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Yellow foxtail (*Setaria pumila*) reduces establishment of alfalfa interseeded into corn

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Abstract

Interseeding alfalfa (*Medicago sativa* L.) into corn (*Zea mays* L.) is a novel approach that increases the production of high-quality forage and reduces the risk of nutrient and soil loss from cropland. Annual grass weeds like yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.] can reduce the success of alfalfa establishment and are difficult to manage in the interseeding system. This study evaluated ground cover, fall biomass, and fall plant density of interseeded alfalfa in response to varying populations of *S. pumila*. Our goal was to identify a threshold for initiating control of annual grasses to ensure good establishment of alfalfa in this intercropping system. Ground cover of interseeded alfalfa growing under corn declined as *S. pumila* density increased from 0 to 125 plants m^{-2} in July, August, and October with the sharpest decline in August (up to a 70% reduction in alfalfa cover). This reduction in ground cover was associated with a decline in postestablishment shoot and root mass and a reduction in alfalfa plant density from 246 to 146 plants m^{-2} in October. Results suggest that June *S. pumila* populations should be kept to less than 50 plants m^{-2} to obtain recommended fall alfalfa densities of 200 plants m^{-2} that are needed to maximize alfalfa yield the following year. This research provides crucial information to practitioners on when annual grass management is needed to ensure successful alfalfa establishment in this interseeded system.

Introduction

Milk production by dairy cows is highly dependent on diets formulated with high-quality forages. Historically, corn (*Zea mays* L.) silage and alfalfa (*Medicago sativa* L.) have served as the primary sources of forage for dairy cows in the U.S. Midwest (Gillespie 2023; Kellogg et al. 2001). In Wisconsin, corn silage and alfalfa were the top two forage crops harvested in 2023, occupying 25% of the state's cropland (1.6 and 1.3 million ha, respectively) (USDA-NASS 2024). Fields are often rotated between alfalfa and corn to provide multiple production benefits such as increased forage yield of both crops, reduced pest populations, improved soil health and nutrient retention, and reduced reliance on nitrogen fertilizers (Huggins et al. 2001; Kanwar et al. 2005; Olmstead and Brummer 2008; Russelle 2014; Sanford et al. 2021). Inclusion of alfalfa in corn silage–based diets also benefits cattle health and productivity (Brito and Broderick 2006; Lopes et al. 2015).

Despite these benefits, land devoted to alfalfa production has decreased within the United States. Nationally, the area of alfalfa harvested (hay and haylage) has decreased by 32% over the past 15 yr, whereas harvested corn silage increased by 15% (USDA-NASS 2024). Reductions in alfalfa harvested in Wisconsin were even greater than the national average, declining by 44% between 2009 and 2023 (USDA-NASS 2024). Alfalfa plantings have typically been replaced with less diverse production systems based on annual row crops such as corn silage, corn grown for grain, or soybean [*Glycine max* (L.) Merr.] (Blum 2020; Zulauf 2018). Increasing the area where alfalfa is grown would improve the sustainability of crop and dairy production, which is aligned with national and Wisconsin priorities (Innovation Center of U.S. Dairy 2023; Wisconsin DATCP and UW System 2019).

Establishment of alfalfa by interseeding into corn silage is a novel cropping system that could be used to increase alfalfa usage on farms while providing multiple environmental benefits. In this system, alfalfa is established under a corn silage companion crop that is capable of producing up to 4.4-fold more high-quality forage than conventionally spring-seeded alfalfa. During its establishment, interseeded alfalfa serves as a cover crop before and after corn silage harvest and then is harvested in subsequent years, producing forage of comparable yield as alfalfa established by conventional methods (Grabber 2016; Grabber et al. 2021b, 2024). As a result, the interseeding system bypasses the low-yielding establishment year typical of conventionally spring-seeded alfalfa. In addition to improved yield, this interseeding approach has been shown to improve the profitability of alfalfa–corn silage rotations (Berti et al. 2021; Osterholz et al. 2020). Environmental benefits from this approach include reduced soil erosion, nutrient runoff (Osterholz et al. 2019), and nitrate leaching (Osterholz et al. 2021b) compared with corn silage monocultures. Because of these benefits, there are multiple efforts underway aiming to increase adoption of this interseeding system in the U.S. Midwest.

Although this intercropping system provides economic and environmental benefits, stands of interseeded alfalfa can fail due to excessive competition from the corn silage companion crop and from defoliation and necrosis caused by disease and insect pests. These issues can be managed by planting corn silage at moderate populations, by early seeding of well-adapted alfalfa varieties, and by application of prohexadione-calcium growth retardant and foliar fungicide and insecticide (Grabber et al. 2021b, 2023; Osterholz et al. 2018). Observations across multiple studies by our group, however, suggest high populations of annual grasses such as yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.] might also contribute to poor establishment of interseeded alfalfa. Poor alfalfa establishment due to competition with high populations of annual grasses such as *S. pumila* has been reported as a concern by others (Norris and Ayres 1991). Maintaining control of weeds during establishment is important, as Chu et al. (2022) found a weed-free period of 394 growing degree days (GDD) was needed to maximize alfalfa establishment and productivity. While herbicides (e.g., acetochlor) have been identified to control early-emerging weeds, including annual grasses, and resulted in the successful establishment of alfalfa (Osterholz et al. 2021a), they are applied near planting and are not effective in controlling annual grasses that emerge later in the season. Roundup Ready® (RR) corn and alfalfa hybrids used in conjunction with glyphosate are an effective tool to provide annual grass control in the alfalfa–corn interseeding system when applied postemergence. However, concern exists surrounding reliance on the repeated use of a single active ingredient, as it increases the risk of selecting for herbicide-resistant populations. Additionally, many of the alfalfa cultivars that are best adapted to the interseeding system are conventional, for which postemergence glyphosate is not an option (Grabber et al. 2021a).

Weed densities are frequently used as decision support tools for weed management (Larson et al. 2016). While the effects of annual grasses on the establishment of interseeded alfalfa is poorly understood, its impact is likely density dependent, and identifying its threshold is crucial to inform management decisions. In this study, the effect of annual grass weed density on the establishment of alfalfa interseeded into corn silage was evaluated during 2023 at two locations in southern Wisconsin. *Setaria pumila* was selected as the model annual grass, as this late-emerging species is abundant in agronomic fields in Wisconsin (Fickett et al. 2013a, 2013b). Specifically, the impact *S. pumila* density in June had on summer ground cover, fall plant density, and fall biomass of interseeded alfalfa during the establishment year was evaluated.

Materials and Methods

Site Description

Field experiments were implemented in southern Wisconsin in 2023 at the Lancaster Agricultural Research Station (LARS; 42.82°N, 90.78°W) and the U.S. Department of Agriculture Dairy Forage Research Center at Prairie du Sac (PDS; 43.35°N, 89.76°W). In the 2 yr before experiment initiation, sites were planted with corn (LARS) or soybean followed by corn (PDS). Weed community composition included giant foxtail [*Setaria faberi* Herrm.], common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters (*Chenopodium album* L.), dandelion (*Taraxacum officinale* F.H. Wigg.), and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] at LARS. At the PDS site, *C. album* and redroot pigweed (*Amaranthus retroflexus* L.) were the only common species.

Soil types were Fayette (fine-silty, mixed, superactive, mesic Typic Hapludalfs) at LARS and Richwood (fine-silty, mixed, superactive, mesic Typic Argiudolls) at PDS. Before experiment establishment, soil samples were collected for characterization. Soil at LARS had 6.6 pH, 2.1% organic matter, 25 mg P kg $^{-1}$, 106 mg K kg^{-1}, and 0.99 g cm^{-3} soil bulk density. Soil at PDS had 6.4 pH, 2.8% organic matter, 33 mg P kg^{-1}, 173 mg K kg^{-1}, and 0.94 g cm^{-3} soil bulk density. Total rainfall and average temperatures for 2023 were obtained from nearby weather stations at Lancaster and Sauk City. GDD were calculated summing the difference between average daily temperature and alfalfa's base temperature (5 C; Sharratt et al. 1989), starting on the first day after planting. Values were averaged across both locations, as data were pooled for analysis.

Experiment Establishment and Management

Field trials in both locations were planted without tillage (no-till). Plots received glyphosate at 1 kg ae ha^{-1} (Roundup PowerMax®, 540 g ai L^{-1}, Bayer (Monheim am Rhein, Germany)) in the last week of April before planting to control any weeds that had emerged. Based on soil analysis results, fertilizer was broadcast preplant at rates of 202 kg N, 52 kg P, and 344 kg K ha^{-1} on April 27 at LAR and on April 29 at PDS. Corn (ARL:P0529Q; Pioneer Seed Company (Johnston, IA); LARS:BO4RR11Q-N804Q; Brevant (Indianapolis, IN)) was planted on May 4 at LARS and May 9 at PDS. Alfalfa (431RRLH; Farm Science Company) was interseeded in four rows between each corn row the following day (May 5 at LARS and May 10 at PDS) as described by Grabber et al. (2021b). The seed rate of corn and alfalfa was 74,100 seeds ha^{-1} and 18 kg PLS ha^{-1}, respectively, with a row spacing of 0.76 m for corn and 0.15 m for alfalfa.

Treatments and Experimental Design

Initially, target treatments were seven *S. pumila* density ranges (0, 5 to 15, 30 to 50, 70 to 90, 91 to 100, 101 to 120, and >120 plants m^{-2}) established in 3 m by 7.6 m plots arranged in a randomized complete block design with four replicates. To achieve these treatments, *S. pumila* seeds (75% germination rate) were broadcast on May 5 at LARS and on May 10 at PDS immediately before alfalfa planting. Weed control in weed-free treatments was achieved by applying glyphosate at 1 kg ae ha^{-1} (Roundup PowerMax®, 540 g ai L^{-1}, Bayer) when alfalfa had four trifoliate leaves. At 25 d after planting alfalfa (DAPa), three 0.76 m^2 sampling areas were marked within the center interrow of each plot for subsequent measures. In each sampling area, *S. pumila* plants were counted and thinned by hand weeding to achieve target densities. This procedure started at 30 DAPa and was repeated biweekly thereafter until the end of June. Annual grasses that were not *S. pumila* and broadleaf weeds were also removed during this time frame. Two irrigation events (64 and 38 mm) were applied at 31 DAPa (June 11) and 77 DAPa (July 27) at PDS due to the unusually dry spring, but no irrigation was applied in LARS due to infrastructure constraints.

Table 1. Monthly 2023 weather and 30-yr historical averages at Lancaster (LARS) and Prairie du Sac (PDS) Wisconsin during the growing season.[a]

	LARS				PDS					
	Temperature		Precipitation		Temperature		Precipitation and irrigation[b]			
Month	Historical average	2023	Historical average	2023	Historical average	2023	Historical average	2023	Irrigation	Total
	C		mm		C		mm			
April	8.0	8.9	90	79	7.2	7.7	100	111		111
May	14.4	15.7	114	45	14.1	15.1	108	26		26
June	19.8	20.8	142	61	19.7	20.2	149	19	64	83
July	21.7	21.7	133	113	21.7	21.5	113	101	38	139
August	20.7	21.7	102	35	20.5	21.1	113	45		45
September	16.6	18.3	108	57	16.4	18.1	93	65		65
October	9.6	11.1	79	86	9.6	10.2	75	79		79
November	2.1	2.7	52	4	2.3	2.4	53	12		12
Total	—	—	820	480	—	—	804	457	102	559

[a]Sources: National Weather Service, https://www.weather.gov/wrh/Climate?wfo=arx (accessed: March 5, 2024); Wisconet, Wisconsin's Environmental Mesonet, https://wisconet.wisc.edu (accessed: July 21, 2024).
[b]Irrigation was available at PDS only; total volume of water, consisting of precipitation plus irrigation, is presented for that location.

To control alfalfa foliar diseases, plots were treated with fluxapyroxad at 48.6 g ai ha^{-1} and pyraclostrobin, and 97.4g ai ha^{-1} (Priaxor®, BASF (Research Triangle Park, NC)) at 75 DAPa in both locations when the corn canopy was beginning to close (V10-V12 growth stages). Lambda-cyhalothrin at 18.2 g ai ha^{-1} (Warrior II®, Syngenta (Greensboro, NC)) was mixed with fungicide and applied at PDS to control potato leaf hopper (*Empoasca fabae*), but no insecticides were needed at LARS, as potato leaf hopper was not present.

Measurements

Corn Silage

Silage biomass was harvested on September 12 and 5 at LARS and PDS, respectively, and yields were estimated at the field level at each location. Four representative areas (23 m^2) were harvested within each location to determine silage dry matter (DM) yield. This was done by measuring fresh weights, oven-drying samples until constant weight at 60 C, and reweighing to calculate percentage moisture. Moisture was averaged over the four samples within each location (64% and 63% in LARS and PDS, respectively) and used to correct field-level silage yield to a DM basis.

Alfalfa and Setaria pumila

All measurements for alfalfa and *S. pumila* were conducted within the 0.76×0.76 m^2 sampling area determined at 25 DAPa. At 35 DAPa, *S. pumila* density (plants m^{-2}) was estimated. At 40, 70, 105, and 160 DAPa, percentage alfalfa and *S. pumila* cover were visually estimated. To document initial alfalfa establishment, alfalfa plants (plants m^{-2}) were counted in early summer (40 DAPa) within one sampling area within each plot at each site ($n = 28$). To ensure individual plants were identified, all alfalfa plants within sampling areas were destructively harvested by uprooting plants to a depth of 20 cm. Alfalfa plant density was counted in all remaining sampling areas within each plot at each site after corn silage harvest (160 DAPa; $n = 56$). At this time, alfalfa crowns were collected and then separated into shoot and root biomass. These samples were then dried at 105 C until constant weight and then weighed to determine alfalfa shoot and root biomass accumulation at the end of the growing season.

Modeling and Statistical Analysis

Setaria pumila populations varied substantially within plots and did not conform to target treatment densities. Therefore, *S. pumila* populations were considered continuous rather than categorical treatments (Kohmann et al. 2018). Regression analysis was used with the *nls()* function to determine the relationships between alfalfa response variables and *S. pumila* density using the R Studio platform (R Development Core Team 2021). Data across sites were visually inspected and considered similar in their responses among sites, and therefore were pooled for analysis. *Setaria pumila* density was chosen as the determinant variable, as this is a common metric used to assess weed competition and results are easily adopted by stakeholders. For all responses, three models were compared: linear, two-parameter concave, and linear plateau, all commonly used with establishment and yield experiments (Larson et al. 2016; McCartor and Rouquette 1977; Ratkowsky 1990). The best model was selected based on a combination of visual assessment of residuals, normality, and root-mean-square error (RMSE).

Percentage alfalfa cover at 40, 70, 105, and 160 DAPa was best described by a two-parameter concave model shown in Equation 1 (Ratkowsky 1990):

$$f(x) = 1/(a + bx) \quad [1]$$

where $1/a$ is the percent alfalfa cover when *S. pumila* population is zero, b is the rate of decline of alfalfa cover with increasing *S. pumila* density, and x is *S. pumila* density in June. Fall alfalfa plant density and shoot and root biomass at 160 DAPa were fit to a linear plateau model (McCartor and Rouquette 1977) described in Equation 2:

$$f(x) = a - b(x - c) \text{if } x \leq c; \text{otherwise}, f(x) = c \quad [2]$$

where a is the point where alfalfa plant density, shoot, or root biomass reached a plateau, b is the rate of change in those responses as *S. pumila* density increased (before reaching the plateau), c is the *S. pumila* density at the join point of the linear and plateau response of alfalfa, and x is *S. pumila* density in June.

Results and Discussion

Temperature and precipitation during the growing season (April to November) were atypical at LARS and PDS (Table 1). Average monthly temperature was 5% to 16% and 3% to 10% greater than the 30-yr average at LARS and PDS, respectively, except July, which had temperatures similar to the 30-yr average. Monthly precipitation was below average at both locations, and the total for the growing season amounted to only about 57% of the normal expected at LARS and PDS. With irrigation applied at PDS in June and July, the precipitation deficit was partially offset, and the total for the growing season reached 70% of the expected normal. Corn silage yields at LARS and PDS were 7.5 and 16.7 Mg ha^{-1}, respectively. In prior studies, yields of corn silage grown with interseeded alfalfa typically approached or exceeded 20 Mg ha^{-1} at these sites under near-normal precipitation (Grabber, 2016, 2021b, 2023, 2024; Osterholz et al. 2018).

Relationship between June *Setaria pumila* Density and Alfalfa Cover

Setaria pumila density in June ranged from 0 to 460 plants m^{-2}. This resulted in different amounts of *S. pumila* cover, which ranged from 0% to 95% throughout the growing season. A concave function best described the relationship between *S. pumila* density and alfalfa cover in July, August, and October (Figure 1), for which all parameters were significant ($P < 0.0001$; RMSE = 12, 17, and 13, respectively). However, the parameter that describes change in alfalfa cover relative to *S. pumila* density *b* in June was not significant ($P = 0.109$) and approached zero, suggesting *S. pumila* competition was not limiting alfalfa growth at that time. Chu et al. (2022) reported similar results, as the critical period for weed control for alfalfa did not begin until late June in a weed competition study using Japanese millet [*Echinochloa esculenta* (A. Braun) H. Scholz] as a surrogate for annual weeds in alfalfa interseeded systems in Michigan.

Competition between *S. pumila* and alfalfa was evident between July and October, as the *b* parameter that described change in alfalfa cover relative to *S. pumila* density was significant ($P < 0.0001$; Figure 1). The effect of June *S. pumila* density on alfalfa cover was greatest in August, when the *b* parameter was nearly 3-fold greater than in July. The pronounced increase in the *b* parameter during August likely occurred because the adverse effects of abiotic and biotic stress on stand loss and defoliation of interseeded alfalfa are most pronounced in late July and August when plants are subjected maximal shading from corn and defoliated from foliar disease and insects (Grabber et al. 2021a, 2021b, 2023). These effects declined by October, because corn silage harvest in early September alleviated competition and *S. pumila* did not resprout after harvest. This allowed surviving alfalfa plants, especially those subjected to additional stress from moderate to high populations of *S. pumila*, to regrow and increase ground cover. While the relationship between *S. pumila* density and alfalfa cover was significant, alfalfa plant density is recommended for evaluating successful alfalfa establishment, as the degree of stand loss and defoliation of interseeded alfalfa during late July and August and regrowth following silage harvest are highly dependent on other factors that cannot always be controlled (Grabber et al. 2021b, 2023; Osterholz et al. 2021b). In particular, alfalfa leaf defoliation (often caused by potato leaf hopper and alfalfa foliar diseases) and fall precipitation have been identified as important factors for alfalfa plant survival during this time frame (Grabber et al. 2021b). These factors make it difficult to rely solely on cover as an assessment of establishment success.

Relationship between June *Setaria pumila* Density and Fall Alfalfa Density

June *S. pumila* density impacted alfalfa density in October. A linear plateau function best described the relationship of all three

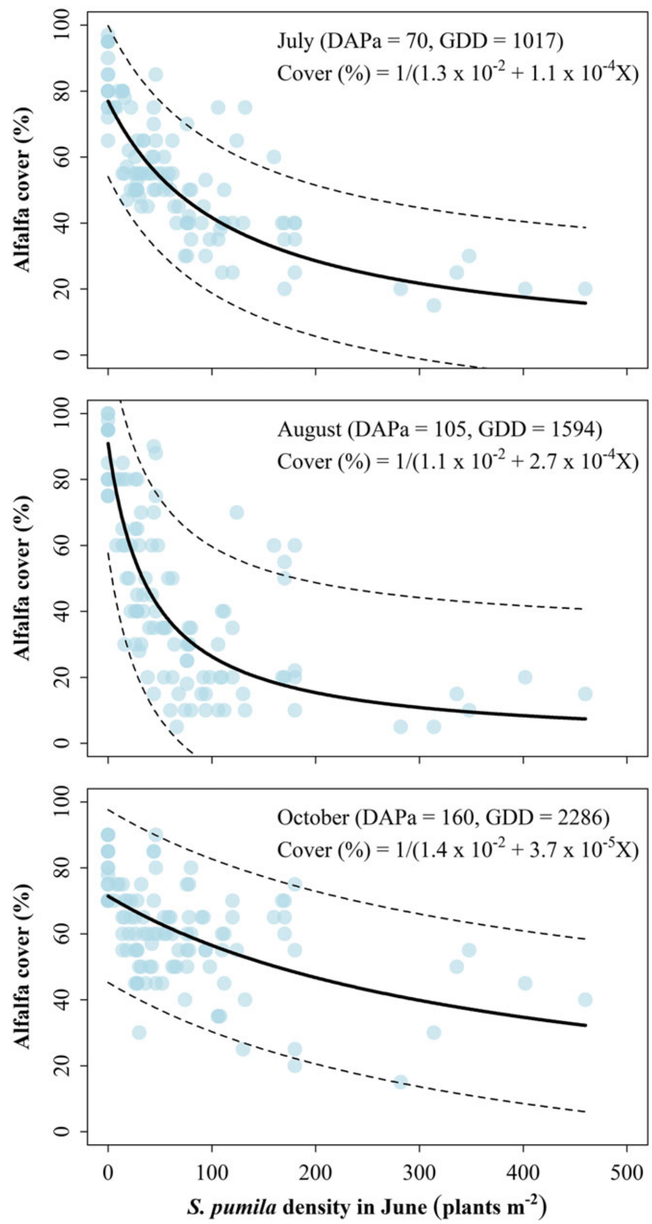

Figure 1. Effect of *Setaria pumila* density in June (*x*) on alfalfa cover at 70, 105, and 160 d after planting alfalfa (DAPa) (July, August, and October, respectively). Points represent measured responses; the continuous line represents the response estimated by the regression model; and the dashed lines represent the 95% confidence interval. The relationship between alfalfa ground cover and June *S. pumila* density was established using data pooled across two locations ($n = 112$). GDD, growing degree days.

models evaluated (RMSE = 49), with all parameters significant ($P < 0.0001$; Figure 2). Alfalfa density when no weeds were present was 242 plants m^{-2} and decreased linearly with increasing June *S. pumila* density by 0.8 alfalfa plants m^{-2} for every 1 *S. pumila* plant m^{-2}. However, when June *S. pumila* density was ≥125 plants m^{-2}, the alfalfa population remained constant (145 plants m^{-2}) under the relatively dry growing conditions of this experiment.

Adequate alfalfa plant density is critical to maximize productivity in alfalfa interseeded systems. In solo-seeded alfalfa fields, densities >107 plants m^{-2} are required to maximize forage harvested (Sheaffer et al. 2023). Several studies suggest that alfalfa plant densities >200 plants m^{-2} are required by the fall of the

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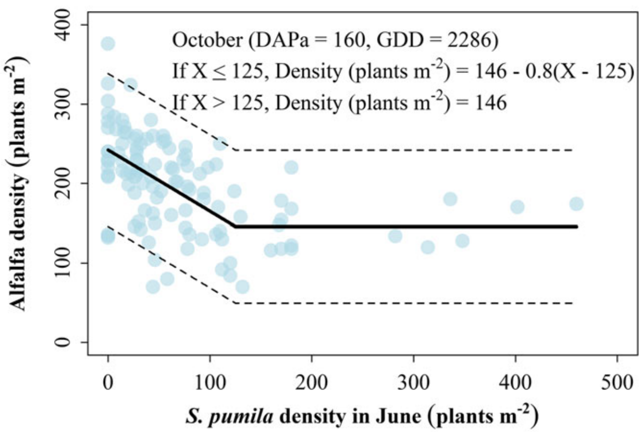

Figure 2. Relationship between *Setaria pumila* plant density in June (*x*) and alfalfa plant density in October (160 d after planting alfalfa [DAPa]). Points represent measured responses; the continuous line represents the response estimated by the regression model; and the dashed lines represent the 95% confidence interval. Data were pooled across two locations ($n = 112$). GDD, growing degree days.

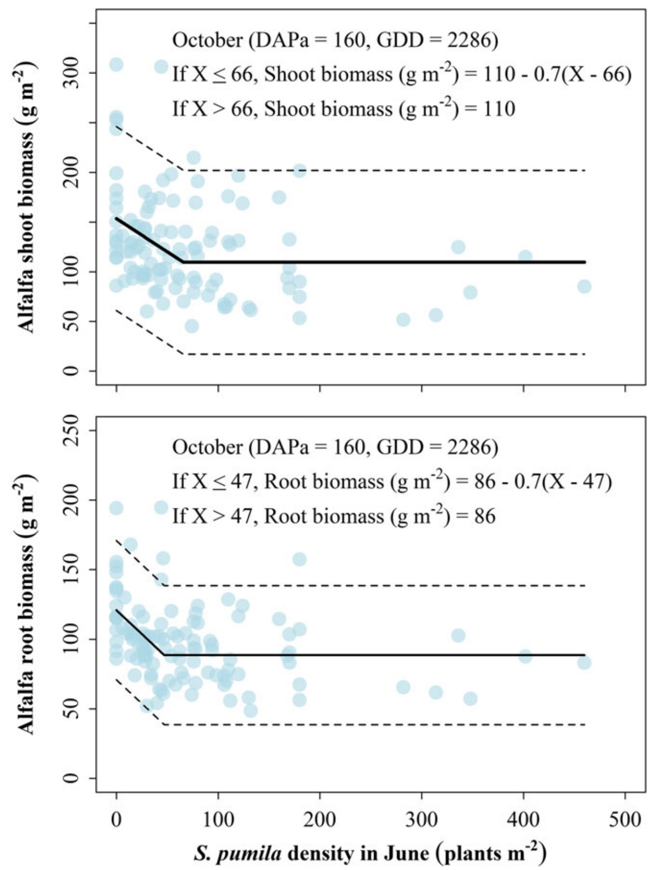

Figure 3. The relationship between *Setaria pumila* plant density in June (*x*) and alfalfa shoot and root biomass in October (160 d after planting alfalfa [DAPa]). Points represent measured responses; the continuous line represents the response estimated by the regression model; and the dashed lines represent the 95% confidence interval. Data were pooled across two locations ($n = 112$). GDD, growing degree days.

establishment year to maximize first-cut yield the following year (Grabber et al. 2021b, 2024; Osterholz et al. 2021a). In our study, fall plant density of alfalfa exceeded 200 plants m^{-2} at *S. pumila* densities <50 plants m^{-2}. This threshold is similar to Zhou et al.'s (1992) finding that *S. pumila* densities needed to be <32 plants m^{-2} to ensure successful establishment of solo-seeded alfalfa. As noted earlier, this study was conducted under relatively dry growing conditions; thus, *S. pumila* effects on alfalfa establishment may differ if intercropping is carried out under normal to wet growing conditions. Weed densities are frequently used as decision support tools for weed management (Larson et al. 2016) due to their easy adoption by crop consultants and farmers. We recommend assessing *S. pumila* density at 35 to 45 DAPa (June in Wisconsin) to determine whether additional weed management activities are needed to ensure successful establishment of alfalfa in this interseeded system.

Relationship between Setaria pumila Density and Fall Alfalfa Shoot and Root Biomass

Similar to alfalfa plant density at October, the relationship between June *S. pumila* density and alfalfa shoot and root biomass were best described by a linear plateau function (RMSE = 47 and 26, respectively), in which all parameters were significant ($P < 0.0001$; Figure 3). Shoot and root biomass both decreased by 0.7 g m^{-2} for each *S. pumila* plant m^{-2}. Shoot biomass decreased until *S. pumila* density reached 66 plants m^{-2}, while root biomass declined and plateaued at *S. pumila* densities of 47 plants m^{-2}. While removal of alfalfa shoots during corn silage harvest and its regrowth likely impacted these observations, these results suggest shoot biomass is more sensitive than root biomass at higher *S. pumila* densities in this system. The impact from *S. pumila* was on alfalfa plant survival, because surviving plants had similar root or shoot biomass per plant regardless of the *S. pumila* density (data not shown).

Results provide strong evidence that annual grasses, estimated using *S. pumila*, can reduce establishment of alfalfa interseeded into corn silage. As *S. pumila* populations increased in June, alfalfa responded by sharply reducing ground cover during July and August when competition from corn and foliar damage from disease and insects are also typically most pronounced in this intercropping system. *Setaria pumila* densities above 50 plants m^{-2} in June reduced postestablishment stand density of alfalfa in October to less than 200 plants m^{-2}, a level previously established as a benchmark for maximizing first-cut yield of alfalfa the following year. This suggests weed control efforts should be initiated if annual grass populations exceed 50 plants m^{-2} in June. Several options exist for annual grass weed management in the interseeded system, including preemergence applications of residual herbicides (alachlor, pendimethalin) or postemergence applications of glyphosate (in RR systems only) (Osterholz et al. 2021a). Additional herbicide options need to be explored for controlling annual grasses that emerge after alfalfa emergence as no options are currently registered for use for late-season annual grass applications in this system if conventional alfalfa varieties are used. *Setaria pumila* populations need to be below the 50 plants m^{-2} threshold before July, as reductions to alfalfa cover were observed in this time frame (Figure 1). Because environmental conditions can affect alfalfa establishment in the interseeded system, additional research is needed to confirm the validity of this threshold. Despite this limitation, our results provide information to crop consultants and farmers as to the level and timing of management of a difficult to control and impactful weed species in this interseeded system.

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