

## ORIGINAL ARTICLE

## Forage &amp; Grazinglands

# Intercropping in maize silage versus solo-seeding for alfalfa establishment in Wisconsin and Idaho

John H. Grabber<sup>1</sup>  | David L. Bjorneberg<sup>2</sup> | Christopher W. Rogers<sup>2</sup> 

<sup>1</sup>USDA-ARS, U.S. Dairy Forage Research Center, Madison, Wisconsin, USA

<sup>2</sup>USDA-ARS, Northwest Irrigation and Soils Research, Kimberly, Idaho, USA

### Correspondence

John H. Grabber, USDA-ARS, U.S. Dairy Forage Research Center, 1925 Linden Drive, Madison, WI 53706, USA.

Email: [John.Grabber@usda.gov](mailto:John.Grabber@usda.gov).

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### Abstract

Alfalfa (*Medicago sativa* L.) intercropping with maize (*Zea mays* L.) silage is being developed in the northern United States to improve the profitability and environmental sustainability of forage production. This study, conducted under rainfed conditions in Wisconsin and semiarid irrigated conditions in Idaho, compared the establishment of alfalfa and dry matter yield of four intercropping systems to three conventional systems. The former systems included alfalfa interseeded at planting or the vegetative emergence (VE) stage of maize and grown with or without prohexadione growth retardant. The latter systems included alfalfa seeded in spring, summer-seeded after barley (*Hordeum vulgare* L.), or late summer-seeded after maize silage. Spring seeded and interseeded alfalfa in Wisconsin also received foliar fungicide and insecticide during establishment. During alfalfa establishment, yield of intercropped maize silage was 1.8- to 4.4-fold greater than spring-seeded alfalfa. Compared to spring-seeded alfalfa, interseeded alfalfa had similar or somewhat lower stand density but similar first cut yield the following year, provided that intercropped maize was harvested near September 1 to allow ample alfalfa fall regrowth. Shifting interseeding from maize planting to the VE stage decreased early-season alfalfa growth, but improved maize silage yield, with minor effects on alfalfa fall growth, stand density, and first cut yield. Prohexadione application had little impact on establishment or yield of interseeded alfalfa. While having high plant density, alfalfa seeded after barley or especially maize had less fall growth and low first cut yield. Overall, alfalfa establishment and yield of intercropping systems compared favorably with conventional systems.

## 1 | INTRODUCTION

Cropland devoted to alfalfa (*Medicago sativa* L.) production in the United States has declined over the last 40 years to approximately 7 million ha (USDA National Agricultural

Statistics Service, 2023). Among other factors, this decline occurred because maize (*Zea mays* L.) silage has become the primary source of forage for dairy cattle and other ruminant livestock. One reason for this shift is the relatively low yield of alfalfa compared to maize silage (Marten et al., 2017). In cold temperate regions of the United States, alfalfa is typically solo-seeded in spring and dry matter yield during the

**Abbreviation:** PHD, prohexadione; VE, vegetative emergence.

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establishment year is especially low, often being one-half that of subsequent full-production years. Spring seeding small grain, grass, or legume forages with alfalfa modestly improves total yield, but this depresses alfalfa production and overall forage quality during the establishment year (Wiersma et al., 1999). Alternatively, alfalfa can be sown during the summer in the northern United States after small grain harvest and during late summer or early fall in the southern United States after harvest of longer season crops such as maize silage (Undersander et al., 2015). The success of seedings made late during the growing season is, however, highly dependent on favorable growing conditions. Specifically, adequate soil moisture and heat units are needed in the late summer and fall to ensure rapid germination and adequate seedling growth of alfalfa prior to winter (Hall, 1995).

One way to bypass the low yielding establishment year of alfalfa and to increase farm profitability would be to interseed alfalfa into maize silage (Berti, Lukaszewsky et al., 2021; Osterholz, Renz et al., 2020). In this system, maize silage serves as a high-quality and high yielding forage companion crop for alfalfa. During establishment, interseeded alfalfa initially serves as a highly effective cover crop to reduce soil and nutrient loss during and after maize production and then it is brought into full forage production in subsequent years (Grabber, Smith et al., 2021; Osterholz et al., 2019; Osterholz et al., 2021a).

While many management aspects of this alfalfa-maize silage intercropping system have been investigated in recent years (e.g., Grabber, Osterholz, et al., 2021; Grabber, Smith et al., 2021; Osterholz, Dias et al., 2020; Osterholz et al., 2021b), alfalfa establishment by interseeding into maize silage has not been compared to conventional spring and summer seeding systems. An experiment designed to compare these systems was carried out in Wisconsin and Idaho, two major northern latitude alfalfa producing states that represent the rainfed Midwestern region and the irrigated Western Intermountain region of the United States. In this paper, we compared stand characteristics and initial dry matter yield of alfalfa during its establishment in four intercropping systems in comparison to three solo-seeding systems for alfalfa.

## 2 | MATERIALS AND METHODS

### 2.1 | Sites, soil characteristics, fertilizer applications, and irrigation

Independent experiments were initiated in 2020 and in 2021 on a Richwood silt-loam soil (fine-silty, mixed, superactive, mesic Typic Argiudolls) near Prairie du Sac, WI (43° 20' N, 89° 43' W) and a Portneuf silt-loam soil (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids) near Kimberly, ID (42° 32' N, 114° 20' W). The crop preceding the

### Core Ideas

- Delaying alfalfa interseeding until the vegetative emergence stage improved maize silage companion crop yield.
- During alfalfa establishment, intercropped maize silage had up to 4.4-fold greater yield than solo-seeded alfalfa.
- Early harvest of maize silage improved fall growth and first cut yield of interseeded alfalfa after establishment.
- Interseeded and spring solo-seeded alfalfa had comparable stand density and first cut yield after establishment.
- Summer solo-seeded alfalfa had relatively poor fall growth and first cut yield after establishment.

study was maize silage for both Wisconsin and Idaho. The fall prior to planting in Wisconsin, soil sampled for the 2020 and 2021 seedings had respective pH values of 7.0 and 6.5, Bray-1 P levels of 17 and 21 mg kg<sup>-1</sup>, and K levels of 161 and 107 mg kg<sup>-1</sup>. Soil sampled in the spring prior to planting in Idaho contained 24 mg kg<sup>-1</sup> NO<sub>3</sub>-N, 5.4 mg kg<sup>-1</sup> NH<sub>4</sub>-N, and 10.8 mg kg<sup>-1</sup> Olsen P in 2020 and 23 mg kg<sup>-1</sup> NO<sub>3</sub>-N, 4.8 mg kg<sup>-1</sup> NH<sub>4</sub>-N, and 10.7 mg kg<sup>-1</sup> Olsen P in 2021; soil pH typically ranges from 8.0 to 8.3.

Sites in Wisconsin were amended 6 months prior to planting with broadcast applications of 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 300 kg ha<sup>-1</sup> of K<sub>2</sub>O, 28 kg ha<sup>-1</sup> of S, and 2.2 kg ha<sup>-1</sup> of B for the 2020 seeding and 140 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 336 kg ha<sup>-1</sup> of K<sub>2</sub>O, 28 kg ha<sup>-1</sup> of S, 2.2 kg ha<sup>-1</sup> of B, and 340 kg ha<sup>-1</sup> of pelletized lime for the 2021 seeding. Nitrogen fertilizer was broadcast-applied at 84 kg N ha<sup>-1</sup> just before seeding barley (*Hordeum vulgare* L.) and at 168 kg N ha<sup>-1</sup> just before planting maize. Maize also received starter fertilizer containing 56-23-23-7 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, and S in a band applied 5 cm alongside and 5 cm below seed at planting. Following alfalfa establishment, broadcast fertilizer applications included 140 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 340 kg ha<sup>-1</sup> of K<sub>2</sub>O, 28 kg ha<sup>-1</sup> of S, 2.2 kg ha<sup>-1</sup> of B, and 340 kg ha<sup>-1</sup> of pelletized lime for the 2020 seeding and 185 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 490 kg ha<sup>-1</sup> of K<sub>2</sub>O, 42 kg ha<sup>-1</sup> of S, 3.8 kg ha<sup>-1</sup> of B, and 500 kg ha<sup>-1</sup> of pelletized lime for the 2021 seeding. In Idaho, 45 kg ha<sup>-1</sup> of N and 110 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> were applied to the entire field in 2020 before seeding barley. An additional 55 kg ha<sup>-1</sup> N was applied with the planter when maize was seeded. Similarly in 2021, 70 kg ha<sup>-1</sup> of N and 145 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> were applied to the entire field before seeding barley with no additional nitrogen fertilizer applied to the maize.

Due to limited rainfall experiments in Idaho were irrigated starting within a few days of planting maize in early May to ensure good germination and then scheduled according to estimated crop water usage until early October based on data provided by a local weather station (AgriMet, U.S. Bureau of Reclamation). A solid-set sprinkler system was used to uniformly irrigate all plots approximately once each week. Irrigation was scheduled to meet the greatest water use, typically maize, without excessively irrigating the other crops. Sprinklers on barley plots were plugged for one or two irrigations in August to allow for harvest, while maize and alfalfa continued to receive irrigation.

## 2.2 | Cropping systems and management

The following cropping systems for alfalfa establishment were evaluated in both states:

1. Maize silage interseeded early with alfalfa at maize planting.
2. Identical to system 1 but with prohexadione (PHD) growth retardant sprayed onto alfalfa to enhance seedling survival (Grabber, Smith et al., 2021; Grabber, Dias, & Renz, 2023).
3. Maize silage interseeded late with alfalfa at the vegetative emergence (VE) stage of maize.
4. Identical to system 3 but with PHD sprayed onto alfalfa seedlings.
5. Spring-seeded alfalfa.
6. Maize silage followed by spring-seeded alfalfa in Year 2.
7. Spring barley grown for grain and straw, followed by summer-seeded alfalfa.
8. Maize silage followed by late summer- or early fall-seeded alfalfa.

Cropping systems were assigned to plots according to a randomized complete block design with four replications. Plot size was 3.1 m × 9.1 m in Wisconsin and 12.2 m × 12.2 m in Idaho to match the spacing of the sprinkler irrigation system. Glyphosate-resistant maize hybrids were planted in 2020 and 2021 between April 28 and May 5 in plots and surrounding borders using a 0.76-m row spacing to obtain final populations near 84,000 plants ha<sup>-1</sup> in Wisconsin and 94,000 plants ha<sup>-1</sup> in Idaho. In Wisconsin, a 106-day hybrid (A636-56) was grown for cropping systems 1–4 and 6, while an 88- or 89-day hybrid (NK9227 or A629-22) was grown prior to cropping system 8. In Idaho, a single 91-day hybrid (P9188) was grown both years for cropping systems 1–4, 6, and 8. Barley (variety not stated) was sown in Wisconsin at 120 kg ha<sup>-1</sup> on May 2, 2020 and April 29, 2021. In Idaho, barley (Moravian 69) was planted at 112 kg ha<sup>-1</sup> on April 15, 2020 and April 2, 2021.

Glyphosate-resistant alfalfa FSG431RRLH (fall dormancy 4) in Wisconsin and 55VR08 (fall dormancy 5) in Idaho were

drilled at a 15-cm row spacing at a seeding rate of 18 kg ha<sup>-1</sup>. Alfalfa in cropping systems 1–4 in both states was interseeded immediately after maize planting or 10–15 days later at the VE stage of maize. AlSeeding dates for cropping systems 5, 7, and 8 in Wisconsin were May 2, 2020 and April 29, 2021 for spring-seeded alfalfa; August 12, 2020 and July 28, 2021 for summer-seeded alfalfa after barley; and August 26, 2020 and August 25, 2021 for late summer-seeded alfalfa after maize silage. In Idaho, seeding dates for cropping systems 5, 7, and 8 were April 28, 2020 and May 6, 2021 for spring-seeded alfalfa; August 20, 2020 and August 16, 2021 for summer-seeded alfalfa after barley; and September 25, 2020 and September 21, 2021 for early fall-seeded alfalfa after maize silage. Alfalfa in cropping system 6 was spring seeded in year 2 on April 26, 2021 and April 20, 2022 in Wisconsin and on May 8, 2021 and April 29, 2022 in Idaho.

Glyphosate was the primary herbicide used in both states for weed control, but in Wisconsin, encapsulated acetochlor was also applied after planting spring seeded and interseeded alfalfa (Osterholz, Dias et al., 2020). The growth-retardant PHD-calcium was sprayed at 0 or 0.28 kg a.i. ha<sup>-1</sup> onto interseeded alfalfa seedlings in mid-June (Grabber, Dias, & Renz, 2023). In Wisconsin, 0.147 kg a.i. ha<sup>-1</sup> of fungicide (fluxapyroxad and pyraclostrobin) and 0.018 kg a.i. ha<sup>-1</sup> of insecticide (lambda-cyhalothrin) were also applied during establishment to spring-seeded and interseeded alfalfa in late June or early July to suppress foliar disease and insect pests and to enhance seedling vigor and survival (Grabber, Smith et al., 2021; Grabber, Dias, & Renz, 2023).

## 2.3 | Data collection

During the establishment year, spring-seeded alfalfa was harvested at the bud to early flowering growth stage three times in Wisconsin (July 1, July 28, and September 1, 2020 and on July 1, July 26, and August 26, 2021) and two times in Idaho (July 16 and September 19, 2020 and on July 14 and August 23, 2021). Interseeded alfalfa was not harvested or clipped during intercropping with maize and only maize silage yield was determined in the establishment year. Mature barley grain and straw were harvested on July 28, 2020 and July 26, 2021 in Wisconsin and on August 13, 2020 and August 6, 2021 in Idaho. In Wisconsin, dry conditions prior to harvest accelerated dry down of maize, especially the 106-day hybrid; as a result, maize silage was harvested earlier than normal at whole plant moisture contents ranging from 60% to 65% on August 26, 2020 and August 24, 2021 for cropping system 8 and on August 27, 2020 and September 2, 2021 for cropping systems 1–4 and 6. In Idaho the 91-day maize hybrid was harvested for maize silage from cropping systems 1–4, 6, and 8 at whole plant moisture contents ranging from 55% to 65% on September 16, 2020 and September 2, 2021; relatively warm temperatures in June and July contributed to the earlier

maturation and harvest of maize in 2021. The year following establishment, the first cutting of alfalfa from cropping systems 1–4, 5, 7 and 8 were harvested at the bud stage on May 25, 2021 and June 2, 2022 in Wisconsin. In Idaho, the first cut of alfalfa was harvested at the bud stage on June 1, 2021 and at the early flowering stage on June 8, 2022. Harvests of all crops were made from a 1.5-m-wide strip down the middle of plots using self-propelled plot harvesters equipped with weigh bins and load cells. Cutting height above soil level was 20 cm for maize and 8 cm for alfalfa and barley. Subsamples of harvested crops were oven-dried at 55°C to determine dry matter content. Dry matter yield was calculated from weights of freshly harvested crops and dry matter estimates.

Plant height of alfalfa from ground level to the uppermost extended leaf tip was determined at six locations per plot near the V5 stage and the V10 stage of maize and at the end of the establishment year. Plant height was measured on June 8, June 29, and October 13, 2020 and on June 3, June 23, and October 19, 2021 in Wisconsin and on June 5, July 1, and October 16, 2020 and on June 16, July 7, and October 18, 2021 in Idaho.

Plant density of alfalfa was measured by removing soil along a 30-cm length of eight rows and counting individual plants. Plant density during the establishment year was measured on June 17 and October 14, 2020 and on June 8 and October 20, 2021 in Wisconsin and on June 14 and September 30, 2020 and on June 30 and October 21, 2021 in Idaho. Prior to first harvest, plant density was determined on April 15, 2021 and April 25, 2022 in Wisconsin but in Idaho, stem density was determined on April 27, 2021 and April 29, 2022 by counting the total number of stems >10 cm in height in a 0.2 m<sup>2</sup> area in each plot.

## 2.4 | Statistical analyses

Due to several differences in cropping system management and data collection methods, Wisconsin and Idaho results were analyzed separately at  $\alpha = 0.05$  using PROC MIXED (SAS Institute) with seeding year, cropping system, and their interaction considered as fixed effects and with block within seeding year considered as a random effect. Alfalfa plant height in both states and plant density in Wisconsin were analyzed as repeated measures using CSH or CS covariance structures. Plots of residuals and influence diagnostics were used to assess homogeneity of variance and to detect possible outliers. In several cases, a single observation (studentized residual > 4) was removed from the dataset. To obtain homogeneity of variance, some analyses were run with heterogeneous variance models or with data that were square root or log transformed. A significant cropping system  $\times$  year interaction occurred for most response variables, so a slice statement was used to compare least square means of cropping systems within years. The LSMEANS statement with CL

and DIFF options was used to generate 95% confidence intervals and to compare least square means of significant main effects and year effects within significant cropping system  $\times$  year interactions. Differences among treatments described in Section 3 were significant at  $p \leq 0.05$ .

Multiple linear regression models for first harvest dry matter yield of alfalfa following establishment were developed using seeding year and both original and linear transformed data (Sit & Poulin-Costello, 1994) collected on fall plant height, fall plant density, or spring stem density following establishment as potential independent predictors in PROC REG (SAS Institute). Stepwise selection with an entry level of  $p \leq 0.1$  and an exit level of  $p \leq 0.05$  were used to develop the model. Assumptions of a linear relationship between dependent and independent variables, homoscedasticity and multicollinearity in the final model were evaluated and found to not be violated.

## 3 | RESULTS AND DISCUSSION

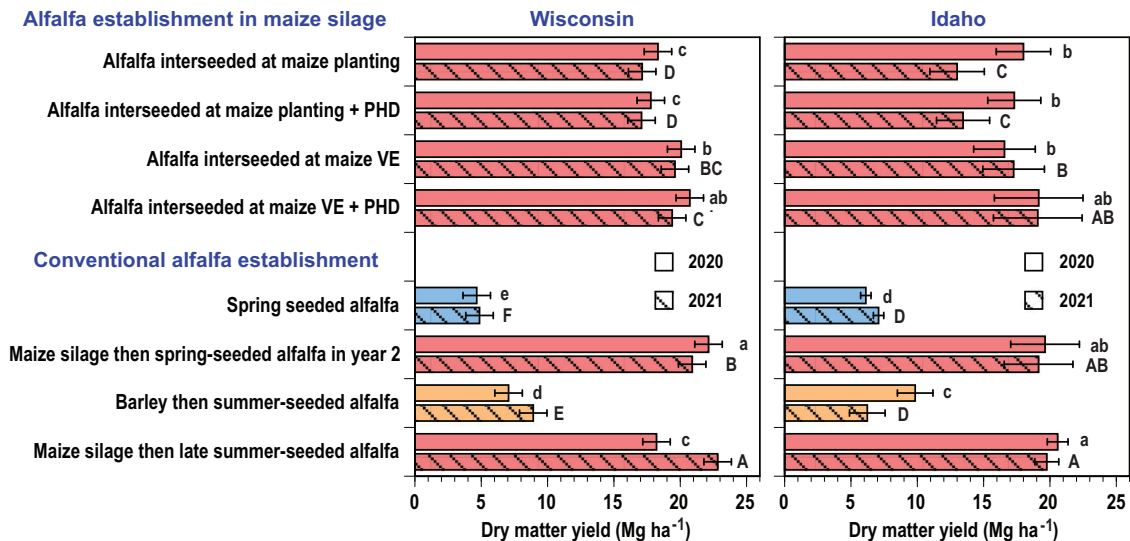
### 3.1 | Growing conditions during alfalfa establishment

During alfalfa establishment, temperatures during the April to October growing season at Prairie du Sac, WI, were generally close to long-term averages, but 2.8°C below normal in October 2020 and 3.3°C above normal in October 2021 (Table S1). Total precipitation from April to October was 785 mm in 2020, close to the long-term average of 740 mm, but rainfall was below normal in April and above normal in July, September, and October. During 2021, conditions were relatively dry throughout the year, and total rainfall was 203 mm below normal during the growing season.

At Kimberly, ID, alfalfa establishment during the April through October growing season occurred under near-normal temperatures during 2020 and for much of 2021 but in the latter year, temperatures were approximately 4°C above normal in June and July (Table S2). The Idaho site was irrigated because it receives limited precipitation; long-term total rainfall averages only 143 mm from April through October. Precipitation was below normal throughout most of the 2020 growing season and totaled only 74 mm. During 2021, rainfall was below normal from April through June but total precipitation was close to normal due to above-average rainfall in October.

### 3.2 | Crop biomass harvested during the alfalfa establishment year

Crops harvested during establishment of alfalfa in year 1 were maize silage (cropping systems 1–4, 6, and 8), alfalfa



**FIGURE 1** Dry matter yield of maize silage (red), alfalfa (blue), or barley straw plus grain (orange) harvested during alfalfa establishment in maize silage versus conventional methods in 2020 and 2021. Alfalfa was interseeded immediately after maize planting or at the vegetative emergence (VE) growth stage of maize and grown without or with prohexadione (+PHD) treatment. Least square means within states and years with no common lowercase letter for 2020 or uppercase letter for 2021 are significantly different at  $p \leq 0.05$ . Capped lines indicate 95% confidence intervals.

(cropping system 5), and barley grain plus straw (cropping system 7). Dry matter yield of harvested crop biomass in both states was influenced by a seeding year  $\times$  cropping treatment interaction (Figure 1). The interaction in Wisconsin was largely driven by substantial differences between seeding years in the yield of the short-season maize hybrid grown prior to a late-summer seeding of alfalfa. In Idaho, the interaction primarily occurred

because yield of maize silage interseeded early with alfalfa and of barley grain plus straw grown prior to summer-seeded alfalfa were greater in 2020 than in 2021.

For both years in Wisconsin and 1 year in Idaho, the yield of maize silage interseeded early with alfalfa was lower than maize silage grown alone prior to spring seeding of alfalfa in year 2 (Figure 1). In these cases, delaying interseeding of alfalfa from maize planting to later at the maize VE stage substantially improved yield of maize silage. Dry matter yield of maize silage interseeded with alfalfa was, however, approximately two- to fourfold greater than the dry matter yield of spring-seeded alfalfa or barley grain plus straw.

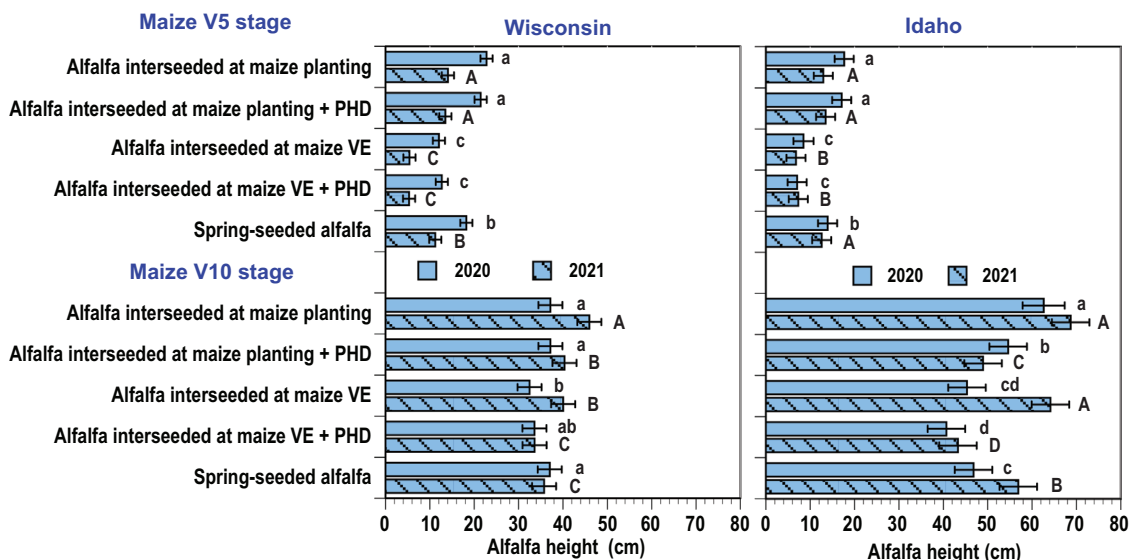
Overall, our results are consistent with previous intercropping studies that found maize yield was frequently reduced by early interseeding of alfalfa but not influenced by a directed spray application of PHD on alfalfa (Berti, Lukaschewsky et al., 2021; Berti, Cecchin et al., 2021; Osterholz et al., 2018). Our current study, however, suggests delaying interseeding of alfalfa until the VE stage can be used as a management technique to reduce yield drag on the maize silage companion crop. Recent findings from other studies suggest delaying interseeding of alfalfa until the V2 or V3 stage can further reduce or eliminate yield drag on maize but this can result

in alfalfa stand failure if competition from maize and disease pressure on alfalfa are especially high during the growing season (Grabber et al., unpublished results).

### 3.3 | Plant height of alfalfa during establishment

Initial growth of interseeded and spring-seeded alfalfa was assessed by measuring alfalfa plant height in early June to mid-June when maize reached the V5 stage and in late June to early July when maize reached the V10 stage. Plant height was influenced by a relatively complex seeding year  $\times$  sampling date  $\times$  cropping system interaction in both states that was caused in part by the application of PHD growth retardant on interseeded alfalfa between the V5 and V10 growth stage of maize (Figure 2). In most cases, plant height at the V5 stage in both states was greatest for early interseeded alfalfa, intermediate for spring-seeded alfalfa, and lowest for late interseeded alfalfa. At the V10 stage, interseeded alfalfa not treated with PHD was taller than spring-seeded alfalfa for one seeding year in Wisconsin and for both seeding years in Idaho. Treatment with PHD soon after the V5 stage reduced plant height of early and late interseeded alfalfa at the V10 stage during one seeding year in Wisconsin and during both seeding years in Idaho.

Overall, early-season growth of intercropped alfalfa was enhanced by early planting, and possibly by high N fertilization rates in Wisconsin and shading by maize. Stimulation of alfalfa growth by N fertilization was reported in another Wisconsin intercropping study (Osterholz et al., 2023).



**FIGURE 2** Plant height of interseeded alfalfa and spring solo-seeded alfalfa during establishment in 2020 and 2021 measured near the V5 and V10 stage of maize. Alfalfa was interseeded immediately after maize planting or at the vegetative emergence (VE) growth stage of maize and grown without or with prohexadione (+PHD) treatment. Least square means within states, maize growth stage, and years with no common lowercase letter for 2020 or uppercase letter for 2021 are significantly different at  $p \leq 0.05$ . Capped lines indicate 95% confidence intervals.

Shading also stimulates etiolation responses by alfalfa (Curran et al., 1993; Lin et al., 2001). Application of PHD growth retardant and delayed interseeding usually reduced alfalfa plant height, but only the latter treatment was associated with improved yield of the maize silage companion crop, presumably because late seeding reduced the overall growth and early season competitiveness of alfalfa. By contrast, PHD treatment has little effect on leaf growth but reduces stem growth and enhances root growth in alfalfa (Grabber, Osterholz et al., 2021; Grabber, Riday et al., 2023) and these morphological shifts apparently had no impact on alfalfa's competitive effect on maize under the conditions of these studies.

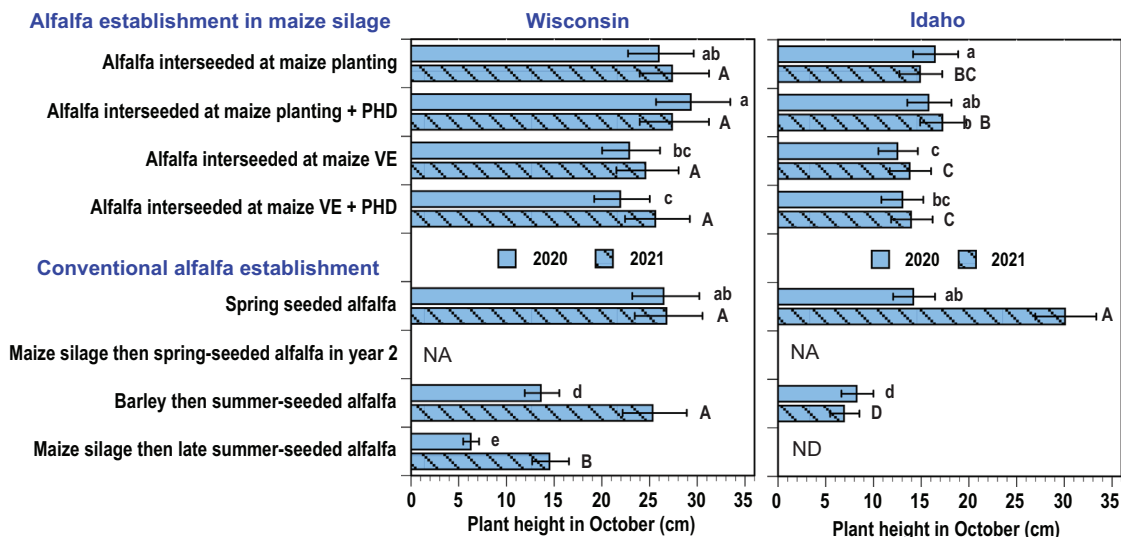
Fall growth of alfalfa in all cropping systems, as assessed by measuring plant height in October prior to a killing frost, was influenced by a seeding year  $\times$  cropping treatment interaction in both states (Figure 3). In most cases, differences in plant height between spring-seeded alfalfa and early interseeded alfalfa were small or not significant in both states. The main exception was Idaho during the fall of 2021, where spring-seeded alfalfa had 1.9-fold greater plant height than early interseeded alfalfa. This difference in plant height resulted from a longer fall regrowth period after the final harvest of spring-seeded alfalfa in late August 2021 compared to mid-September in 2020. In some cases, shifting interseeding from maize planting to the VE stage reduced plant height in the fall, but application of PHD had no effect. In most cases, alfalfa planted after barley or maize silage had substantially lower plant height than interseeded or spring-seeded alfalfa and this could be attributed to the former treatments having a much shorter growth period. The exception was alfalfa sown after barley during 2021 in Wisconsin, which had a similar plant

height as interseeded or spring-seeded alfalfa. In this case, relatively early planting after barley followed by 107 mm of rainfall over a 2-week period favored quick germination and fall growth of summer-seeded alfalfa. Due to delayed emergence and minimal plant development, fall plant heights were not taken for alfalfa sown after maize silage in Idaho.

Spring-solo seeding of alfalfa is preferred in the northern United States, but summer seedings can be successfully established and produce high yield if planted relatively early and if sufficient soil moisture is available to promote rapid seedling growth before winter (Hall, 1995; Undersander et al., 2015). Hall (1995) in Pennsylvania noted that plant height of summer-seeded alfalfa should be at least 7.5- to 10-cm tall in the fall to ensure good winter survival and forage production the following year.

### 3.4 | Stand density of alfalfa during establishment

Initial stand density of alfalfa in cropping systems was assessed in June for interseeded and spring-seeded alfalfa and in October for alfalfa seeded after barley or maize silage. Initial stand density was influenced by a seeding year  $\times$  cropping system interaction in Wisconsin and by a cropping system main effect in Idaho. In Wisconsin during 2020, interseeded and spring-seeded alfalfa had a similar average stand density (452 plants  $m^{-2}$ ) that was greater than alfalfa seeded after barley (266 plants  $m^{-2}$ ), while stand density of alfalfa seeded after maize (366 plants  $m^{-2}$ ) did not differ from the former cropping treatments. During 2021, stand density of



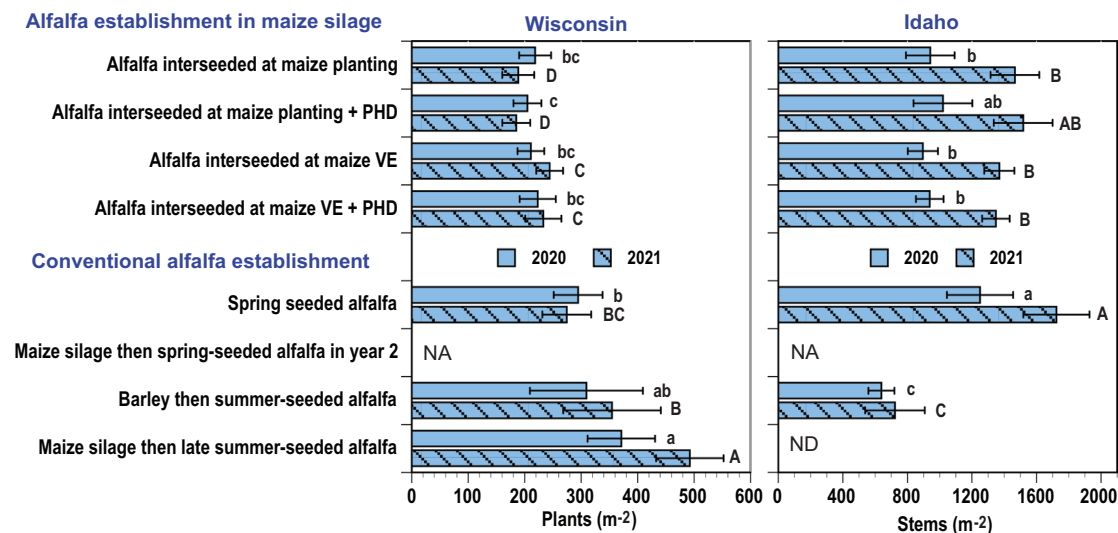
**FIGURE 3** Fall plant height of alfalfa following establishment in maize silage versus conventional methods in 2020 and 2021. Alfalfa was interseeded immediately after maize planting or at the vegetative emergence (VE) growth stage of maize and grown without or with prohexadione (+PHD) treatment. Back transformed least square means within states and years with no common lowercase letter for 2020 or uppercase letter for 2021 are significantly different at  $p \leq 0.05$ . Capped lines indicate 95% confidence intervals. NA, not applicable; ND, not determined.

late summer-seeded alfalfa after maize (532 plants  $m^{-2}$ ) was greater than the average stand density of alfalfa in all other cropping treatments (358 plants  $m^{-2}$ ). In Idaho, the average stand density of interseeded and spring-seeded alfalfa was greater than alfalfa seeded after barley (615 vs. 503 plants  $m^{-2}$ ). Stand counts of alfalfa seeded after maize in Idaho were not taken in October due to delayed emergence and minimal plant development. Overall, initial establishment of stands was more consistent with interseeded and spring-seeded alfalfa than with alfalfa seeded after barley or maize silage.

Following establishment, stand density of alfalfa was influenced by a seeding year  $\times$  cropping system interaction in both states (Figure 4). The interaction in Wisconsin was mainly due to a greater plant density of summer-seeded alfalfa after maize in 2021 than in 2020. Plant counts taken during October and the following April in Wisconsin gave similar estimates of stand density, indicating all cropping systems had excellent winter survival of alfalfa (data not shown). In general, plant density in Wisconsin was greatest for late summer-seeded alfalfa following maize, intermediate for summer-seeded alfalfa after barley and spring-seeded alfalfa, and lowest for interseeded alfalfa systems (Figure 4). The relatively low plant density of interseeded alfalfa

could be attributed to stands being seeded earlier and subjected to competition from a companion crop during establishment (Rehm et al., 1998; Sulc et al., 1993). However, based on published recommendations (Grabber, Smith et al., 2021; Tesar & Marble, 1988), all cropping systems in Wisconsin appeared to have sufficient plant density to support high yield of alfalfa during the first full production year.

In Idaho, visual assessments and counts made in selected plots indicated all cropping systems had high plant counts ( $>300$  plants  $m^{-2}$ ) exceeding recommendations for alfalfa following establishment (Grabber, Smith et al., 2021; Tesar & Marble, 1988). Therefore, alfalfa stand density prior to the first full production year was assessed by taking stem counts in late April. The seeding year  $\times$  cropping system interaction in Idaho occurred because stem density of interseeded and spring-seeded alfalfa was greater in 2021 than in 2020, while seeding year had no effect on summer-seeded alfalfa after barley. Overall, stem density was greatest for spring-seeded alfalfa, intermediate for interseeded alfalfa, and lowest for alfalfa seeded after barley (Figure 4), but alfalfa in all cropping systems exceeded the minimum recommended density of 600 stems  $m^{-2}$  (Undersander et al., 2015). Alfalfa seeded in early fall after maize visually had high plant density but insufficient plant development ( $<10$ -cm tall) the following April for estimating stem counts. Previous studies indicate stem density of spring-seed alfalfa is initially greater than alfalfa sown with a small grain or maize companion crop during establishment, but differences among establishment methods often decline as stands mature (Hoy et al., 2002; Patel et al., 2021; Roberts et al., 2023). To our knowledge, comparisons of stem density for spring- versus summer-seeded alfalfa have not been reported in the literature. Based on published recommendations (Grabber, Smith et al., 2021; Undersander et al., 2015), stem density in April was sufficient for spring-seeded and interseeded alfalfa to support high forage production, but marginal for alfalfa seeded after barley. By contrast, the absence of countable stems for alfalfa seeded in early fall after maize suggests this



**FIGURE 4** Plant density of alfalfa in Wisconsin (averaged across fall and spring sampling dates) and spring stem density in Idaho following alfalfa establishment in maize silage versus conventional methods in 2020 and 2021. Alfalfa was interseeded immediately after maize planting or at the vegetative emergence (VE) growth stage of maize and grown without or with prohexadione (+PHD) treatment. Least square means within states and years with no common lowercase letter for 2020 or uppercase letter for 2021 are significantly different at  $p \leq 0.05$ . Capped lines indicate 95% confidence intervals. NA, not applicable; ND, not determined.

system would have low forage yield potential the following year.

The timing of interseeding after maize planting and the application of PHD had little or no effect on alfalfa stand density in either state (Figure 4). Application of PHD often improves plant survival of interseeded alfalfa in Wisconsin (Grabber, Smith et al., 2021; Osterholz et al., 2018), but other studies carried out under normal to dry growing conditions in the Eastern and Midwestern United States also found that stand density of alfalfa intercropped with maize was in some cases lower than spring-seeded alfalfa or not responsive to PHD treatment (Berti, Lukaschewsky et al., 2021; Berti, Cecchin et al., 2021; Grabber, Osterholz et al., 2021; Grabber, Dias, & Renz, 2023; Patel et al., 2021).

### 3.5 | First cut yield of alfalfa following establishment

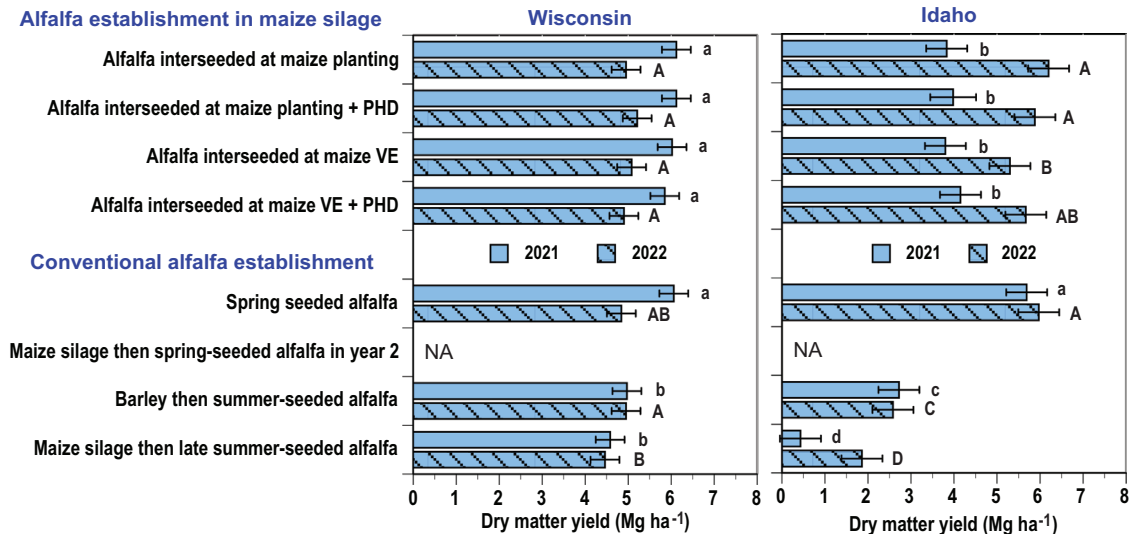
The effect of cropping system on alfalfa establishment was further evaluated by measuring first cut yield of alfalfa at the beginning of the first full production year. Dry matter yield of alfalfa in Wisconsin and Idaho was influenced by a seeding year  $\times$  cropping system interaction (Figure 5). Seeding year mainly influenced yields of interseeded alfalfa in both states and yields of spring-seeded alfalfa in Wisconsin and early fall -seeded alfalfa after maize in Idaho. In most cases, yield of interseeded alfalfa was similar to spring-seeded alfalfa for 2021 and 2022 in

Wisconsin and for 2022 in Idaho; in these trials, the maize silage companion crop was harvested the prior year near September 1. By contrast, the average yield of interseeded alfalfa for 2021 in Idaho was 1.75 Mg ha<sup>-1</sup> lower than spring-seeded alfalfa and, in this case, the maize silage companion crop was harvested in mid-September of 2020. A concurrent harvest timing study in Wisconsin also found that shifting maize harvest from September 1 to September 15 reduced dry matter yield of interseeded alfalfa the following year (Grabber et al., unpublished results). Depressed yield of alfalfa during the first full production year has also been reported for early interseeded alfalfa established in fall harvested maize grain systems (Berti, Cecchin et al., 2021; Patel et al., 2021).

In the current study, the timing of alfalfa interseeding and the application of PHD under normal to dry conditions in Wisconsin and semiarid irrigated conditions in Idaho did not consistently impact subsequent forage yield (Figure 5). Other studies carried out under normal to dry establishment conditions have also found that first production year yield of interseeded alfalfa was not responsive to PHD treatment (Berti, Lukaschewsky et al., 2021; Berti, Cecchin et al., 2021; Patel et al., 2021). By contrast, PHD often improves yield of interseeded alfalfa in Wisconsin by improving alfalfa establishment under difficult growing conditions (e.g., Grabber, Smith et al., 2021).

In Wisconsin, average first cut yield of spring-seeded and interseeded alfalfa in 2021 was 1.25 Mg ha<sup>-1</sup> greater than summer-seeded alfalfa following barley or maize silage, but in 2022, differences among these systems were small or not





**FIGURE 5** First cut yield of alfalfa in 2021 and 2022 at the start of the first full production year following alfalfa establishment in maize silage versus conventional methods in 2020 and 2021. Alfalfa was interseeded immediately after maize planting or at the vegetative emergence (VE) growth stage of maize and grown without or with prohexadione (+PHD) treatment. Least square means within states and years with no common lowercase letter for 2020 or uppercase letter for 2021 are significantly different at  $p < 0.05$ . Capped lines indicate 95% confidence intervals. NA, not applicable.

significant (Figure 5). By contrast, in Idaho, yield of spring-seeded alfalfa in 2021 and 2022 was, in turn, 3.0 and 3.4 Mg ha<sup>-1</sup> greater than summer-seeded alfalfa after barley and 5.3 and 4.1 Mg ha<sup>-1</sup> greater than early fall-seeded alfalfa after maize silage. Reductions in first cut yield of alfalfa planted after harvest of barley or short-season maize silage in Wisconsin or after harvest of barley in Idaho occurred even though seedings were carried out prior to the cutoff date of September 1 recommended in Extension publications (Shewmaker, 2005; Undersander et al., 2015). Prior studies also reported that forage yield of summer-seeded alfalfa during the first full production year was less than spring-seeded alfalfa (Buxton and Wedin, 1970; Grabber, 2009). Although winters are relatively mild with low risk of winterkill in southcentral Idaho, seeding alfalfa in late September after maize substantially limited fall seedling development and first cut alfalfa yield the following year.

### 3.6 | Relationship of first cut yield with yield alfalfa growth and stand density

Multiple regression was used to examine linearized relationships (Sit & Poulin-Costello, 1994) between first cut dry matter yield of alfalfa and the predictor variables of fall plant height, stand density, and seeding year. First cut yield was highly related to the fall plant height of alfalfa according to the following models: Wisconsin yield  $\ln(\text{Mg ha}^{-1}) = 1.120 + 0.207\ln(\text{cm}) - 0.188(\text{seeding year})$ ,  $R^2 = 0.80$ ,  $p < 0.001$ ,  $N = 56$ ; Idaho yield  $\ln(\text{Mg ha}^{-1}) = 1.988 - 7.623(1/\text{cm}) + 0.209(\text{seeding year})$ ,  $R^2 = 0.68$ ,  $p < 0.001$ ,  $N = 48$ . In these

models, the 2020 and 2021 seeding years were coded as 0 and 1, respectively. Fall plant height accounted for 48% and 58% of the variation in the Wisconsin and Idaho models, respectively, indicating year and other undefined factors also had major impacts on first cut yield. Based on Sit and Poulin-Costello (1994), the non-linearized form of the relationships was a power function in Wisconsin and a Type III exponential function in Idaho. Both consisted of a concave up curve with a decreasing slope, meaning that first cut yield increased at a declining rate as fall plant height increased. When included in the analysis, plant density following establishment was not selected as a significant predictor variable in the Wisconsin model, suggesting plant density in all cropping systems was above the threshold needed to maximize alfalfa yield. By contrast, including spring stem density following establishment displaced both fall plant height and seeding year from the Idaho model to give the following relationship: yield  $\ln(\text{Mg ha}^{-1}) = 2.335 - 857.5(1/\text{stems m}^{-2})$ ,  $R^2 = 0.79$ ,  $p < 0.001$ ,  $N = 45$ ; here the non-linearized form was again a Type III exponential function where first cut yield increased at a declining rate as stem density increased. Results in both states indicate plant vigor following establishment, when measured as fall plant height or spring stem density, had significant impacts on subsequent first cutting alfalfa forage yield. In all cases, the main drivers of the regression curves were summer- or late summer-seeded alfalfa systems that had relatively low first cut yield with short fall plant stature or low spring stem density. These findings were in broad agreement with Hall (1995), who suggested fall growth of summer-seeded alfalfa should exceed 7.5–10 cm in height to ensure both good winter survival and forage production the following year.

To maximize first cut yield, however, our results suggest seeding and harvest management during the establishment year should be done in a manner that will favor sufficient stand density and ample fall growth of alfalfa prior to a killing frost. Further work is needed to unveil the physiological drivers of high dry matter yield following alfalfa establishment.

## 4 | CONCLUSIONS

Under normal to dry rainfed conditions in Wisconsin and semiarid irrigated conditions in Idaho, the intercropping system provided much greater dry matter yield of maize silage during alfalfa establishment than conventionally spring-seeded and harvested alfalfa. Shifting interseeding from maize planting to the VE stage reduced early-season growth of alfalfa and improved maize silage yield, but in general, all interseeding systems and spring-seeded alfalfa had good to excellent plant or stem density following establishment. This suggests interseeding alfalfa at the VE stage could be used as a management technique to improve maize silage yield in the intercropping system. After establishment, first cut yield of spring seeded and interseeded alfalfa was similar when maize silage in the intercropping system was harvested near September 1 to allow ample time for alfalfa regrowth prior to winter. Alfalfa seeded after barley or maize silage had relatively high plant density after establishment but fall growth and first cut yield the following year were usually much lower than interseeded or spring-seeded alfalfa. Regression analysis of data from all cropping systems indicated high plant vigor of alfalfa following establishment (measured as fall plant height or spring stem density) favored high first cut yield of alfalfa at the start of the first full production year. These results, along with findings from other studies, suggest early harvest of maize in the intercropping system along with early planting of summer-seeded alfalfa should be practiced to favor ample fall growth and high yield of alfalfa the following year. As in other studies conducted under normal to dry growing conditions, PHD treatment of interseeded alfalfa seedlings had little or no impact on alfalfa establishment or subsequent yield. Overall, the establishment of alfalfa and dry matter yield of the maize silage companion crop in the intercropping system compared favorably with solo-seeding methods for alfalfa establishment. We will continue to monitor alfalfa yield from this experiment and carry out other work aimed at improving the performance of this intercropping system, especially under wet growing conditions that currently lead to poor establishment and low subsequent yield of interseeded alfalfa in humid, cold-temperate regions.

## AUTHOR CONTRIBUTIONS

**John H. Grabber:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology;

project administration; resources; supervision; writing—original draft. **David L. Bjorneberg:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; writing—review and editing. **Christopher W. Rogers:** Conceptualization; data curation; investigation; methodology; supervision; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## ORCID

John H. Grabber  <https://orcid.org/0000-0002-1384-2341>

Christopher W. Rogers  <https://orcid.org/0000-0002-1989-1582>

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