

## Article

# Establishment of Alfalfa Intercropped under Corn in Response to Varying Rates of Prohexadione with or without Fungicide Plus Insecticide

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**Abstract:** Establishment of interseeded alfalfa (*Medicago sativa* L.) under corn (*Zea mays* L.) silage is enhanced with foliar applications of prohexadione (PHD) followed by fungicide plus insecticide (FI), but the lowest effective rates must be determined. We evaluated stand characteristics of alfalfa interseeded into corn at Arlington, Wisconsin, USA in response to PHD applied at 0 to 0.423 kg a.e. ha<sup>-1</sup> followed two weeks later with FI (none vs. 0.147 kg a.i. ha<sup>-1</sup> fluxapyroxad-pyraclostrobin plus 0.018 kg a.i. ha<sup>-1</sup> lambda-cyhalothrin). Application of PHD reduced etiolation, while FI treatment increased plant health and vigor. Following corn harvest, non-treated alfalfa stands averaged 4.2 plants m<sup>-2</sup> and 1.2% groundcover under wet growing conditions in 2019 compared with 71.3 plants m<sup>-2</sup> and 15.9% groundcover under normal growing conditions in 2020. Stand density in 2019 reached 130 plants m<sup>-2</sup> but failed to plateau with combined PHD-FI treatments, while in 2020, stand density averaged 177 plants m<sup>-2</sup> with FI regardless of the PHD rate. Alfalfa groundcover plateaued at 63% in 2019 and 71% in 2020 when 0.16 to 0.30 kg a.e. PHD ha<sup>-1</sup> was applied prior to FI. The results indicate that FI enables excellent alfalfa establishment under normal conditions, but both PHD and FI should be applied during wet growing conditions.



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**Keywords:** lucerne; maize; intercropping; growth retardant; pesticide

## 1. Introduction

In cold temperate regions of the USA, low dry-matter yields of alfalfa during its seeding year have contributed to a continued shift away from more diversified crop rotations based on this perennial legume toward forage production largely dominated by continuous annual cropping of corn grown for silage. One approach for reversing this trend would be to seed and establish alfalfa with a relatively high-yielding corn silage companion crop. In this system, corn is typically planted during early May, and alfalfa is interseeded immediately or up to the VE growth stage of the corn. After the corn silage is harvested in early September, the alfalfa completes its establishment before winter, and then full-forage production of alfalfa commences the following spring [1]. As highlighted in a recent review [2], intercropping alfalfa in a corn silage companion crop can provide multiple production and environmental benefits, including reduced weed growth, soil erosion, and nutrient loss from cropland and increased overall dry-matter yield and profitability of forage crop rotations.

Key aspects for successfully implementing this system have been identified in recent years [1], and among these are agrichemical treatments for enhancing plant vigor and health of the alfalfa growing under the corn canopy [3]. Early studies focused solely on utilizing relatively high rates of the gibberellin inhibitor PHD because of its low toxicity and reported ability to reduce stem elongation, stimulate root growth, and increase photosynthesis and stress resistance of various crops [4–7]. Directed spray applications of PHD onto 10 to

25 cm tall interseeded alfalfa, made in mid-June at the onset of rapid growth, reduced stem elongation and chlorosis due to shading and often improved seedling survival under corn [1,8–10]. These findings led to the labeling of PHD for use in this intercropping system. But such applications of PHD are costly and proved ineffective for preventing excessive stand loss of alfalfa under corn during growing seasons with above average precipitation and prolonged wet growing conditions [9]. In this situation, damp conditions and shading under the corn canopy led to extensive foliar disease, defoliation, and stand loss of the interseeded alfalfa prior to corn harvest. Work in solo-seeded alfalfa production systems [11–13] suggests that timely application of fungicides could be useful for reducing foliar disease and defoliation and for improving the vigor of interseeded alfalfa during its establishment under corn, particularly under damp growing conditions. Stress from high populations of insect pests such as potato leafhoppers (PLH, *Empoasca fabae* Harris) might also contribute to stand failure of interseeded alfalfa, but this might be remedied by using PLH-resistant varieties or applying insecticides [14–16].

Based on these observations, we recently conducted a trial to test the efficacy of applying FI after PHD for improving the canopy health and seedling survival of alfalfa established under corn [3]. The applications utilized labeled rates of the pesticides for alfalfa and were timed just before the corn canopy closure, which, based on multiyear observations in Wisconsin, is the typical onset of foliar disease and PLH damage of alfalfa. This sequential treatment proved to be a breakthrough for improving the establishment of alfalfa under corn during both normal and wet growing conditions. As PHD is expensive compared with other agrichemicals, further work is needed to determine the lowest effective rate of PHD to apply in conjunction with FI. Therefore, the objectives of this study were to (1) further examine foliar and plant density responses of interseeded alfalfa to factorial combinations of PHD and FI, and (2) define the lowest effective rate of PHD to apply prior to FI to facilitate good establishment of interseeded alfalfa under corn.

## 2. Materials and Methods

### 2.1. Sites and Crop Management

Independent experiments were initiated in 2019 and 2020 at the Arlington Agricultural Research Station (43°18' N, 89°20' W) on a Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls). The Arlington Station was chosen for this study because previous multilocation intercropping studies [8,17] indicated conditions at this location fostered relatively high levels of foliar damage and death of alfalfa seedlings under corn. Corn silage was grown prior to the 2019 study, and soybeans were grown prior to the 2020 study. The fall prior to planting, the soils at the 2019 and 2020 sites had respective pH values of 6.5 and 6.8, Bray-1 P levels of 20 and 24 mg kg<sup>-1</sup>, and K levels of 112 and 104 mg kg<sup>-1</sup>. Prior to planting, the sites were amended with lime, 200 kg N ha<sup>-1</sup>, and P, K, B, and S containing fertilizers based on Wisconsin nutrient recommendations for corn silage production and alfalfa establishment [18]. Weeds were controlled with broadcast applications of glyphosate plus microencapsulated acetochlor sprayed immediately after alfalfa seeding, followed four weeks later by a broadcast application of bromoxynil [19].

Each year, four rows of corn in a 0.76 m row spacing were planted with a no-till drill into 3 m wide by 6 m long whole plots according to a randomized complete block design with four replications. Corn borders at least 3 m wide were planted around the perimeter of the experimental sites to provide uniform shading of the interseeded alfalfa during establishment. A 105 d maturity corn hybrid, 'A635-54 STXRIB' (Agrigold, St. Francisville, IL, USA), having multiple modes of protection from above- and below-ground insect pests, was planted at 86,500 seed ha<sup>-1</sup> on 13 May 2019 and 82,800 seed ha<sup>-1</sup> on 11 May 2020. According to product literature, the hybrid has medium-tall plant height, upright leaves, semi-flex ears, and good silage yield potential and agronomic characteristics. Two alfalfa varieties (PLH-resistant '55H94' and non-PLH-resistant 'Hybriforce 3420', Corteva Agriscience, Johnston, IA, USA) were randomly assigned to two 1.5 m wide by 6 m long subplots within each whole plot. Rhizobia-inoculated and fungicide-treated alfalfa seeds

of each variety were planted in a 16.5 cm row spacing at 18 kg live seed ha<sup>-1</sup> on 14 May 2019 and 12 May 2020 using a no-till drill. Previous studies in Wisconsin demonstrated that both alfalfa varieties consistently have above average plant survival when interseeded into corn silage [8].

Alfalfa in whole plots was treated with six rates of 'Kudos' PHD (Fine Americas, Walnut Grove, CA, USA) applied at 0, 0.065, 0.130, 0.195, 0.260, or 0.423 kg a.e. ha<sup>-1</sup>, followed approximately two weeks later with FI. The FI treatment was a mixture of 0.147 kg a.i. ha<sup>-1</sup> 'Priaxor' fungicide (fluxapyroxad and pyraclostrobin, BASF, Beaumont, TX, USA) and 0.018 kg a.i. ha<sup>-1</sup> of 'Warrior II' insecticide (lambda-cyhalothrin, Syngenta, Greensboro, NC, USA). Additional whole plots were treated with 0, 0.130, 0.260, or 0.425 kg a.e. ha<sup>-1</sup> of PHD without a subsequent FI treatment. Solutions of PHD or FI were applied in a spray volume of 187 L ha<sup>-1</sup> using a CO<sub>2</sub> pressurized backpack sprayer equipped with flat-tip nozzles (Teejet XR11005VS or 8002E, Teejet Technologies, Glendale Heights, IL, USA). Solutions of PHD were prepared and applied with a directed application to the alfalfa as previously described [20]. Applications of PHD were made on 20 June 2019 at average plant heights of 21 cm for alfalfa and 33 cm for corn and on 18 June 2020 at average plant heights of 25 cm for alfalfa and 59 cm for corn. Applications of FI were broadcast applied over whole plots on 4 July 2019 at average plant heights of 36 cm for alfalfa and 105 cm for corn and on 30 June 2020 at average plant heights of 32 cm for alfalfa and 126 cm for corn. Application rates and timing for PHD and FI were based on previous research [3,9,10] and on the pesticide product labels for alfalfa and corn.

Corn was chopped at a cutting height of 15 cm and removed from the plots on 17 September 2019 and 18 September 2020 using a research plot harvester. Chopped corn from three whole plots per block was weighed and subsamples were dried at 55 °C in a forced draft oven to provide an estimate of dry-matter yield for the experimental sites. Interseeded alfalfa was not clipped or harvested during its establishment under corn.

## 2.2. Alfalfa Measurements and Statistical Analyses

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 11 July 2019 and 17 July 2020. Visual ratings of the alfalfa leaves for injury (disease plus insect damage) were taken on 19 July 2019 and 27 July 2020. A visual rating of percent defoliation was also taken on 10 August 2020. Sweep net data between mid-June and mid-August for PLH populations on alfalfa fields near the research site were provided by cooperators listed in the acknowledgments. Alfalfa plant density and groundcover following corn harvest were determined for the 2019 seedings on 18 October 2019 and 20 April 2020 and for the 2020 seedings on 9 October 2020 and 12 April 2021. Groundcover was estimated visually, while plant density was determined by removing soil along 60 cm lengths of four rows with a trowel and counting the crowns of individual plants.

Data were analyzed using PROC MIXED (SAS Institute, Cary, NC, USA). Data for plant height, foliar damage, groundcover, and plant density of alfalfa were analyzed according to a split-plot factorial design with four replications. Fixed effect factors included seeding year (2019 and 2020), whole plot combinations of PHD (0, 0.130, 0.260, or 0.425 kg a.e. ha<sup>-1</sup>) and FI (with and without), and subplots of two alfalfa varieties (55H94 and 3420). Block (year) and block (year) × PHD × FI were considered random effects. The timing of plant density counts (fall vs. spring) and its interactions with other fixed effects were included in the model as a repeated measure using an AR(1) covariance structure to assess the extent of stand loss due to winterkill. Because herbage growth is highly dependent on growing conditions, groundcover estimates were averaged across the fall and spring observations prior to analysis. Homogeneity of variance and possible outliers (studentized residuals > 4) were assessed with residual plots and influence diagnostics, and in several cases, up to 1% of observations were removed as severe outliers from the dataset. Data transformations used to obtain homogeneity of variance included  $(Y + 0.5)^{1/2}$  for alfalfa plant density and arcsin for percent groundcover and percent foliar damage; back transformed means are

reported in this publication. When  $F$ -tests were significant ( $p \leq 0.05$ ), then least square means of fixed effects were compared at  $p = 0.05$  using pdiff or slice statements. Analysis of variance results and treatment differences described in Section 3 were significant at  $p \leq 0.05$ .

Alfalfa plant density and groundcover data averaged across sampling times were also analyzed with a sequential (Type 1) polynomial regression approach [21] using PROC MIXED. In this analysis, the quantitative factor was PHD applied at six rates (0, 0.065, 0.130, 0.195, 0.260, or 0.423 kg a.e. ha<sup>-1</sup>) followed by FI. The qualitative factors of year and alfalfa variety and their interactions were considered fixed effects. Interactions between PHD rate and fixed effects were used to test for unique slopes for individual years or alfalfa varieties. Block (year) and block (year)  $\times$  PHD plus FI were considered random effects. Based on the polynomial regression results, groundcover observations from each block and alfalfa variety averaged across sampling times were fit by year to a quadratic plus plateau regression model using PROC NLIN (SAS Institute, Cary, NC, USA). The model was used to estimate the value of the plateau and the join point where PHD applied with FI maximized groundcover. Regression models described in Section 3 were significant at  $p \leq 0.05$ .

### 3. Results and Discussion

#### 3.1. Weather Conditions, Corn Silage Competitiveness, and PLH Populations

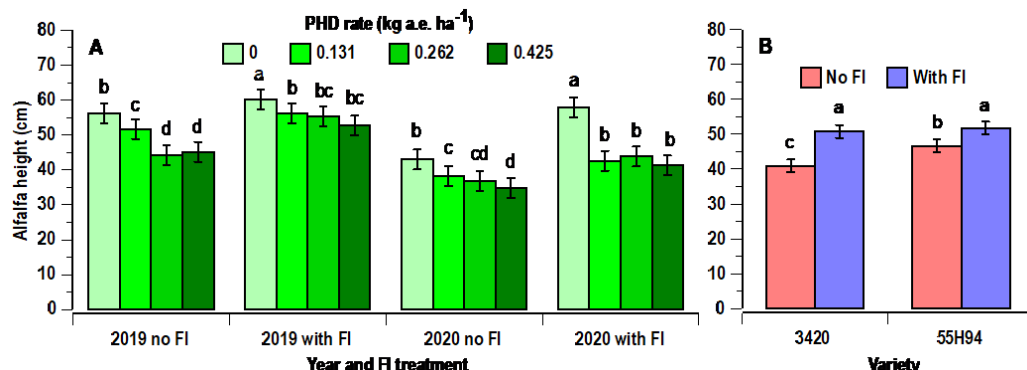
Average monthly temperature and precipitation for 2019 to 2020 at Arlington, Wisconsin are reported in Table S1. During alfalfa establishment from May through October, temperatures were generally close to normal, averaging 16.7 °C in 2019 and 16.6 °C in 2020. Precipitation during this period totaled 842 mm in 2019 and 660 mm in 2020, 41% and 11%, respectively, above normal. Excess precipitation occurred in May and in July through October during 2019, while precipitation during 2020 was evenly distributed throughout the growing season. Alfalfa was interseeded into a corn companion crop that had average harvest populations of 81,100 plants ha<sup>-1</sup> in 2019 and 78,500 plants ha<sup>-1</sup> in 2020. Corn silage dry-matter yields averaged 20.0 Mg ha<sup>-1</sup> in 2019 and 20.1 Mg ha<sup>-1</sup> in 2020. Populations of PLH in alfalfa fields near the research sites peaked in early July at 0.8 and 0.65 insects per sweep in 2019 and 2020, respectively.

Overall, the establishment of interseeded alfalfa occurred under near normal temperatures with excessive precipitation in 2019 and near normal precipitation in 2020. Corn populations and yields in both years were moderate and suitable for good to excellent establishment of interseeded alfalfa treated with PHD followed by FI [3]. Previous work [10,13,22–24] found that directed spray applications of prohexadione on alfalfa and broadcast applications of fungicide and insecticide had little or no impact on corn silage yield. Prior to and 6 weeks following the application of FI, populations of PLH near the research sites were below the economic thresholds set for insecticide treatment of alfalfa over 20 cm in height [25].

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

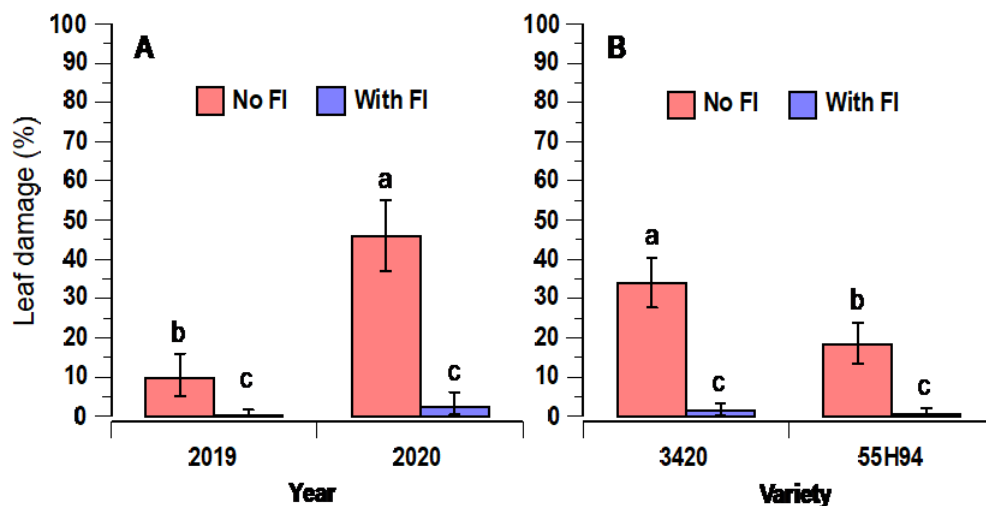
Applications of PHD and FI were made about 5 and 7 weeks, respectively, after seeding the alfalfa. Alfalfa plant height, measured in mid-July approximately 9 weeks after seeding, was influenced by a seeding year  $\times$  alfalfa variety interaction. Average plant height of 55H94 exceeded that of 3420 in 2019 (55.3 vs. 50.1 cm), but in 2020 both varieties were shorter and had a similar average plant height of 42.3 cm. Alfalfa plant height was also influenced by a seeding year  $\times$  PHD rate  $\times$  FI treatment interaction and by an alfalfa variety  $\times$  FI treatment interaction. Application of PHD reduced plant height, but the response differed between years and FI treatments (Figure 1A). In most cases, the growth reduction was most pronounced with the lowest rate of PHD (0.131 kg a.e. ha<sup>-1</sup>) applied to the alfalfa. Alfalfa responds to shading by elongating its stems, and previous studies also found that PHD reduced the plant height of alfalfa growing under corn [3,10]. Conversely, treatment with FI increased the average plant height of both alfalfa varieties (Figure 1B),

but the response of the 3420 was greater than that of the 55H94. Both varieties had similar plant height with FI treatment, but without FI treatment, the height of the 3420 was less than that of the 55H94.



**Figure 1.** Plant height of interseeded alfalfa in mid-July. (A) Seeding year × fungicide plus insecticide (FI) × prohexadione (PHD) rate interaction averaged across alfalfa varieties. Least square means within years with no common letter are significantly different ( $p = 0.05$ ). (B) Alfalfa variety × FI interaction averaged across seeding years and PHD rates. Least square means with no common letter are significantly different ( $p = 0.05$ ). Bars indicate 95% confidence intervals of least square means.

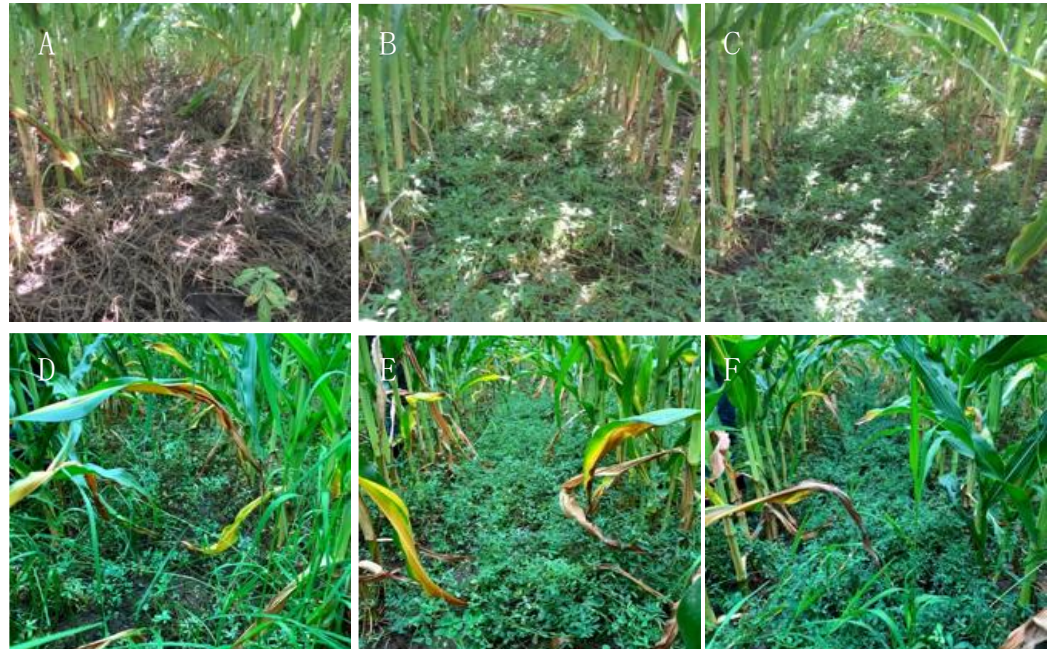
Alfalfa leaf damage was primarily influenced by interactions of seeding year × FI treatment and variety × FI treatment (Figure 2). In the absence of FI treatment, average leaf damage was greater in 2020 than in 2019 (Figure 2A) and greater in variety 3420 than in variety 55H94 (Figure 2B). Differences between seeding years could in part be attributed to damage ratings being performed earlier in 2019 than in 2020 (mid- vs. late July). The application of FI reduced leaf damage in July to negligible levels in both seeding years and in both varieties. Defoliation in early August 2020 averaged 48% without FI and 6% with FI treatment.



**Figure 2.** Leaf damage of alfalfa in mid-July 2019 and late July 2020. (A) Seeding year × fungicide plus insecticide (FI) interaction averaged across prohexadione rates and alfalfa varieties. (B) Alfalfa variety × FI interaction averaged across seeding years and prohexadione rates. Back-transformed least square means in both panels with no common letter are significantly different ( $p = 0.05$ ). Bars indicate back-transformed 95% confidence intervals of least square means.

While not assessed by visual ratings in 2019, the photographs in Figure 3 suggest defoliation of alfalfa grown without FI was more severe in 2019 than in 2020. The photographs also illustrate how the application of FI improved foliar health and reduced defoliation

of interseeded alfalfa. Similar results were observed in our previous study when FI was applied after PHD [3]. Treatment with PHD had comparatively little effect on foliar damage of alfalfa (data not shown), which is in contrast with our earlier study where PHD treatment increased foliar damage of alfalfa that was subjected to high pressure from PLH [3]. The alfalfa variety and PHD rate also had little effect on defoliation in 2020 (data not shown).



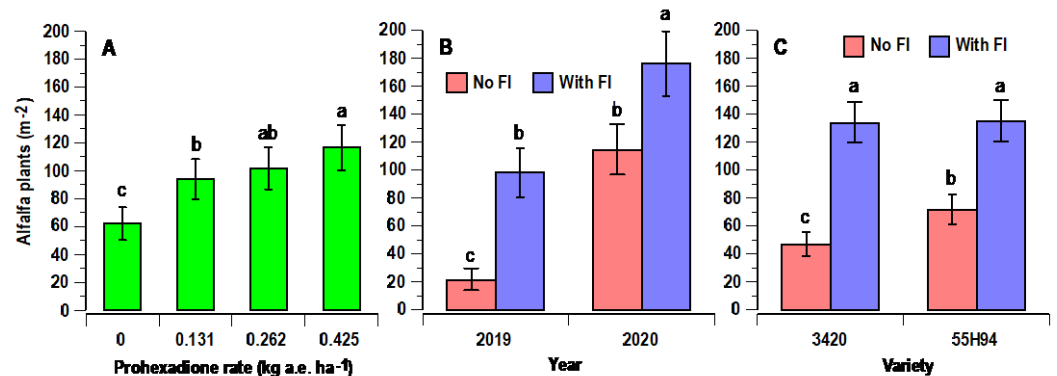
**Figure 3.** Photographs of interseeded alfalfa variety 55H94 under corn taken on 15 August during the 2019 seeding year (A–C) and 10 August during the 2020 seeding year (D–F). Agrichemical treatments consisted of a non-treated control (A,D), fungicide plus insecticide (B,E), and prohexadione at 0.262 kg a.e. ha<sup>-1</sup> followed by fungicide plus insecticide (C,F).

### 3.3. Stand Density and Groundcover of Alfalfa Following Establishment

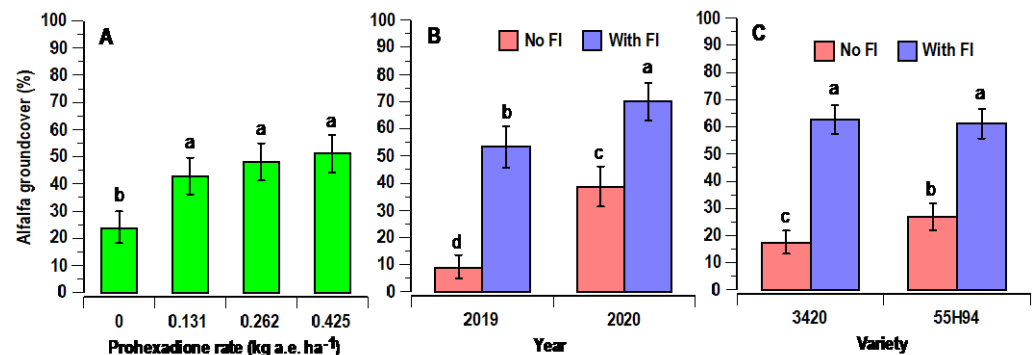
Alfalfa plant density after establishment under corn was primarily influenced by the main effects of sampling time and PHD rate, and by interactions between seeding year  $\times$  FI treatment and variety  $\times$  FI treatment. When averaged across other factors, stand density declined from 101 plants m<sup>-2</sup> in October to 84 plants m<sup>-2</sup> in April, indicating some stand loss occurred due to winterkill. Treatment with 0.423 kg a.e. ha<sup>-1</sup> of PHD increased the average stand density by 88% compared with non-treated controls (from 62 to 117 plants m<sup>-2</sup>); increases in stand density were most pronounced with the lowest rate of PHD applied to alfalfa (Figure 4A). Stand density without FI treatment averaged only 21 plants m<sup>-2</sup> in 2019 compared with 114 plants m<sup>-2</sup> in 2020, but treatment with FI increased the average stand density by 4.7-fold in 2019 and by 1.5-fold in 2020 (Figure 4B). Without FI treatment, 3420 had lower average stand density than 55H94, but FI treatment increased the stand density of both varieties to comparable levels (Figure 4C). Stand density was also influenced by a seeding year  $\times$  variety interaction. Average stand density was much lower in 2019 than in 2020, but the difference was slightly less pronounced for variety 55H94 (62 vs. 150 plants m<sup>-2</sup>) than for variety 3420 (44 vs. 138 plants m<sup>-2</sup>).

Groundcover of alfalfa, averaged across the fall and spring observation periods, was also influenced by the main effect of PHD rate and interactions between seeding year  $\times$  FI treatment and variety  $\times$  FI treatment. Treatment with 0.423 kg a.e. ha<sup>-1</sup> of PHD doubled the groundcover compared with non-treated controls (51 vs. 24%). In contrast with plant density, significant gains in groundcover were observed only with the lowest rate of PHD applied to alfalfa (Figure 5A). Alfalfa groundcover without FI treatment averaged only 9% in 2019 compared with 39% in 2020, but treatment with FI increased groundcover by 6.1-fold in 2019 and by 1.8-fold in 2020 (Figure 5B). Without FI treatment, 3420 had lower

groundcover than 55H94, but FI treatment increased the groundcover of both varieties to similar levels (Figure 5C).



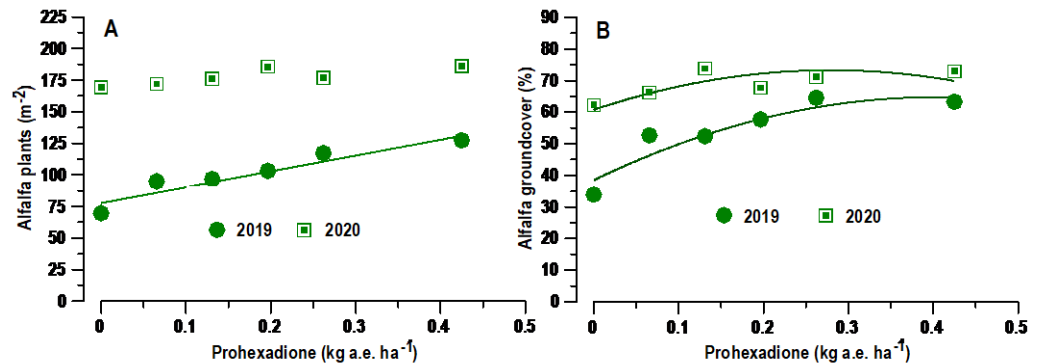
**Figure 4.** Plant density of established alfalfa after harvest of the corn silage companion crop, averaged across fall and spring sampling periods. (A) Prohexadione rate main effect averaged across seeding years, fungicide and insecticide (FI) rates, and varieties. (B) Seeding year  $\times$  FI interaction averaged across prohexadione rates and varieties. (C) Alfalfa variety  $\times$  FI treatment interaction averaged across seeding years and prohexadione rates. Back-transformed least square means with no common letter are significantly different ( $p = 0.05$ ). Bars indicate back-transformed 95% confidence intervals of least square means.



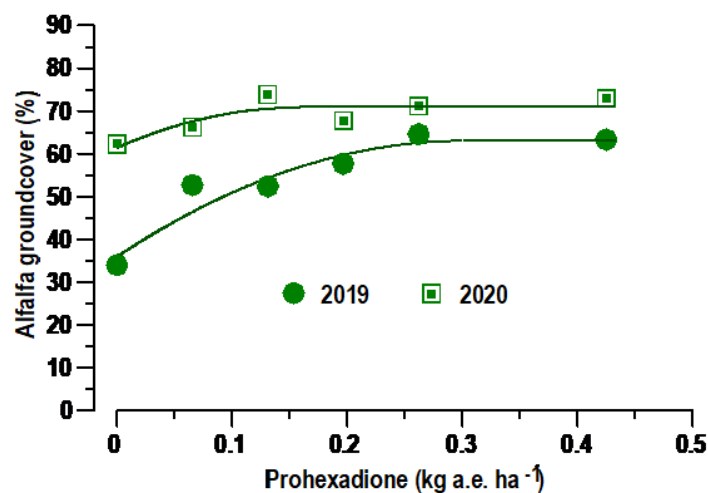
**Figure 5.** Groundcover of established alfalfa after harvest of the corn companion crop, averaged across fall and spring observation periods. (A) Prohexadione rate main effect averaged across seeding years, fungicide and insecticide (FI) rates, and varieties. (B) Seeding year  $\times$  FI interaction averaged across prohexadione rates and varieties. (C) Alfalfa variety  $\times$  FI treatment interaction averaged across seeding years and prohexadione rates. Back-transformed least square means with no common letter are significantly different ( $p = 0.05$ ). Bars indicate back-transformed 95% confidence intervals of least square means.

Data averaged across the fall and spring observation periods were then fit using regression models to assess the response of alfalfa plant density and groundcover following establishment to varying rates of PHD applied prior to FI (Figure 6). Polynomial regression indicated intercepts and slopes of both response variables differed by seeding year but were similar for both alfalfa varieties. Average stand density increased linearly from 78 to 130 plants  $m^{-2}$  in 2019 as the PHD rate was increased from 0 to 0.425 kg a.e.  $ha^{-1}$ , but in 2020, stand density averaged 177 plants  $m^{-2}$  with FI, regardless of the rate of PHD application (Figure 6A). Average groundcover increased quadratically at a declining rate from 38 to 65% in 2019 and from 61 to 70% in 2020 as the PHD rate was increased from 0 to 0.425 kg a.e.  $ha^{-1}$  (Figure 6B). By contrast, plant density and groundcover of alfalfa established without any agrichemical treatments averaged only 4.2 plants  $m^{-2}$  and 1.2% in 2019 compared with 71.3 plants  $m^{-2}$  and 15.9% in 2020. Based on the polynomial regression results, alfalfa groundcover data averaged across the fall and spring observation

periods were then fit by year to a segmented quadratic-plateau model (Figure 7). The model estimated groundcover reached a plateau of 63% at a PHD rate of 0.30 a.e. ha<sup>-1</sup> in 2019. In 2020, groundcover reached a plateau of 71% at a PHD rate of 0.16 a.e. ha<sup>-1</sup>.



**Figure 6.** Mean values and fitted regressions for alfalfa (A) plant density and (B) groundcover in response to the rate of prohexadione applied prior to FI treatment. The models for plant density were  $Y = 77.7 (\pm 6.9) + 125.1 (\pm 40.3)X$  in 2019 and  $Y = 176.6$  in 2020. The models for groundcover were  $Y = 38.5 (\pm 4.4) + 130.1 (\pm 22.3)X - 160.63 (\pm 45.9)X^2$  in 2019 and  $Y = 60.8 (\pm 4.4) + 89.7 (\pm 22.3)X - 160.63 (\pm 45.9)X^2$  in 2020. Intercepts and linear regression slopes for plant density and groundcover differed between years ( $p \leq 0.05$ ) but were not influenced by alfalfa variety.



**Figure 7.** Mean values and quadratic-plateau regression curves for alfalfa groundcover in response to the rate of prohexadione applied prior to FI treatment. The model for 2019 was  $Y = 35.9 (\pm 4.7) + 180.2 (\pm 74.3)X - 301.8 (\pm 236.3)X^2$  with a prohexadione join point of 0.30 kg a.e. ha<sup>-1</sup> and a groundcover plateau of 62.8%. The model for 2020 was  $Y = 61.4 (\pm 2.3) + 118.7 (\pm 70.7)X - 374.6 (\pm 409.5)X^2$  with a prohexadione join point of 0.16 kg a.e. ha<sup>-1</sup> and a groundcover plateau of 70.8%.

In general, the factorial analyses indicated that gains in plant density and groundcover in response to increasing rates of PHD were consistent across seeding years. Treatment with FI also increased plant density and groundcover, but the response was much larger in 2019 than in 2020. Large seeding-year effects were also observed in regression analyses; however, the response of plant density and groundcover to increasing rates of PHD applied prior to FI treatment were much more pronounced in 2019 than in 2020. When established under relatively wet growing conditions and a moderate corn population near 80,000 plants ha<sup>-1</sup>, average stand density of alfalfa reached only 130 plants m<sup>-2</sup> in 2019 when the highest rate of PHD was applied prior to FI treatment. This treatment combination produced a comparable alfalfa plant density in a prior study that was carried out under wet growing conditions and approximately the same corn population [3]. Conversely, under near-normal



growing conditions in 2020, an average stand density of 177 plants  $m^{-2}$  was obtained with FI regardless of the rate of PHD applied. Other studies have reported that stand density of interseeded alfalfa following corn silage harvest was not affected by PHD treatment under normal to dry growing conditions [8,22]. When considered together, these findings indicate that FI treatment is sufficient to ensure excellent establishment of alfalfa under moderately competitive corn during normal to dry growing conditions. Under wet growing conditions, application of PHD in addition to FI will improve alfalfa survival under corn, but stands following establishment may still be less than the 140 to 200 plants  $m^{-2}$  needed for high first-year forage production [9,10,26].

We also found that gains in plant density and groundcover in response to increasing rates of PHD were consistent for both alfalfa varieties, whereas gains due to FI treatment were larger in the non-leafhopper-resistant variety 3420 than in the leafhopper-resistant variety 55H94. Regression analyses also indicated that plant density and groundcover responses to combined PHD and FI applications were similar for both alfalfa varieties. Varietal responses in plant density to PHD and to sequential PHD-FI treatments can vary among environments, but differences among varieties tend to be most pronounced under wet growing conditions [3,8]. A previous attempt to associate reported disease or insect resistance traits of varieties with the establishment of interseeded alfalfa was unsuccessful [8]. However, the clear benefits of fungicide and insecticide treatments on seedling health, vigor, and survival, especially under wet growing conditions, suggest that disease and perhaps insect pests play a large role in determining the success of alfalfa establishment under corn. Breeding new alfalfa varieties with greater survival under corn is one option for improving establishment in this system [17]. Further work to identify more effective agrichemical treatments and other improved management practices is also needed to ensure interseeding can be successfully realized under a wide range of production environments.

#### 4. Conclusions

Non-treated interseeded alfalfa failed to establish under moderately competitive corn during unusually wet conditions in 2019, but poor stands (71.3 plants  $m^{-2}$  and 15.9% groundcover) were obtained following establishment under normal growing conditions in 2020. Application of the PHD growth retardant reduced etiolation, while FI reduced foliar damage and increased the vigor of alfalfa growing under corn. Responses to FI were, however, more pronounced during wet conditions in 2019, and this treatment also eliminated inherent varietal differences in foliar damage, vigor, and plant survival. Under these conditions, moderate to high rates of PHD followed by FI were required to obtain good stands of alfalfa (130 plants  $m^{-2}$  and 65% groundcover). Conversely, under normal growing conditions in 2020, close to optimal stands (177 plants  $m^{-2}$  and 70% groundcover) for forage production were obtained solely by applying FI. When these findings are considered with other work, it appears that excellent establishment of alfalfa under moderately competitive corn can be obtained during normal to dry growing conditions with FI treatment, while combined PHD and FI treatments are currently required to obtain reasonably good stands during wet growing conditions. The development of more effective agrichemical treatments, better suited alfalfa varieties, and other improved production practices will further ensure interseeding can be successfully implemented under the most challenging growing conditions.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13112823/s1>, Table S1: Monthly total precipitation (mm) and average temperature ( $^{\circ}C$ ) near Arlington, Wisconsin USA relative to 30-year means from 1991 to 2020 (NOAA, [www.climate.gov](http://www.climate.gov)).

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