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Defoliation impacts on smooth bromegrass (*Bromus inermus* Leyss.) outgrowth and axillary bud production.

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Abstract

Smooth bromegrass (*Bromus inermis* Leyss.) is an introduced, perennial, cool-season invasive grass that has invaded native rangelands in the Great Plains. Defoliation at specific growth stages may reduce the abundance of *B. inermis* but information is limited about when this should occur. Between 2018 and 2020 we assessed how defoliation at four different phenological stages influenced the amount of outgrowth on *B. inermis* tillers near Mandan, North Dakota, USA. In three replicated plots we evaluated axillary buds, tillers, and rhizome outgrowth. Each year, 10 tillers per plot were defoliated at a height of 5 cm at each of the following phenological stages: 1) once in the vegetative stage; 2) twice in the vegetative stage, 3) once in the elongation stage, 4) once in the reproductive stage, and an undefoliated control. Individual tillers were collected in the fall following defoliation and processed in the laboratory. The total number of crown positions were determined for each tiller and outgrowth (tillers and rhizomes) and number of axillary buds were counted on each tiller. A double staining technique was used to determine active, dead and dormant axillary buds. Tillers defoliated twice in the vegetative stage had less outgrowth per tiller than tillers defoliated once in the reproductive stage or the undefoliated controls (new outgrowth of 1.2, 2.2, and 1.8 per tiller for twice vegetatively, reproductive and control respectively). Results show that defoliating tillers in the elongation or reproductive stage resulted in nearly complete tiller mortality. Our data suggest managers who wish to reduce *B. inermis* abundance should focus on defoliating it twice in the vegetative stage and avoid defoliating *B. inermis* in the reproductive stage to limit outgrowth.

Keywords: Axillary buds, vegetative outgrowth, phenology and defoliation, invasive plants,

Management Implications:

Our study provides important insights into control strategies for *Bromus inermis*. Previous studies suggested that defoliation in the elongation stage would provide maximum reduction in outgrowth and persistence. Instead, we found that defoliating *B. inermis* twice in the vegetative stage minimized the number of tillers and rhizomes produced over the season or remaining alive into the fall compared to undefoliated *B. inermis* tillers or those defoliated in the reproductive stage (R). Therefore, managers wishing to reduce *B. inermis* abundance should avoid defoliation during the reproductive stage (R) and instead try to increase defoliation opportunities in the vegetative stage (V2). However, *B. inermis* has a large amount of meristematic potential available for future productivity regardless of defoliation treatment, which is a concern for control efforts since axillary bud dynamics are closely tied to above-ground tiller dynamics **(Ott and Hartnett 2015)**. The large meristematic potential suggests multiple years of defoliation may be needed to reduce the abundance of *B. inermis* on these pastures.

We looked at the impact of defoliation timing on a single tiller and did not include the potential impacts on daughter tillers produced via rhizome. Bam et al. **(2024)** found that nearly 50% of new tillers may occur from rhizomes which may indicate that treatment impacts on a single tiller could differ from the effect of a treatment on a population of tillers. Also, because of the destructive harvest for evaluation of axillary buds, different parent tillers were defoliated each year. Repeated application of a particular defoliation treatment over time may change the persistence of *B. inermis*. Other variables affecting treatment response, which were not studied, including selective herbivory **(Schmitz 2008)** plant community composition or other factors **(Willson and Stubbendieck 2000)**. Still, this field based study provides needed information for designing future grazing studies looking at the effect of defoliation timing on *B. inermis*.

Introduction:

Smooth bromegrass (*Bromus inermis* Leyss.) is an introduced, perennial, cool-season invasive grass that was planted to stabilize cultivated soils in the western United States (Vogel 2004). *B. inermis* is often used for pasture in the Midwest and northcentral US because of its ability to survive drought, excellent winter hardiness and good yield potential (Vogel et al. 1996). Over time, *B. inermis* escaped and invaded native grasslands in the northern Great Plains. A study of

US Fish and Wildlife refugees in the northern Great Plains found that *B. inermis* made up to 45-49% of the vegetation (Grant et al. 2009). The consequences of this invasion include negative impacts to soil and hydrological properties, the population dynamics of native arthropods and native grass abundance (Palit and De Keyser 2022). These impacts highlight the negative effect that *B. inermis* invasion has on native rangelands in the northern Great Plains (Grant et al. 2020).

*B. inermis*Multiple efforts have been made to develop control strategies for *B. inermis*. A review of the impacts of *B. inermis* invasion suggested that both mowing and grazing could weaken *B. inermis* plants (Salesman and Jessica 2011). A single defoliation of *B. inermis* in the vegetative stage reduced the total tillers plant^{-1} in a greenhouse study (Bam et al. 2022). Mowing and raking reduced the abundance of *B. inermis* on idle native prairies in North Dakota (Hendrickson and Lund 2010). In Kansas, Harmoney (2007) found that grazing reduced *B. inermis* on upland soils while in North Dakota, early season grazing (mid-May) of native rangelands reduced *B. inermis* abundance (Hendrickson et al. 2020). Heavy grazing by horses for two consecutive years reduced *B. inermis* biomass compared to untreated controls (Stacy et al. 2005).

Despite the potential impact of defoliation as a tool to control *B. inermis*, there have been differing reports regarding how the timing of defoliation impacts *B. inermis* persistence. Eastin et al. (1964) and Lawrence and Ashford (1969) reported that defoliating *B. inermis* in early growth stages reduced yield. However, for controlling *B. inermis*, through burning (Willson and Stubbendieck 2000) or grazing (C. Dixon, personal communication), the 5 leaf stage corresponding with the early elongation stage (Preister et al. 2019), has been suggested as the critical phenological stage. Biligetu and Coulman (2010) indicated that defoliating *B. inermis* in the vegetative stage resulted in a 14% increase in tiller numbers but defoliating in the stem elongation stage reduced tiller numbers by 51%. Yield of *B. inermis* was also reduced when it was grazed by horses in the elongation stage (Stacy et al. 2005).

One of the problems with controlling *B. inermis* is its competitiveness (Palit and De Keyser 2022). Like many clonal grasses, *B. inermis* often relies on vegetative growth to increase tiller density (Palit and De Keyser 2022). Vegetative growth originates from axillary buds which are the source of up to 99% of the new shoots emerging in grasslands (Benson and Hartnett 2006). Growth from axillary buds provides an efficient mechanism for perennial grasses to persist or sustain their populations even during periods of disturbance (Ott et al. 2017, 2019). *B. inermis* had more axillary buds per tiller and initiated more buds than western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve] (Bam et al. 2024) which may enhance its competitive ability. However, in both warm-season (Mullahey et al. 1991) and cool-season grasses (Becker et al. 1997) defoliation at specific growth stages had impacted axillary bud numbers and activity. Bam et al. (2022) found that a single defoliation of *Pascopyrum smithii* in the vegetative stage increased bud dormancy.

Overall, the literature suggests 1) grazing or defoliation has the potential to reduce *B. inermis* abundance and 2) defoliation at certain growth stages may maximize this effect. If defoliation affects the number or outgrowth of axillary buds, it could provide an indication on the future effectiveness of the defoliation treatment. Therefore, we implemented a study to determine how defoliation of *B. inermis* by grazing or mowing should be timed to minimize its persistence. We defoliated individual *B. inermis* tillers in a field setting at different growth stages to determine the number of axillary buds and outgrowth. We hypothesized that defoliation in the culm elongation stage would result in a reduction of both outgrowth and axillary buds.

Methods

Site Description:

The study was initiated in May 2018 at the USDA-ARS Northern Great Plains Research Laboratory (NGPRL) located near Mandan, North Dakota (46.767085° -100.909112°). The study sites were located on loamy ecological sites (Sedivec and Printz 2012) on Temvik-Wilton soils (fine-silty, mixed superactive, frigid Typic and Pachic Haplustolls) (Liebig et al. 2006). Precipitation in both 2017 and 2020 was less than the long-term average (1913-2023), whereas the precipitation for 2018 and 2019 was greater than the long-term average (Figure 1). The precipitation received in September 2019 was the second highest on record for the month.

The study took place within three sites that had not been grazed since 1981. Each site was a 40m x 25m exclosure, fenced with a 3-wire barbed wire fence, preventing livestock from grazing but potentially allowing some wildlife grazing (deer and small mammals). All study sites were located within 0.4 km from each other.

A previous study on the pastures in which the study sites were located found the surrounding pasture was dominated by Kentucky bluegrass (*Poa pratensis* L.) and *B. inermis* but with native grasses of western wheatgrass, purple threeawn (*Aristida purpurea* Nutt.) and needleandthread [*Nassella viridula* (Trin.) Barkworth] also being present (Hendrickson et al. 2020). They reported over 20 different forb species with field pussytoes (*Antennaria neglecta* Greene) being the most abundant, and western snowberry (*Symphoricarpos occidentalis* Hook.) and leadplant (*Amorpha canescens* Pursh.) in lesser amounts. Visual evaluation of the study sites indicated greater *B. inermis* abundance within the exclosed study sites than in the surrounding pastures.

Tiller emergence in *B. inermis* starts in the early spring (Otfinowski et al. 2007) and tillers begin to elongate and flower in summer (Lamp 1952; Otfinowski et al. 2007). Tiller emergence can begin again after anthesis but often these tillers do not elongate (Lamp 1952) until the following spring (Lamp 1952; Otfinowski et al. 2007). Most tillers which elevate their apices in the fall do not survive the winter (Otfinowski et al. 2007).

Field Procedures:

In the spring (May) of 2018, 2019 and 2020, each study site had 50 *B. inermis* tillers randomly assigned to 5 different defoliation treatments (10 tillers per treatment). Tillers were at least 1 m apart from each other. Spacing was based on Otfiowski et al. (2007) who found *B. inermis* rhizomes may expand up to 83 cm into adjacent native prairie over 2 growing seasons. The tillers were defoliated by clipping at a height of 5 cm. Defoliation treatments were implemented 1) once in the vegetative stage (V1); 2) twice in the vegetative stage (V2); 3) once in the elongation stage (E); 4) once in the reproductive stage (R) and 5) an undefoliated control (C). Colored wires were placed at the base of each tiller to determine the defoliation treatment and assist in relocating the tiller in the fall. *B. inermis* is rhizomatous and only the tiller marked by the wire was defoliated during the clipping treatment.

The 50 *B. inermis* plants per site were destructively harvested in the fall (Oct-Nov) after senescence. Tillers were located using the colored wire placed on them in the spring and marked at the base with nail polish for further identification. A 5 cm diameter area was excavated around the marked tiller to a depth of approximately 3.5 cm. Excess dirt was removed if possible and the tiller was placed in a Ziplock bag with a moist paper towel, then stored at 4°C until processed. Each tiller was processed within 10 days of harvesting.

Laboratory Procedures:

During processing tillers were washed, and leaves were removed until only the crown remained. Since each phytomer has the potential to form an axillary bud, phytomers that were above the soil surface were excluded from processing. In addition, the rhizomatous nature of *B. inermis* can make it difficult to differentiate between rhizome and crown. Examination of the tillers showed a lengthening of the internode near the base of the plant on the rhizome. Therefore, we chose this lengthening as the point to differentiate between rhizome and crown.

Crowns were examined under a binocular microscope and crown positions (i.e., either outgrowth or potential bud location) were classified into categories. Categories included 1) live tillers, 2) dead tillers, 3) axillary buds, 4) missing buds, 5) rhizomes, and 6) leaf scars (Table 1; Figure 2). Leaf scars are crown positions where the existence of leaf attachment suggests that an axillary bud exists but there is no evidence of a bud (Figure 2). A bud was considered to have transitioned into a tiller or rhizome (Figure 2) when either portion could be identified as having emerged from the soil surface or there was visual evidence of leaf formation. Missing buds were crown positions where there was evidence of a bud, but it was removed during processing. We considered the point where the crown separated from the rhizome as the spot where the node lengthened below the crown.

Crowns that had axillary buds and/or missing buds were placed into vials with a 0.1% (w/v) solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 24 hours at 20°C. Vials were wrapped in aluminum foil and placed in a cabinet. After 24 hours, axillary buds and/or missing buds were examined for a red stain indicating activity. If no red stain was present, tillers were placed in a solution of 0.25% (w/v) solution of Evans Blue for 20 minutes. A deep blue stain indicated the axillary bud was dead. If buds were not stained with either red or blue, they were considered dormant (Busso et al. 1989; Hendrickson and Briske 1997).

After crowns were processed and activity evaluated, crown positions were further grouped into categories. These categories were used to further understand *B. inermis* responses to defoliation. For a list of categories and their descriptions and acronyms, see Table 1.

Data analysis:

The experiment was an RCBD that was blocked by site. Each treatment was replicated 10 times in each block each year. Since each tiller within a site had a treatment randomly assigned to it, each tiller was considered an experimental unit. Data were analyzed using the GLIMMIX Procedure in SAS® 9.4 (SAS/STAT 15.1, © 2016 by SAS Institute Inc., Cary, NC, USA,) with the fixed effects of year and treatment and their interactions. Because the response variables were primary count data, the model used a Poisson distribution to evaluate the error terms. In 2019 and 2020, but not in 2018, we also evaluated the mortality of the defoliated parent tillers marked during the treatment phase. To evaluate the mortality of the defoliated marked tillers in 2019 and 2020, the number of dead marked tillers was divided by the total marked tillers within each defoliation treatment in each block. Because there were two mutually exclusive outcomes (the tiller was either live or dead), data were modeled using a binomial distribution with the GLIMMIX Procedure. Least squares mean differences were determined using the LSMEANS option with the Tukey-Kramer adjustment at an $\alpha=0.10$ unless otherwise noted.

Most of the parent tillers (77%) did not have any dead buds so we did not further analyze the effect of treatment or year on dead buds. Although most parent tillers did not have dormant axillary buds, we combined both active and dormant axillary buds into a live bud category since both had the ability to produce new outgrowth. The number of live buds per tiller was assessed for overdispersion of the response, and two processes were determined to be in effect. For the live bud response, when the bud was not present, the only outcome possible was a zero, and when the bud was present, the response was a count process of total number of buds present. Thus, a zero-inflated model was determined as the most robust approach here. The live bud response was analyzed using the GENMOD Procedure (see SAS reference from above). The two parts of this zero-inflated model are a binary model, which is a logit distribution to model which of the two processes the zero outcome is associated with (i.e. the probability of having buds or not having buds) and a count model, in this case, a negative binomial model, to model the count process (which only exists in the presence of buds).

Results and Discussion:

B. inermis is one of the major invasive grasses on rangelands in the northern Great Plains and there are few available control options. We rejected our null hypothesis that defoliating *B. inermis* in the culm elongation stage would reduce the number of axillary buds and total outgrowth (tillers and rhizomes). We found when parent tillers were defoliated in the elongation stage, the number of tillers and rhizomes produced by the parent tiller was similar to the undefoliated controls. Instead, defoliating twice in the vegetative stage was the most promising strategy to reduce the amount of outgrowth produced per tiller. Defoliating *B. inermis* in the reproductive stage was the least effective treatment and resulted in the highest number of crown positions, total number of tillers and rhizomes produced over the growing season and number of tillers and rhizomes remaining active at harvest.

The growth stage when the parent tiller was defoliated affected the number of crown positions ($F_{4,\ 438}$ = 2.63; P=0.034); the number of tillers and rhizomes produced by a defoliated tiller ($F_{4,438}$ = 6.70; P=<0.0001) and the number of tillers and rhizomes still viable at the end of the growing season ($F_{4,\ 438}$=4.02; P=0.0033) (Table 2). Year impacted the number of tillers and rhizomes produced by a defoliated tiller ($F_{2,438}$=9.13; P=0.0001) and the number of rhizomes and tillers still viable at the end of the growing season ($F_{2,438}$=13.34; P=<0.0001) but not the total number of crown positions ($F_{2,438}$=1.39; P=0.2509). However, there was not a year by growth stage when defoliated for number of crown positions ($F_{8,\ 438}$=0.83; P=0.5793), the number of tillers and rhizomes produced by the defoliated tiller ($F_{8,438}$=0.96; P=0.4651) or the number of tillers and rhizomes still alive at the end of the growing season ($F_{8,438}$=0.68; P=0.7073).

In a greenhouse study, Bam et al. (2022) found that a single defoliation in the vegetative stage produced a minor but significant reduction in whole plant tiller and rhizome numbers in *B. inermis* and western wheatgrass. In our field study, a single defoliation of the parent tiller in the vegetative stage (V1) produced a slight and insignificant reduction in the total number of tillers and rhizomes produced by the parent tiller (TOTOUT) or those tillers and rhizomes that remained active in the fall (ACTOUT) (Table 2). However, defoliating the parent tiller twice in the vegetative stage (V2) did reduce the number of tillers and rhizomes produced (TOTOUT and ACTOUT) compared to undefoliated control (Table 2). *B. inermis* generally has two tiller cohorts during the growing season (Lamp 1952); although, Mitchell et al. (1998) reported three periods in eastern Nebraska. They also reported that depending on the year, *Bromis inermis* tiller populations could be primarily vegetative until mid- to late-May. The population structure of *B. inermis* early in the growing season may provide opportunities to apply the V2 treatment. The V2 treatment may mimic the more intensive defoliation by sheep, cattle or mowing that has been previously been reported to reduce tiller density in *B. inermis* (Brown et al. 2024).

The five-leaf stage (Willson and Stubbendieck 2000) has previously been suggested as the optimum time to apply management treatments such as burning or grazing. As Preister et al. (2019) noted, the five-leaf stage is well correlated with the early elongation stage in *B. inermis*. However, our results did not support using this stage as a management guide. We found that defoliating the parent tiller in the elongation stage did not reduce either the total number of tillers and rhizomes produced per tiller or the number remaining active in the fall, compared to the undefoliated controls (Table 2). Willson and Stubbendieck (2000) developed their recommendations from studies in areas with greater precipitation (eastern Nebraska and western Minnesota) which may explain the different responses in our two studies. They also highlighted the importance of species composition (i.e. having enough desired species) to achieving desired results from control measures.

As previously indicated, our field study found defoliating the tiller twice in the vegetative stage (V2) was the most promising defoliation treatment for limiting outgrowth on *B. inermis* tillers (Table 2) because, compared to the undefoliated controls, defoliation at this stage reduced the number of tillers and rhizomes produced over the entire season (TOTOUT) and the number of tillers and rhizomes remaining active into the fall (ACTOUT). In contrast, defoliating the parent tiller in the reproductive stage (R) resulted in an increase in the total number of crown position (TOT) compared to most other treatments except the V1 treatment and greater total outgrowth (TOTOUT) and greater active outgrowth (ACTOUT) than the V2 defoliation treatment. Paulsen and Smith (1969) also noted an increase in tillers per *B. inermis* stem grown *in vitro* after the plant initiated reproductive development but Biligetu and Coulman (2010) reported lower tiller densities when *B. inermis* was defoliated after elongation. However, they did not defoliate tillers at growth stages later than stem elongation. Tiller emergence in *B. inermis* tends to occur in the spring and after anthesis (Lamp 1952). While defoliation of the parent tillers in the reproductive (R) stage occurred before anthesis, it may have been close enough to anthesis not to affect outgrowth.

The increased outgrowth when the parent tillers were defoliated at the reproductive stage suggests that apical dominance may be regulating outgrowth in these grasses (Kebrom 2017). However, the mechanism regulating apical dominance are complex and recently research focus has switched from apical dominance being regulated by auxin to regulation by novel classes of plant hormones and sugars (Beveridge et al. 2023). Regardless of the mechanism, our data suggests that management strategies focusing on reducing the *B. inermis* outgrowth should avoid defoliating *B. inermis* in the reproductive stage.

When tillers were defoliated in the reproductive stage, they also had more rhizomes per tiller than if they were defoliated in the elongation stage (P=0.0011) or twice in the vegetative stage (P=0.0019) (Figure 3A). Defoliating parent tillers twice in the vegetative stage also resulted in fewer tillers per tiller than when parent tillers were defoliated in the elongation stage (P=0.0273, Figure 3A). Changes in the numbers of tillers and rhizomes can indicate a change in below-ground growth strategy from ‘phalanx’ strategy with tightly grouped tillers to a ’guerilla’ strategy with more widely spaced stems (Lovett-Doust 1981). The increase of rhizomes when *B. inermis* is defoliated during the reproductive stage may help it spread and invade new areas (Bam et al. 2024).

There were differences between years in the number of tillers and rhizomes produced by an individual tiller (Figure 3B) We found that the wettest year (2019) maximized the number of rhizomes but the driest year (2020) had the greatest number of tillers parent per tiller. There may be several factors leading to the increase of tillers versus rhizomes in 2020. The autumn of 2019, especially September, was very wet and the increased precipitation may have increased tiller emergence the following spring. Eastin et al. (1964) had reported that tillering in *B. inermis* ceased by the elongation phase and the increased soil moisture the previous autumn and into the spring may have benefited tillers more than rhizomes. New tillers that emerged in 2020 may have developed the previous autumn (Lamp 1952) but rhizomes are not initiated until plants are older such as the four-leaf stage (Otfnowski et al. 2006).

The distribution of precipitation may have also affected the number of tillers and rhizomes. While both 2018 and 2019 had similar amounts of annual precipitation (Figure 1) the distribution was different with 2018 having greater precipitation in June, July and August but 2019 had greater precipitation in May and September. In a greenhouse study, Bam et al.(2022) found that an intermediate watering regime (every 8 days) produced more rhizomes on *B. inermis* than an infrequent watering (every 16 days). Therefore, changes in precipitation frequency may have affected the numerical distribution of tillers and rhizomes.

Total and active outgrowth per tiller was also affected by year. Total and active outgrowth (Table 2) were both lower in 2018 than in 2019 and 2020. This was unexpected since 2020 had the lowest precipitation of any of the three sampling years (Figure 1). However, tying outgrowth to precipitation has been difficult in cool-season grasses. Mitchell et al. (1998) suggested that increased tiller numbers in intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey] was due to increased precipitation but changes in early season tiller numbers between years for *B. inermis* was driven by temperature. Hendrickson and Berdahl (2002) found tiller numbers were not impacted by water stress in intermediate wheatgrass or Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski]. For our study, precipitation during the previous year may have affected tiller outgrowth as has been reported for warm-season grasses (Hendrickson et al. 2000). Therefore, the lower outgrowth per tiller in 2018 may reflect lower precipitation in 2017 and the higher outgrowth in 2020 could be a result of increased precipitation in the fall of 2019 (Figure 1). In 2020, tillers more than rhizomes contributed to increased outgrowth (Figure 3B). Bam et al. (2022) found that outgrowth can be affected by decreased precipitation frequency. In their greenhouse study, *B. inermis* plants watered less frequently produced fewer total tillers and rhizomes than plants watered more frequency despite all treatments receiving the same amount of water.

One other possibility with the different moisture distributions between years, is a change in the age of the tiller population. For example, precipitation in the fall of 2019 followed by a dry summer in 2020 may have resulted in a majority of the tiller population for 2020 being initiated in the fall of 2019 (see (Lamp 1952). Whereas, in 2018, the dry 2017 followed by a more normal 2018 may have resulted in tillers being initiated in the spring of 2018. The tiller population in 2020 would have been ‘older’ with potentially more time to produce outgrowth than the ‘younger’ tiller population in 2018.

The number of tillers and rhizomes per tiller that remained alive at harvest (ACTOUT) can give insight into whether the population of *B. inermis* tillers is increasing or decreasing similar to a tiller replacement ratio (Briske and Hendrickson 1998; Olson and Richards 1988). There were differences in ACTOUT between defoliation treatments and between years (Table 2). ACTOUT responded similarly to parent tiller defoliation treatments as TOTOUT with the greatest number of tillers or rhizomes still alive at the end of the growing season occurring when *B. inermis* was defoliated in the reproductive stage (Table 2). The lowest value of ACTOUT was 1.1 when the tillers were defoliated twice in the vegetative stage (V2). A tiller replacement ratio > 1 suggests that a population is growing (Hendrickson et al. 2005). Because ACTOUT was > 1 for all treatments, this suggests that none of the defoliation treatments were sufficient to reduce the population of *B. inermis* (Table 2).

Our study focused on the outgrowth from a single tiller. However, under certain conditions, *B. inermis* may produce nearly half of new tillers from the nodes and tips of rhizomes (Bam et al. 2024). If the new tillers arising from rhizomes are not defoliated or defoliated at a different time than the original tiller, it could affect the affect the abundance of smooth bromegrass on a per unit area basis. The age of axillary buds can also affect outgrowth. Generally, younger buds located at the distal end of the crown are more likely to produce outgrowth (Hendrickson and Briske 1997). In our study, axillary buds located on rhizomes or new tillers may be younger and more likely to grow out than axillary buds on the original defoliated tiller. Since we focused on the outgrowth from a single tiller, we did not measure the impact of defoliation on these younger axillary buds.

The number of axillary buds or bud banks are an important mechanism regulating population dynamics in perennial grasses (Ott et al. 2019). A majority of our tillers did not have dormant axillary buds (66%) and we did not see clear trends in the impact of defoliation treatment or year. Therefore, we combined active buds and dormant buds into a category called live buds since both could potentially produce outgrowth. After combining the categories, approximately 13% of the parent tillers did not have any active or dormant buds. This was generally caused when a crown position did not form an axillary bud but other indicators (i.e., leaf attachment scars) suggesting the potential for forming an axillary bud (Hendrickson and Briske 1997). Overdispersion of the tillers without any active or dormant buds revealed that analysis of this data was most appropriate with a zero-inflated model. The probability that a parent tiller would not have any live buds was not affected by defoliation treatment ($P \leq 0.05$). However, the undefoliated controls (C) had fewer live buds than did any of the defoliation treatments (Figure 3).

Mullahey et al. (1991) found defoliation at different time periods reduced bud densities in two warm-season perennial grass species and Becker et al. (1997) found that defoliating two cool-season tussock grasses after the elongation stage reduced active axillary buds. However, an evaluation of clipping frequency on crested wheatgrass (*Agropyron cristatum* [(L.) Gaertn.] and hybrid bromegrass (*Bromus inermus* x *Bromus riparius*) reported that there were no differences in axillary bud number based on defoliation frequency (Fan et al. 2022). In a greenhouse study, a single defoliation of *B. inermis* increased axillary bud dormancy (Bam et al. 2022). While we did not see trends in dormant axillary buds, we found fewer active and dormant buds on our undefoliated controls compared to the defoliated tillers. Undefoliated tillers had fewer crown positions but more outgrowth resulting in fewer live axillary buds per tiller (Figure 4). Because axillary buds are the basis for future population growth (Murphy and Briske 1992; Ott et al. 2019), knowing there are fewer live axillary buds per tiller on the undefoliated tillers may provide opportunities for future burning or grazing scenarios. However, even the undefoliated control tillers had an average of 4.7 axillary buds per tiller (Figure 4) which still provides more than adequate meristematic potential for future growth.

In 2019 and 2020, we also evaluated the direct mortality of the parent tillers when defoliated at different growth stages. Defoliation at different growth stages did impact the survivorship of the parent tiller (P=0.0002). Individual tiller mortality ranged from 99 ± 2% for tillers defoliated in the R stage to 15±5% for the undefoliated tillers in the C treatment. Tillers defoliated in the R stage (99 ± 2%) and E stage (99 ± 4%) had similar mortalities but their mortalities were greater than for tillers defoliated in the V2 stage (75 ± 6%). Mortality for tillers defoliated in the R, E and V2 stages was also different than tillers defoliated in the V1 stage (22 ± 6%) and undefoliated tillers in the C treatment (15 ± 5 %).

Geater mortality for tillers defoliated at later growth stages is unsurprising considering that the shoot apex was elevated and removed with the defoliation. Removal of this apex causes the tiller to die (Nelson and Moore 2020) which explains the nearly 100% tiller mortality when defoliated in the elongation or reproductive stage. However, although parent tillers had greater mortality when defoliated at later growth stages, they also had greater outgrowth. Outgrowth that remained alive until the end of the growing season (ACTOUT) averaged 1.8 for parent tillers defoliated in the reproductive stage and 1.4 for tillers defoliated in the elongation stage (Table 2). This suggests that every tiller that died after being defoliated in the reproductive or elongation stage was not only replaced but had additional outgrowth (0.8 and 0.4 outgrowth per tiller for reproductive and elongation respectively) that remained alive until the end of the growing season.

The objective of this study was to determine how defoliating *B. inermis* at different growth stages

would affect outgrowth and number of axillary buds on the crown. We did focus on defoliating single tillers at each growth stage and assessed the outgrowth arising from those single tillers. This may have affected our results in that 1) *B. inermis* populations are made up of a wide variety of morphological growth stages (Mitchell et al. 1998); 2) many new tillers are initiated by rhizomes (Bam et al. 2024); and selective herbivory (Schmitz 2008) and other factors (Willson and Stubbendieck 2000).

Still this study provides valuable baseline information needed to further research into controlling *B. inermis*. In semi-arid regions, more emphasis should be focused on defoliating *B. inermis* in the vegetative stage rather than at later growth stages. Our data also suggest that because of *B. inermis*'s meristematic potential (i.e. outgrowth and axillary buds), its population can continue to increase even after defoliation treatment. This suggests that multiple defoliation events over several years may be necessary to reduce its abundance. Additional demographic research into the number and timing of defoliation could provide further insights into controlling *B. inermis*.

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Table 1. Category, description and acronym of different groupings of axillary buds and outgrowth used in analyses.

Category	Description	Acronym
Total Crown Positions	Live Tillers + Dead Tillers + Active Axillary Buds + Dormant Axillary Buds + Missing Axillary Buds + Rhizomes + Leaf Scars	TOT
Total Outgrowth	Live Tillers + Dead Tillers + Rhizomes	TOTOUT
Active Outgrowth	Live Tillers + Rhizomes	ACTOUT
Live Buds	Active Axillary Buds + Active Missing Axillary Buds + Dormant Axillary Buds	LIVE

Table 2. Mean number of positions for total crown positions (TOT), total number of crown positions with tillers or rhizomes (TOTOUT) and total number of crown positions with live tillers or rhizomes (ACTOUT) for different defoliation times and years. Defoliation treatments were 1) undefoliated or control tillers (C), 2) tillers defoliated in the elongation stage (E), 3) tillers defoliated in the reproductive stage (R), 4) tillers defoliated once in the vegetative stage (V1) and 5) tillers defoliated twice in the vegetative stage (V2). Standard errors of the means are in parentheses next to means. Means with different letters within a column indicate differences between treatment or years at P≤ 0.10.

Defoliation Treatment	TOT	TOTOUT	ACTOUT
C	6.1 (0.4) b	1.8 (0.1) ab	1.6 (0.1) a
E	6.5 (0.4) b	1.6 (0.1) bc	1.4 (0.1) ab
R	7.9 (0.4) a	2.2 (0.1) a	1.8 (0.1) a
V1	6.8 (0.4) ab	1.5 (0.1) bc	1.4 (0.1) ab
V2	6.1 (0.4) b	1.2 (0.1) c	1.1 (0.1) b
Year			
2018	6.3 (0.3) a	1.3 (0.1) b	1.0 (0.1) b
2019	6.6 (0.3) a	1.8 (0.1) a	1.6 (0.1) a
2020	7.1 (0.3) a	2.0 (0.1) a	1.8 (0.1) a

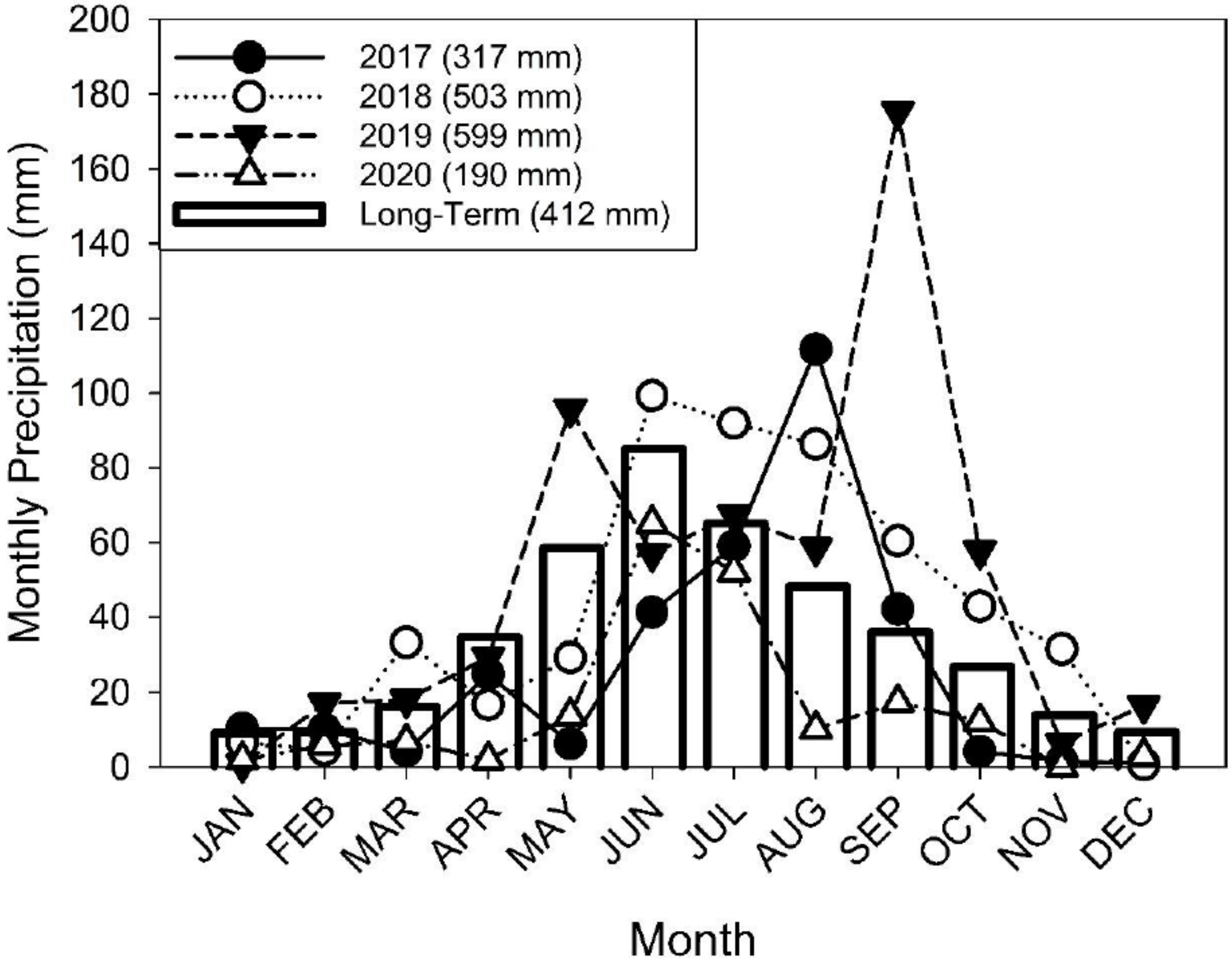

Figure 1. Average monthly precipitation at the Northern Great Plains Research Laboratory (NGPRL) for 2017-2020 and the long-term (1913-2020) average. Precipitation from April – October was gathered from an North Dakota Automated Weather Network station (NDAWN). Precipitation for the remaining months and the long-term average were taken from a US Weather Service station located 5 km north of the study site.

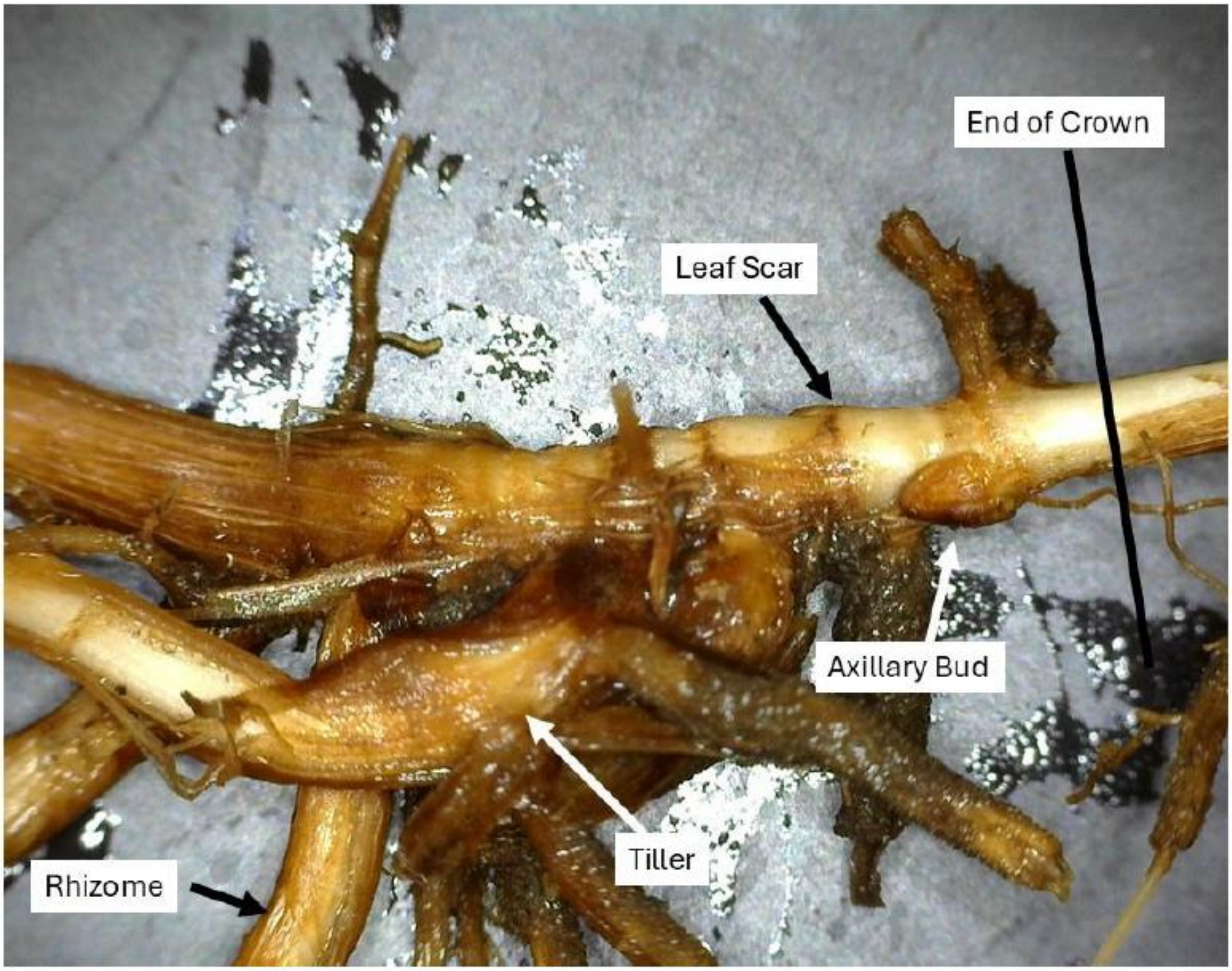

Figure 2. A crown base showing an axillary bud, leaf scar, tiller, rhizome and the point where the crown was separated from the rhizome (End of Crown).

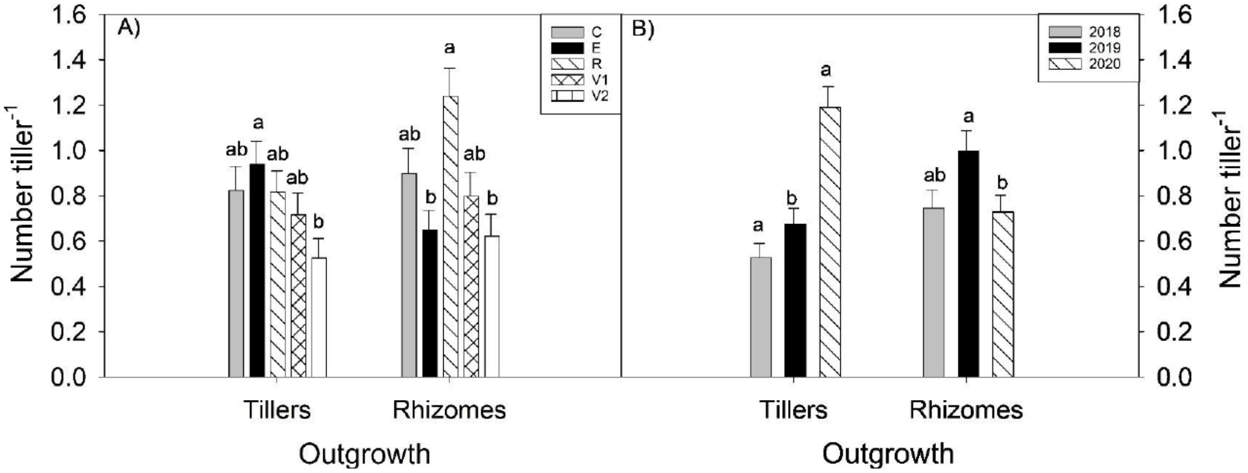

Figure 3. The number of tillers and rhizomes produced per tiller by A) defoliation treatment and B) by year. Defoliation treatments were 1) undefoliated controls (C), 2) defoliated in the elongation stage (E), 3) defoliated in the reproductive stage (R), 4) defoliated once in the vegetative stage (V1) and 5) defoliated twice in the vegetative stage (V2).

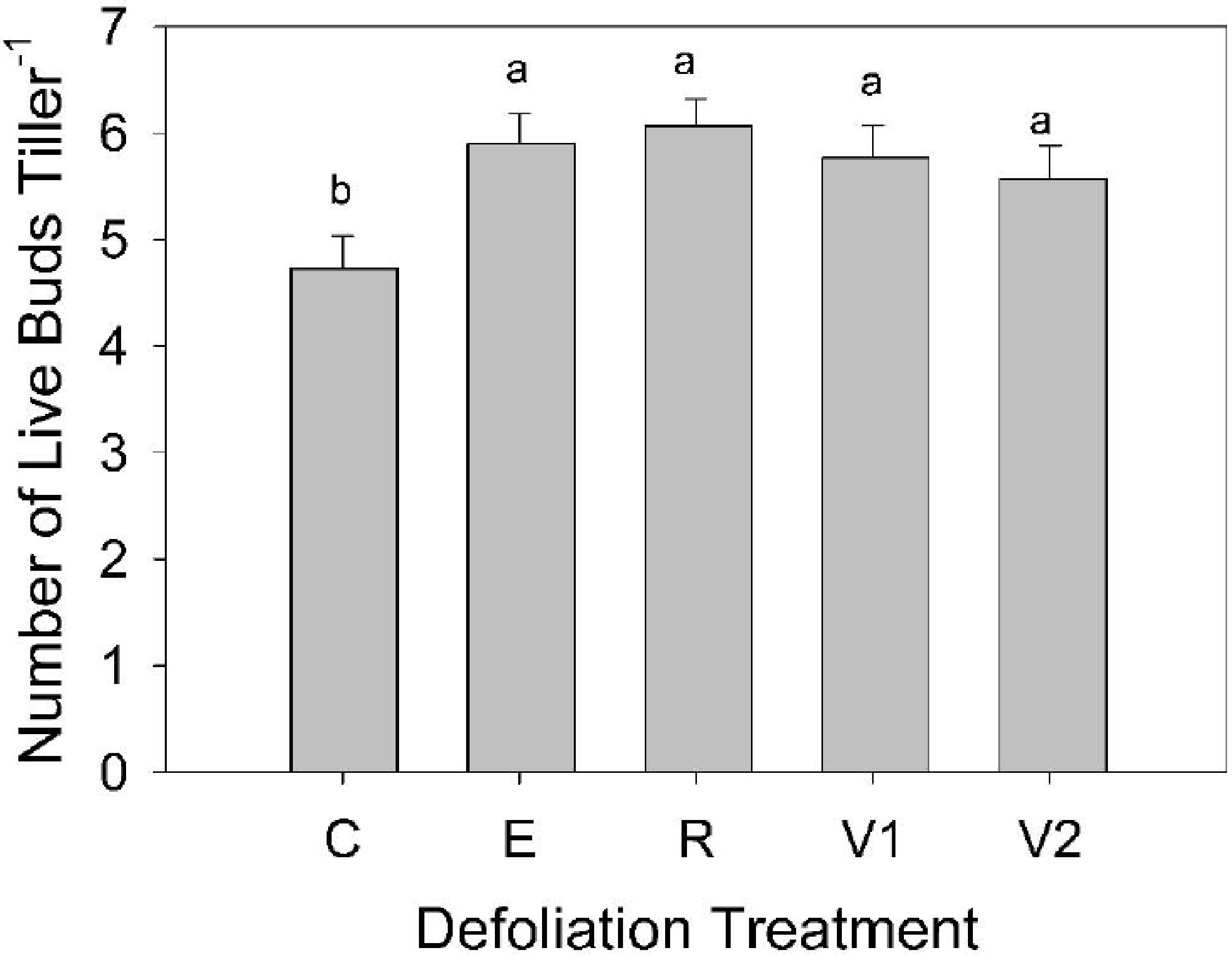

Figure 4 The number of live axillary buds (active + dormant) per tiller for each defoliation treatment. Defoliation treatments were 1) undefoliated controls (C), 2) defoliated in the elongation stage (E), 3) defoliated in the reproductive stage (R), 4) defoliated once in the vegetative stage (V1) and 5) defoliated twice in the vegetative stage (V2).