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# Establishment and First Year Yield of Interseeded Alfalfa as Influenced by Corn Plant Density and Treatment with Prohexadione, Fungicide and Insecticide

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**Abstract:** Interseeding alfalfa (*Medicago sativa* L.) into a silage corn (*Zea mays* L.) companion crop can increase the yield and profitability of forage production and reduce the risk of nutrient and soil loss from cropland, but unreliable establishment of alfalfa hampers the adoption of this practice on dairy farms. This study evaluated plant survival, foliar health, and dry matter yields of two alfalfa varieties when established in corn sown at populations ranging from about 47,500 to 100,000 plants per ha$^{-1}$ and when treated with prohexadione (PHD), PHD followed by fungicide and insecticide (PHD-FI), or not treated with agrichemicals. The plant density of alfalfa during establishment was adversely impacted by above average precipitation and high corn populations, but substantially improved by PHD-FI treatment, which limited alfalfa etiolation, disease, and defoliation. First-cut dry-matter yields of interseeded alfalfa after corn were maximized at a stand density of approximately 200 plants m$^{-2}$ or 850 stems m$^{-2}$ and total first year yield exceeded conventionally spring-seeded alfalfa by 59 to 75%. Overall, our results indicated that PHD-FI treatment promoted good establishment and subsequent forage production of interseeded alfalfa. Applications of PHD-FI must, however, be fine-tuned, and additional management practices must be developed to ensure both good yields of corn silage and reliable establishment of interseeded alfalfa, especially during wet growing conditions.

**Keywords:** lucerne; maize; intercropping; growth retardant; pesticide; common leaf spot; potato leaf hopper; photosynthetically active radiation; foliar senescence; biomass production

## 1. Introduction

Growing corn silage in rotation with alfalfa provides a blend of forage that enhances milk production while providing other benefits, including lower inputs for fertilizer nitrogen and pest control as well as improved soil quality and soil conservation. Alfalfa usage by dairy farms is, however, declining, partly because corn produces greater dry matter yields of forage [1,2]. The yields of solo-seeded alfalfa during establishment are particularly poor, typically only one-half that of subsequent full-production years in the northern USA [3,4]. Seeding small grains or other commonly used companion crops with alfalfa can modestly improve forage production during the establishment year, but forage quality is often reduced [5–7]. One way to circumvent the low establishment year yields of alfalfa would be to use corn silage as a relatively high-yielding and high-quality companion crop. In this system, alfalfa is interseeded to serve as a highly effective cover crop for corn, and then it is brought into full forage production the following year. The successful interseeding of alfalfa reduces the risk of soil and nutrient loss from cropland, suppresses weeds, and increases the overall forage yields and profitability of corn silage–alfalfa rotations [8–15]. Full realization of these benefits will, however, require the reliable establishment of alfalfa while maintaining relatively high yields of the corn companion crop [10].

During our studies in Wisconsin, we found that planting alfalfa with a drill at normal seeding rates immediately after corn planting, followed by a foliar application of the gibberellin inhibitor prohexadione (PHD) improved the establishment of interseeded alfalfa [8,9]. Subsequent studies found that alfalfa establishment was often further enhanced by seeding a well-suited alfalfa variety in conjunction with PHD treatment [16]. These practices, however, failed to provide satisfactory establishment when above-average precipitation and intensive management favored foliar disease and the defoliation of interseeded alfalfa and high yields of corn silage [16]. Stress on interseeded alfalfa may further be exacerbated by potato leafhopper (PLH, *Empoasca fabae* Harris) feeding after corn canopy closure (J.H. Grabber, et al., unpublished observations). Studies with conventionally sown alfalfa indicate that well-timed foliar fungicide and insecticide (FI) treatments can, in some situations, be highly effective for reducing leaf damage and defoliation and improving alfalfa vigor and yield [17–20], but the utility of these treatments for improving the establishment of interseeded alfalfa has not been examined. Limited work by our group also suggests that stand density of interseeded alfalfa can be improved by reducing corn populations from the current norms of around 85,000 to approximately 60,000 plants ha$^{-1}$ [9], but gains in alfalfa establishment by this approach must be balanced against reductions in corn silage yield that impact the profitability of forage production [10]. Therefore, the objective of this study was to determine if foliar applications of PHD followed by FI would improve the establishment and first-year yield of two well-adapted interseeded alfalfa varieties subjected to varying levels of competition and shading stress from a corn silage companion crop sown at five plant populations.

## 2. Materials and Methods

### 2.1. Sites and Crop Establishment

A two-year experiment was conducted twice at a USDA research facility near Prairie du Sac, Wisconsin, USA (43°20′ N, 89°43′ W). During 2017 and 2018, the experiment was carried out at a site with a Pillot silt loam soil (fine–silty over sandy or sandy–skeletal, mixed, superactive, mesic Typic Argiudolls). During 2018 and 2019, the experiment was again conducted on a Richwood silt loam soil (fine–silty, mixed, superactive, mesic Typic Argiudolls). Monthly precipitation and average temperatures from 2017 to 2019 are reported in Table S1. Corn silage was grown at both sites during the two years prior to the experiments. The fall prior to planting, the soils at the respective sites had pH values of 7.0 and 6.7, Bray-1 P levels of 19 and 24 mg kg$^{-1}$ and K levels of 133 and 125 mg kg$^{-1}$, which were considered optimal for corn and alfalfa production in Wisconsin [21]. Each year, sites were amended with broadcast applications of P-, K-, B-, and S-containing fertilizers according to recommendations for corn silage and alfalfa production in Wisconsin [21]. Corn was fertilized with 224 kg N ha$^{-1}$, with 40% as urea banded in starter fertilizer, along with 13 kg ha$^{-1}$ of $P_2O_5$ and 16 kg ha$^{-1}$ of $K_2O$, and the balance of N was side-dressed as urease-inhibitor-treated urea at the V6 growth stage. Weeds were controlled by 1.12 kg a.e. ha$^{-1}$ of glyphosate mixed with 1.26 kg a.i. ha$^{-1}$ of microencapsulated acetochlor sprayed to the soil surface immediately after alfalfa seeding, followed by 0.28 kg a.i. ha$^{-1}$ of bromoxynil sprayed four weeks after corn planting [14].

Eight rows of corn were planted in a north–south direction into 6.1 m wide by 32 m long whole plots according to a randomized complete block design with four replications using a no-till drill with a 0.76 m row spacing. Corn borders at least 3 m wide were planted around the perimeter of the experimental sites to provide uniform shading of interseeded alfalfa during establishment. The glyphosate-resistant corn hybrid 'Agrigold 6267' (102-d maturity) was planted at populations of 51,000, 68,400, 83,000, 100,100, and 111,700 seed ha$^{-1}$ on 10 May 2017 to obtain harvest populations of 46,600, 65,900, 79,000, 95,500, and 105,900 plants ha$^{-1}$. 'Agrigold 6348' (104-d maturity) was planted at 48,200, 64,500, 79,600, 91,900, and 106,200 seed ha$^{-1}$ on 30 April 2018 to obtain harvest populations of 44,200, 61,200, 72,900, 85,100, and 98,600 plants ha$^{-1}$. The differing seed sizes of the hybrids and constraints on drill settings prevented identical populations from being sown

both years. According to product literature (Agrigold, St. Francisville, IL, USA) both hybrids had medium or medium–tall plant height, semi-upright leaves, semi-flex ears, and very good silage yield potential and agronomic characteristics. Each corn population whole plot contained six subplots that were formed by overlaying two 3.05 m wide by 32 m long alfalfa variety strips with three 6.1 m wide and 7.6 m long agrichemical treatment strips. Alfalfa variety and agrichemical treatment strips were randomly assigned to each whole plot. Agrichemical treatments were separated by 4.6 m borders. Rhizobia-inoculated and fungicide-treated seed of PLH-resistant '55H94' and non-PLH-resistant 'Hybriforce 3420' alfalfa (Corteva Agriscience, Johnston, IA, USA) were planted at 18 kg live seed ha$^{-1}$ on 12 May 2017 and 7 May 2018 using a no-till drill with a 16.5 cm row spacing. Previous studies in Wisconsin demonstrated that both alfalfa varieties consistently had above average plant survival when interseeded into intensively managed corn [16]. Agrichemical treatments included (1) a nontreated control, (2) PHD applied approximately five weeks after seeding, and (3) PHD followed by FI respectively applied about five and seven weeks after seeding. The timing of PHD application was based on previous research [8,9], while FI application was carried out prior to full corn canopy closure and just before the expected onset of foliar disease and damage from PLH. The 'Kudos' formulation of PHD (Fine Americas, Walnut Grove, CA, USA) was applied at 0.425 kg a.e. ha$^{-1}$ when alfalfa was 30-cm tall on 20 June 17 and 11 June 2018 using a backpack sprayer equipped with drop nozzles that maximized deposition to alfalfa but minimized spray distribution to corn. Solutions of PHD were prepared and applied as previously described [22]. The FI treatment consisted of 'Priaxor' fungicide (fluxapyroxad and pyraclostrobin, BASF, Beaumont, TX, USA) at 0.147 kg a.i. ha$^{-1}$ mixed with 'Besiege' insecticide (chlorantraniliprole and lambda-cyhalothrin, Syngenta, Greensboro, NC, USA) at 0.088 kg a.i. ha$^{-1}$. FI was applied using a 3.05 m wide overhead boom attached to a $CO_2$-pressurized backpack sprayer calibrated to deliver 187 L ha$^{-1}$ when corn height to the uppermost leaf was 172 cm on 5 July 2017 and 156 cm on 25 June 2018. Interseeded alfalfa grown without agrichemical treatment under the highest corn population underwent complete stand failure during the summer of 2017 and 2018, so these subplots were reseeded the following spring during the third week of April to provide an estimate of dry matter yield for spring-seeded alfalfa following corn. Weeds during alfalfa production were suppressed using spray applications of clethodim at 0.14 kg a.i. ha$^{-1}$ and imazethapyr at 0.07 kg a.e. ha$^{-1}$.

### 2.2. Measurements and Statistical Analyses

Approximately two to three weeks after PHD application, alfalfa plant height was measured from the soil surface to the tip of the uppermost leaf of live shoots at eight locations per subplot on 13 July 2017 and 25 June 2018; lodged plants were held upright for measurement. An overall assessment of initial alfalfa plant density prior to corn canopy closure was determined in randomly selected subplots on 27 June 2017 and 25 June 2018 by removing soil along four rows with a trowel and counting the taproots of individual plants in a 60 cm length of each row. The penetration of photosynthetically active radiation (PAR) to interseeded alfalfa was measured at approximately the V14 and R3 growth stages at two locations within in each agrichemical treatment strip on 13 July and 16 August in 2017 and on 5 July and 9 August in 2018 using an Accupar LP-80 ceptometer (Meter, Pullman, WA, USA) with the PAR sensor placed diagonally across the corn interrow area just above the alfalfa canopy. A second PAR sensor was placed above the corn canopy to measure incoming radiation. Ratings of alfalfa for percent stem defoliation and percent leaf necrosis from common leaf spot (*Pseudopeziza medicaginis*, Lib.) were taken on 19 July and 2 August 2017 and on 11 July and 26 July 2018, approximately two and four weeks after FI application. Ratings of percent leaves with necrosis from PLH feeding were also taken about two weeks after FI application. Ratings were performed visually using standard area diagrams to aid estimates of leaf necrosis. After removing corn borders, two central rows of corn plants in each subplot were chopped and weighed on 8 September 2017 and 10 September 2018 using a research plot harvester set at a cutting height of 15-cm.

Interseeded alfalfa was not clipped or harvested during its establishment in corn. Alfalfa plant density after establishment was determined in all subplots in mid-October in 2017 and 2018, approximately four weeks after corn silage harvest, using the trowel method described above. The following spring, alfalfa stems greater than 5 cm tall were counted on 7 May 2017 and 24 April 2018 in three 0.25 m$^2$ locations per subplot. A 1.5 m by 6 m strip of first-year alfalfa after corn in the center of each subplot was harvested and weighed at the late vegetative to early flowering growth stages near 30 May, 28 June, 28 July, and 30 August during 2018 and 2019 using a research plot harvester set at a cutting height of 5 cm. The alfalfa established by interseeding was harvested at all four dates, while conventional spring-seeded alfalfa was harvested on the latter three dates. Subsamples of freshly chopped corn and harvested alfalfa were weighed before and after heating in a forced-draft oven at 55 °C to estimate dry matter content and crop yields.

Data collected from alfalfa (plant height, defoliation, leaf necrosis and plant density) and corn (dry matter, dry matter yield, and PAR penetration) were analyzed by a sequential (Type 1) polynomial regression approach [23] using PROC MIXED (SAS Institute, Cary, NC, USA). The quantitative factor of corn population and the qualitative factors of year, agrichemical treatment, and alfalfa variety and their interactions were considered fixed effects. Block(year), block(year) X corn population, block(year) X corn population X agrichemical treatment, and block(year) X corn population X alfalfa variety were considered random effects. PAR penetration data were analyzed as a repeated measure using a compound symmetry covariance structure. Residual plots and influence diagnostics were used to assess the homogeneity of variance and identify possible outliers (studentized residuals > 3.5) and, in several cases, up to 1.5% of observations were removed from the dataset. Due to unequal variances, datasets were often analyzed using heterogenous variance models or transformed by $Y^{1/2}$, $(Y + 0.5)^{1/2}$, or the logit function. If *F*-tests were significant ($p \leq 0.05$), then least square means of fixed effects were compared at $p = 0.05$ using Tukey's multiple comparison method. A multiple linear regression model for alfalfa plant density after corn harvest was developed using the data collected from alfalfa (plant height, defoliation, and leaf necrosis from common leaf spot and PLH feeding), corn (actual plant population, dry matter yield and PAR penetration), weather (average monthly precipitation and temperature from May through October), and alfalfa variety as potential independent predictors in PROC REG (SAS Institute, Cary, NC, USA). Stepwise selection with an entry level of $p \leq 0.001$ and an exit level of $p \leq 0.01$ was 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. Dry matter yield responses of alfalfa to plant and stem density were analyzed by PROC REG (SAS Institute, Cary, NC, USA) using a quadratic plus plateau regression model to estimate the value of the yield plateau and join point of plant or stem density where maximum yield was achieved. Back-transformed means are reported, and treatment differences described in the Results and Discussion were significant at $p \leq 0.05$.

## 3. Results and Discussion

### 3.1. Weather Conditions during Crop Production

During the primary period of interseeded alfalfa establishment from May through October, temperatures and precipitation were generally near normal in 2017, but with relatively wet conditions in July and dry conditions in August and September (Table S1). Temperatures were 6% above normal and precipitation was 47% above normal during establishment of interseeded alfalfa during 2018, with excess precipitation concentrated in May and in August through October. The primary period of first-year alfalfa forage production from May through August occurred under temperatures that were 8% above normal in 2018 and near normal in 2019, while precipitation during this period was 40 and 21% above normal in 2018 and 2019, respectively.

### 3.2. Characteristics of Corn during Establishment of Interseeded Alfalfa

Penetration of PAR through the corn canopy at approximately the V14 stage in July and the R3 stage in August quadratically declined as corn population increased and was influenced by a year X sampling date X corn population interaction. PAR penetration in 2017 exceeded 2018 and declined both years from July to August. Differences in PAR penetration were, however, most pronounced in July, declining from 23.9 to 7.6% in 2017 and from 14.3 to 4.0% in 2018 as corn population increased (Figure 1). In August, PAR penetration declined from 13.3 to 3.9% in 2017 and from 10.2 to 2.8% in 2018 as corn population increased. Dry matter yields of corn silage in September were affected by a year X corn population interaction; yields averaged 19.21 Mg ha$^{-1}$ in 2017 and were not influenced by corn population, but, in 2018, yields increased quadratically from 17.5 to 23.0 Mg ha$^{-1}$ as corn population increased (Figure 1). Other studies have also noted that PAR interception and corn yields are often, but not always, positively associated with corn population and responses often differ among growth environments and hybrids [24–30]. Greater PAR interception and silage yields in 2018 compared to 2017 could partly be attributed to earlier planting, warmer temperatures, and more abundant precipitation in 2018, which would favor more rapid corn canopy development and biomass accumulation. Corn maturity and silage yield are positively associated with whole-plant dry-matter content and grain development [31], and, in our study, dry matter content from the lowest to the highest corn population increased from 41.5 to 43.3% in 2018, but declined from 32.9 to 31.3% in 2017. This suggests that the unresponsiveness of silage yield to plant population in 2017 was partly related to corn being less mature at harvest. In addition, product literature (Agrigold, St. Francisville, IL, USA) indicated dry matter yields of the hybrid we used in 2017 was less responsive to corn population than the hybrid we used in 2018. Corn silage yields also differed between agrichemical treatments and were greater in nontreated controls (20.7 Mg ha$^{-1}$) than in PHD and PHD-FI treatments (19.9 Mg ha$^{-1}$). While further study is needed, reductions in corn yield associated with PHD or PHD-FI treatment might be associated with the improved establishment and competitiveness of interseeded alfalfa, which is described below. Uptake of the PHD growth retardant might also reduce corn silage yield, but this issue was limited by our use of drop nozzles to direct spray away from corn and onto interseeded alfalfa [8,9].
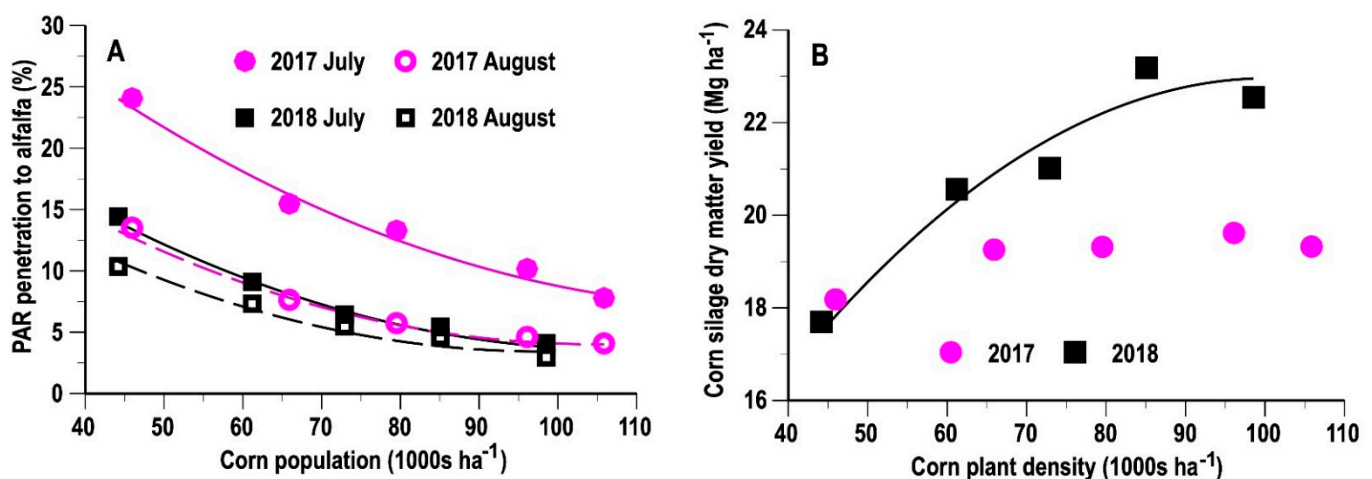


**Figure 1.** Penetration of photosynthetically active radiation (PAR) through the corn canopy to interseeded alfalfa in July and August (**A**) and corn silage yield in September (**B**) as influenced by production year and corn plant population. Data were averaged across alfalfa varieties and agrichemical treatments. Regression intercepts and slopes of PAR measured in July and in August and of corn yield differed between years (*p* = 0.05).

### 3.3. Plant Height and Foliar Health of Interseeded Alfalfa

The growth retardant PHD was applied to 30 cm tall alfalfa in mid-June, and FI was applied two weeks later. Alfalfa plant heights, measured one week after FI application in 2017 and concurrently with FI application in 2018, were influenced by a year X agrichemical treatment X alfalfa variety interaction. The application of PHD and PHD-FI substantially reduced plant height both years relative to the nontreated control, but in 2017, PHD-FI-treated alfalfa was slightly taller than PHD-treated alfalfa and 55H94 was also taller than Hybriforce 3420 (Figure 2). A year X agrichemical treatment X corn population interaction also affected alfalfa height because increasing the corn population had no effect on the nontreated control and PHD-treated alfalfa, whereas the heights of PHD-FI treated alfalfa were linearly reduced by 5 cm in 2017 but linearly increased by 4 cm in 2018 (data not shown). The differing responses by year, particularly for the PHD-FI treatment, could partly be attributed to the timing of height measurements relative to FI application, but overall PHD treatment was the dominant factor influencing alfalfa plant stature in this study. Similar growth-retardant effects of PHD have been observed in previous studies with interseeded alfalfa [9,16,22].
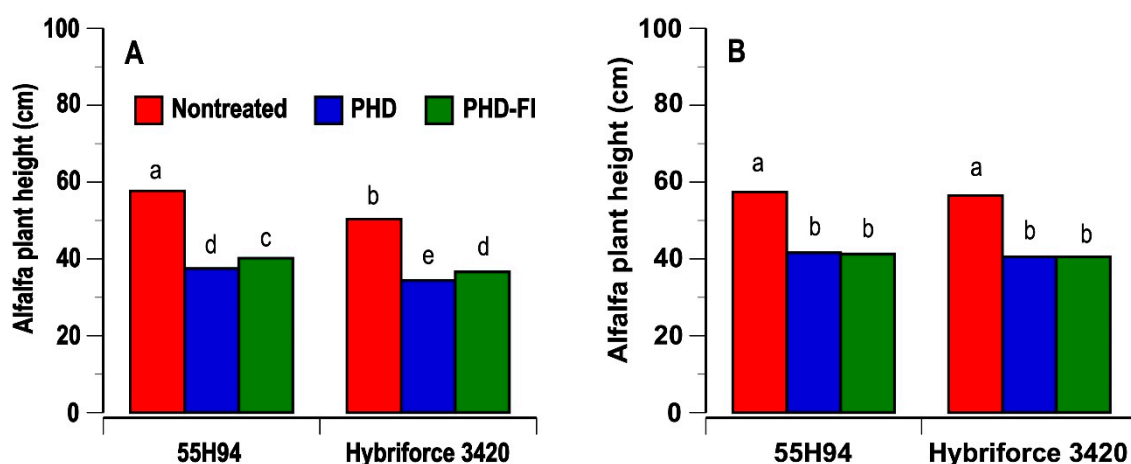


**Figure 2.** Plant height of interseeded alfalfa in 2017 (**A**) and 2018 (**B**), as influenced by variety and agrichemical treatments. Agrichemical treatments applied to alfalfa during establishment consisted of a nontreated control, prohexadione (PHD), and prohexadione followed by fungicide and insecticide (PHD-FI). Data were collected about 2.5 weeks after PHD application and were averaged across corn populations. Least square means within each panel with no common letter are significantly different ($p = 0.05$).

Alfalfa defoliation and leaf disease ratings in mid-July, taken approximately four weeks after PHD application and two weeks after FI application, were influenced by a year X agrichemical treatment interaction (Figure 3). As anticipated, the application of PHD-FI substantially reduced defoliation and the extent of damage from common leaf spot on the remaining leaves relative to the nontreated control, while alfalfa treated only with PHD had intermediate ratings. Defoliation in mid-July was more extensive in 2018 than in 2017, while the converse occurred for necrosis from common leaf spot. Necrosis from PLH feeding in 2017 was reduced by sequential PHD-FI treatment relative to the nontreated control, but it was unexpectedly increased by PHD treatment. By contrast, necrosis from PLH feeding was very low for all agrichemical treatments in 2018. While further direct confirmation is needed, reductions in defoliation and common leaf spot damage associated with PHD treatment of alfalfa might be related to the thickening of epidermal cell walls and the biosynthesis of biocidal compounds, or to a more open canopy that improved the air circulation and penetration of fungicidal treatments [32]. Epidermal thickening and biocidal compounds produced in conjunction with PHD treatment can also deter feeding by insect pests [32], but our results suggest that PHD treatment may increase PLH feeding or the hypersensitive response of alfalfa.
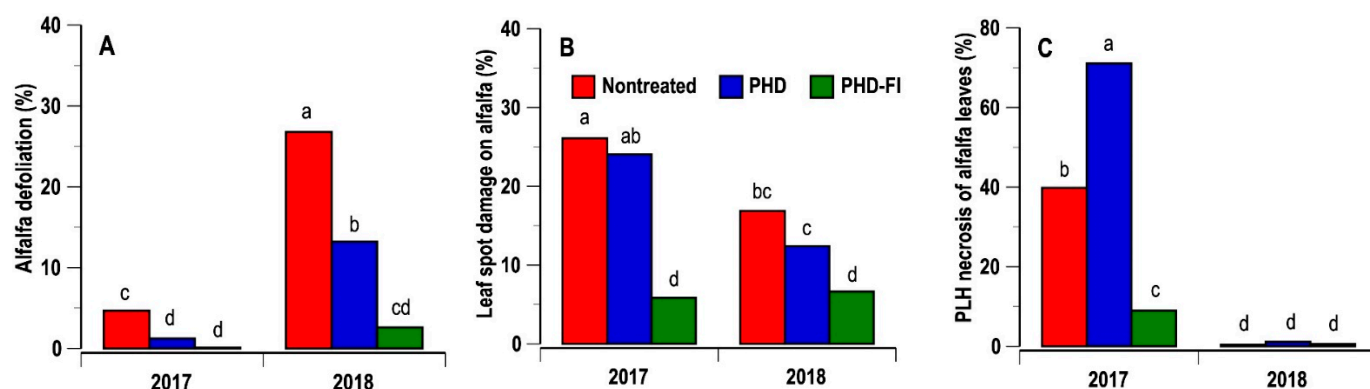
**Figure 3.** Production year and agrichemical treatment impacts on alfalfa defoliation (**A**) and damage of remaining leaves by common leaf spot (**B**) and potato leafhopper (PLH, **C**). Agrichemical treatments applied to alfalfa during establishment included a nontreated control, prohexadione (PHD), and prohexadione followed by fungicide and insecticide (PHD-FI). Data were collected two weeks after application of fungicide and insecticide and were averaged across alfalfa varieties and corn populations. Least square means with no common letter are significantly different ($p$ = 0.05).

The canopy health of alfalfa in mid-July was also influenced by a year X alfalfa variety interaction. In 2017, both alfalfa varieties had similar levels of defoliation (average of 1.6%) and necrosis from common leaf spot (average of 17.2%) but, in 2018, Hybriforce 3420 had less defoliation (8.5 vs. 16.5%) and common leaf spot necrosis (9.3 vs. 14.3%) than 55H94. As expected, leaf necrosis in mid-July of 2017 from PLH feeding was lower in the resistant variety, 55H94, than in the susceptible variety, Hybriforce 3420 (27.1 vs. 50.7%). Both varieties had similarly low levels of leaf necrosis in-mid July of 2018 (average of 0.5%), apparently due to the minimal feeding by PLH that year. Corn population only influenced canopy health via an interaction with alfalfa variety for PLH necrosis, which averaged at 6% for 55H94 but declined from 16.8 to 7.7% for Hybriforce 3420 as corn population increased. This suggests that PLH feeding on the susceptible variety Hybriforce 3420 may have been deterred at higher corn populations.

Alfalfa defoliation in late July or early August, approximately four weeks after FI treatment, was influenced by a corn population X agrichemical treatment interaction and a year X alfalfa variety interaction. Defoliation linearly increased as corn population increased, but it was substantially lower for PHD-FI treated alfalfa than for PHD-treated or nontreated alfalfa (Figure 4). Defoliation four weeks after FI treatment was surprisingly similar in both years, given the contrasting levels of precipitation received each year during July and the differing degrees of defoliation and leaf necrosis observed earlier in mid-July. In 2017 both alfalfa varieties had similar levels of defoliation (average of 62.3%), but in 2018 defoliation of 55H94 was greater than Hybriforce 3420 (75.7 vs. 66.9%). The effect of agrichemical treatments on the canopy characteristics of alfalfa during 2017 is shown in Figure 5.

### 3.4. Plant Density of Interseeded Alfalfa

Alfalfa plant density determined in late June exceeded 500 plants m$^{-2}$ in 2017 and 2018, indicating excellent initial establishment under corn in both years. In October, one month following corn silage harvest, average stand density of 55H94 exceeded Hybriforce 3420 (97 vs. 85 plants m$^{-2}$) although both varieties responded similarly to agrichemical treatment and corn population. Averaged across varieties, October stand density ranged from 0.6 to 353 plants m$^{-2}$ after corn silage harvest due to a substantial year X corn population X agrichemical treatment interaction (Figure 6). Nontreated alfalfa had the lowest stand density, which quadratically declined from 156 to 5 plants m$^{-2}$ as corn population increased in 2017, but averaged only 6.6 plants m$^{-2}$ across corn populations in 2018. Treatment with PHD and especially PHD-FI improved plant density to a greater degree in 2017 than in 2018, but alfalfa experienced a linear decline in plant survival in both years as corn population increased. As illustrated in Figure 7, stands of interseeded alfalfa in late October were

uniform and vigorous in 2017 when PHD or PHD-FI were applied, but, in 2018, plant distribution and growth were variable even with these agrichemical treatments. Most mortality of interseeded alfalfa occurs between late July and corn silage harvest, and appears to be exacerbated by above-average precipitation that favors vigorous competition from corn [8,9,16]. Improved survival of interseeded alfalfa under corn following PHD treatment has consistently been observed in Wisconsin, but not in other locations in the USA [8,9,15,16,22,33]. The current study is the first to demonstrate that foliar applications of PHD followed by FI substantially improves the stand density of interseeded alfalfa, but the results from 2018 indicate this treatment will not always prevent the excessive plant mortality of alfalfa during its establishment under corn.
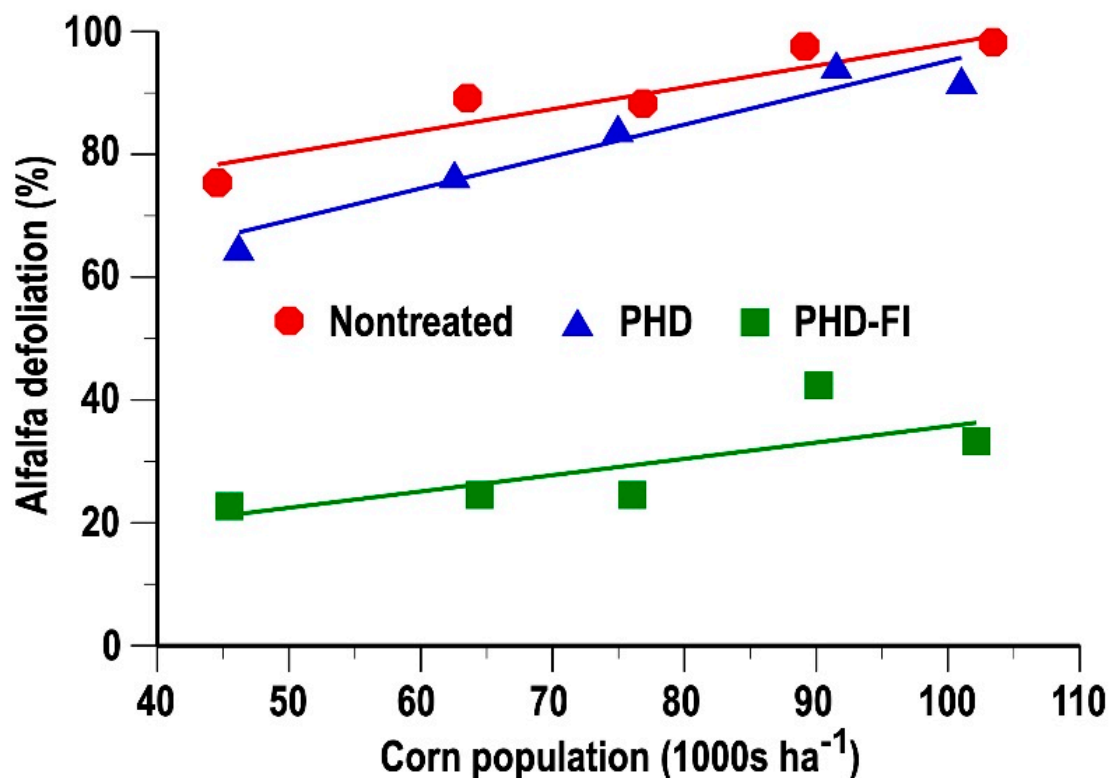


**Figure 4.** Defoliation of alfalfa four weeks after fungicide and insecticide application as affected by corn population and agrichemical treatments. Agrichemical treatments applied to alfalfa during establishment consisted of a nontreated control, prohexadione (PHD), and prohexadione, followed by fungicide and insecticide (PHD-FI). Data were averaged across alfalfa varieties and production years. Regression intercepts and slopes differed between agrichemical treatments (*p* = 0.05).
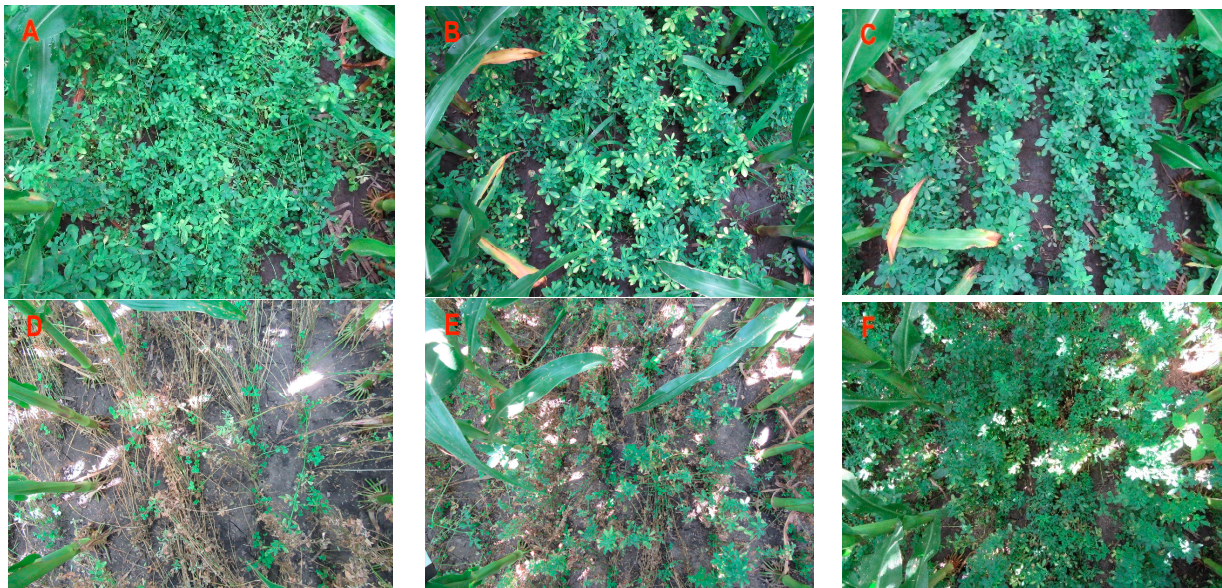
**Figure 5.** Photographs of interseeded alfalfa taken at the time of defoliation and leaf necrosis ratings on 19 July 2017 (**A**–**C**) and 2 August 2017 (**D**–**F**). Agrichemical treatments consisted of a nontreated control (**A**,**D**), prohexadione (**B**,**E**), and prohexadione, followed by fungicide and insecticide (**C**,**F**). Corn population was approximately 79,000 plants ha$^{-1}$ at harvest in 2017.
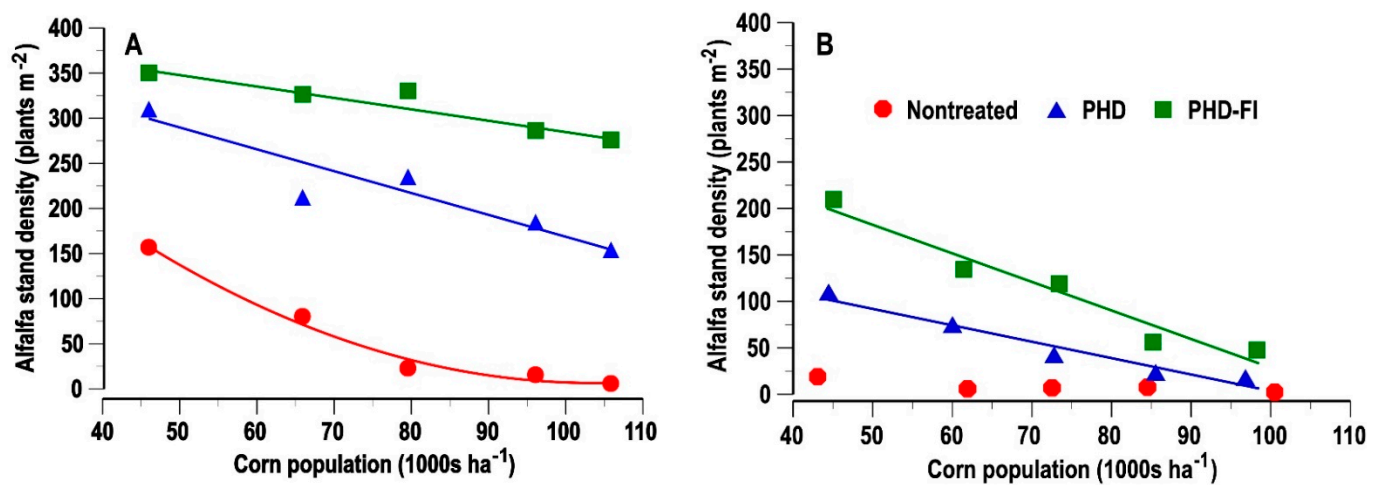


**Figure 6.** Corn population and agrichemical treatments effects on plant density of interseeded alfalfa in October 2017 (**A**) and October 2018 (**B**) following corn silage harvest. Agrichemical treatments applied to interseeded alfalfa included a nontreated control, prohexadione (PHD), and prohexadione followed by fungicide and insecticide (PHD-FI). Data were averaged across alfalfa varieties. Regression intercepts and/or slopes differed between agrichemical treatments ($p = 0.05$).
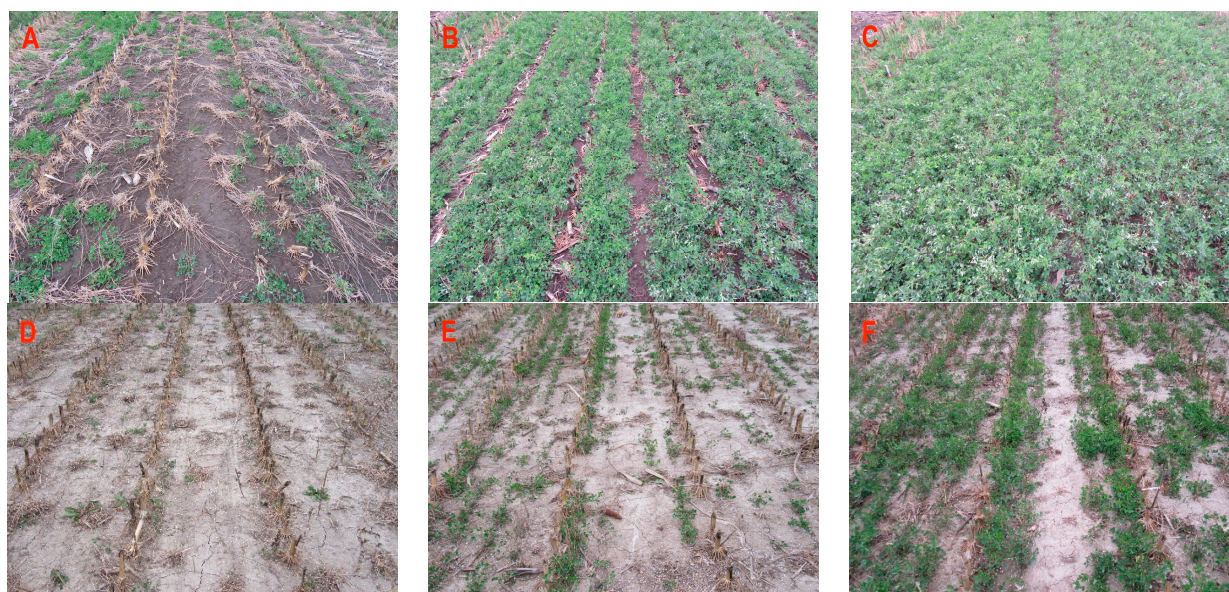
**Figure 7.** Photographs of interseeded alfalfa in late October 2017 (**A**–**C**) and late October 2018 (**D**–**F**) approximately seven weeks after corn silage harvest. Agrichemical treatments applied to alfalfa during establishment consisted of a nontreated control (**A**,**D**), prohexadione (**B**,**E**), and prohexadione, followed by fungicide and insecticide (**C**,**F**). Corn population was approximately 79,000 plants ha$^{-1}$ at harvest in 2017 and 72,900 plants ha$^{-1}$ at harvest in 2018.

### 3.5. Relationship of Alfalfa Stand Density to Corn and Alfalfa Characteristics

Multiple linear regression indicated that plant density of interseeded alfalfa in October following corn harvest had the following relationship to categorical and continuous variables: Alfalfa plants m$^{-2}$ = 1038 − 1.074(% defoliation at four weeks after FI application) − 3.270(average monthly precipitation in mm from May to October) − 4.925(alfalfa height in cm at two to three weeks post-PHD application) − 0.0017(actual corn population at harvest in plants ha$^{-1}$) − 42.255(alfalfa variety) − 2.338(% common leaf spot at two weeks after FI application), $N$ = 220, $R^2$ = 0.80, $p < 0.001$, with alfalfa varieties 55H94 and Hybriforce 3420, coded as 0 or 1, respectively. Based on partial $R^2$ values, the continuous predictor variables of defoliation, precipitation, alfalfa height, corn population, and common leaf spot accounted for 36.1, 27.8, 7.5, 5.0, and 1.8%, respectively, of the variation in alfalfa plant density after establishment. The categorical predictor of alfalfa variety accounted for 2.3% of the variation in alfalfa plant density. The model did not account for 20% of the variation in alfalfa stand density, and so further work during the period of maximal plant mortality from late July until corn silage harvest might prove useful for the identification of additional factors that influence the establishment of interseeded alfalfa.

Overall, the multiple linear regression analysis indicated alfalfa plant density in October after corn harvest was negatively associated with foliar disease (defoliation and common leaf spot), precipitation, alfalfa plant height, and corn plant density. As noted above, the application of FI improved leaf retention and overall canopy health, while the application of PHD restricted the unproductive etiolation of alfalfa under corn. As noted above, average monthly precipitation was near normal in 2017, but 47% above normal in 2018, and its strong negative effect on alfalfa stand density in October was consistent with observations from previous interseeding studies in corn [8,9,16]. An inverse relationship between alfalfa plant survival and companion crop plant density (or to related factors such as PAR interception or leaf area index) was also noted in previous interseeding studies with corn or small grains [9,34,35]. Alfalfa stand density in October was not associated with corn silage yield or with defoliation and PLH necrosis two weeks after FI application. However, other studies with conventionally sown alfalfa indicated that feeding by PLH substantially reduced plant growth and seedling survival [36,37]. This suggests additional

work is needed to clarify the appropriate circumstances for applying insecticide to aid the survival and subsequent production of interseeded alfalfa.

The growth-altering and canopy-health-promoting effects of PHD-FI treatment combined with the negative impact of corn plant density suggests that the survival of interseeded alfalfa is largely determined by its capacity to sustain photosynthate production, storage, and utilization when grown under the corn canopy. In addition to moderate corn populations, other management factors that might favor the establishment of interseeded alfalfa would likely be the same as those favoring weed growth under corn. This would include planting corn in more widely spaced rows and using slower-developing corn hybrids that have a short stature, upright leaves, and a lower leaf area index [38,39]. Because interseeded alfalfa suppresses weed growth, implementing these practices should not excerbate weed issues in corn [14]. Further work is, however, needed to refine corn management practices that will ensure both the reliable establishment of interseeded alfalfa and good corn silage yields that are necessary for this system to be profitable for farms [10]. Additional studies are also needed to determine if lower, more cost-effective rates of PHD can be used in conjunction with FI treatment, and if the timing of these treatments can be further optimized to further improve the establishment of interseeded alfalfa.

### 3.6. Relationship of Alfalfa Dry Matter Yield to Stand Density

Dry matter yields of interseeded alfalfa on an individual plot basis in the first full production year after corn responded in a quadratic-plateau manner to stand density, whether measured as plants m$^{-2}$ in the fall of the prior establishment year or as stems m$^{-2}$, measured in early spring prior to the first harvest. First cut yield plateaued at a stand density of 200 plants m$^{-2}$ and approximately 850 stems m$^{-2}$, but the yield plateaued at 5.5 Mg ha$^{-1}$ in 2017 compared to 3.5 Mg ha$^{-1}$ in 2018 (Figure 8). Although the first cutting occurred on the same calendar date each year, visual assessment at harvest indicated that plants were at the late bud stage in 2018 vs. the late vegetative stage in 2019. Slower development and lower yield of alfalfa in 2019 could be attributed to the depressed growth during the prior fall and to relatively cool growing conditions in May prior to the first harvest (Figure 7 and Table S1). When a quadratic-plateau model is fit to data from a prior interseeding study in southcentral Wisconsin [16], first-cut yield of alfalfa plateaued at 5.6 Mg ha$^{-1}$ at a stand density of 209 plants m$^{-2}$ measured in the fall and at 180 plants m$^{-2}$ in the spring measured at the time of the first-cut harvest. As observed in studies of conventionally established alfalfa [3,40,41], the plant density of interseeded alfalfa declined between the fall of the establishment year and the fall of the first production year, and plant loss was most pronounced for stands with the highest plant densities following establishment (data not shown). As a result, the relationships between dry matter yield and plant or stem density measured after establishment were less consistent for the second, third, and fourth harvests, taken during the first full production year (data not shown). The relationships between dry matter yield and plant or stem density exhibited a high degree of scatter within all harvests, in part because herbicides failed to control all weeds at very low alfalfa plant densities (data not shown) and because other factors such as mass per stem have a substantial influence on alfalfa productivity [42,43]. When considered together, the results from this and previous work suggest that a stand density of roughly 200 plants m$^{-2}$ or about 850 stems m$^{-2}$ following establishment would be a desirable target for maximizing the first-cut yield of interseeded alfalfa during the first full production year in southcentral Wisconsin. The actual yields of alfalfa will, however, be dependent on a number of factors such as crop management practices, soil fertility, and growth environment, in addition to plant or stem density [42–44].
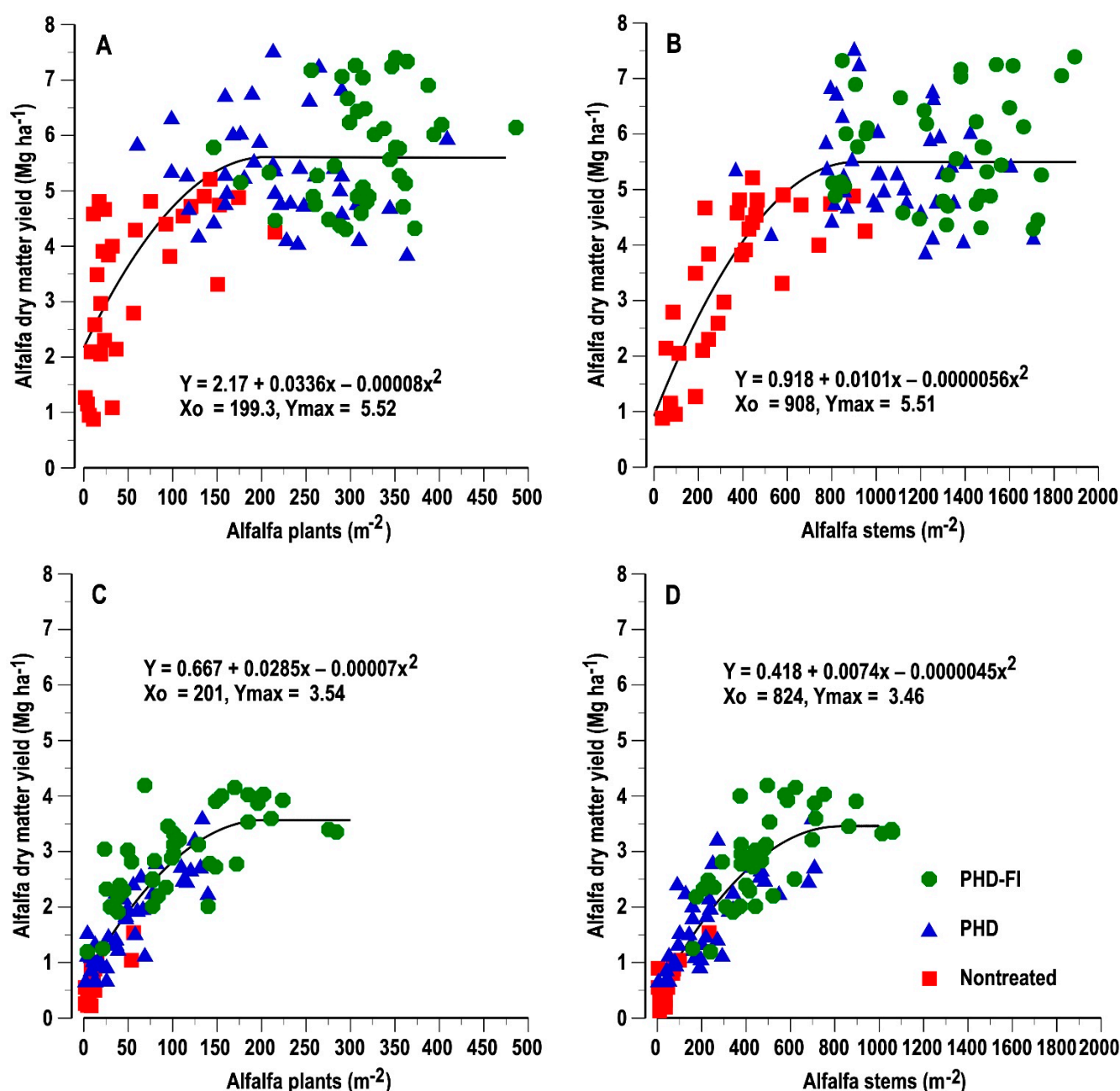
**Figure 8.** Relationship of first-cut dry-matter yield vs. plant or stem density of alfalfa interseeded into corn during 2017 and harvested in 2018 (**A**,**B**) and interseeded into corn in 2018 and harvested in 2019 (**C**,**D**). Agrichemical treatments applied during alfalfa establishment included a nontreated control, prohexadione (PHD), and prohexadione, followed by fungicide and insecticide (PHD-FI). Regressions were significant at *p* < 0.001.

Total alfalfa yield from four harvests in 2018 plateaued at 13.7 Mg ha$^{-1}$, with 227 plants m$^{-2}$ and 1023 stems m$^{-2}$. By contrast, total alfalfa yield from four harvests in 2019 plateaued at 11.0 Mg ha$^{-1}$, with 140 plants m$^{-2}$ and with 657 stems m$^{-2}$ (data not shown) and, as noted above, the differences in yield between years were mainly associated with the first harvest. For comparison, establishment-year yields from three harvests of conventionally spring-seeded alfalfa planted after corn averaged 7.9 ± 0.47 Mg ha$^{-1}$ in 2018 and 6.9 ± 1.05 Mg ha$^{-1}$ in 2019. Consequently, successful interseeding increased first-year yield of alfalfa following corn by 73% in 2018 and 59% in 2019. Previous studies in Wisconsin found that stand densities ranging from 130 to 320 plants m$^{-2}$ for interseeded alfalfa treated with PHD produced 10.2 to 12.7 Mg ha$^{-1}$ of dry matter during the first full

production year, exceeding the establishment year yields of conventionally spring-seeded alfalfa after corn by from 60 to 130% [8,9,45]. By comparison, recent studies in cold temperate regions of the USA with conventionally spring-seeded alfalfa found that a stand density above 100 plants m$^{-2}$ in the fall of the establishment year usually maximized total dry matter yields of alfalfa during the following first full production year [3,41]. Our findings are more in line with older studies that indicated approximately 140 to 250 plants m$^{-2}$ would be needed after the conventional establishment of spring-seeded alfalfa to maximize yields during the first full production year [46].

## 4. Conclusions

We found that foliar applications of PHD followed by FI supported the superior establishment of interseeded alfalfa in southern Wisconsin by limiting stem etiolation, leaf disease, and defoliation of alfalfa under corn. Alfalfa establishment was also favored by lower corn populations. These management practices, however, reduced corn silage yields and were not sufficient to prevent excessive mortality of interseeded alfalfa during one growing season with excessive precipitation. Following establishment in corn, first-cut dry matter yields of interseeded alfalfa the following year were maximized at a stand density of approximately 200 plants m$^{-2}$ or 850 stems m$^{-2}$, and the total yields from four cuttings were from 59 to 75% greater than three cuttings from conventionally spring-seeded alfalfa after corn. Further work is needed to define the optimal rate and timing of PHD-FI application on interseeded alfalfa. It would also be desirable to identify and attempt to ameliorate factors that contribute to the excessive mortality of interseeded alfalfa during relatively wet growing conditions and to develop additional management practices that will ensure both the reliable establishment of interseeded alfalfa and good yields of corn silage.

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