution were related to differences in bud longevity, quality, and control of dormancy. These differences in individual bud characteristics indicated different roles of the bud bank in each species. Although both *A. gerardii* and *D. oligosanthes* were inactive over the winter season, *D. oligosanthes* also reduced its activity during the peak temperatures of summer due to its $C_3$ photosynthetic pathway. *Dichanthelium oligosanthes*, which tillers in the fall, used buds to survive over this short summer dormancy period and juvenile tillers to survive over the longer winter dormancy period. In contrast, *A. gerardii* used its buds to overwinter. Thus, the bud bank characteristics of each species were determined by the bud's role in the life history of the plant. Knowledge of bud bank dynamics, as it offers insight into the control of grass regeneration and ultimately regulation of ANPP, will be useful in understanding the underlying mechanisms by which management practices and environmental change can alter perennial grasslands.


## Introduction

Grassland ecosystems and many of their processes and functions are defined by their primary vegetation, grass. Although grass recruitment from both seed and vegetative bud occurs, the persistence of perennial grasslands strongly relies on vegetative reproduction via tiller

recruitment from the bud bank (Benson and Hartnett, 2006). While the roles and dynamics of seed banks have been thoroughly studied (Baskin and Baskin, 1998), our understanding of bud banks is in its infancy.

The current understanding of prairie grass bud bank production, longevity and phenology is limited to inferences gleaned from plant community-level studies, typically from a single census of the bud bank during the dormant season, (Benson et al., 2004; Dalgleish and Hartnett 2006, 2009) and a few studies focused at the population level (Cable, 1971; Noble et al., 1979; Mueller and Richards, 1986; Busso et al., 1989; Mullahey et al., 1991; Busso et al., 1993; Hendrickson and Briske, 1997; Hartnett et al., 2006; N'guessan, 2007; Dalgleish et al., 2008). In all these studies, seasonal bud bank sampling was the most intensive sampling conducted except for one study in which bi- and tri-weekly sampling occurred during the growing season (Dalgleish et al., 2008). Although tiller dynamics are inherently associated with the bud bank, population studies of tillering trends often begin at tiller emergence without consideration of previous stages such as bud production, dormancy, and outgrowth (e.g., Robson, 1968; Butler and Briske, 1988; Olson and Richards 1988a, 1988b). When buds have been taken into consideration, their date of natality was usually unknown (e.g., Mueller and Richards, 1986; Hendrickson and Briske 1997). The effects of disturbances and environmental conditions on bud bank size and viability have been the primary focus of published studies while basic bud bank dynamics under ambient conditions or the ecological consequences of the maintenance of a dormant bud bank have not been well documented. Studies have compared grass and forb bud bank densities in grassland communities subject to grazing or fire (Benson and Hartnett, 2004; Dalgleish and Hartnett, 2009) and across a precipitation gradient (Dalgleish and Hartnett, 2006). However, community-level bud bank measurements, or annual censuses of the bud bank densities, do not offer a full understanding of the dynamics within the bud bank because they are composed of multiple species with potentially varying bud longevities, productivities, developmental stages, and phenologies. The underlying mechanisms driving changes in bud bank dynamics in response to disturbance or changes in environmental conditions and the bud bank's effect on aboveground grass populations will become more apparent with an increased understanding of basic bud bank ecology.

Growth forms (e.g., caespitose, rhizomatous) and aboveground phenologies of grasses likely are a consequence of their basic bud bank ecology. Plant architecture is largely

determined by patterns of bud placement and outgrowth. Variation in bud bank dynamics and their spatial arrangement creates variation in plant architecture which can help explain plant dispersal capabilities in the field. Yet the morphology and development patterns of vegetative buds have been described for very few grass species. Photosynthetic pathway (e.g., $C_3$ vs. $C_4$) plays a major role in determining the optimal growing temperature range of a species and ultimately its growth period, flowering phenology, and geographic distribution. Since these two photosynthetic guilds differ in growth phenology, bud bank phenology likely differs between the two guilds as well.

Foundational knowledge of bud bank dynamics of species with different growth forms and photosynthetic pathways can provide insight into the mechanisms that drive population, community, and ecosystem processes in grasslands. Comprehension of clonal plant population dynamics are dependent on understanding the demography of meristems (Noble et al., 1979). If we understand how a species' bud bank functions phenologically, we can then predict how the timing and action of disturbances, such as grazing or fire, or fluctuations in temperature or precipitation might affect the species' bud bank and hence its tiller population dynamics. Dispersal of perennial species throughout the grassland vegetation matrix can be further explained by the interspecific plasticity in plant architecture which is dependent on patterns of bud placement and outgrowth. Tracking bud fate will allow vegetative reproductive potential to be linked to vegetative reproductive success. In addition to linking the belowground bud bank to aboveground vegetation processes, detailed bud bank studies conducted similar to studies of the seed bank (e.g., Sarukhan, 1974) can facilitate further comparison of the ecological consequences of variation in these two means of plant propagation.

Therefore, the primary objectives of this study were to: 1) Describe the bud and tiller developmental stages and morphology of a representative $C_3$ and $C_4$ perennial grass 2) Compare the temporal bud bank dynamics and bud longevity of a representative $C_3$ and $C_4$ grass 3) Relate the bud bank dynamics of each grass to its tiller dynamics and 4) Examine bud production of different annual cohorts within each species. This is the first detailed study describing bud developmental stages and examining bud bank dynamics of any grass species.

# Materials and Methods

## *Site Description*

The study was conducted at Konza Prairie Biological Station (KPBS), a 3,487-ha tallgrass prairie preserve, which is located in the Flint Hills region of northeastern Kansas (39º05'N, 96º35'W). The hilly topography and limestone bedrock results in shallow, rocky upland soils (Udic Argiustolls, Florence series), steep slopes, and deep lowland soils (Pachic Argiustolls, Tully series). The vegetation composition at KPBS is dominated by warm season ($C_4$) grasses, such as *Andropogon gerardii* Vitman (big bluestem), *Sorghastrum nutans* (L.) Nash (Indiangrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem). Subdominant vegetation includes cool season ($C_3$) grasses, composites, legumes and other forbs, and a few woody species. Due to the continental climate at KPBS, the majority of the mean annual precipitation (835mm) falls during the warm, wet springs and summers. KPBS is divided into multiple experimental units, each defined as a single watershed. Each watershed contains upland, slope, and lowland topographical positions and has both a grazing and fire regimen assigned to it. Fire regimes include 1-, 2-,4-, 10-, and 20- year fire return intervals and grazing regimes include grazed or ungrazed by bison. Two replicate watersheds (KPBS unit 4A- 47ha and KPBS unit 4B-135 ha), each with an ungrazed and 4-year spring fire return interval regimen, were chosen for this study. At the beginning of this study in fall 2007, it had been one year and two years since last fire on watershed 4A and 4B, respectively. Vegetation at these intermediate fire return interval sites are dominated by grass with both $C_3$ and $C_4$ grasses being readily abundant. In annually burned prairie, $C_4$ grasses dominate and $C_3$ grasses are less abundant.

## *Field Sampling*

*Andropogon gerardii* is a warm season ($C_4$), stout, short-rhizomatous, dominant perennial grass that grows from 60cm to 140cm in height. Flowering in the Great Plains occurs from July to October (Great Plains Flora Association, 1986). Due to the rhizomatous growth form of *A. gerardii* and intermingling of tillers from different genets, discrete genets are very difficult to identify. *Dichanthelium oligosanthes* (Schult.) Gould (Scribner's panicum) is a cool season ($C_3$) sub-dominant caespitose perennial grass that grows from 10cm to 70cm in height. Flowering occurs in the Great Plains from April to June although branching secondary panicles may bloom until the fall (Great Plains Flora Association, 1986). Genets of *D. oligosanthes* are easy to

determine because of its tufted growth form. These two species were chosen for this study because they were the most frequently encountered native grasses on KPBS within their respective photosynthetic pathway guilds. Bud banks of $C_3$ and $C_4$ species would be predicted to differ phenologically because the activity and growth of $C_3$ species are highest in the spring and fall whereas the activity of $C_4$ species peak in the summer. *Andropogon gerardii* and *D. oligosanthes* also differed in their respective rhizomatous and tufted growth forms. Therefore, by using *A. gerardii* and *D. oligosanthes* as representative species, the bud bank consequences of variant growth forms and photosynthetic pathways could be considered.

At each of five upland sites on each watershed, individuals of *A. gerardii* and *D. oligosanthes* were sampled and marked in 2007 at the end of their respective flowering periods (August for *A. gerardii* and June for *D. oligosanthes*). Sites on each watershed were selected to be as distant from one another as possible while remaining on upland soils. On watershed 4A and 4B, sites were separated by an average 90 ± 13 meters and 208 ± 40 meters, respectively. An individual of *A. gerardii* consisted of a flowering tiller with all neighboring tillers and associated belowground parts within a 15cm diameter circle. A *D. oligosanthes* individual consisted of an entire genet. Hereafter, sampled *A. gerardii* and *D. oligosanthes* individuals will be referred to as "plants." Each plant was marked by encircling its base with a wire ring and its tillers were each counted and encircled with a small wire ring. Plants of *D. oligosanthes* were marked in early June and again in early October while *A. gerardii* was marked in October.

The number of tillers per sampled plant was counted when *A. gerardii* and *D. oligosanthes* were marked. The 2007 tiller counts per plant did not change between the June 2007 and October 2007 markings of *D. oligosanthes* as there was no observed decomposition of tillers during the summer. Tiller cohorts from before 2007 were not distinguishable from one another and were no longer visible aboveground for *A. gerardii* and *D. oligosanthes* during fall 2007. Therefore, all residual pre-2007 tiller bases of *A. gerardii* were counted when plants were harvested and the soil surrounding their belowground parts was washed away. Residual pre-2007 tiller bases of *D. oligosanthes* were not counted as they had decomposed.

For each species, ten plants, one from each site on each watershed, were harvested biweekly starting on September 18, 2007 for *D. oligosanthes* and on October 16, 2007 for *A. gerardii*. This biweekly sampling continued through July 2008 when the next generation of tillers of both species had flowered. This subsequent 2008 set of plants was similarly marked

with large rings as done in 2007. However, 2007 tillers were no longer present aboveground and could not be counted or marked with rings. Residual 2007 tillers of *A. gerardii* were distinguishable belowground from pre-2007 residual tillers by their healthy lutescent coloring and their leaf remains often found at the distal portion of their residual tiller base. Residual 2007 tillers of *D. oligosanthes* were identified by their short tiller stems protruding from their dead tiller base. The current 2008 generation of tillers of both species were easily identified for the remainder of the study and, therefore, were not counted or marked. Biweekly harvesting continued until each species fully senesced aboveground. *Andropogon gerardii* and *D. oligosanthes* were last sampled on October 20, 2008 and December 2, 2008 respectively.

Plants were harvested by excavating to a 7cm depth for *D. oligosanthes* and a 15cm depth for *A. gerardii*. All interconnected belowground rhizomes of both species occur within these specified soil depths and were collected as part of the plant. Any rhizomes of *A. gerardii* that extended outside the ring encircling the plant were severed. Since *D. oligosanthes* is a tufted grass, the entire plant was able to be harvested without severing belowground connections. Following harvest, each plant was washed to remove soil and other species found with it were discarded.

## *Lab Methods*

Buds and tillers were counted, assessed to be living or dead, and classified by developmental stage and hierarchical level. Buds and tillers were examined using a dissecting scope with magnifications between 7 and 25x.

### *Andropogon gerardii*

Parent tillers were classified by their cohort (pre-2007, 2007 or 2008 recruits) and as flowering or vegetative. As observed in other grass species (Hendrickson and Briske, 1997), buds of *A. gerardii* have greater longevity than their parent tillers, remaining viable up to three years. These longer lived buds exist on the base of their residual parent tiller. These residual tiller bases can be identified to year of recruitment as described above (Plate 1.1A,C). Flowering tillers were differentiated from vegetative tillers by the presence of a flowering head. Bolting tillers were considered vegetative tillers since tillers were not considered to be flowering until their seed head was exposed. In this study's observations, bolting tillers always completed their development into a flowering tiller. Since vegetative tillers in *A. gerardii* are culmless, residual

2007 tiller bases could be identified as flowering tillers by the increased diameter of their base due to the culm development and the larger size of their buds.

Rhizomes and buds borne on them were counted separately from tillers and their buds. Rhizomes were composed of multiple internodes extending laterally longer than 3cm. The majority of newly emerging tillers arose at the base of the parental tiller either from buds directly at the parental tiller's internode or buds that elongated as short rhizomes composed of one internode of 3cm or less. Fewer tillers emerged from the apices of rhizomes.

***Dichanthelium oligosanthes***

A hierarchical order of bud development occurs in *D. oligosanthes* with existing buds and juvenile tillers producing axillary buds (Chapter 3). Buds themselves are composed of multiple tiny phytomers. If meristematic portions of these phytomers are active and grow, fully formed buds of higher order can be found. This hierarchy has four levels at which buds or juvenile tillers, the propagule supply, may be produced: primary (arising on mature tiller internodes), secondary (arising on primary buds and juvenile tillers), tertiary (arising on secondary buds and juvenile tillers), and quaternary (arising on tertiary buds and juvenile tillers). This bud hierarchy was not discovered until September 2008 and is discussed in detail in chapter 3.

Mature tiller senescence was recorded during the fall and early winter sampling periods of 2007 and 2008 (Plate 1.1D). Survival of mature tillers in 2007 and 2008 was calculated as the proportion of mature tillers conducting photosynthesis in any leaf. A more sensitive index was also used in 2008 that allowed a mature tiller to be partially senesced when less than half of its leaves were conducting photosynthesis.

## *Analysis*

Using the bud and tiller classifications of both species, the number of buds per tiller was calculated for each plant according to developmental bud stage and adult tiller generation. Lab tiller counts closely resembled field tiller counts and were therefore used when calculating buds per tiller. Proportions of total buds and tillers found on a plant were calculated according to developmental stage. Overall bud production per tiller of *A. gerardii* includes all dormant and active buds. Overall bud production per mature tiller of *D. oligosanthes* includes all dormant, active, and photosynthesizing buds whereas overall propagule production per mature tiller of *D. oligosanthes* includes all bud stages and juvenile tillers.

Plant developmental processes are often regulated by temperature, and soil temperature has been shown to be a regulator of belowground processes such as root growth (Kaspar and Bland, 1992). Therefore, bud and tiller production and development were observed in relation to daily soil temperature data taken at 2cm depths by the Konza Prairie LTER program.

# Results

## *Andropogon gerardii*

### *Description of bud and tiller developmental stages*

In *A. gerardii*, three main bud developmental stages were identified, characterized by their coloration and size (Plate 1.2). (1) Developing buds are deltoid to lanceolate in shape and white with hirsute margins. The abaxial surface of the bud molds to the curvature of the parent tiller's base. (2) Developed dormant buds are larger in basal girth than developing buds which yields a conical or plano-convex bud shape. A brown prophyll surrounds the bud. Developed dormant buds are from 2.5mm to 9.0mm in height and from 1.0mm to 3.5mm in basal width. (3) Active buds are characterized by a deep fuchsia color and sometimes elongation beyond the tip of the prophyll. Active buds transition into tillers when they begin photosynthesis at their tips and continue elongation. At this developmental stage, the young tillers are several centimeters in height with developed but unfolded leaf blades. As the tillers age, the leaf blades unfold from the apex but remain ascending. For this study, dormant bud, active bud, and tiller were the three developmental categories that were used. Therefore, developing buds (1) and developed dormant buds (2) were grouped as dormant buds. Dead buds, easily identified by their soft or mealy brown interiors, and dead 2008 residual tillers were also counted (Plate 1.1B). Adult 2008 tillers that had been killed by herbivory or causes other than natural senescence were considered dead 2008 residual tillers.

### *General life cycle of a bud*

Bud natality began on six-week-old tillers at the end of May and was concluded within 10 weeks. These young buds joined older age classes of dormant buds produced in previous years to form the overwintering dormant bud bank. In late March, active buds were recruited from the dormant bud bank and subsequently tillered in late April. A subset of the vegetative

tillers flowered in late July and all tillers senesced by the end of October. Unless severe herbivory or other factors caused the death of a mature tiller during the growing season requiring the outgrowth of a dormant bud to replace it, the transition of dormant buds to active buds and then to tillers was rare at any other time. The median life cycle of a tiller took 16.3 months to complete in which it spent 9.8 months of its life as a dormant bud, 1.3 months as an active bud, and 5.2 months as a tiller.

### *Overall bud production and dynamics*

#### *Pre-2007 and 2007 cohorts*

The study of *A. gerardii* began in October 2007 and ended in October 2008. This allowed assessment of the 2007-8 contribution of various bud cohorts, including those buds initiated in 2007 and those prior to 2007, to the 2007-8 overwintering bud bank, bud transition to 2008 tiller, and 2008 tiller senescence (Figure 1.1). In the 2007-8 overwintering bud bank, the pre-2007 cohort maintained fewer buds (1.99 ± 0.17 dormant buds/tiller) than the 2007 bud cohort (7.24 ± 0.33 dormant buds/tiller; Figure 1.1A). Although fewer buds were maintained per pre-2007 tiller than 2007 tiller, more residual pre-2007 tillers than 2007 tillers (5.3 ± 0.4 pre-2007 tillers/ 2007 tillers) were present per plant over the entire time of the study. Residual pre-2007 tillers varied significantly in their contribution of dormant buds (range of 0 to 11 buds/residual tiller). Although active buds were recruited from both pre-2007 and 2007 cohorts, dormant buds from the pre-2007 cohort (0.12 ± 0.02 active buds/tiller) were recruited to a much lower extent than 2007 dormant buds (2.38 ± 0.20 active buds/tiller; Figure 1.1B). Based on the ratio of active buds to tiller within each cohort, transition from active bud to tiller was quite successful since 2008 tillers per pre-2007 and 2007 tillers averaged 0.17 ± 0.05 and 2.28 ± 0.13 respectively (Figure 1.1C). A small number of active buds died between June 4$^{th}$ and August 13$^{th}$ in both pre-2007 (0.02 ± 0.01 active buds/tiller) and 2007 (0.19 ± 0.06 active buds/tiller) cohorts. Among the buds at the base of a parent tiller, the order of emergence occurred from the acropetal to the basipetal direction with the most distal bud occasionally remaining dormant.

In the 2008 growing season, *A. gerardii* more than adequately replaced its previous season's tillers as at least two tillers were produced for every 2007 tiller (Figure 1.1C). Tiller production above the population maintenance level of one 2008 tiller per 2007 tiller correlated with the increased biomass production observed on Konza Prairie in 2008. Annually spring

burned watersheds on Konza Prairie had grass biomass production in 2008 that was 1.5x the long term average. Of those 2008 tillers produced from the 2007 bud cohort, 14 ± 4% of them became flowering tillers in late summer (Figure 1.1D,E). An individual 2007 tiller had the capacity of producing multiple flowering 2008 tillers. After dormant bud activation and tiller emergence in March and April, a low amount of dormant bud mortality occurred within the 2007 bud cohort during the summer starting in June, reducing the number of live buds and their established tillers from 7.46 ± 0.12 per tiller in spring and early summer to 5.77 ± 0.12 per tiller in late summer and fall (Figure 1.2). Herbivory of dormant buds was not evident at this time of year. Rhizomes made a small contribution to the bud bank (0.83 ± 0.10 dormant buds on rhizome/ 2007 tiller) and none of their dormant buds were ever observed transitioning to the active bud or tiller stages.

Within the bud bank as a whole, the pre-2007 and 2007 bud cohorts were similar in phenology but varied in the proportion of buds in different developmental stages (Figure 1.3). The pre-2007 bud bank transitioned a lower proportion of its buds from the dormant to active stage. On average, 13 ± 1% of the pre-2007 bud bank and 34 ± 1% of the 2007 bud bank were released from dormancy. However, after floral initiation, a larger percentage of the pre-2007 cohort's vegetative tillers (32 ± 5%) became flowering tillers than the 2007 cohort's vegetative tillers (19 ± 2%; Figure 1.3). When dividing the established tillers produced in 2008 according to the bud cohort from which they originated, approximately one-third (35 ± 3%) of them originated from the pre-2007 bud cohort.

***2008 cohort***

Tillers originating in 2008 began producing new buds in May after they were six weeks old. Bud production was initially rapid with tillers accumulating 67% of their final dormant bud production in four weeks but decelerated and plateaued at 8.21 ± 0.17 buds/tiller by the end of twelve weeks (Figure 1.4). Dead buds were rarely found on 2008 tillers (0.09 ± 0.02 dead buds/tiller).

As fall approached, 2008 adult tillers began to senesce aboveground. However, some of these tillers died due to herbivory or causes other than natural senescence. Herbivory by caterpillars occurred on actively growing apical meristems of 2008 tillers, especially in July and August. Caterpillars preferred bypassing the outer, more accessible 2008 cohort of axillary buds

and created tunnels into the core of the tiller to consume the apical meristem of the growing tiller. In some cases when the apical meristem was destroyed on a 2008 tiller, a new tiller was recruited from one of its younger 2008 dormant buds. Although some tillers experienced herbivory, naturally senescent tillers were much more common in the ungrazed conditions of this experiment (Figure 1.1C,F). These damaged tillers, or dead residual tillers, appeared to contribute smaller numbers of buds (3.45 ± 0.38 dormant buds/tiller) to the bud bank than those tillers that die naturally during this same time period (8.21 ± 0.17 dormant buds/tiller).

***Bud activity in relation to soil temperature***

The activation of buds in the spring tracked changes in soil temperature. When soil temperatures were consistently above freezing, dormant buds began their transition into active buds and ultimately tillers (Figure 1.5). Tiller recruitment was completed before the warmer summer temperatures occurred. Tillers produced in 2008 completely senesced aboveground in October before soil temperatures dropped below 11º C.

## *Dichanthelium oligosanthes*

***Description of bud and tiller developmental stages***

In *D. oligosanthes*, three bud developmental stages were identified, each with a characteristic bud size, shape, and photosynthetic activity (Plate 1.3A-F). (1) Dormant buds have a rotund to deltoid complanate shape. If they are large enough to have an abaxial surface, it rests appressed to the parent tiller. Dormant buds are usually as wide as they are tall and can range in height from 0.30mm to 2.10mm and in width from 0.30mm to 1.40mm. Their coloring is either all white or deep fuchsia with a white tip. Overall, dormant buds tend to be smaller than all other bud classes and have box-like dimensions. (2) Active buds have a conical shape with a well-defined apex. Their height ranges from 1.90mm to 9.80mm and their basal width ranges from 1.01mm to 2.20mm. Active buds are white at their apex and fuchsia at their base. (3) Photosynthesizing buds are round to conical in shape with a yellow to green coloration at their apex. They typically range in height from 3.0mm to 12.30mm and width from 0.70mm to 2.20mm. Dead buds were also identified by their soft, brown interiors.

Two tiller developmental stages were identified and distinguished by phytomer size and leaf blades (Plate 1.3G-I). (1) Juvenile tillers have at least one and no more than three leaf

blades open perpendicular to their bud tip. These young tillers conduct photosynthesis in their leaves and apex. Phytomers of juvenile tillers are usually less than 1cm from ligule to node. (2) Mature tillers have more than three leaf blades open perpendicular to the stem axis. Mature tillers have distinctly elongated phytomers measuring at least 2cm from ligule to node. Mature tillers have proportionally larger and longer leaf blades than juvenile tillers. Juvenile tillers are considered dead if their interior is brown and adult tillers are considered dead if their aboveground leaves have fully senesced.

***General life cycle of a bud***

Production of dormant buds began in late April to early May and continued throughout the growing season. During most of the summer, new dormant buds were continually produced and existing buds transitioned to active buds. However, dormant bud production and dormant bud transition to active bud continued simultaneously but at a slower rate from late June until August. Active buds transitioned to photosynthesizing buds starting in late summer. In September and October, photosynthesizing buds quickly transitioned into juvenile tillers. Juvenile tillers survived over the winter and grew out into mature tillers in May of the following year while buds that had overwintered died by July. Mature spring tillers fully senesced by December. Assuming that a bud originated in May in the dormant stage, overwintered as a juvenile tiller, and became a mature tiller the following spring, its life span is approximately 19 months. Buds that were produced later in the growing season have shorter life spans (approximately 15 months). In contrast with the synchrony in bud development observed in *A. gerardii*, buds of multiple ages and stages occurred in *D. oligosanthes*.

***Overall bud production and dynamics***

***2007 cohort***

When our study began in fall 2007, mature 2007 tillers had finished their production of buds and juvenile tillers (Figure 1.6A-D). The overwintering propagule supply for next season was thus composed primarily of juvenile tillers (4.36 ± 0.42 juvenile tillers/tiller) and active buds (3.23 ± 0.33 active buds/tiller) while dormant buds (1.05 ± 0.14 dormant buds/tiller) and photosynthesizing buds (0.68 ± 0.26 photosyn. buds/tiller) were present in small numbers. When considering the bud bank collectively, an asynchrony in bud development was evident

(Figure 1.7A). Phytomers of juvenile tillers elongated in late April to become mature 2008 tillers. A mature 2007 tiller produced an average of 2.81 ± 0.20 mature 2008 tillers over the growing season which meant that the majority of overwintering juvenile tillers successfully transitioned to mature tillers in 2008. The increased grass biomass production on Konza Prairie in 2008 may be reflected in the tiller production above the population maintenance level of one 2008 tiller per 2007 tiller Shortly after mature tiller recruitment in 2008, the 2007 bud cohort declined as evidenced by an increase in observed dead buds (Figure 1.6F). By July 2008, the 2007 bud cohort had almost completely died or had become tillers (Figure 1.7A). Almost all tillers were determinate in their flowering.

***2008 cohort***

Buds of the 2008 cohort were continuously produced throughout the growing season (Figure 1.8). In spring 2008, two-week old mature tillers began producing dormant buds in early May and some of these dormant buds began transitioning to active buds in late May (Figure 1.8A,B). In order to maintain a dormant bud bank, overall dormant bud production continued as dormant buds transitioned to higher developmental stages. Although a few photosynthesizing buds were observed in June, photosynthesizing bud production rapidly increased beginning in mid-August (Figure 1.8C). Photosynthesizing buds transitioned into juvenile tillers starting in early August and their production peaked in early November (Figure 1.8C,D). During this time, an average tiller gained one juvenile tiller every 20 days. Bud mortality was not notable until December. The majority of the 2008-9 overwintering propagule supply for the 2009 tiller population was in the dormant bud and active bud stages with smaller numbers in the photosynthesizing bud and juvenile tiller stages (Figure 1.7B).

During the winter months, dormant bud abundance differed greatly between the 2007 and 2008 cohorts. In the 2008 winter bud bank, dormant bud counts were very high (11.21 ± 1.45 dormant buds/2008 tiller) when compared with the winter bud bank of 2007 (1.05 ± 0.14 dormant buds/2007 tiller). The differences of bud production between years may partially be explained by differences in bud production but also observer bias. Observer knowledge increased during the study enabling the dissector to detect the smallest dormant buds and dormant bud production on juvenile tillers in the 2008 bud cohort. Dormant buds residing on juvenile tillers did account for 55 ± 5% of the dormant buds produced in the 2008 winter bud

bank. Therefore, conclusions about the interannual variability of *D. oligosanthes* cannot be firmly reached with these data.

***Bud activity in relation to soil temperature***

Soil temperature was correlated with bud phenology and production of the cool-season grass *D. oligosanthes* by creating two main activity periods, spring and fall. In the spring of 2008, juvenile tillers transitioned to mature tillers a month after soil temperatures were consistently above freezing (Figure 1.9). Mature 2008 tillers began producing buds in June and had reached maturity before peak summer temperatures. Bud bank activity was reduced in the 2007 and 2008 bud cohorts during the peak 2008 summer soil temperatures (Figure 1.10). When soil temperatures were consistently above 22º C, the 2007 bud and juvenile tiller cohort diminished and the 2008 bud cohort production was slowed. The overall bud bank and the overall dormant bud bank were smallest in late May to July because the 2007 cohort of buds was rapidly dying and the 2008 bud cohort was just beginning its production (Figure 1.6A-C, 1.8A-C).

During the fall activity period, the 2008 bud cohort transitioned to higher levels of development than reached in the spring. Photosynthesizing buds and juvenile tillers of the 2008 cohort were not produced until mid-August and increased in production as soil temperatures cooled (Figure 1.8C,D). A regression of mature tiller survival against average weekly soil temperatures showed that declining soil temperatures in the fall strongly correlated with mature 2008 tiller senescence (Regression, $r^2$=0.85, $p<0.0001$, $y=0.06x-0.16$). Tiller senescence began at temperatures below 20º C and was completed around 3º C. In both 2007 and 2008, juvenile tiller natality increased as mature tiller mortality increased in the fall (Figure 1.11). Thus, soil temperature not only related to mature tiller senescence but also juvenile tiller natality.

Because there were two active periods for *D. oligosanthes*, there were also two bud dormancy periods, summer and winter. Bud development in the 2008 cohort was slowed and bud death in the 2007 cohort occurred rapidly when temperatures were too high during the summer. Bud production was halted for a longer period of time, although extensive bud mortality did not occur, when temperatures were too low during the winter. The summer dormant season bud bank (5.78 ± 0.47 buds/tiller including 2007 and 2008 cohorts of buds) was smaller than either the 2007 or 2008 winter season propagule supply (9.32 ± 0.87 buds and juvenile tillers/ 2007 tiller, 20.88 ± 1.75 buds and juvenile tillers/2008 tiller). The summer bud

bank consisted of only dormant and active buds unlike the winter propagule supply which also had photosynthesizing buds and juvenile tillers. Therefore, *D. oligosanthes* maintained a larger propagule supply over the longer winter dormant season leading up to spring mature tiller recruitment than over the shorter summer dormant season.

### *Bud bank phenology comparison of A. gerardii and D. oligosanthes*

Development pattern and timing leading to tiller maturity and senescence differed between the two grass species. In late April, both species had individuals who reached the mature tiller stage. Dormant buds of *A. gerardii* had transitioned to active buds in late March and completed their active bud development phase before juvenile tillers of *D. oligosanthes* began elongation to become mature tillers. Although *D. oligosanthes* overwintered as juvenile tillers, by late April they were no further along in their tiller development than *A. gerardii*. However, *D. oligosanthes* did begin bud production on its mature tillers two weeks before *A. gerardii*. While timing of mature tiller natality was similar between the two species, tiller mortality occurred earlier in *A. gerardii*. The aboveground leaves of *A. gerardii* fully senesced by mid-October whereas full senescence of *D. oligosanthes* did not occur until soil temperatures neared freezing in December.

The strict synchrony in stage transitions observed in *A. gerardii* was not seen in *D. oligosanthes* (Figure 1.12). When combining all cohorts of buds to study overall bud bank dynamics, *A. gerardii* only transitioned buds from dormancy in the spring. Although *D. oligosanthes* only recruited mature tillers in the spring as *A. gerardii* did, its dormant buds continually transitioned into active buds all year long and active buds transitioned to photosynthesizing buds and juvenile tillers in the fall. Therefore, *D. oligosanthes* maintained active stages of development over longer periods of time than *A. gerardii* resulting in interspecific differences in the composition of the overwintering bud banks. *Dichanthelium oligosanthes* retained large numbers of dormant and active buds all year long causing photosynthesizing buds and tiller stages to drive changes in its bud bank developmental stage composition (Figure 1.12B).

Buds from multiple cohorts contributed to the bud bank of *A. gerardii* whereas a single bud cohort comprised the bud bank of *D. oligosanthes* at any one time (Figure 1.13). A complete generational bud bank turnover occurred in *D. oligosanthes* during June and July while

*A. gerardii* continually maintained multiple bud cohorts. Because a bud cohort's contribution to the overall bud bank size diminishes with age, the most recently formed cohort of *A. gerardii* buds comprises the majority of the bud bank. Therefore, a single bud cohort contributed to the annual regeneration of aboveground tillers in *D. oligosanthes* while multiple bud cohorts did in *A. gerardii*.

## Discussion

### *Andropogon gerardii*

The bud bank of *A. gerardii* consisted of multiple cohorts. Each cohort of dormant buds was produced during spring and summer and buds are capable of living at least 2.5 years. The synchronous bud development phenology of *A. gerardii* was observed across all cohorts although cohorts varied in overall abundance and proportion of buds recruited to tiller each season. Each spring, individuals from all bud cohorts broke dormancy and transitioned to tiller as soil temperatures increased above freezing. Buds, regardless of age, remained in the dormant stage at other times of the year. Although older cohorts had smaller numbers of buds per tiller and transitioned lower proportions of their dormant buds to tillers than the youngest bud cohort, older cohorts had larger numbers of residual tillers per plant which enabled them to still contribute one-third of the 2008 tiller population.

Tiller recruitment from dormant buds was highly successful within all cohorts as almost all buds that were activated became tillers. As a result of low dormant bud mortality of the 2007 bud cohort and rapid bud production of the 2008 bud cohort, dormant buds that had died in the older cohort were more than adequately replaced.

Bud production is closely tied to tiller growth. Grass tillers are modular units comprised of multiple phytomers (Etter, 1951; Harper, 1981; Briske, 1991; Evert, 2006). Each phytomer consists of an internode, leaf sheath, leaf blade, and axillary bud (Briske, 1991; Evert, 2006) and forms nodes with the connecting phytomers above and beneath it (Sharman, 1942). As a tiller grows, its apical meristem continually adds phytomers by leaving behind regions of actively dividing cells. Each region is used to produce the leaf blade, leaf sheath, internode, and axillary bud of a phytomer (Sharman, 1942; Etter, 1951; Langer, 1972; Briske, 1991; Evert, 2006). The bud is the last structure formed of the phytomer, after the leaf and the internode (Sharman, 1942; Etter, 1951). Rapid production of buds of *A. gerardii* in June coincided with the peak time of

tiller growth. The continual addition of full sized phytomers to attain more leaf area also added buds at the same rate. Because grasses condense their nodes at the base of the tiller only exposing their leaves aboveground during vegetative growth, buds accumulate belowground (Hyder, 1972; Jewiss, 1972). As *A. gerardii* tillers reached their peak mid-summer vegetative size, bud production slowed. Since rhizomes of *A. gerardii* have few internodes and, therefore, few buds, they were unable to significantly contribute to the overall bud bank.

The basal accumulation of primary buds is permanently stopped when a tiller flowers. Upon floral induction, the apical meristem switches production of vegetative buds to production of spikelet buds (Sharman, 1947; Langer, 1972). The vegetative phytomers, which still exist as primordia after the apical meristem has been induced to flower, grow with elongated internodes, collectively forming the culm (Hyder, 1972), which raises the seed head for wind dispersal. Although these remaining phytomers may still produce vegetative buds, these buds usually become aerial buds instead of basal buds due to this internode elongation.

Lepidopteran caterpillars consumed apical meristems of actively growing tillers in July and August by eating tunnels through the tiller base and bypassing the recently produced buds on the tiller. Two potential hypotheses for this selective grazing are: (1) the apical meristem contains more nutrients than buds because it is active whereas the buds are dormant or (2) the outer scale of the axillary buds deters herbivores. Flowering in July and August during peak herbivory allowed some tillers to avoid death by transforming their apical meristem into a flowering head and removing it from easy ground access to the canopy. Although tillers exposed to grazing do not proactively produce more tillers to compensate for grazing effects (Hendrickson and Briske, 1997), a herbivorized tiller can undergo compensatory growth by producing a new tiller. When the apical meristem had been consumed in *A. gerardii*, a recently formed bud near the apex of the herbivorized tiller base grew out as a replacement tiller. Prairie that has undergone large herbivore grazing pressure over several years had lower grass bud densities likely due to tiller death before bud production was complete (Dalgleish and Hartnett, 2009). Bud production per tiller was reduced in *A. gerardii* tillers whose apical meristem was consumed by caterpillars. Grazing in late May to early June could strongly affect bud bank densities of *A. gerardii* since bud production of *A. gerardii* primarily occurs at this time and large grazers prefer the younger shoots which provide a key portion of the bud bank for the following season. Since the bud is the last component formed in a phytomer, death of its leaf

may inhibit further development of the phytomer's bud and ultimately bud production of the tiller.

***Bud dormancy***

The synchronous behavior and persistence of the bud bank of *A. gerardii* depends on the control of bud dormancy. Bud dormancy in grasses is dependent on three factors and their interactions: 1) environmental conditions, 2) the parent tiller (including apical dominance effects), and 3) the internal conditions within the bud itself. Applying the classifications used for seed dormancy (Harper, 1957; Nikolaeva, 1977; Baskin and Baskin, 1998), bud dormancy may be maintained by endogenous factors (from within the solitary bud), exogenous factors (from plant structures outside the bud, such as the parent tiller), or external environmental factors (enforced dormancy). Buds can be under multiple dormancy controls at the same time since the environment, exogenous, and endogenous factors can act independently of one another.

When considering both the youngest cohort formed during the growing season and the older cohorts from which tillers were recruited in the spring, multiple types of bud dormancy were evident in *A. gerardii* over its annual cycle. Overwintering dormant buds near the apex of the residual tiller base were released from enforced dormancy when soil temperature and other environmental conditions required for tiller growth were satisfied in the spring. However, dormant buds at lower positions failed to grow out into tillers and remained dormant as observed in other studies (McIntyre 1967, 1970, 1972; Mueller and Richards, 1986). Environmental conditions did not appear to inhibit bud outgrowth during the growing season as newly formed buds were able to grow out after herbivory of the apical meristem. Therefore, both the newly formed buds and the older dormant buds must be under innate dormancy caused by either endogenous or exogenous factors rather than enforced dormancy.

Exogenous bud dormancy can be maintained by mechanical or chemical mechanisms (following seed bank classifications; Nikolaeva, 1997; Baskin and Baskin, 1998). Buds can be mechanically restrained from outgrowth by the tightly enclosing leaf sheaths of the parent tiller as observed in wheat (Williams et al., 1975). Buds on a tiller base would be released acropetally from this dormancy over time as leaf senescence would start with the lowermost leaves.

Buds on actively growing tillers are in exogenous chemical dormancy since they are subordinate to the apical meristem that formed them. Apical dominance is mediated by auxin, other hormones, or a nutrient source-sink gradient, or a combination of these hypotheses (Cline,

1991; McIntyre, 2001; Tomlinson and O'Connor 2004). Apical dominance is removed when the apical meristem is destroyed as demonstrated by the outgrowth in *A. gerardii* of one or two buds upon caterpillar herbivory of the apical meristem. The remaining dormant buds could be subjected to the apical dominance of the newly formed tillers from distal buds (Richards et al., 1988) and therefore, remain in exogenous chemical dormancy. However, the distance of an apical meristem's influence has not been determined for grasses. By the end of the growing season, the apical meristem has senesced on both vegetative and flowering *A. gerardii* tillers. Therefore, overwintering buds are no longer under exogenous chemical dormancy by means of apical dominance. This may be evidenced by the large synchronous tiller recruitment from these overwintering buds observed in the spring upon release from enforced dormancy.

Perennial grasses are typically composed of many interconnected tillers, such that the tiller that determines exogenous bud dormancy could itself be influenced by neighboring tillers or environmental conditions. In a grazing study on *Agropyron desertorum*, resource sharing influenced new tiller growth but not new tiller emergence suggesting that neighboring tillers do not strongly affect bud dormancy (Olson and Richards, 1988b). Temperature, nitrogen, and precipitation can affect overall plant vigor and transpiration rates which could in turn affect the apical dominance mechanisms whether nutrient or hormonal.

During the growing season, endogenous-caused dormancy is evident in dormant *A. gerardii* buds on residual tiller bases which do not have an actively growing tiller. These buds cannot be in enforced dormancy as the conditions for growth are adequate. Also, there is no obvious exogenous factor inducing their dormancy because no apical meristem is present and leaves have senesced. These well-developed dormant buds of older cohorts from previous seasons are most likely under endogenous physiological dormancy (following Nikolaeva, 1977; Baskin and Baskin, 1998). Endogenous physiological dormancy, whether caused by low metabolism, inhibitor presence, or lack of promoters, may be influenced by bud age and size. Due to the growth habit of grasses, a gradient of bud ages occurs along the tiller base with buds decreasing in age from base to apex. Well-developed dormant buds increase in size from the base to the apex of the tiller base (Mueller and Richards, 1986; Busso et al., 1989). Distal buds on the tiller base are the most likely to grow out (Mitchell, 1953; McIntyre, 1967, 1970, 1972; Mueller and Richards, 1986; Hendrickson and Briske, 1997). Therefore, the youngest and usually the largest buds grow out to become tillers. Older buds may need to overcome stronger

physiologically constraints than younger buds because they are dormant for longer periods of time. Smaller buds may have a more difficult time obtaining the resources necessary to grow out due to residual competition effects resulting from residual effects of apical dominance. Environmental conditions at the time of bud formation could also impact the quality of the buds. The existence of multiple gradients, including size, age, nutrient, and hormonal gradients, along the tiller base make it difficult to distinguish the reason why position affects bud outgrowth. Bud size may be affected by the nutritional and hormonal factors of apical dominance. Although internal bud physiology is somewhat independent of parent tiller physiology, their interactions with one another can affect bud dormancy.

### *Dichanthelium oligosanthes*

The continuous production and developmental transitioning of *D. oligosanthes* buds resulted in asynchrony in bud developmental stages. *Dichanthelium oligosanthes* produced dormant buds throughout the growing season like *A. gerardii*. However, the hierarchical bud production exhibited in *D. oligosanthes* enabled it to have increased rates of dormant bud production later in the season. In addition, its dormant buds were continually transitioning to higher developmental stages throughout the growing season.

An annual turnover of the bud bank of *D. oligosanthes* occurred. Bud longevity was limited to about one year with all buds dying in June regardless of whether they were born in the early or late portion of the previous summer. Therefore, tiller recruitment occurred exclusively from a single cohort every year. The next cohort of mature tillers began bud production before all buds of the previous cohort had died.

Two dormant seasons occurred in the annual cycle of *D. oligosanthes*. Over the summer dormant season, the propagule supply was comprised of only buds whereas buds and juvenile tillers persisted over the winter dormant season. Juvenile tiller natality was closely associated with mature tiller senescence in the fall. In turn, mature tiller senescence was correlated with decreasing temperature. Thus, *D. oligosanthes* maintained tillers throughout the whole year in either juvenile or mature stages. In the spring, about two-thirds of the overwintering juvenile tillers successfully transitioned into mature tillers but the overwintering buds died after the juvenile tillers had become mature tillers.

***Bud dormancy***

In the spring, dormant buds of the previous year's cohort remained in endogenous or enforced dormancy until their death in early summer. Since their parent tillers were dead, the dormant buds were no longer under exogenous dormancy. This older dormant bud cohort died within a month of mature tiller recruitment. Therefore, the older cohort may have been held in enforced dormancy almost to its death since tiller activity within the plant did not begin until shortly before the older bud cohort's death. When the environmental conditions were slightly favorable for growth, the well-developed dormant buds were unable to respond. Since these buds were well-developed at this point, endogenous physiological dormancy was more likely than morphological or morphophysiological dormancy (following seed bank classifications; Nikolaeva, 1977; Baskin and Baskin, 1998).

In both the spring and the fall, some buds of the current season's cohort were maintained in exogenous or endogenous dormancy but many dormant buds, especially in the fall, lost their dormancy and readily transitioned to higher developmental stages. Primary dormant buds started production in the spring when juvenile tillers began to grow out into mature tillers. Therefore, most dormant buds were under exogenous dormancy. These young buds, which were not fully developed, were likely under endogenous morphological or morphophysiological dormancy as well. By late spring, several of the dormant buds would be free from endogenous morphological or morphophysiological and exogenous mechanical dormancy because the buds would be fully developed and the tiller's leaves would have aged, relaxing their constrictive presence on the buds. Since the flowering heads of *D. oligosanthes* senesce before summer and *D. oligosanthes* is determinate in its flowering, apical dominance (i.e., exogenous chemical dormancy) was lost before buds entered enforced dormancy in the summer allowing time for well-developed dormant buds to transition to active buds. However, apical dominance must not have always successfully conferred dormancy on all buds as a few dormant buds of *D. oligosanthes* transitioned into active buds before the mature tillers flowered in the spring. During peak summer temperatures, most buds appeared to be in enforced dormancy. Following the summer resting period, a third of the primary buds remained dormant to the end of the growing season. Any apical dominance exerted by primary buds or juvenile tillers with active apical meristems must have been weak since a large percentage (66%) of primary buds did transition to higher developmental levels (Chapter 3). The early removal of exogenous dormancy conferred by

mature tillers and the lack of endogenous dormancy enabled many primary dormant buds to develop into juvenile tillers before the winter dormant season began effectively placing buds under enforced dormancy.

### *Comparison of A. gerardii and D. oligosanthes*

*A. gerardii* and *D. oligosanthes* differed in bud longevity, bud development timing and synchrony, and type of overwintering propagule supply. Buds of *A. gerardii* lived up to 1.5 years longer and were able to remain dormant for longer periods of time than buds of *D. oligosanthes*. Thus, bud banks of *A. gerardii* were more well-established and contained multiple bud cohorts while the bud bank of *D. oligosanthes* was almost always comprised of a single cohort. The shorter bud dormancy of *D. oligosanthes* resulted in an asynchrony in bud bank development over time whereas *A. gerardii* had temporal synchrony in bud bank development. Although both species began bud production at similar times in the spring, *D. oligosanthes* continued at a high rate of bud production in the fall after bud production of *A. gerardii* had ceased. The ability of *D. oligosanthes* to produce buds later in the growing season was primarily due to its higher-order bud development capabilities which *A. gerardii* lacked (Chapter 3). The overwintering propagule supply of *A. gerardii* consisted solely of dormant buds whereas both active and dormant buds and juvenile tillers comprised the overwintering propagule supply of *D. oligosanthes*. However, in the spring, *D. oligosanthes* only transitioned juvenile tillers into mature tillers whereas the dormant buds of *A. gerardii* were the source of its tillers. Dormant and active buds of *D. oligosanthes* played a larger role in population persistence over the summer than over the winter as they were the only propagule types present over the summer dormant season.

*Dichanthelium oligosanthes* and *A. gerardii* differed in their aboveground phenology since they had different photosynthetic pathways and different optimum temperatures for growth. Photorespiration increases with temperature in $C_3$ plants (Ehleringer and Bjorkman, 1977). $C_4$ plants also have a higher water use efficiency (WUE) than $C_3$ plants, and their carboxylating enzyme has a higher affinity for $CO_2$, enabling them to maintain photosynthetic rigor at high temperatures (Pearcy et al., 1981; Barbour et al., 1987; Begon et al., 1996). *Andropogon gerardii* tiller emergence in late spring and flowering in July and August followed the characteristic growth period of a $C_4$ plant. Tillers of *D. oligosanthes* flowered in the spring

before peak summer temperatures were reached, and juvenile tiller production occurred during the fall's cool temperatures. Overwintering as a juvenile tiller did not give *D. oligosanthes* an advantage of tillering earlier than *A. gerardii* but it could help *D. oligosanthes* complete flowering and necessary bud production before the onset of its summer dormant season. Dormant buds of *A. gerardii* were initiated early enough in the spring to insure that tillers would be fully formed to take advantage of the long daylengths during the summer. Mature tillers of *D. oligosanthes* senesced later in the fall than tillers of *A. gerardii* and did not finish until soil temperatures neared 0°C. Therefore, tiller natality, flowering, and mortality, varied between the $C_3$ grass *D. oligosanthes* and the $C_4$ grass *A. gerardii*. $C_3$ and $C_4$ belowground bud bank processes, such as bud production and development, primarily coincide with their respective aboveground tiller development. The majority of *A. gerardii* buds were produced in late spring shortly after transitioning some of its dormant buds to active buds while *D. oligosanthes* produced and transitioned buds to higher developmental stages in early spring and fall. Bud activity was reduced in the cool season grass *D. oligosanthes* during the peak summer temperatures and bud mortality of *D. oligosanthes* was highest at the start of the $C_3$ summer dormant period. The bud bank phenology of each grass was highly dependent on warm- or cool-season grass growth phenology.

The buds of *A. gerardii* and *D. oligosanthes* served two different roles in the life cycle of each species. The buds of *D. oligosanthes* provided a dormant propagule supply over its summer dormant season from which more buds and juvenile tillers were produced in the fall. Although some buds survived over the winter, they did not contribute to the spring recruitment of mature tillers, unlike the buds of *A. gerardii* which overwintered and contributed to spring tiller production. Therefore, because of the multiple growing seasons of *D. oligosanthes* and its overwintering of juvenile tillers, buds are only needed over short periods of time (i.e., the summer) whereas *A. gerardii* requires buds to live at least one year in order to survive over the winter until the next growing season. The different minimum bud longevity requirements of *A. gerardii* and *D. oligosanthes* were reflected in visual differences in bud quality. Dormant buds of *D. oligosanthes* were smaller than those of *A. gerardii*, keeping with the smaller stature of *D. oligosanthes*, and were also more fragile and fleshy almost always without matured protective prophylls. The high bud quality of *A. gerardii* buds may enable them to live multiple years resulting in multiple cohorts comprising its bud bank. Bud longevity and quality may also be

dependent on the rhizome and tiller base longevity and quality of the species as buds cannot live longer than the rhizome or tiller base to which they are attached (Noble et al., 1979). The belowground tiller bases of *D. oligosanthes* were quite decomposed around the time when their cohort of buds died in the summer. Bud longevity and bud quality, as they relate to bud dormancy, affect the dynamics within the population bud bank.

The differences in bud longevity and bud quality encountered in the representative $C_3$ and $C_4$ grass species of this study may reflect a more wide-spread general trend between $C_3$ and $C_4$ grasses in North America. Buds of the $C_4$ grasses *Hilaria belangeri* and *Bouteloua curtipendula*, which are found in more arid grasslands, can overwinter and live at least 1.5 and 2 years respectively (Hendrickson and Briske, 1997). *Panicum virgatum* and *S. scoparium*, two mesic prairie $C_4$ grasses, only overwintered as dormant buds and appeared to have hardy buds (Ott, in prep). Other mesic prairie $C_3$ grasses such as *Elymus canadensis, Koeleria macrantha*, and *Poa pratensis* as well as *Carex* spp. overwinter as tillers and may have similar short-term bud longevity as *D. oligosanthes* (Ott, unpublished data). The $C_3$ grasses *Agropyron spicatum* and *A. desertorum*, which are found in more arid grasslands, also overwinter as tillers (Mueller and Richards, 1986). The bud longevity and quality of $C_3$ and $C_4$ grasses may determine the prevalent tiller life cycles in the photosynthetic guilds of grasses.

***Linking bud banks to higher ecosystem processes***

Although the $C_4$ grass *A. gerardii* and the $C_3$ grass *D. oligosanthes* differ in their timing of tiller recruitment and bud activity, both species undergo the same basic transitions between the bud and tiller populations (Figure 1.14). Annual tiller production of a perennial grass (Figure 1.14 arrow I) is determined by the number of buds in the bud bank, the proportion of these buds which are non-dormant, and the proportion of tillers that successfully establish after bud emergence. Interannual variability in tiller production is primarily a result of the environment's influence on bud dormancy during each growing season. Annual bud production (Figure 1.14 arrow II) is determined by the growing season's tiller production and bud number produced per tiller. Changes in annual bud production are primarily a result of changes in tiller number rather than changes in bud number produced per tiller. This enables the dormant proportion of each cohort when it is first produced to be similar to every other cohort when they are first produced. Thus, the interannual variation in dormant proportion of buds is mainly dependent on environmental conditions rather than the architectural organization of the buds in the bud bank.

The annual bud production (Figure 1.14 arrow II) is added to the dormant buds remaining from previous seasons (Figure 1.14 arrow III) to create the overall bud bank. Some buds from previous seasons may die before the next tiller recruitment period (Figure 1.14 arrow IV). In the case of *D. oligosanthes* and potentially other $C_3$ grasses, all the buds that remain dormant will die before the next tiller recruitment period (Figure 1.14B).

There is a lag effect of past tiller production on current tiller production because past tiller production determines the number of buds present for current tiller production (Figure 1.14). Therefore, environmental conditions of past seasons through their effect on tiller production of past seasons via bud dormancy can affect current tiller production. The longevity and intensity of the lag effect is dependent on bud longevity and bud production per tiller. In the case of *A. gerardii* with bud longevity of at least 2.5 years, lag effects of one bud cohort could exist longer than one year but would diminish each year as the cohort's bud supply was depleted (Figure 1.14A). However, species like *D. oligosanthes* may have a shorter lag effect because buds rarely survive past one year (Figure 1.14B). Populations with longer-lived buds may confer stability to the aboveground population similar to longer-lived seed banks. If bud production per tiller was low and bud emergence was high during the first tiller recruitment opportunity for the tiller's buds, the lag effect would be reduced because fewer to no buds would be left for future periods of tiller recruitment.

Because of endogenous and exogenous bud dormancy, not all buds in the bud bank are available for recruitment as tillers. Therefore, the total number of non-dormant buds per area determines the production potential of grasslands rather than the total number of buds per area. Since the environment influences bud dormancy and therefore bud availability and tiller recruitment, the bud bank mediates the effect environmental conditions have on grassland tiller productivity and thus ANPP. In summary, bud dormancy is an important factor in regulating grassland productivity.

***Contribution of buds to growth form***

Spatial distribution of buds varied between the two species because of differences in internode elongation and hierarchical bud formation. *Andropogon gerardii* typically elongates one internode approximately 3cm away from the parent tiller before forming a new tiller with condensed internodes. Over time with multiple cohorts of tillers and rhizomes, *A. gerardii* can disperse throughout the prairie. Each successive cohort of tillers tends to disperse in the same

general direction enabling directed movement over time because buds are oriented to begin growth in the same direction as their parent rhizome or tiller. The rhizomatous grass *P. pratensis* exhibits this same pattern (Etter, 1951). Since some *A. gerardii* buds survive from earlier cohorts on the parent tiller, buds on new tillers expanding out from the parent tiller disperse the population while older buds retain the population's ability to persist in the general vicinity even after the parent tiller's buds die. Internodes fail to elongate before tiller production in *D. oligosanthes* creating a tufted growth form. The clumped spatial organization of the buds of *D. oligosanthes* is exacerbated by its higher-order bud formation creating a large number of tightly packed buds. In summary, the spatial bud distribution of *D. oligosanthes* is more clumped than *A. gerardii* due to its lack of internode elongation and its hierarchical bud formation.

### *Application*

Since tiller recruitment from the bud bank is an important mechanism by which perennial grassland productivity responds to environmental variability, understanding the bud bank and its phenology provides explanatory and predictive power of how grasslands will respond to various pressures, such as climate change or grazing. By linking bud dynamics to tiller dynamics, impacts on the belowground bud bank can be translated into impacts on aboveground tillering and ANPP. Since bud production is tied to tiller growth, grazing at certain times may more greatly impact the bud bank size than other times. Livestock management decisions need to be made while considering the tradeoff between providing current forage and future forage production, which is dependent on the bud bank.

The bud bank dynamics of the representative $C_3$ and $C_4$ grasses used in this study can be the starting point to provide insight into some of the general mechanisms of grass cover change on prairies under different land management regimes. On Konza Prairie, $C_3$ grass cover increases in mown areas, replacing $C_4$ grasses. Mowing occurs in late July and early August after the flowering period of the $C_3$ grasses and during or near the end of the $C_4$ flowering period. Mowing only removes apical dominance in those species that have culmed tillers whether vegetative or flowering. In the case of the $C_3$ *D. oligosanthes*, exogenous and endogenous bud dormancy controls are very lax so increased light resources and the onset of the cooler fall temperatures easily enable bud outgrowth after the mid-summer mowing. Since *D. oligosanthes* is fairly determinate in flowering, removal of apices that have not senesced, by mowing would

only aid dormant bud outgrowth. At the time of mowing, $C_4$ grasses have already created their dormant bud banks and, unless their apical dominance is removed, will remain under endogenous and exogenous dormancy until winter when buds will also be under enforced dormancy. The dominant grasses of tallgrass prairie, such as *A. gerardii, S. scoparium,* and *S. nutans,* have low proportions of flowering tillers and, therefore, will not have large losses in apical dominance to enable buds to break dormancy.

$C_3$ grass abundance declines while $C_4$ grass abundance increases on Konza Prairie watersheds with increasing fire frequency (Gibson and Hulbert, 1987; Hartnett and Fay, 1998). Burning typically occurs in the spring when the dormant buds of *A. gerardii* are beginning to emerge but before the juvenile tillers of *D. oligosanthes* begin bud production of their own. Therefore, the fire kills the juvenile tillers, the source of new buds for *D. oligosanthes,* and likely many of the more developed buds near the surface of the ground. In contrast, *A. gerardii* buds are still in the process of approaching the ground surface and, if they are aboveground during the fire, they have more dormant buds from which to recruit tillers. Vegetative tillers of other $C_3$ grasses such as *E. canadensis, P. pratensis,* and *K. macrantha* are present over the winter and would be killed in the early spring burns (Ott, personal observation). In tillering studies of *A. desertorum, A. spicatum,* and *Festuca arundinacea,* overwintering tillers of these $C_3$ grasses were noted (Robson, 1968; Mueller and Richard, 1986). Even if spring burns did not directly destroy the bud banks of these $C_3$ grasses, they would put these species at a competitive disadvantage to $C_4$ grasses. $C_3$ grasses would have to grow from buds at the same time the $C_4$ grasses were growing out from buds but the $C_3$ growth would be constrained by the onset of the summer dormancy period. If the majority of $C_3$ grasses are susceptible to fire, it would explain the diminished returns of burning in the northern Great Plains where $C_3$ grasses are dominant. Interspecific variation in bud placement within the soil layer is independent of photosynthetic pathway but can also greatly affect bud survival of fire. Buds of *D. oligosanthes* are located much shallower than *A. gerardii* and would be more susceptible to fire as a few centimeters of soil can make a large difference in bud survival.

Since plants are often restricted in their timing and ability to disperse large distances, effects of climate change, such as the predicted shifting of $C_3$ dominance to $C_4$ dominance in the northern Great Plains (Epstein et al., 2002) and the earlier onset of spring growth (Schwartz et al., 2006), will be mediated through the bud bank in perennial grasslands by directly altering

enforced dormancy and indirectly altering endogenous and exogenous dormancy. For example, increasing temperatures will alter the bud bank phenology of both $C_3$ and $C_4$ grasses by lengthening the summer dormant season of $C_3$ grasses and shortening the winter dormant season of both $C_3$ and $C_4$ grasses. In the northern Great Plains, the lengthening of the summer dormant season of $C_3$ grasses would enable $C_4$ grasses to become more dominant. The longer summer dormant period could stress the $C_3$ bud bank which is the over-summer propagule supply and the longer fall active growing period could enable fall tillers to develop beyond the juvenile stage. Bud emergence of $C_4$ grasses and tiller growth of $C_3$ grasses would begin earlier in the spring. Since the growing season would be longer for $C_4$ grasses, a second tiller generation could commence before the end of the growing season but it would be uncertain whether these tillers could overwinter and complete growth the following spring. The responses of the bud bank to climate change and grazing pressures ultimately determine the effect these pressures have on grassland productivity.

Buds are mediators of grassland productivity. Since belowground buds are the primary source of tillers in tallgrass prairie (Benson and Hartnett, 2006) and variation in aboveground net primary production (ANPP) is primarily due to changes in tiller number rather than tiller size (Hartnett and Fay, 1998), variation in ANPP depends upon tiller recruitment from the bud bank. Therefore, further insight can be gained into the mechanisms regulating annual ANPP and producing interannual variability in ANPP on perennial grasslands by understanding the dynamics of bud and tiller production. Belowground demographic processes such as bud bank dynamics should be considered in models and management of grassland since they drive tiller populations and aboveground production (Tomlinson, 1974). This study described the morphology, development, and dynamics of buds of only one representative $C_3$ and $C_4$ grass species. Demographic studies of additional species will be needed to ascertain whether these patterns apply generally to these two guilds.

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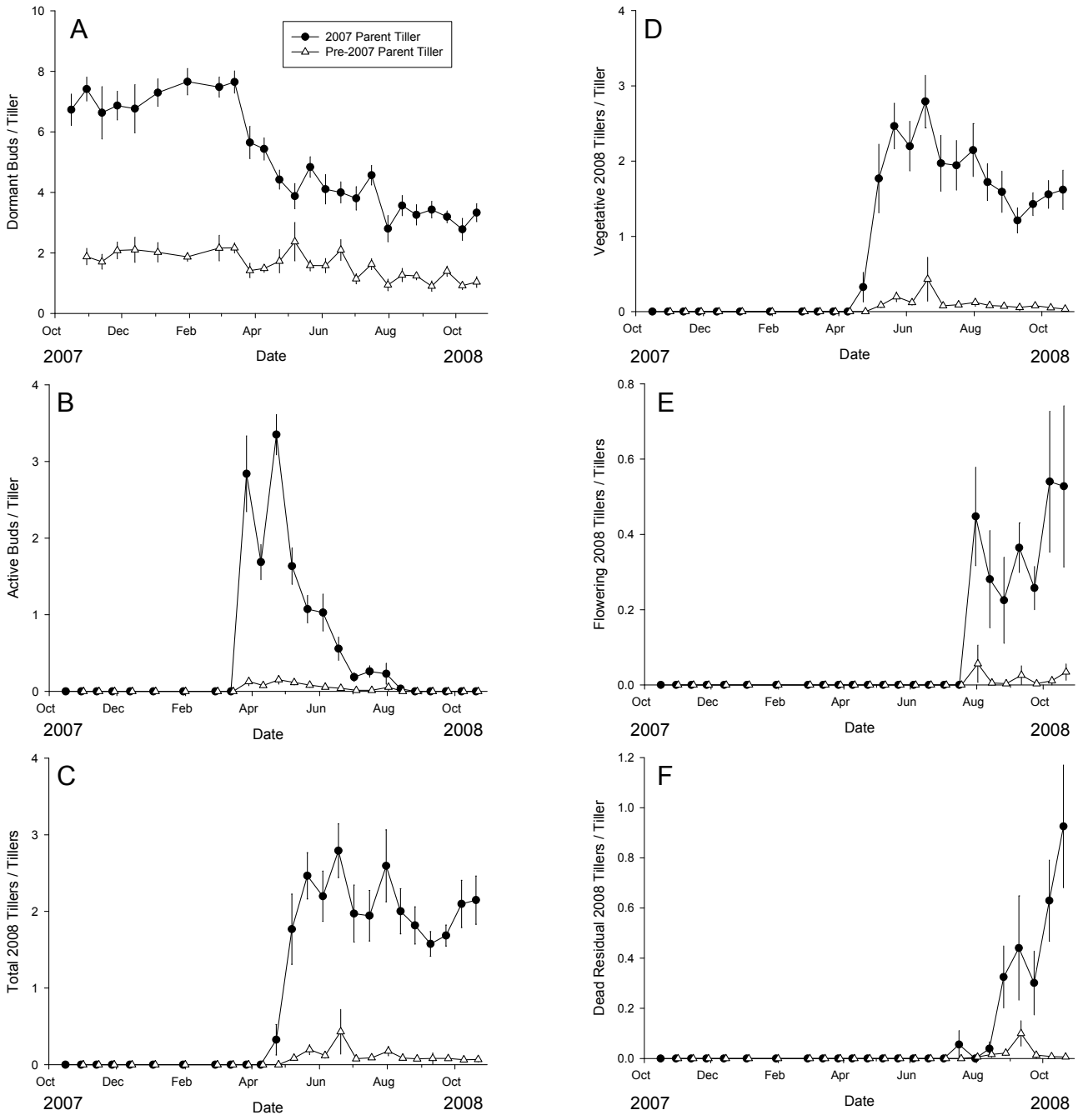


**Figure 1.1 Bud and tiller production of *Andropogon gerardii* by 2007 and pre-2007 tillers from October 2007 to October 2008.** A significant proportion of dormant buds (A) transitioned into active buds (B) which then became 2008 tillers (C). 2008 tillers either remained vegetative (D) or became flowering tillers (E). During the end of the growing season, mortality of 2008 tillers occurred due to herbivory or causes other than natural senescence (F). Scaling on the y-axis varies for each graph. Error bars are ±1 SE of the mean.

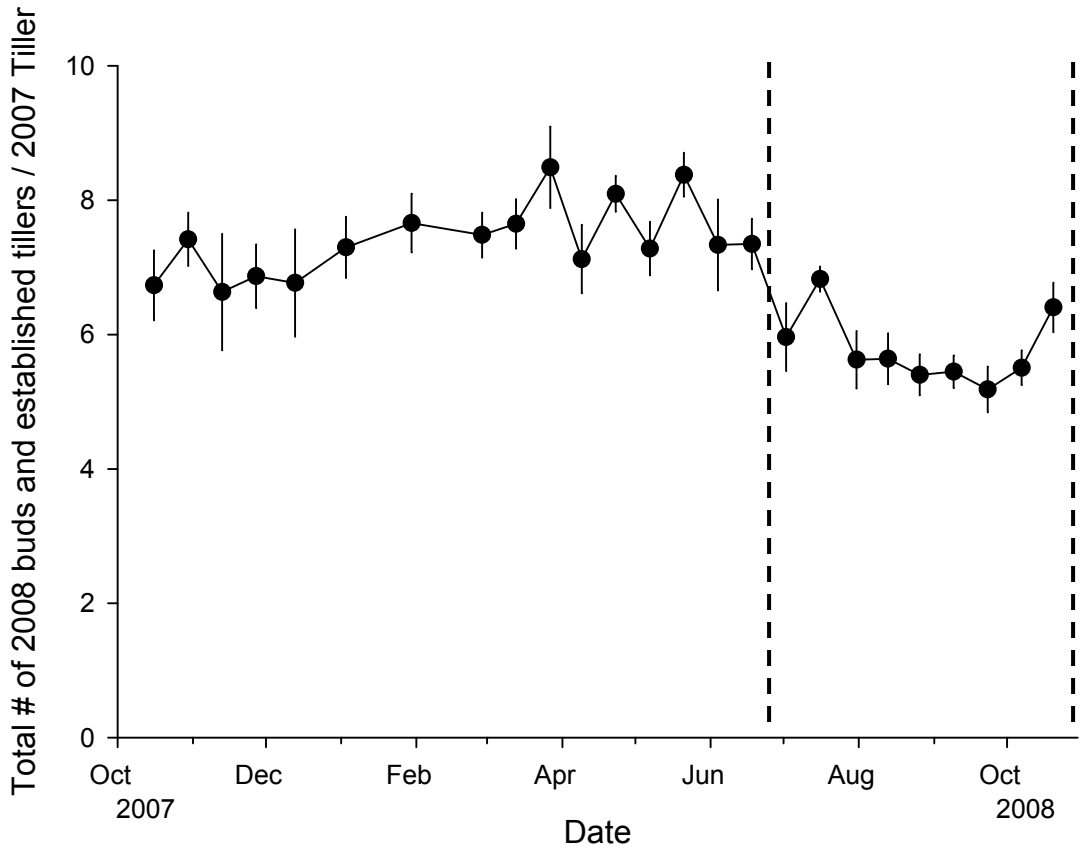


**Figure 1.2 Mortality of *A.* gerardii buds**. Bud mortality occurred in late June to August (between the dashed lines). The total of buds and buds that grew out into tillers from 2007 tillers in 2008 remained constant until June 2008. Tiller mortality was negligable as dead residual tillers were counted as part of the total established tillers. No additional dormant buds were produced on mature 2007 tillers once they had senesced in October 2007. Error bars are ± 1 SE of the mean.

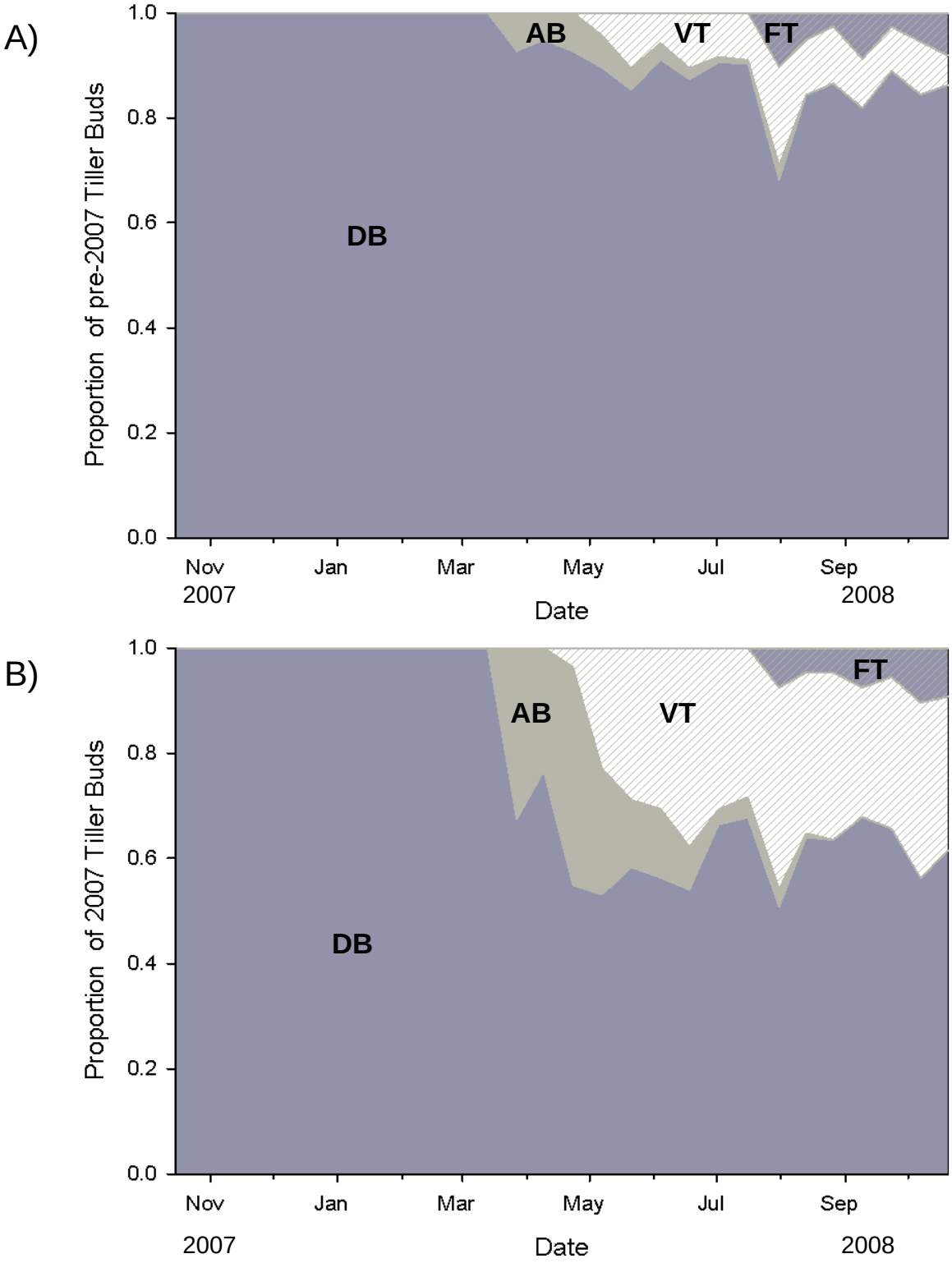


**Figure 1.3 Fate of buds produced on A) pre-2007 and B) 2007 mature tillers of *A. gerardii* between October 2007 and October 2008.** Buds were either dormant (DB) or active (AB) or had grown out into vegetative (VT) or flowering (FT) tillers. Bud and tiller stages are represented by solid colors and a hatched texture respectively.

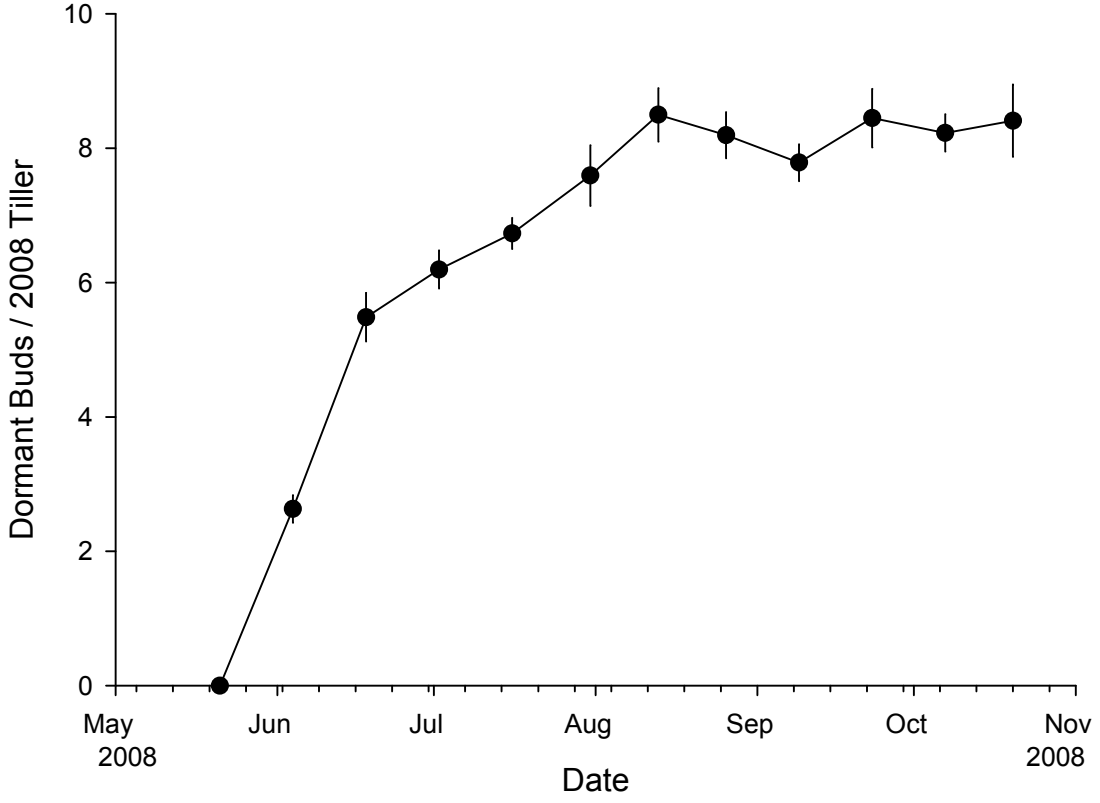


**Figure 1.4 Production of dormant buds by 2008 tillers of *A. gerardii*.** Error bars are ± 1 SE of the mean.

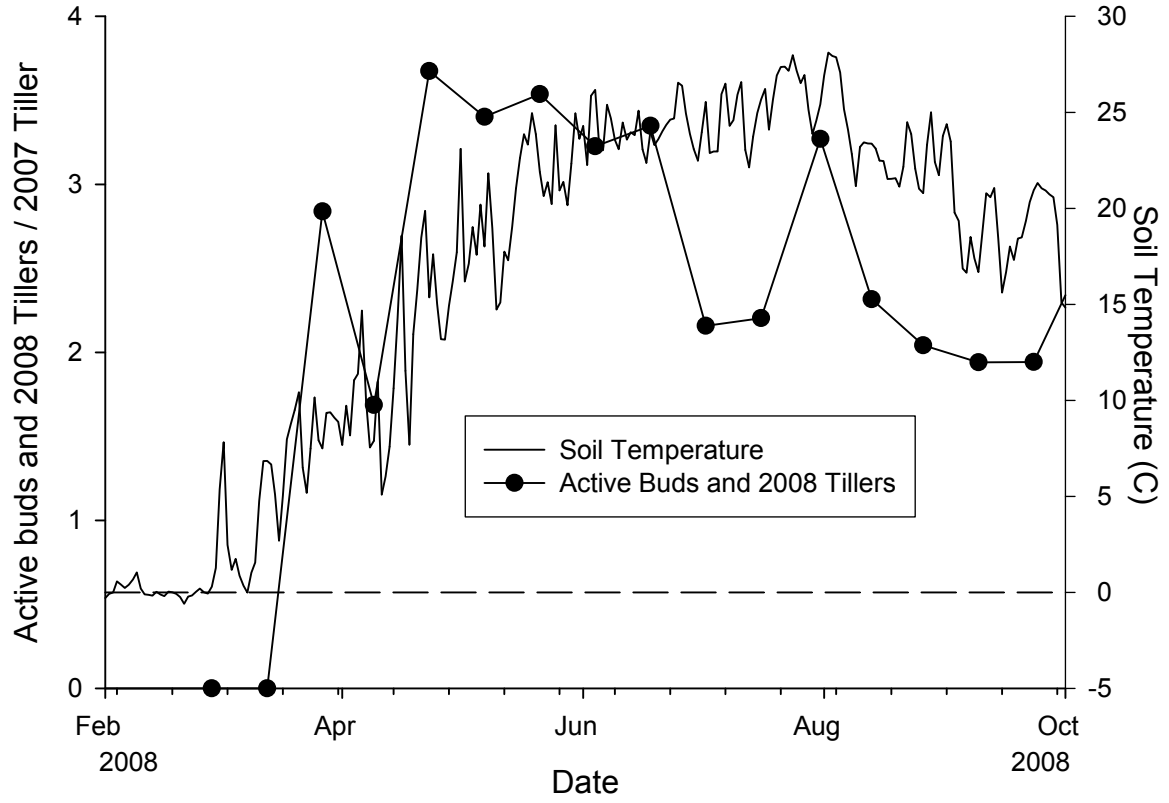


**Figure 1.5 Relationship of *A. gerardii* bud activation and soil temperature during 2008**. Soil temperatures were taken at 2cm depths. The dashed reference line indicates freezing point.

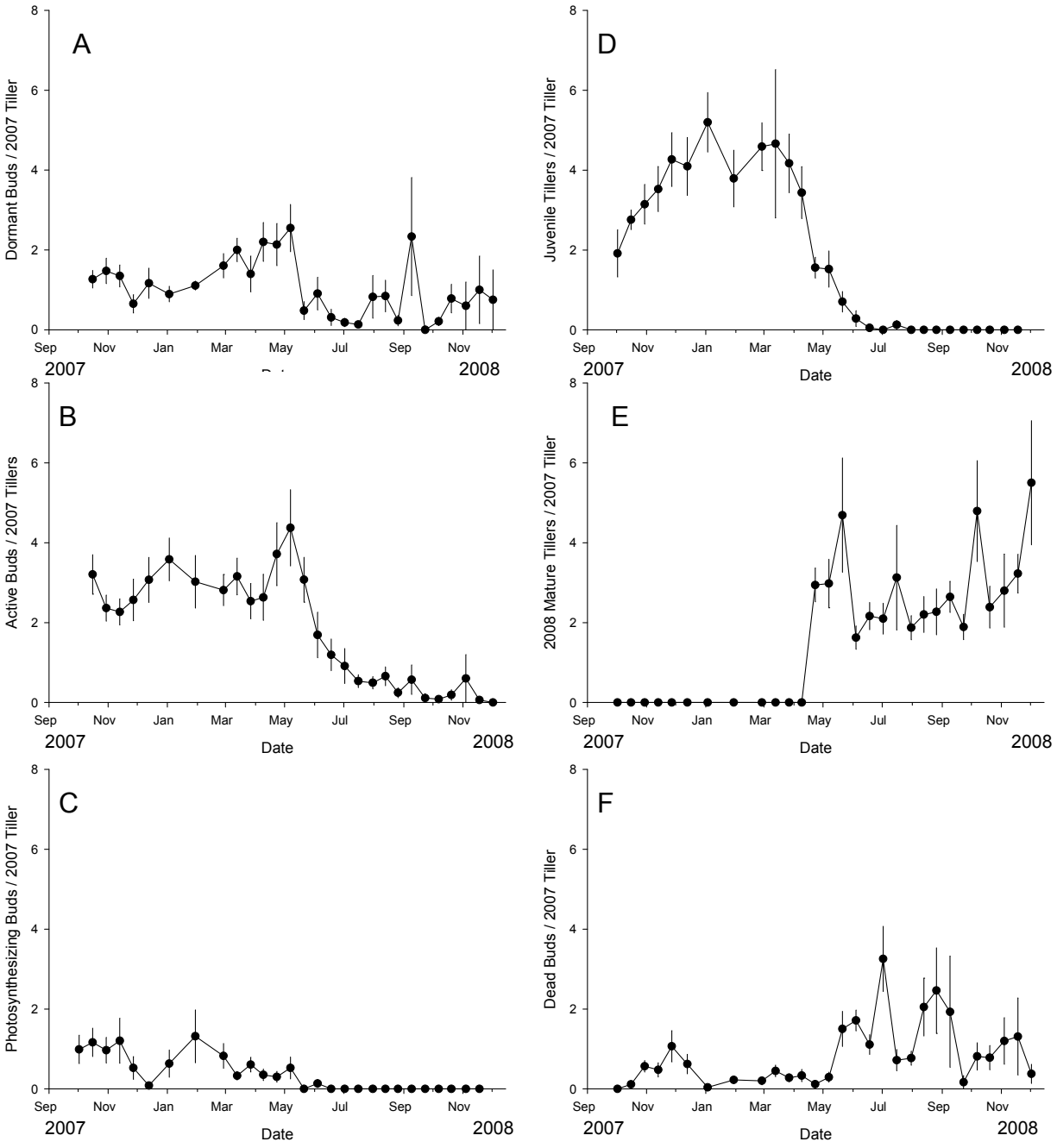


**Figure 1.6 Bud and tiller production of *D. oligosanthes* by 2007 mature tillers from September 2007 to December 2008.** Dormant (A), active (B), and photosynthesizing (C) bud amounts declined in May and contributed to the increase in dead buds following May (F). Many juvenile tillers (D) transitioned into 2008 mature tillers (E). Error bars are ±1 SE of the mean.

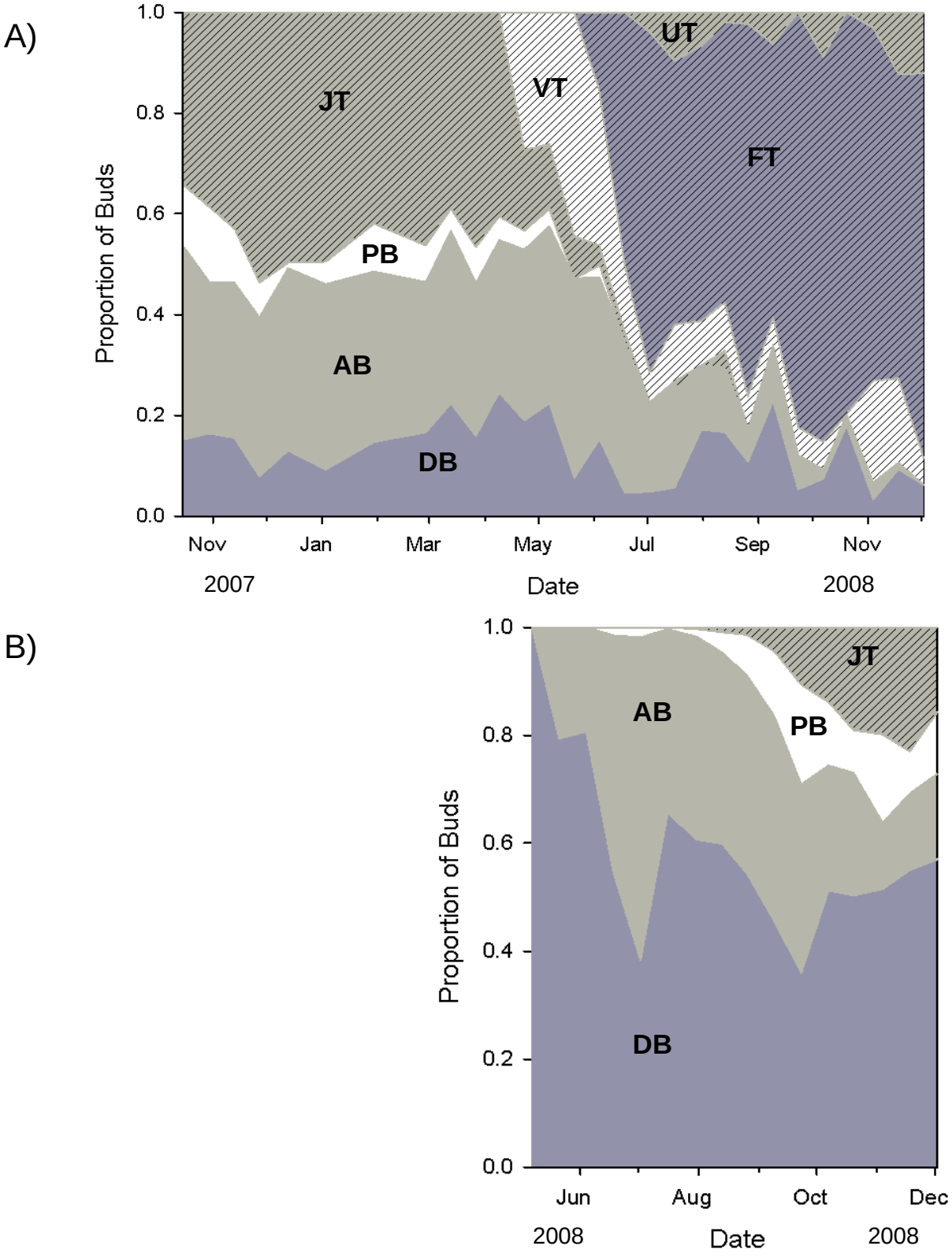


**Figure 1.7 Fate of buds produced on A) 2007 and B) 2008 mature tillers of *D. oligosanthes*.** Buds were either dormant (DB), active (AB), or photosynthesizing (PB) or had grown out into juvenile (JT), vegetative (VT), or flowering (FT) tillers. The flowering status of some tillers was unknown (UT) due to culm damage. Bud and tiller stages are represented with solid colors and a hatched texture respectively.

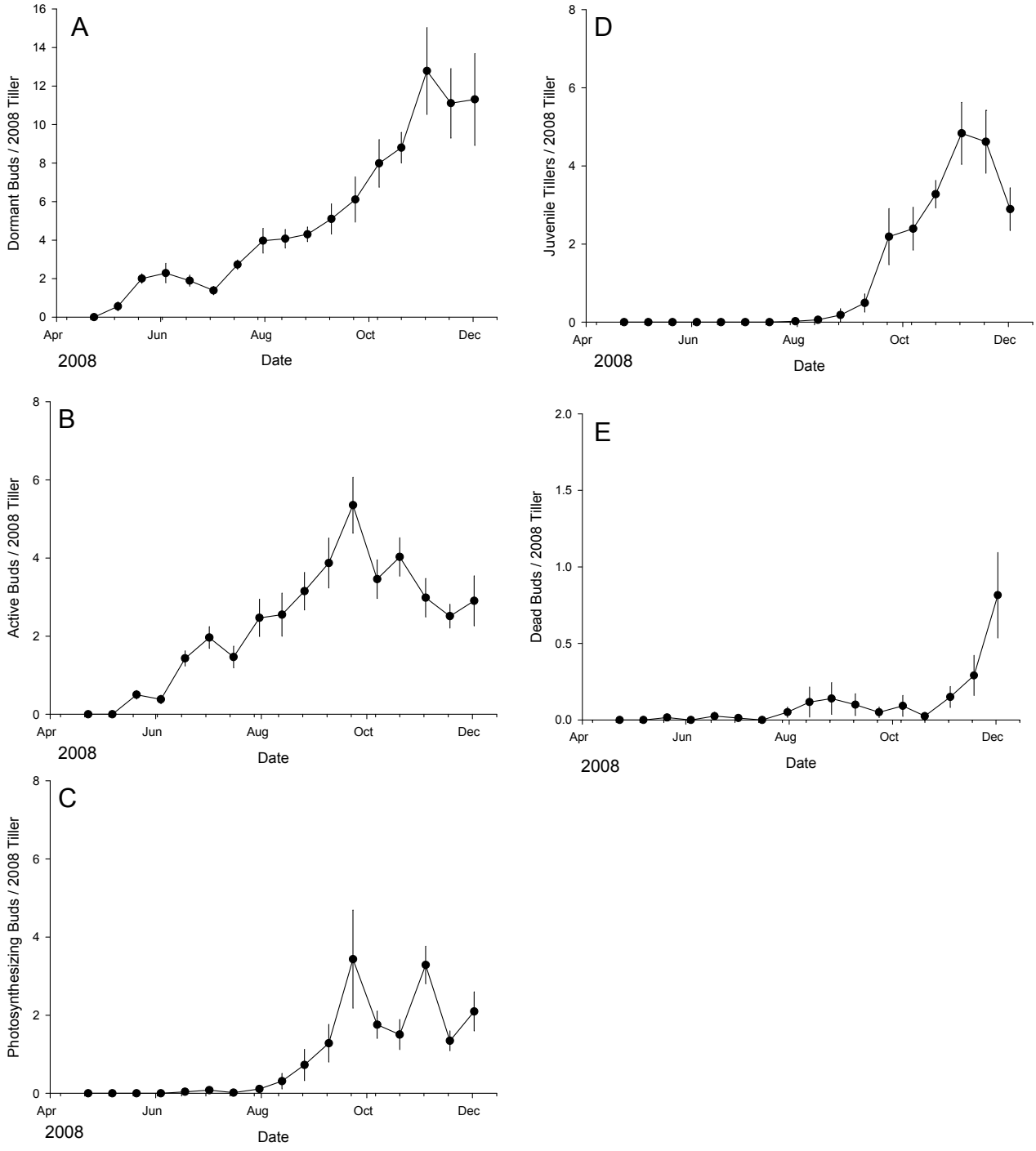


**Figure 1.8 Bud and tiller production of *D. oligosanthes* by 2008 mature tillers**. *Dichanthelium oligosanthes* increased production of dormant buds (A) as some of them transitioned to higher developmental stages, such as active (B) and photosynthesizing (C) buds and juvenile tillers (D). This was also a result of the hierarchical method of bud production that occurred in *D. oligosanthes.* Bud mortality remained low (E). Scaling on the y-axis varies for each graph. Error bars are ±1 SE of the mean.

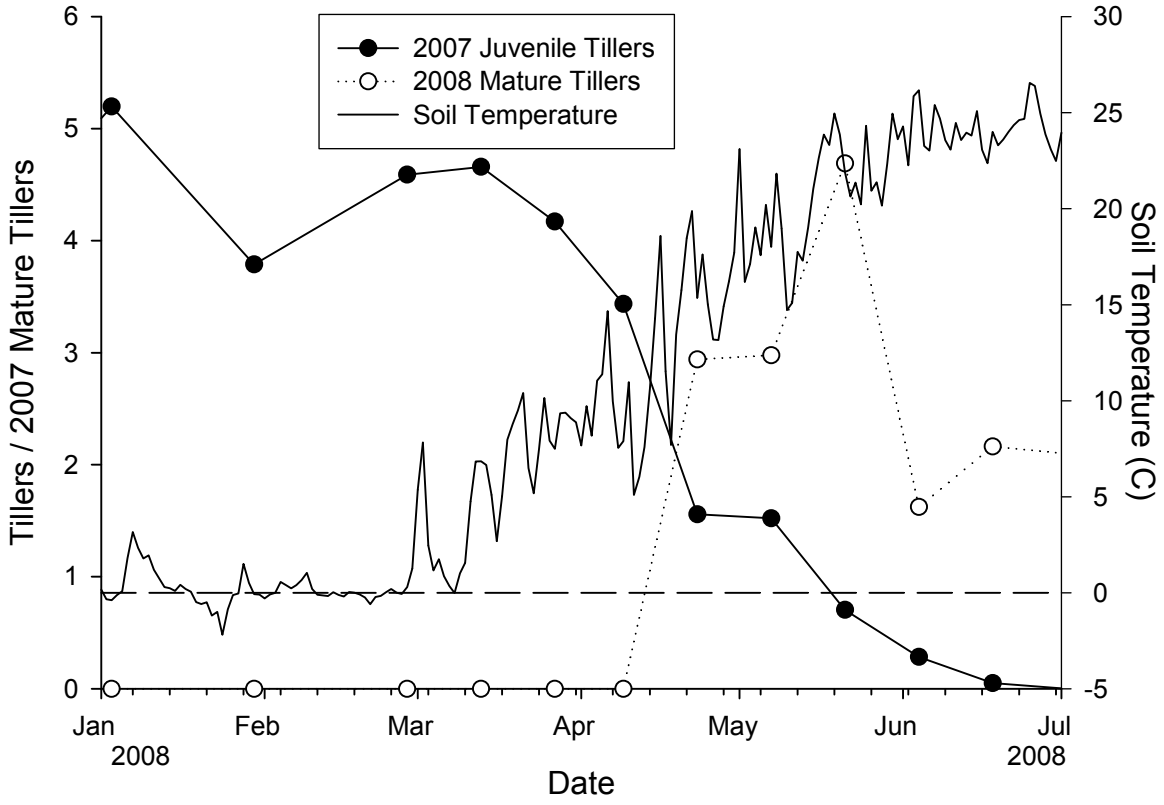


**Figure 1.9 Spring turnover of 2007 juvenile tillers to mature 2008 tillers of *D. oligosanthes* in relation to soil temperature.** Soil temperature was taken at 2cm depths. The dashed reference line indicates freezing point.

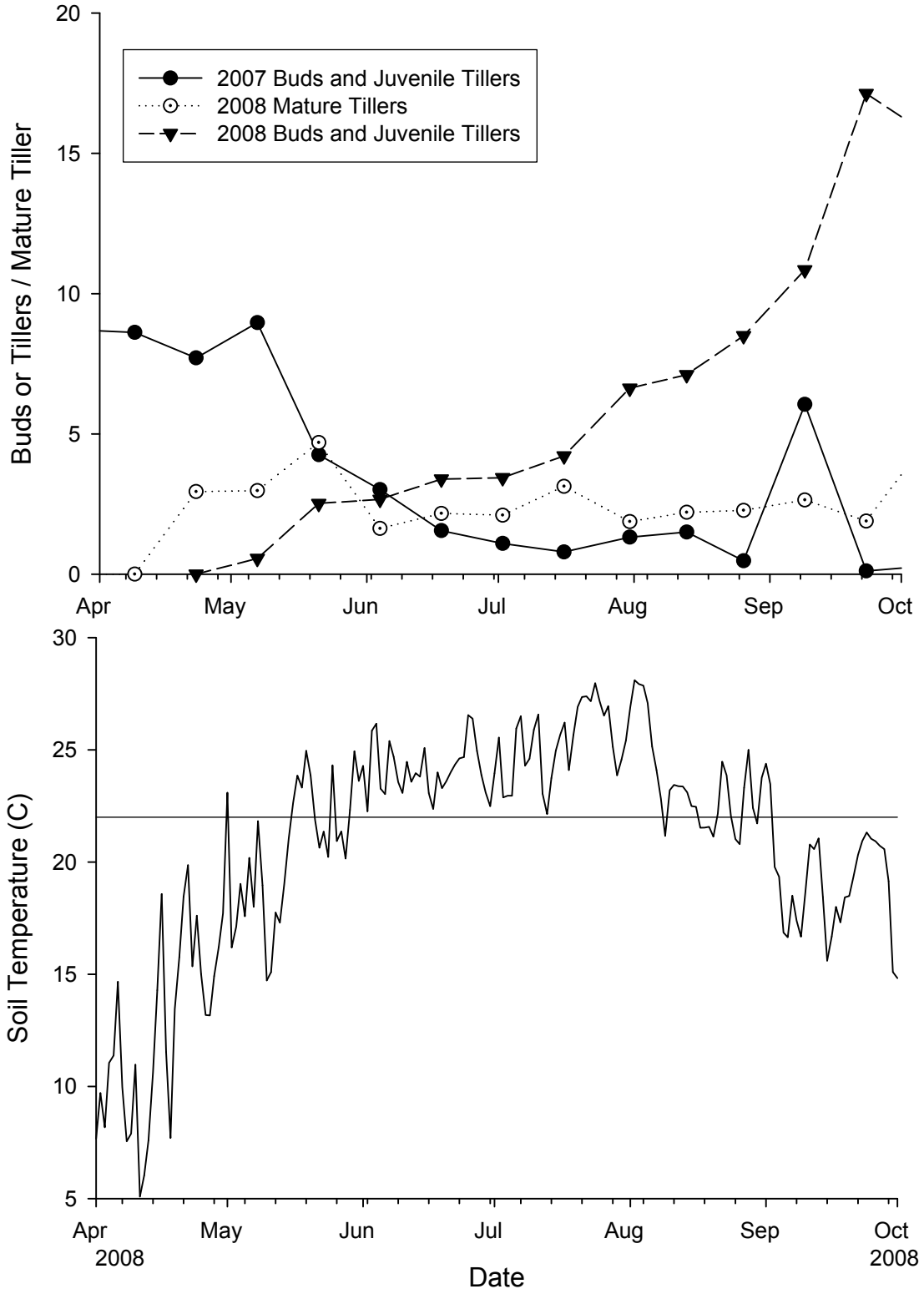


**Figure 1.10 Summer Dormant Season of *D. oligosanthes***. Total buds and juvenile tillers of 2007 and 2008 decreased in activity during peak soil temperatures. Mature 2008 tillers were formed before the peak soil temperatures. A reference line for 22ºC is positioned on the lower panel. Soil temperatures which occur above 22ºC coincided with reduced activity of *D. oligosanthes.*

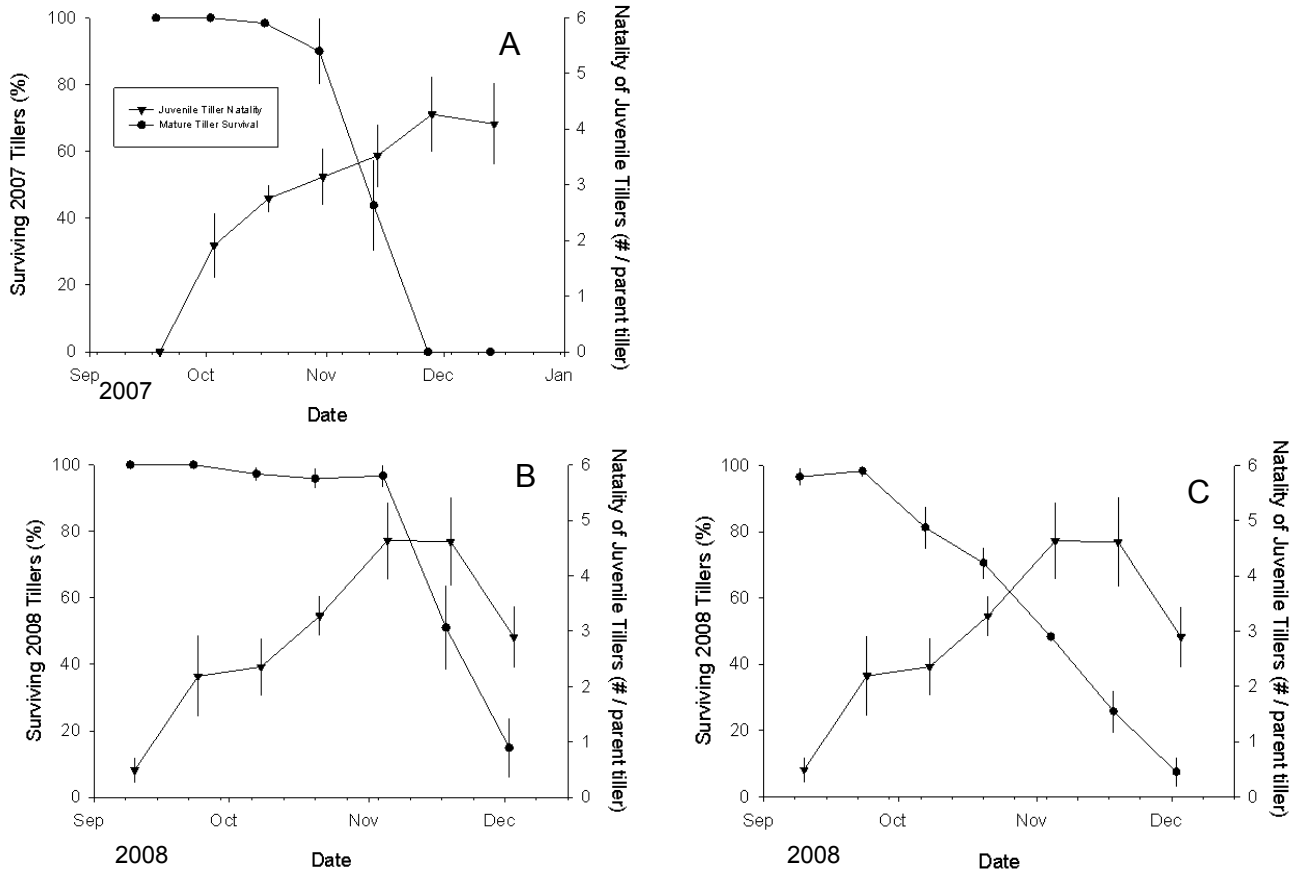


**Figure 1.11 Fall turnover of mature tillers to juvenile tillers by *D. oligosanthes*** in A) 2007 and B&C) 2008. Survival of adult tillers in 2007 and 2008 was calculated as the proportion of adult tillers conducting photosynthesis in any leaf (A&B). A more sensitive index was also used in 2008 that allowed an adult tiller to be partially senesced when less than half of its leaves conducting photosynthesis (C).

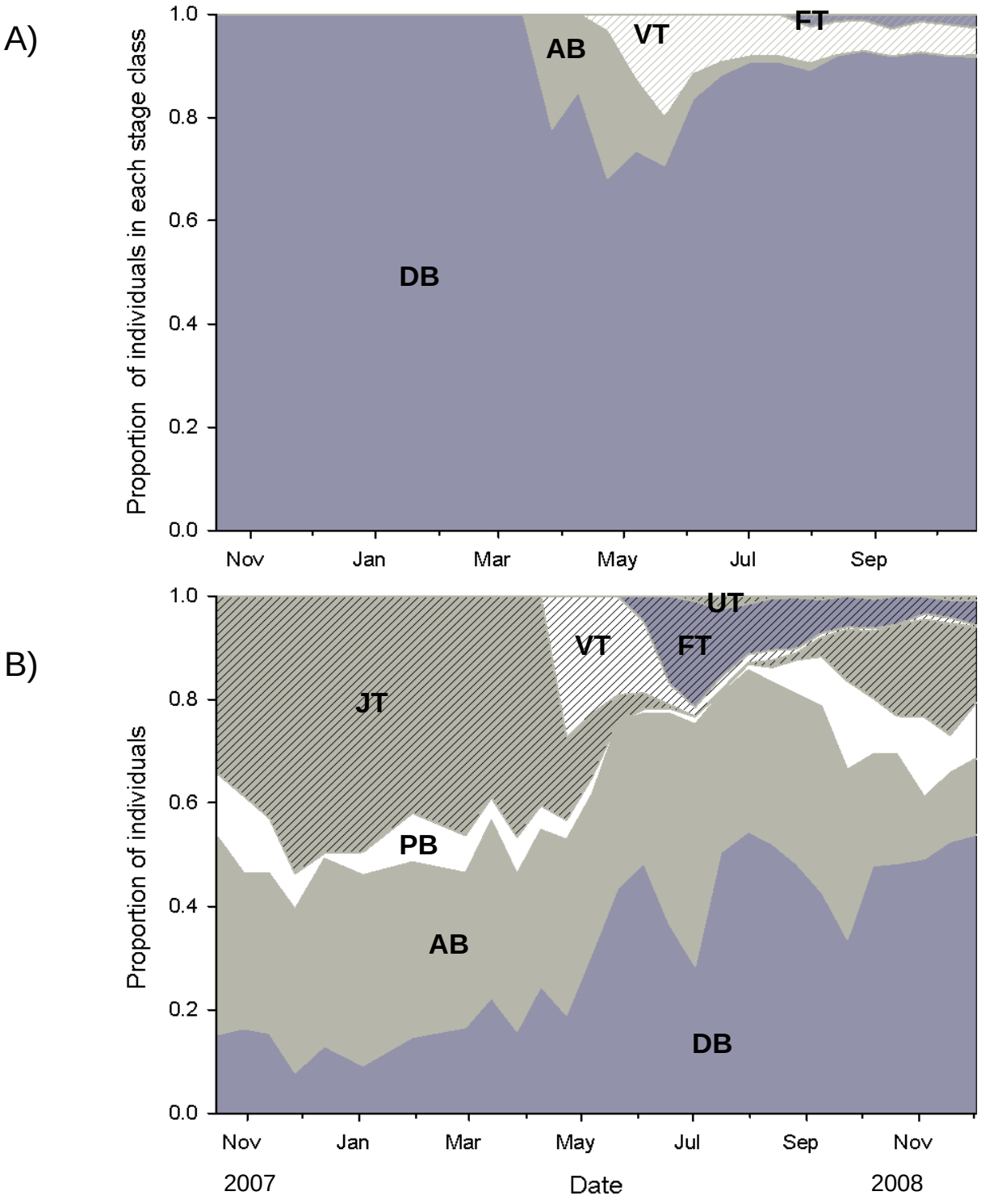


**Figure 1.12 Overall bud bank and tiller dynamics of A) *A. gerardii* and B) *D. oligosanthes.*** All generations of buds and tillers are included and are divided according to stage class. Buds were either dormant (DB), active (AB), or photosynthesizing (PB) or had grown out into juvenile (JT), vegetative (VT), or flowering (FT) tillers. The flowering status of some mature tillers was unknown (UT) due to culm damage. Photosynthesizing buds, juvenile tillers, and unknown tillers only occurred for *D. oligosanthes*. Bud and tiller stages are represented with solid and hatched textures respectively.

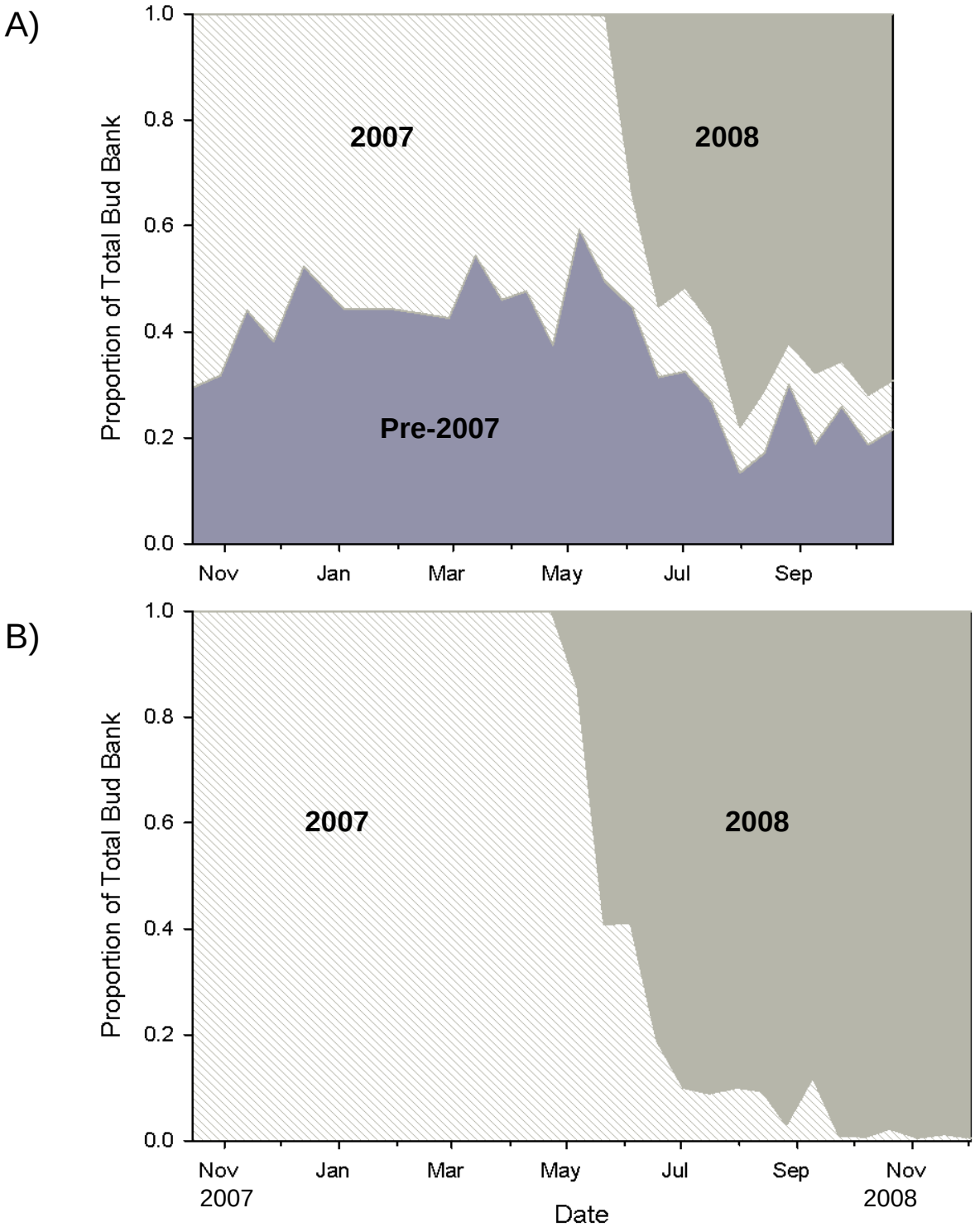


**Figure 1.13 Proportion of buds belonging to different age classes of A) *A. gerardii* and B) *D. oligosanthes.*** Ages of buds initiated before 2007 were grouped as a pre-2007 age class. Juvenile tillers were considered part of the bud bank for *D. oligosanthes* as they are the key transitioning stage to mature tillers. Juvenile tiller is not a developmental stage specified for *A. gerardii*.

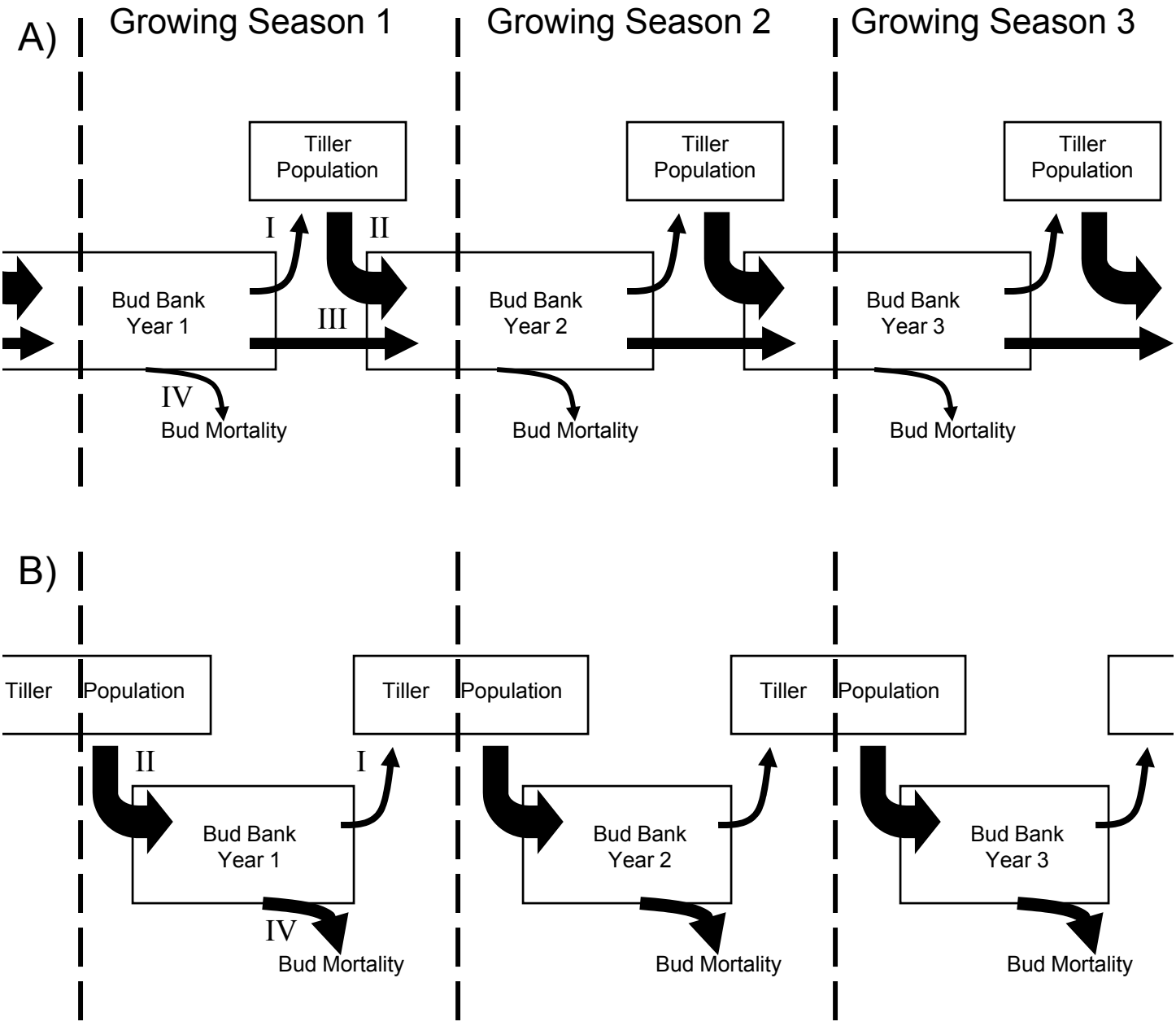


**Figure 1.14 Conceptual diagram of the dynamic relationship between the aboveground tiller population and the bud bank over multiple years for A) *Andropogon gerardii*, a representative $C_4$ grass and B) *Dichanthelium oligosanthes*, a representative $C_3$ grass**. Roman numerals indicate the major transitions discussed.

**Plate 1.1 Bud and tiller development in relation to plant architecture.**

A- Three generations of *A. gerardii* tillers. Senesced 2007 tillers retained their aboveground leaves and remained attached to residual 2006 tillers (leafless terminating structures near the base of the 2007 tillers). These residual 2006 tillers remained attached to their residual 2005 tiller (lower left).

B- Dead dormant bud of *A. gerardii*. 12.5x. December 13, 2007. The dead bud had a mealy interior.

C- Tiller production of *A. gerardii* by previous year's residual tiller. The 2007 tiller produced two 2008 tillers.

D- Senesced mature tillers of *D. oligosanthes* with juvenile tillers. By the beginning of winter, the aboveground mature tillers have senesced and the juvenile tillers, which will become the next generation of mature tillers, have formed.


A


B


C


D

**Plate 1.2 Bud developmenetal stages of *Andropogon gerardii***

A- Developing dormant bud. 20x. July 2, 2008. The developing dormant bud was forming on a newly recruited 2008 tiller.
B- Developing dormant bud and developed dormant bud. 7.1x. June 18, 2008. The younger developing dormant bud was superior to the older developed dormant bud with its darkened prophyll as they grow on a 2008 tiller.
C- Developed dormant buds. 7.1x. August 13, 2008. The most basal buds on the 2008 tiller were developed.
D- Overwintered developed dormant buds. 7.1x. March 13, 2008. Developed dormant buds before transitioning into the active bud stage on 2007 tiller. One dormant bud with the prophyll removed reveals the solid white meristematic bud interior.
E- Active bud. 12.5x. November 8, 2007. Fuschia coloring signaled initiation of bud in the active state on a 2007 tiller.
F- Active bud. 10x. April 10, 2008. Full fushia coloration of the bud occurred before extensive elongation past the prophyll on a 2007 tiller.
G- Active bud. 7.1x. March 27, 2008. Elongation of the bud began during the active bud stage on a 2007 tiller.
H- Active bud. April 28, 2008. Active buds continued to elongate.
I- 2008 tillers. July 8, 2008. Tillers arose from active buds.


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**Plate 1.3 Bud developmental stages of *Dichanthelium oligosanthes***

A- Young dormant bud. 12.5x. May 21, 2008. Dormant buds began as rotund protuberances. This dormant bud was located at the base of a mature 2008 tiller.
B- Dormant buds. 10x. July 16, 2008. Dormant buds maintained a basal fushia coloring with white apices. At times, they would have brown coverings.
C- Dormant buds. 12.5x. August 13, 2008. Dormant buds have a rotund to deltoid shape.
D- Active bud with a secondary dormant bud. 12.5x. August 26, 2008. Primary buds, such as this active bud, were capable of producing secondary buds.
E- Active buds. 10x. August 26, 2008. Active buds have a conical form with a distinctive apex.
F- Photosynthesizing and active buds. 7.1x. August 26, 2008. Photosynthesizing buds began photosynthesis at their apices.
G- Photosynthesizing bud and juvenile tiller. 7.1x. November 27, 2007. The juvenile tiller in the background has developed its first distinctive leaf and is therefore, no longer a photosynthesizing bud as seen in the foreground.
H- Juvenile tiller. 7.1x. September 18, 2007. A juvenile tiller was defined as having at least one leaf (as seen here) and no more than three leaves.
I- Mature tiller. May 9, 2008. Mature tillers have elongated phytomers and many leaves.


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# CHAPTER 2 - Bud production and dynamics of flowering and vegetative tillers of *Andropogon gerardii* Vitman.


## Abstract

Perennial grasses undergo both vegetative reproduction via belowground buds and sexual reproduction via seed in order to maintain their aboveground tiller populations. Given limited resource availability to a plant, a tradeoff between these two modes of reproduction would be expected. However, the ontogeny of the tiller could affect its ability to allocate between these two modes of reproduction. Vegetative bud production and dynamics as well as tiller production was examined biweekly for one year on vegetative and flowering tillers of *Andropogon gerardii*. Although vegetative and flowering tillers had similar bud phenology, flowering tillers produced equal or larger numbers of buds per tiller and transitioned a larger proportion of their buds to tiller than vegetative tillers. Therefore, a tradeoff was not evident between sexual and vegetative reproduction. A size threshold for floral induction likely exists resulting in flowering tillers initially having more buds per tiller than vegetative tillers. Since flowering results in cessation of belowground bud production, vegetative tillers may continue to add buds belowground under good growing conditions thus accumulating a similar number of buds as flowering tillers by the end of the season. The increased bud outgrowth on flowering tillers could be a result of their larger bud size or more distantly removed apical meristem than vegetative tillers. Plant development can place significant constraints on tradeoffs between the modes of reproduction in perennial grasses and could affect the plasticity in plant reproductive allocation, which may be especially important in the context of global environmental change.


## Introduction

In perennial grasses, populations persist through vegetative reproduction as new tillers are recruited from belowground buds. In tallgrass prairie, >99% of tiller recruitment occurs from belowground buds rather than seed (Benson and Hartnett, 2006). However, perennial grasses do invest in sexual reproduction through flowering and seed production. There is value to both modes of reproduction in plants. Vegetative reproduction enables plants to respond quickly to their surrounding environmental conditions and to herbivory through the active connections of buds to their parent plant. Vegetative offspring typically have much higher survivorship and

growth rates than seedlings (Benson and Hartnett, 2006). Yet, reproduction by seed enables long distance dispersal and reduces local intraspecific competition. Some successful tiller recruitment from seed benefits perennials because it guarantees introduction of genetic novelty into the population. Although genetic novelty as a result of accumulating mutations in the seed bank has been documented (Levin, 1990), the potential of accumulating somatic mutations during bud development, and thus producing novel genotypes, is unexplored.

Tradeoffs between vegetative and sexual reproduction would be expected within a plant. Flowering tillers might be assumed to have lower vegetative reproductive capacity than vegetative tillers since resources are being allocated to sexual reproduction. However, the ontogeny of the tiller may place developmental constraints on the tradeoff between sexual and vegetative reproduction. All tillers begin as vegetative tillers and subsequently some or all, depending on the species, transition into flowering tillers before they senesce (Hyder, 1972; Jewiss, 1972). Developmental timing of vegetative bud production in relation to flower production may determine the relative allocation of resources to the two reproductive modes (Watson and Casper, 1984). Allocation to each reproductive strategy may be predetermined within a species and, thus, vary among species.

A study was designed to examine the bud and tiller production of flowering versus vegetative tillers of the dominant tallgrass prairie species *A. gerardii*. Thus, the expected negative impact flowering would have on bud production could be examined as well as the implication this would have on future tillering from flowering tillers. Although the general bud bank dynamics of *A. gerardii* have been closely examined (Chapter 1), knowledge of how flowering and vegetative tillers contribute to population persistence will further understanding of this species. Therefore, the primary objectives of this study were to compare the bud production, dynamics, and tiller production of vegetative and flowering tillers of *A. gerardii*.

## Materials and Methods

### *Site Description*

The study was conducted at Konza Prairie Biological Station (KPBS), a 3,487-ha tallgrass prairie preserve located in the Flint Hills region of northeastern Kansas (39º05’N, 96º35’W). The hilly topography and limestone bedrock resulted in shallow, rocky upland soils (Udic Argiustolls, Florence series), steep slopes, and deep lowland soils (Pachic Argiustolls,

Tully series). The vegetation composition at KPBS is dominated by warm season ($C_4$) grasses, such as *Andropogon gerardii* Vitman (big bluestem), *Sorghastrum nutans* (L.) Nash (Indiangrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem). Subdominant vegetation includes cool season ($C_3$) grasses, composites, legumes and other forbs, and a few woody species. Due to the continental climate at KPBS, the majority of the mean annual precipitation (835mm) falls during the warm, wet springs and summers. KPBS is divided into multiple experimental units, each defined as a single watershed. Each watershed contains upland, slope, and lowland topographical positions and has both a grazing and fire regimen assigned to it. Fire regimes include 1-, 2-,4-, 10-, and 20- year fire return intervals and grazing regimes include grazed or ungrazed by bison. Two replicate watersheds (KPBS unit 4A- 47ha and KPBS unit 4B-135 ha), each with an ungrazed and 4-year spring fire return interval regimen, were chosen for this study. At the beginning of this study in fall 2007, it had been one year and two years since last fire on watershed 4A and 4B, respectively. Vegetation at these intermediate fire return interval sites are dominated by grass with both $C_3$ and $C_4$ grasses being readily abundant.

### *Field Sampling*

*A. gerardii* is a warm season ($C_4$), stout, short-rhizomatous, dominant perennial grass which grows from 60cm to 140cm in height and is considered a keystone grass species of the tallgrass prairie. Flowering of *A. gerardii* in the Great Plains occurs from July to October (Great Plains Flora Association, 1986). Since *A. gerardii* is indeterminate in its flowering, vegetative tillers exist throughout the growing season. Due to the rhizomatous growth form of *A. gerardii* and intermingling of tillers from different genets, discrete genets are very difficult to identify in the field.

At each of five upland sites on each watershed, individuals of *A. gerardii* were sampled and marked after the conclusion of its flowering period. Sites on each watershed were selected to be as distant from one another as possible while remaining on upland soils. On watershed 4A and 4B, sites were separated by an average 90 ± 13 meters and 208 ± 40 meters respectively. An *A. gerardii* individual consisted of a flowering tiller with all neighboring tillers and associated belowground parts within a 15cm diameter circle. Hereafter, sampled *A. gerardii* individuals

will be referred to as "plants." In October 2007, each plant was marked by encircling its base with a wire ring and its 2007 tillers were each counted and encircled with a small wire ring.

Ten plants, one from each site on each watershed, were harvested biweekly starting on October 16, 2007. This biweekly sampling continued through July 2008 when the next generation of tillers had flowered. This subsequent 2008 set of plants was similarly marked with large rings as done in 2007. However, 2007 tillers were no longer present aboveground and could not be counted or marked with rings. Therefore, residual 2007 tiller bases of *A. gerardii* were counted when plants were harvested and the soil surrounding their belowground parts was washed away. Residual 2007 tillers of *A. gerardii* were distinguishable belowground from 2006 residual tillers by their healthy lutescent coloring and their leaf remains often found at the distal portion of their residual tiller base. The current 2008 generation tillers were easily identified for the remainder of the study and, therefore, were not counted or encircled with small rings. Biweekly harvesting continued until complete aboveground senescence. *Andropogon gerardii* was last sampled on October 20, 2008.

Plants were harvested by excavating to a 15cm depth. All interconnected and belowground stems occur within this specified soil depth and were collected as part of the plant. Any belowground stems of *A. gerardii* that extended outside the ring encircling the plant were severed. Following harvest, each plant was washed to remove soil and other species found with it were discarded.

### *Lab Methods*

Tillers and basal buds were counted, assessed to be living or dead, and classified by developmental stage. Buds and tillers were examined using a dissecting scope with magnifications between 7 and 25x. Dormant bud, active bud, and tiller were the three developmental categories that were used in this study following the classifications of chapter 1. Dead buds were easily identified by their soft or mealy brown interiors.

Parent tillers were classified by their age (2007 or 2008 recruits) and as flowering or vegetative. As observed in other grass species (Hendrickson and Briske, 1997), buds of *A. gerardii* have greater longevity than their parent tillers, remaining viable up to three years (Chapter 1). These longer lived buds exist on their residual parent tiller bases. These residual tiller bases can be identified to year of recruitment as described above (see pictures in Chapter

1). Flowering tillers were differentiated from vegetative tillers by the presence of a flowering head. Tillers were not considered to be flowering until their seed head was exposed since it was unknown *a priori* whether a bolting tiller would successfully flower. Therefore, bolting tillers were considered vegetative tillers to create consistency in data collection. In this study's observations, bolting tillers always completed their development into a flowering tiller. Since vegetative tillers in *A. gerardii* are culmless, residual 2007 tiller bases could be identified as flowering tillers by the increased diameter of their base due to the culm and the larger size of their buds.

### *Analysis*

Using the bud and tiller classifications of both species, the number of buds per tiller was calculated for each plant according to developmental bud stage, adult tiller generation, and adult flowering status. Lab tiller counts closely resembled field tiller counts and were therefore used when calculating buds per tiller. Proportions of total buds and tillers found on a plant were calculated according to developmental stage. Overall bud production per tiller of *A. gerardii* includes all dormant and active buds.

## Results

Flowering and vegetative tillers differed in their bud production but had similar phenology (Figure 2.1). Flowering 2007 tillers had significantly more dormant buds (8.64 ± 0.17 dormant buds/tiller) than vegetative 2007 tillers (6.82 ±0.19 dormant buds/tiller) during the 2007-8 winter dormant season (ANOVA, $F=49.75$, $p<0.0001$). During the 2008 spring when active buds emerged and grew into tillers, a larger proportion of dormant buds broke dormancy on flowering tillers (64%) than on vegetative tillers (38%; Figure 2.1AB). After this transitioning period, 3.15 ± 0.13 dormant buds per flowering tiller and 4.25 ± 0.14 dormant buds per vegetative tiller remained over the rest of the growing season (Figure 2.1A). Therefore, on a per tiller basis, flowering 2007 tillers produced more 2008 tillers than did vegetative 2007 tillers (3.00 ± 0.15 2008 tillers/flowering tiller, 1.66 ± 0.09 2008 tillers/vegetative tiller; Figure 2.1C). Although buds on flowering tillers have a higher likelihood of becoming tillers than buds on vegetative tillers, flowering tillers made up a smaller proportion (0.34 ± 0.05) of a plant's 2007 tiller population than vegetative tillers (0.66 ± 0.05) based on data from October sampling dates.

When 2008 tillers flowered in mid-August, their bud production, which had begun in early June, was near completion (Figure 2.2). Flowering tillers initially had higher dormant bud numbers than vegetative tillers (8.88 ± 0.22 buds/flowering tiller, 7.57 ± 0.18 buds/vegetative tiller). However, by late September, a difference in dormant bud number between 2008 flowering and vegetative tillers no longer existed (8.14 ± 0.43 buds/flowering tiller, 8.23 ± 0.18 buds/vegetative tiller, Figure 2.2). The proportion of flowering tillers per 2008 plant during October sampling dates (0.30 ± 0.05 flowering tillers/total tillers) was similar to 2007.

Interannual variation in buds produced per tiller was assessed by comparing the 2007 and 2008 cohort's total bud production at the end of the growing season (i.e. the overwintering bud bank) in which they were first produced. The overwintering bud numbers per tiller of the 2008 cohort in 2008-9 (8.21 ± 0.17 buds/tiller) was significantly larger than the 2007-8 overwintering bud numbers per tiller of the 2007 cohort (7.24 ± 0.33 buds/tiller; ANOVA, $F=8.21$, $p=0.005$). The increased number of the 2008 cohort's 2008-9 winter buds per tiller when compared to numbers of the 2007 cohort's 2007-8 winter buds per tiller was driven by the higher bud production on the 2008 vegetative tillers than on 2007 vegetative tillers.

## Discussion

A tradeoff between sexual and vegetative reproduction was not evident within *A. gerardii* as tillers that allocated resources to flowering and seed production also produced more buds. This coincides with findings for *Sporobolous heterolepis* and *Koeleria macrantha* (Dalgleish et al., 2008). Flowering tillers not only produced more buds but transitioned a larger proportion of these buds to tiller than vegetative tillers did. Although differences in bud production of flowering and vegetative tillers were apparent in both the 2007 and 2008 cohorts, these differences disappeared in the 2008 cohort during the end of the 2008 growing season. Bud production on vegetative tillers varied more interannually than on flowering tillers. Despite differences in bud production, bud bank phenology was very similar between vegetative and flowering tillers.

Dormant bud production is inherently tied with tiller growth (Chapter 1). Upon flowering, the apical meristem switches production of vegetative buds to production of spikelet buds (Langer, 1972; Sharman, 1947). The vegetative phytomers, which still exist as primordia after the apical meristem has been induced to flower, grow with elongated internodes,

collectively forming the culm (Hyder, 1972), which raises the seed head for wind dispersal. Although these remaining phytomers may still produce vegetative buds, these buds become aerial buds rather than basal buds due to this internode elongation. Thus, the basal accumulation of primary buds is permanently stopped when a tiller flowers. Therefore, a tiller will always produce basal buds independently of flowering as vegetative bud natality always precedes floral induction. Since apical meristems first produce vegetative buds and then transition to producing the flowering head, it would be expected that allocation to seed reproduction would be more variable and more closely related to resource availability than would bud production.

Flowering tillers typically produced a greater number of dormant basal buds than vegetative tillers in *A. gerardii*. In addition to photoperiod, temperature, and other environmental cues, tillers may have to reach a size threshold before floral initiation (Langer, 1972). If the larger tillers with more leaves, or phytomers, were recruited to flower, flowering tillers would have higher bud counts than vegetative tillers. In *Agropyron repens,* floral induction of shoots which had not reached their minimum leaf number failed when the proper photoperiod and environmental requirements for flowering were met (Sharman, 1947). As each phytomer produces both a leaf and a bud (Briske, 1991; Evert, 2006), flowering tillers of *A. repens* likely had higher bud counts than tillers that were unable to flower because they had not met the threshold size. Under good conditions early in the growing season before floral induction, more tillers would be expected to meet or exceed the minimum size threshold and flower. However, good growing conditions after flowering may eliminate the belowground bud production discrepancy between flowering and vegetative tillers. Those vegetative tillers which did not meet the size threshold at the time of floral induction could continue to produce basal vegetative buds, albeit at a slow rate, until the end of the growing season, unlike flowering tillers. As a result, vegetative and flowering tillers could have similar production of buds especially during good growing conditions. This might explain the convergence of vegetative and flowering tiller bud numbers at the end of the 2008 growing season which resulted in the overall higher bud production per tiller in 2008 than 2007. Grass biomass production on annually burned watersheds at Konza Prairie Biological Station in 2008 was 1.5x the long term average grass biomass whereas 2007 was near the long term average. Thus, the good growing conditions of 2008 enabled vegetative tillers to have similar bud production to flowering tillers but the

growing conditions of 2007 did not enable vegetative tillers to match the bud production of the flowering tillers.

In lieu of the size threshold explanation for higher bud production of flowering tillers, the transition from vegetative culmless tillers to flowering culmed tillers, which occurs in *A. gerardii,* offers another explanation. When a tiller flowers, its most basipetal elongated phytomer's internode base along with its axillary bud would be located belowground, with the rest of the condensed nodes it had produced before becoming a culmed flowering tiller. It would leave behind a well-developed dormant bud at its internode base, thus increasing the belowground bud count, while the same bud primordium on a vegetative tiller would remain under-developed and may die when the apical meristem dies if it is not fully developed. The average difference between flowering and vegetative tillers was around one bud. If this hypothesis holds true, a species with culmed vegetative tillers would have similar bud production on both flowering and vegetative tillers. However, this hypothesis fails to explain how the 2008 vegetative and flowering tillers converged on the same average buds per tiller by the end of the growing season.

Sexual reproduction indirectly aided vegetative reproduction potential in that a larger proportion of buds on flowering tillers than on vegetative tillers transitioned to tillers. The youngest, most distally located buds on the tiller base are the most likely to grow out (Mitchell, 1953; McIntyre, 1970; Cable, 1971; Mueller and Richards, 1986). Potential explanations for why flowering tillers activate greater numbers of distal buds than vegetative tillers ultimately deal with the controls of apical dominance and individual bud characteristics (i.e. exogenous and endogenous dormancy). Two main differences between flowering and vegetative tillers are: 1) the location of the apical meristem and 2) bud size. 1) When a tiller flowers, the apical meristem is removed a great distance away from the vegetative buds it formed belowground. In the following growing season after the parent tiller's senescence, apical dominance may exert less of a residual influence on the more distantly removed flowering tiller buds than buds on vegetative tillers which had an apical meristem in close proximity. There are some inherent problems with this explanation since apical dominance would suggest that distal buds would be more inhibited than proximal buds. However, the mechanisms of apical dominance are not fully resolved (Cline, 1991). 2) Buds are added acropetally along the tiller base leaving the most active and youngest buds near the apex. Well-developed buds increase in size from the basipetal to the

acropetal positions along the tiller base (Mueller and Richards, 1986; Busso et al., 1989). Although a study correlating bud size with increased tillering has not been conducted, distal buds, which are more apt to grow out, are larger than proximal buds. Flowering tillers tend to have thicker tiller bases than vegetative tillers due to their culm formation which lead to larger buds near the tiller base. Since these larger buds had an increased period of metabolic activity before the winter dormant season, they may be reactivated more quickly in the following growing season. Although the mechanism of increased bud production in flowering tillers is undetermined, a flowering tiller contributes more to vegetative reproduction in the following year than a vegetative tiller by producing a larger numbers of buds per tiller and transitioning more buds to tiller.

A set tradeoff between sexual and vegetative propagation may exist for each species or for a specific population of a species. This tradeoff would not be based on resource allocation but based on developmental allocation (Watson, 1984; Watson and Casper, 1984). As discussed above, basal vegetative bud production ceases upon floral induction. The developmental turning point from vegetative to sexual bud production is determined by the flowering requirements of the individual species. African perennial grass species tend to have lower bud production and higher flowering effort than North American species (Hartnett et al., 2006). These African species may have a low minimum size threshold for flowering, or their other flowering requirements are easily met early in the development of the tiller, resulting in the low bud production per tiller but high flowering effort. The reverse could be true for the North American species. Thus, flowering indirectly affects bud production per tiller at the species level. Changes in resource availability would only alter the growth rate of tillers, subsequently changing the proportion of tillers that are able to meet the flowering requirements by the time flowering cues occur for the species, instead of altering the set number of basal buds produced per flowering tiller.

Plant development and morphology may act as a constraining factor in more areas than reproductive allocation hypotheses of the grasses. Total leaf surface area which is a factor in determining overall plant photosynthetic rates could be constrained within a grass species by the number of phytomers the species is able to produce. Proportion of flowering tillers in some grass species appears to be determined by whether or not plants are required to reach a minimum

production of phytomers (i.e., plant development) before flowering (Sharman, 1947; Langer, 1972).

In addition to considering plant development when examining plant responses and characteristics, plant development should be considered in the context of global change. The expression of current life history traits of grasses may change because of alterations in growing season length due to increasing temperatures, while flowering cues such as photoperiod may remain unchanged. If floral induction commences at its usual time but the growing season is extended, the continued growth of vegetative tillers after other tillers have flowered would continue for longer periods of time than presently observed. Thus, vegetative tillers would be able to produce more buds per tiller than flowering tillers. Global change has the potential of altering so many factors of the environment that it can be difficult to comprehend how plant development will constrain or enable plant responsiveness. However, plant development should be taken into consideration when predicting how plants will respond during these rapid environmental changes.

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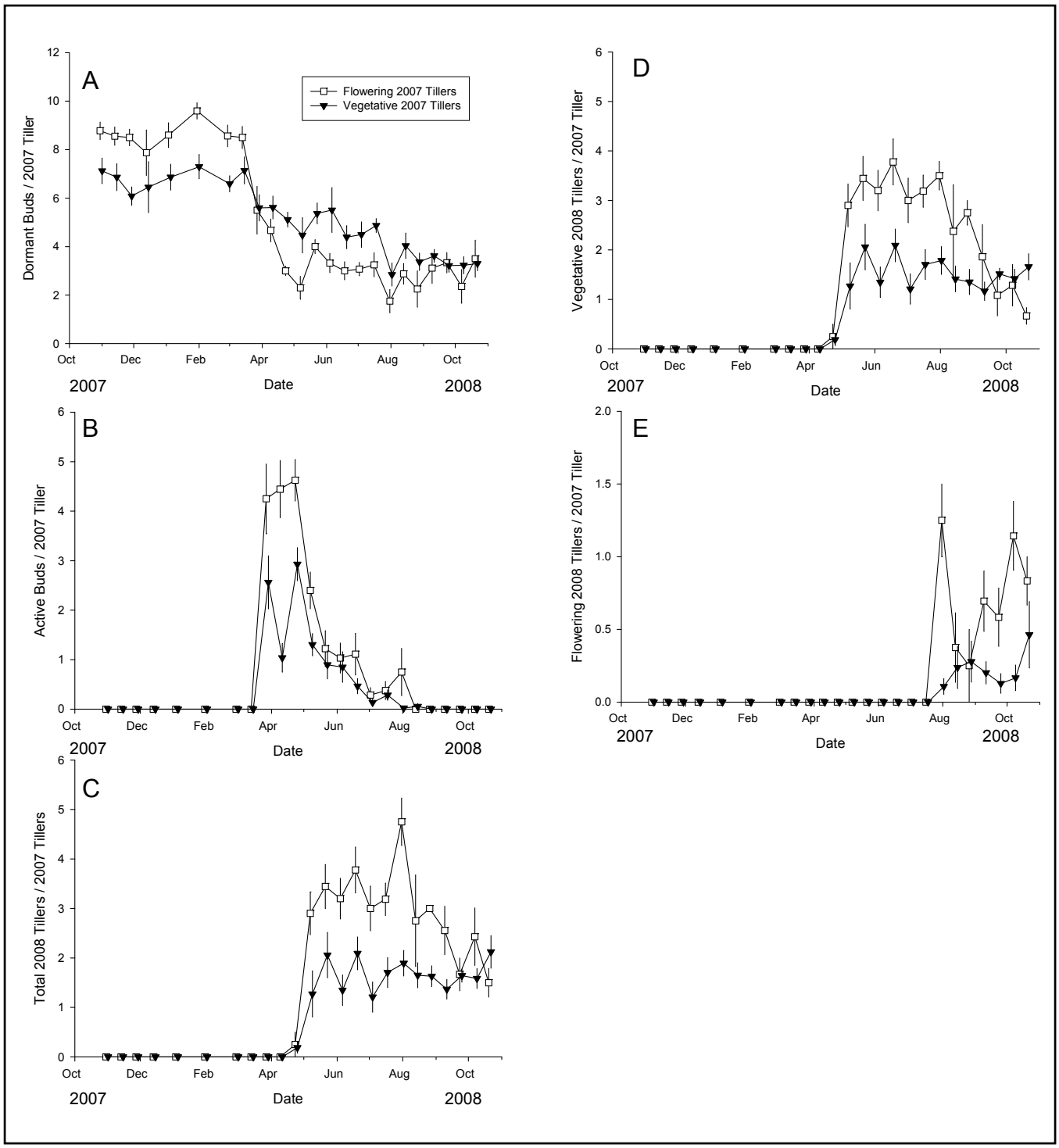


**Figure 2.1 Comparison of bud and tiller production by vegetative and flowering *A. gerardii* 2007 tillers from October 2007 to October 2008.** A) Dormant Buds B) Active Buds C) Total 2008 Tillers D) Vegetative 2008 Tillers E) Flowering 2008 Tillers. Error bars are ±1 SE of the mean.

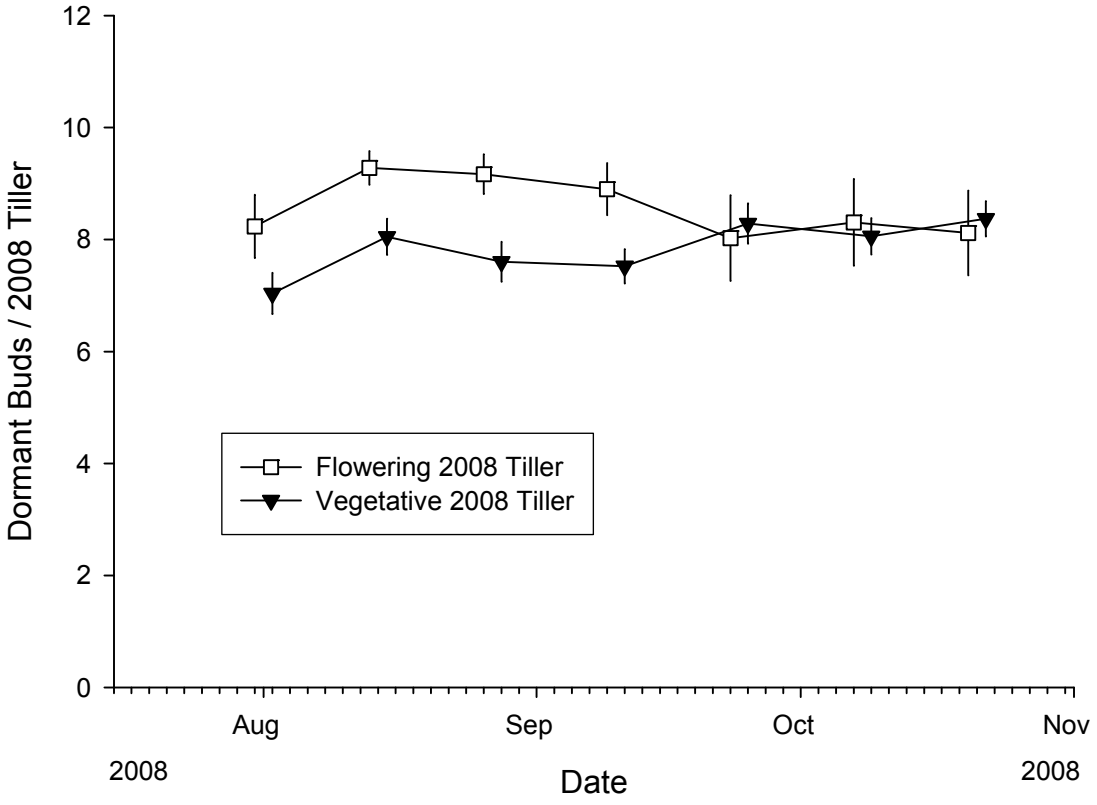


**Figure 2.2 Comparison of dormant bud production by vegetative and flowering 2008 tillers.** Tillers began flowering in late July. Error bars are ±1 SE of the mean.

# *CHAPTER 3* - Higher-Order Bud Production by *Dichanthelium oligosanthes* (Schult.) Gould


## Abstract

Since the majority of tiller recruitment on tallgrass prairie occurs from the belowground bud bank, it is important to understand how buds are produced and the dynamics and structure of bud populations. Most perennial grass buds are assumed to be produced directly from the parent tiller's apical meristem. However, higher-order bud production has been observed in a few grass species including *Dichanthelium oligosanthes* where secondary, tertiary, and quaternary bud branching occurs. Higher-order grass bud production and dynamics have not been studied and their ecological consequences have not been considered. Therefore, the role of higher-order bud production and its dynamics were examined in *D. oligosanthes* during its period of higher-order bud production in the fall. Higher-order bud production greatly multiplied the tiller population growth potential. Bud production and dynamics varied across hierarchical levels. The highest bud production per mature tiller occurred as secondary buds, which were produced on primary buds. The developmental stage and hierarchical level of the parent bud determined the number and developmental stage of the propagules it produced. Juvenile tillers arose in equal numbers from both primary and secondary buds. Higher-order bud production enabled bud production to occur independently of mature tiller growth making it possible for the plant to be flexible in its life history. Limited exogenous chemical and endogenous physiological bud dormancy enabled higher-order bud production to occur and created asynchrony in the bud development of this species. The overwintering bud bank primarily comprised of higher-order buds provided insurance against failure of juvenile tillers to overwinter. Higher-order bud production also increased the spatial occupancy ability of a plant in a given growing season. Higher-order bud production is a vital characteristic in some grasses that has been often overlooked and should be taken into consideration in future studies of grass production.


## Introduction

In perennial grasslands, >99% of tiller recruitment occurs from belowground buds rather than seeds (Benson and Hartnett, 2006). Therefore, the study of bud production is important for understanding the potential for the annual regeneration of the grassland community. Grasses

form the majority of the belowground plant community bud bank reflecting the aboveground dominance of grasses (Dalgleish and Hartnett, 2009). Most of this grass bud production has been assumed to be primary bud production, in which buds are produced directly from the mature parent tiller. However, *Dichanthelium oligosanthes* has been observed to produce higher-order buds arising from the primary bud. Thus, not all grass buds are produced directly from apical meristems of mature tillers as previously assumed.

Higher-order bud production in the grasses has only been mentioned in the semi-desert perennial *Trichachne californica* (Cable, 1971) and the dynamics and production of higher-order buds have never been examined in detail in any grass species. Higher-order (i.e. hierarchical) bud production also potentially depends on lower-order bud development and transition to tiller. Certain developmental stages of buds may be more likely to produce higher-order buds than others. Since *D. oligosanthes* produces juvenile tillers from buds during hierarchical bud production in the fall, hierarchical juvenile tiller production may also occur. Gaining insight into how higher-order bud production functions as well as its limitations will help in understanding the role it has in bud bank dynamics and vegetative grass regeneration on prairies. Therefore, the objectives of this study were to examine 1) the dynamics of higher-order bud and juvenile tiller production, 2) the general trends of bud developmental stages and juvenile tiller occurrence at different hierarchical levels, 3) how a bud's developmental stage and hierarchical order determines its bud production, and 4) the contribution primary buds versus higher order buds make to tiller recruitment.

## Materials and Methods

### *Site Description*

The study was conducted at Konza Prairie Biological Station (KPBS), a 3,487-ha tallgrass prairie preserve, which is located in the Flint Hills region of northeastern Kansas (39º05'N, 96º35'W). The hilly topography and limestone bedrock resulted in shallow, rocky upland soils (Udic Argiustolls, Florence series), steep slopes, and deep lowland soils (Pachic Argiustolls, Tully series). The vegetation composition at KPBS is dominated by warm season ($C_4$) grasses, such as *Andropogon gerardii* Vitman (big bluestem), *Sorghastrum nutans* (L.) Nash (Indiangrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem). Subdominant vegetation includes cool season ($C_3$) grasses, composites, legumes and other forbs, and a few

woody species. Due to the continental climate at KPBS, the majority of the mean annual precipitation (835mm) falls during the warm, wet springs and summers. KPBS is divided into multiple experimental units, each defined as a single watershed. Each watershed contains upland, slope, and lowland topographical positions and has both a grazing and fire regimen assigned to it. Fire regimes include 1-, 2-,4-, 10-, and 20- year fire return intervals and grazing regimes include grazed or ungrazed by bison. Two replicate watersheds (KPBS unit 4A- 47ha and KPBS unit 4B-135 ha), each with an ungrazed and 4-year spring fire return interval regimen, were chosen for this study. At the beginning of this study in fall 2007, it had been one year and two years since last fire on watershed 4A and 4B, respectively. Vegetation at these intermediate fire return interval sites are dominated by grass with both $C_3$ and $C_4$ grasses being readily abundant.

### *Field Sampling*

*Dichanthelium oligosanthes* (Schult.) Gould (Scribner's panicum) is a cool season ($C_3$) sub-dominant caespitose perennial grass which grows from 10cm to 70cm in height. Flowering occurs in the Great Plains from April to June although branching secondary panicles may bloom until the fall (Great Plains Flora Association,. 1986). Genets of *D. oligosanthes* are easy to determine because of its tufted growth form.

At each of five upland sites on each watershed, individuals of *D. oligosanthes* were sampled and marked in 2008 after its flowering period. Sites on each watershed were selected to be as distant from one another as possible while remaining on upland soils. On watershed 4A and 4B, sites were separated by an average 90 ± 13 meters and 208 ± 40 meters respectively. A *D. oligosanthes* individual consisted of an entire genet. Hereafter, sampled *D. oligosanthes* individuals will be referred to as "plants." In July 2008, each plant was marked by encircling its base with a wire ring and its tillers were counted.

Ten plants, one from each site on each watershed, were harvested biweekly starting on September 9$^{th}$, 2008. Biweekly harvesting continued until December 2$^{nd}$, 2008 when *D. oligosanthes* had fully senesced aboveground. Plants were harvested by excavating to a 7cm depth. All interconnected and belowground stems of *D. oligosanthes* occur within this specified soil depth and were collected as part of the plant. Following harvest, each plant was washed to remove soil and other species found with it were discarded.

### *Lab Methods*

Buds and tillers were counted, assessed to be living or dead, and classified by developmental stage and hierarchical level. Buds and tillers were examined using a dissecting scope with magnifications between 7 and 25x. Buds were identified as dormant, active, or photosynthesizing and tillers were identified as juvenile or mature following the classification of chapter 1. Dead buds were also identified by their soft, brown mealy interiors. Juvenile tillers were considered dead if their interior is brown and adult tillers were considered dead if their aboveground leaves have fully senesced.

A hierarchical order of bud development occurs in *D. oligosanthes* with existing buds and juvenile tillers producing axillary buds (Plate 3.1). This hierarchy has four levels at which buds or juvenile tillers, the propagule supply, may be produced: primary (arising on mature tiller internodes), secondary (arising on primary buds and juvenile tillers), tertiary (arising on secondary buds and juvenile tillers), and quaternary (arising on tertiary buds and juvenile tillers).

### *Analysis*

Using the bud and tiller classifications, buds per tiller was calculated for each plant according to developmental bud stage and hierarchical level. Lab tiller counts closely resembled field tiller counts and were therefore used when calculating buds per tiller. Proportions of total buds and juvenile tillers found on a plant were also calculated according to developmental stage and hierarchical level. Overall bud production per mature tiller included all dormant, active, and photosynthesizing buds whereas overall propagule production per mature tiller included all bud stages and juvenile tillers.

## Results

The capacity of buds and juvenile tillers of *D. oligosanthes* to produce higher order buds and juvenile tillers greatly multiplied the propagule production potential of each mature tiller and hence the tiller population growth potential (Figure 3.1). The higher order bud and juvenile tiller branching enabled *D. oligosanthes* to produce up to 20.88 ± 1.75 overwintering propagules per mature 2008 tiller, which was 4.5x higher than if it had been limited to primary bud and juvenile tiller production (Table 3.1). Propagules with dead parent buds or juvenile tillers were able to survive and independently contributed to the propagule population.

Overall propagule production and its timing varied across hierarchical levels. Secondary propagule production was greater than primary, tertiary, or quaternary propagule production (Table 3.1). Since larger proportions of primary propagules than other hierarchical levels produced propagules, overall secondary propagule production was higher than other hierarchical levels. In the overwintering 2008 cohort, 61 ± 4% of primary propagules and 18 ± 2% of secondary propagules had produced secondary and tertiary propagules respectively. Propagules with both lower hierarchical and higher developmental status were most likely to produce higher order propagules (Table 3.2). While primary propagule production had stopped by September, secondary propagule production was only about halfway completed (Figure 3.2). Secondary propagule production continued until early November coinciding with tertiary propagule production which began in September and ended in early November (Figure 3.2).

Presence of each developmental stage varied among the hierarchical levels of the 2008 overwintering propagule supply. Primary and secondary propagule supplies were comprised of different proportions of each developmental stage than tertiary and quaternary propagule supplies. Primary and secondary hierarchical levels maintained large portions of their propagules as dormant buds and juvenile tillers (Table 3.3). A large percentage of the tertiary propagule supply was composed of dormant buds and lacked the large juvenile tiller component observed in the primary and secondary hierarchical levels (Table 3.3). As a result, tertiary propagules contributed most strongly to the dormant bud bank than any other developmental stage. Because tertiary propagules were not produced until September or later, they had inadequate time to develop into further stages before the end of the growing season. Quaternary propagules, which occurred infrequently, all remained at the dormant bud stage (Table 3.3).

The parent propagule's development stage and hierarchical level influenced the number of propagules it produced and developmental stages of the propagules. Secondary propagules were mainly located on primary active and photosynthesizing buds in September (Figure 3.1). Since the primary active buds and photosynthesizing buds continued their development, the majority of secondary propagules were located on primary juvenile tillers by December (Figure 3.1, Table 3.4). The average primary juvenile tiller which bore secondary propagules supported more propagules than primary buds which bore secondary propagules (Table 3.5). Secondary propagules were commonly at the same or earlier developmental stages than the primary propagule from which they arose (Table 3.6). Secondary juvenile tillers, which originated as

secondary buds, were more likely than secondary buds to support tertiary propagules (86 ± 4% of tertiary propagules arose from secondary juvenile tillers in December). However, secondary active buds, photosynthesizing buds, and juvenile tillers that did support tertiary propagules bore similar amounts of propagules (Table 3.7). Tertiary propagules were only at the same or earlier developmental stages as their parent secondary propagule (Table 3.8). In summary, primary and secondary juvenile tillers maintained a large proportion of propagules because juvenile tillers were the second most abundant propagule in the overwintering propagule supply, were more likely to produce propagules than bud developmental stages, and were able to have equal or higher production of propagule progeny than bud developmental stages.

Fall juvenile tillers are the source of next year's tiller population. Primary and secondary juvenile tiller formation began in September and increased in similar numbers until November when production slowed (Figure 3.3). Secondary juvenile tillers were slightly more abundant (2.22 ± 0.26 juvenile tillers/2008 tiller) than primary juvenile tillers (1.74 ± 0.16 juvenile tillers/2008 tiller, stats) entering the winter dormant season starting in November. When considering juvenile tiller production at all hierarchical levels, juvenile tillers were usually the progeny of mature or juvenile tillers because all primary juvenile tillers originated on mature tillers, the majority (78 ± 5%) of secondary juvenile tillers originated on primary juvenile tillers, and all tertiary juvenile tillers originated on secondary juvenile tillers.

## Discussion

Higher-order bud production is linked to growth of the parent bud or juvenile tiller just as primary bud production is linked to growth of the mature tiller. As primary buds are released from dormancy, they become apical meristems with small phytomers of their own. From these meristematic regions of each small phytomer, a higher-order bud is formed (Sharman, 1942; Etter, 1951; Langer, 1972; Briske, 1991; Evert, 2006). Although it was possible that low numbers of higher order buds were formed in the spring, the majority of higher-order bud production occurred following the summer dormant season of *D. oligosanthes*. Since most of the mature tillers flowered by June, primary bud production ceased as the apical meristem was used to create the flowering head rather than more phytomers (Sharman, 1947; Langer, 1972). Summer only slowed or momentarily stopped bud growth and production (Chapter 1). When favorable environmental conditions occurred in the fall, primary buds continued to develop and

subsequently produced additional buds as many buds were not under exogenous or endogenous dormancy (Chapter 1). Evidence of this fall activation of buds also occurs aboveground as culm bud outgrowth creates branches on the upper portions of mature tillers (Great Plains Flora Association, 1986). Normally, timing of bud production is limited to when a tiller is vegetatively growing. However, hierarchical bud production enables buds to be produced independently of the timing of mature tiller growth.

Higher order dormant buds were under similar dormancy controls as primary dormant buds. Dormant buds were found at all hierarchical levels and usually occurred on non-dormant buds and juvenile tillers. In the fall as higher order dormant buds were formed, the leaf primordia of non-dormant buds and the leaves of juvenile tillers placed exogenous mechanical dormancy upon the young buds. These developing dormant buds could also have been under endogenous morphological or morphophysiological dormancy. Since the non-dormant buds and juvenile tillers were actively growing, their active apical meristems would be able to exert exogenous chemical dormancy in the form of apical dominance over dormant buds. However, many dormant buds located on actively growing parent buds or juvenile tillers were still able to grow out indicating that apical dominance was weak and that many buds were not under endogenous control. Those buds that did remain dormant were either under endogenous physiological dormancy or were impacted enough by the apical meristem to be under exogenous chemical dormancy. Until the enforced dormancy of winter occurred, some higher order buds were able to continue development. Limited exogenous chemical and endogenous physiological dormancy contributed to the ability of *D. oligosanthes* to produce higher order buds and, with the limited dormancy of primary buds, created asynchrony in bud development of *D. oligosanthes*. The brief longevity of the buds of *D. oligosanthes* may be a result of its limited bud dormancy (Chapter 1).

Since dormant buds would not be actively growing and thus producing buds, the very low percentage of dormant buds of *D. oligosanthes* which did produce buds must be considered. Non-dormant buds likely have higher metabolism, due to their continuous expansion in height and girth, which enables their phytomer units to grow and produce buds. Although buds appear dormant, evidence suggests that most buds are always slowly growing (Williams et al., 1975). In a bud only inhibited by apical dominance, a slow rate of bud elongation along with high metabolic activity could be expected (Cline, 1991). Thus, the higher metabolism in some

dormant buds without endogenous dormancy may enable their small active phytomers to produce higher-order buds. Secondary dormant bud production has been observed on primary dormant buds of *S. scoparium* (Ott, personal observation) and on enlarged basal buds of *T. californica* (Cable, 1971). *S. scoparium* sometimes has slow dormant bud elongation but still maintains the bud within its prophyll (Ott, personal observation). Interestingly, both of these species and *D. oligosanthes* are caespitose grasses. Although internode length determines the growth form of a grass, higher-order bud production can assist in maintaining this growth form (Chapter 1). The increased metabolism and lack of endogenous dormancy of enlarging basal buds only under exogenous chemical dormancy may help explain hierarchical bud production on dormant buds.

Higher-order bud production aided mature tiller recruitment and constituted a large portion of the overwintering bud bank. Since mature tillers were recruited from juvenile tillers, mature tiller recruitment depended on hierarchical propagule production since more than half of the juvenile tillers originated as higher order buds. Primary and secondary juvenile tillers may or may not differ in their probability of establishing as mature tillers but should be studied in the future. However, even if primary juvenile tillers did not directly contribute to the mature tiller populations, they supported a large number of secondary juvenile tillers that could transition to mature tillers. In addition to enabling mature tiller success, juvenile tillers maintained high bud production which strongly contributed to the overwintering bud bank. The overwintering bud bank, which was largely composed of higher order buds, was insurance against failure of juvenile tillers to survive overwinter. Thus, higher order buds and especially juvenile tillers, which created many of those buds, facilitated the persistence of *D. oligosanthes* either through contributions to the overwintering bud bank or to mature tillers.

Higher-order bud production plays a noteworthy role in the overall bud production of *D. oligosanthes* and provides impetus to examine more species that show higher-order bud production. If a perennial grass species only produces primary buds, dispersal of its tillers is determined by the internode elongation before those buds become tillers. Higher order buds provide a way for a species to increase its spatial occupancy in a single growing season. Thus far, higher-order bud production has only been observed in caespitose grasses. Those caespitose grasses with large amounts of higher-order bud production may be less susceptible to fragmentation since tillers from higher order buds could help increase the tiller density of the bunchgrass. This would be true even more so in species with long-lived dormant buds.

Higher-order bud production in species that occur over a wide range of latitudes may vary. Hierarchical bud production in *D. oligosanthes* ceased because of the onset of winter. Thus, shorter growing seasons likely inhibit hierarchical bud production whereas longer growing seasons may enhance hierarchical bud production. Those species which undergo hierarchical bud production may be more adapted to regions with longer growing seasons. Therefore, higher-order bud production could be another plant characteristic that helps explain the distribution of certain species of grasses. The ecological role of higher-order bud production will continue to be elucidated as more species with this trait are examined.

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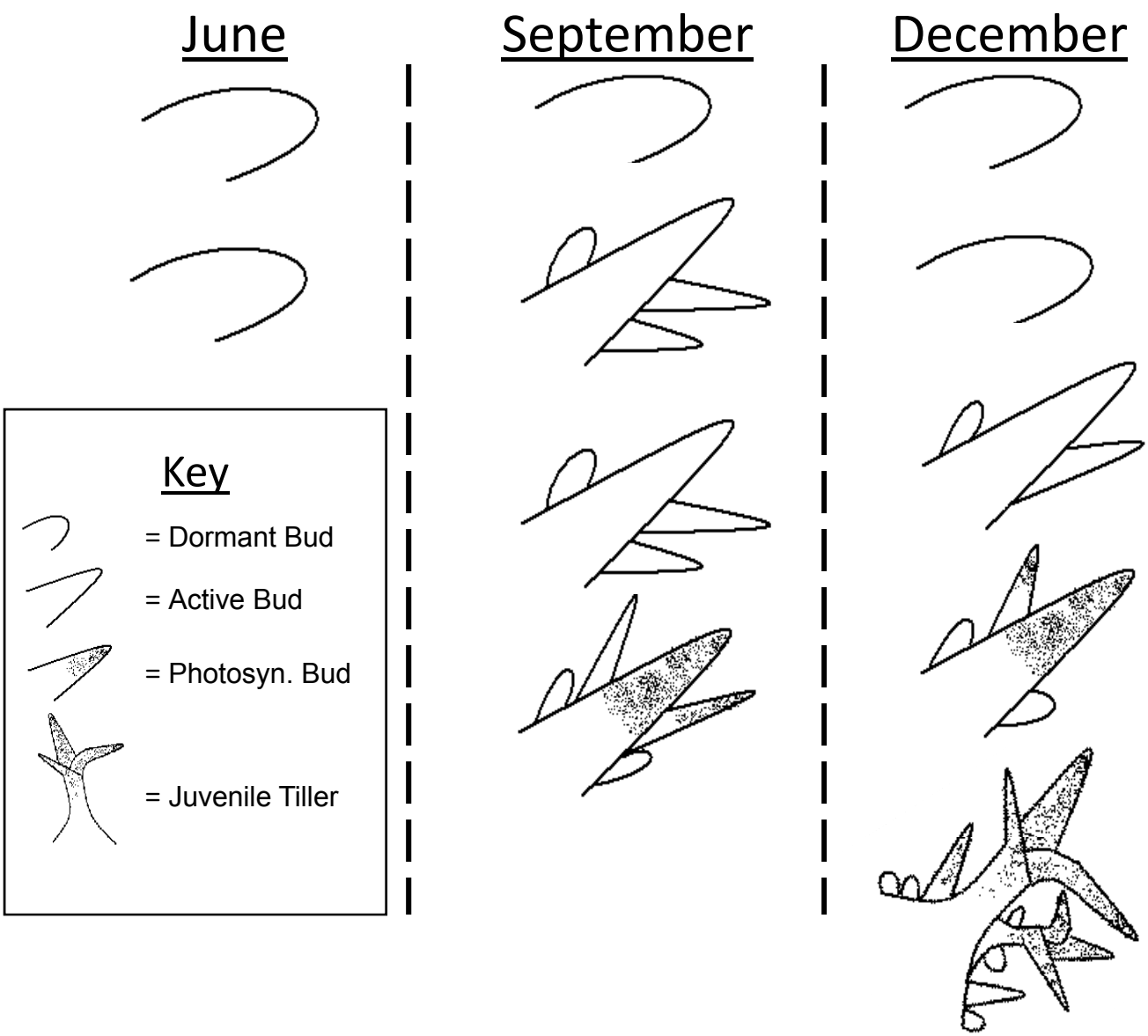



**Figure 3.1 Average hierarchical development of buds per mature 2008 tiller over a growing season.** See Appendix A for raw data. June averages are based on Chapter 1 data.

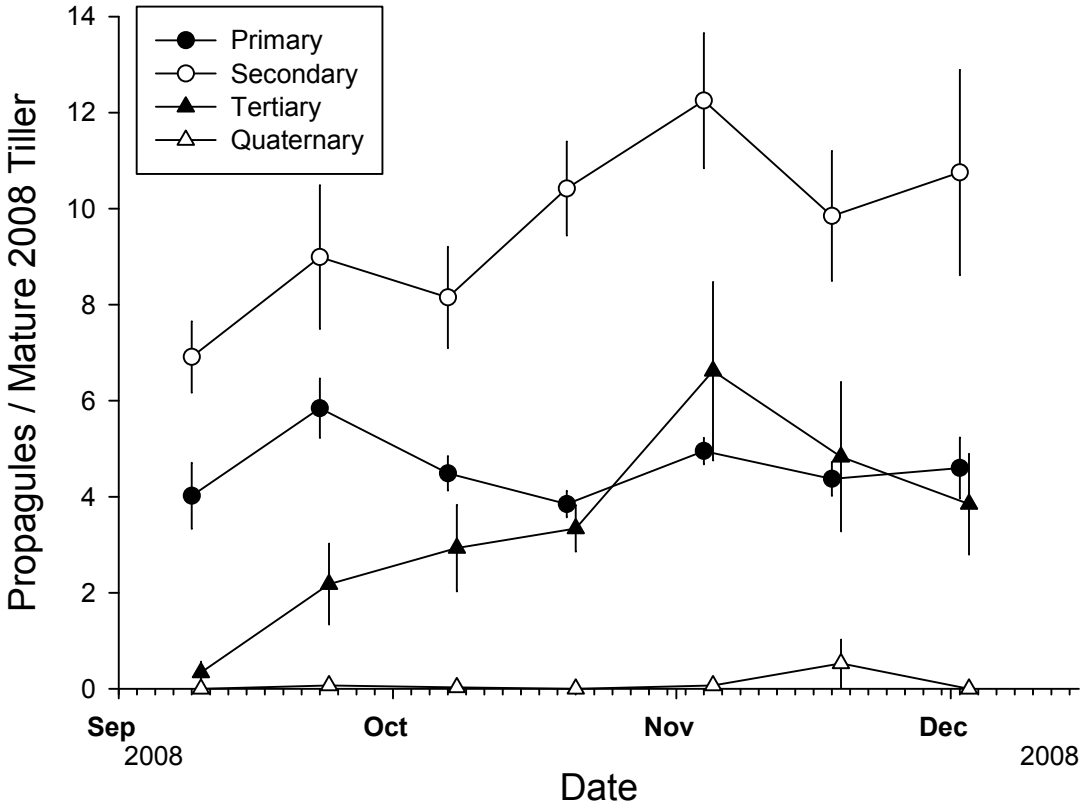



**Figure 3.2 Propagule supply (buds and juvenile tillers) by hierarchical level**. Error bars are ±1 SE of the mean.

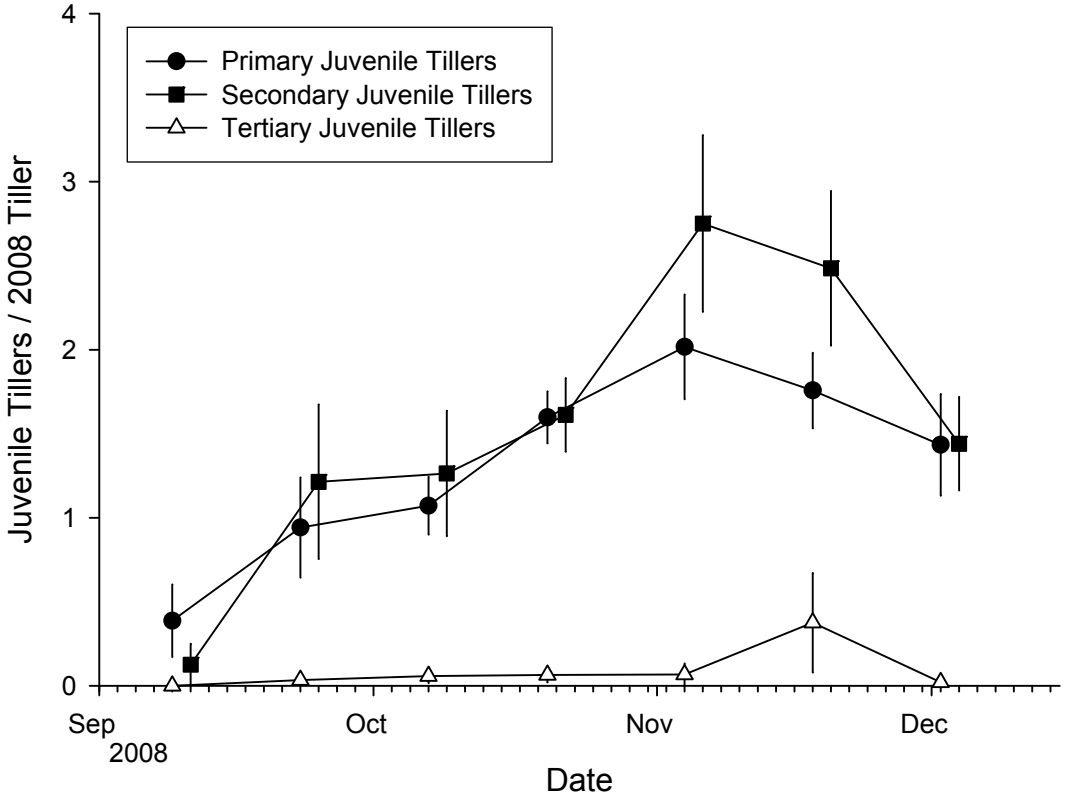



**Figure 3.3 Fall 2008 juvenile tiller production according to hierarchical level**. Secondary and tertiary juvenile tillers originated as secondary and tertiary buds located on primary and secondary buds respectively. Primary juvenile tillers were located directly on the mature 2008 tiller since they originated as primary buds. Error bars represent ±1SE of the mean.

**Table 3.1 Overwintering propagule supply according to hierarchical level in 2008-2009.** Propagule supply includes buds and juvenile tillers and is per mature 2008 tiller. Error is ±1SE of the mean.

| Hierarchical Level | Propagule Supply |
|---|---|
| Primary | 4.64 ± 0.25 |
| Secondary | 10.95 ± 0.95 |
| Tertiary | 5.10 ± 0.88 |
| Quaternary | 0.20 ± 0.17 |

**Table 3.2 Percentage of all primary and secondary propagules at each developmental stage that produced at least one higher order propagule (whether buds or juvenile tillers) by the end of the 2008 growing season.**

| | | **Developmental Stage** | | | |
|---|---|---|---|---|---|
| | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| **Hierarchical Level** | Primary | 5 ± 3% | 59 ± 7% | 83 ± 5% | 97 ± 3% |
| | Secondary | 0.2 ± 0.1% | 5 ± 2% | 13 ± 4% | 73 ± 5% |

**Table 3.3 Developmental stage composition of the 2008 bud cohort according to each hierarchical level at the end of the 2008 growing season.** Each hierarchical level was divided up according to developmental stage.

| | **Hierarchical Level** | | | | |
|---|---|---|---|---|---|
| **Developmental Stage** | | Primary | Secondary | Tertiary | Quaternary |
| | Dormant Bud | 33 ± 4% | 50 ± 2% | 88 ± 2% | 100% |
| | Active Bud | 16 ± 2% | 15 ± 1% | 6 ± 1% | 0% |
| | Photosyn. Bud | 12 ± 3% | 15 ± 2% | 4 ± 1% | 0% |
| | Juvenile Tiller | 39 ± 3% | 20 ± 2% | 2 ± 1% | 0% |

**Table 3.4 Percentage of total secondary or tertiary propagules located on parents of different developmental stages.**

| | **Parent Propagules** | | | |
|---|---|---|---|---|
| | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| **% secondary propagules on** | 2 ± 1 | 11 ± 2 | 16 ± 3 | 71 ± 3 |
| **% tertiary propagules on** | 0.3 ± 0.2 | 3 ± 1 | 11 ± 3 | 86 ± 4 |

**Table 3.5 Average number of secondary propagules per primary propagule.** Only primary propagules that had secondary propagules were considered.

| **Primary Propagule Developmental Stage** | **Secondary Propagules / Primary Propagule** |
|---|---|
| Dormant Bud | 1.56 ± 0.15 |
| Active Bud | 2.41 ± 0.17 |
| Photosyn. Bud | 3.29 ± 0.15 |
| Juvenile Tiller | 4.66 ± 0.16 |

**Table 3.6 Percentage of secondary propagules according to developmental stage that were produced from primary parents of each developmental stage.** This only assessed primary propagules that did produce propagules.

| | **Secondary Developmental Stage** | | | | |
|---|---|---|---|---|---|
| **Primary Parent Developmental Stage** | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| | Dormant Bud | 86 ± 9% | 0% | 14 ± 9% | 0% |
| | Active Bud | 65 ± 8% | 16 ± 4% | 5 ± 3% | 14 ± 7% |
| | Photosyn. Bud | 50 ± 5% | 9 ± 2% | 27 ± 6% | 14 ± 4% |
| | Juvenile Tiller | 48 ± 3% | 15 ± 2% | 13 ± 2% | 24 ± 3% |

**Table 3.7 Average number of tertiary propagules per secondary propagule.** Only secondary propagules that had tertiary propagules were considered.

| **Secondary Propagule Developmental Stage** | **Tertiary Propagules / Secondary Propagule** |
|---|---|
| Dormant Bud | 1.00 ± 0 |
| Active Bud | 1.96 ± 0.26 |
| Photosyn. Bud | 2.14 ± 0.21 |
| Juvenile Tiller | 2.39 ± 0.12 |

**Table 3.8 Percentage of tertiary propagules according to developmental stage that were produced from secondary parents of each developmental stage.** This only assessed sesecondary propagules that did produce tertiary propagules.

| | **Tertiary Developmental Stage** | | | | |
|---|---|---|---|---|---|
| **Secondary Parent Developmental Stage** | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| | Dormant Bud | 100 ± 0% | 0% | 0% | 0% |
| | Active Bud | 93 ± 7% | 7 ± 7% | 0% | 0% |
| | Photosyn. Bud | 87 ± 10% | 7 ± 7% | 6 ± 4% | 0% |
| | Juvenile Tiller | 90 ± 2% | 5 ± 1% | 3 ± 1% | 2 ± 0.1% |

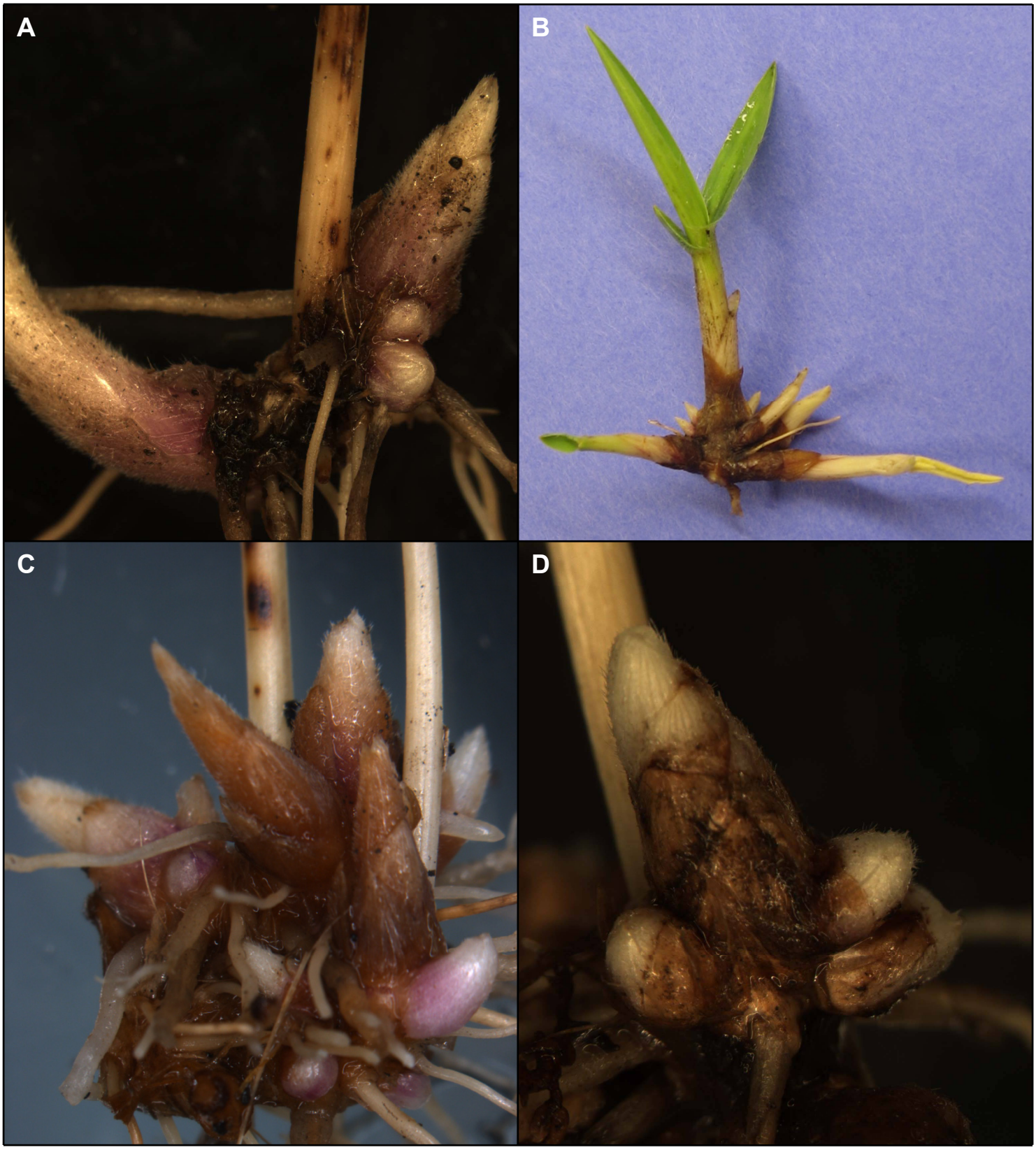
**Plate 3.1 Higher-order bud development of *Dichanthelium oligosanthes***

A- Primary and secondary buds of *D. oligosanthes*. 7.1x. September 18, 2007. The primary active bud supports two secondary dormant buds.

B- Primary juvenile tiller of *D. oligosanthes* with its bud progeny. The central juvenile tiller was attached to a mature tiller base identifying it as a primary bud. The secondary buds which surround it are attached to the primary juvenile bud. These secondary buds potentially have tertiary buds that are not visible in this photograph.

C- Caespitose growth pattern of *D. oligosanthes*. 7.1x. August 26, 2008. The lack of internode elongation before tillering in addition to higher-order bud production creates a dense clump of buds at the base of the two tillers.

D- Hierarchical structure of *D. oligosanthes*. 10x. October 30, 2007. The primary active bud, attached to the mature tiller, supported three secondary dormant buds.


A
B
C
D

# Appendix A - Higher-order bud production details for September 9th and December 2nd, 2008

Averages and percentages from September 9th, 2008 (Figures. A.1-A.5) and December 2nd, 2008 (Figures.A.6-A.10) were used to create Figure 3.1.

**Table A.1 Average primary propagules per mature tiller on September 9th, 2008.**

| Primary Propagule | Propagules / mature tiller |
|---|---|
| Dormant Bud | 1.34 ± 0.68 |
| Active Bud | 1.68 ± 0.34 |
| Photosyn. Bud | 0.61 ± 0.30 |
| Juvenile Tiller | 0.39 ± 0.22 |

**Table A.2 Percentage of primary propagules that have at least one secondary propagule on September 9th, 2008.** Primary propagules are divided according to developmental stage.

| Primary Propagule Developmental Stage | % of Prim. Propagules with Sec. Propagules |
|---|---|
| Dormant Bud | 0 % |
| Active Bud | 69 ± 11% |
| Photosyn. Bud | 53 ± 23% |
| Juvenile Tiller | 0% |

**Table A.3 Average secondary buds and juvenile tillers per primary buds and juvenile tillers on September 9th, 2008.**

| | | Secondary Propagule type per primary parent bud type | | | |
|---|---|---|---|---|---|
| | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| **Primary Parent Bud** | Dormant Bud | 0 | 0 | 0 | 0 |
| | Active Bud | 1.21 ± 0.13 | 1.66 ± 0.38 | 0.02 ± 0.02 | 0.08 ± 0.08 |
| | Photosyn. Bud | 1.5 ± 0.29 | 0.83 ± 0.44 | 0.5 ± 0.29 | 0 |
| | Juvenile Tiller | 0 | 0 | 0 | 0 |

**Table A.4 Percentage of secondary propagules that have at least one tertiary propagule on September 9th, 2008.** Secondary propagules are divided according to developmental stage.

| Secondary Propagule Developmental Stage | % of Sec. Propagules with Tert. Propagules |
|---|---|
| Dormant Bud | 0 % |
| Active Bud | 1 ± 1% |
| Photosyn. Bud | 0 |
| Juvenile Tiller | 71% (no SE) |

**Table A.5 Average tertiary buds and juvenile tillers per secondary primary buds and juvenile tillers on September 9th, 2008.**

| | | Tertiary Propagule type per Secondary parent bud | | | |
|---|---|---|---|---|---|
| | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| Secondary Parent Bud | Dormant Bud | 0 | 0 | 0 | 0 |
| | Active Bud | 3 (no SE) | 0 | 0 | 0 |
| | Photosyn. Bud | 0 | 0 | 0 | 0 |
| | Juvenile Tiller | 2.2 (no SE) | 0 | 0 | 0 |

**Table A.6 Average primary propagules per mature tiller on December 2nd, 2008.**

| Primary Propagule | Propagules / mature tiller |
|---|---|
| Dormant Bud | 1.93 ± 0.43 |
| Active Bud | 0.60 ± 0.12 |
| Photosyn. Bud | 0.64 ± 0.19 |
| Juvenile Tiller | 1.43 ± 0.30 |

**Table A.7 Percentage of primary propagules that have at least one secondary propagule on December 2nd, 2008.** Primary propagules are divided according to developmental stage.

| Primary Propagule Developmental Stage | % of Prim Propagules with Sec. Propagules |
|---|---|
| Dormant Bud | 1.9 ± 1.5% |
| Active Bud | 71 ± 13% |
| Photosyn. Bud | 89 ± 3% |
| Juvenile Tiller | 96 ± 3% |

**Table A.8 Average secondary buds and juvenile tillers per primary buds and juvenile tillers on December 2nd, 2008.**

| | | Secondary Propagule type per primary parent bud type | | | |
|---|---|---|---|---|---|
| | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| **Primary Parent Bud** | Dormant Bud | 1 ± 0 | 0 | 0.25 ± 0.25 | 0 |
| | Active Bud | 1.33 ± 0.16 | 1.12 ± 0.51 | 0.32 ± 0.14 | 0.31 ± 0.28 |
| | Photosyn. Bud | 1.95 ± 0.27 | 0.39 ± 0.16 | 0.60 ± 0.15 | 0.41 ± 0.10 |
| | Juvenile Tiller | 2.64 ± 0.32 | 0.74 ± 0.13 | 0.63 ± 0.16 | 0.74 ± 0.13 |

**Table A.9 Percentage of secondary propagules that have at least one tertiary propaguleon December 2nd, 2008.** Secondary propagules are divided according to developmental stage.

| **Secondary Propagule Developmental Stage** | **% of Sec Propagules with Tert. Propagules** |
|---|---|
| Dormant Bud | 0.6 ± 0.4 |
| Active Bud | 0.006 ± 0.006 |
| Photosyn. Bud | 11 ± 5 |
| Juvenile Tiller | 74 ± 7 |

**Table A.10 Average tertiary buds and juvenile tillers per secondary primary buds and juvenile tillers on December 2nd, 2008.**

| | | Tertiary Propagule type per Secondary parent bud | | | |
|---|---|---|---|---|---|
| | | Dormant Bud | Active Bud | Photosyn. Bud | Juvenile Tiller |
| **Secondary Parent Bud** | Dormant Bud | 1 ± 0 | 0 | 0 | 0 |
| | Active Bud | 1 (no SE) | 0 | 1 (no SE) | 0 |
| | Photosyn. Bud | 3.25 ± 1.01 | 0 | 0 | 0 |
| | Juvenile Tiller | 2.35 ± 0.31 | 0.20 ± 0.06 | 0.06 ± 0.04 | 0.02 ± 0.02 |