EVALUATING INTERSEEDING CORN AND ALFALFA IMPACTS ON THE CRITICAL PERIOD OF WEED CONTROL, WEED POPULATION DYNAMICS, AND PHYSIOLOGICAL PLANT COMPETITION

By

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ABSTRACT

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Interseeding corn and alfalfa allows farmers to replace the low yielding alfalfa establishment year with corn silage, while simultaneously establishing alfalfa. Field experiments were conducted to 1) identify the critical period of weed control (CPWC), 2) analyze the role light plays (upright vs. pendulum corn hybrid light interception) in the CPWC, 3) determine interseeding and weed competition effects on corn-alfalfa yield and quality, and 4) evaluate weed population dynamics. The CPWC was determined to be 303 and 369 GDD for interseeded corn and alfalfa. Duration of weed competition reduced corn silage yield and quality and alfalfa yield; however, it did not reduce alfalfa quality. Weed seed viability decreased in the interseeded system compared to monoculture corn system. In general, interseeding these two crops will result in limited light quantity and changes in light quality; thus, it is important to understand the possible underlying stress mechanisms interseeded corn and alfalfa use to detoxify light stress. Therefore, to further understand the stress experienced by plants in the interseeded system, it is important to: 1) determine reactive oxygen species (ROS) responses in interseeded corn and alfalfa, which was addressed in a greenhouse study evaluating ROS scavenging enzymes including glutathione *S*-transferase, dehydrascorbate reductase, and superoxide dismutase activity. Overall, ROS scavenging enzyme rates were modified by level and duration of plant competition.

Dedicated to my Biggest Cheer Section:
Philip Chu
James Drumm
Lynette Drumm
Kristen Drumm
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Introduction

Current projections for global food production state, by 2050 food production needs to double compared to 2009 production rates to meet the increasing demand of a growing global population (Northoff, 2009). There are many ways to achieve this demand, including using resources more efficiently, specifically land (Foley, 2014). One possible solution to increasing production on land already dedicated to agriculture is interseeded cropping systems. Interseeding corn silage and alfalfa can help meet this production demand. To further understand the importance of interseeded systems and their fit in increasing agriculture production we first need to understand how the current agriculture system uses these two crops and how this system could be modified to increase production goals. Corn (*Zea mays*) silage and alfalfa (*Medicago sativa*) are extremely different in terms of physiology; however, they are similar in their importance to the dairy industry due to economic, environmental, and animal health reasons.

Economic Aspects of Corn and Alfalfa

Low milk prices have driven farmers to consider more economically sustainable production methods of corn and alfalfa (Novakovic and Wolf, 2018). Low profit margins are due to high labor requirements, perishability of the milk, large milk storage requirements, and the fact that the fixed assets, cows, are tied specifically to dairy farmers (Novakovic and Wolf, 2018). Milk cooperatives, further restrict these margins as they handle about 82% of the milk produced in the United States (US) and they can regulate milk prices and dump milk (Novakovic and Wolf, 2018).

Borton et al. (1997) constructed a forage model that predicted the highest economic return, based on profit alone, for dairy farmers, is an alfalfa system that utilizes alfalfa and corn as a feed source compared to a continuous corn silage system. The model determined the best use of labor and manure resources came from utilizing a forage ratio of one-third to two-thirds corn silage to alfalfa. Furthermore, the addition of alfalfa feed in the system increased economic returns compared to a system that only utilized corn silage. Adoption of two feed sources is hindered by farmers preference to store one feed source over two, even though two feed sources can result in higher yield from dairy cows resulting in greater profits (Coléno et al., 2002). Furthermore, Coléno et al. (2002) theorized this preference resulted from the advantage that less equipment is needed to produce one feed source as opposed to two. In Iowa, the most profitable crop rotation was corn-soybean (*Glycine max*)-followed by three years of alfalfa, compared to rotations such as corn-soybean, corn-soybean-alfalfa, and corn-soybean-followed by two years of alfalfa (Olmstead and Brummer, 2008). A slight increase in labor and additional machinery costs were reported for the corn-soybean-three years of alfalfa rotation; however, this rotation was 43% more profitable than the corn-soybean rotation with government payouts (Olmstead and Brummer, 2008). This demonstrates there are not only economic benefits to the use of two feed sources for a struggling industry, but there is also evidence demonstrating that incorporation of two feed sources can result in environmental benefits.

Environmental Aspects of Corn and Alfalfa

Continuous monocultures are not a recommended practice as they can create environmental problems. Systems that lack diversity are less able to cope with extreme weather variability and can exacerbate current problems present in the system (Roesch-McNally et al., 2018). Problems specifically in corn monocultures result from runoff and tile drainage contamination with nutrients, like nitrates and phosphorus, which are used in excess amounts in the Corn Belt region of the Midwestern U.S. (Gast et al., 1978; Kladivko et al., 1991; Logan et al., 1994; Logan et al., 1980). Runoff from this region has been linked to the hypoxia zone in the Gulf of Mexico (Donner and Kucharik, 2008). To decrease nitrogen contamination by 30% the Mississippi River & Gulf of Mexico Watershed Nutrient Task Force (2001) outlined that increased cropping system diversification is needed beyond the use of monoculture corn and corn-soybean rotations.

Diversification can help mitigate the impact of corn monocultures on the environmental problems outlined above. The adoption of a single year of alfalfa into continuous corn rotations resulted in yield increases of 19% (Pikul et al., 2005) to 84% (Crookston et al., 1991) compared to monoculture corn. In general, the addition of legume crops helps decrease the amount of nitrogen (N) the following corn needs in the rotation. However, not all legumes provide the same N benefits, for example soybeans reduce the amount of N needed following corn by only 0-25%; in contrast, alfalfa can decrease the N requirement by 80-85% (Lamond et al., 1988). This attests to alfalfa's ability to fix nine times more N than soybeans; therefore, alfalfa contributes an 18-20 kg ha^{-1} larger N credit than soybean to the following corn crop in the rotation (Francis et al., 1990). Corn synthetic fertilizer demand decrease with the adoption of alfalfa; therefore, decreasing the cost of production for the farmer (Olmstead and Brummer, 2008). Overall, cropping system diversification enhances ecosystem services that help decrease the need for synthetic inputs (Davis et al., 2012).

Dairy Cow Health and Feed Sources

Corn grown in monoculture has multiple applications, ranging from ethanol production to a source of feed. Corn gained prominence as a feed source in the 1960s (Barnes et al., 1988) due to the lower cost per unit energy to produce compared to forage legumes (Knaus, 2016). This reduction in cost led farmers to start mixing corn silage into historically legume dominated feed rations for dairy cows, which resulted in maximizing feed intake and milk performance. In recent years, farmers have adopted corn silage as the sole feed source (Knaus, 2016). This adoption of continuous corn silage led to a balancing act for farmers trying to: 1) maximize feed intake and milk performance, 2) increase nutrient and energy density of the cow by adding corn silage, while 3) attempting to minimize the negative effects from corn silage on the animal digestive system and metabolic processes (Knaus, 2016). Brito and Broderick (2006) reported that cows fed a high ratio of corn silage to forages (37% corn silage:13% alfalfa) yielded at least 2.0 kg less milk per day than cows that were fed a lower ratio of corn silage (24% corn silage:27% alfalfa; 10% corn silage:40% alfalfa), while holding crude protein constant across ratios. Increasing alfalfa and decreasing corn silage resulted in increased dry matter intake and milk yield increased linearly as corn silage was replaced with alfalfa. In addition to measuring milk yield, Brito and Broderick (2006) measured N excretion and found that the combination of corn and alfalfa resulted in less N excreted, which is better for the environment and signals high N utilization within the animal (Huhtanen et al., 2008).

Weed Control in Silage Corn

As the demand for corn silage increased, continuous corn cropping systems increased. Monoculture corn systems have resulted in environmental problems (as stated above), and an overreliance on herbicides as the only source of weed control. Herbicide use increased in crop production because it was more economical and often more effective than other control measures. For example, mechanical weed control in a corn field requires 16.43 hours ha^{-1} and costs \$138.65 ha^{-1}, chemical weed control requires 4.48 hours ha^{-1} and costs \$27.08 ha^{-1}, and genetic + chemical weed control results require 3.08 hours ha^{-1} and costs \$20.68 ha^{-1} (Swinton and Van Deynze, 2017). Herbicides that can be applied preemergence in corn silage include *S*-

metolachlor, atrazine, isoxaflutole, flufenacet, clopyralid, and many others (Johnson et al., 2012). Postemergence herbicide options include glyphosate (in glyphosate resistant corn hybrids), rimsulfuron, *S*-metolachlor, benoxacor, dicamba, diflufenzopyr, nicosulfuron, mesotrione, atrazine, and prosulfuron, in addition to many others (Stewart et al., 2012). Overreliance on herbicides has resulted in selection for herbicide resistant weeds and lack of effective weed control. When weeds are not controlled, they have the ability to compete for water, nutrients, and light, which reduce silage corn yield. In 2019, 137,593 ha were dedicated to corn silage in Michigan and the average yield was 41,472 kg ha^{-1} (USDA, 2019). Weeds are estimated to be the most serious threat to crop production as they result in 270 billion dollars in economic loss due to lower yields, cost of weed control, and contamination of the harvested product (Pimentel et al., 2005).

In addition to chemical weed control, there are many ways to modify crop-weed competition including modifying light interception. Light interception can be modified by utilizing high plant populations, planting more aggressive fast growing hybrids, and planting hybrids with differing leaf architecture (Toler et al., 1999). Fast growing aggressive corn hybrids intercept disproportionately more light than weeds, leading to reductions in weed growth (Toler et al., 1999). Furthermore, corn hybrids that have the ability to intercept more light due to leaf architecture can reduce weed competition. There are three main types of corn hybrid leaf architecture: upright, semi-pendulum, and pendulum. Upright corn hybrids have erect, narrow leaf angles, pendulum corn hybrids have horizontal, wider leaf angles, and semi-pendulum corn hybrids have leaf angles in between those of upright and pendulum (Stewart et al., 2003). Historically, corn hybrids had pendulum leaf angles and were planted at low plant populations. The pendulum hybrids have a wide leaf distribution resulting in increased light interception, directly contributing to increased yield (Stewart et al., 2003). In efforts to increase yield, plant populations were increased and hybrids with upright leaf angles were selected by breeders to tolerate higher plant populations (Duvick, 2005; Mickelson et al., 2002). The leaf angle is controlled by genetic factors (Mickelson et al., 2002) and environmental ones, driven by photomorphogenic responses (Fellner et al., 2003). Overall, hybrids that have upright leaf angles demonstrate yield benefits in simulated (Duncan, 1971) and field studies (Pepper et al., 1977).

Corn leaf angles directly affect light use efficiency of the crop. Hikosaka and Hirose (1997) found that upright leaf architecture allows more light to penetrate the lower leaf canopy resulting in the maximum light capture. The even distribution of light throughout the canopy results in higher biomass accumulation, and greater yield compared to a pendulum leaf architecture. However, more light penetrating to lower depths of the crop canopy could result in more light reaching the weeds also leading to reductions in yield (Hikosaka and Hirose, 1997). Leaf angle also influences radiation use efficiency and subsequent crop growth rate, specifically upright leaf angles are more efficient than pendulum leaf angles (Duncan et al., 1967).

Sankula et al. (2004) conducted research evaluating weed control in upright and pendulum corn hybrids in irrigated and non-irrigated environments. Under irrigation, weed density was not affected by leaf architecture; however, end of the season weed biomass was negatively impacted. Shading from the pendulum leaf angle reduced weed biomass by 73-90% in year one and 20-47% in year two of the study compared to the upright leaf angle. Pendulum leaf architecture reduced light transmission to the lower canopy of weeds by 50% compared to the upright leaf for both years under irrigation. Under non-irrigation conditions, there was no difference in leaf architecture treatments on weed density and biomass. These findings highlight hybrid leaf architecture can have an suppressive effect on weed growth when water is not a limiting factor (Sankula et al., 2004). In addition to corn, weed suppression has been achieved with the use of varieties with differing leaf architecture in potato (*Solanum tuberosum*), cotton (*Gossypium hirsutum*), and soybean (Callaway, 1992).

In addition to herbicides and leaf architecture, timing of weed control also plays a large role in corn silage production. One method to determine when weed control should occur is defining the critical period of weed control (CPWC). The CPWC is defined as the period in the crop growth cycle in which weeds must be controlled to prevent yield loss (Nieto et al., 1968). CPWC is identified by removing and adding weeds at specific timings, this allows weeds to compete with the crop for a given period of time. The critical timing of weed removal (CTWR) is the amount of time the crop can tolerate early season weed competition before an unacceptable yield loss. The critical weed free period (CWFP) is the minimum weed free period required to prevent an unacceptable yield loss. In order to have uniform crop-weed competition, weeds are seeded across treatments (Knezevic et al., 2002). Numerous CPWC studies have been conducted in grain corn across a wide variety of locations, systems, and years. The CPWC is often location and site-specific (Hall et al., 1992). Halford et al. (2001) conducted a CPWC study from 1995 to 1997, in Ontario, Canada and reported that the critical period started between 14 to 18 days after emergence and ended between 24 to 46 days after emergence using a locally harvested weed seed mixture at a seeding rate of 1 kg ha^{-1}. Page et al. (2012) conducted a CPWC study at three locations, University of Guelph, Ohio State University, and Colorado State University, in 2009 and 2010, using winter wheat as a surrogate weed seeded at 150 seeds m^{-2}. The CPWC was identified as the first leaf tip to the third leaf tip of corn and reported that the longer winter wheat was allowed to compete, the larger decrease in corn yield was observed. Williams et al. (2006) identified the CPWC in Illinois for sweet corn to be from 160 (V4) to 320 (V8) growing degree days (GDD).

Alfalfa

Alfalfa was the first forage crop to be domesticated in the world (Bolton et al., 1972). Alfalfa was introduced in 1736 when colonists brought it to the U.S. to feed horses, although it was not successful until it was introduced into the Western U.S. because of its adaption to dry irrigated soils (Barnes et al., 1988). Alfalfa is a highly important forage legume and is a popular source of hay for dairy farmers (Baxter et al., 2017). In the U.S., nine biotypes of alfalfa constitute the foundational germplasm for breeding (Barnes et al., 1988). In Michigan, there were 303,514 ha planted in 2019 with average yields of 2,903 kg ha^{-1} (USDA, 2019).

Alfalfa is an environmentally friendly and economical crop to grow as outlined above; however, there are still barriers to adoption. Olmstead and Brummer (2008) conducted a survey of Iowa farmers and identified lack of adoption stems from four categories: lack of available markets, time constraints, and culture. Furthermore, many states would benefit from the inclusion of alfalfa in their current rotation to alleviate environmental problems in current rotations, but adoption is limited due to market demand. In addition, the lack of adoption stems from the time constraints from off-farm employment, the increased need for labor, and the need for new equipment not used in current corn-soybean rotations (Olmstead and Brummer, 2008).

Weed Control in Alfalfa

Weed control in alfalfa falls into two phases, the seeding or establishment year and the following full production years. During the establishment year, alfalfa is vulnerable to weeds due to slow growth (Dillehay and Curran, 2010). Specifically, alfalfa yield in the first cutting can decrease by 18 to 33% due to weed competition. Companion crops, like oats, can be seeded to help improve alfalfa establishment by 5% when seeded at the correct rate (Lanini et al., 1991).

Overall, as oat biomass increases there is a linear decrease in alfalfa biomass. Once alfalfa is established it is very competitive with weeds (Summers, 1998). However, under extreme weed pressure first cutting yields can decrease up to 80% (Cosgrove and Barrett, 1987; Ott et al., 1989).

In addition to yield reductions, historically weeds were thought to reduce protein content and overall alfalfa quality (Cords, 1973; Klemme and Albrecht, 1952). However, recent studies have evaluated weed and alfalfa quality and found that the many weed species have the same if not better nutrient quality as alfalfa (Dutt et al., 1982; Marten and Andersen, 1975; Marten et al., 1987). Specifically, Marten and Andersen (1975) reported that redroot pigweed (*Amaranthus retroflexus*), common lambsquarters (*Chenopodium album*), and common ragweed (*Ambrosia artemisiifolia*) have similar in-vitro digestible dry matter, acid detergent fiber, neutral detergent fiber, and crude protein as alfalfa harvested at the same time. One of the few weeds that are known to decrease the nutrient value of forages is yellow rocket (*Barbarea vulgaris arcuate*) (Dutt et al., 1982). Interestingly, Canada thistle (*Cirsium arvense*) has a higher amount of crude protein compared to alfalfa, but it is not palatable to animals due to the physical deterrents (spines) along the plant (Marten et al., 1987). However, weeds can also be toxic to livestock, such as common groundsel (*Senecio vulgaris*) and many other species (Puschner, 2000). Therefore, controlling weeds is an important factor to ensure palatable, high quality, and high yielding alfalfa.

Alfalfa weed control was revolutionized in 1998 with the first transformation to confer glyphosate resistance in alfalfa (Dillehay and Curran, 2010), as this allowed for low cost broad spectrum herbicidal weed control without damaging alfalfa. Historically, cultural and mechanical weed control methods for alfalfa were to delay the first cutting, which can compromise alfalfa quality, or delay seeding and till early season weeds, but this may delay future harvests (McCann et al., 2006).

Herbicide options prior to glyphosate resistant alfalfa are actively utilized. Preplant incorporated herbicide options include EPTC for grass and broadleaf control, however alfalfa injury risk is high if not correctly incorporated. Trifluralin, a preemergence herbicide, controls annual grasses and some annual broadleaf weeds and rarely causes injury to alfalfa (Undersander et al., 2011). Postemergence control options include bromoxynil, 2,4-DB, pendimethalin, imazethapyr, and glyphosate (Undersander et al., 2011). 2,4-DB is utilized as a postemergence herbicide for broadleaf control and sethoxydim or fluazifop-butyl for grass control in alfalfa (Fischer et al., 1988). Glyphosate is a widespread option for postemergence weed control because other chemical applications require alfalfa fields to not be harvested for 30 days after application. Glyphosate is considered a once in a lifetime discovery, due to its unique site of action, systemic translocation, low cost, reduced toxicology, environmental profile, the development of resistant crops allowing for more selectivity, and the low cost to produce (Duke and Powles, 2008).

Postemergence herbicide application timing plays a large role in yield for corn silage and alfalfa. Unlike corn, the CPWC for alfalfa has not been thoroughly researched. This may be due to the perennial nature of the crop, historically alfalfa has been viewed to offer some cultural weed control (Dillehay and Curran, 2010) or the lack of postemergence weed control options (Undersander et al., 2011). One CPWC study conducted in Pennsylvania during 2004-2006, reported the CPWC for the first harvest started at 0.5 trifoliate (97 GDD) and ended at 7 trifoliate leaf stage (862 GDD) (Dillehay et al., 2011). Furthermore, as weed density increased the duration alfalfa could withstand weed competition decreased (Dillehay et al., 2011), due to increased competition for vital resources including water, nutrients, and light.

Allelopathy may also play a role in weed control in alfalfa. Leaf extracts from alfalfa inhibited barnyardgrass root growth at a concentration of 40 g L^{-1} (Chon et al., 2002). Interestingly, the same study reported that leaf extracts from alfalfa had no impact on barnyardgrass hypocotyl growth. The allelopathic chemical responsible for reductions in barnyardgrass root growth is coumarin, a toxic phenolic compound that is an alfalfa leaf exudate from decomposition (Chon et al., 2002). The degree of root inhibition is concentration dependent, 10 g L^{-1} and 20 g L^{-1} of leaf extracts reduced barnyardgrass root growth by 70% and 80%, respectively.

Unlike in corn, little research has been conducted about interspecific light competition in alfalfa; however, research has been conducted evaluating intraspecific light competition in alfalfa. Alfalfa grown under low light conditions with low red to far-red ratios (R:FR), produced less biomass than alfalfa grown under ambient light conditions (Baldissera et al., 2014). Furthermore, Baldissera et al. (2014) reported that shading from intraspecific competition, due to higher planting densities, resulted in later flowering. Flowering is associated with poorer forage quality, thus decreasing the R:FR ratio may result in a higher quality alfalfa; however, the decrease in light quality results in a lower ratio of leaf to stem biomass (Fick and Mueller, 1989). A better understanding is needed on how light could affect alfalfa yield and quality, which could be evaluated by interseeding alfalfa and corn together.

Interseeding Corn Silage and Alfalfa

As outlined above, incorporating corn silage and alfalfa in the same system can have environmental and economic benefits; one method to achieve this is interseeding these two crops together. Interseeding is planting two crops at the same time. Interseeding alfalfa and silage corn is not a new idea, the first paper published about interseeding these two crops was in 1955 (Schaller and Larson, 1955). This initial paper evaluated alfalfa as a cover crop in between corn rows, with the goal of introducing corn into the rotation. Schaller and Larson (1955) reported that alfalfa stands could be established when corn row spacing was greater than 150 cm and the seeding rate was less than 60,000 seeds ha^{-1}. Abdin et al. (1998) reported that interseeding cover crops, such as fall rye (*Secale cereale*), hairy vetch (*Vicia villosa*), red clover (*Trifolium pratense*) + ryegrass (*Lolium perennis*), white clover (*Trifolium repens*) + ryegrass, and black medic (*Medicago lupilina*) into grain corn near corn planting was achievable and had minimal effect on corn yield. However, the cover crops were difficult to establish and provided little to no weed control (Abdin et al., 1998).

Current studies have evaluated agronomic practices to optimize the alfalfa corn silage interseeded system. Grabber (2016) assessed how plant growth regulators (PGRs) could be used to help offset competition between corn and alfalfa in the establishment year. Interseeded with corn, alfalfa biomass decreased 18-24% with the use of the prohexadione-calcium (PGR), but increased alfalfa biomass by 65-114% after corn harvest compared to untreated plots. Prohexadione-calcium application also increased alfalfa seedling survival 57% when applied at 2.4 kg ha^{-1} compared to untreated plots (Grabber, 2016). In total, regardless of PGR application there was a 12% increase in total dry matter yield in the interseeded system compared to the monoculture systems of both crops. In contrast, Osterholz et al. (2018) reported that PGR application decreased corn silage yield by 9% in the interseeded system compared to the monoculture corn system. This may be due to the unintentional application of the PGR to the corn. However, prohexadione-calcium application increased alfalfa yield by 4% following establishment (Osterholz et al., 2018). Additionally, Grabber et al. reported that alfalfa varieties that had leaf hopper resistance and glyphosate resistance resulted in higher alfalfa establishment when interseeded with corn and alfalfa.

In addition to yield benefits, interseeded corn silage and alfalfa increases ground cover by 52% compared to monoculture corn (Osterholz et al., 2019). Interseeding reduced total runoff volume by 63% compared to runoff from monoculture corn, thus reducing the loss of total N by 74%, and total phosphorus by 37% compared to the monoculture corn system. Therefore, interseeding alfalfa into corn silage substantially improved the soil and water conservation outcomes of corn silage production (Osterholz et al., 2019).

Finally, interseeding can have positive impacts of farm profitability. Osterholz et al. (2020b) determined through the use of models, that interseeding increased profitability by 15% compared to the conventional rotation of corn silage and alfalfa. Although corn silage yield may be reduced in the interseeded system, model projections found that interseeded corn yield loss could reach 20% and net profits were still equal to the most profitable conventional rotation of corn and alfalfa. Overall, interseeding corn silage and alfalfa has yield, economic, and environmental benefits.

To achieve these benefits weeds must be controlled in this system. Herbicide options are limited when interseeding, due to planting a grass and legume crop together and impacts on herbicide selectivity. To evaluate herbicide options Osterholz et al. (2020a) conducted research on postemergence and preemergence herbicides. This study evaluated herbicides to determine crop safety and weed control. Four weeks after treatment (WAT), 0.27 kg ha^{-1} mesotrione applied postemergence resulted in an 87% decrease in weed cover compared to the untreated (weedy) control. At 8 WAT, glyphosate and mesotrione decreased weed cover by 96% and 76%, respectively, compared to the untreated control (Osterholz et al., 2020a). Although mesotrione provided consistent weed control applications resulted in alfalfa injury including stunting, malformed new leaves, necrosis, and chlorosis. Injury ratings from mesotrione ranged from 34% to 74% depending on rate applied. Additional herbicides evaluated that provided weed control similar to glyphosate were bromoxynil and 2,4-DB postemergence. The other 11 herbicides tested pre- and postemergence either provided poor weed control or were injurious to the crop. Interestingly without herbicide application interseeding suppressed weeds by 65-70% compared to monoculture corn without herbicide application (Osterholz et al., 2020a). Therefore, interseeding itself may be providing additional weed control benefits when compared to monoculture corn systems.

Interseeding Modifies Weed Competition

Interseeding corn and alfalfa increases the amount of interspecific competition, which can impact weed phenology and modify the maternal environment in which weed seeds are produced. One method to study interspecific competition in the corn silage alfalfa interseeded system is using surrogate weed species sown at known densities. A weed surrogate is a cultivated crop that has weedy characteristics. Japanese millet (*Echinochloa esculenta*) has been used as a surrogate weed to help determine the CPWC in alfalfa in previous studies (Dillehay et al., 2011) and is in the same family as barnyardgrass (*Echinochloa crus-galli*).

Barnyardgrass is a C_4 weed, which has allowed it to adapt and become a problematic weed worldwide (Roy et al., 2000). In eastern North America, barnyardgrass occurs from Florida, U.S. to Quebec, Canada (Hitchcock and Chase, 1951). Barnyardgrass is a summer annual, allohexaploid weed, that can self-pollinate (Honěk and Martinková, 1996). Barnyardgrass produces seeds at a terminal panicle, approximately 10-40 cm in length (Roy et al., 2000). The panicles color varies between green and purple, possess individual spikelets that develop with a terminal awn between 2-10 mm and varies in number of seeds produced (Roy et al., 2000). Barnyardgrass is estimated to produce 2,000 to 100,000 seeds plant^{-1} grown in competition with corn (Bosnic and Swanton, 1997). Under ideal conditions with no crop competition and unlimited resources, it is estimated that a single plant can produce one million seeds (Bagavathiannan et al., 2011). Chauhan (2013) reported in competition with rice barnyardgrass produced 2,977 seeds plant^{-1} under 100% light, 1,134 seeds plant^{-1} under 50% light, and 682 seeds plant^{-1} under 25% light.

Barnyardgrass seed dormancy is variable and prolonged depending on location, year, and environmental factors that dictate dormancy including rainfall and temperature (Bagavathiannan et al., 2011). Prolonged dormancy in barnyardgrass is advantageous as individuals within the population can remain in the seedbank and avoid weed control tactics (Bagavathiannan et al., 2011). Dormancy may be tied to environmental condition and seed age as Martinkova et al. (2006) found seed emergence is more uniform in one year old stratified seed compared to eight year old stratified seed. In addition to temperature requirements, phytochrome dependency may also play a role in seed dormancy (Taylorson and Dinola, 1989). Taylorson and Dinola (1989) reported that germination of barnyardgrass increased by 63% when exposed to high R:FR light for 5 minutes compared to seeds that were germinated in the dark. Furthermore, primary dormancy is broken in newly dispersed seeds with alternating temperatures of 35°C for four days and 46°C for one hour under ample soil moisture (Taylorson and Dinola, 1989). Breaking dormancy in barnyardgrass outlined above is dictated by many environmental factors, but long-term seed persistence is based on the ability of the seed to repair its DNA overtime (Martinez-Ghersa et al., 1997).

Genetic and geographic diversity in barnyardgrass populations dictates the maternal environment in which the seed is produced and subsequent dormancy characteristics (Honěk and Martinková, 1996). The maternal environment is determined by the growing conditions of the maternal parent, including inter- and intraspecific competition during plant growth. Kirkpatrick and Lande (1989) defined the maternal environment as a sum of genetic and non-genetic effects, that consists of environmental and maternal effects, which can drive evolutionary responses to selection. For example, barnyardgrass flag leaf protein concentration was 50% greater when parental plants were grown under a controlled greenhouse environment than when parental plants were grown under field conditions (Potvin and Charest, 1991). Similar findings were reported for fountain grass (*Pennisetum setaceum*), offspring from plants originating from three different states, Hawaii, Arizona, and California, differed in their water use efficiency, suggesting that genetic and environmental maternal differences play large roles in offspring physiology (Poulin et al., 2007). Furthermore, competition during seed production affects offspring success, maternal plants that experience high levels of competition produce smaller seeds which leads to a reduction in competitive ability of the offspring the following season during establishment (Espeland and Hammond, 2013). These studies demonstrate that maternal effects influence seed size, nutrient partitioning, and overall competitive ability.

Barnyardgrass competition reduces crop yield, and the magnitude of this reduction is dictated by density and emergence timing. In corn, 30 barnyardgrass seedlings emerging at the 3-leaf corn stage resulted in a 14% yield loss compared to a 4% loss if seedlings emerged later at the 7-leaf stage (Bosnic and Swanton, 1997). Emergence timing also modifies barnyardgrass seed production, if seedlings emerged at the 3-leaf corn stage plants produced 14,440-34,600 seeds m^{-2}, however if seedlings emerged one stage later at the 4-leaf stage seed production was reduced by 91% (Bosnic and Swanton, 1997). Wild proso millet (*Panicum miliaceum*) in competition with short maturing corn hybrids produced 66% more seeds and the following germination was 12.5% greater than the millet plants grown in competition with longer maturing corn hybrids (Williams et al., 2012). In addition to direct crop competition, cropping system diversity also modifies weed density and biomass. For example, barnyardgrass seedling density was 60-80% less in a corn-alfalfa rotation compared to continuous corn (Clay and Aguilar, 1998). Broadleaf weed biomass in the establishment year of alfalfa was approximately 10% of the total biomass and the following year it was reduced to 0.1%, 0.6%, and 3.6 % for the first, second, and third cuttings, respectively (Clay and Aguilar, 1998). A similar trend was observed with grassy weed biomass, in the establishment year grassy weeds were 36% of the total biomass; however, the following year, the grass biomass accounted for less than 0.4% and 17% of the total biomass for the first and fourth cuttings, respectively (Clay and Aguilar, 1998).

Shade Avoidance Response and Physiological Changes

Light in a cropping system can come from the sun and from light reflected off neighboring plants. Sunlight is necessary for photosynthesis and biomass accumulation, leading to plant competition for this limited resource. Light competition can result in physiological and morphological modifications of growth habits due to the quality of light being received. Shade avoidance is triggered by the perception of differences in light quality, specifically a reduction of R:FR light due to neighboring vegetation, which results in a morphological response (Ciolfi et al., 2013). Shade avoidance impacts corn growth and development via delays in leaf emergence and an increase in duration of vegetative growth due to light reflected from neighboring weeds (Cerrudo et al., 2012). Specifically, corn will alter physiological and phenological development in the presence of weed competition (Afifi and Swanton, 2012). For example, when corn was exposed to low R:FR light height was reduced. Furthermore, this early season shade avoidance response reduced the corn plants ability to tolerate other stressors, including weed competition later in the season (Afifi and Swanton, 2012). Extensive research has been performed evaluating monoculture corn competition with weeds and resulting physiological changes resulting from shade avoidance; however, literature is lacking for alfalfa.

Crop plants respond to stress from weed competition in many ways outlined above, from reductions in growth to changes in phenology and ultimately reductions in yield. Overall, stress is defined as abiotic and biotic conditions that prevent the plant from achieving its full genetic potential (Mhamdi and Van Breusegem, 2018). Plants do not have the ability to move; therefore, they modify their metabolism in ways that allow them to detoxify stress and continue to grow (Mhamdi and Van Breusegem, 2018). Plants have many morphological and physiological responses to stress; one physiological response is producing reactive oxygen species (ROS). ROS is a group of molecules from molecular oxygen which are formed by redox reactions or electron excitement within the plant (Sies and Jones, 2020). A build-up of ROS in the cell can result in damage to DNA, RNA, and proteins, and may result in cell death. Forms of ROS in plants include singlet oxygen, superoxide, hydroxyl, and most common, hydrogen peroxide. ROS are natural byproducts of mitochondrial respiration, photosynthesis in chloroplasts, and photorespiratory reactions (Mhamdi and Van Breusegem, 2018). ROS can act as a signal for other metabolites and phytochromes in plants. These signals help plants preform many functions, including germination, meristem development, plant cell death, and expansion (Mhamdi and Van Breusegem, 2018). Due to the natural development of ROS, plants have evolved mechanisms to detoxify and modify the concentration of ROS within cells, minimizing negative impacts (Mhamdi and Van Breusegem, 2018). Many enzymes play roles in ROS detoxification, including catalases, ascorbate peroxidase, glutathione *S*-transferases, peroxiredoxins (Mhamdi and Van

Breusegem, 2018), and anthocyanins (Afifi and Swanton, 2012).

Afifi and Swanton (2012) demonstrated that corn grown hydroponically, with no limiting resources except light, resulted in morphological and physiological changes under weed competition. Corn plants grown under competitive weed treatments exhibited a shade avoidance response due to modifications in the ratio of R:FR light. The lower R:FR light produced from weeds and the use of far-red light filters resulted in corn plants with elongated stems, delayed leaf emergence, and changes in physiology. Furthermore, hydrogen peroxide production in corn was 84% greater under weed competition and 63% greater under light filter treatments compared to weed free or normal light treatments (Afifi and Swanton, 2012). In soybean, under far-red enriched treatments ROS increased upon initial emergence, but concentrations decreased over time compared to ambient light treatments (Mckenzie-Gopsill et al., 2016). Additionally, superoxide dismutase, an enzyme that helps break down potentially harmful oxygen molecules, expression was 1.75 times greater at the hypocotyl stage, 2.75 at the cotyledon stage, and 0.5 at the unifoliate stage of soybeans compared to the non-light treated controls. These findings suggest plants have the ability to change morphological and physiological responses even at emergence in response to changes in light quality.

Objectives

Interseeding corn silage and alfalfa results in a system that is more ecologically and environmentally sustainable than a monoculture system of corn. Interseeding could provide weed control, which may be driven by light competition within the system. Research is needed to identify when weed control should occur, how weeds interact, and what are the biochemical responses of competition in this interseeded system. Therefore, the main questions this thesis seeks to address are:

1. What is the critical period of weed control in the interseeded system of corn silage and alfalfa?
2. Are there any additional weed control benefits that can result from the interseeding of corn silage and alfalfa?
3. How is corn, alfalfa, and weed physiology modified by interseeding competition?

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CHAPTER II

INFLUENCE OF WEEDS AND LIGHT DISTRIBUTION ON THE CRITICAL PERIOD OF WEED CONTROL AND FORAGE QUALITY COMPONENTS OF INTERSEEDED CORN AND ALFALFA

Abstract

Alfalfa acres in Michigan are declining due to reliance on corn silage as a continuous feed source, partly due to low alfalfa yield in the establishment year; however, by interseeding corn and alfalfa farmers can replace the alfalfa establishment year with corn silage, while simultaneously establishing alfalfa. A randomized split-block field study was conducted in East Lansing, Michigan over three years (2019-2021) with the goal to determine the critical period of weed control (CPWC) in the interseeded corn silage and alfalfa system with two corn hybrids with differing leaf architecture (pendulum vs. upright) and impacts on corn silage and alfalfa yield and quality. Whole plots were assigned to corn hybrids and subplots were assigned to surrogate weed (Japanese millet) duration of competition treatments. Weed-free and weedy plots were included as controls. At the end of the interseeding year, corn silage was harvested while alfalfa was harvested the following year. The CPWC, Japanese millet biological data, and corn-alfalfa quality and yield were analyzed in R. The CPWC was 303 and 369 growing degree days in corn and alfalfa, respectively. Japanese millet seed production decreased by 67% in the pendulum corn hybrid compared to the upright corn hybrid in 2020. Japanese millet seed viability decreased by 46% in the interseeded system compared to a corn monoculture system. Corn silage yield did not decrease when grown with alfalfa in both 2019 and 2020, but was negatively impacted when in competition with weeds. Extended duration of weed competition negatively impacted corn silage quality; however, did not have an effect on alfalfa first cutting quality. Identification of the CPWC, evaluation of quality parameters, and alfalfa yield in the

interseeded system will hopefully increase adoption and interest in other interseeded systems that have the ability to mitigate environmental and economic issues developing in agriculture.

Introduction

Alfalfa (*Medicago sativa*), a highly important forage legume for dairy farmers, is the third-ranked field crop in Michigan by value (Baxter et al., 2017; USDA, 2019; USDA, 2020). Besides its benefits as a source of feed, alfalfa has the ability to improve crop yields and reduce crop loss via increased rotational diversity, reduced fertilizer use, improved soil quality, and reduced soil nutrient loss (Davis et al., 2012; Olmstead and Brummer, 2008). However, alfalfa acreage is on the decline due to an increase in use of corn as the single source of forage in the dairy industry (Barnes et al., 1988; Knaus, 2016). Additionally, establishment year yields of spring seeded alfalfa are low compared to following seasons further reducing acreage (Stanger and Lauer, 2008). The increased use of corn for feed has resulted in economic (Borton et al., 1997; Novakovic and Wolf, 2018), environmental (Logan et al., 1994; Logan et al., 1980; Roesch-McNally et al., 2018), and animal health issues (Brito and Broderick, 2006; Huhtanen et al., 2008; Knaus, 2016). Furthermore, the increased usage of corn silage resulted in the subsequent overreliance on herbicides within the same site of action thus selecting for herbicide resistant weeds (Stewart et al., 2012), which can increase yield losses due to plant competition for the limited resources of water, nutrients, and light (Pimentel et al., 2005). Thus, to encourage the use of alfalfa, new approaches are needed to increase alfalfa yield during the establishment year. One option to improve system yield during alfalfa establishment is to interseed alfalfa into corn silage, thus substituting corn yield for the low yield typical of establishment year alfalfa (Grabber, 2016). Corn and alfalfa interseeded together can improve economic, environmental, and animal health outcomes (Brito and Broderick, 2006; Knaus, 2016; Osterholz et al., 2019; Osterholz et al., 2020b).

Interseeding corn and alfalfa can have positive impacts on farm economics, as Osterholz et al. (2020b) reported interseeding increased profitability by 15% compared to the traditional rotation of corn and alfalfa commonly found on dairy farms. Also, research has shown that interseeding increased ground cover by 52% and reduced total runoff volume by 63% compared to monoculture corn (Osterholz et al., 2019), which can help decrease the amount of contaminated water sourced from farm fields (Kladivko et al., 1991; Logan et al., 1994; Logan et al., 1980). Furthermore, interseeding corn silage and alfalfa provides two sources of feed for dairy cows, resulting in decreased health issues documented with corn only diets (Knaus, 2016) and an increase in milk yield of 2.0 kg day^{-1} compared to corn only diets (Brito and Broderick, 2006).

To establish interseeded alfalfa while maintaining high corn silage yield, weeds must be controlled. Weed control in this system can be difficult due to few selective herbicides available when planting legume and grass crops together. Herbicide based weed control options were investigated by Osterholz et al. (2020a), who concluded that mesotrione, glyphosate, bromoxynil, and 2,4-DB applied postemergence resulted in 76-96% weed control; however, all herbicide listed above, except glyphosate, resulted in unacceptable alfalfa injury. Although glyphosate applications provided acceptable weed control both corn and alfalfa varieties must contain glyphosate resistance traits. However, there has been a lack of adoption of glyphosate resistant alfalfa due to grower preference and market availability (Putnam et al., 2016). Although various levels of weed control can be achieved with the use of herbicides, Osterholz et al. (2020a) also noted interseeding suppressed weeds by 65-70% without herbicide application compared to monoculture corn.

Alfalfa and corn seedling success is strongly related to the duration of weed competition termed the critical period of weed control (CPWC) (Knezevic et al., 2002). The CPWC is defined by the period in the crop cycle in which weeds must be controlled to prevent major yield losses (Nieto et al., 1968). Yield can be maximized if weeds are controlled during this critical period via postemergence herbicide application or other weed control options (Halford et al., 2001; Knezevic et al., 2002). The CPWC for corn has been well documented and reports of the CPWC in corn ranges from VE to V8 (Page et al., 2012; Williams, 2006) and is highly influenced by agronomic practices and environmental variables (Hall et al., 1992). The CPWC in alfalfa is not well understood. One study conducted in Pennsylvania found the optimal time for weed removal is between the 0.5 trifoliate [97 growing degree days (GDD)] and 7 trifoliate leaf stage (862 GDD) (Dillehay et al., 2011). Currently, the CPWC is unknown for many interseeded systems.

The CPWC can be modified by changing competition for shared resources, such as light. By utilizing corn hybrids with differing leaf architecture, light penetrating to the lower canopy is modified. Leaf angles can effect crop canopy closure and allows for selectivity for more or less light to reach the lower canopy (Callaway, 1992). Corn hybrids have a range of leaf angles, specifically, in the 1930s pendulum leaf architecture dominated (Tian et al., 2011). Pendulum hybrids have a wider leaf angle. Recently corn hybrids are dominated by upright architecture, which is a narrower leaf angle (Tian et al., 2011). Under irrigation, competition from pendulum corn hybrids reduced weed biomass by 73-90% compared to upright hybrids (Sankula et al., 2004). Overall, the pendulum leaf architecture reduced light transmission to the lower canopy of weeds by 50% compared to the upright hybrid. Increased light penetration will play two roles in the interseeding system by 1) allowing more light for alfalfa to grow and establish prior to corn canopy closure and 2) allowing more light for weeds to grow thus potentially increasing the

CPWC.

Overall, interseeding corn silage and alfalfa has the ability to alleviate economic, environmental, and animal health issues that exist in the current corn monoculture practices on dairy farms and increase alfalfa acres. Understanding the role of light in this system and subsequent impacts on the duration of the CPWC is vital information needed to increase adoption, economic, and environmental benefits of interseeding. Therefore, the objectives of this research were to: 1) identify the CPWC for the interseeded system, 2) determine how changes in light quantity achieved by utilizing corn hybrids with differing leaf architecture (pendulum vs. upright) modifies the CPWC, and 3) determine how weed competition impacts interseeded corn silage and alfalfa yield and quality.

Materials and Methods

Field experiments to determine the CPWC in the interseeded system of corn silage and alfalfa were initiated in 2019 and 2020 at the Michigan State University (MSU) Plant Pathology Farm in East Lansing, Michigan (42.68 ° N, 84.50 ° W). The interseeded experiment was arranged as a split-plot randomized complete block design with four replications. Whole plots were assigned to one of two corn hybrids and subplots were assigned to weed addition or removal times. Corn and alfalfa were planted in soils prepared with a fall chisel plow, followed by two passes with a soil finisher, and one pass with a soil cultipacker in the following spring. Fertilizer was applied using a preplant incorporated urea (46-0-0) at 168 kg ha^{-1} followed by 112 L ha^{-1} of 16-16-16 (N-P-K) applied at corn planting. Plots were 3 m by 9.1 m in 2019 and 3 m by 8.8 m in 2020, with each plot having four rows of corn spaced 76 cm.

Two corn hybrids of differing leaf architecture, upright or pendulum, were planted on June 4, 2019, and May 28, 2020, using a 91 cm drill, 76 cm row width**,** and a seeding rate of

89,100 seeds ha^{-1}. The upright corn hybrid, G89A09 (Golden Harvest, Minnetonka, MN), has a narrow leaf angle. The pendulum corn hybrid, G90Y04 (Golden Harvest, Minnetonka, MN), has a wider leaf angle. Glyphosate-tolerant alfalfa was planted on the same day as the corn. In 2019, the alfalfa consisted of two different varieties DKA4051 (Bayer, St. Louis, MO) for replications one-three and FSG430LHRR (Allied Seed, Nampa, ID) for replication four, due to inadequate DKA4051 seed reserves. In 2020, the alfalfa variety was FSG431LHRR (Allied Seed, Nampa, ID) for all replications. In both years, the alfalfa was planted in the same direction as the corn, with a John Deere tow drill with row width set at 19 cm with 17 openers at a seeding rate of 2.94 kg ha^{-1} resulting in four alfalfa rows in between each corn row. Soil samples were taken at the end of the seeding year (hereafter year one) of the studies (2019 and 2020) and at the end of the second year (hereafter year two) of the studies (2020 and 2021) (Table 2.1).

To identify the critical period of weed control (CPWC) weed addition or removal timing treatments were randomly assigned to subplots. To identify the CPWC two components within the period need to be assessed, the critical timing of weed removal (CTWR) and the critical weed free period (CWFP) (Knezevic and Datta, 2015). To identify the CTWR, Japanese millet (*Echinochloa esculenta*), a surrogate weed, was planted at 120 seeds m^{-2}, and removed with glyphosate (1.6 L ha^{-1}) at approximately 180 (two weeks after planting, WAP), 352 (four WAP), 532 (six WAP), or 711 (eight WAP) corn growing degree days (GDD) after planting (Table 2.2). To identify the CWFP, plots were kept weed free 180 (two WAP), 352 (four WAP), 532 (six WAP), or 711 (eight WAP) corn GDD after planting at which time, Japanese millet was planted at the same rate outlined above (Table 2.3). Controls consisted of corn only and corn-alfalfa plots left weedy or weed free for the duration of the experiment. Weather and precipitation data were obtained throughout the growing seasons using Enviro-weather Network from the weather station within 1 kilometer of the study location (Table 2.3).

To assess the effects of interseeding and weed competition stress on perennial alfalfa performance, each experimental run was conducted for two years. In year one, data was collected at each timing (Table 2.2). Two, 1-m^2 (2019) and 0.5-m^2 (2020) quadrats were randomly assigned within each plot to evaluate alfalfa percent cover and millet percent cover. Millet density was collected in the quadrats two weeks after seeding. Data collected on corn stage, corn height, and corn leaf angle were taken on 10 (2019) and 5 (2020) tagged corn plants. Corn canopy closure was measured using a SunScan (Dynamax Inc, Huston, TX) with a measurement taken in the center of two corn rows of each plot at solar noon under full sun. Corn was harvested at approximately half-milk line, which occurred on September 19, 2019, and September 2, 2020, with a self-propelled 152 cm header. Sub-samples were taken to determine percent moisture and feed quality. Corn biomass was dried for 3 days at 66° C and a 328 g sample was ground with Christy mill with a 1-mm screen prior to near infrared reflectance (NIR) analysis. Percent moisture was assessed by weighing a subsample approximately 440 g wet and then weighing it again after 3 days of drying at 66° C. Japanese millet biomass and seed production was collected within the quadrats a week before corn harvest. Japanese millet biomass was dried for three days at 66° C, then dry weight was recorded. Japanese millet seed was processed by hand, seed count was calculated via linear regression (2019) or by hand (2020), and seed viability was assessed via tetrazolium testing (Analysts et al., 2010).

In year two, data was collected before each alfalfa harvest. Alfalfa was harvested four times during year two at one-tenth bloom using a 92 cm Carter Harvester at the height of 7.6 cm. Measurements taken prior to each harvest included alfalfa maturity (1 row, 57 cm) and quality (3 rows, 57 cm). The maturity samples were rated according to Kalu and Fick (1981). Alfalfa biomass for quality analysis (115 g sample) was dried following the same methods as the corn and ground with a Wiley mill with a 4-mm screen and Udy mill with a 1-mm screen prior to NIR analysis. Nutritive value of corn silage and alfalfa was measured using near infrared reflectance spectroscopy (Model DS2500, Foss, Eden Prairie, MN) with the 2020 calibration set for corn silage and alfalfa from the NIRS Consortium (NIRSC, Berea, KY).

Statistical Analysis

Surrogate Weed: Japanese Millet

Japanese millet density, biomass, seed production, and seed viability was analyzed by linear mixed effect models in R (R, 2020) with the lme4 package. Corn hybrid and duration of weed competition were considered fixed effects and replication was considered a random effect. Differences in means were assessed using Tukey's HSD post hoc test in the rcompanion package in R (R, 2020).

Corn

Years was analyzed separately after examining the side-by-side box plots of the residuals and conducting Levene's test for unequal variances. Normality assumptions were checked by examining normality histograms. To identify the CPWC in corn silage data were analyzed using the drc package in R (R, 2020) following the methods outlined in Knezevic and Datta (2015). To estimate a 5% acceptable yield loss a four parameter, log logistic model was fit to corn silage yield data for the 2019 and 2020 CTWR and 2019 CTWR (Table 2.4; Equation 1). The 2020 CWFP was fit to the Weibull type two four parameter model (Table 2.4; Equation 3). A four-parameter log logistic model was used to estimate a 25%, and 50% reduction in corn height in 2019 (Table 2.4; Equation 1). A Weibull type two four parameter model was used to estimate a 25%, and 50% reduction in corn height in 2020 (Table 2.4; Equation 3). A three-parameter log logistic model was used to estimate when 50%, and 90% light interception occurred (Table 2.4; Equation 3). All models used were chosen using the drc modelFit function in R (Table 2.4) (Knezevic and Datta, 2015; R, 2020).

$$Y = c + \frac{d - c}{1 + \exp\left(b(\log(x) - \log(e))\right)} \qquad [1]$$

Equation 1 is for the log logistic model with four parameters where *Y* is the response variable, *x* is the accumulated growing degree days, *d* is the upper limit, *c* is the lower limit, *b* is the relative slope around *e*, and *e* is the inflection point (Ritz et al., 2006).

$$Y = \frac{d}{1 + \exp\left[b(logx - e)\right]} \qquad [2]$$

Equation 2 is the three parameter log logistic model where *Y* is the response variable, *x* is the accumulated growing degree days, *d* is the upper limit, *b* is the relative slope around *e*, and *e* is the inflection point (Ritz et al., 2006).

$$Y = \gamma x^{(\gamma - 1)} \exp(-(x^{\gamma})) \qquad [3]$$

Equation 3 is the Weibull type two four parameter probability density function, where *Y* is the response variable, *x* is the accumulated growing degree days, and γ is the shape parameter. In the type two Weibull function, *x* is greater or equal to zero and γ is greater than zero (Ritz et al., 2006).

To analyze the impact interseeding and weed competition had on corn silage yield, leaf angle, quality parameters, and milk yield were analyzed using linear mixed effects model in R (R, 2020) with the lme4 package. Milk yield was calculated based on Milk 2006 developed by Undersander et al. (2006), which utilizes corn silage quality data and yield to calculate the amount of milk a cow weighing approximately 613 kg would produce. Corn hybrid and weed addition or removal timings were considered fixed effects and replication was considered a random effect. Differences in means were assessed using Tukey's HSD post hoc test in the rcompanion package in R (R, 2020).

Alfalfa

Years was analyzed separately after examining the side-by-side box plots of the residuals and conducting Levene's test for unequal variances. Normality assumptions were checked by examining normality histograms. To identify the CPWC based on alfalfa yield, the drc package in R (2020) was used following the methods outlined in Knezevic and Datta (2015). To estimate a 5% acceptable yield loss a four parameter, log logistic model was fit to alfalfa yield CTWR data for the first cutting for both experimental repeats (Equation 2; Table 2.4). The alfalfa yield CWFP model was fit to a Weibull type two four parameter model (Equation 3).

To analyze the impact that effects of interseeding, corn hybrids, and weed competition had on alfalfa yield, biomass, stem and crown counts, maturity scores, quality parameters, milk yield, and percent cover were analyzed using linear mixed effects model in R (R, 2020) with the lme4 package. Milk yield was calculated based on Milk 2016 developed by Undersander et al. (2016), which utilizes alfalfa forage quality data and yield to calculate the amount of milk a cow weighing approximately 613 kg would produce. Corn hybrid and weed addition or removal timings were considered fixed effects and replication was considered a random effect. Differences in means were assessed using Tukey's HSD post hoc test in the rcompanion package in R (R, 2020).

Results and Discussion

Surrogate Weed: Japanese Millet

Density

Density was recorded 14 days after Japanese millet seeding. Japanese millet density differed between years (p<0.0001); therefore, years were analyzed separately. In 2019, duration of weed competition modified density ($p<0.0001$), while corn hybrid did not, therefore density is averaged over corn hybrids ($p=0.08$). Japanese millet density did not differ between the corn monoculture weedy treatment (UTC) and interseeded weedy treatment (UTCA) (Table 2.7). Weed free after two weeks (WF2) had 62% less weeds than weed removal at 2 weeks (R2) (Table 2.5). There was no difference in Japanese millet density in weed free for 4 weeks (WF4) and weed removal at 4 weeks (R4) with 120.5 and 167.7 plants (m^{-2}), respectively. Weed removal at 6 weeks (R6) had 99% more Japanese millet than weed free for 6 weeks (WF6). Weed free for 8 weeks (WF8) had no Japanese millet establish, while weed removal at 8 weeks (R8) had 181.13 plants (m^{-2}) (Table 2.5).

In 2020, Japanese millet density was modified by a two-way interaction between corn hybrid and duration of weed competition ($p=0.04$). UTC and UTCA treatments in both the pendulum and upright corn hybrids had similar densities (Table 2.5). WF2 and R2 density did not differ between hybrids (Table 2.5). WF4 and R4 density was similar between hybrids (Table 2.5). For the pendulum hybrid, density was 99% greater in R6 compared to WF6 (Table 2.5). There were no differences in density between the upright hybrid R6 and WF6 treatments (Table 2.5). The R8 and WF8 density for the pendulum hybrid were not different (Table 2.5). However, within the upright hybrid, WF8 density was 97% less than the R8 Japanese millet density (Table 2.5).

Differences between years for weed density may have to do with the differences in the amount of precipitation received (Table 2.2). For example, precipitation in June 2020 was 41.14 mm less than June 2019 (Table 2.2). June 2020 also had less rain compared to the 30-year average, which was 73.67 mm and 87.6 mm, respectively. The later weed addition timings are less dense due to lack of precipitation during establishment (Table 2.2) and also lack of a prepared soil bed, as the alfalfa being present in the lower canopy challenged the use of a rake to establish it as used in previous studies (Evans et al., 2003).

Biomass

Japanese millet (surrogate weed) biomass differed between 2019 and 2020 ($p<0.0001$). In 2019, duration of weed control modified biomass ($p<0.0001$), while corn hybrid did not, therefore biomass is averaged over corn hybrids ($p=0.5$). In 2019, there was no difference in weed biomass amongst UTCA and UTC, ranging from 734-748 g (m^{-2}) (Table 2.6). Furthermore, there was no difference in surrogate weed biomass amongst any of the weed addition (WF) or removal (R) timings in 2019 (Table 2.6). In 2020, duration of weed control modified biomass ($p<0.0001$), while corn hybrid did not, therefore biomass is averaged over corn hybrids ($p=0.1$). UTCA biomass was 31% greater than UTC biomass in 2020. Additionally, surrogate weed biomass was similar across many of the weed addition (WF) or removal (R) timings in 2020 (Table 2.6).

The differences between years may have been driven by precipitation differences during Japanese millet establishment, for example precipitation in July 2020 was 16.77 mm less than July 2019 (Table 2.2). July and August in 2019 and 2020 had less rain compared to the 30-year average (Table 2.2). The lack corn hybrid effect on Japanese millet biomass is contrary to previously published results, in which weed biomass was reduced by 73-90% when grown in competition with a pendulum corn hybrid compared to the upright corn hybrid under irrigation (Sankula et al., 2004). Differences in our study and that of Sankula et al., (2004) may be driven by differences in irrigation, ours was rainfed, and weed species present, our study evaluated one grass, while they evaluated multiple broadleaf and grass weeds. Furthermore, the seedbed became less conducive to new seed establishment as the season progressed. Additionally, the highly competitive interseeded environment may have resulted in poor weed establishment, as a similar study found interseeded corn and alfalfa suppressed weed biomass by 65-70% (Osterholz et al., 2020a).

Seed Production and Viability

Japanese millet, a surrogate weed, seed production differed between years (p<0.0001); therefore, years were analyzed separately. In 2019, there was no difference in Japanese millet seed production between UTC and UTCA (p=0.1). Furthermore, averaged across cropping systems there was no difference in Japanese millet seed production when plants were grown in competition with pendulum or upright corn hybrids (p=0.7; Figure 2.1a). Similar to 2019, in 2020, there was no difference in Japanese millet seed production between monoculture or interseeded corn and alfalfa cropping systems (p=0.5). However, in 2020 competition from pendulum corn hybrids reduced Japanese millet seed production by 67% compared to the upright hybrid averaged over cropping systems (p=0.03; Figure 2.1b). Interestingly, Japanese millet seed production decreased by 94% in 2020 compared to 2019 (Figure 2.1a-b).

The 2019 results are similar to those of Sankula et al. (2004), who reported that under irrigated and non-irrigated treatments, weed seed production did not differ between weeds in competition with pendulum or upright corn hybrids. Similar to the 2020 results, Wild proso millet (*Panicum miliaceum*) in competition with short maturing, less competitive architecture corn hybrids produced 66% more seeds than the millet plants grown in competition with longer maturing, more competitive architecture corn hybrids (Williams et al., 2012). Additionally, the light differences induced by corn leaf angles may have played a role, Chauhan (2013) reported that barnyard grass seed production decreased as light decreased under rice (*Oryza sativa*) competition. The differences between years may have been driven by precipitation differences during Japanese millet establishment, for example precipitation in June 2020 was 41.14 mm less than June 2019 (Table 2.2). June 2020 also had less rain compared to the 30-year average, which was 73.67 mm and 87.6 mm, respectively.

Japanese millet seed viability did not differ between years (p=0.85); therefore, years were combined for analysis. Competition from corn hybrids with differing leaf architecture had no impact of surrogate weed seed viability (p=0.3), therefore it was averaged over for analysis. However, surrogate weed seed viability decreased by 46% in the interseeded treatment compared to the monoculture corn treatment (p=0.02; Figure 2.2), averaged across years and corn hybrids. The decrease in viable weed seeds may be due to differences in the rate of seed maturation under highly competitive interseeded maternal environments vs. less competitive monoculture maternal environments. For example, Powell amaranth (*Amaranthus powellii*) seed viability decreased by 10% when grown in competition with broccoli (*Brassica oleracea*) and interseeded winter rye (*Secale cereale*) compared to broccoli monocultures (Brainard et al., 2005). Wild proso millet seed germination was 12.5% greater when in competition with short maturing, less competitive architecture corn hybrids than millet plants grown in competition with longer maturing, more competitive architecture corn hybrids (Williams et al., 2012). Targeting the weed seedbank is essential to sustainable weed management systems (Garrison et al., 2014), although weed seed rain did not differ between interseeded and monoculture treatments in our study, surrogate weed seed viability was reduced by 46% suggesting this interseeded corn and alfalfa system can provide cultural weed control via a more competitive environment.

Corn

Critical Period of Weed Control

The critical period of weed control (CPWC) is made up of two components the critical timing of weed removal (CTWR) and the critical weed free period (CWFP). The CTWR did not differ between years (p=0.9) and hybrids (p=0.1) although the CWFP differed amongst years (p=0.0001), therefore years were analyzed separately for both periods. Averaged across hybrids, the estimated 5% acceptable yield loss for the CTWR was found to be 303 (62) GDDs for both 2019 and 2020 (Figure 2.3a-b). In 2019 the CWFP did not differ amongst hybrids (p=0.3), thus averaged across hybrids the 5% acceptable yield loss for the CWFP was estimated to be greater than the study duration (1,130 GDD; data not shown). In 2020, the CWFP differed between hybrids (p<0.0001), although estimates for both hybrid were greater than the duration of the study, which was 1,115 GDD (data not shown).

Results from this study suggest that interseeding corn and alfalfa can buffer weed competition for 303 GDD after planting after which time yield steadily declines if weeds are allowed to compete longer. For context, in 2019 and 2020, 303 GDD was approximately July 6 and June 28, respectively. Furthermore, due to the highly competitive nature of the interseeded system, weed control may only have to occur once at the CTWR, given that later emerging weeds had little impact on yield. Previous research from Ontario, Canada reported the CTWR for monoculture corn was 14 to 18 days after emergence (Halford et al., 2001). Based on the days after corn emergence, the CTWR would have been between 234 to 282 GDD, which is slightly less than the 303 GDD. Other studies have reported the CTWR began at the first leaf tip stage (Page et al., 2012), which occurs at corn emergence (90 GDD). This is considerably earlier than the 303 GDD to reach the CTWR in the interseeded study. Given these differences, it is important to evaluate the CTWR in a site, species, and cropping system specific manner. Overall, interseeding corn and alfalfa resulted in a longer CTWR period compared to monoculture corn studies resulting in an increased window for postemergence weed control.

Leaf Angles

Leaf angles differed between years (p<0.0001); therefore, years were analyzed separately. In 2019, the interaction between corn hybrid and weed competition duration was significant (p=0.02). The pendulum corn hybrid, which has a wider leaf angle, was 5 degrees greater than the upright corn hybrid, which is a narrower leaf angle, for the UTC in 2019 (Table 2.7). In 2019 within pendulum hybrids, leaf angle did not differ between any WF and R treatments (Table 2.7). In 2019 within upright hybrids, leaf angle decreased by 1 degree in the R8 compared to the R4 treatment (Table 2.7). In 2019, when comparing across hybrids within each duration of weed control treatment, there were no differences (Table 2.7). In both pendulum and upright corn hybrids, there were no differences between the WFC, WFCA, UTC, and UTCA treatments (Table 2.7).

In 2020, main effects of hybrid and weed competition duration modified leaf angle (p=0.0002; p<0.0001). The pendulum corn hybrid's leaf angle was 4% greater than the upright corn hybrid averaged across duration of weed control in 2020 (Table 2.7). In 2020, the pendulum WFC leaf angle is 3.4 degrees less than UTC (Table 2.7). A similar decrease occurs in the upright hybrid, where WFC is 4.2 degrees less than UTC (Table 2.7). This trend is not observed in the WFCA and UTCA treatments for both pendulum and upright corn hybrids (Table 2.7). Within a corn hybrid there were no difference between WFC and WFCA treatments in 2020 (Table 2.7).

Interestingly, regardless of hybrid or study year, there were no differences in leaf angles between the WFC and WFCA within each hybrid, suggesting that the addition of alfalfa has no impact on leaf angle; however, the presence of weeds in the interseeded system reduced leaf angles (Table 2.7). The pendulum hybrid leaf angle was greater than the upright hybrid when averaged over duration of weed competition for both 2019 and 2020. The two-way interaction between corn hybrid and duration of weed competition in 2019 suggests that leaf angle changes based on the type of hybrid and duration of weed competition the hybrid is experiencing. Furthermore, main effects of corn hybrid and duration of weed competition modified leaf angle in 2020, thus suggesting that leaf angle changes based on the duration of weed competition as well. Changes in leaf angles may be attributed to shade avoidance, which is driven by competition for light and the sensing of light off neighboring plants (Afifi and Swanton, 2012; Cerrudo et al., 2012; Ciolfi et al., 2013). Shade avoidance response has been well documented in corn, resulting in phenological and physiological alterations such as the reduction in height and changes in corn crop maturity rates (Afifi and Swanton, 2012), when exposed to artificial red to far red light. Stem elongation can also occur in crops as a shade avoidance response, which can negatively affect yield due to partitioning of nutrients towards height and not the harvestable organs (Boccalandro et al., 2003; Carriedo et al., 2016; Robson et al., 1996). Leaf angle differences can have a direct impact on biomass accumulation (Hikosaka and Hirose, 1997). Leaf angles are genetically predetermined but can be modified via the environment (Fellner et al., 2003) which is supported from results of our study. Overall, how interseeded corn changes its leaf angle in relation to weed competition could play a crucial role in integrated weed management in this system (Callaway, 1992; Sankula et al., 2004).

Canopy Closure

The rate of canopy closure differed between years (p<0.0001). In 2019, there was no difference in the number of GDD required to reach 50% canopy closure between pendulum or upright corn hybrids grown under WFC or WFCA competition (Table 2.8). In 2019, there was no difference in the number of GDD required to reach a 50% canopy closure between pendulum or upright corn hybrids grown under UTC and UTCA competition (Table 2.8). WFCA and WF2 reached 50% canopy closure approximately 118 GDD sooner than the UTC and UTCA within the pendulum hybrid. The pendulum R8 treatment reach 50% canopy closure 208 and 234 GDD sooner than the pendulum WFCA and WF2, respectively (Table 2.8). Within the pendulum hybrid 50% canopy closure was achieved approximately 212 GDD sooner in R8 than the WFC, WF4, WF6, and WF8 (Table 2.8). In the upright WFC, WFCA, WF2, R2, WF4, WF6, and WF8 50% canopy closure was achieved approximately 351 GDD, which was 208 GDD later than the R8 pendulum treatment (Table 2.8). In 2019, 90% canopy closure was achieved 250 GDD sooner in the WFC pendulum compared to the WFCA pendulum treatment (Table 2.8). R4 and R6 upright treatments reached 90% canopy closure 409 GDD later than the WFC pendulum treatment (Table 2.8). In 2019, pendulum R4 and R6 treatments and upright R4 and R6 treatments had a 90% canopy closure greater than the corn harvest date; therefore, they are estimated to never reach 90% canopy closure. In 2020, WF8 pendulum treatment reached 50% canopy closure 150 GDD earlier than the UTCA upright treatment (Table 2.8). There were no significant differences between 90% canopy closure rates in 2020 (Table 2.8).

The differences between years may have resulted from the differences in precipitation during the two study years (Table 2.2), which influenced weed establishment resulting in more weeds in 2019 than in 2020. Thus, potentially changing the magnitude of the shade avoidance signal the corn received, which can alter height and maturity timing in corn (Afifi and Swanton, 2012). Further research is needed on how shade avoidance can change leaf architecture and canopy closure timing, especially in interseeded systems.

Corn Height

The GDD required to reach a 25% and 50% reduction in corn height differed between years ($p<0.0001$). In 2019, pendulum and upright corn hybrids, required the same number of GDD to reach a 25% reduction in height when grown under WFC and WFCA (Table 2.9). Furthermore, in 2019, pendulum and upright corn hybrids, required the same number of GDD to reach a 25% reduction in height when grown under UTC and UTCA (Table 2.9). R4 reached a 25% reduction in height 60 GDD later in the pendulum hybrid then in the upright hybrid in 2019 (Table 2.9). R4 reaches a 50% reduction in height in the pendulum treatment 91 GDDs later than the WFC (Table 2.9). All treatments except pendulum R4 do not differ for number of GDD required to reach a 50% reduction in height in 2019 (Table 2.9).

In 2020, there was no difference in the number of GDD required to reach a 25% reduction in corn height when pendulum corn hybrids were grown under WFC, WFCA, UTC, and UTCA treatments (Table 2.9). In contrast upright corn hybrids grown in UTC reached a 25% reduction in corn height 44 GDD earlier than the UTCA in 2020 (Table 2.9). The pendulum hybrid WFC treatment reached a 50% reduction in height 32 GDD sooner than the UTCA treatment (Table 2.9). Additionally, there were no difference in the number of GDD required to reach a 50% reduction in corn height between WFC and WFCA treatments (Table 2.9). Interestingly, the pendulum R6 treatment reached a 50% reduction in corn height 33 GDD later than the WFC pendulum in 2020 (Table 2.9).

Similar to other parameters measured differences between years may be due to differences in weed establishment (Table 2.9), resulting in more weeds in 2019 compared to 2020. Many studies support the result that early season weed competition decreases overall corn height (Carey and Kells, 1995). Therefore, to ensure high yielding corn in this study it would be important to remove weeds by 303 GDD as identified via CPWC (Figure 2.3a-b). The differences between years can be attributed to less precipitation in 2020 compared to 2019 and the 30-year average (Table 2.2). Furthermore, corn height is reduced under drought conditions (Shahbazi et al., 2021).

Weed Free Corn Yield

Weed free yield differed between 2019 and 2020 (p<0.0001). In 2019, there was no difference in weed free corn yield between hybrids or cropping systems (p=0.7, p=0.5, Figure 2.4a). In 2020, there was no difference in weed free corn yield between hybrids or cropping systems (p=0.5, p=0.5, Figure 2.4b). Even though corn yield differed significantly based on the presence of weeds at specific times (Figure 2.3a-b), the presence of interseeded alfalfa did not reduce corn yield in either study year averaged over corn hybrid (Figure 2.4a-b). This is in contrast to the 9% corn yield decrease reported by Osterholz et al. (2018) when comparing monoculture corn to interseeded corn and alfalfa.

Corn Silage Quality

Corn silage quality parameters: crude protein (CP), neutral detergent fiber (NDF), neutral detergent fiber digestibility after 48 hours (NDFD 48), and starch all differed between years (p<0.0001); therefore, years were analyzed separately for each parameter. In 2019 there was no impact of corn hybrid on CP (p=0.12). CP concentration decreased by 10% under WFCA compared to the WFC system in 2019, averaged across hybrids. In 2019 there was no difference in CP between UTC and UTCA, averaged across hybrids (Figure 2.5). In 2020 there was no impact of corn hybrid on CP (p=0.06). CP concentrations were the same between the WFC, WFCA, and UTC, averaged across hybrids (Figure 2.5). UTCA CP concentration decreased by 13% compared to the WFCA in 2020, averaged across hybrids (Figure 2.5). CP concentration also decreased by 14 and 18% in R6 and R8, respectively, in 2020 compared to the WFCA, averaged across hybrids (Figure 2.5). Similar to our study Pilipavicius et al. (2003) demonstrated that weed competition decreased CP by 11% in corn silage compared to weed free treatments. In contrast to our study, Iqbal et al. (2018) reported sorghum CP concentration increased by 15% when intercropped with forage legumes. An increase of 11-51% of CP was observed when corn was intercropped with other plants compared to a corn monoculture system (Lithourgidis et al., 2011).

In 2019, NDF did not differ between hybrids (p=0.1). NDF concentrations changed with the duration of weed competition (p<0.0001). NDF increased by 20% in UTCA compared to the WFCA in 2019, averaged across hybrids (Figure 2.6). The lack of significant differences between the WFCA and WFC treatments suggest that the alfalfa had no effect on corn NDF (Figure 2.6). NDF also increased by 22% under R8 compared to WFCA in 2019, averaged across hybrids (Figure 2.6). In 2020, NDF did not differ between hybrids or duration of weed competition (p=0.1; p=0.07). Overall, NDF helps determine the digestibility of the plant to be consumed by livestock, so the increase in NDF observed in the weedy treatments, negatively impacted the plants ability to be digested by animals (Carita et al., 2016; Contreras-Govea et al., 2009).

In 2019, main effects of corn hybrid and duration of weed competition impacted NDFD 48 (p=0.0008; p<0.0001). NDFD 48 was slightly less in the upright corn hybrid with 68% (0.27) compared to the pendulum corn hybrid with 69% (0.3), averaged across durations of weed competition (data not shown). In 2019, NDFD 48 concentrations increased by 4.5%, 5.5%, and 4.4% in treatment R4, R6, and R8, compared to the WFCA treatment, averaged across hybrids (Figure 2.7). There was no difference in NDFD 48 between duration of weed competition treatments in 2020 (p=0.4; Figure 2.7). The documented increase in NDFD 48 concentration as corn was in competition with weeds for longer durations may be related to lignin concentration, reductions in lignin content can increase the digestibility of the forage (Gerhardt et al., 1994; Oliver et al., 2004).

In 2019, main effects of corn hybrid and duration of weed competition impacted starch (p<0.0001; p<0.0001). In 2019, starch concentration was 9% less in the pendulum hybrid compared to the upright corn hybrid, averaged across durations of weed competition (data not shown). Starch did not differ between WFC and WFCA, averaged across hybrids (Figure 2.8). In 2019, starch content decreased with the increase in the duration of weed competition (Figure 2.8). For example, starch decreased by 0.6, 22, 44 and 34% in R4, R6, R8, and UTCA, respectively, compared to WFCA (Figure 2.8).

In 2020, the main effects of corn hybrid impacted starch content (p=0.003); however, duration of weed control did not have an impact (p=0.18). In 2020, starch concentration was 7% less in the pendulum hybrid compared to the upright corn hybrid, averaged over duration of weed competition treatments (data not shown). In 2020, there were no differences between the WF and R treatments (Figure 2.8). Starch concentration is highly dependent on corn hybrid selection (Ferraretto et al., 2015). Starch concentration in corn decreased by 11% in an intercropped system with faba bean (*Vicia faba*) when weeds were present compared to no weeds (Stoltz and Nadeau, 2014), which is similar to the 2019 results, where starch concentration decreased in the WFCA treatment compared to the WFC. As a whole, corn silage quality data suggest that weed removal timing has an effect on CP, NDF, NDFD 48, and starch concentration. However, the addition of alfalfa under weed free conditions does have an impact on starch concentration in 2019, but not in 2020, however all other quality parameters were not negatively impacted by the presence of alfalfa.

Corn Milk Yield

Estimated milk yield differed between years (p<0.0001). There was no impact of corn hybrids on milk yield resulting in hybrids being combined for the analysis (p=0.2), however the duration of weed competition had an effect on milk yield in 2019 (p<0.0001). There were no differences between WFC and WFCA milk yield in 2019. Milk yield decreased by 37% in UTCA compared WFCA in 2019 (Figure 2.9). In 2019, R4, R6, and R8 decreased milk yield by 24, 32, and 36% compared to WFCA (Figure 2.9). In 2020, there was no impact of corn hybrids on milk yield (p=0.3); therefore, it was averaged over for the analysis, however the duration of weed competition had an effect on milk yield (p=0.02). In 2020, the UTCA milk yield decreased by 28% compared to UTC (Figure 2.9). In 2020, WFC and WFCA did not differ in milk yield (Figure 2.9). The lack of milk yield differences between the WFCA and WFC for both 2019 and 2020 suggests that interseeding does not decrease milk yield. Decreases in milk yield as duration of weed competition increased is supported by Cox et al. (2005) who reported similar results, that weeds competing until late vegetative and early reproductive stages in corn resulted in a decrease in milk yield. Cox et al. (2005) also reported that drought conditions can decrease milk yield, which helps explain the differences between years in our study (Table 2.2), as precipitation in June 2020 was 41.14 mm less than June 2019 (Table 2.2). June 2020 also had less rain compared to the 30-year average, which was 73.67 mm and 87.6 mm, respectively.

Alfalfa

Critical Period of Weed Control

The critical period of weed control is made up of two components the critical timing of weed removal (CTWR) and the critical weed free period (CWFP). The CTWR was not significantly different between year or corn hybrid for the estimated 5% acceptable yield loss (p=0.4; p=0.6), therefore study years and corn hybrids were combined for analysis. The 5% acceptable yield loss was estimated to be 369 (123) GDD for the CTWR (Figure 2.10). The CWFP was not significantly different between year or hybrid for the estimated 5% acceptable yield loss (p=0.9; p=0.9), therefore study years and corn hybrids were combined for analysis. The CWFP was estimated to be at 394 (201) GDD for a 5% acceptable yield loss (Figure 2.10).

Consequently, the CPWC starts at 369 GDD and ends at 394 GDD in the interseeded establishment year to maximize first cutting alfalfa yield the following season. For context, 369 GDD was on June 30, 2019, and June 22, 2020, and 394 GDD was on July 2, 2019, and June 24, 2020. These GDD align with the removal time of 303 GDD provided by the corn silage CPWC analysis (Figure 2.3). The standard errors for the alfalfa CTWR and CWFP overlap, therefore weed removal will only have to occur once within this period. The CTWR for the alfalfa is different than Dillehay et al. (2011), which concluded CTWR was 97 GDD and the CWFP was 862 GDD in the establishment year. The CTWR and CWFP is much later in the interseeded study, which may be due to increased competition from interseeded corn and alfalfa against weeds. Overall, the CPWC has not be thoroughly researched in alfalfa due to the perennial nature and consistent cutting schedule (Dillehay and Curran, 2010), so the differences in CTWR may additionally be due to differences in weed densities, climate, or site between the previously published study and our study (Dillehay et al., 2011; Hall et al., 1992). Our results support prior research that found interseeded corn silage and alfalfa had the inherent ability to suppress weeds more than monoculture corn or alfalfa (Osterholz et al., 2020a). The underpinnings of this inherent ability to suppress weeds may be linked to the later CPWC start and finish identified in this study.

Percent Cover

Establishment year percent cover did not differ between 2019 and 2020; therefore, years were analyzed together (p=0.8). Establishment year percent cover was modified by a two-way interaction between corn hybrid and duration of weed competition (p=0.005). Percent cover decreased by 74 and 69% in the UTCA compared to the WFCA for the pendulum and upright hybrids, respectively (Table 2.10). There was no difference in alfalfa percent cover between the pendulum and upright corn hybrids under UTCA, R6, and R8 (Table 2.10). All WF timings were similar to the WFCA treatment in the pendulum and upright (Table 2.10). This is contrary to our original hypothesis that the upright hybrid would increase alfalfa percent cover by allowing more light to reach the alfalfa; however, the alfalfa may be experiencing an increase in light competition resulting in a shade avoidance response (Ciolfi et al., 2013). The trend is similar across hybrids, where a decrease in percent over begins around the R4 treatment and continues under R6 and R8, which supports the idea that weed removal should occur around 369-394 GDD in the establishment year to have an acceptable alfalfa yield in the first cutting the following year.

Stem and Crown Count

Stem counts collected in the fall of the establishment year different between years (p<0.0001). In 2019 corn hybrids did not affect stem counts, so it was averaged over for the analysis (p=0.4); however, the effect of duration of weed competition did have an effect (p<0.0001). There were no difference in alfalfa stem counts between any of the WF treatments. Furthermore, the only treatment that reduced alfalfa stem counts was the R8 and UTCA treatments which reduced stem counts by 57-69% in 2019 compared to all other treatments (Figure 2.11). In 2020, corn hybrids did not affect stem counts (p=0.5), but the effect of duration of weed competition did have an effect (p=0.02); therefore, hybrid was averaged over for the analysis. Alfalfa stem counts in 2020 were reduced by 42 and 34% for the R8 treatment compared to R2 and R4 treatments (Figure 2.11). Interestingly in 2020 there was no difference in alfalfa stem counts between WFCA and UTCA. Stem counts collected in the fall of 2020 were approximately 18% less than the alfalfa stems counted in 2019 averaged over corn hybrid and duration of weed competition (Figure 2.11). Alfalfa stem counts are a nondestructive measurement that can assist in predicting the ability of the alfalfa stand to overwinter and be productive the following year (Undersander and Cosgrove, 2007). Stem counts reported by Undersander and Cosgrove (2007) for monoculture spring seeded alfalfa were 430 stems m^{-2}, which was 126 and 270 stems m^{-2} more than what was counted in the weed free treatments in 2019 and 2020, respectively, suggesting that interseeding may reduce alfalfa stem production. However, alfalfa stem counts do not equal the actual number of alfalfa plants; therefore, destructive alfalfa crown counts were also taken at the end of the second year of each study.

As this is an ongoing experiment, 2021 crown counts have not yet been taken. In 2020 crown count data was collected and the duration of weed competition impacted crown counts (p<0.0001). Corn hybrid did not affect alfalfa crown count and was averaged over for the analysis (p=0.3). Crown count decreased by 44% when comparing the WFCA to UTCA (Figure 2.12). Crown count also decreased in R8 treatment by 36% compared to WFCA treatment. There was no difference in alfalfa crown count between UTCA and R8 treatments.

Alfalfa Biomass

Alfalfa biomass harvested in the fall of the interseeding year, approximately 4 weeks after corn harvest, and biomass collected during the first cutting the following year, were significantly different between years (p<0.0001). Duration of weed competition modified alfalfa fall biomass in 2019 (p<0.0001), but there was no impact of corn hybrid on alfalfa fall biomass therefore it was averaged over for the analysis (p=0.7). Alfalfa fall biomass decreased by 95 and 87% in the R6 and R8 compared to WFCA (Table 2.11). There was no difference in fall biomass between UTCA, R6, and R8 treatments. Duration of weed competition modified alfalfa fall biomass in 2020 (p=0.01), but there was no impact of corn hybrid on alfalfa fall biomass therefore it was averaged over for the analysis (p=0.7). In 2020, fall biomass decreased by 46% under R8 compared to R4.

Alfalfa spring biomass was significantly different between years (p<0.0001). In 2020, hybrid had no effect on spring biomass; therefore, averaged over for the analysis (p=0.9), but duration of weed competition did (p=0.0004). Alfalfa spring biomass was 48 and 47% less in the UTCA and R8 compared to R4 for the study established in 2019 (Table 2.11). In 2021, there was no difference in alfalfa spring biomass between corn hybrid or duration of weed competition treatments (p=0.7; p=0.09).

When comparing fall alfalfa biomass to the spring, biomass increased by 62 and 71% in 2019 to 2020 and 2020 to 2021, respectively, for the weed free treatment (Table 2.11). However, the increase in alfalfa biomass observed from fall to spring, does not make up the alfalfa biomass differences due to weed treatments (Table 2.11). The effects of extreme weed competition in the establishment year are present the following season and weed removal should occur before the eight-week timing in the interseeded system to improve alfalfa biomass the year following interseeding. Additionally alfalfa grown under low R:FR light, such as that reflected from the corn in the establishment year, has resulted in less biomass than alfalfa grown in ambient light observed in previous studies (Baldissera et al., 2014). This could also be a factor driving differences between spring and fall biomass.

Maturity Score

Mean stage by count (MSC) was significantly different between the first cuttings taken in 2020 and 2021 ($p<0.0001$); however, hybrid and duration of weed competition was not ($p=0.8$; $p=0.3$). MSC for the first alfalfa harvest in 2020 was 2.15 (0.02) and in 2021 was 2.42 (0.05) averaged over corn hybrid and duration of weed competition. Differences in MSC between years suggests that environmental conditions between establishment years caused differences in maturation rates (Table 2.2). All four cutting in 2020 were evaluated and MSC differed between cuttings in 2020 ($p<0.0001$), but not hybrid or duration of weed competition ($p=0.9$; $p=0.2$) and was averaged over both main effects for analysis. MSC was highest in the second cutting, then decreased by 31, 17, and 41% in first, third, and fourth cutting, respectively (Figure 2.13). The differences between the first and second cutting may be due to increased competition in the establishment year, which can result in later flowering (Baldissera et al., 2014). The decrease in alfalfa maturity score is similar to the trend reported in Kalu and Fick (1981), where the MSC decreased as the number of cuttings increased. Alfalfa maturity effects quality: therefore, it is important to harvest the alfalfa when there are more vegetative plants in the field than flowering ones, which occurred across all our cuttings as they were less than 4 MSC (Kalu and Fick, 1983).

Alfalfa Yield

First cutting alfalfa yield did not differ between years, therefore years were averaged over for the analysis ($p=0.1$). Furthermore, first cutting alfalfa yield did not differ between hybrids so it was averaged over for the analysis ($p=0.8$), but did differ between duration of weed competition ($p<0.0001$). UTCA reduced alfalfa yield by 44% compared to WFCA (Figure 2.14). Furthermore, weed competition for eight weeks (R8) reduced alfalfa yield by 31% compared to the WFCA (Figure 2.14). Similar to other parameters measured there was no difference in alfalfa yield between WF treatments. Furthermore, there was no difference in alfalfa yield between UTCA and R8 treatments (Figure 2.14).

Alfalfa harvested in 2020 was significantly different between cuttings ($p<0.0001$), therefore cuttings were analyzed separately. The corn hybrid had no effect on alfalfa yield for the first, second, third, and fourth cuttings ($p=0.4$; $p=0.4$; $p=0.4$; $p=0.5$), therefore it was averaged over for analysis. For the first and third cuttings, duration of weed competition was significant ($p<0.0001$; $p=0.004$). For the second and fourth cutting duration of weed competition was not significant ($p=0.2$; $p=0.2$). Within the first cutting, UTCA and R8 decreased alfalfa yield by 47 and 40%, respectively, compared to WFCA (Figure 2.15). In the third cutting, UTCA alfalfa yield decreased by 23% compared to WFCA (Figure 2.15).

For the first and third cuttings of the alfalfa, yield was influenced by the presences of weeds. This is slightly different then previously reported alfalfa yields, where weeds had no impact on the following years alfalfa yields (Hoy et al., 2002). Differences between yield may be due to differences in the crown count, as both decreased around the R8 treatment (Figure 2.12). Cosgrove and Barret (1987) reported that long term alfalfa yield is dependent on the ability of alfalfa to establish itself in the first year. Therefore, long-term alfalfa yield is dependent on the ability of alfalfa to establish during the first year, it is important to remove weeds before the eight-week timing, which would occur with the utilization of the CPWC.

Alfalfa Forage Quality

Crude protein (CP) for the first cutting was significantly different between years ($p<0.0001$). In 2020 there was no difference in CP between duration of weed competition treatments resulting in the average of these treatments in the analysis ($p=0.09$), however corn hybrid modified CP ($p=0.04$). Alfalfa in competition with upright corn hybrids resulted in a 2%

decrease in CP for the first cutting in 2020 compared to the pendulum hybrid effects (Figure 2.16). In 2021, there was no difference in CP between duration of weed competition or corn hybrid treatments (p=0.9; p=0.5). The differences between years may be due to differences in precipitation (Table 2.2) or weed biomass (Table 2.7). Crude protein concentrations for the first cutting is similar to the historical figure that ranges from 15% to 17% (Popovic et al., 2001). The lack of effect the duration of weed competition had on CP concentration is interesting as previous studies have shown that weed competition can decrease CP concentration (Cords, 1973; Klemme and Albrecht, 1952).

Neutral detergent fiber (NDF) was not significant between years (p=0.001). NDF concentration in 2020 was affected by duration of weed competition (p<0.0001) and not by corn hybrid (p=0.3). NDF concentration decreased by 13% in UTCA compared to WFCA in 2020 (Figure 2.17). In 2021, alfalfa NDF concentration was not affected by effect of hybrid or duration of weed competition (p=0.6; p=0.3). NDF concentrations ranging from 40-65% are acceptable for animal feed (Undersander et al., 2011). The majority of NDF concentrations are higher than 40%, except the UTCA treatment 2020, therefore all other weed free and removal are acceptable for animal feed.

Neutral detergent fiber digestibility after 48 hours (NDFD 48) was significantly different in the first cuttings between years (p<0.0001). In 2020, duration of weed competition modified NDFD 48 (p=0.003); however, there were no differences between hybrids, therefore corn hybrids were averaged for the analysis (p=0.6). In 2020, NDFD 48 increased by 5% with UTCA compared to R2, WF2, and WF8 (Figure 2.18). In 2021, effect of hybrid and duration of weed competition were not significant (p=0.3; p=0.5). NDFD 48 concentrations ranging from 35-50% are acceptable for animal feed, all NDFD 48 reported in this study are within that acceptable range (Undersander et al., 2011).

Acid detergent fiber (ADF) concentrations were significantly different in the first cutting between years (p<0.0001). In 2020, ADF concentration differed between the duration of weed competition treatments (p<0.0001), but not corn hybrids therefore for the analysis hybrid was averaged over (p=0.5). The UTCA has approximately 12% less ADF compared to the WF2, R2, WF4, R4, WF6, and WF8 treatments in 2020 (Figure 2.19). In 2021, there were no differences across the weed duration treatments or corn hybrids (p=0.2; p=05). However, all ADF levels were less than 41% in both years (Figure 2.19), which is considered the point at which the ADF can be harder for the animal to digest; therefore, all alfalfa from the first cuttings would be acceptable for feed.

Alfalfa ash concentration differed between 2020 and 2021 for the first cutting (p<0.0001). In 2020, ash concentration was modified by the duration of weed competition (p=0.01), but not by corn hybrid (p=0.8). Ash was 2% less in WF2 compared to the WFCA treatment, averaged across hybrids in 2020. In 2021, hybrid and duration of weed competition did not have an effect on ash concentration (p=0.09; p=0.2). When comparing the first cutting of each year, ash was slightly less on average in 2020 compared to 2021 (Figure 2.20).

Relative forage quality (RFQ), a statistic calculated using alfalfa quality parameters was not different between years (p=0.001). RFQ was not significant for main effect of corn hybrid; therefore, results are averaged over the effect of hybrid in 2020 (p=0.4). However, RFQ was modified by the duration of weed competition (p<0.0001) in 2020. RFQ increased by approximately 17% for the UTCA compared to the WFCA, WF2, R2, WF4, R4, WF6, and WF8 treatments in 2020 (Figure 2.21). In 2021, corn hybrids and duration of weed competition did not impact RFQ (p=06; p=0.5). RFQ for all addition and removal timings fall within 125-151 except for the UTCA in 2020, which is greater than 151 (Undersander et al., 2011). Overall, the effects of weed competition and corn hybrids had minimal effects on alfalfa quality as it all fell within normal alfalfa feed parameters.

Alfalfa Milk Yield

Within the first cutting, milk yield did not differ between years, therefore years were averaged over for the analysis (p=0.4). The effect of corn hybrid did not vary milk yield, so it was averaged over in the analysis (p=0.7); however, milk yield was different between duration of weed competition treatments (p<0.0001). Weed competition for the duration of the experiment (UTCA) reduced milk yield by 41% compared to the WFCA (Figure 2.22). Weed competition for eight weeks (R8) reduced milk yield by 30% compared to the WFCA (Figure 2.22). Interestingly, milk yield did not differ between the R8 and UTCA treatments (Figure 2.22).

Alfalfa harvested in 2020 was significantly different between cuttings (p<0.0001), therefore cuttings were analyzed separately. For the first, second, third, and fourth cuttings, hybrid did not affect alfalfa milk yield (p=0.4; p=0.2; p=0.9; p=0.7), therefore it was averaged over for the analysis. However, duration of weed competition did impact first and third cutting milk yield (p<0.0001; p=0.02), but not the second and fourth cuttings (p=0.1; p=0.5). Alfalfa milk yield in the first cutting decreased by 42 and 36% in the UTCA and R8, respectively, compared to WFCA (Figure 2.23). In the third cutting alfalfa milk yield decreased by 21% in the UTCA compared to the WFCA (Figure 2.23).

Interseeding corn silage and alfalfa has the ability to positively impact economic, environmental, and animal health issues. Additionally, with the identification of the CPWC adoption of interseeding may increase. Hybrids did not have an effect on the CPWC; however, they did decrease weed seed production in 2020. Seed viability decreased by 46% in the interseeded system compared to the monoculture corn. Corn yield and quality did not decrease due to interseeding with alfalfa; however, the presence of weeds for extended periods of time resulted in decreases in corn yield and quality. This is in contrast to previous studies evaluating interseeded corn and alfalfa which documented a 9% decrease in interseeded corn yield (Osterholz et al., 2018). Alfalfa yield for the first cutting was impacted by the effects of weeds in the establishment year. This may be due to lack of establishment in the weedy treatments as supported by the stem count, crown count, and percent cover data. Alfalfa quality for the first cuttings in 2020 and 2021 were not impacted by the presence of weeds during the establishment year, which resulted in the milk yield only being affected by the yield components, not quality.

Future research should be targeted to increase the understanding of interseeding systems and how they interact with pests in the field. Such as, how weed species diversity changes in an interseeded system of corn silage and alfalfa compared to monoculture systems of corn and alfalfa. Additionally, it would be interesting to evaluate weed seed dynamics further as the interseeding system has the ability to decrease weed seed viability. In addition, identifying the amount of interspecific competition that elicits a shade avoidance response by the cash crop, as seen in the corn hybrids in this study, could assist in selecting a seeding rate for alfalfa that could optimize alfalfa biomass and cash crop yield. Identification of the critical period of weed control, evaluation of quality parameters, and alfalfa yield in the interseeded system will hopefully increase adoption and increase interest in other systems that have the ability to mitigate environmental and economic issues developing in agriculture.

APPENDIX

Table 2.1. Soil characteristics (soil type, pH, organic matter) of the interseeded corn silage and alfalfa two-year field study location (2019-2021) taken in the fall of each year.

Year	Timeline	Soil Type	pH	Organic Matter (%)
2019	Establishment Year	Loam	5.9	3.0
2020	Second Year	Loam	6.0	2.6
2020	Establishment Year	Sandy Clay Loam	6.6	2.1
2021	Second Year[1]	-	-	-

[1] Second year soil samples have yet to be collected, due to the ongoing nature of this experiment

Table 2.2. Monthly precipitation totals at the study location in East Lansing, MI for 2019-2021.

	2019	2020	2021	30-year average[2]
Month	Precipitation (mm)			
May	85.08[1]	108.97	24.37	85.3
June	114.81	73.67	176.5	87.6
July	58.42	41.65	95	72.1
August	18.28	69.34	-	82
September	92.46	108.72	-	88.9
Total	369.05	402.35	-	415.9

[1] Precipitation data collected from Michigan Enviro-Weather station within 1 kilometer of the study location

[2] Monthly 30-year average precipitation for Lansing, MI, data retrieved from NOAA Nation Centers for Environmental Information DOI:10.7289/V5PN93J

Table 2.3. Dates and cumulative growing degree days (GDD) estimates starting at planting for Japanese millet (surrogate weed) addition and removal, corn and alfalfa planting, and harvests for an interseeded field study of corn silage and alfalfa replicated two times over three years (2019-2021).

	Experimental Repeat 1			Experimental Repeat 2		
	Date	Alfalfa GDD (5°C)	Corn GDD (10°C)	Date	Alfalfa GDD (5°C)	Corn GDD (10°C)
Planting	6/4/2019	0	0	5/28/2020	0	0
Duration of Weed Competition						
WF or R 2[2]	6/26/2019	293.4	180.5	6/17/2020	279.9	181.3
WF or R 4	7/11/2021	571.9	384.0	6/29/2020	479.4	320.8
WF or R 6	7/23/2019	793.5	545.6	7/13/2020	747.3	518.7
WF or R 8	8/6/2019	1027.7	709.8	7/28/2020	1017.4	713.8
10[3]	8/20/2019	1260.3	872.4	8/11/2020	1233.3	860.3
Harvests						
Corn	9/19/2019	1666.6	1130.8	9/2/2020	1598.7	1115.9
Fall Alfalfa	10/25/2019	1968.3		10/14/2020	1982.5	
First Cutting	6/4/2020	2519.6		6/4/2021	2731.3	
Second Cutting[1]	7/7/2020	3050.7				
Third Cutting	8/6/2020	3563.0				
Fourth Cutting	9/14/2020	4147.8				

[1] For the 2nd run of this experiment, not all alfalfa cuttings have occurred, due to the ongoing nature of this experiment.

[2] WF or R 2=Weed free or removal of weeds 2 weeks after planting; WF or R 4=Weed free or removal of weeds 4 weeks after planting; WF or R 6=Weed free or removal of weeds 6 weeks after planting; WF or R 8=Weed free or removal of weeds 8 weeks after planting

[3] Data collected approximately 2 weeks after the last weed addition and removal timings

Table 2.4. List of models used for field growth parameters and critical period of weed control parameters. Models were chose using the modelFit function in R (2020).

Crop	Year	Data	Model Used	ModelFit value
Corn	2019	CTWR[1]	LL.4	p=0.5
		CWFP	LL2.4	p=0.7
	2020	CTWR	LL.4	p=0.5
		CWFP	W2.4	p=0.4
	2019	Height	LL.4	p=0[2]
	2020	Height	W2.4	p=0[2]
	2019 and 2020	Canopy Closure	LL.3	p=0.002[2]
Alfalfa	2020 and 2021	CTWR	LL.4	p=0.4
		CWFP	W2.4	p=0.5

[1] CTWR= Critical timing of weed removal; CWFP= Critical weed free period

[2] Models with low p-values were checked to provide realistic estimates

Table 2.5. Mean (SE) Japanese millet (surrogate weed) density (m^{-2}) for the duration of weed competition with two corn hybrids with different leaf architecture (pendulum vs. upright) in a two-year field experiment (2019-2020). Density was recorded 14 days after Japanese millet seeding.

Duration of Weed Competition	2019	2020	
		Pendulum	Upright
	Plants (m^{-2})		
WFC[1]	0.00 (0.0) d[2]	0.00 (0.0) A[3]	0.00 (0.0) A
UTC	172.06 (12.3) a	23.38 (4.0) ABC	17.13 (2.5) AB
WFCA	0.00 (0.0) d	0.00 (0.0) A	0.00 (0.0) A
UTCA	184.63 (10.2) a	36.13 (9.4) ABC	30.88 (9.9) ABC
R2	178.75 (11.2) a	14.38 (2.8) AB	29.00 (13.2) ABC
WF2	66.50 (4.3) c	13.13 (6.8) AB	24.75 (12.1) ABC
R4	167.69 (9.8) ab	29.38 (5.9) ABC	42.38 (7.3) BC
WF4	120.50 (19.4) b	10.00 (6.7) AB	9.88 (3.5) AB
R6	194.75 (14.3) a	55.25 (9.9) C	20.00 (4.7) ABC
WF6	1.13 (0.7) d	0.50 (0.3) A	0.50 (0.3) A
R8	181.13 (15.4) a	24.00 (9.7) ABC	38.75 (9.3) BC
WF8	0.00 (0.0) d	0.38 (0.2) A	0.88 (0.4) A

[1] UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means followed by the same lowercase letter are not significantly different for main effect of duration of weed competition in 2019 ($p \geq 0.05$).

[3] Means followed by the same capital letter are not significantly different for the two-way interaction between corn hybrid and duration of weed competition in 2020 ($p \geq 0.05$).

Table 2.6. Mean (SE) Japanese millet (surrogate weed) biomass (g m^{-2}) for differing durations of weed competition with two corn hybrids with different leaf architecture (pendulum vs. upright) in a two-year field experiment (2019-2020).

Duration of Weed Competition [1]	2019	2020
	g (m^{-2})	
WFC	0.00 (0.0) a[2]	0.00 (0.0) a
UTC	748.36 (65.2) b	51.95 (7.2) b
WFCA	0.00 (0.0) a	0.00 (0.0) a
UTCA	734.54 (35.5) b	75.82 (16.0) c
WF2	3.92 (1.6) a	64.75 (16.4) ac
R2	0.00 (0.0) a	0.00 (0.0) a
WF4	18.52 (17.9) a	3.79 (1.7) a
R4	0.00 (0.0) a	0.00 (0.0) a
WF6	0.00 (0.0) a	0.00 (0.0) a
R6	0.00 (0.0) a	0.00 (0.0) a
WF8	0.00 (0.0) a	0.00 (0.0) a
R8	0.00 (0.0) a	0.00 (0.0) a

[1] UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means within a year followed by the same lowercase letter are not significantly different for main effect of duration of weed competition, averaged across corm hybrid ($p \geq 0.05$).

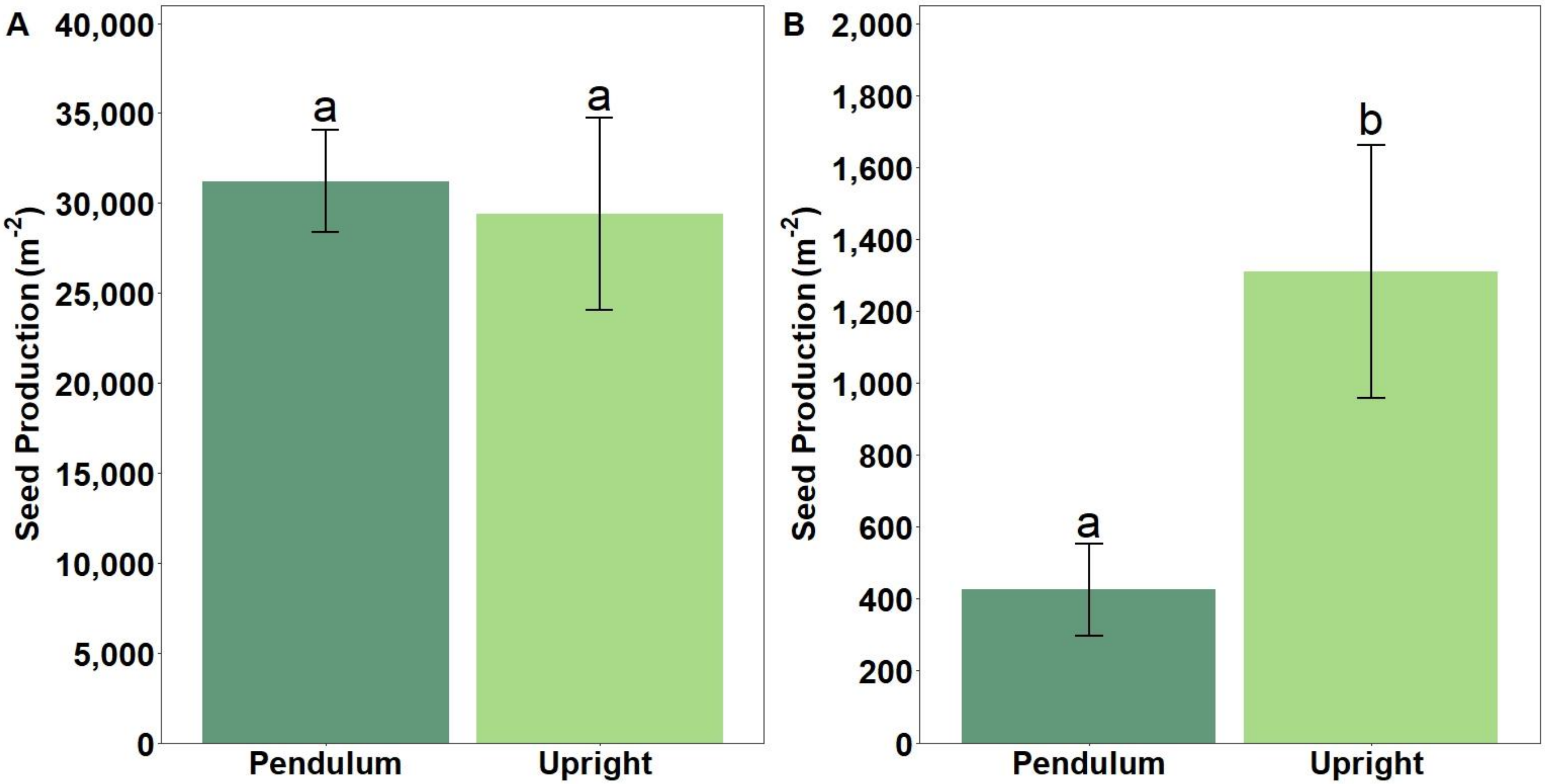

Figure 2.1. Mean (SE) Japanese millet (surrogate weed) seed production impacted by corn hybrids with different leaf architecture, pendulum or upright, in a two-year field study in 2019 (A, scale 40,000) and 2020 (B, scale 2,000), averaged over monoculture and corn-alfalfa interseeded systems. Bars labeled by the same lowercase letter in the same year are not statistically different for main effect of corn hybrid ($p \geq 0.05$).

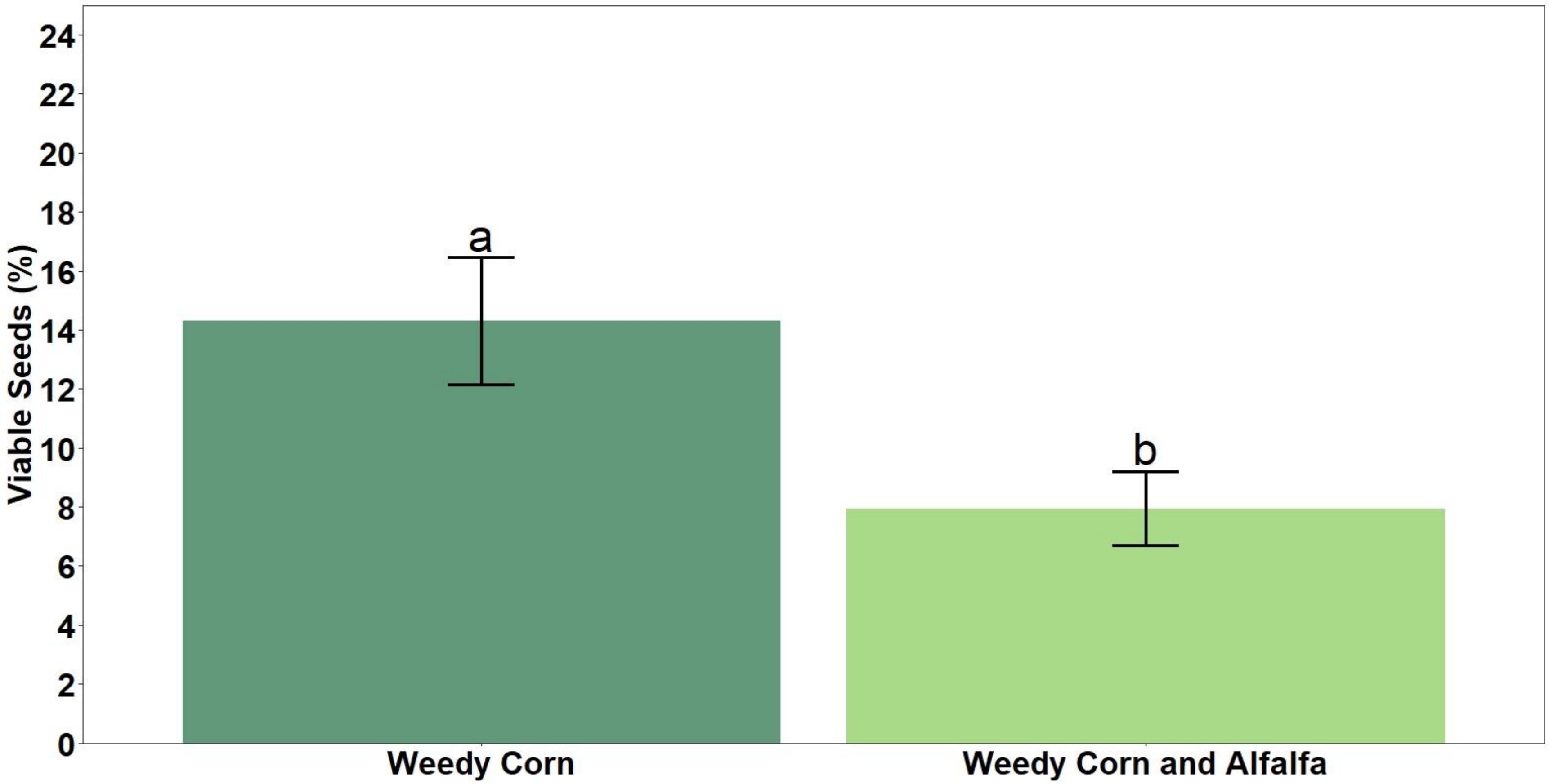

Figure 2.2. Mean (SE) Japanese millet (surrogate weed) seed viability impacted by interseeding corn and alfalfa and corn monoculture systems, averaged across years (2019-2020) and corn hybrids (pendulum and upright) in a two-year field experiment (2019-2020). Bars labeled by the same lowercase letter are not statistically different for main effect of duration of weed competition ($p \geq 0.05$).

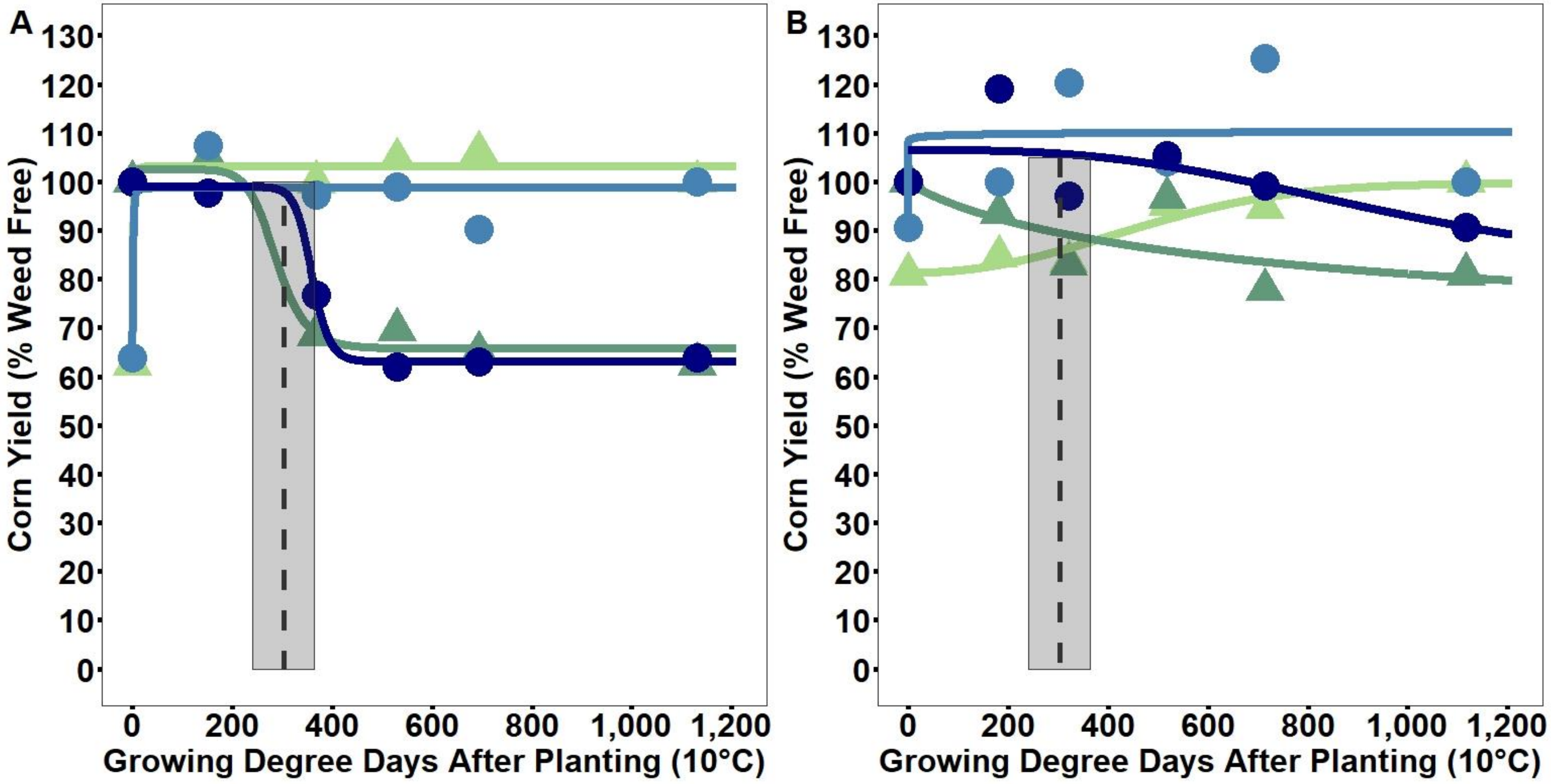

Figure 2.3. Interseeded corn silage yield as a percentage of the weed free control over the critical duration of weedy treatments with differing leaf architecture, pendulum (blue, ●) or upright (green, ▲), for 2019 (A) and 2020 (B). In weedy treatments, weeds emerged with the crop and were then removed at different dates, creating the critical timing of weed removal (dark colors). In weed free treatments, weeds were added later in the crop, creating the critical weed free period (light colors). An untreated and a weed-free check were included within these treatments. The critical timing of weed removal are based on a 5% acceptable yield loss, averaged over hybrids ($p \geq 0.05$), is denoted by the dashed vertical line (black) and the boxes denote the standard error of those estimates. The critical weed free period estimates are not shown, because they were greater than harvest date. Points represent observed mean values; lines represent the fitted models calculated by the drc package in R (2020).

Table 2.7. Mean (SE) leaf angles of two corn hybrids with differing leaf architecture (pendulum vs. upright), under differing durations of Japanese millet (surrogate weed) competition, across a two-year field study (2019-2020).

	2019		2020	
Duration of weed competition[1]	Pendulum	Upright	Pendulum	Upright
	Leaf Angle (°)			
WFC	30.74 (1.1) a[2]	28.70 (1.4) abcde	35.50 (1.2) A[3]d[4]	33.95 (0.6) Bd
WFCA	32.80 (0.8) abcde	30.90 (1.2) bde	34.75 (0.6) Aabcd	31.80 (0.4) Babcd
UTC	35.78 (0.5) ac	30.48 (0.7) be	38.89 (0.7) Aabc	38.15 (0.7) Babc
UTCA	35.18 (0.8) abcde	29.70 (1.1) abcde	36.11 (0.8) Aa	34.55 (0.4) Ba
WF2	32.63 (1.1) abcde	30.98 (1.0) abcde	35.10 (0.5) Aab	32.20 (0.5) Bab
R2	34.46 (0.8) abcde	33.55 (0.9) abcde	38.40 (0.6) Aab	37.25 (0.6) Bab
WF4	35.87 (0.6) acd	31.03 (1.1) abcde	38.20 (0.5) Acd	34.90 (0.6) Bcd
R4	35.45 (0.6) acd	30.05 (0.8) abcd	38.00 (0.7) Aabcd	36.70 (0.7) Babcd
WF6	33.49 (0.7) a	32.40 (1.0) abcde	35.70 (0.5) Abcd	31.42 (1.0) Bbcd
R6	34.60 (0.8) abcde	34.03 (1.1) be	35.40 (0.4) Aab	36.89 (0.5) Bab
WF8	33.00 (0.8) ac	28.93 (1.1) bcde	32.85 (0.7) Acd	34.50 (0.6) Bcd
R8	30.58 (0.7) abcde	28.55 (0.9) e	34.35 (0.9) Aab	32.80 (0.7) Bab

[1] UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means within 2019 followed by the same lowercase letter are not significantly different for interaction of duration of weed competition and corn hybrid ($p \geq 0.01$)

[3] Means within 2020 followed by the same capital letter are not significantly different for main effect of corn hybrid, averaged over the duration of weed competition treatments ($p \geq 0.01$).

[4] Means within 2020 followed by the same lowercase letter are not significantly different for main effect of duration of weed control, averaged over corn hybrids ($p \geq 0.01$).

Table 2.8. Mean (SE) accumulated growing degree days (GDD) required to reach 50 and 90% canopy closure under differing durations of Japanese millet (surrogate weed) competition and two corn hybrids with different leaf architecture (pendulum vs. upright) in a two-year field study (2019-2020). Percent reductions and probability values were calculated by ED and EDcomp functions, respectively, in the drc package in R (2020).

		2019		2020	
Hybrid	Duration of weed competition[1]	50%	90%	50%	90%
		GDD			
Pendulum	WFC	350.28 (17.3) bc[2]	721.39 (47.0) ac	338.15 (38.5) ab	>1,116 a
	UTC	258.11 (32.4) ab	1,114.14 (151.0) abcdef	365.37 (31.9) ab	938.10 (205.3) a
	WFCA	397.03 (21.8) c	971.71 (71.4) bdef	356.39 (31.4) ab	937.63 (220.7) a
	UTCA	281.12 (24.6) ab	851.22 (86.9) abcde	367.98 (52.3) ab	>1,116 a
	WF2	377.04 (18.9) c	812.82 (51.8) abcd	404.16 (41.1) ab	>1,116 a
	R2	349.71 (18.5) abc	778.90 (54.6) ab	395.88 (35.1) ab	1,083.76 (277.1) a
	WF4	365.08 (19.5) bc	844.22 (60.1) abcd	359.20 (32.9) ab	985.44 (243.7) a
	R4	207.81 (70.0) abc	>1,131 cdef	421.32 (33.9) ab	1,079.26 (271.3) a
	WF6	350.29 (18.2) bc	771.31 (54.1) abcd	355.05 (37.6) ab	>1,116 a
	R6	267.79 (37.3) abc	>1,131 cdef	303.14 (63.8) ab	>1,116 a
	WF8	351.61 (19.1) bc	797.62 (55.4) abcd	321.63 (26.3) a	713.85 (124.0) a
	R8	142.26 (48.9) a	1,108.31 (257.6) abcdef	381.09 (37.5) ab	>1,116 a
Upright	WFC	357.80 (17.0) bc	719.98 (45.8) abcdef	350.91 (39.4) ab	>1,116 a
	UTC	240.11 (31.5) abc	936.27 (124.6) abcdef	370.61 (39.5) ab	>1,116 a
	WFCA	323.42 (16.4) bc	660.99 (48.2) abcdef	379.43 (33.6) ab	1,039.75 (265.2) a
	UTCA	283.85 (22.1) abc	760.20 (69.0) abcdef	471.17 (39.3) b	>1,116 a
	WF2	344.11 (16.4) bc	697.73 (49.0) abcdef	422.36 (35.3) ab	>1,116 a
	R2	358.03 (18.8) bc	811.23 (57.8) abcd	348.84 (30.9) ab	905.54 (205.1) a
	WF4	359.27 (19.1) bc	832.63 (62.1) abcdef	345.58 (32.2) ab	916.47 (203.1) a
	R4	280.99 (37.1) abc	>1,131 f	427.72 (39.3) ab	>1,116 a
	WF6	356.76 (20.2) bc	874.45 (67.1) abcdef	343.31 (35.6) ab	1,052.64 (297.9) a
	R6	222.29 (44.4) abc	>1,131 ef	408.42 (48.0) ab	>1,116 a
	WF8	364.97 (18.3) bc	826.37 (61.7) abcdef	367.81 (50.2) ab	>1,116 a

Table 2.8 (cont'd)

Hybrid	Duration of weed competition[1]	2019		2020	
		50%	90%	50%	90%
		GDD			
Upright	R8	274.24 (27.5) abc	944.13 (106.2) abcdef	409.20 (31.8) ab	991.43 (220.0) a

[1] UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means within a year followed by the same letter are not significantly different for interaction between hybrid and duration of weed competition ($p \geq 0.001$)

[3] GDD estimate exceeds harvest GDD, which was 1,131 and 1,116 GDD in 2019 and 2020, respectively.

Table 2.9. Mean (SE) accumulated growing degree days (GDD) required to reduce height by 25 and 50% under differing durations of Japanese millet (surrogate weed) competition and two corn hybrids with different leaf architecture (pendulum vs. upright) in a two-year field study (2019-2020). Percent reductions and probability values were calculated by ED and EDcomp functions, respectively, in the drc package in R (2020).

Hybrid	Duration of weed competition[1]	2019		2020	
		25%	50%	25%	50%
		GDD			
Pendulum	WFC	486.07 (7.3) abc	569.86 (6.9) abc	452.56 (12.4) ac	531.37 (7.9) c
	UTC	455.33 (14.9) e	607.71 (36.2) abcd	462.30 (11.0) abc	538.16 (7.4) abc
	WFCA	505.40 (7.4) abc	581.06 (6.7) abc	465.54 (12.0) abc	541.32 (8.4) abc
	UTCA	498.69 (13.9) abcdefg	648.63 (33.7) ad	492.01 (9.5) ab	563.42 (9.0) ab
	WF2	505.85 (6.5) abc	583.07 (6.4) abc	478.37 (10.8) abc	555.46 (8.9) abc
	R2	505.71 (6.9) abc	579.06 (6.1) abc	461.99 (12.8) abc	545.93 (9.7) abc
	WF4	513.86 (6.5) bc	583.84 (6.4) ab	470.98 (11.2) abc	548.24 (8.7) abc
	R4	545.22 (10.0) d	660.80 (28.1) d	483.76 (10.4) abc	558.19 (9.0) abc
	WF6	503.18 (7.5) abc	581.83 (7.2) abc	473.55 (10.9) abc	548.09 (8.4) abc
	R6	480.12 (20.6) aefg	651.88 (50.3) abcd	493.21 (9.9) ab	565.20 (9.3) a
	WF8	504.28 (6.9) abc	582.10 (6.7) abc	474.58 (11.3) abc	546.13 (8.2) abc
	R8	472.11 (15.6) efg	625.98 (38.2) abcd	492.10 (13.2) abc	572.43 (11.4) a
Upright	WFC	475.32 (7.4) abefg	561.45 (6.9) c	445.01 (12.4) c	530.49 (8.5) c
	UTC	459.15 (16.1) ef	618.09 (40.1) abcd	449.81 (12.1) c	532.60 (8.0) bc
	WFCA	487.36 (8.2) abcg	565.94 (6.0) bc	463.84 (11.7) abc	540.13 (8.1) abc
	UTCA	474.01 (8.8) abcdefg	591.14 (15.9) abc	493.55 (10.2) b	560.06 (9.7) abc
	WF2	498.21 (7.5) abc	575.33 (6.3) abc	494.27 (10.9) b	565.83 (10.1) a
	R2	504.74 (8.2) bc	572.33 (5.6) abc	462.03 (11.6) abc	542.72 (8.5) abc
	WF4	493.44 (8.7) abc	571.64 (6.6) abc	462.09 (10.1) abc	538.51 (6.9) abc
	R4	500.87 (10.5) cd	600.93 (14.9) a	470.29 (11.0) abc	551.50 (8.5) abc
	WF6	501.54 (8.4) abc	572.23 (5.9) abc	470.30 (10.6) abc	545.94 (7.9) abc
	R6	461.19 (15.8) ef	616.72 (39.4) abcd	485.61 (11.0) abc	559.47 (9.5) abc
	WF8	502.69 (9.7) bc	570.06 (6.2) abc	462.49 (11.0) abc	538.71 (7.5) abc

Table 2.9 (cont'd)

Hybrid	Duration of weed competition[1]	2019		2020	
		25%	50%	25%	50%
		GDD			
Upright	R8	490.34 (9.7) abcfg	617.68 (21.7) ad	480.90 (9.8) abc	552.47 (7.9) abc

[1] UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means within a year followed by the same letter are not significantly different for interaction between hybrid and duration of weed competition ($p \leq 0.01$)

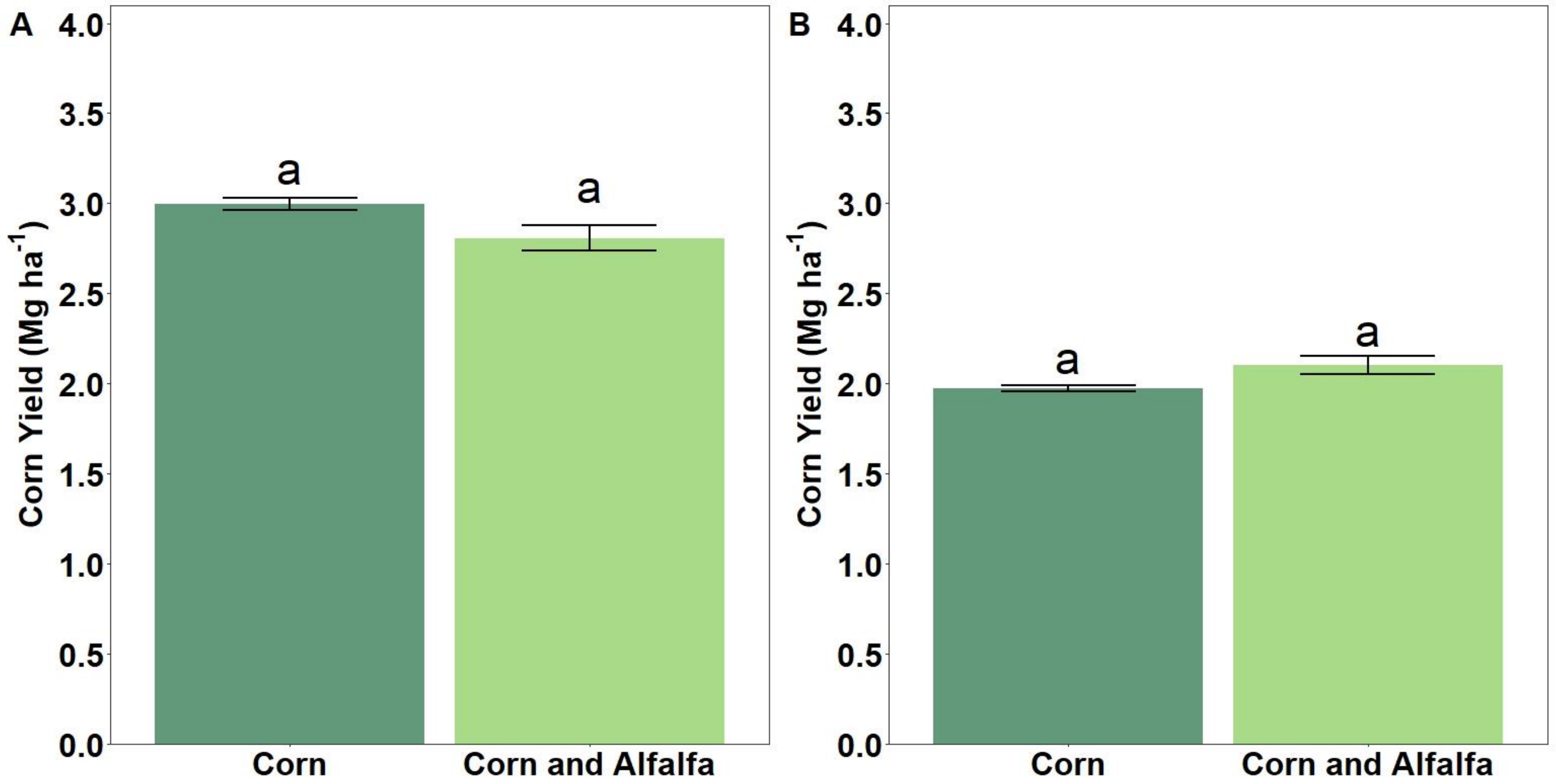

Figure 2.4. Mean (SE) corn silage dry biomass yield under weed free interseeded corn and alfalfa or weed free corn monoculture systems averaged across two corn hybrids of different leaf architecture (upright or pendulum) for a two-year field experiment, 2019 (A) and 2020 (B). Bars labeled by the same letter within a year are not statistically different for main effect of cropping system ($p \geq 0.05$).

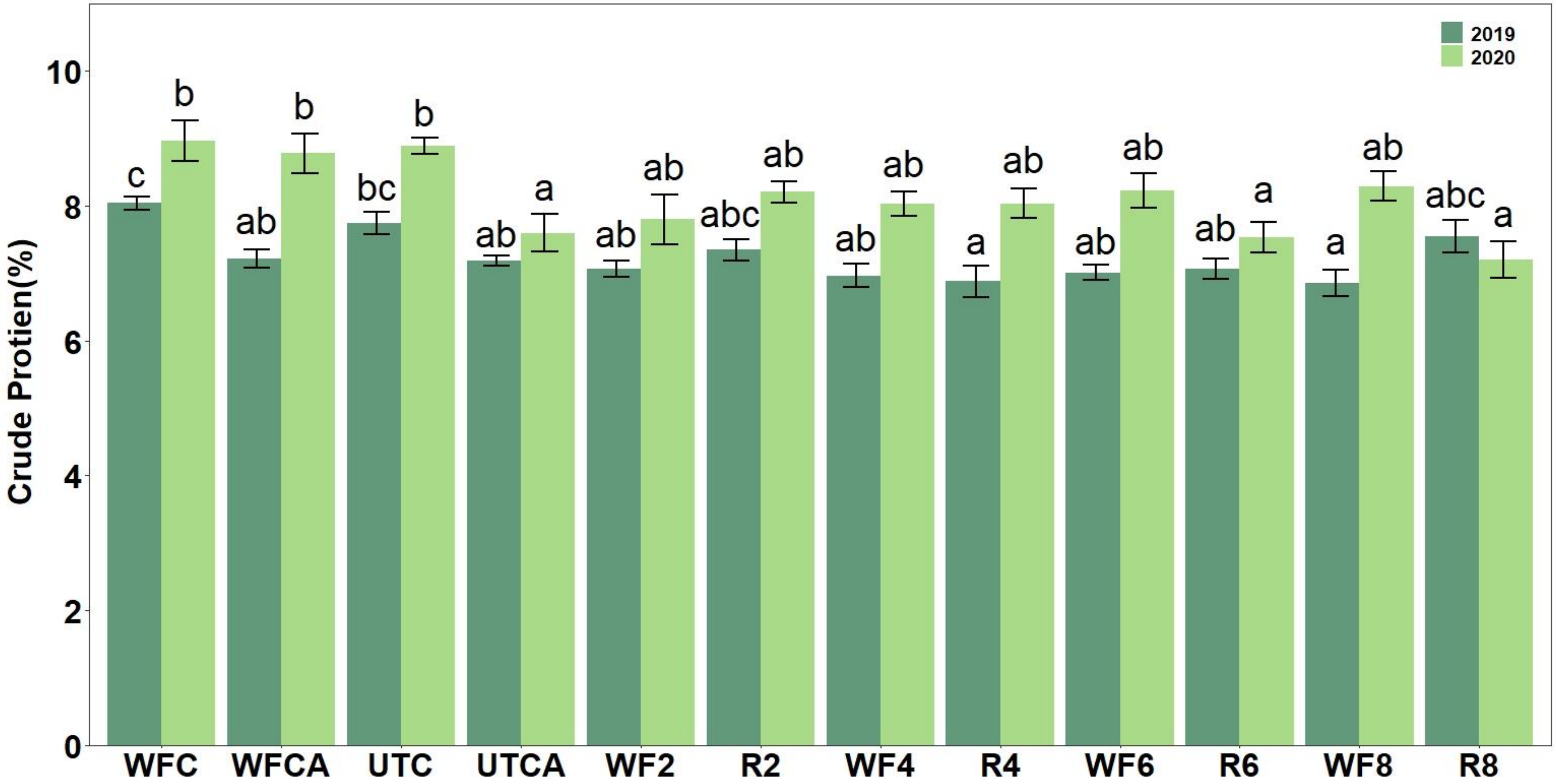

Figure 2.5. Mean corn crude protein under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

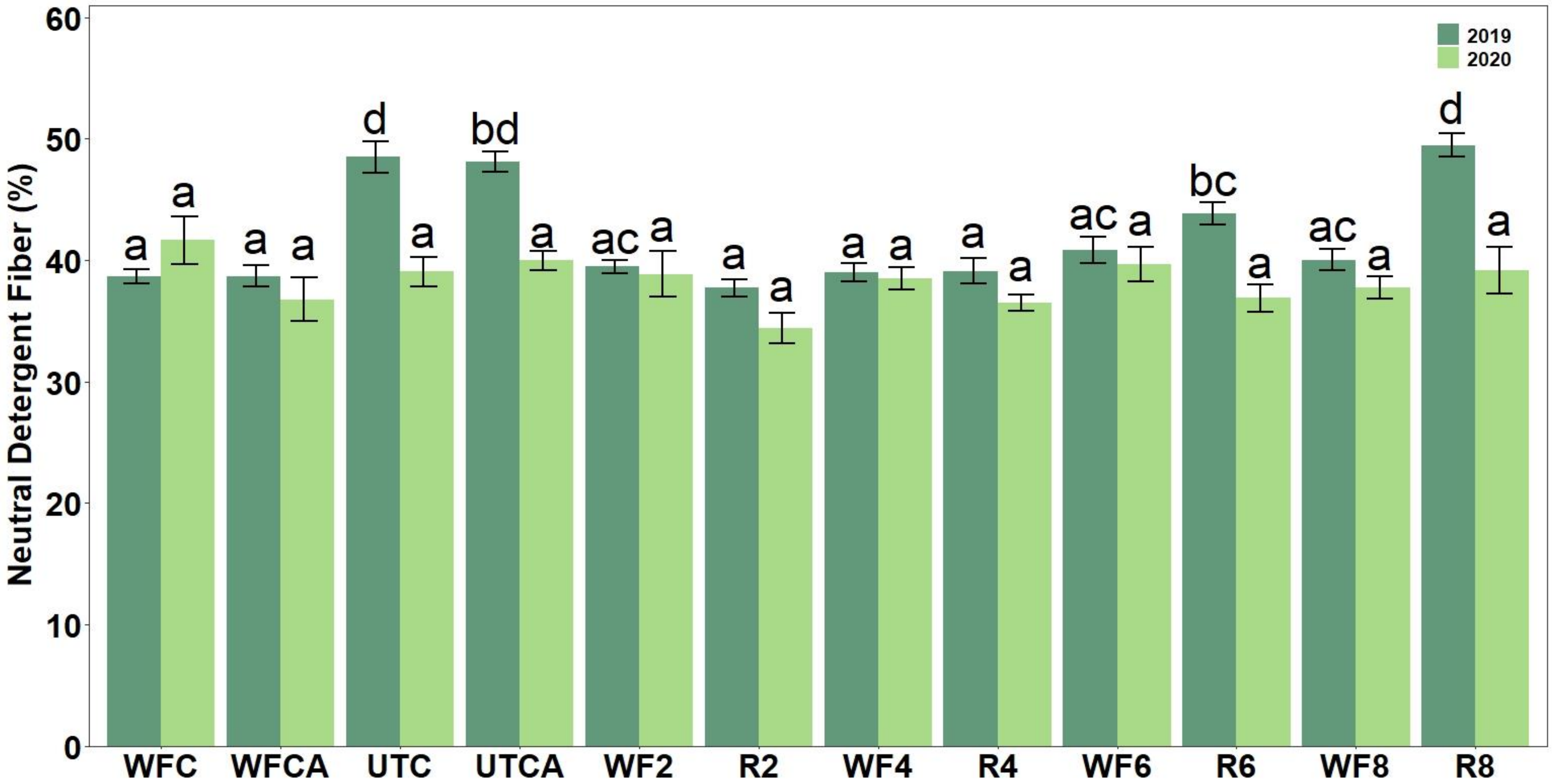

Figure 2.6. Mean corn neutral detergent fiber under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

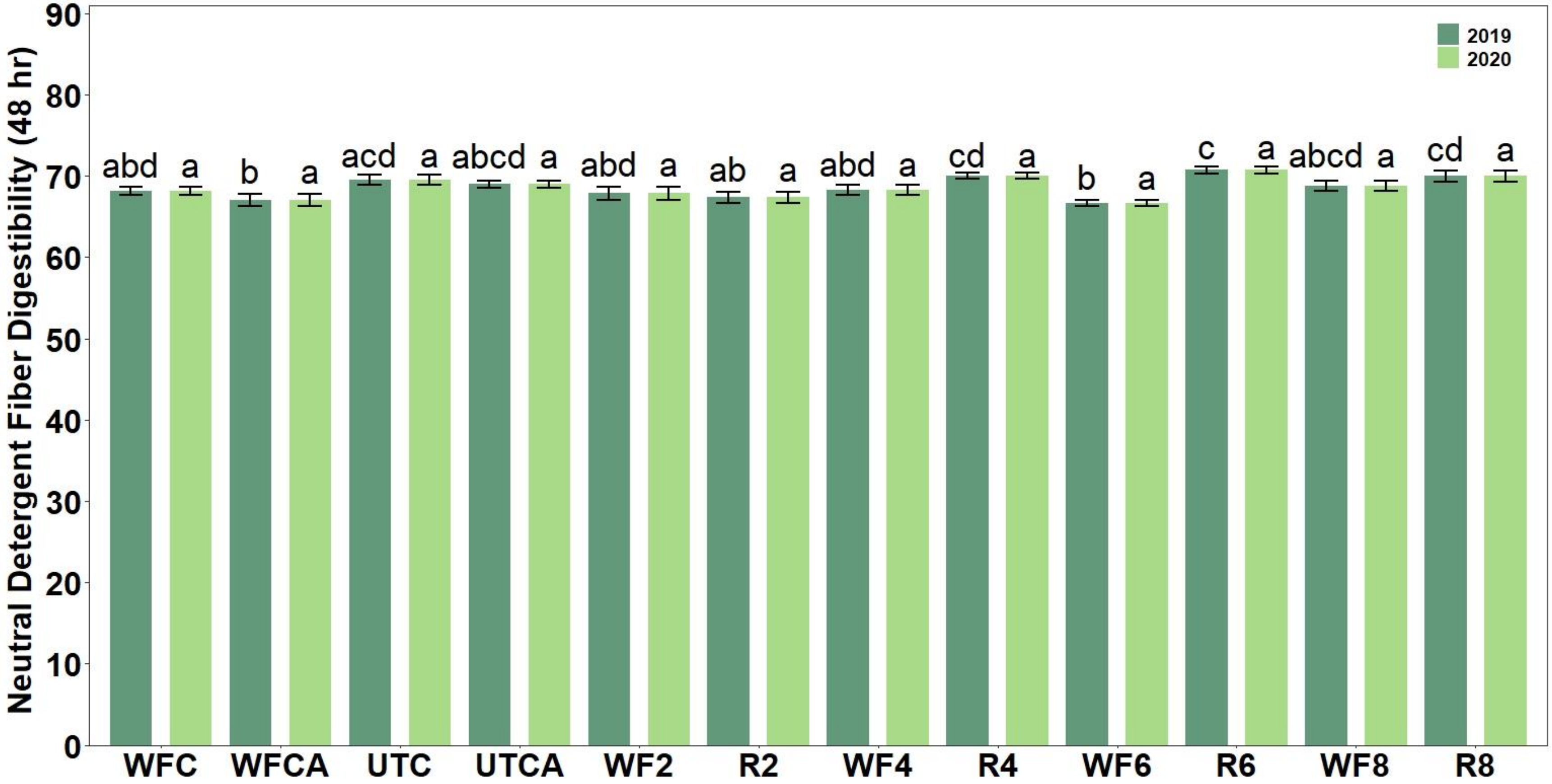

Figure 2.7. Mean corn neutral detergent fiber digestibility after 48 hours (48 hr) under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

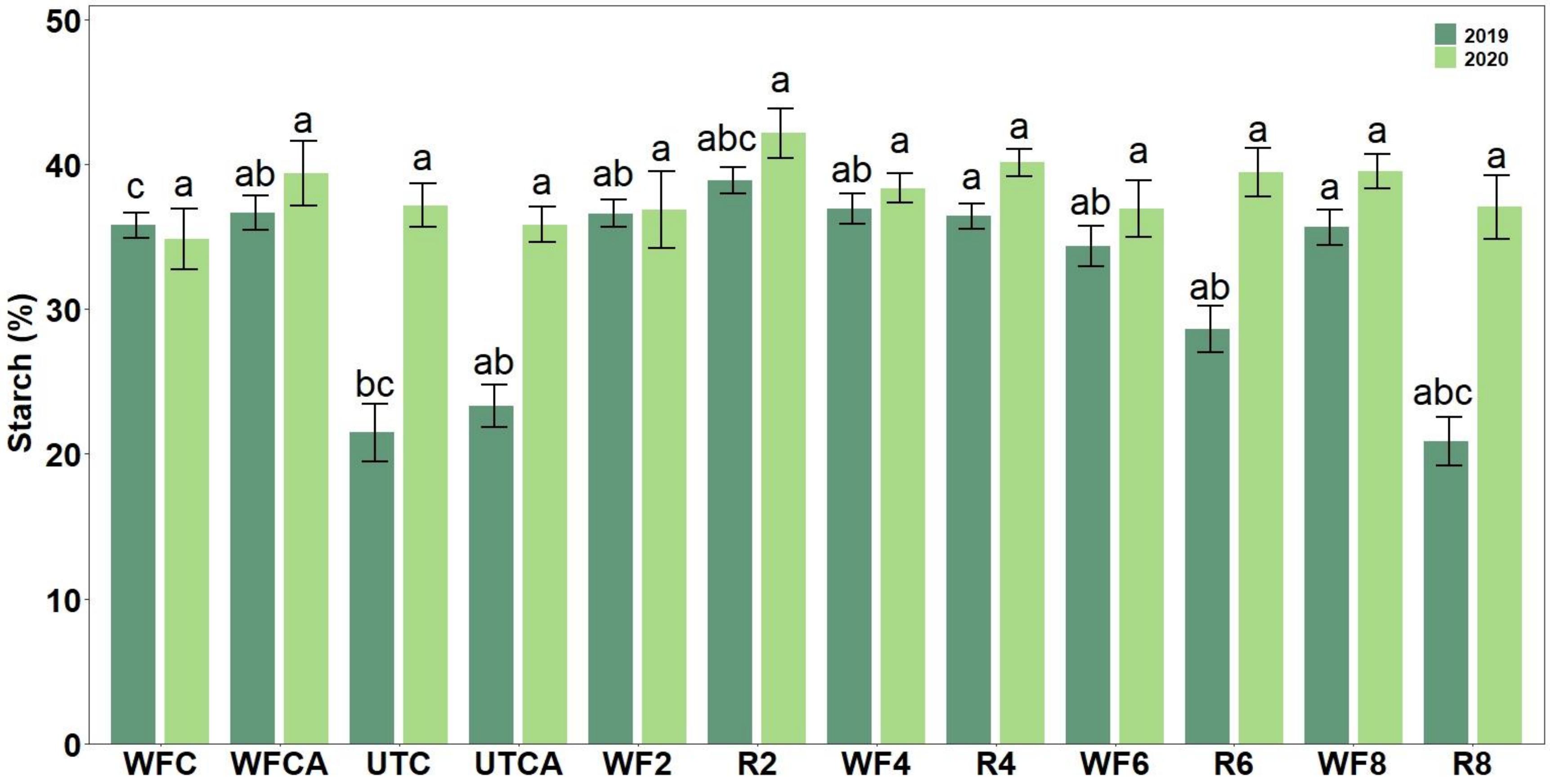

Figure 2.8. Mean corn starch under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

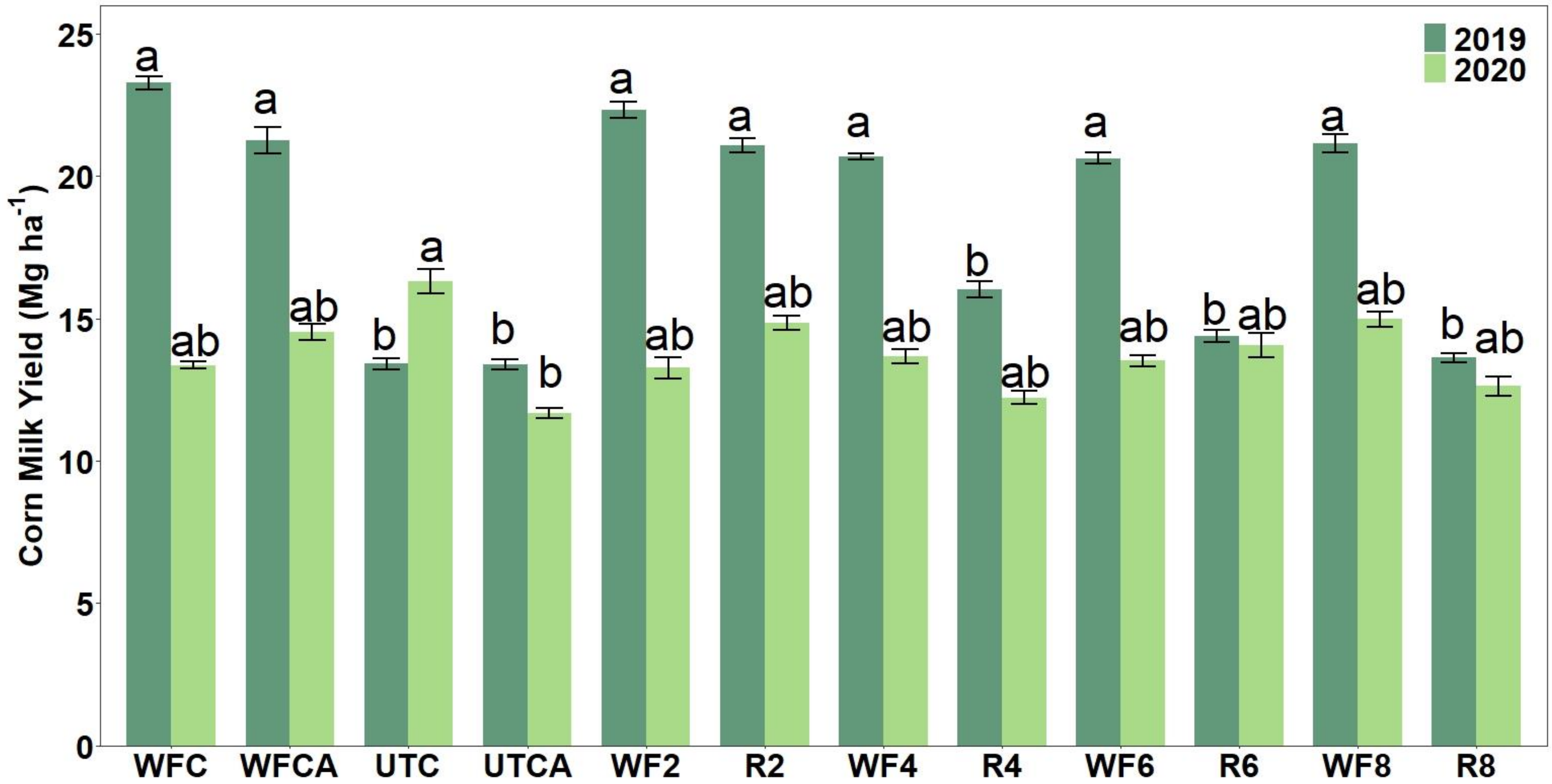

Figure 2.9. Estimated mean corn milk yield under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

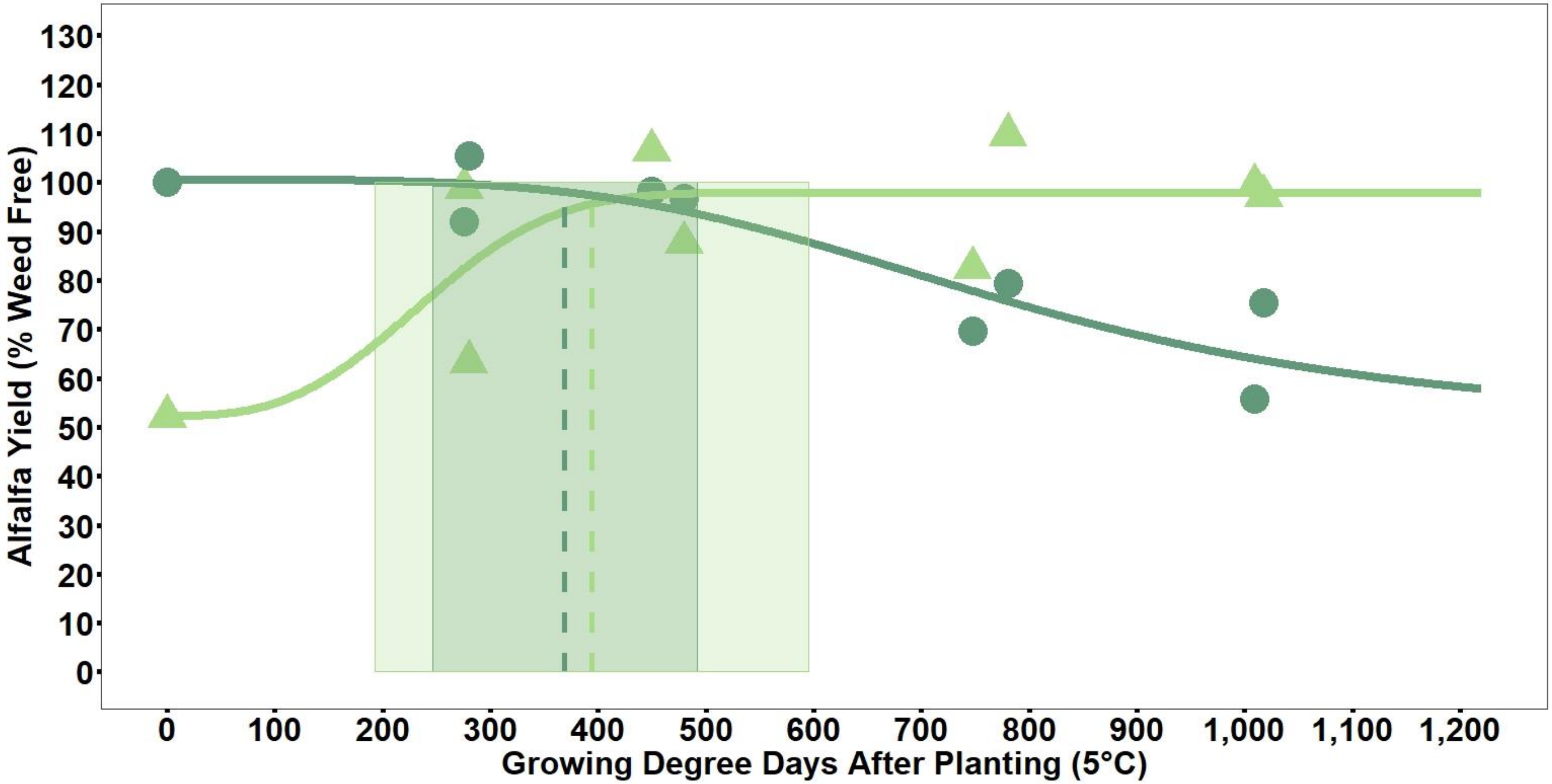

Figure 2.10. Interseeded alfalfa yield for the first cutting as a percentage of the weed free control over the critical duration of weedy treatments averaged over corn hybrid (pendulum and upright), for a two-year study (2020-2021). Interseeding corn and alfalfa was established in 2019 and 2020, (establishment years) and alfalfa was harvested the following season, 2020 and 2021. In weedy treatments, weeds emerged with the crop and were then removed at different dates, creating the critical timing of weed removal (dark colors, ●). In weed free treatments, weeds were added later in the crop, creating the critical weed free period (light colors, ▲). An untreated and a weed-free check were included within these treatments. The critical period times are based on a 5% acceptable yield loss is denoted by the dashed vertical lines, averaged over years and effect of corn hybrid, and the boxes denote the standard error for each of the GDD estimates. Points represent observed mean values; lines represent the fitted models calculated by the drc package in R (2020).

Table 2.10. Mean (SE) alfalfa percent cover under differing durations of Japanese millet (surrogate weed) competition and averaged over two corn hybrids with different leaf architecture (pendulum vs. upright) during the establishment year for a two-year study (2019-2020) taken 4 weeks before corn harvest averaged over study year. Interseeding corn and alfalfa was established in 2019 and 2020, (establishment years) and alfalfa was harvested the following season, 2020 and 2021, respectively.

Duration of Weed Control[1]	Pendulum	Upright
	Percent Cover (%)	
WFCA	59.06 (4.4) ae[2]	53.44 (5.1) ae
UTCA	15.31 (3.5) d	16.56 (4.1) d
WF2	47.19 (6.3) a	46.88 (6.6) a
R2	55.63 (4.3) a	56.56 (4.3) a
WF4	53.75 (4.3) bce	45.63 (5.3) ae
R4	38.44 (2.0) bc	43.13 (2.5) b
WF6	52.81 (4.3) ae	49.50 (5.3) ae
R6	14.38 (2.4) d	22.81 (3.8) d
WF8	56.25 (5.0) ace	50.31 (3.2) ae
R8	18.44 (3.1) d	14.06 (3.6) d

[1] UTCA= Weedy corn and alfalfa; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means within a hybrid followed by the same lowercase letter are not significantly different for a two-way interaction between duration of weed control and hybrid ($p \geq 0.05$)

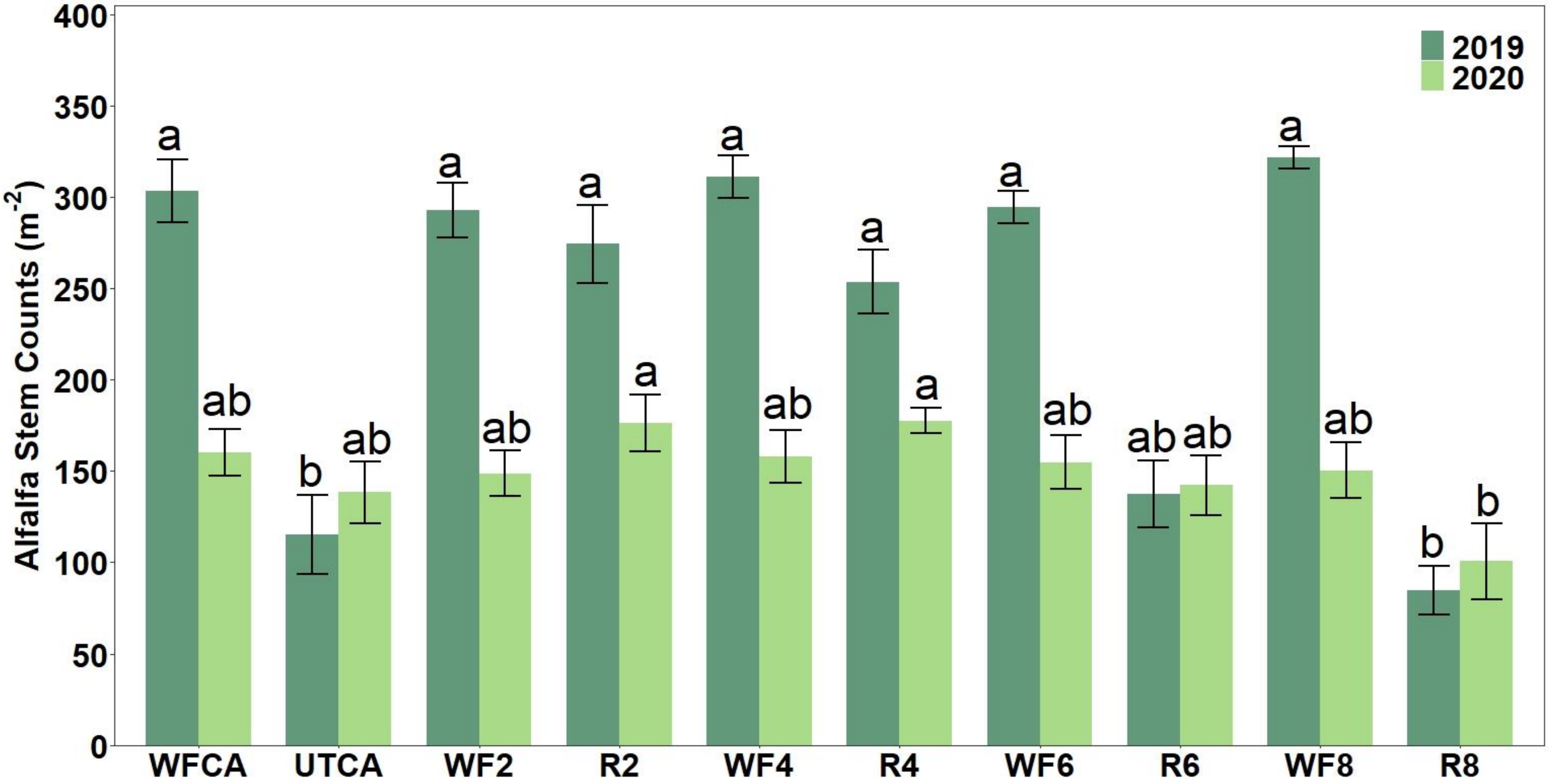

Figure 2.11. Means alfalfa stem counts, collected in the fall of the establishment year, under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

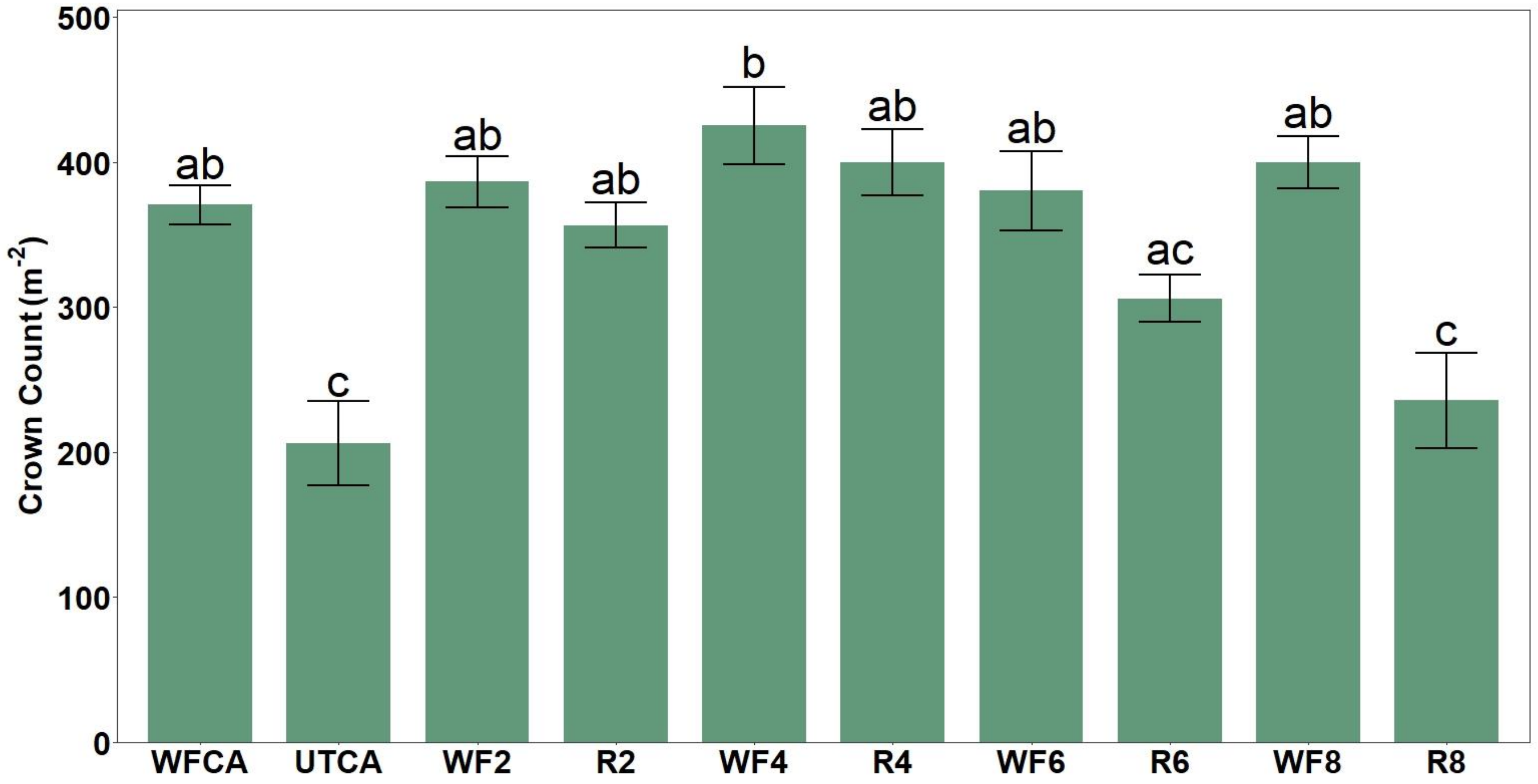

Figure 2.12. Mean alfalfa crown counts, collected in the fall of the second year, under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

Table 2.11. Mean (SE) alfalfa biomass harvested in the fall after corn harvest and the following spring, averaged over main effect of corn hybrid (pendulum and upright), for a two-year field experiment (2019-2020) for the effects of Japanese millet (surrogate weed) duration of competition. Interseeded corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively.

	Fall		Spring	
Duration of weed competition[1]	2019	2020	2020	2021
	g (m^{-2})			
WFCA	21.24 (2.0) a[2]	12.69 (1.5) ab	56.43 (6.8) ab	45.27 (5.1) a
UTCA	3.48 (0.9) b	9.69 (2.0) ab	38.85 (6.7) b	32.14 (5.0) a
WF2	20.17 (1.6) a	9.30 (0.4) ab	62.82 (5.3) ab	44.43 (5.4) a
R2	19.73 (1.2) a	13.04 (1.1) ab	62.11 (6.0) ab	58.86 (4.6) a
WF4	18.98 (0.9) a	11.12 (1.1) ab	58.46 (3.3) ab	45.05 (3.2) a
R4	16.60 (1.8) a	15.18 (1.3) a	75.56 (4.1) a	49.66 (4.9) a
WF6	20.09 (0.9) a	10.16 (1.2) ab	62.15 (4.6) ab	37.62 (7.2) a
R6	6.18 (1.2) b	10.11 (1.7) ab	56.48 (4.8) ab	36.63 (7.0) a
WF8	20.02 (0.9) a	12.60 (1.6) ab	59.50 (5.3) ab	53.97 (6.5) a
R8	2.72 (0.7) b	7.65 (2.0) b	39.83 (6.1) b	44.13 (6.5) a

[1] UTCA= Weedy corn and alfalfa; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting

[2] Means within a season (Fall or Spring) and year (2019, 2020, or 2021) followed by the same letter are not significantly different for the main effect of duration of weed competition ($p \leq 0.05$)

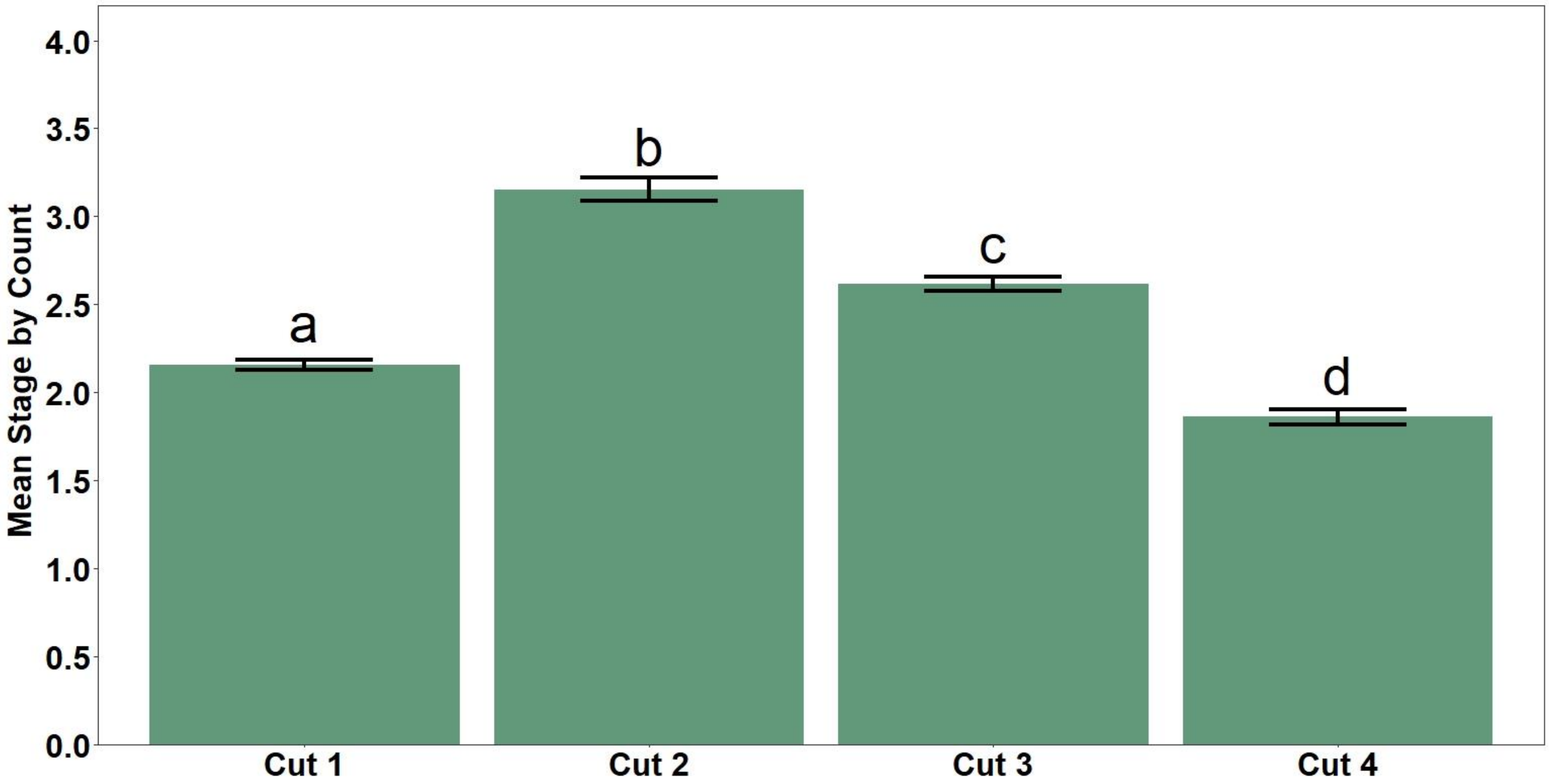

Figure 2.13. Mean alfalfa stage count collected before each alfalfa cutting in the second year after the 2019 establishment year, averaged over the effects of different Japanese millet (surrogate weed) duration of weed competition, two corn hybrids with different leaf architecture (pendulum and upright) in the first year (2019) of a two-year field study (2019-2020). Interseeding corn and alfalfa was established in 2019 and alfalfa was harvested the following season, 2020. Bars with the same lowercase letter are not significantly different for the main effect of cutting ($p \geq 0.05$).

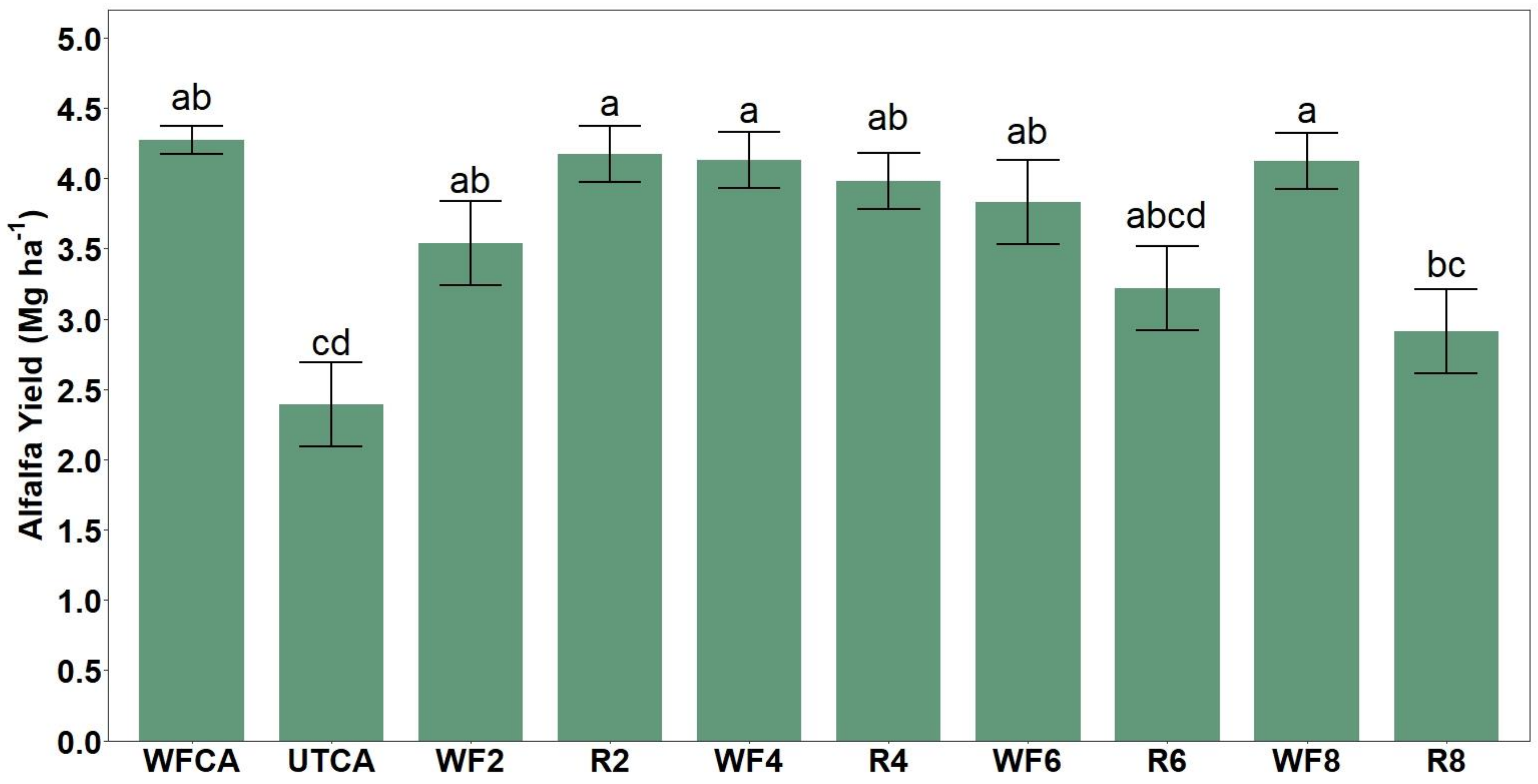

Figure 2.14. Mean first cutting alfalfa dry biomass yield under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) and years in a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

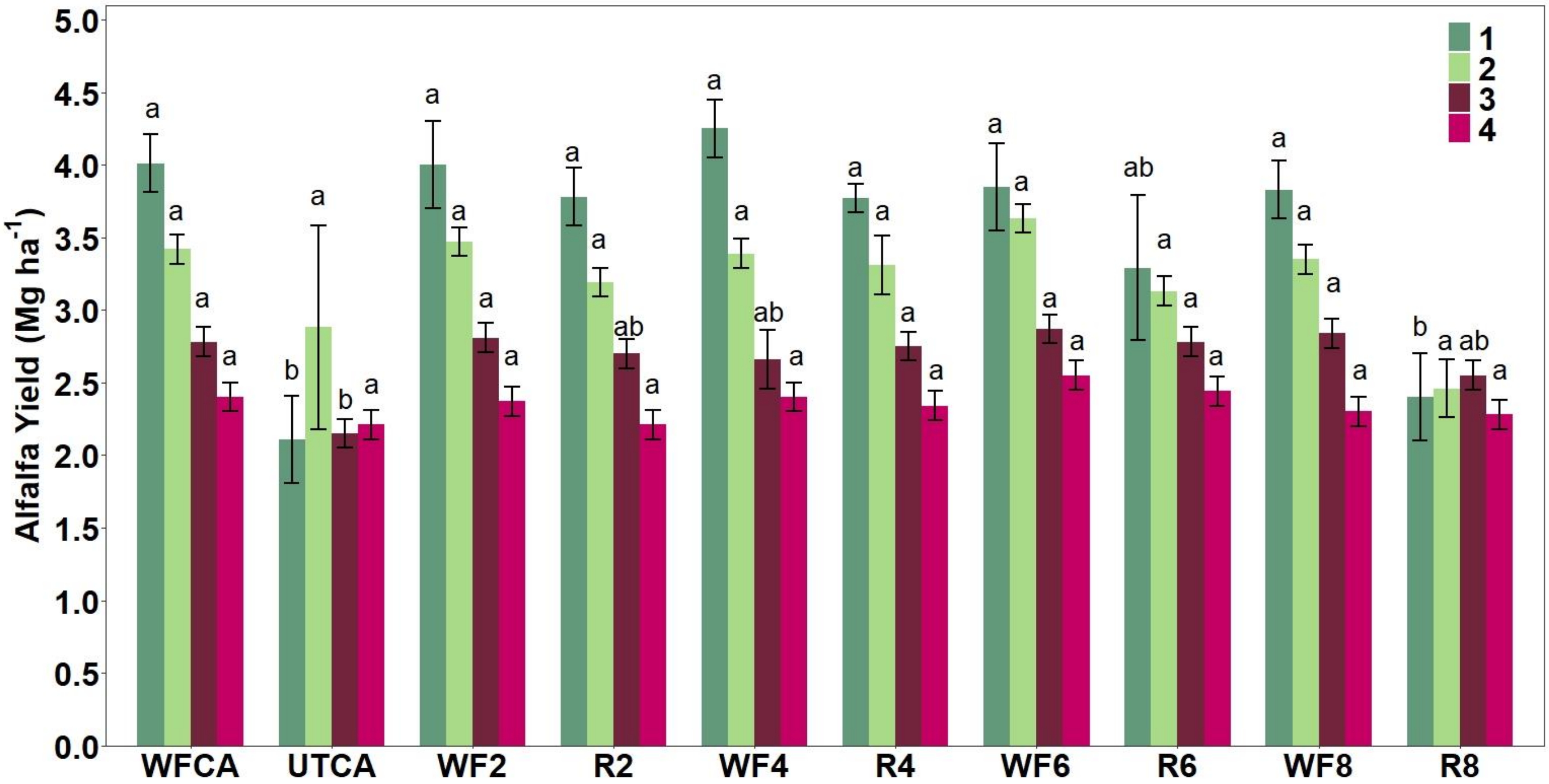

Figure 2.15. Mean alfalfa dry biomass yield under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for main effect of the duration of weed competition within that cutting ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019, and alfalfa was harvested the following season, 2020. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

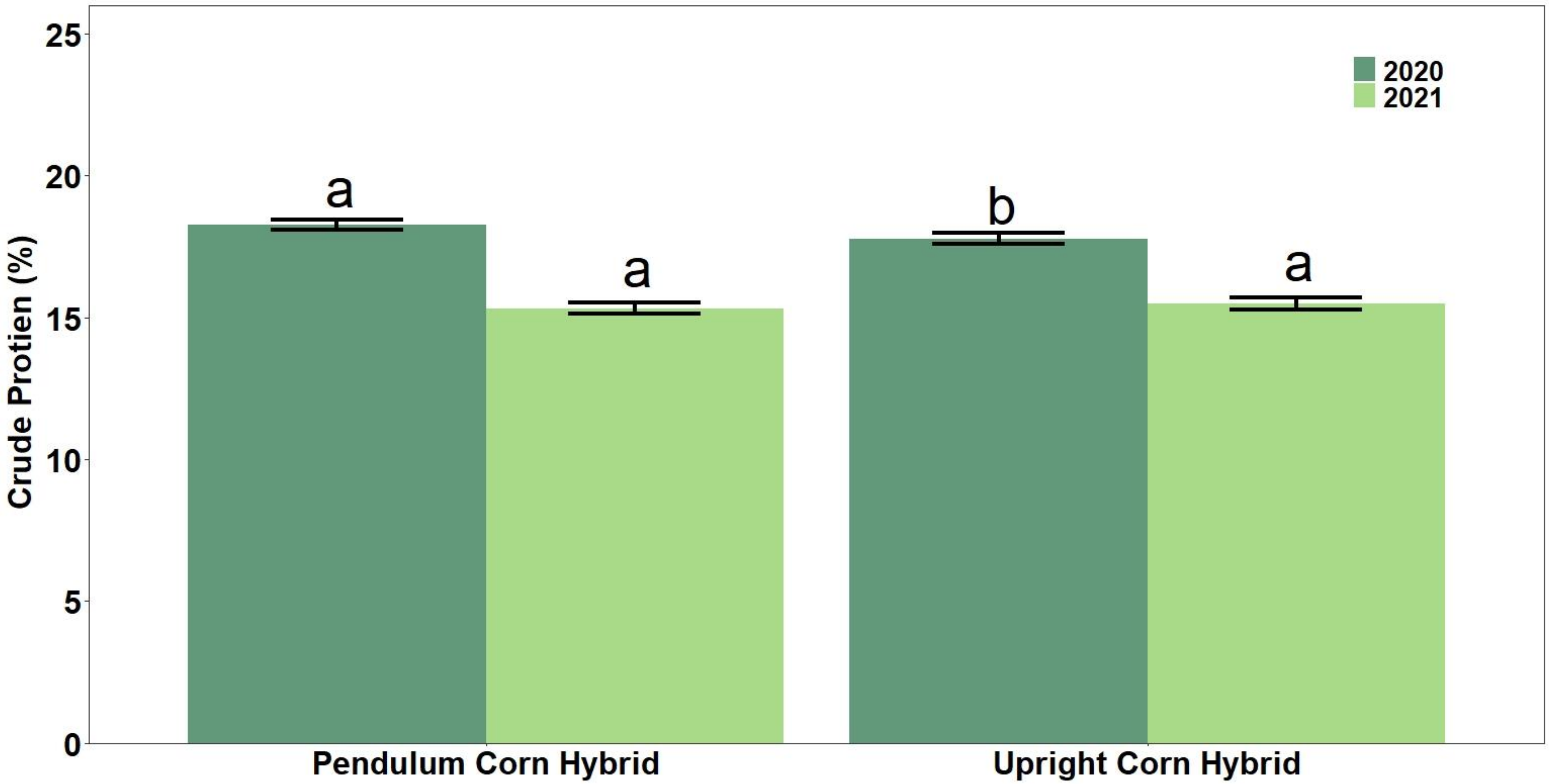

Figure 2.16. Mean alfalfa crude protein concentration for the first cutting of alfalfa in the year after the establishment year (2019-2020), averaged over the effects of different Japanese millet (surrogate weed) duration of weed competition in a two-year field study (2019-2020). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. Bars with the same lowercase letter and color are not significantly different for the main effect of hybrid within in that year ($p \geq 0.05$).

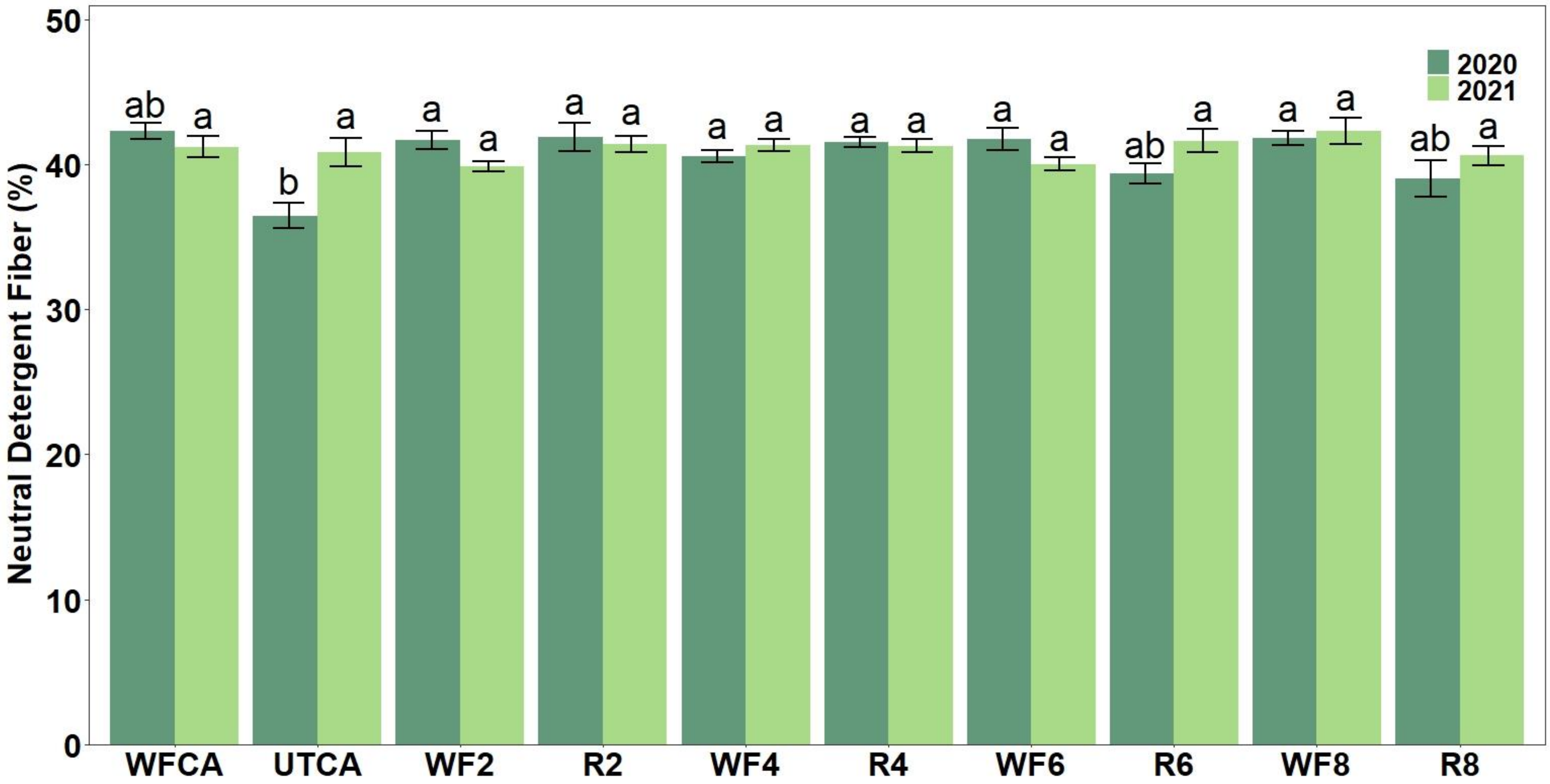

Figure 2.17. Mean alfalfa neutral detergent fiber under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

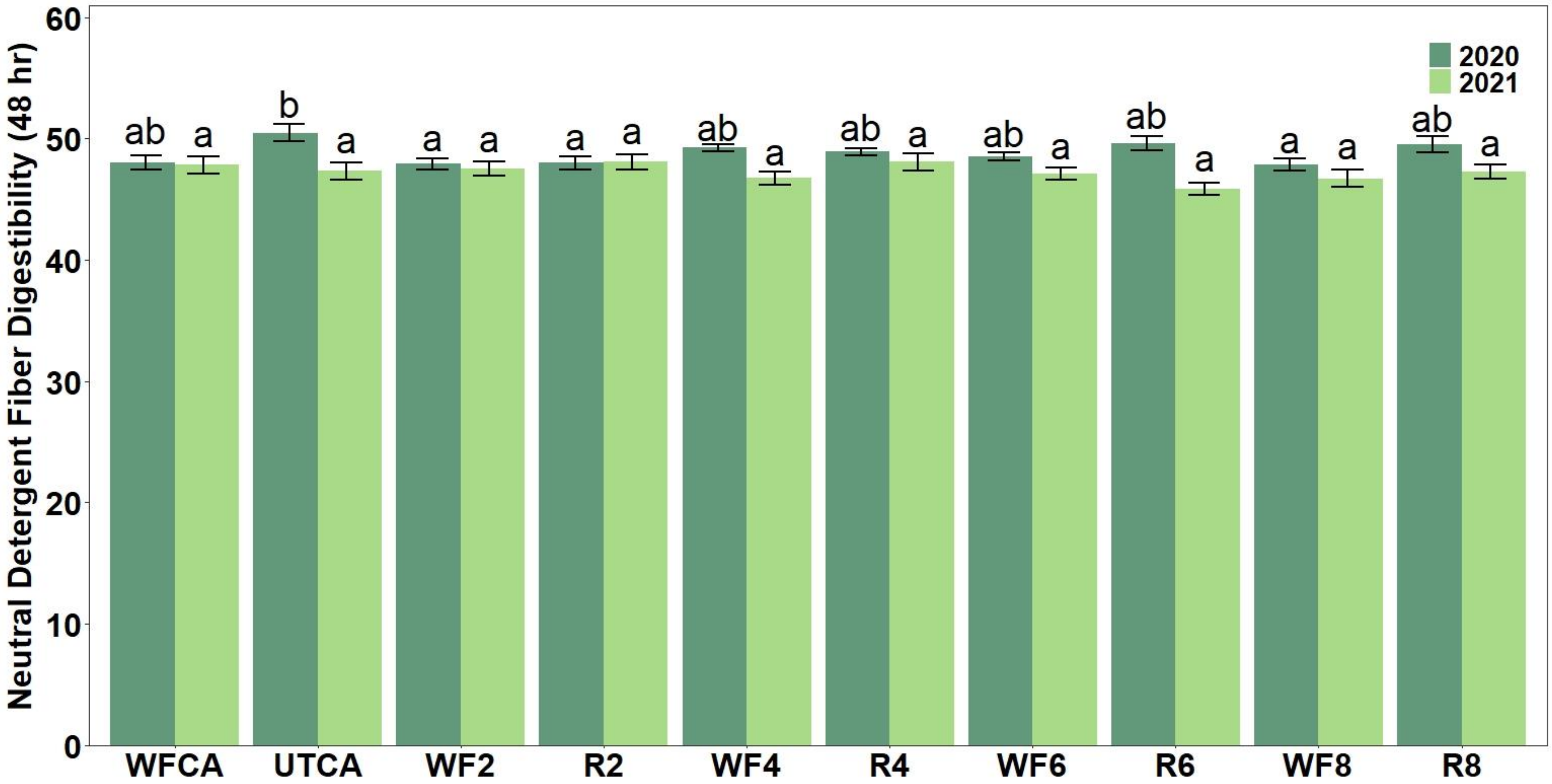

Figure 2.18. Mean alfalfa neutral detergent fiber digestibility after 48 hours under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

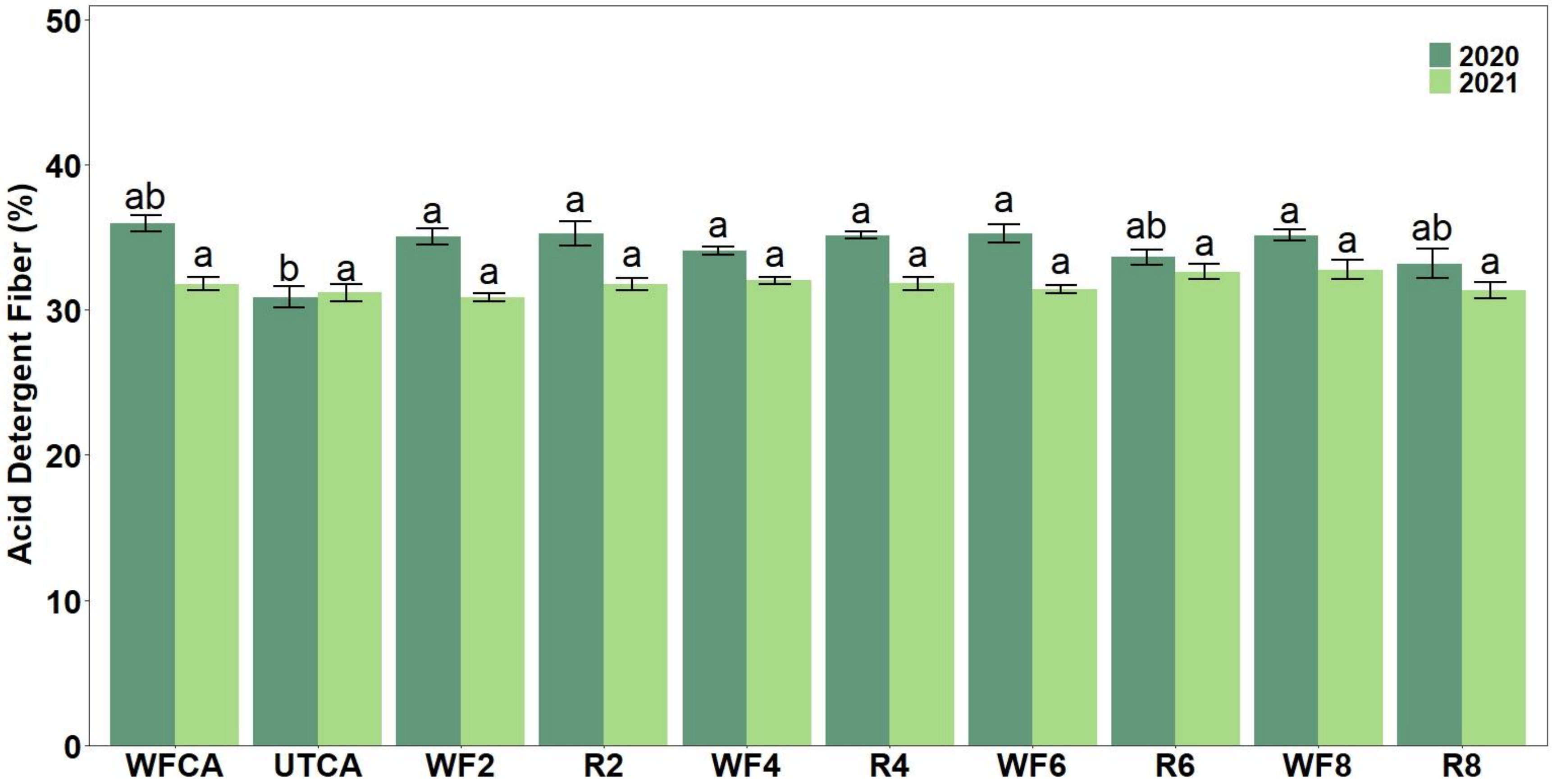

Figure 2.19. Mean alfalfa acid detergent fiber under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

Figure 2.20. Mean alfalfa ash concentration under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

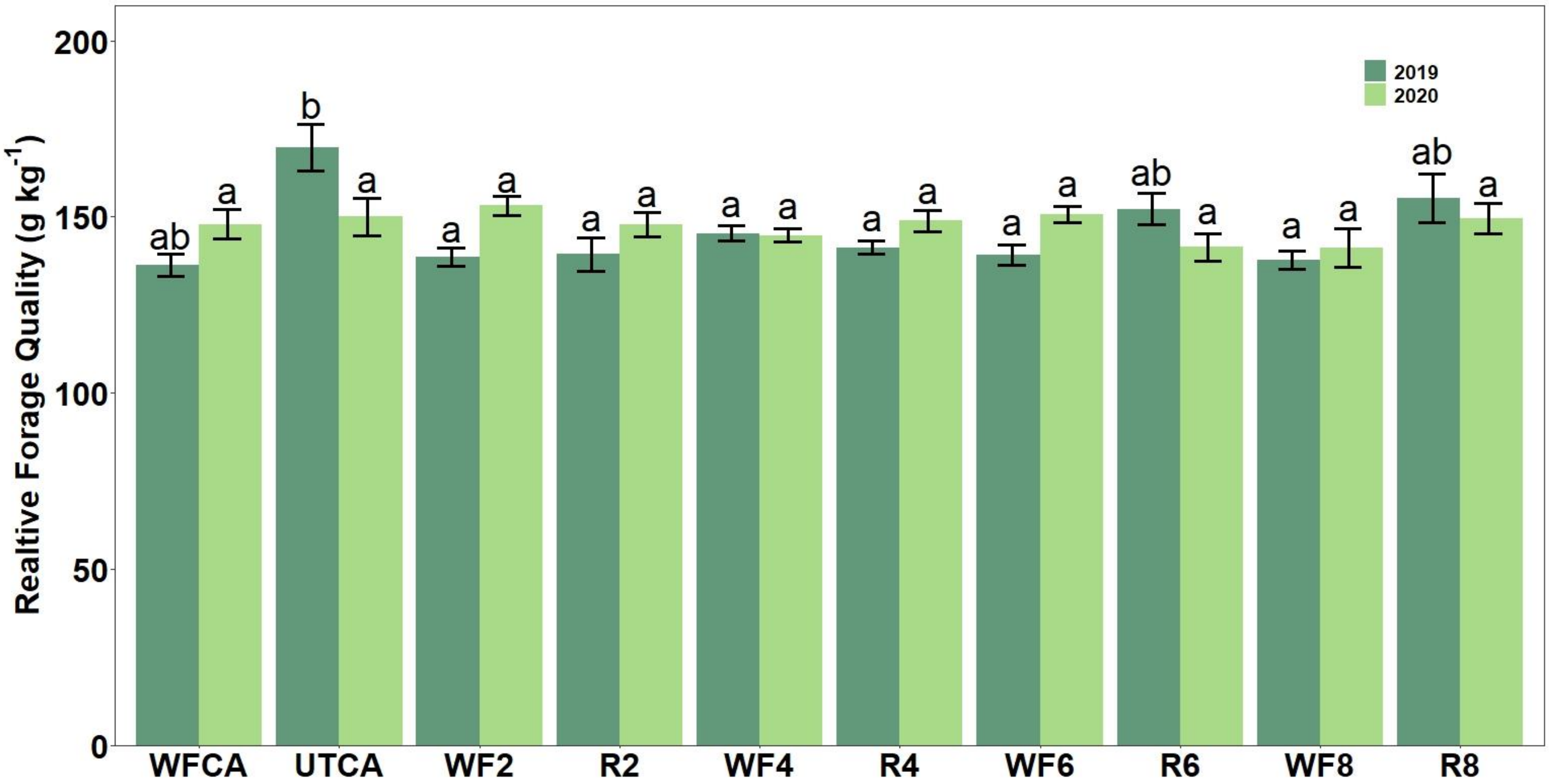

Figure 2.21. Mean alfalfa relative forage quality under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

Figure 2.22. Mean first cutting alfalfa milk yield under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) and years in a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and 2020, and alfalfa was harvested the following season, 2020 and 2021, respectively. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

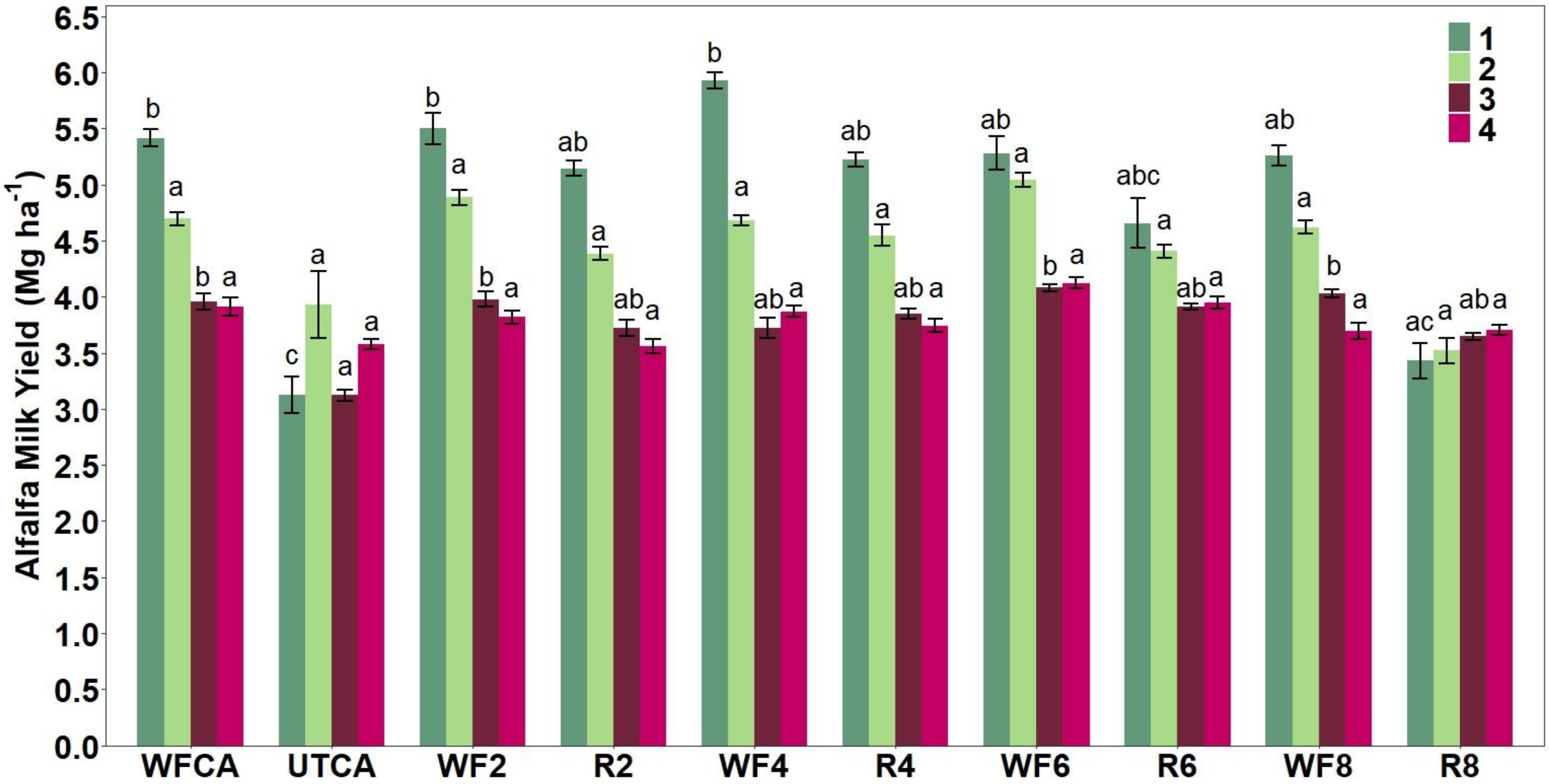

Figure 2.23. Mean alfalfa milk yield under differing durations of Japanese millet (surrogate weed) competition averaged over two corn hybrids with different leaf architecture (upright or pendulum) for a two-year field experiment (2019-2020). Bars with the same color with the same lowercase letter are not significantly different for the main effect of duration of weed competition within that cutting ($p \geq 0.05$). Interseeding corn and alfalfa was established in 2019 and alfalfa was harvested the following season, 2020. UTC= Weedy corn; UTCA= Weedy corn and alfalfa; WFC= Weed free corn; WFCA= Weed free corn and alfalfa; R2= Removal of weeds at 2 weeks after planting; WF2= Weed free for 2 weeks after planting; R4= Removal of weeds at 4 weeks after planting; WF4= Weed free for 4 weeks after planting; R6= Removal of weeds at 6 weeks after planting; WF6= Weed free for 6 weeks after planting; R8= Removal of weeds at 8 weeks after planting; WF8= Weed free for 8 weeks after planting.

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CHAPTER III

INTERSEEDING CORN AND ALFALFA AS AN ECOLOGICAL FORM OF WEED CONTROL: A DEMOGRAPHIC MODEL

Abstract

Interseeding alfalfa and corn silage may increase alfalfa acres that are decreasing in Michigan, provide environmental benefits, and possibly provide weed suppression. A two-year split plot field study with four replications was conducted in East Lansing, MI (2019-2020) to assess weed population dynamics in an alfalfa-corn silage interseeded system. Whole plots were assigned to one of two corn silage hybrids with different leaf architecture, pendulum (PH) or upright (UH) to assess impacts of light penetration on weed dynamics. Subplots consisted of Japanese millet, surrogate weed, presence, or absence. Japanese millet seed production was collected at the end of the season and viability assessed. The following year soil samples were collected to assess overwinter seed survival. Data were analyzed using linear mixed effect models in R. Differences in means were separated using Tukey's HSD. Japanese millet seed production differed between years ($p<0.001$); therefore, years were analyzed separately. In 2019, seed production did not differ between hybrid; however, in 2020, seed production decreased in the PH by 68% compared to the UH ($p=0.04$). Interseeding decreased weed seed viability by 46% compared to monoculture corn. Demographic data was used to create a stochastic density dependent population dynamics model in R to evaluate long-term impacts of interseeding on weed populations. Within the model, interseeding decreased Japanese millet seed production by 55% compared to the monoculture treatments in 2019. Seed production and growth rates crashed rapidly in 2020 due to lack of precipitation during weed establishment. Seed production was the most elastic parameter for the 2019 PH interseeded system. Interseeding corn and alfalfa has the

ability to control weeds by limiting the number of viable seeds produced. Furthermore, management tactics that reduce seed production and overwinter seed survival will enhance weed control benefits proved from interseeding corn and alfalfa.

Introduction

Weeds cause approximately 270 billion dollars annually in economic loss due to lower crop yields, the cost of weed control, and contamination of the harvested crop (Pimentel et al., 2005). Weed control costs are dependent on the type of weed control and the cropping system it is occurring in. In corn systems on average, mechanical weed control requires 16.43 hours ha^{-1} and costs \$138.65 ha^{-1}, chemical weed control requires 4.48 hours ha^{-1} and costs \$27.08 ha^{-1}, and genetic (e.g. herbicide resistant crop traits) plus chemical weed control requires 3.08 hours ha^{-1} and costs \$20.68 ha^{-1} (Swinton and Van Deynze, 2017). Weed control is increasingly difficult and costly with the evolution of herbicide resistant weeds. To date, there are 263 weed species identified to be resistant to herbicides, with resistance to 23 out of 26 sites of action (Heap, 2021). Even with the increase in herbicide resistant weeds, there has been little to no new identification of novel herbicide modes of action (Qu et al., 2021).

In addition to the increase in herbicide resistant weeds, environmental issues have arisen due to monoculture practices (Logan et al., 1994; Logan et al., 1980; Roesch-McNally et al., 2018). Monoculture corn systems in the Corn Belt region of the Midwestern U.S. have resulted in contamination of water sources via runoff and tile drainage from nutrients such as nitrates and phosphorus (Kladivko et al., 1991; Logan et al., 1994; Logan et al., 1980). This contamination has resulted in a hypoxia zone in the Gulf of Mexico (Donner and Kucharik, 2008). It is important to look into more diverse cropping systems, which could counteract the environmental issues and provide other forms of weed control to help combat the increase in herbicide resistant weeds and environmental issues that arise from monoculture systems. Furthermore, diverse agriculture systems could assist in coping with extreme weather variability, which is predicted to become more prevalent in the future (Roesch-McNally et al., 2018).

The increase in herbicide resistant weed populations, negative environmental consequences of monoculture cropping systems, and climate change projections have resulted in a renewed interest in agriculture systems that could help offset these consequences. One way to reduce the impact of these problems is by increasing cropping system diversity via interseeding. Interseeding is defined as planting two crops at the same time, which increases crop diversity spatially and can provide ecosystem services (Didon et al., 2014; Samedani et al., 2014). Alfalfa interseeded into corn has the ability to increase diversity and provide ecosystem services. By interseeding these crops together, alfalfa acres can increase, which are currently declining (Baxter et al., 2017; Grabber, 2016; USDA, 2020). In addition, farmers can use corn silage as a source of animal feed to substitute the typical low yielding establishment year of alfalfa and use alfalfa in the following seasons, which diversifies feed sources and improves animal health (Brito and Broderick, 2006; Stanger and Lauer, 2008).

Osterholz et al. (2019) showed that interseeding corn and alfalfa increased ground cover by 52% and reduced total runoff volume by 63% compared to monoculture corn, which can help protect waterways and reduce nutrient loss. Interseeding corn silage and alfalfa can have a positive impact on the economics of dairy farms, by increasing profitability by 15% compared to the traditional rotation of corn and alfalfa found commonly on dairy farms (Osterholz et al., 2020b). Interseeding corn silage and alfalfa could contribute to a more resilient system by decreasing the amount of herbicides applied based on its inherent ability to suppress weed biomass by 65-70% without the use of herbicides compared to monoculture corn (Osterholz et al., 2020a). However, interseeding grain and legume crops can provide a unique challenge to chemical and mechanical weed control, as shown by Osterholz et al. (2020a) conclusion that only glyphosate provided sufficient weed control and crop safety compared to eight other herbicides tested. Even though glyphosate can provide weed control in this interseeded system, with the rise in glyphosate resistant weeds (Beckie, 2014; Walsh and Powles, 2014) it is important to evaluate the interseeded system's ability for ecological weed control.

Although there has been limited research on ecological weed control provided from interseeding alfalfa and corn, there has been an increase in research to suppress weeds via cover crops. The use of cover crops between two cash crops results in an increased duration of soil cover as either a living crop or dead mulch, depending on management and diversification of the crop rotation (Büchi et al., 2020). Fall planted cover crops directly compete for water, nutrients, and light with winter annual weeds, whose life cycles overlap and leftover residues from termination of the cover crop can influence the growth rate of the summer annual weed species (Ryan et al., 2011; Teasdale et al., 2003). Both direct competition and leftover residues from the cover crops can reduce the need for herbicides (Alonso-Ayuso et al., 2018; Brust et al., 2014; Teasdale and Mohler, 2000). The level of weed suppression is highly dependent on the type of cover crop species used (Akemo et al., 2000; Baraibar et al., 2018; Hayden et al., 2012), as confirmed in a meta-analysis of previous literature, grass cover crops provide better weed suppression than broadleaf cover crops (Osipitan et al., 2019), which may be due to deterioration differences (Campiglia et al., 2012; Hayden et al., 2012; Mennan et al., 2006; Ruffo and Bollero, 2003). Furthermore, the later the cover crops are terminated by herbicide application, disking, mowing, rolling, or undercutting, the greater the ability of the cover crop to suppress weeds (Osipitan et al., 2019). Overall, the ability of cover crops to provide weed control is very dependent on location and management decisions, such as cover crop species, seeding rate, season it is sown in, termination date, planting date of the cash crop, tillage system, and other weed control practices (Osipitan et al., 2019).

One management option to increase the amount of time cover crops can suppress weeds via competition is interseeding cover crops with cash crops (Gage and Schwartz-Lazaro, 2019). Youngerman et al. (2018) reported that increases in corn density and cover crop biomass resulted in a decrease in weed biomass and density due to enhanced light interception by the corn and cover crops in the interseeded system. Furthermore, Brooker et al. (2020) reported annual ryegrass, a cover crop that can overwinter, interseeded into corn resulted in a 44% decrease in weed biomass the following spring, while interseeded crimson clover (*Trifolium incarnatum*) and oilseed radish (*Raphanus sativus*), had no impact on spring weed biomass due to lack of overwinter survival. Suggesting that interseeding has the ability to suppress weeds when the duration of living cover is increased via winter hardy cover crops.

The increase in competition for light, water, and nutrients between the weeds, cover crops, and cash crops from interseeding changes the maternal environment of weed seed production. The maternal environment can influence seed viability, dormancy, and survival in the seedbank (Williams et al., 2012). In a shaded maternal environment, such as a system with cover crops or interseeded systems, seeds produced may be immature or exhibit less dormancy than those exposed to full sunlight (Brainard et al., 2005). A shaded environment can result in a wetter microenvironment more suitable to fungi and bacteria, which could increase infection rates and decreased seed survival in the seedbank (Juroszek and Von Tiedemann, 2011). Overall, immature seed, the increase in fungi and bacteria, and the increase in light captured by the crop can contribute to weed suppression (Callaway, 1992). A way to increase shade in a corn system besides increasing the number of plants present via cover crops, is hybrid selection based on leaf architecture. Corn hybrids have a range of leaf angles, driven by breeding selection (Tian et al., 2011). The two extremes of corn leaf architecture are pendulum, which has a wider leaf angle, capturing more light and upright, which has a narrower leaf angle, capturing less light (Callaway, 1992; Tian et al., 2011). Sankula et al. (2004) reported that competition from pendulum corn hybrids reduced weed biomass by 73-90% compared to the upright corn hybrid under irrigation. In total, ecological weed management can be achieved by using cropping systems that have the ability to outcompete weeds.

Although there has been some research about the possibilities of weed suppression or management from interseeding crops and cover crops together, there has been little to no research about weed seed dynamics in an interseeded system of corn and alfalfa, which are both cash crops. Therefore, the objectives of this research were to: 1) determine the effect of interseeding corn silage and alfalfa has on weed seed production, germinability, and viability, and 2) predict the long-term effects of interseeding corn silage and alfalfa has on weed population dynamics.

Materials and Methods

Japanese millet (*Echinochloa esculenta*) population dynamics in an interseeded system of corn silage and alfalfa were studied in a two-year experiment (2019-2020) conducted at the Michigan State University (MSU) Plant Pathology Farm in East Lansing, Michigan (42.68 ° N, 84.50 ° W). The study followed a split-plot randomized complete block design with four replications. Whole plots were assigned to one of two corn hybrids with differing leaf architecture, pendulum, wider leaf angle, or upright, narrower leaf angle. Subplots were assigned to presence or absence of alfalfa and Japanese millet, a surrogate weed, to ensure uniform planting populations. Japanese millet was planted at 120 seeds m^{-2}. Corn and alfalfa were planted in soils prepared with a fall chisel plow, followed by two passes with the soil finisher, and one pass of the soil cultipacker in the spring. Urea fertilizer was applied preplant incorporated (46-0-

0) at 168 kg ha^{-1} followed by 18 L ha^{-1} of 16-16-16 (N-P-K) applied at corn planting. Plots were 3 m by 9.1 m in 2019 and 3 m by 8.8 m in 2020. The corn was planted on June 4, 2019, and May 28, 2020, using a 91cm drill, row width at 76 cm**,** and a seeding rate of 89,100 seeds ha^{-1}. The upright corn hybrid, G89A09 (Golden Harvest, Minnetonka, MN), has a narrow leaf angle. The pendulum corn hybrid, G90Y04 (Golden Harvest, Minnetonka, MN), has a wider leaf angle. Glyphosate tolerant alfalfa was planted on the same days as the corn with four rows in between each corn row and the alfalfa rows were spaced 19.05 cm apart. A John Deere tow drill with row width set at 19 cm with 17 openers at a seeding rate of 2.94 kg ha^{-1}. In 2019, the alfalfa consisted of two different varieties DKA4051 (Bayer, St. Louis, MO) for replications one-three and FSG430LHRR (DLF Pickseed, Halsey, OR) for replication four, due to inadequate DKA4051 seed reserves. In 2020, the alfalfa variety was FSG431LHRR for all replications. In addition, monoculture corn plots were included as controls.

Japanese millet seedlings were count two weeks after germination in 1 m^2 (2019) and 0.5 m^2 (2020) permanently installed quadrates per subplot. Once mature, the seeds were harvested from quadrats, placed in a paper bag, and stored at 5°C until processed. After harvest, seed panicles were counted, seeds were threshed, and weighted. Seed count was obtained by regression analysis in Japanese millet harvested in 2019 (Table 3.1) and hand counted from seed harvested in 2020. Once the seeds were counted, a subsample of 50 seeds in 2019 and 25 seeds in 2020 was taken and tested with tetrazolium to determine viability following the methods of (Analysts et al., 2010).

The following spring, ten soil samples were taken from the weedy controls to assess Japanese millet overwinter seed survival, as this was the introduction of Japanese millet to the field, the original seeding number was 120 seeds m^{-2}. Soil samples were taken at ten random locations in each subplot with a handheld bulb planter (7.6 cm in diameter, 11.4 cm deep) following methods outline in (Burns et al., 2018). The soil samples were place in flats (53.34 cm X 26.67 cm) with a base of 1.3 cm of peat and perlite mixture (SureMix, Michigan Grower Products, Inc, Galesburg, MI) at the bottom to ensure adequate water retention. The samples were grown for 42 days in the greenhouse, with temperature set to 27°C (diurnal range 25-29°C) and 16-hour photoperiod. Emergence of Japanese millet was counted and removed every two days. Once emergence had ceased, the soil was sieved and seeds were chemical enumerated following methods outlined in Malone et al. (1967). After the enumeration, the seeds were counted, and viability was tested following the methods of (Analysts et al., 2010).

Statistical Analysis

The overall impacts of competition from interseeded alfalfa and corn hybrids with differing leaf architecture on weed seed production, viability, and overwintered seeds were analyzed using lmer function (R, 2020). Differences in means were further investigated using Tukey's HSD post hoc tests.

A stochastic density dependent model was constructed using a series of difference equations to project Japanese millet populations forward 20 years under monoculture or interseeding treatments for each corn hybrid. The model parameters included: seedling emergence, seedling establishment, seedling survival, number of mature plants, asymptomatic density dependent seeds produced per plant, overwinter seedbank survival, and proportion of seed remaining in the field (Table 3.2; equations in Table 3.2 build sequentially to form the annual projection equation presented in the last line of the table). Proportion of the seed remaining in the field was recorded to be 50 for giant foxtail in a corn system in Illinois (Davis, 2006). Other parameters were fitted using means and associated standard errors from data obtained in this experiment. Nonlinear least-squares estimates of the asymptotic density dependent parameters for seeds produced per plant (i, a) were fit using the nls function in R (R, 2020).

The population model was constructed under two cropping systems, interseeded or monoculture, within each system populations were projected for each corn hybrid, pendulum, or upright leaf angles, for each year the experiment 2019 or 2020 with a starting Japanese millet seedbank population of 120 seeds. This process was repeated over 20 years which constitutes one simulation. The population projection was modelled for 10 simulations for each treatment combination.

Elasticity analysis was used to quantify the proportional change in the Japanese millet seedbank resulting from a proportional change in vital rates (Caswell, 2000). Elasticities were calculated as:

$$E_{ij} = \frac{a_{ij}}{\mathrm{SB}} \frac{\delta \mathrm{SB}}{\delta a_{ij}} \qquad [1]$$

Where E_{ij} is the elasticity of the seedbank (SB) to the proportional change in the parameter a_{ij} (Caswell, 2000) where i and j represent rows (years) and columns (treatments). Elasticity analysis was performed using the population dynamics model described above modelled for 10 simulations.

To assess the overall impacts of interseeding and corn hybrid leaf angle on the dN/dt (the change in the number of individuals [dN] per change in time [dt]) for each Japanese millet populations, a linear mixed effect model was constructed with fixed effects for the two-way interaction between cropping system and hybrid with random effect for replication. The model was fitted using the lmer function (R, 2020) . Differences in means were further investigated using Tukey's HSD post hoc test in R (R, 2020).

Results and Discussion

Japanese Millet in Season Results

Seed Production

Japanese millet, a surrogate weed, seed production differed between years ($p<0.0001$), therefore years were analyzed separately. In 2019, there was no difference in Japanese millet seed production between monoculture corn or interseeded corn and alfalfa cropping systems ($p=0.1$). Furthermore, averaged across cropping systems there was no difference in Japanese millet seed production when plants were grown in competition with pendulum or upright corn hybrids ($p=0.7$; Figure 3.1). Similar to 2019, in 2020, there was no difference in Japanese millet seed production between monoculture corn or interseeded corn and alfalfa cropping systems ($p=0.5$). However, in 2020 competition from pendulum corn hybrids reduced Japanese millet seed production by 67% compared to the upright hybrid ($p=0.03$; Figure 3.1). Interestingly, Japanese millet seed production decreased by 94% in 2020 compared to 2019 (Figure 3.1).

Our 2019 results are similar to those of Sankula et al., (2004) who reported that under irrigated and non-irrigated treatments, weed seed production did not differ between weeds in competition with pendulum or upright corn hybrids. Similar to the 2020 results, Wild proso millet (*Panicum miliaceum*) in competition with short maturing, less competitive architecture corn hybrids produced 66% more seeds than the millet plants grown in competition with longer maturing, more competitive architecture corn hybrids (Williams et al., 2012). The differences between years may have to do with precipitation differences during Japanese millet establishment, for example precipitation in June 2020 was 41.14 mm less than June 2019 (Table 3.1). June 2020 also had less precipitation compared to the 30-year average, which was 73.67

mm and 87.6 mm, respectively (Table 3.1). Therefore, during years with less precipitation planting pendulum corn hybrids may be beneficial at reducing the weed seedbank.

Seed Viability

There was no difference in Japanese millet seed viability between years (2019-2020) and corn hybrids (p=0.85; p=0.3); therefore, year and hybrids were combined for the analysis. Interestingly, surrogate weed seed viability decreased by 46% in the interseeded treatment compared to the monoculture corn treatment (p=0.02; Figure 3.2). The observed decrease in the number of viable weed seeds may be attributed to changes in the maternal environment the seeds were produced in. For example, changes in light distribution throughout the canopy and moisture differences leads to different microenvironments, which can influence seed viability (Brainard et al., 2005; Juroszek and Von Tiedemann, 2011; Williams et al., 2012). Powell amaranth (*Amaranthus powellii*) seed viability was decreased by 10% when grown in competition with broccoli (*Brassica oleracea*) and winter rye (*Secale cereale*) compared to broccoli alone (Brainard et al., 2005). Seed germination of Wild proso millet in competition with short maturing, less competitive architecture corn hybrids was 12.5% greater than the millet plants grown in competition with longer maturing, more competitive architecture corn hybrids (Williams et al., 2012). Both Brainard et al. (2005) and Williams et al. (2012) documented decreases in weed seed viability by increasing competition; therefore, changing the microenvironment the seeds are produced in. Interseeding corn and alfalfa can provide weed control by targeting the weed seedbank, which will assist in the goal of creating a more sustainable weed management system (Garrison et al., 2014).

Overwinter Seed Survival

Japanese millet overwinter seed survival differed between 2019 and 2020 (p=0.04);

however, corn hybrid and cropping system did not affect overwinter seed survival (p=0.1; p=0.3; Figure 3.3). In 2019, overwinter seed survival was 93% less than in 2020 averaged over hybrid and cropping systems (Figure 3.3). The reduction in 2019 may be attributed to increased competition during seed production, which impacts offspring success by resulting in smaller seeds leading to a reduction in competitive ability of the offspring the following season during establishment (Espeland and Hammond, 2013). Crop and weed dormancy is tied tightly to favorable environmental conditions, such as precipitation; however, crops are bred to easily break dormancy while weeds use dormancy as a method to reproduce through time (Roberts, 1984). Although Japanese millet has weedy characteristics similar to barnyardgrass, it was originally bred to be a crop. The crop like nature resulted in all Japanese millet seed to germinate in 2019, increasing intraspecific competition. The reverse occurred in 2020 with less precipitation resulting in less intraspecific competition. Furthermore, reductions in overwinter seed survival are also related to seed viability and the ability to produce mature seeds, which is often reduced in highly competitive crop environments (Brainard et al., 2005).

Projection Model

Population Growth Rate

In 2019, there was no difference in Japanese millet population growth rates (λ) for plants grown in competition with monoculture pendulum (λ = 0.98 (0.007)) or upright (λ = 0.97 (0.007)) corn hybrids (p=0.2; Figure 3.4). Furthermore, both growth rates for these treatment combinations do not contain one, therefore populations are declining slowly. Japanese millet population growth rates were similar in the pendulum (λ = 0.94 (0.005)) and upright (λ = 0.94 (0.005)) interseeded cropping systems (Figure 3.4; p=0.2). Interseeded populations are declining slowly, as the growth rate does not include one. Overall, interseeding decreased the Japanese millet population 3% faster than the monoculture system did based on 2019 data averaged over hybrid ($p<0.0001$).

In 2020, there was no difference in Japanese millet population growth rates for plants grown in competition with monoculture pendulum corn (λ = 0.11 (0.01)), monoculture upright corn (λ= 0.12 (0.008)), and interseeded upright corn and alfalfa (λ = 0.10 (0.02)) (Figure 3.4). In contrast, the Japanese millet population growth rate for plants grown in competition with interseeded pendulum corn and alfalfa (λ = 0.03 (0.008)) was 72, 75, and 70% less than plants grown in competition with monoculture pendulum corn, monoculture upright corn, and interseeded upright corn and alfalfa (Figure 3.4). All 2020 growth rate models crashed to a growth rate of zero by the 5th modeled year (Figure 3.4).

Differences between years can be attributed to the significant differences between precipitation observed in the start model year, which changed the number of seeds produced in 2020 compared to 2019 (Table 3.3; Figure 3.1). Overall, in years with reduced precipitation during weed establishment similar to 2020, planting hybrids with differing leaf architecture nor interseeding had appreciable positive impacts at reducing Japanese millet population growth (Figure 3.4). However, in years with ample precipitation like 2019 interseeding corn and alfalfa regardless of hybrid choice had positive impacts at reducing Japanese millet population growth compared to monoculture treatments (Figure 3.4). As both 2019 and 2020 had lower precipitation than the 30-year average, it is possible that compounding water and interseeding stress has the ability to decrease Japanese millet population growth (Figure 3.4).

Seed Production

Seed production projections for the 20th modeled year differed between the establishment years ($p<0.0001$; Figure 3.5). In 2019, the interaction between cropping system and hybrid was significant for seed production ($p<0.0001$). In 2019, Japanese millet seed production under the pendulum corn monoculture system had consistently higher seed production than the upright monoculture, pendulum interseeded, or upright interseeded systems (Figure 3.5). There was no difference in seed production amongst upright or pendulum interseeded systems (Figure 3.5). Pendulum seed production was 46% greater than upright seed production in the monoculture systems (Figure 3.5). Seed production (2019, 20th modeled year) decreased in the interseeded pendulum and upright treatments by 67% and 64% compared to the monoculture pendulum corn treatment (Figure 3.5). Seed production decreased (2019, 20th modeled year) decreased in the interseeded pendulum and upright treatments by 40% and 33% compared to the monoculture upright corn treatment (Figure 3.5).

The 2020 seed production projections all crashed before the 5th modeled year (Figure 3.5). Differences between years can be attributed changes in precipitation observed in the start model year, which changed the number of seeds produced in 2020 compared to 2019 (Table 3.3; Figure 3.1). The differences between the 2019 corn upright vs. pendulum monoculture treatments are contrary to previous studies, which showed that seed production decreased under more shaded environment (Sankula et al., 2004; Williams et al., 2012), which the pendulum hybrid should provide. Overall, reductions in modeled seed production under the interseeded corn and alfalfa cropping system suggests that interseeding has the ability to decrease seed production long-term.

Elasticity

Elasticity analysis can help determine the stage within the Japanese millet life cycle that will be the most vulnerable to weed management and thus will have the largest negative effect on projected population growth. By calculating elasticity, based on the Japanese millet seed production in the 20th model year, the vital rates with the largest impact on population growth are equal to one (Table 3.2). In 2019, the most elastic vital parameters were asymptomatic density dependent seed production for the pendulum and upright monoculture corn hybrids treatments (Table 3.4). For the pendulum corn hybrid interseeded system treatment, the most elastic parameter was maximum seed production (Table 3.4). For the upright corn hybrid interseeded system treatment, the most elastic vital parameter was sr, which is the proportion of seed remaining in the field and is a proxy for seed predation (Table 3.2). Overall, 2019 elasticity values suggested that the best target for management should be seed production and seed survival. In 2020, all seed production data equaled zero before the 20th modeled year, hence all elasticity values are zero (Table 3.2). Overall, our data supports a central theme of the important of targeting the weed seedbank for integrated weed management (Espeland and Hammond, 2013; Garrison et al., 2014).

d*N*/d*t*

The d*N*/d*t* differed between years ($p<0.0001$), therefore years were analyzed separately. In 2019, a two-way interaction occurred between corn hybrid and cropping system ($p<0.0001$). There was no difference in d*N*/d*t* between the upright corn monoculture and upright interseeded systems (Table 3.5). However, Japanese millet d*N*/d*t* decreased by 1,341%, 1,019%, and 1,329% when in competition with pendulum interseeded, upright corn monoculture, and upright interseeded, respectively, compared to the pendulum monoculture system (Table 3.3). In 2020, the main effect of hybrid modified d*N*/d*t* ($p=0.04$; Table 3.3); however, the cropping system did not ($p=0.6$). Averaged across cropping systems the d*N*/d*t* for the pendulum corn hybrid is slightly less than the upright corn hybrid (Table 3.3).

As resistant weeds become more prevalent ecological forms of weed control will need to be employed such as interseeding investigated in this study. Interseeding corn and alfalfa has been found to suppress weed biomass by 65-70% without the use of herbicides (Osterholz et al., 2020a), which can have long-term impacts on weed populations. Our study evaluated weed population dynamics further and found that interseeding corn silage and alfalfa has the ability to decrease Japanese millet seed viability compared to a monoculture corn system. Reductions in seed viability led to less weed seed additions to the seedbank; therefore, decreasing weed populations in subsequent seasons. The projected model for Japanese millet, surrogate weed, growth rate and seed production, decreased in the interseeded system compared to the monoculture system, which suggests that long-term interseeding has the ability to reduce the weed seedbank. This is further supported by the elasticity analysis in which targeting density dependent seed production and the number of seeds entering into the seedbank will have the largest negative impact on population growth. Management to decrease the number of seed produced would include removing weeds before they produce seeds via chemical or non-chemical control options, such as glyphosate or tillage; however, if weeds escape from initial control weed seed harvest technologies and would assist in decreasing weed seedbank additions. Additionally, the increase competition from interseeding corn and alfalfa, which resulted in a decrease of seed viability may assist in the decrease of seedbank additions. Weed seedbank management is a foundational principle of integrated weed management (Espeland and Hammond, 2013; Garrison et al., 2014). Interseeding corn and alfalfa has the potential to provide weed seedbank management, which could be exploited further in the future with the utilization of more competitive corn hybrids.

Future research should evaluate weed seed dynamics in a more traditional rotation found on dairy farms, such as corn-corn-alfalfa for four years compared to the interseeded system of corn silage and alfalfa. Additionally, it would be interesting to evaluate seed predation differences between the two different rotations, as more ground cover is thought to increase weed seed predation (Bartel and Orrock, 2020). Weed species and diversity should be evaluated in the interseeded system, not just a surrogate weed alone, as the ability of the surrogate weed and other weeds to exit and enter dormancy is different. Additionally, weed species interact differently depending on the envrioment and cropping system which may modify the results of this study. As weed pressure increases due to climatic issues and herbicide resistance continues to evolve at a fast pace, alternative forms of weed control and suppression are needed, and interseeding crops, like corn and alfalfa, could provide sustainable ecological weed management.

APPENDIX

Table 3.1. Linear regression equations and r^2 values for Japanese millet seed production.

Hybrid	Treatment	Equation	r^2
Upright	Corn and Alfalfa	$y=1177.3x+33.404$	0.9712
	Corn	$y=561.74x+3.2561$	0.9949
Pendulum	Corn and Alfalfa	$y=1087.1x+24.576$	0.9836
	Corn	$y=683.32x+28.288$	0.9840

Table 3.2. Abbreviations and estimators for Japanese millet parameters used in population dynamics model. [*]

Parameter	Abbreviation	Equation
Emergence	estab	number of seedlings/120
Seedling density	sdl	seedbank density*estab
Seedling survival	mature	sdl*(mature plants/ number of seedlings)
Maximum seed production (m^2)	sppM	sppmax*(1-(i*mature)/(1+(i*mature)/a)) [¥]
Seeds produced per a population	SP	sppM*mature
Seeds in next spring's seedbank	Seeds	((Seeds-sdl) + SP*sr) *ssrv[§]

[*] Equations in the table build sequentially to form the annual projection equation presented in the last line of the table

[¥] sppmax maximum carrying capacity; i-a, asymptomatic desnity dependent shape parameters

[§] sr proportion of seed remaining in the field, a proxy for seed predation (Davis, 2006); ssrv seed viability

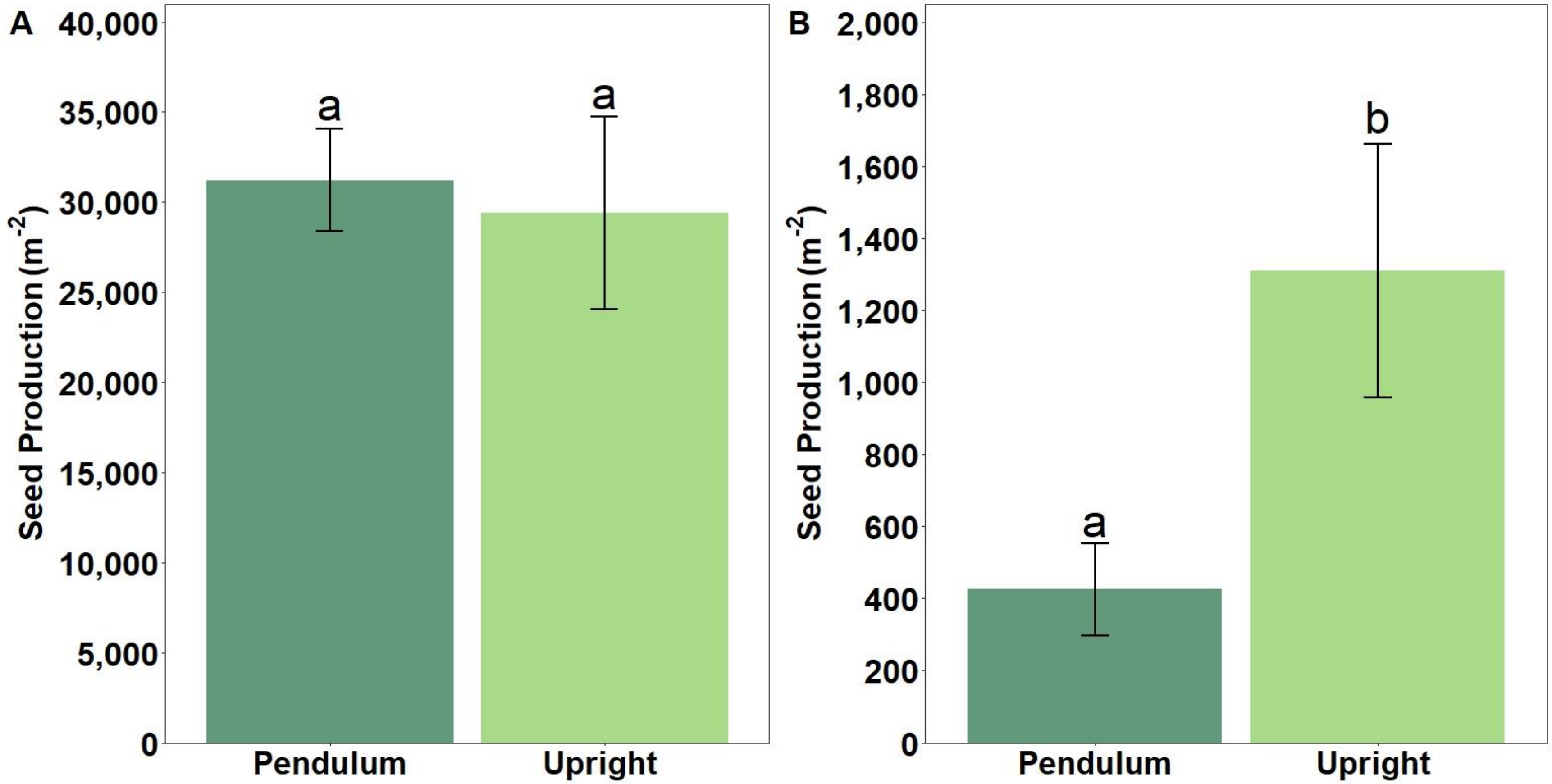

Figure 3.1. Mean (SE) Japanese millet (surrogate weed) seed production impacted by corn hybrids with different leaf architecture, pendulum or upright, in a two-year field study in 2019 (A, scale 40,000) and 2020 (B, scale 2,000), averaged over monoculture corn and corn-alfalfa interseeded systems. Bars labeled by the same lowercase letter with years are not statistically different ($p \geq 0.05$).

Table 3.3. Monthly precipitation at the study location in East Lansing, MI for 2019, 2020, and 2021.

Month	2019	2020	2021	30-year average[2]
	Precipitation (mm)			
May	85.08[1]	108.97	24.37	85.3
June	114.81	73.67	176.5	87.6
July	58.42	41.65	95.0	72.1
August	18.28	69.34	-	82
September	92.46	108.72	-	88.9
Total	369.05	402.35	-	415.9

[1] Precipitation data collected from Michigan Enviro-Weather station within 1 kilometer of the study location DOI:10.7289/V5PN93J

[2] Monthly 30-year average precipitation for Lansing, MI, data retrieved from NOAA Nation Centers for Environmental Information

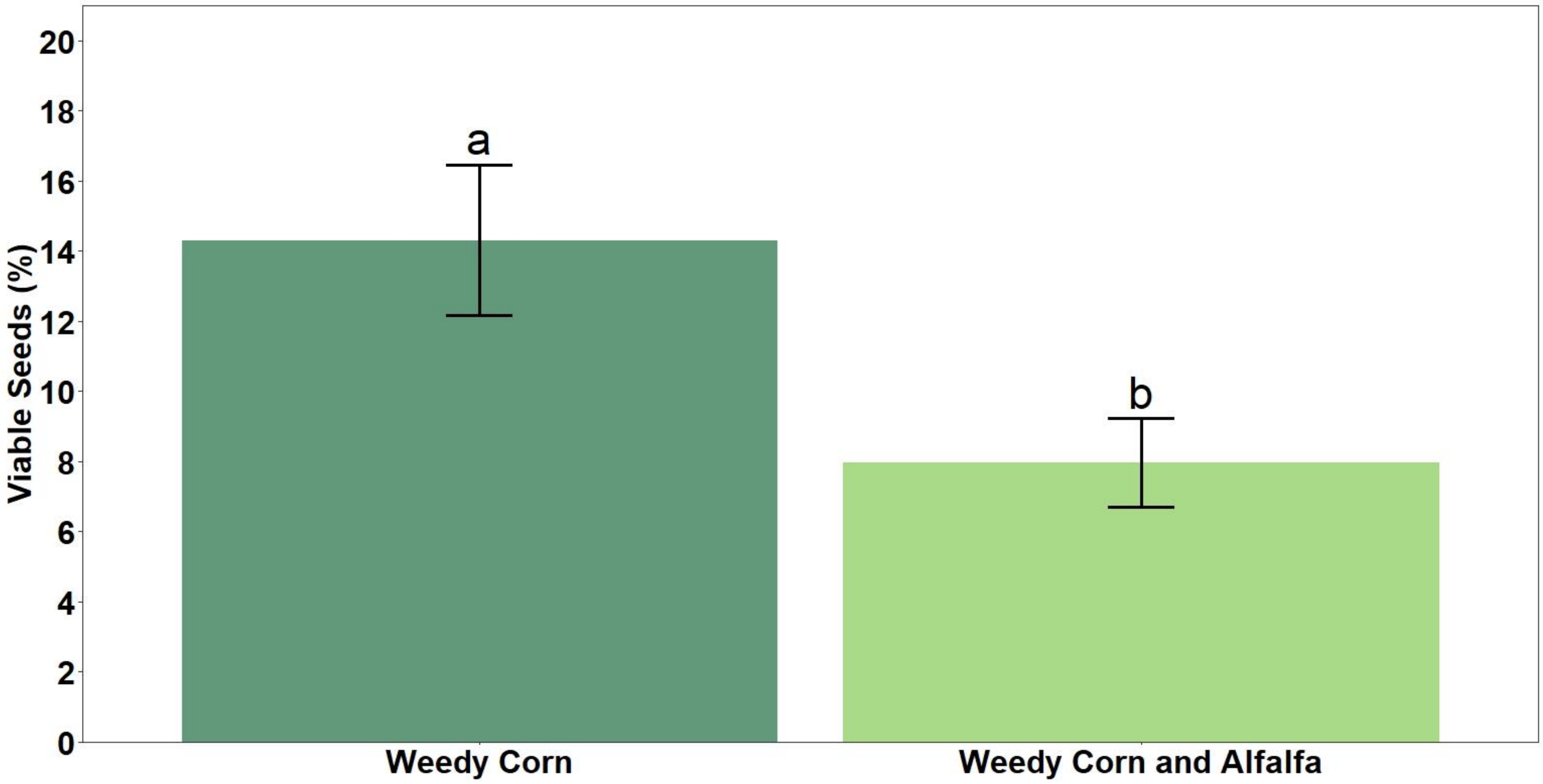

Figure 3.2. Mean (SE) Japanese millet (surrogate weed) seed viability under monoculture corn and corn-alfalfa interseeded systems averaged across years (2019-2020) and corn hybrids with differing leaf architecture (pendulum and upright) in a two-year field study (2019-2020). Bars labeled by the same lowercase letter are not statistically different ($p \geq 0.05$).

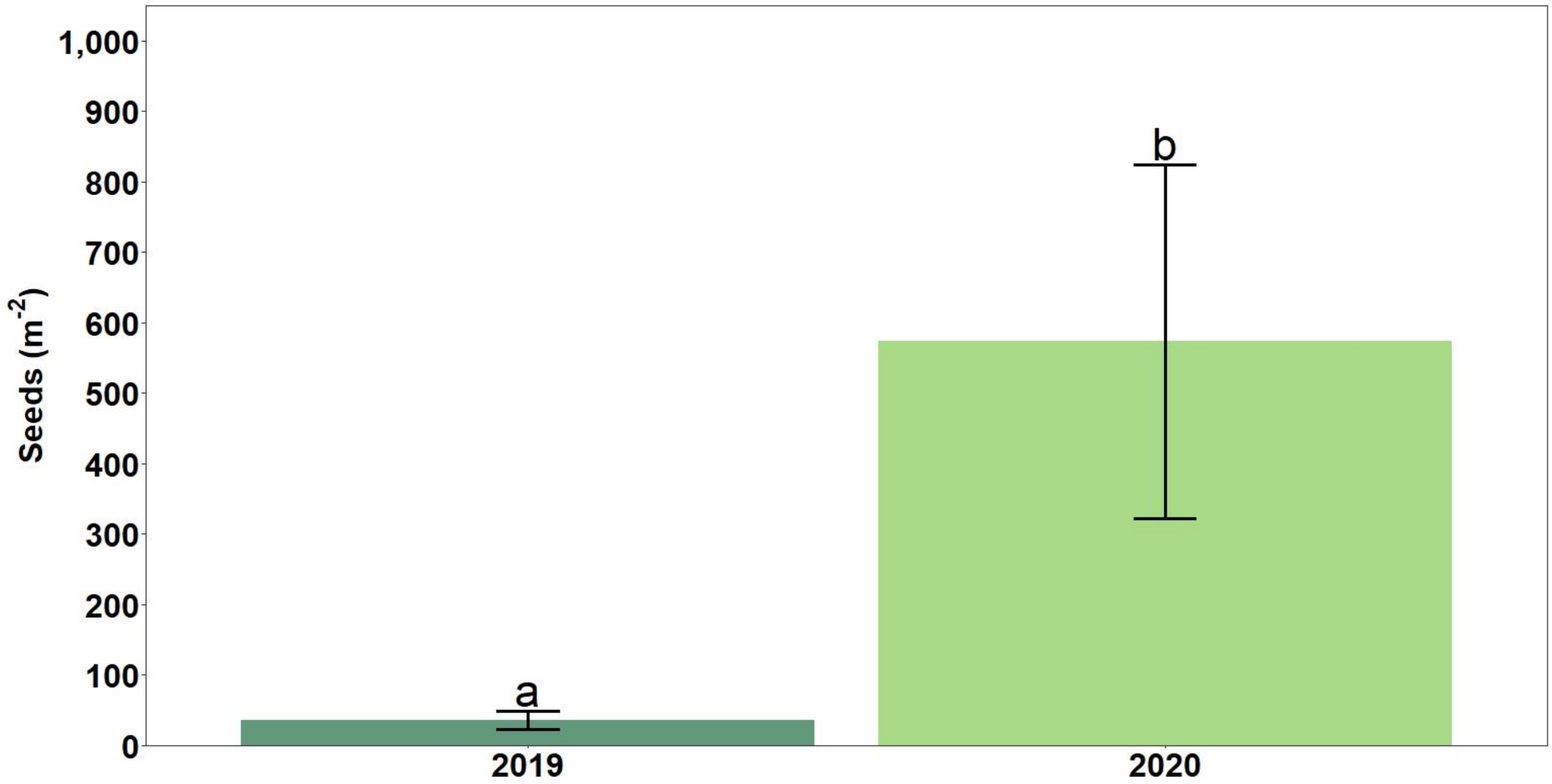

Figure 3.3. Mean (SE) Japanese millet (surrogate weed) overwinter survival in 2019 and 2020, averaged across monoculture corn and corn-alfalfa interseeded systems and corn hybrids with differing leaf architecture (pendulum and upright) in a two-year field study (2019-2020). Bars labeled by the same lowercase letter are not statistically different ($p \geq 0.05$).

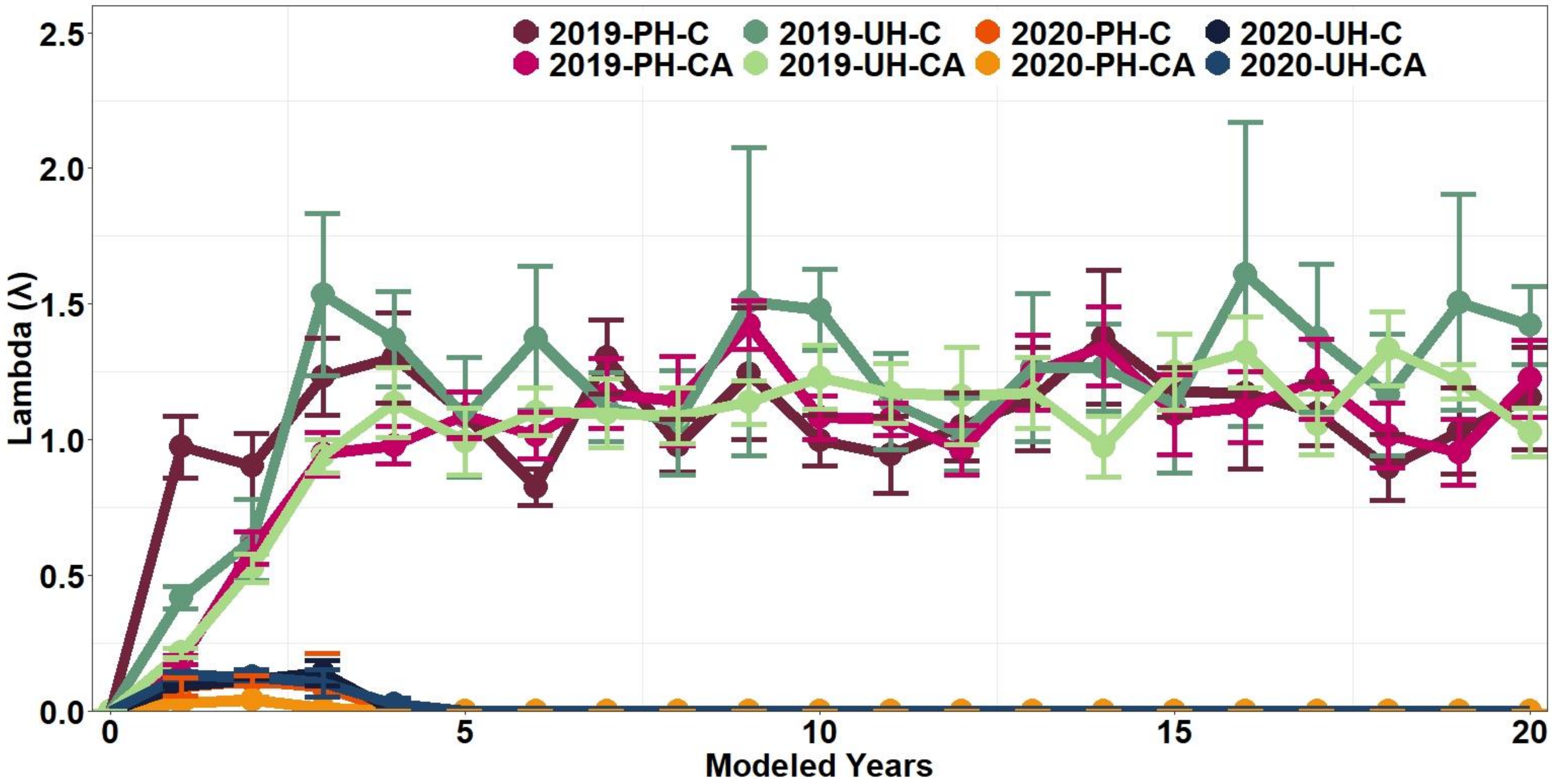

Figure 3.4. Mean modeled population growth rate (λ) projections for Japanese millet (surrogate weed) under two corn hybrids with different leaf architecture, pendulum hybrid (PH) or upright hybrid (UH), and two cropping systems, corn monoculture (C) or interseeding corn and alfalfa (CA) based on data collected in a two-year field study (2019-2020).

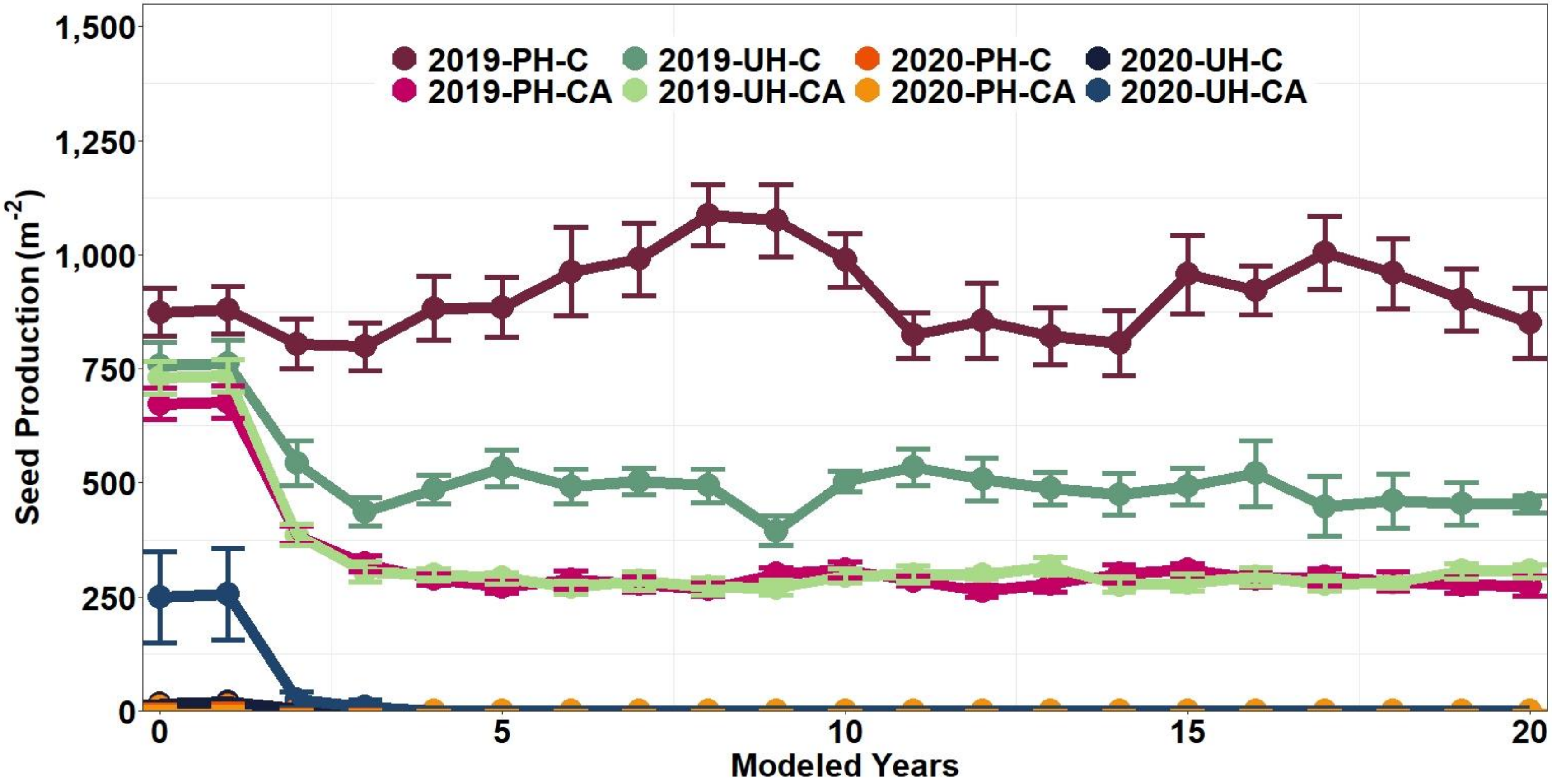

Figure 3.5. Mean modeled seed production for Japanese millet (surrogate weed) under two corn hybrids with different leaf architecture, pendulum hybrid (PH) or upright hybrid (UH), and two cropping environments, corn monoculture (C) and interseeding corn and alfalfa (CA) based on data collected in a two-year field study (2019-2020).

Table 3.4. Seedbank elasticity of Japanese millet (surrogate weed) under two corn hybrids with different leaf architecture, pendulum or upright, and two cropping environments, monoculture corn and interseeded corn and alfalfa calculated based on the results of a 20-year projection model from data collected in a two-year field study (2019-2020).

Year	Hybrid	Cropping System	estab[a]	sdls	sppmax	i	a	ssrv	sr
2019	Pendulum	Corn	2.69E-05	9.95E-06	1.93E-05	6.81E-05	**1.00**	1.73E-04	4.76E-05
		Corn and Alfalfa	5.77E-01	2.06E-01	**1.00**	4.62E-01	8.95E-01	4.23E-01	3.32E-01
	Upright	Corn	7.48E-01	2.12E-01	1.24E-02	6.56E-01	**1.00**	8.46E-01	1.03E-01
		Corn and Alfalfa	2.74E-01	5.32E-01	9.47E-01	9.35E-01	8.76E-01	6.39E-01	**1.00**
2020	Pendulum	Corn	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Corn and Alfalfa	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Upright	Corn	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Corn and Alfalfa	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] estab=seedling establishment; sdls=seedling survival; sppmax=maximum seed production; i=shape parameter in asymptotic density dependent seed production function; a=shape parameter in asymptotic density dependent seed production function; ssrv=seed survival in seedbank; sr=proportion of seed remaining in the field, a proxy for seed predation

Table 3.5. Japanese millet (surrogate weed) mean d*N*/d*t* (SE) under two corn hybrids with different leaf architecture, pendulum or upright, and two different cropping systems monoculture corn or interseeding corn and alfalfa based on the results of a 20-year projection model from data collected in a two-year field study (2019-2020).

Hybrid	Cropping System	2019	2020
		d*N*/d*t*	
Pendulum	Corn	1.8 (16.31) A[1]	-24.4 (17.18) A[2]a[3]
	Corn and Alfalfa	-22.35 (15.2) B	-24.5 (18.57) Aa
Upright	Corn	-16.55 (12.89) B	-24.38 (17.03) Ba
	Corn and Alfalfa	-22.13 (14.61) B	-24.33 (16.13) Ba

[1] Means within 2019 followed by the same capital letter are not significantly different for a two-way interaction between hybrid and cropping system ($p \geq 0.1$).

[2] Means within 2020 followed by the same capital letter are not significantly different for main effect of hybrid ($p \geq 0.1$).

[3] Means within 2020 followed by the same capital letter are not significantly different for main effect of cropping system ($p \geq 0.1$).

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CHAPTER IV

ROLE OF REACTIVE OXYGEN SPECIES SCAVENGING ENZYMES IN EARLY INTERSPECIFIC PLANT COMPETITION

Abstract

Scavenging enzymes help detoxify reactive oxygen species (ROS) within plants that are produced in response to stress. Plant stress results from plant competition for resources, such as light, water, and nutrients. A greenhouse study to determine corn and alfalfa stress levels when grown in competition together or with a surrogate weed, Japanese millet was conducted in East Lansing, Michigan. To achieve the overarching goal of understanding how interseeded plant competition modifies stress on a physiological level, corn, alfalfa, and Japanese millet were grown alone, in two-, or three-way combinations and harvested 7, 10, or 16 days after planting (DAP). Once harvested, plants were assayed for glutathione *S*-transferase (GST), dehydrascorbate reductase (DHAR), and superoxide dismutase (SOD) levels. Corn had a greater GST reaction rate compared to Japanese millet and alfalfa grown in three-way competition across all DAP. Japanese millet had a similar reaction rate across 7, 10, and 16 DAP when grown in competition with corn and alfalfa. Alfalfa had a negative GST reaction rate for all DAP when grown in the three-way plant competition with corn and Japanese millet. Therefore, corn and Japanese millet detoxify ROS via GST activity more than alfalfa. DHAR activity was often minimal, however, DHAR rates were often greatest 7 DAP for alfalfa and corn grown alone and declined 10 and 16 DAP. Japanese millet in competition with alfalfa and corn had negligible DHAR activity. There was no difference in SOD enzyme activity amongst treatments. Differences between species and DAP suggests that scavenging enzymes roles may change based on age and plant type. Overall, interseeding and weed competition stress is species and duration of plant competition specific. Further categorization of scavenging enzymes roles in each plant

species is needed in order to reduce stress levels to optimize interseeded corn and alfalfa systems.

Introduction

Inter- and intraspecific competition for shared resources including water, nutrients, and light have profound impacts on plant development in agricultural systems. Resource competition can result in changes in morphology and phenology of crops and weeds (Ballaré and Casal, 2000). Changes in light quality and quantity can have an effect on early season weed and crop competition (Staniforth, 1957; Thomas and Allison, 1975). Light quantity is determined by the amount of light from the sun and light quality can be driven by the amount of red to far red (R:FR) light in the lower canopy due to light reflecting from neighboring plants (Rajcan and Swanton, 2001). Solar radiation determines crop yield due to multiple factors including its primary role in photosynthesis and subsequent transpiration rates, which drives soil moisture and nutrient uptake (Ballaré and Casal, 2000). As light plays an important role in plant functions, when quality or quantity changes it can have major impacts.

Light is perceived in plants by photoreceptors, which in turn change photomorphogenic responses. Changes in photomorphogenic response can alter the way plants capture and compete for resources (Ballaré and Casal, 2000). One photomorphogenic, morphological response is shade avoidance, which is defined as the plants' perceived changes of R:FR light which acts as a notification of neighboring vegetation. The change in light quality can result in the display of physical responses such as stem and leaf elongation (Ciolfi et al., 2013). Light stress triggering shade avoidance responses ultimately lead to reductions in growth, changes in phenology, and finally reductions in yield. For example, early season corn and weed competition has a linear effect on root growth in maize, as weeds reflect light to the above ground biomass root growth decreases (Afifi and Swanton, 2011). In general, photomorphogenic responses occur as early as seed germination and can change throughout the life cycle of the plant.

Competition for light can also result in a physiological plant response with the production of reactive oxygen species (ROS). ROS is a group of molecules derived from molecular oxygen, which are formed by reduction-oxidation reactions or electron excitement within the plant (Sies and Jones, 2020). Elevated ROS levels in the cell can result in oxidative damage to DNA, RNA, and proteins and may cause cell death. ROS in plants include singlet oxygen, superoxide anion radicals, hydroxyl radicals, and the most common hydrogen peroxide. ROS is generated in a plant in response to stress, which can trigger pleiotropic metabolic responses such as signaling for other metabolites, phytochromes, and changes in gene expression (Dietz, 2011; Mignolet-Spruyt et al., 2016; Noctor and Foyer, 2016). In addition to roles in stress response, ROS signaling helps plants perform many functions, such as germination, meristem development, plant cell death, and expansion (De Simone et al., 2017; Diaz-Vivancos et al., 2015; Menon and Goswami, 2007).

As ROS is a requirement for the plant to survive, it is important that plants have a way to break ROS down to prevent negative reactions within the plant. ROS can be broken down by enzymatic and non-enzymatic reactions to achieve homeostasis including: catalases, ascorbate peroxidase, glutathione *S*-transferases, peroxiredoxins, thioredoxin-dependent peroxiredoxins, anthocyanins, and superoxidase dismutase (Afifi and Swanton, 2012; Bela et al., 2015; Dixon and Edwards, 2010; Fridovich, 1997; Iqbal et al., 2006; Noctor et al., 2012; Richards et al., 2015; Triantaphylidès and Havaux, 2009). The detoxification of ROS allows the plant to continue to survive and thrive.

Plants growing under non-ideal conditions accumulate ROS to generate a stress response. Previous research has evaluated how light stress can induce increases in ROS concentrations and evaluated enzymes used to detoxify them (Afifi and Swanton, 2012; Mckenzie-Gopsill et al., 2016). Afifi et al. (2012) demonstrated corn grown in a hydroponic system, with no limiting resources except light, were morphologically and physiologically different when grown under weed competition. Corn plants grown under competitive weed treatments exhibited a shade avoidance response due to modifications in the ratio of R:FR light. The lower R:FR light produced from weeds resulted in corn plants with elongated stems, delayed leaf emergence, and changes in physiology. Furthermore, hydrogen peroxide production in corn was 84% greater under weed competition and 63% greater under light filter treatments, which increased the amount of R:FR light, compared to the weed free or ambient light treatments. Gao et al. (2020) reported that shade stress increased proteins production with roles in stress, defense, and detoxification in corn plants by 20%. In soybean (*Glycine max*), Mckenzie et al. (2016) reported an upregulation of ROS and superoxide dismutase upon initial emergence, but concentrations decreased over time in the FR light enriched environment compared to the non-light treated controls. Piasecki et al. (2018a) reported a sustained increase in hydrogen peroxide concentration in soybean with increased densities of volunteer corn; however, in a similar study with dry bean (*Phaseolus vulgaris*), hydrogen peroxide levels stabilized after 21 days after emergence (Piasecki et al., 2018b). Furthermore, soybean plants grown under early season competition from weeds reflecting low R:FR light resulted in reduced nodulation, height, and biomass later in the season compared to soybeans without weed competition and light exposure (Gal et al., 2015). Competition between wheat (*Triticum aestivum*) and annual ryegrass (*Lolium multiflorum*), lead to increase levels of hydrogen peroxide in wheat compared to wheat grown alone throughout the whole growing season (Agostinetto et al., 2017). Overall, crops that are exposed to light competition during earlier phenological stages have an increase in ROS concentration and activity; however, the long-term response is dependent on crop and the amount/type of competition being experienced.

Morphological changes within plants due to early season light competition can influence the plants ability to cope with other environmental stressors (Afifi and Swanton, 2012). Cropping system diversity can help agriculture systems tolerate environmental stressors (Roesch-McNally et al., 2018). Cropping system diversity can be achieved by interseeding crops together; however, little is understood on how plants experience stress in these systems. Previous studies have investigated the relationship of light between monoculture crop-weed interactions; however, it is also important to determine the interactions occurring between crops and weeds in the more diverse interseeded systems. Interseeding corn and alfalfa can increase crop diversity and has many economic and environmental benefits (Osterholz et al., 2019; Osterholz et al., 2020). Interseeding these two crops will result in limited light quantity and changes in light quality; thus, it is important to understand the possible underlying stress mechanisms that can drive these changes. Therefore, to better understand the stress experienced by plants in the interseeded system, it is important to 1) determine ROS responses in interseeded corn and alfalfa and 2) evaluate if the ROS stress response is different between crop-crop interactions compared to crop-weed interactions in this system.

Material and Methods

Plant Material

Greenhouse experiments were conducted for 16 days at Michigan State University in East Lansing, MI. The study followed a completely randomized design with four replications. Treatments included six levels of corn, alfalfa, and Japanese millet (*Echinochloa esculenta*) (surrogate weed) plant densities: 1:0:0, 0:4:0, 1:4:0, 1:0:4, 0:4:4, and 1:4:4. The corn silage hybrid utilized in this study was G89A09 (Golden Harvest, Minnetonka, MN). The alfalfa hybrid utilized in this study was FSG430LHRR (DLF Pickseed, Halsey, OR). Japanese millet seed was purchased from La Crosse Seed (La Crosse, WI). For all treatments containing corn, corn was planted into the center of each pot. For alfalfa treatments four seeds were planted in a square approximately 3 cm from the edge of the pot. Four Japanese millet seeds were planted in a square, perpendicular to the alfalfa in shared treatments, 3 cm from the edge of the pot. All three crops were planted on the same day during mid-morning to reduce variability. Greenhouse temperature was set to 27°C (diurnal range 25-29°C) temperature and 16-hour photoperiod. The plants were grown in 15.3 cm (849.5 cm^3) pot with a peat and perlite mixture (SureMix, Michigan Grower Products, Inc, Galesburg, MI). Plants were watered daily. Plants were harvested 7, 10, and 16 days after planting during mid-morning at the soil line, placed in liquid nitrogen, and stored in a -80°C freezer until use. Plants were ground with the use of a Tissue Lyser II (Qiagen, Hilden, Germany) for 90 s.

Enzyme Assays

Protein Extraction

Approximately 250 g of ground plant tissue were suspended in a cold extraction buffer, containing 0.1 M tris HCl (pH 7.5), 2 mM EDTA, 1 mM DTT, 1 mM PMSF, and 5% PVPP. Samples were vortexed until homogenized, then filtered through Mira cloth (EMD Millipore, NY) in a 30 s centrifuge 3000 rpm at 21°C. Filtrates were centrifuged 15,000 rpm for 10 minutes at 4°C, the leftover supernatant was subjected to ammonium sulfate (0-80%) precipitation for one hour, and then the samples were centrifuged for 15 minutes at 15,000 rpm at 4°C. The resulting pellet was resuspended in potassium phosphate buffer (pH 7, 50 mM) then desalted via Zeba™ Spin Desalting Columns (Thermo Fisher Scientific, Waltham, MA). Total protein concentration was determined based on Bradford (1976). Once concentration was determined, samples were stored in -80°C for further analysis.

Dehydrascorbate Reductase Assay

Dehydrascorbate reductase (DHAR) activity was determined as described by Dixon et al. (2002) in 90 mM Potassium Phosphate buffer (PPB, 6.5 pH), containing 5mM glutathione, 0.5 mM of dehydrascorbate, and 15 ug of protein extract. The blank contained all compounds listed above except dehydrascorbate. Enzyme activity was determined by measuring the increase in absorbance at 256 nm over 120 s at 21°C.

Glutathione *S*-transferase Assay

Glutathione *S*-transferase (GST) activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was determined as described in Habig et al. (1974) in 200 mM PPB (7.6 pH) containing 2 mM glutathione, 40 mM CDNB, and 30 ug of plant protein. The blank contained all compounds listed above except CDNB. Enzyme activity was determined by measuring the increase in absorbance at 340 nm over 300 s at 21°C.

Superoxide Dismutase Assay

Superoxide dismutase (SOD) activity was determined as described in Dhindsa et al. (1981) with 200 mM of methionine, 0.26 mM nitro blue tetrazolium chloride, 15 mM EDTA, riboflavin 30 mM, sodium carbonate 672 mM, 338 mM PPB (7.6 pH), and 30 ug of protein extract. Reactions were kept under light for 10 mins, then absorbance was recorded at 560 nm. The reaction mixture without plant extract and irradiation served as the blank.

Statistical Analysis

GST and DHAR activity were determined by using linear regression to assess the influences of plant species, competition, and harvest timings by using the lm function in R (R, 2020). SOD activity and plant biomass was analyzed by creating a linear mixed effects model with the lmer function from the lme4 package in R (R, 2020). Plant competition, species, and harvest timing were considered fixed effects, and rep was considered a random effect. Differences in means were investigated using Tukey's HSD post hoc test in the emmeans package in R (R, 2020).

Results and Discussion

Glutathione S-transferase

Expression levels differ over time

GST levels were impacted by a three-way interaction between plant combination, assay plant, and harvest date ($p<0.0001$). Corn alone reaction rate was greatest 7 DAP, then decreased by 135% 10 DAP compared to 7 DAP (Table 4.1). Corn GST reaction rate 16 DAP increased by 313% compared to 10 DAP, but was 32% lower than 7 DAP (Table 4.1). When alfalfa was grown alone, GST reaction levels were negative 7, 10, and 16 DAP; however, when assayed 7 DAP reaction rates were 47 and 46% greater than reaction rates 10 and 16 DAP (Table 4.1). The corn GST reaction rate, grown in corn and alfalfa combination, was greatest 7 DAP, then decreased by 57 and 45% 7 and 10 DAP, respectively. In the alfalfa and corn combination, alfalfa was negative for all three harvest times, with the 16 DAP having the lowest reaction rate compared to 7 and 10 DAP (Table 4.1). Corn grown with Japanese millet reaction rate was greatest 10 DAP, which did not differ from 7 DAP. The corn GST reaction rate 16 DAP was 6.3 and 6.8 times less than 7 and 10 DAP (Table 4.1). Japanese millet grown in competition with corn had a positive reaction rate 7 and 16 DAP; however, reaction rate was negative 10 DAP (Table 4.1). Japanese millet grown in competition with corn GST reaction rates 10 and 16 DAP were 112 and 67% less than 7 DAP (Table 4.1). Alfalfa in competition with Japanese millet had a positive reaction rate 7 DAP, but negative 10 or 16 DAP (Table 4.1). Furthermore, the 7 DAP reaction rate for alfalfa was 228 and 245% greater than 10 and 16 DAP, respectively (Table 4.1).

Japanese millet in competition with alfalfa had a negative reaction rate 7 and 16 DAP planting; however, the reaction rate was positive 10 DAP (Table 4.1). The reaction rate 10 DAP was 317 and 321% greater than the reaction rate 7 and 16 DAP, respectively (Table 4.1). Corn grown in the three-way competitive environment had similar GST reaction rates 7, 10, and 16 DAP. Japanese millet follows the same trend grown in competition with corn and alfalfa in which the GST reaction rate is similar between 7,10, and 16 DAP (Table 4.1). Alfalfa grown in competition with Japanese millet and corn was negative across all harvest times. Alfalfa in competition with corn and Japanese millet 16 DAP GST reaction rate was 204 and 270% less than the reaction rate 7 DAP and 10 DAP (Table 4.1).

Plant competition modifies GST reaction levels

GST reaction rates when corn was grown alone was greater than all other GST reaction rates 7 DAP. Furthermore, this reaction rate was the greatest when compared to all other reaction rates across DAP for any plant combination (Table 4.1). When corn was grown in competition with alfalfa, Japanese millet, or alfalfa-Japanese millet 7 DAP GST reaction rates decreased by 50%, 58%, and 41% when compared to the corn only treatment, respectively (Table 4.1). When corn and alfalfa were grown together, alfalfa GST reactions rates were 200% less than corn GST reaction rates 7 DAP (Table 4.1). When corn and Japanese millet were grown together, corn GST scavenging enzyme reactions rates were two times greater than Japanese millet reaction rates 7 DAP (Table 4.1). When alfalfa and Japanese millet were grown together, alfalfa GST reaction rates were 200% greater than Japanese millet reaction rates, suggesting alfalfa plants can buffer Japanese millet (surrogate weed) competition without the presence of corn 7 DAP (Table 4.1). When corn was grown in competition with alfalfa and Japanese millet, corn GST reaction rates were the greatest, specifically 130 and 140% greater than the alfalfa and Japanese millet grown in the same three-way plant competition 7 DAP (Table 4.1). Interestingly, GST reaction rates were similar when alfalfa was grown alone compared to alfalfa grown in competition with Japanese millet and corn 7 DAP (Table 4.1).

The corn reaction rate was 123 and 74% greater than the alfalfa and Japanese millet, reaction rate, respectively, when all three are grown in competition together (Table 4.1). Alfalfa grown alone had a similar GST reaction rate as alfalfa grown in competition with Japanese millet 10 DAP (Table 4.1). Japanese millet grown in competition with alfalfa and corn had a similar reaction rate as the Japanese millet grown in competition with alfalfa 10 DAP (Table 4.1). Corn when grown in competition with alfalfa or with Japanese millet had similar rates and were 267 and 179% greater than corn grown alone 10 DAP (Table 4.1). Japanese millet grown in competition with alfalfa had a reaction rate 2.7 times greater than the alfalfa grown alone 10 DAP (Table 4.1). Interestingly, all alfalfa grown 10 DAP had a negative reaction rate.

Corn grown alone had the greatest GST reaction rate of all other plants assayed in the 16 DAP harvest time. The GST reaction rate when corn was grown alone was 64, 91, and 35% greater than corn grown with alfalfa, corn grown with Japanese millet, and corn grown with Japanese millet and alfalfa, respectively 16 DAP (Table 4.1). Alfalfa grown in competition with corn had the greatest reaction rate for all other alfalfa grown 16 DAP; however, all alfalfa grown 16 DAP had a negative reaction rate (Table 4.1). Alfalfa grown alone 16 DAP had a similar reaction rate as the alfalfa grown in competition with the corn and Japanese millet; however, these reaction rates differed from the alfalfa grown with corn or with Japanese millet, which had similar reaction rates (Table 4.1). Corn and Japanese millet had a similar reaction rate when grown in competition with each other 16 DAP. GST reaction rates of Japanese millet grown with corn were 628 and 85% greater than Japanese millet grown with alfalfa or with corn and alfalfa

16 DAP (Table 4.1). Thus, suggesting that at 16 DAP alfalfa induces a high stress response from the surrogate weed, Japanese millet. Corn had a greater reaction rate than alfalfa when grown in competition together 16 DAP. Corn had the greatest reaction rate when all three plants were grown together, with it being 204 and 97% greater than the alfalfa and Japanese millet GST reaction rates (Table 4.1).

Corn plants grown in the three-way plant combination 7 DAP, was not different from the corn only reaction rate 16 DAP (Table 4.1). Japanese millet grown in competition with alfalfa and corn 10 DAP GST reaction rates were similar for alfalfa and Japanese millet (Table 4.2). Alfalfa GST reaction rates when grown in competition with corn 7 DAP are similar to the alfalfa alone reaction rate 10 and 16 DAP (Table 4.1). A similar trend is observed 10 DAP, where the alfalfa reaction rate grown in competition with Japanese millet was similar to the alfalfa grown alone 10 and 16 DAP (Table 4.1). Corn grown in competition with alfalfa and Japanese millet 7 and 10 DAP resulted in a similar GST reaction rates to the corn only reaction rate 16 DAP (Table 4.1). Japanese millet grown in competition with corn and alfalfa 7 DAP had a similar reaction rate as the Japanese millet grown in competition with corn 10 DAP and 16 DAP (Table 4.1). The different levels of competition having a similar reaction rate as the following DAP one competition level less suggests that the plants experiencing stress are potentially aging faster due to the increase in competition.

GST is a ubiquitous scavenging enzyme within plants (Bartling et al., 1993; Dixon and Edwards, 2009; Wagner et al., 2002); therefore, it reacts to all types of stress within the plant. Additionally, the majority negative reaction rates for alfalfa supports the idea that alfalfa does not produce GST as a scavenging enzymes readily to compensate with stress within the timeframe assessed in this study. Interestingly, corn had a greater GST reaction rate compared to

Japanese millet and alfalfa grown in three-way competition across all DAP. This suggests that corn upregulates GST enzyme activity quickly to buffer stress when compared to alfalfa and Japanese millet. Reactive oxygen species can play a role in cell development and death, which may explain the similarities between plants grown in competition and alone (Dietz, 2011; Mignolet-Spruyt et al., 2016; Noctor et al., 2012); therefore, the GST enzyme may play a role in delaying cell death, but not stopping cell aging of the plant experiencing increased stress. Although this is only one detoxifying enzyme within the plant, the response to stress may be multifaceted.

Dehydrascorbate Reductase

Expression levels differ over time

A three-way interaction between plant combination, assay plant, and DAP was significant for DHAR (p<0.0001). Corn grown alone had a positive DHAR reaction rate 7 DAP; however, it is negative 10 and 16 DAP. Furthermore, the reaction rate was similar between 10 and 16 DAP and was 122 and 114% less than 7 DAP. Alfalfa DHAR reaction rates followed a similar pattern as the corn alone did, with the DHAR reaction rate being negative 10 and 16 DAP, while positive 7 DAP. Specifically, the 7 DAP DHAR reaction rate was 972 and 1,080% greater than 10 and 16 DAP. Corn grown in combination with alfalfa was negative for all three DAP, and 7 DAP DHAR reaction rate was 11.3 and 14.18 times greater than the reaction rate 10 and 16 DAP. For alfalfa grown with corn, the DHAR reaction rate was negative 7 DAP, however, positive 10 and 16 DAP. Specifically, when alfalfa was grown with corn the DHAR reaction rate 10 DAP was 216 and 57% greater than 7 and 16 DAP. Corn and Japanese millet grown together both had positive reaction rates 7 and 10 DAP; however, both had negative reaction rates by 16 DAP. The corn grown in competition with Japanese millet reaction rate was 58 and 276% greater

10 DAP compared to 7 and 16 DAP, respectively. Japanese millet reaction rates were 2.8 and 40.5 times greater 7 DAP compared to 10 and 16 DAP when grown in competition with corn. Alfalfa grown in competition with Japanese millet had the greatest reaction rate 16 DAP which is similar to the reaction rate 7 DAP. Furthermore, alfalfa grown in competition with Japanese millet DHAR reaction rate 10 DAP was 283 and 312% less than 7 and 16 DAP, respectively. Japanese millet grown in competition with alfalfa had a positive reaction rate 7 and 10 DAP and a negative 16 DAP. Japanese millet grown in competition with alfalfa 7 and 16 DAP was 9.1 and 3.2 times less than 10 DAP reaction rate. Corn grown in competition with Japanese millet and alfalfa DHAR reaction rates were positive 7, 10, and 16 DAP. However, corn grown in competition with Japanese millet and alfalfa DHAR reaction rate was greatest 7 DAP, then decreased by 60 and 73% 10 and 16 DAP. Alfalfa grown in competition with corn and Japanese millet had a negative reaction rate 7 and 16 DAP, however, it was positive for 10 DAP. The negative reaction rates were similar 7 and 16 DAP. Japanese millet grown in competition with alfalfa and corn was negative for all three harvest times, with the greatest reaction rate being 7 DAP. Japanese millet grown in competition with alfalfa and corn DHAR reaction rates 10 and 16 DAP were 237 and 140% less than the 7 DAP.

Plant competition modifies DHAR reaction levels

Corn grown alone or in competition with alfalfa and Japanese millet 7 DAP DHAR reaction rates were similar. However, the greatest corn DHAR reaction rate was when corn was grown alone (Table 4.2). The reaction rate for corn alone decreased by 108, 70, and 4% from corn grown in combination with alfalfa, Japanese millet, and in the three-way competitive environment with Japanese millet and alfalfa 7 DAP (Table 4.2). The greatest reaction rate for alfalfa occurred when grown in competition with Japanese millet 7 DAP, which was 5.2 times greater than alfalfa grown alone 7 DAP (Table 4.2). When corn and alfalfa were grown in competition with each other, the corn reaction rate was 218% greater than the alfalfa reaction rate 7 DAP. Within the three-way plant competitive environment, Japanese millet and alfalfa reaction rates were 129 and 167% less than the corn reaction rate 7 DAP (Table 4.2). Japanese millet had a 6.75 times greater reaction rate than the corn when grown in competition together 7 DAP. When alfalfa and Japanese millet were grown together, the alfalfa DHAR reaction rate was 7.5 times greater than the Japanese millet DHAR reaction rate (Table 4.2).

The greatest alfalfa DHAR reaction rate occurred when grown in competition with corn compared to all other alfalfa harvested 10 DAP. Corn reaction rate was the greatest when grown with Japanese millet and the DHAR reaction rate was negative when grown alone 10 DAP (Table 4.2). Corn and Japanese millet grown alone 10 DAP had a similar reaction rate as when grown together 10 DAP (Table 4.2). Additionally, corn grown with Japanese millet had a similar reaction rate as corn grown in the three-way plant combination of corn, alfalfa, and Japanese millet. However, Japanese millet grown with corn and with corn and alfalfa were 50 and 168% less than the Japanese millet grown with the alfalfa 10 DAP (Table 4.2). Alfalfa had a positive and 157% greater reaction rate than corn when grown together 10 DAP. Additionally, in the three-way plant competition environment, alfalfa had a positive reaction rate, which is contrary to the alfalfa grown alone which was negative 10 DAP (Table 4.2).

Corn had the greatest reaction rate 16 DAP when grown in competition with alfalfa and Japanese millet, as it was 158, 603, and 583% greater than corn grown alone, with alfalfa, or with Japanese millet, 16 DAP respectively (Table 4.2). Alfalfa grown with Japanese millet DHAR reaction rate was 110, 63, and 134% greater than alfalfa grown alone, with corn, or with corn and Japanese millet 16 DAP (Table 4.2). Alfalfa grown with Japanese millet or with corn had a positive reaction rate, while alfalfa grown alone or with corn and Japanese millet was negative 16 DAP. Japanese millet reaction rates were similar when grown in competition with alfalfa or with corn and alfalfa 16 DAP (Table 4.2). The corn reaction rate was 238 and 370% greater than the alfalfa and Japanese millet reaction rates when all three plants were grown in competition together 16 DAP (Table 4.2).

Alfalfa grown in competition with corn 7 DAP had a similar reaction rate as the alfalfa grown alone 10 and 16 DAP (Table 4.2). Corn grown in competition with alfalfa had a similar trend of reaction rates 7 DAP being similar to corn grown alone 10 and 16 DAP (Table 4.2). Alfalfa grown in competition with corn and Japanese millet 7 DAP had a similar reaction rate as alfalfa grown in competition with Japanese millet and alfalfa grown alone 10 DAP and alfalfa grown alone for 16 DAP (Table 4.2). Japanese millet in competition with corn and alfalfa harvested 10 DAP had a similar reaction rate as Japanese millet grown in competition with alfalfa 16 DAP (Table 4.2). The similarities across time and different plant combinations may be driven by plant competition increasing plant maturity over time. Specifically in corn shade avoidance responses speed up corn maturity rates (Afifi and Swanton, 2012). This is similar to the observations made in the GST assay, which suggests that the upregulation of enzymes may be related to plant age (Dietz, 2011; Mignolet-Spruyt et al., 2016; Noctor and Foyer, 2016). As plant competition increases, the upregulation of enzymes may limit cell death, but not cell aging.

DHAR functions as a scavenging enzyme that react to various types of stress, specifically involved in chloroplasts redox stress (Ushimaru et al., 2006). Alfalfa and corn grown alone have a similar trend positive reaction rates 7 DAP, but negative reaction rates 10 and 16 DAP in the DHAR assay. However, these trends are not exhibited in the plants grown in competition, for example, corn grown in competition with Japanese millet and alfalfa had a positive reaction rate for all DAP. The differences between DHAR and GST assay may be driven by the different roles the enzymes play within plants and the inherent concentration of scavenging enzymes in early season competition may differ. This study provides foundational knowledge that GST and DHAR enzymes change over time and are modified by level of competition, further detailed categorization of these enzymes roles within each of these plants under interseeded environments is warranted.

Superoxide Dismutase

Superoxide dismutase (SOD) absorbance did not differ amongst treatments (p=0.4; Figure 4.1). This is contrary to previous studies that reported SOD was upregulated upon emergence in soybeans when exposed to R:FR light and then decreased over time (Mckenzie-Gopsill et al., 2016). Superoxides are commonly generated under high photosynthetic activity and oxidative stress, which triggers the expression of SOD (Bowler et al., 1992). SOD activity did not change between level of plant competition, assay plant, or duration of competition, perhaps due to lack of high photosynthetic activity and oxidative stress.

Scavenging enzymes used to detoxify ROS differ between crops and weeds, based on species, plant age, and the environment they were grown in. We observed this in the GST and DHAR assays noted by the three-way interaction between assay plant, plant combinations, and DAP. Positive enzymatic reaction rates in the GST and DHAR assay have two implications, 1) the plant generated more GST or DHAR to detoxify the stress or 2) GST or DHAR levels are constitutively expressed at a greater rate without stress and plants are already primed to deal with the additional stressors in this study. Negative reaction rates have three implications, 1) plants do not perceive the stress, 2) plants perceives the stress but has no way to compensate for it, or 3) plants use different ROS scavenging enzymes not assayed in this study to detoxify stress.

Overall, for each harvest, plants grown in competition had different reaction rates for GST and DHAR, than the plant grown alone; therefore, plant competition in this study changes the enzymatic reaction rate no matter what the plants were grown in competition with.

In the GST assay, Japanese millet had a similar reaction rate across DAP when grown in competition with corn and alfalfa. Corn had the same trend with the GST reaction rate being similar across harvest dates when grown in competition with alfalfa and Japanese millet. However, the greatest GST reaction rate across all DAP was in corn in the three-way plant competition combination. Alfalfa had a negative GST reaction rate for all DAP when grown in the three-way plant combination. Therefore, corn and Japanese millet are compensating for ROS better than the alfalfa and at a constant level across DAP. Previous research has shown that GST activity is often greater in crops compared to weeds (Hatton et al., 1996), our corn results support this finding, however the alfalfa results do not. Lack of GST activity in alfalfa may be due to the low tolerance for competition alfalfa can withstand in the establishment year (Dillehay et al., 2011). Alternatively, the lack of GST activity in alfalfa may be due to other enzymes playing a role to detoxify ROS stress. DHAR activity was often minimal, however, DHAR rates were often greatest 7 DAP for alfalfa and corn grown alone and steadily declined 10 and 16 DAP. This trend suggests that DHAR activity decreases with age in alfalfa and corn. Japanese millet in competition with alfalfa and corn had negligible DHAR activity, which is supported by Hatton et al. (1996) conclusions that weeds have less ROS scavenging enzymes than crops.

Crops interpret competition from weeds and other crops at a molecular level, resulting in changes to ROS enzymatic activity. These changes are linked to interspecific stress, supported by our results in which plants grown in competition had different enzymatic rates than plants grown alone. The similarities between SOD activity across treatments suggests that SOD activity is not differentially regulated by the treatments evaluated in this study. Alternatively, as SOD is a large family of enzymes, our assay may have not been sensitive enough to detect changes.

Future research should target narrowing the role of these enzymes in interseeded systems. With further understanding of how corn and alfalfa perceive stress from each other, there is the possibility to modify interseeded systems to increase competition resulting in an unsuitable environment for weeds. Furthermore, understanding physiological stress responses can result in breeding or selection efforts for plants that can tolerate changes in light quality or quantity, which occurs in an interseeded system. Additionally, as plants grown in competition had an enzymatic response similar to older plants grown alone, there is the possibility to research the role of ROS in plant maturity. Reactive oxygen species and their scavenging enzymes are essential to many plant functions, one of them being produced as a resultant of stress, which can change based on the level of competition the plants are experiencing. Therefore, in the case of interseeding corn and alfalfa, both crops experience stress at a molecular level when interseeded together, which is different depending on the presence of weeds as well. Overall, this study lays the foundation to optimize interseeded systems by understanding how stress is perceived and detoxified in these highly competitive environments.

APPENDIX

Table 4.1. Mean (SE) glutathione *S*-transferase (GST) enzymatic reaction rates for plants grown in different plant combinations of corn, alfalfa, and Japanese millet (surrogate weed) at three different harvest times 7, 10, and 16 days after planting.

Plant Combination	Assay Plant	Days after Planting		
		7	10	16
		µmol/ml/min		
Corn	Corn	2.398 (0.18) s[1]	-0.851 (0.19) cfl	1.818 (0.19) m
Alfalfa	Alfalfa	-0.692 (0.19) abc	-1.308 (0.19) gh	-1.288 (0.19) g
Corn - Alfalfa	Corn	1.185 (0.19) de	0.508 (0.19) kq	0.651 (0.19) jk
Corn - Alfalfa	Alfalfa	-1.268 (0.19) fgh	-0.797 (0.19) bcl	-1.993 (0.19) r
Corn - Japanese Millet	Corn	0.991 (0.19) dij	1.071 (0.19) dei	0.157 (0.20) opq
Corn - Japanese Millet	Japanese Millet	0.703 (0.19) ijk	-0.085 (0.20) no	0.228 (0.20) opq
Alfalfa - Japanese Millet	Alfalfa	1.170 (0.19) de	-1.506 (0.19) gh	-1.700 (0.19) hr
Alfalfa - Japanese Millet	Japanese Millet	-1.186 (0.19) fgl	0.546 (0.19) kq	-1.208 (0.19) fg
Corn - Alfalfa - Japanese Millet	Corn	1.403 (0.19) dem	1.430 (0.19) em	1.176 (0.19) de
Corn - Alfalfa - Japanese Millet	Alfalfa	-0.402 (0.20) abn	-0.331 (0.20) an	-1.226 (0.19) fg
Corn - Alfalfa - Japanese Millet	Japanese Millet	0.007 (0.20) nop	0.364 (0.20) kpq	0.034 (0.20) nop

[1] Means followed by the same lowercase letter are not significantly different for the three-way interaction of plant combination, assay plant, and harvest date ($p \geq 0.05$).

Table 4.2. Mean (SE) dehydrascorbate reductase (DHAR) enzymatic reaction rates for plants grown in different plant combinations of corn, alfalfa, and Japanese millet (surrogate weed) at three different harvest times 7, 10, and 16 days after planting.

Plant Combination	Assay Plant	Days after Planting 7	 10	 16
		μmol/ml/min		
Corn	Corn	0.123 (0.02) jk[1]	-0.027 (0.02) bcfln	-0.018 (0.02) abcfn
Alfalfa	Alfalfa	0.025 (0.02) abcde	-0.218 (0.02) gp	-0.245 (0.02) g
Corn - Alfalfa	Corn	-0.011 (0.02) abcdf	-0.124 (0.02) mq	-0.156 (0.02) pq
Corn - Alfalfa	Alfalfa	-0.251 (0.02) g	0.216 (0.02) ir	0.092 (0.02) hj
Corn - Japanese Millet	Corn	0.036 (0.02) adeh	0.085 (0.02) ehj	-0.150 (0.02) q
Corn - Japanese Millet	Japanese Millet	0.243 (0.02) i	0.086 (0.02) ehj	-0.006 (0.02) abcdf
Alfalfa - Japanese Millet	Alfalfa	0.143 (0.02) jk	-0.078 (0.02) lmno	0.166 (0.02) kr
Alfalfa - Japanese Millet	Japanese Millet	0.019 (0.02) abcde	0.173 (0.02) kr	-0.077 (0.02) lmno
Corn - Alfalfa - Japanese Millet	Corn	0.118 (0.02) jk	0.047 (0.02) deh	0.031 (0.02) acdeh
Corn - Alfalfa - Japanese Millet	Alfalfa	-0.080 (0.02) lmno	0.129 (0.02) jk	-0.057 (0.02) flno
Corn - Alfalfa - Japanese Millet	Japanese Millet	-0.035 (0.02) bfln	-0.118 (0.02) moq	-0.084 (0.02) lmo

[1] Means followed by the same lowercase letter are not significantly different for the three-way interaction of plant combination, assay plant, and harvest date ($p \geq 0.05$).

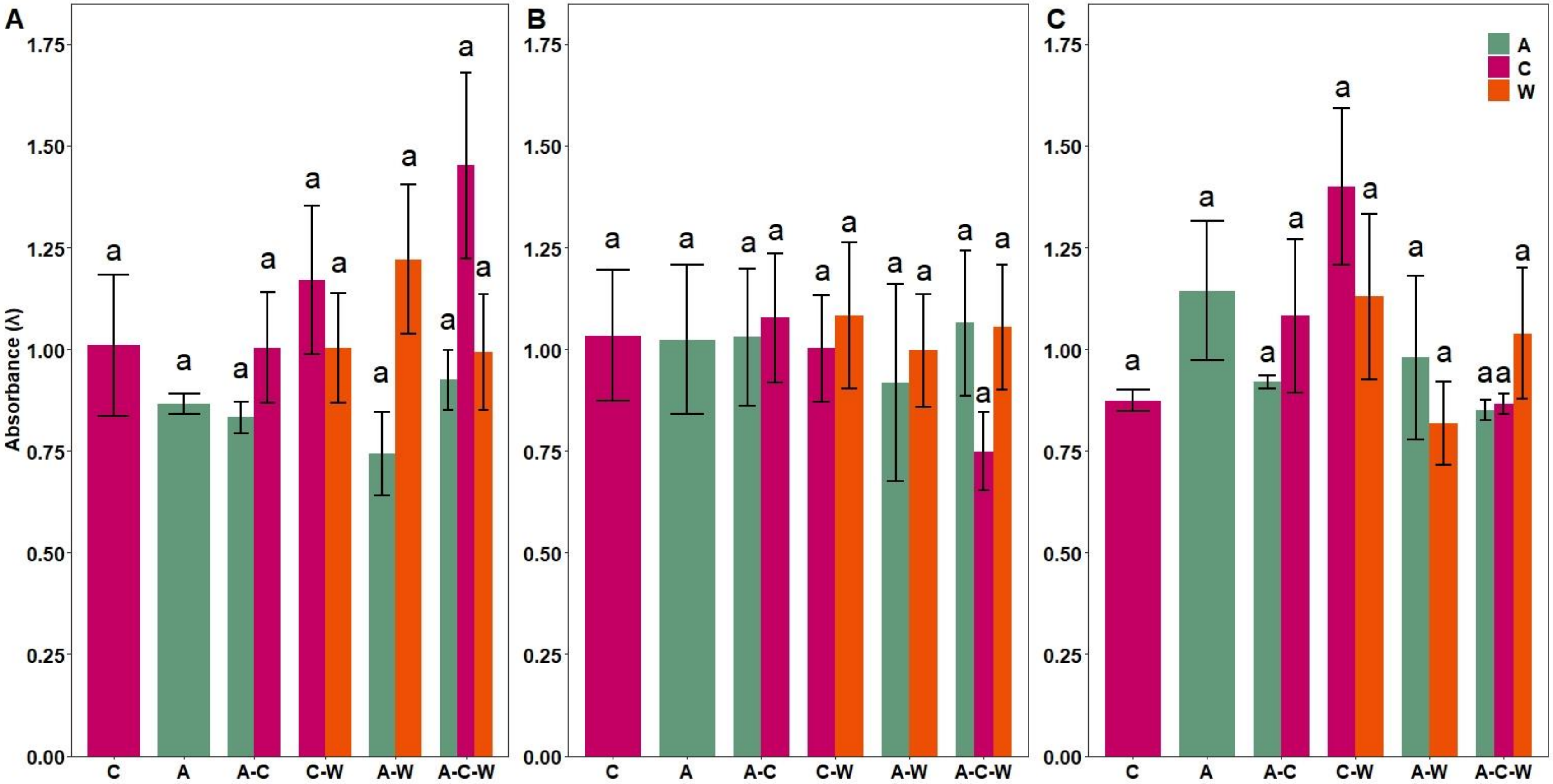

Figure 4.1. Mean (SE) superoxidase dismutase absorbance (λ) for plants grown in different plant combinations of corn (C), alfalfa (A), and Japanese millet (surrogate weed, W) at three different harvest times 7 (A), 10 (B), and 16 (C) days after planting. Means followed by the same lowercase letter are not significantly different for the three-way interaction of plant combination, assay plant, and harvest date ($p \geq 0.05$).

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